REVIEWS IN Aquaculture

*Reviews in Aquaculture* (2017) **0**, 1–36 doi: 10.1111/raq.12200

# Astaxanthin as feed supplement in aquatic animals

Keng Chin Lim[1], Fatimah Md. Yusoff[1,2], Mohamed Shariff[3] and Mohd Salleh Kamarudin[1]

1 Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Selangor, Malaysia
2 Laboratory of Marine Biotechnology,Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia
3 Aquatic Animal Health Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, Malaysia

**Correspondence**
Fatimah Md. Yusoff, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Emails: fatimahyus@gmail.com and fatimamy@upm.edu.my

Received 25 November 2016; accepted 30 March 2017.

## Abstract

Astaxanthin is a high value keto-carotenoid pigment renowned for its commercial application in various industries comprising aquaculture, food, cosmetic, nutraceutical and pharmaceutical. Among the verified bio-resources of astaxanthin are red yeast *Phaffia rhodozyma* and green alga *Haematococcus pluvialis*. The supreme antioxidant property of astaxanthin reveals its tremendous potential to offer manifold health benefits among aquatic animals which is a key driving factor triggering the upsurge in global demand for the pigment. Numerous scientific researches devoted over a number of years have persistently demonstrated the instrumental role of astaxanthin in targeting several animal health conditions. This review article evaluates the current best available evidence to judge the beneficial usage of astaxanthin in aquaculture industry. Most apparent is the profound effect on pigmentation, where astaxanthin is frequently utilized as an additive in formulated diets to boost and improve the coloration of many aquatic animal species, and subsequently product quality and price. Moreover, the wide range of other physiological benefits that this biological pigment confers to these animals is also presented which include various improvements in survival, growth performance, reproductive capacity, stress tolerance, disease resistance and immune-related gene expression.

**Key words:** astaxanthin, carotenoid, disease resistance, growth, pigmentation, reproductive performance.

## Introduction

Carotenoids (tetraterpenoids) comprise the structurally diverse group of over 700 organic lipid-soluble pigments that are exclusively produced within plants, phytoplankton, algae, bacteria and some fungi. These pigments dominate in providing the broad variety of conspicuous hues (red, orange and yellow) attributed to many aquatic animals, fruits and leaves of plants. Among the best-known and well-studied carotenoids include astaxanthin (red), β-carotene (orange), canthaxanthin (orange-red), fucoxanthin (brown), zeaxanthin (yellow-red) and lutein (greenish-yellow) (Britton 1995; Eonseon *et al.* 2003; Dufosse *et al.* 2005; Sajilata *et al.* 2008; Goh *et al.* 2009; Foo *et al.* 2015a, b, 2017a,b). The aforementioned pigments are closely related to one another; thus, they exhibit many of the functional metabolic and physiological characteristics of carotenoids (Guerin *et al.* 2003; Britton 2008). Astaxanthin (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) is an oxidized form of β-carotene being widely distributed in nature and largely discovered in the marine environment (Lorenz & Cysewski 2000; Li *et al.* 2011; Markou & Nerantzis 2013; Zhang *et al.* 2014). This carotenoid pigment is abundant in the flesh of salmonids, carapace of many crustaceans (e.g. shrimp, crabs, lobsters and crayfish) and also in other marine organisms such as microbes (Johnson & An 1991; Asker *et al.* 2012) and microalgae (Lorenz & Cysewski 2000; Stewart *et al.* 2008; Fassett & Coombes 2011; Han *et al.* 2013; Begum *et al.* 2016). Aquatic animals generally exhibit poor ability to biochemically synthesize astaxanthin *de novo* and thus require diets containing astaxanthin to acquire the appropriate coloration (Nakano *et al.* 1995; Lorenz & Cysewski 2000; Moriel *et al.* 2005; Liu *et al.* 2006; Kim *et al.* 2006). Naturally, the carotenoid pigment astaxanthin is primarily biosynthesized in microalgae within the food chain at the primary production level. Microalgae are then consumed by crustaceans, zooplankton or insects that amass the astaxanthin and in turn transferred to the higher trophic levels when ingested by fish and other aquatic animals. Kuhn and Soerensen (1938) stated that astaxanthin was first isolated from lobster in 1937. According to Yuan *et al.* (2002), several organisms that have been recognized to biosynthesized astaxanthin consist mainly of *Haematococcus pluvialis*, *Chlorococcum* sp., *Chlorella zofingiensis*, *Xanthophyllomyces dendrorhous* (red yeast) and the marine *Agrobacterium aurantiacum*. Among the exploited sources, *Haematococcus pluvialis* was identified to contain the highest level of astaxanthin (>30 g of astaxanthin $kg^{-1}$ dry biomass) in nature and has provoked considerable interest as natural and inexpensive source of astaxanthin (Boussiba *et al.* 1999; Boussiba 2000; Panis & Carreon 2016).

