Carotenoids
Volume 4: Natural Functions

Chapter 12

Carotenoids in Aquaculture: Fish and Crustaceans

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A. Introduction

This Chapter deals with selected topics on the use of carotenoids for colouration in aquaculture and incudes examples from ecological studies which support our understanding of functions and actions of carotenoids and colouration in fishes and crustaceans. Animal colours may be physical or structural in origin [1], *e.g.* Tyndall blues and iridescent diffraction colours, or they may be due to pigments, including carotenoids (*Chapter 10*).

Many marine and freshwater animals, including fish and crustaceans, owe their bright colouration to carotenoids. In captivity, such animals require a diet supplemented with carotenoids to obtain a colour that is typical for the species and to meet other requirements.

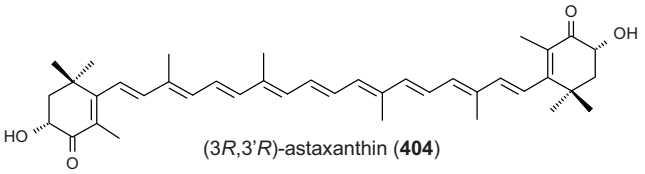
As discussed in *Chapter 11*, natural animal colouration conveys information to other individuals *via,* for instance, carotenoid-based sexual signals that influence mate choice [2] or warning colours that deter predators [3] and thus play an important role in certain ecological interactions. For humans, colours in food elicit in the consumer psychological and physiological expectations based on experience, tradition and customs, and are linked to anticipated quality [4]. The colour of a food item is a cue used to form judgements about desirability.

Carotenoids have been used extensively as additives to provide food colours, either by applying them to foods directly, or by supplying them indirectly in the diets (feed) of animals that are used for food [5]. The conspicuous colouration of most seafood is due to carotenoids

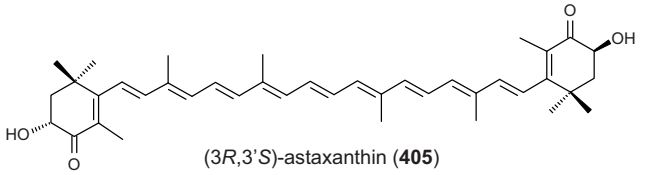
[6]. Most of the applications to farmed species involve indirect colouration. External colouration is important to the ornamental fish/animal hobbyist and the farming industry that supplies them. Carotenoids should be included in the diet of many ornamental species to avoid the dull colouration that would otherwise be acquired by many animals kept in captivity.

Capture fisheries are not expanding, but food fish production still grew at an annual rate of 3.1% in the period between 1987 and 1997, driven by aquaculture, which showed a global increase of 11.2% [7]. In 1997, production of high-value finfish (certain salmonid and sparid fish species) represented only 5% of the total aquaculture production, but generated 39% of the export revenue; 76% of the production was in developed countries. In contrast, developing countries accounted for 98% of the production of crustaceans. Flesh colour is among the most important quality parameters for salmonid fishes [8], and pigment feeding is regarded as the most important management practice for successful marketing of farmed Atlantic salmon *(Salmo salar)* [9]. The difficulty of obtaining natural colouration in captivity has been a bottleneck for successful commercialization of other species such as red seabream (*Chrysophrys major*), gilthead seabream (*Sparus aurata*), and red porgy (*Pagrus pagrus*).

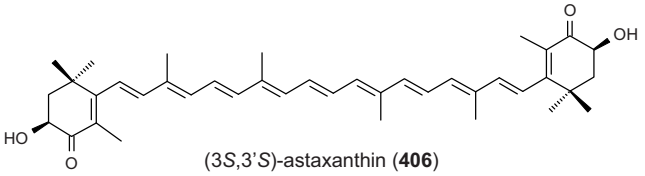
Astaxanthin (**404-406**) and canthaxanthin (**380**), either alone or in combination, are the carotenoids most commonly used for pigmentation in farming of aquatic animals.



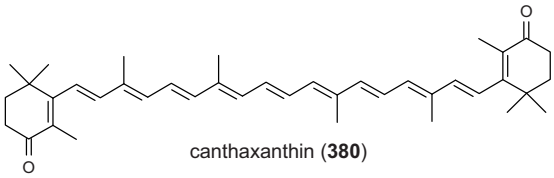
(3*R*,3'*R*)-astaxanthin (**404**)



(3*R*,3'*S*)-astaxanthin (**405**)



(3*S*,3'*S*)-astaxanthin (**406**)



canthaxanthin (**380**)

This Chapter covers developments in research on carotenoid pigmentation in fish and crustaceans in general and highlights collected experiments on aquacultured species during the past decade. The main focus is on muscle of salmonid fishes and integumentary pigmentation of certain other fish species and crustaceans. Comprehensive reviews have dealt with carotenoid distribution and biochemistry in animals [10], and marine animal carotenoids [11,12]. Earlier literature on carotenoid pigmentation in aquaculture is treated in previous reviews [6,13-19]. The absorption and metabolism of carotenoids in fishes and crustaceans was treated in *Volume 3, Chapter 7* [20]; only more recent developments are reviewed here.