Astaxanthin is primarily used as a pigmentation source in aquaculture and dietary supplements in food industry as well as applications in nutraceuticals and pharmaceuticals (Dufosse *et al.* 2005; Dutta *et al.* 2005; Higuera-Ciapara *et al.* 2006; Li *et al.* 2011; Begum *et al.* 2016). This carotenoid pigment is best known as an essential aquacultural feed additive for imparting the pinkish-red coloration to the flesh of salmons, trouts, ornamental fish, shrimp, lobsters and crayfish resulting in a better quality and acceptance in the consumer market (Lorenz & Cysewski 2000; Guerin *et al.* 2003; Cysewski & Lorenz 2004; Li *et al.* 2011; Begum *et al.* 2016). The continued growth of aquaculture industry has generated a massive demand for astaxanthin pigment. Apart from that, as a human dietary supplement, astaxanthin confers the beneficial effects of anti-inflammatory (Nir *et al.* 2002; Lee *et al.* 2003; Choi *et al.* 2008; Pashkow *et al.* 2008; Park *et al.* 2010; Macdermid *et al.* 2012), anti-ageing (Masaki 2010; Kidd 2011; Tominaga *et al.* 2012), immune system boosting (Kishimoto *et al.* 2010; Park *et al.* 2010; Yamashita 2013), anticancer (Smith 1998; Palozza *et al.* 2009; Tanaka *et al.* 2012), sun proofing (O'Connor & O'Brien 1998; Suganuma *et al.* 2010), antidiabetic activities (Maoka & Etoh 2010; Guerra & Otton 2011) and among other various health benefits due to the potent antioxidant activity of this fat-soluble pigment. Astaxanthin exists in various stereoisomers characterized by different configuration of the two hydroxyl groups on the molecule (Fig. 1) (Lorenz & Cysewski 2000; Rufer *et al.* 2006; Ambati *et al.* 2014). The unique features of astaxanthin namely a more polar configuration, ability to be esterified and a higher antioxidant property compared to other carotenoids are largely attributed to its molecular structure with the presence of hydroxyl (OH) and keto-moieties (C=O) on each ionone ring (Martin *et al.* 1999; Hussein *et al.* 2006; Peng *et al.* 2008; Yuan *et al.* 2011; Lin *et al.* 2016). The powerful antioxidant capacity of astaxanthin is correlated with its strong electron-donating capability as reductone to neutralize endogenously produced free radicals (hydrogen peroxide, hydroxyl radicals and superoxide anion) and converting them into more stable products while terminating free radical chain reaction in living organisms (Hussein *et al.* 2006; Ranga Rao *et al.* 2010; Dhankar *et al.* 2012; Guerra *et al.* 2012). In nature, astaxanthin is predominantly esterified with one or two units of fatty acids (monoesters and diesters) or conjugated with proteins, such as in crustacean exoskeleton and salmon muscle, providing stability to the molecule (Turujman *et al.* 1997; Osterlie *et al.* 1999; Coral-Hinostroza & Bjerkeng 2002; Storebakken *et al.* 2004; Higuera-Ciapara *et al.* 2006). Free-ester astaxanthin form is exceptionally vulnerable to oxidation. In *H. pluvialis*, esterified astaxanthin molecules accrued exclusively, biosynthesized virtually in its monoesters (70%), diesters (25%) and free (5%) forms (Sato *et al.* 1998; Lorenz & Cysewski 2000; Denery *et al.* 2004; Peng *et al.* 2008). Thus, the carotenoid pigment astaxanthin has significant potential and important applications in animal health and nutrition, largely ascribed to its astonishing potential in protecting the organism against a broad range of stressors and infectious diseases.