B. Market Issues

Aquaculture is developing, expanding, intensifying and diversifying in most regions of the world, and represented 33.7% of the total world fisheries in 2005 [21]. Due to the lack of growth in capture fisheries since the late 1980s, aquaculture has to meet the growing global demand for aquatic food. According to FAO, global aquaculture production has increased at an average annual growth rate of 8.8% over the past 50 years to 59.4 million tonnes (including plants) with a total value of US$ 70.3 billions by 2004. Of this, China produced almost 70% of the production volume and the rest of Asia and the Pacific region 22% [21]. Of the global production of cultured penaeid shrimp (2.5 million tonnes), oysters (4.3 million tonnes), cyprinid fish (18.3 million tonnes) and plants (13.9 million tonnes), 87% or more is produced in Asia and the Pacific region, whereas about 56% of the farmed salmonid fishes are produced in the northern part of Western Europe. In 2004, the global production of salmonid fishes was about 2 million tonnes, and of crustaceans about 3.7 million tonnes [21]. For Norway, in 2004 the second largest fish exporter after China, sales of salmonid fishes were about 0.65 million tonnes in 2005 [22], but Norwegian fish production from aquaculture is estimated to reach 5 million tonnes by 2020, with Atlantic salmon representing 2 million tonnes [23]. Asia provides more than 50% of the global supply of ornamental freshwater and marine fish, which had an estimated wholesale value of US$ 900 million and a retail value of US$ 3 billion in 2000 [24]. The number of species traded globally is into the thousands, and USA is considered the largest market for ornamental fish [25]. Approximately 90% of the freshwater ornamental fish that are traded are cultured, whereas the marine ornamental species predominantly are collected from the wild [26]. The ornamental animal and plant

trade is expanding, as illustrated by a recent survey of fish species in the upper Paraná River flood plain in Brazil, where more than 83% of the 101 captured taxa were considered ornamental [27]. Capture of marine ornamental fish, especially, raises important issues related to mortality, conservation and trade regulation [28].

Only a small fraction of the total volume of aquacultured species, reared intensively or semi-intensively, require diets supplemented with carotenoids to obtain acceptable colouration. The world market for carotenoids is discussed in *Volume* 5, *Chapter 4*. Astaxanthin is the major carotenoid used to supplement diets, and it would require about 130 tonnes of astaxanthin to feed the global salmonids produced by aquaculture a diet containing 50 mg astaxanthin per kg. About 90% of the astaxanthin is produced by chemical synthesis by two major companies and the current price is in the range from US$ 1500 to US$ 2000 per kg. In comparison, algal products containing astaxanthin esters that are intended for the human market sell for about US$ 5400 and upwards per kg astaxanthin. The recently developed polar water-soluble astaxanthin derivatives such as the disuccinates and diphosphates have interesting properties and may find a future application in aquaculture [29]. Since most of the shrimp and prawn production is extensive or semi-intensive, only a relatively small amount of feed supplemented with carotenoids is used. It is required in intensive production systems, however.

Nutrient requirements for ornamental fish are poorly known [30], and only a few papers are found on the supplementation of various carotenoid sources to such species. Among recent investigations, it was found that inclusion of 8% *Spirulina* was required to obtain optimum colouration of red swordtail (*Xiphophorus helleri*) [31], and that formulated synthetic astaxanthin was a better source than the microalga *Chlorella vulgaris* for skin pigmentation of goldfish (*Carassius auratus*) in terms of total carotenoid content, though different hues may be obtained [32,33]. The global production of tilapia (a common name used for about 70 species in the family Cichlidae) is about 1.8 million tonnes [21]. The red skin colour of the tilapia *Oreochromis niloticus* shows single gene inheritance [34]; marketing has benefited from the introduction and mass selection of red coloured strains [35].