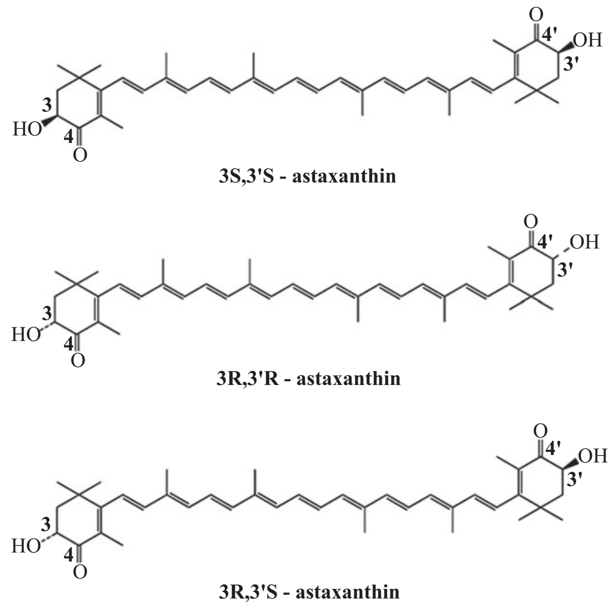


**Figure 1** Various astaxanthin stereoisomers that differ in the configuration of the two hydroxyl groups on the molecule (Guerin *et al.* 2003).

## Sources of astaxanthin

Scientists have been intensively researching on the recovery of astaxanthin from various biological sources in the past few decades as a direct result of the remarkable qualities of this fascinating molecule. The primary natural sources of astaxanthin are relatively simple microorganisms specifically algae, fungi, yeast and bacteria (Table 1). Not a single animal can biochemically synthesize it from scratch, but animals do accumulate astaxanthin in their tissues through

**Table 1** Microbial sources of astaxanthin

| Sources | Astaxanthin (%) on a dry weight basis | References |
|---|---|---|
| Chlorophyceae | | |
| *Botryococcus braunii* | 0.01 | Grung *et al.* (1994) |
| *Chlorococcum* sp. | 0.2 | Zhang *et al.* (1997) |
| *Chlorella zofingiensis* | 0.68 | Orosa *et al.* (2001) |
| | 0.1 | Wang and Peng (2008) |
| *Haematococcus pluvialis* | 4 | Lee and Ding (1994) |
| | 3.8 | Aflalo *et al.* (2007) |
| | 3.8 | Ranga Rao *et al.* (2010) |
| *Neochloris wimmeri* | 0.6 | Orosa *et al.* (2000) |
| *Scenedesmus obliquus* | 0.3 | Qin *et al.* (2008) |
| *Tetraselmis* sp. | 0.23 | Raman and Mohamad (2012) |
| Florideophyceae | | |
| *Catenella repens* | 0.02 | Banerjee *et al.* (2009) |
| Ulvophyceae | | |
| *Enteromorpha intestinalis* | 0.02 | Banerjee *et al.* (2009) |
| *Ulva lactuca* | 0.01 | Banerjee *et al.* (2009) |
| Alphaproteobacteria | | |
| *Agrobacterium aurantiacum* | 0.01 | Yokoyama *et al.* (1995) |
| *Paracoccus carotinifaciens* | 2.2 | EFSA (2007a) |
| Labyrinthulomycetes | | |
| *Thraustochytrium* sp. | 0.2 | Yamaoka (2008) |
| Tremellomycetes | | |
| *Xanthophyllomyces dendrorhous* | 0.5 | Kim *et al.* (2005) |
| | 0.5 | de la Fuente *et al.* (2010) |

the consumption of astaxanthin-containing organisms for the acquisition of attractive coloration. For instance, in marine environments, algae rich in astaxanthin is a food for zooplankton which in turn is ingested by fish (e.g. salmonids) and exoskeleton-bearing creatures (e.g. crabs, crayfish, lobsters, krill and shrimp) at the higher trophic levels. Commercial astaxanthin is essentially derived from either chemical synthesis (Li *et al.* 2011; Milledge 2011) or natural resources such as red yeast *X. dendrorhous* (formerly *Phaffia rhodozyma*) (Johnson & An 2008; Rodriguez-Saiz *et al.* 2010; Hara *et al.* 2014; Dursun & Dalgic 2016) and freshwater microalga *H. pluvialis* (Higuera-Ciapara *et al.* 2006; Han *et al.* 2013; Haque *et al.* 2016; Shang *et al.* 2016; Wang *et al.* 2016). Hitherto, *H. pluvialis* is recognized as one of the most promising sources of natural astaxanthin. More companies are focusing in the production and commercialization of astaxanthin from *H. pluvialis* (Table 2). In addition, muscles of wild and farmed salmonids can be significant sources of astaxanthin (Table 3). However, large variations of astaxanthin contents in the flesh were reported among the wild *Oncorhynchus* species ranging from 3 mg $kg^{-1}$ in chum salmon *Oncorhynchus keta* up to 38 mg $kg^{-1}$ in sockeye salmon *Oncorhynchus nerka* (EFSA, 2005). Astaxanthin concentrations in wild and farmed Atlantic salmon *Salmo salar* were documented as 3–10 mg $kg^{-1}$ flesh and 1–9 mg $kg^{-1}$ flesh, respectively (EFSA 2005). Large trouts (*Oncorhynchus mykiss*) marketed in Europe and Japan contain astaxanthin in the range of 12–25 mg $kg^{-1}$ flesh (EFSA 2005). Thus, wild and farmed salmonid fillets can serve as a good dietary source of natural astaxanthin.

Commercial production of astaxanthin is dominated by synthetically derived astaxanthin (>95%) because it involves lower production costs (approximately US $1000 $kg^{-1}$) compared to the algal alternative (<1%). DSM (Dutch State Mines) and BASF (Baden Aniline and Soda Factory) are the global leading synthetic astaxanthin manufacturers. The total market value of astaxanthin exceeded US$447 million in 2014 with an estimated global market potential of 280 metric tons, and the market price varies usually from US$2500 to 7000 $kg^{-1}$ (Milledge 2011; Borowitzka 2013; Koller *et al.* 2014; Perez-Lopez *et al.* 2014). In spite of this fact, consumer demand for natural products makes the synthetic pigment much less desirable and astaxanthin obtained from biological sources seems to be gaining potential in the global market which is forecasted to surpass US$1.1 billion by 2020 equated to 670 metric tons (Qin *et al.* 2008; Nguyen 2013; Panis & Carreon 2016; Taucher *et al.* 2016).

## Downstream processing and recovery of astaxanthin

Astaxanthin is accumulated predominantly in encysted cells of *H. pluvialis* up to 3–5% on a dry weight basis under unfavourable environmental conditions (Lemoine & Schoefs 2010; Wayama *et al.* 2013; Chekanov *et al.* 2014; Solovchenko 2015). Intact astaxanthin-rich hematocysts (aplanospores) are characterized by thick and resistant cell walls which require mechanical disruption prior to use in order to take full advantage of its bioavailability (Damiani *et al.* 2006; Kaczor & Baranska 2011; Kim *et al.* 2016). Once the cell wall is disrupted, the recovery of astaxanthin is possible via subsequent selective extraction procedure. Many different techniques have been developed over the years to disrupt *H. pluvialis* cells. Destruction of cell wall is usually achieved through physical or mechanical pretreatment and more specifically bead milling and expeller pressing (Mercer & Armenta 2011; Razon & Tan 2011; Zhang *et al.* 2014; Cuellar-Bermudez *et al.* 2015; Kim *et al.* 2016). A bead miller consists of a disruption or milling chamber loaded with tiny grinding beads (e.g. ceramic, glass and steel) that are agitated at high speeds resulting in multiple collisions. The dried biomass is fed in these chambers, and cells are disrupted in the bead collision zones by