C. Importance of Pigmentation

1. Colouration - ecological and evolutionary aspects

Animal colour patterns may serve roles in regulation of temperature, intraspecific communication and evasion of predators. The signalling functions of carotenoids in relation to ecology and evolution are treated in detail in *Chapter 11*. Carotenoid-based colours often have signal effects that have evolved because they elicit responses in the recipients, such as in the decisions of mate choice in guppies [2]. Whereas the coral fish *Hypoplectrus* exhibits extraordinary gene-based colour polymorphism that may drive speciation [36], other species,

such as salmonid fishes, exhibit sexually dimorphic colour patterns. Many prey species have evolved to match their surroundings by adopting colour patterns that represent a more or less random sample of their background [37]. Crustaceans have cryptic colouration. An example is the blue-black of the carapace of lobster *Homarus gammarus* due to astaxanthin-based carotenoproteins (*Chapter 6*).

Sexual selection often relies on carotenoid-based signals. Colourful ornaments are often displayed by males, but occasionally also by females (as with two-spotted gobies [38]) and, as a determinant for sexual selection, play an important role in reproduction. The Pacific salmon (*Oncorhynchus nerka*) displays the most extreme (red) nuptial colour among the salmonid fishes. In a study with various abstract colour models it was shown that males have a strong preference, which apparently is innate, and spawn with models with a red hue [39]. Carotenoid-based ornaments may have evolved because they are limiting due to the scarcity of the carotenoids [40], which may be required for other functions such as provitamin A, immune function or as antioxidants. Thus, in the salmonid fish Arctic charr or char (*Salvelinus alpinus*) the intensity of red integumental colouration was negatively related to lymphocyte counts [41] and may therefore signal immunocompetence. In three-spined stickleback (*Gasterosteus aculeatus*), in which the male is the parental caretaker following spawning, a high astaxanthin intake was associated with increased reproductive investment and a longer lifespan [42]. A lower suceptibility to oxidative stress may explain the increased longevity. As indicated above, a male preference for redder females in two-spotted gobies may have evolved because improved phototaxis in the offspring improves foraging capability [43].

Although the information is not required for studies in evolution *per se*, it would be useful for the carotenoid biochemist to establish the physiological basis for carotenoid signals and why they are working. Controlled feeding trials with carotenoids would facilitate such studies. Poor and unpredictable zygote quality hamper the development of several aquacultured species, notably marine species. It has been suggested that introduction of breeding systems in which broodfish have the opportunity to choose their mates voluntarily (based on, for instance, external carotenoid-based colouration) may improve the offspring quality in terms of survival and growth due to improved genetic compatibility of the parents [44].

2. Salmon muscle colour

Consumers have a preference for pink-coloured salmonid fish products, and associate redder salmon with a fresher fish that has better flavour and higher quality [45]. Consumers are willing to pay significantly more for fillets of Atlantic salmon that are normal or above normal in redness [46,47]. The pleasure of eating salmon is related positively to the red colour which is interpreted as an indicator of quality and superior flavour [48-50]. Older scientific studies apparently supported this notion by indicating a favourable relationship between muscle colouration and flavour attributes in salmonid fishes; more recently, astaxanthin

concentration of raw fillets was found to correlate significantly with more intense smoke odour and less off-odour of the smoked fish [51].

The relationship between colouration and flavour may be rationalized in a number of ways. First, colouration may interact with various sensory perceptions of properties of food commodities or beverages such as flavour intensity [52]. The correspondence between colour and taste, *e.g.* sweetness, may be learned rather than innate, however [53], so that it may be consumer expectations about salmon flavour that are related to colouration. Second, carotenoids may themselves be flavour-active, may be precursors of flavour-active degradation products [54], or may interact with chemical reactions in which flavour-active compounds are produced.

The putative effects of astaxanthin concentration and redness on the intensity of salmon flavour perception have been addressed by tests with patés of salmonid fish [55], including presentation of the samples to the assessor panel under red light to mask the colour differences. Astaxanthin or redness was found to have, at the most, only a minor influence on salmon flavour, which implied that colourants can be applied directly to such products. Astaxanthin utilization can be increased from about 10% to 100% in such instances. Sexual maturation in salmonid fishes is associated with a considerable drop in muscle and whole-body concentrations of astaxanthin and canthaxanthin [56], and induces changes in chemical composition of the muscle that are related to inferior watery and tough texture, and less pronounced odour and flavour [57]. Fallacies are frequently encountered in sensory science due, among other things, to the lesser acuity of the lower senses (taste, smell) compared to vision [58], and previous reports regarding relationships between colour and taste of salmonid fishes could be attributed to factors that not were controlled for, *e.g.* the use of shrimp extracts as astaxanthin source. Traditional opinions regarding the relationship between colour and flavour may instead reflect not the carotenoids themselves but other dietary components that are able to affect flavour, or the sexual maturation status, and may have contributed to current consumer expectations and preferences.