**Table 2** Global commercial manufacturers of *Haematococcus pluvialis*-derived astaxanthin and related products

| Company | Country | Product Name | Description |
|---|---|---|---|
| Regenurex Health Corporation | Canada | Regenurex | Astaxanthin extract in soft-gel capsules; nutraceutical |
| Atacama Bio Natural | Chile | Supreme Asta Oil®; Supreme Asta Powder® | Astaxanthin oleoresin and pure astaxanthin powder; aquaculture and nutraceutical |
| Beijing-Gingko Group Biological Technology Co. Ltd. | China | AstaZine™ | Algae meal, astaxanthin oleoresin and beadlets; aquaculture, nutraceutical and pharmaceutical |
| Jingzhou Natural Astaxanthin Inc. | China | NaturAsta™ | Algae meal, astaxanthin oleoresin; aquaculture, nutraceutical and pharmaceutical |
| Kunming Biogenic Co. Ltd. | China | AstaBio® | Algae meal, astaxanthin oleoresin; aquaculture, nutraceutical and pharmaceutical |
| Wefirst Biotechnology Co. Ltd. | China | AstaFirst™ | Algae meal, astaxanthin oleoresin; aquaculture, nutraceutical and pharmaceutical |
| Algalif | Iceland | Astalif™ | Algae meal, astaxanthin oleoresin; aquaculture, nutraceutical and pharmaceutical |
| Parry Nutraceuticals Ltd. | India | Zanthin® | Astaxanthin extract in beadlets and soft-gel capsules; nutraceutical |
| Evergen Resources | Indonesia | Evergen Astaxanthin | Algae meal, astaxanthin oleoresin; aquaculture and nutraceutical |
| AlgaTechnologies Ltd. | Israel | AstaPure™ | Algae meal, astaxanthin oleoresin and beadlets; aquaculture, nutraceutical and pharmaceutical |
| AstaReal Co. Ltd. | Japan, Sweden, USA | AstaReal® | Astaxanthin extract in oil and powder; nutraceutical and pharmaceutical |
| Algaetech International Sdn. Bhd. | Malaysia | Astaxanthin Premia-EX | Algae meal, astaxanthin oleoresin and soft-gels; aquaculture, nutraceutical and pharmaceutical |
| FEBICO (Far East Bio-Tec. Co. Ltd.) | Taiwan | ORG-ASTA | Astaxanthin extract in medicine capsules; nutraceutical |
| Zestlife | UK | Zestlife Astaxanthin | Astaxanthin extract in soft-gel capsules; nutraceutical |
| Cyanotech Corporation | USA | BioAstin® | Astaxanthin extract in soft-gel capsules; nutraceutical |
| | | Naturose® | Algae meal; colour additive for aquaculture industry |
| Mera Pharmaceuticals Inc. | USA | AstaFactor® | Astaxanthin extract in soft-gel capsules; nutraceutical |
| Stazen Inc. | USA | Stazen® | Astaxanthin extract in soft-gel capsules; nutraceutical |
| Valensa International | USA | Zanthin® | Astaxanthin extract in soft-gel capsules; nutraceutical |

**Table 3** Astaxanthin content of wild and farmed salmonids (EFSA 2005)

| Species | Astaxanthin (mg $kg^{-1}$ flesh) | |
|---|---|---|
| | Wild | Farmed |
| Arctic charr (*Salvelinus alpinus*) | 8.6 | 1–8 |
| Atlantic salmon (*Salmo salar*) | 3–10 | 1–9 |
| Chinook salmon (*Oncorhynchus tshawytscha*) | 5.4 | – |
| Chum salmon (*Oncorhynchus keta)* | 3–5 | – |
| Coho salmon (*Oncorhynchus kisutch*) | 10–21 | – |
| Masu salmon (*Oncorhynchus masou*) | 4.6 | – |
| Pink salmon (*Oncorhynchus gorbuscha*) | 4–7 | – |
| Rainbow trout (*Oncorhynchus mykiss*) | 24 | 12–25 |
| Sockeye salmon (*Oncorhynchus nerka*) | 26–38 | – |