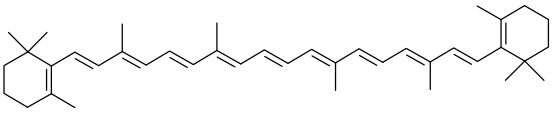
3. Embryonic development and larval growth

Carotenoids in fish eggs and fry and the role of xanthophylls as vitamin A precursors in fish were treated thoroughly in *Volume 3, Chapter 7* [20] and elsewhere [59]. Carotenoids accumulate in the reproductive organs of a wide range of organisms but, in an early assessment, they were found apparently to be unnecessary for normal embryonic development [60]. Until recently, firm knowledge on this topic was hampered by the lack of controlled experiments in which carotenoid supply was the only variable [61].

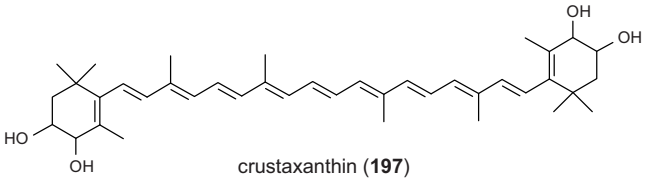
Fertilization rate has often been used as an indicator of egg or embryo quality, but this may not be valid [62]. In Atlantic salmon, supplementation of semi-purified casein/gelatine-based diets with astaxanthin led to improved growth performance in first feeding fry (*ca.* 0.2 g) [63,64], juveniles (*ca.* 1.8 g) [65] and parr (*ca.* 16 g) [66]. In contrast, no effect of feeding

diets supplemented with canthaxanthin to rainbow trout broodstock, before spawning, was found on subsequent growth of fry [67], indicating that any role for this carotenoid in reproduction would be restricted either to long-term sub-clinical effects or to fish exposed to poor fish culture. More recently, in rainbow trout broodstock, maternal dietary supplementation with astaxanthin was found to improve the fertilization rate and percentage of eyed and hatched eggs, and to reduce mortality of eyed eggs. Astaxanthin also has a positive paternal effect on fertilization rate [68].

A pioneering study [69] suggested that all the egg carotenoids of brown trout (*Salmo trutta*) were transferred to the embryo and fry, in which astaxanthin was found predominantly as esters in the integument. Later studies with wild Atlantic salmon, however, showed that about 30% of the egg astaxanthin disappeared during development to fry [70]. The appearance of relatively large amounts of astaxanthin esters at the hatching stage indicates that it is diverted to the integument [71]. In rainbow trout, the loss of egg carotenoids is about 60% when the fish reach the start feeding stage [72]. Since these fishes are not eating at this stage, the carotenoids must have been transformed into colourless metabolites; xanthophylls such as canthaxanthin and astaxanthin serve as vitamin A precursors in salmonid fishes [20]. Astaxanthin is probably not an important vitamin A source at the egg stage whereas it may serve as a precursor at the fry stage when a functional liver has been developed [73]. Irreversible transformation into vitamin A and retinoic acids and subsequent excretion *via* catabolic pathways may be responsible for at least part of the observed loss. In juvenile hybrid tilapia (*Oreochromis niloticus* x *O. aureus*), β-carotene (**3**) may fulfil the vitamin A requirements [74].



β-carotene (**3**)



crustaxanthin (**197**)

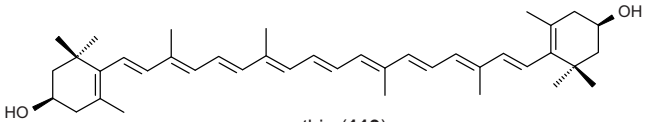
In Arctic charr (*Salvelinus alpinus*) fed a diet supplemented with astaxanthin, the observed ratio between the (3,4,3′,4′-di-*cis*), (3,4-*trans*-3′,4′-*cis*), and (3,4,3′,4′-di-*trans*) isomers of crustaxanthin (**197**) in the ovaries was approximately 2.6:3.1:1 (18-21% of total carotenoids) [75]. This suggests a relatively strongly stereoselective enzymic reduction of astaxanthin to

crustaxanthin in favour of the sterically hindered (3,4-*cis*)-glycolic forms in the Arctic charr. Thus, the role of crustaxanthin in the retinoid metabolism of fish with a reductive carotenoid metabolism requires investigation. No metabolites were detected in the carp (*Cyprinus carpio*) after administration of radioactive crustaxanthin [76], but this species has an oxidative carotenoid metabolism [20]. Also, crustaxanthin is not present in Atlantic salmon [71]. The role of the integumental carotenoids remains to be determined firmly. In addition to serving as a reserve of provitamin A, they may contribute to camouflage, signalling, photoprotection and immunocompetence.

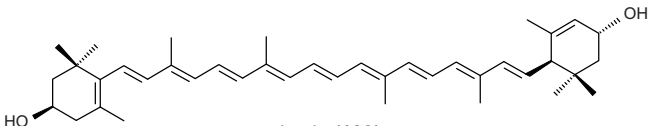
At present there is no knowledge about the biochemical basis of the effects of carotenoids during embryogenesis and later stages in salmonid fishes. However, a gene (*bco1*) from zebrafish (*Danio rerio*) encoding the enzyme responsible for vitamin A formation, β-carotene 15,15´-oxygenase, has been cloned [77] (*Chapter 16*). Targeted gene knockdown resulted in severe malformations of eyes, craniofacial skeleton and pectoral fins during embryonic development. Furthermore, retinoic acid formation, dependent on local formation of retinal *de novo* from provitamin A, appeared to be essential. This firmly establishes a crucial role of carotenoids in the early development of fish. However, the substrate requirements and putative roles of carotene-cleavage enzymes in formation of retinoids in developing embryos of salmonid fishes and other farmed species require investigation. The contribution of astaxanthin to the retinoid pool in eggs and fry of salmonid fishes remains to be determined. Direct effects of intact carotenoids, or metabolites other than vitamin A, should also be considered. Some studies have found carotenoids to have a positive effect on egg production and offspring growth or survival whereas several other studies have failed to detect such effects. This could be due to the choice of parameters. Female two-spotted gobies (*Gobiusculus flavescens*), fed diets supplemented with 135 mg astaxanthin per kg diet, developed a stronger nuptial coloration, were more likely to spawn and produced larvae that had a higher phototactic response than unsupplemented fish [43]. Phototaxis (see *Chapter 10*) is crucial for survival of the larvae. Moreover, commonly reported parameters for reproductive success, such as fertilization and hatching rates, were unaffected.

Broodstock diet formulation is essential in aquaculture for mass producing offspring with good quality [43,78]. Supplementation with 30 mg astaxanthin per kg soft-dry pellets for 5 months before spawning increased total egg production, egg quality and number of normal larvae of yellowtail (*Seriola quinqueradiata*) [79]. Supplementation with paprika powder as an ample source of carotenoids appears to improve larval survival more than supplementation with astaxanthin [80]. It should be noted that, in this species, astaxanthin comprises only about 1% of the total carotenoids of the eggs after the broodstock are fed a diet with astaxanthin, while zeaxanthin (**119**) and lutein (**133**) represent about 90% of the total carotenoids [80]. In striped jack (*Pseudocaranx dentex*), supplementation with 10 mg astaxanthin per kg dry pellets increased the number of eggs produced three-fold [81].

Relatively little is known about the role of dietary carotenoids in crustaceans except for their effect on colouration [82]. Retinoids were not detected in the eggs of the prawn *Penaeus semisulcatus* [83], and a role of carotenoids to provide retinoids is therefore plausible. Supplementation of broodstock diets for the prawn *P. monodon* with astaxanthin improves ovarian development and spawning, which may suggest a role in reproduction [84]. Dietary astaxanthin supplementation (30 - 120 mg/kg) apparently does not affect growth in the spiny lobster (*Panulirus ornatus*) (weight 18 g) [85].



zeaxanthin (**119**)



lutein (**133**)

Nutritionally enriched brine shrimps (*Artemia* spp.) are used as live feed for larval start feeding of marine fish species. Some *Z* isomers of canthaxanthin (**380**) accumulate in the ovaries, eggs and encysted embryos, but not in growing animals [20]. The possible presence of *E/Z* isomers of carotenoids in reproductive tissues of other species of crustaceans or fish has largely been ignored, but a similar geometrical isomer composition of astaxanthin was found in the diet and eggs (*Z* isomers 16% of total astaxanthin) of rainbow trout [86], which indicates that the *Z* isomers are slightly enriched in eggs compared to plasma (in which *Z* isomers comprise 7-8% of total astaxanthin) of fish fed a similar astaxanthin source [87]. The subsequent metabolic fate of these *Z* isomers is not known, but one speculation is that they may serve as precursors for the local formation of (9*Z*)- and (13*Z*)-retinoic acids which are involved in the early events of cell differentiation [88].

It is well recognized that the ratio of optical (*R/S*) isomers of astaxanthin (**404-406**) in the eggs of salmonid fishes is similar to that of the diet [89]. Nothing is presently known about the metabolism of these isomers in the early stages of development.

D. Sources of Dietary Carotenoids

The most important carotenoid sources used in aquaculture are synthetic formulated astaxanthin (**404-406**) and canthaxanthin (**380**) and, as natural astaxanthin sources, the red yeast *Xanthophyllomyces rhodorhous* (formerly *Phaffia rhodozyma*) (3*R*,3′*R*, **404**) and the alga *Haematococcus pluvialis* (3*S*,3′*S*, **406**). Other carotenoid sources with lesser commercial

importance are crustaceans and their by-products for astaxanthin, and microorganisms, mostly algae, producing various carotenoids. Algae are important for direct consumption in shellfish and shrimp aquaculture and are used indirectly as food for live prey fed to fish larvae [90]. Flowers of *Adonis* spp. biosynthesize (3*S*,3′*S*)-astaxanthin (**406**) esters, but there is currently no commercial production of carotenoids from these or other plant sources. The industrial synthesis and formulation of astaxanthin, canthaxanthin and other xanthophylls is covered in *Volume 2, Chapter 3 Part VII*, and natural production by microbial and plant biotechnology in *Volume 5, Chapters 5* and *6*.

Current products containing astaxanthin or canthaxanthin are formulated to contain about 10% carotenoid. To avoid carotenoid loss during extrusion and drying of the feed pellets it is most common to use a cold-water dispersible product that can be applied to the pellets post-extrusion. The corn-starch covered beadlets (size about 400 μm) consist of a matrix, typically lignosulphonate, in which droplets of astaxanthin in antioxidants are embedded. The optical isomer ratio of synthetic astaxanthin is 1:2:1 for the (3*R*,3′*R*)-, (3*R*,3′*S*)- and (3*S*,3′*S*)-isomers, (**404, 405, 406**, respectively), and it contains about 20% *Z*-isomers. Aggregates or crystallites of carotenoids [91] (*Chapter 5*) may form before the fish are fed and this may influence the gastrointestinal absorption rate.

Whereas astaxanthin is more efficiently accumulated in the muscle of rainbow trout than is canthaxanthin [15], the opposite appears to be true for Atlantic salmon [92-94]. When the dietary carotenoid concentration exceeds 30 mg/kg, more canthaxanthin than astaxanthin is taken up into the blood of Atlantic salmon when the inclusion levels are similar. When the diet contains both carotenoids, there is a reduction in the uptake of both carotenoids, the effect being more prominent for astaxanthin [94]. In salmonid fishes, astaxanthin dipalmitate is utilized more poorly than is unesterified astaxanthin [95]. This appears to be true also for natural astaxanthin esters from *H. pluvialis* [96,97]. For skin pigmentation of the sparid fish, red porgy (*Pagrus pagrus*), *H. pluvialis* was utilized more efficiently than a commercially formulated astaxanthin [98].

Mechanical or enzymic disruption of the rigid cell wall of the yeast *X. rhodorhous* is essential for the efficient emptying of the cell content and the utilization of the astaxanthin (the 3*R*,3′*R* isomer, **404**). Although feed production losses of astaxanthin are higher with increasing degree of cell disruption [99], this is more than outweighed by the increased bioavailability of the astaxanthin for muscle pigmentation of rainbow trout [100]. A higher apparent digestibility coefficient (ADC) of astaxanthin (65-70%) is found for Atlantic salmon fed diets supplemented with a modern product of *X. rhodorhous* than for salmon fed the diet supplemented with a formulated synthetic astaxanthin (40%) [101]. This reflected the higher proportion of dietary astaxanthin utilized for muscle pigmentation (6.3%), which was 86% higher than for salmon fed the synthetic formulated astaxanthin. The large difference in utilization warrants further studies into the biochemical basis for intestinal absorption of carotenoids in fishes and the effects of the matrix within which the carotenoids are found. It is important to consider effects of both species and source with respect to carotenoid utilization.

E. Carotenoid Utilization

1. Uptake from the gastrointestinal tract

Carotenoids are poorly utilized by fish, and the retention of astaxanthin in muscle of Atlantic salmon is usually less than 12% [102-105]. One reason for this is the relatively poor absorption of carotenoids from the gut. In general, the factors that influence the bioavailability of carotenoids in humans (*Volume 5, Chapter 7*) are also relevant for fish. In addition, temperature and other environmental factors have to be taken into account for poikilothermic animals.

Determination of apparent digestibility coefficients (ADC) is a classical method for estimating nutrient absorption. In fish, the ADC is often determined by an indirect method that relates concentration of a nutrient in diet and faeces to that of yttrium oxide (Y_2O_3) or another inert digestibility marker supplemented to the diet [106]. Inhibition by phloridzin indicates that absorption of astaxanthin by chinook salmon (*Oncorhynchus tshawytscha*) is an active process [107]. In rainbow trout, however, passive absorption of astaxanthin is indicated by the linear response in blood plasma astaxanthin over a dietary concentration range 12.5-200 mg/kg [108]. Recent evidence for rainbow trout [109,110] also indicates a facilitated uptake from the gastrointestinal tract. Geometrical isomers of astaxanthin had different uptake rates from the intestine (in decreasing order all-*E* >13*Z* >9*Z*), whereas the optical (*R,S*) isomers were taken up to the same extent. The gastrointestinal absorption of astaxanthin into blood is a slow process, as indicated by the relatively high values for T_{max} (time to achieve maximum blood concentration) of about 18 to 30 hours [111-113]. Several factors such as the composition of the diet, the dietary carotenoid content, carotenoid species and molecular linkage (esterification) may influence carotenoid digestibility [14,15,17], as do water temperature and ration size (see below). The ADCs of 4-ketocarotenoids reported in the earlier literature vary widely [14,15]; figures ranging from 4-97% are reported.

It has been common practice to lyophilize faeces samples for digestibility measurements, and high digestibility estimates (>80%) and variation have been reported for freeze-dried faeces samples [114-116]. In part, this variation may be ascribed to the use of different techniques for faeces collection and sample treatment. Breakdown of carotenoids in the faeces during extraction and analysis may explain exceptionally high ADC estimates whereas incomplete extraction may explain unrealistically low values. Low water activity renders carotenoids susceptible to oxidation and degradation. Typical ADC-values for astaxanthin and canthaxanthin range between 35 and 55%, when analyses are performed on frozen non-lyophilized faeces [109,117]. Different values may be found, however, depending on factors such as dose, carotenoid source and type, and diet composition. Thus, a higher dietary lipid level (up to at least 40%) appears to have a positive effect on carotenoid uptake and deposition [118].

Astaxanthin ADC is affected negatively by ration size and, therefore, indirectly by growth [119]. Astaxanthin ADC was 1.5 times higher when Atlantic salmon were fed a low (40%) as opposed to a full ration (100%) but, due to the low feed intake, the total amount of digested astaxanthin was only about 50% of that in fish fed a full ration. Digestibility measurements of astaxanthin, conducted in a commercial sea farm in northern Norway during autumn when the growth rate was very high, indicated that the apparent digestibility of astaxanthin was as low as 14.5% when the fish were fed a full ration but 38% at half the ration [120]. The ADC of astaxanthin is also influenced by temperature, and was about 11% higher in Atlantic salmon kept at 8°C than in ones kept at 12°C [121]. Similarly, Arctic charr reared at 8ºC were significantly redder than fish kept at 12ºC [122,123].

Recent studies with transgenic mice and the fruit fly *Drosophila,* and with human Caco2 cells in culture have revealed the importance of intestinal receptors such as SR-BI for the facilitated uptake of carotenoids (*Volume 5, Chapter 7*). It is considered likely that the situation in fish and crustaceans is similar to this, and that the gastrointestinal uptake, uptake in liver, and ultimately uptake and deposition of dietary carotenoids in the muscle and integumental cells, are governed by receptors and transport proteins. This is an important area for future study.

2. Distribution in muscle and integument

The different types of pigment cells in the skin of poikilothermic vertebrates are referred to as chromatophores, notably xanthophores (yellow) and erythrophores (red) (*Chapter 10*). These have the ability to translocate intracellular pigment organelles, under nervous and endocrine control, thus enabling fish to change colour more rapidly than other vertebrates. The biology of invertebrate and vertebrate integuments has been reviewed [124,125]. The primary pigmentary organelles of xanthophores and erythrophores are carotenoid droplets. Crustaceans have four types of chromatophores, including erythrophores and xanthophores, which are loaded with pigment granules. The red-pigment-concentrating hormone was the first neuropeptide hormone to be characterized and was isolated in 1972 from the shrimp *Pandalus borealis* [126]. Marine invertebrate animals contain carotenoproteins which are carotenoids bound stoichiometrically to proteins (*Chapter 6*).

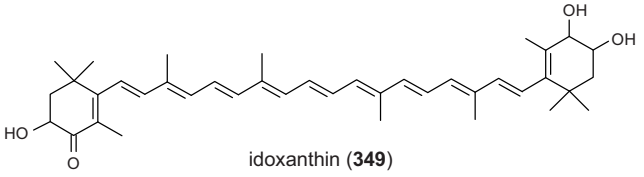
Carotenoids are associated with the protein fraction of the muscle of salmonid fishes, and astaxanthin and canthaxanthin may be combined with the actomyosin complex by non-specific hydrophobic bonds [127-129]. Recently, it was found that α-actinin is the only myofibrillar protein that correlates significantly with astaxanthin binding [130,131]. α-Actinin is a component of the myofibrillar sarcomeric Z-disk which forms the borders of individual sarcomeres of the myofibrils and serves to crosslink opposing thin filaments that interdigitate at the Z-line. Combination studies *in vitro* show that astaxanthin combines, in a close to stoichiometric 1:1 relationship, not only with α-actinin isolated from Atlantic salmon but also with that from the normally unpigmented Atlantic halibut (*Hippoglossus hippoglossus*) [131]. Based on the astaxanthin:actomyosin ratio in muscle of coho salmon

(*Oncorhynchus kisutch*) [128] a theoretical saturation level of nearly 100 mg astaxanthin/kg flesh was indicated for salmonid fishes [132], which is comparable to the highest actual levels (59 mg astaxanthin/kg muscle) reported for sockeye salmon (*O. nerka*) [133]. This is considerably higher than the levels found in large Atlantic salmon where concentrations around 10 mg/kg are found after astaxanthin is fed in the diet [134,135].

The amount of carotenoids that ultimately reach the target tissue(s) can serve as a convenient measure of carotenoid bioavailability. A commonly used measure of carotenoid bioavailability in salmonid fishes is muscle retention (amount of carotenoids deposited in the muscle as a percentage of the ingested amount). The amount of dietary astaxanthin that is utilized for flesh pigmentation rarely exceeds 15% in Atlantic salmon [14] and 18% in rainbow trout [15]. The digestibility and retention of carotenoids are negatively correlated with the dietary carotenoid concentration. The amount retained also depends on fish species, size and growth rate. The muscle retention of dietary astaxanthin, fed at 66 mg/kg diet, was only 3.9% in Atlantic salmon growing from about 0.14 to 0.74 kg [117], but was 30-42% in rainbow trout fed a dietary concentration of about 35 mg astaxanthin/kg diet [109]. This may be explained by extensive metabolic transformation. The muscle tissue carries 93-95% of the total body burden of astaxanthin in Atlantic salmon and rainbow trout [116]. If the percentage of absorbed intact astaxanthin that is excreted *via* the gills and/or urine is close to zero, the results [109] indicate that about 55-67% of the absorbed astaxanthin undergoes metabolic transformation in rainbow trout. Similarly, in Atlantic salmon given various astaxanthin sources at a dietary level of 37-50 mg/kg, the retention of digested astaxanthin ranged from 22.2 to 33.1%. This indicates that about 67% of the astaxanthin that was absorbed by the salmon was transformed metabolically or excreted (not with faeces). Studies are needed to identify these metabolites and their fate.

3. Alternative administration

The bioavailability, determined as the area under the time-concentration curve after a single low dose of intraperitoneally injected astaxanthin (*ca*. 0.5 mg/kg body weight), in Atlantic salmon was about 12-fold higher than for an orally administered dose [113]. A similar difference was obtained after intra-arterial compared to oral administration of [6,7,6′,7′-$^{14}C_4$]-astaxanthin in rainbow trout [136].



idoxanthin (**349**)

The dose-response relationships for astaxanthin *E*/*Z* isomers and the metabolite idoxanthin (**349**) in plasma, muscle, liver, kidney and skin, in fish species that usually have white flesh (Atlantic cod, *Gadus morhua*) or red flesh (Atlantic salmon) were compared following intraperitoneal injection of 100 mg astaxanthin [137]. Astaxanthin concentrations increased linearly in a dose-dependent manner in plasma and muscle of both species, and were highly correlated. Extreme astaxanthin concentrations up to 90 and 50 mg/litre in plasma and 30 and 1 mg/kg in muscle were detected in Atlantic salmon (*ca*. 0.5 kg) and cod, respectively, after 4 weeks. Rapid astaxanthin uptake was also found in rainbow trout [138]. The capacity for muscle binding of astaxanthin is therefore much higher than can be achieved by regular feeding. The linear dose-response showed that muscle astaxanthin-binding capacity had not reached saturation, so there is a higher potential for astaxanthin incorporation. Accumulation of astaxanthin *E/Z* isomers in the various tissues was selective in favour of the *Z*-isomers. Higher astaxanthin levels in plasma, muscle, skin, kidney and liver of Atlantic salmon and Atlantic cod may thus be obtained by intraperitoneal injection than by regular feeding. Differences in uptake mechanisms for cellular incorporation in the muscle may explain the differences in astaxanthin uptake in different fish species. Uptake of carotenoids is easier from the intraperitoneum than the gastrointestinal tract.

F. Conclusion

Although some progress has been made, little is yet known about the genes and proteins involved in carotenoid pigmentation, such as those involved in absorption, transport, uptake, metabolic transformation and their spatial and temporal expression [139]. Determination of the factors that govern the metabolic turnover of carotenoids and the physiological effects of carotenoids and their metabolites in different life-stages of the various species is very important for the aquaculture industry.

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