compaction and shear forces (Jahanshahi *et al.* 2002; Shah *et al.* 2016; Postma *et al.* 2017). Bead mills have been successfully applied for the disintegration of microalgae for the release of intracellular products (Doucha & Livansky 2008; Schwenzfeier *et al.* 2011; Postma *et al.* 2015; Gunerken *et al.* 2016). On the other hand, expeller pressing exerts squeezing force alongside high pressure to rupture tough cell walls. This relatively simple and cost-effective mechanical pressing procedure can greatly reduce the risks of contamination from external sources (Shah *et al.* 2016). The above-mentioned are by far the most widely applied disruption methods to enhance recovery of astaxanthin from *H. pluvialis* on a commercial scale. Following cell disruption, the algal biomass must be quickly dehydrated to avoid quality degradation or spoiling (in only a few hours in a hot climate). The processing is highly specific, but the most common methods developed and practiced for this purpose are freeze-drying (lyophilization), spray-drying, drum-drying and sun-drying (Molina-Grima *et al.* 2003; Mata *et al.* 2010; Uduman *et al.* 2010; Perez-Lopez *et al.* 2014). Finally, the dried product can be directly encapsulated or the astaxanthin extracted to be utilized in feed, pharmaceutical and nutraceutical formulations (Lorenz & Cysewski 2000; Li *et al.* 2011).

Like many carotenoids, astaxanthin is a lipid-soluble (lipophilic) pigment which can be readily dissolved in oils and solvents. Various techniques have been adopted to assist the recovery of astaxanthin from *H. pluvialis* utilizing acids, organic solvents, edible oils, supercritical carbon dioxide (SFE-$CO_2$) as well as microwave-assisted and enzymatic methods. Sarada *et al.* (2006) evaluated the extractability of astaxanthin from *H. pluvialis* with different acid treatments at 70°C and discovered that hydrochloric acid treatment facilitated 86–94% recovery of the pigment without affecting its ester profile. High astaxanthin yield was also noted in different studies employing hydrochloric acid pretreatment (Mendes-Pinto *et al.* 2001; Dong *et al.* 2014). Acidic method was also optimized for the isolation of astaxanthin from *P. rhodozyma* in a few studies (Ni *et al.* 2008; Xiao *et al.* 2009). Zou *et al.* (2013) recorded a relatively high astaxanthin extraction yield of 18 mg $g^{-1}$ from dried *H. pluvialis* biomass using ethanol: ethyl acetate (1:1 v/v) mixture with a brief processing period (2 h). A previous study by Kang and Sim (2008) addressed the use of common vegetable oils (corn, grape seed, olive and soybean) for direct extraction of astaxanthin from *H. pluvialis* with recovery yields of over 88%. Supercritical carbon dioxide coupled with vegetable oil or ethanol as cosolvent has been reported by many workers with promising astaxanthin extraction efficiencies (80–90%) (Machmudah *et al.* 2006; Nobre *et al.* 2006; Thana *et al.* 2008; Fujii 2012; Wang *et al.* 2012). In another study, Sanchez-Camargo *et al.* (2012) demonstrated the extraction of astaxanthin from Brazilian red-spotted shrimp (*Penaeus paulensis*) waste using supercritical carbon dioxide and ethanol mixtures with 65.2% recovery efficiency. Microwave-assisted extraction (MAE) (75°C; 5 min) of astaxanthin from *H. pluvialis* resulted in high astaxanthin recovery (75%) (Ruen-ngam *et al.* 2010), whereas enzymatic approach with the incorporation of specific lytic enzymes produced 70% extractability of astaxanthin (Kobayashi *et al.* 1997). Astaxanthin extraction is likely to remain an active area of research which is expected to stimulate its commercialization from natural resources.

## Astaxanthin in feed processing

Astaxanthin is widely known to possess great sensitivity to heat, intense light and oxidative conditions due to its highly unsaturated molecular structure (Armenta & Guerrero-Legarreta 2009; Anarjan & Tan 2013a; Bustos-Garza *et al.* 2013; de Bruijn *et al.* 2016; Martinez-Delgado *et al.* 2017). The exposure of astaxanthin to a range of these conditions during processing and storage of feed may render the pigment to lose its nutritive and biological desirable properties. Hence, it is extremely necessary that astaxanthin should remain stable upon addition to different feed formulations for maximal efficacy.

Feed manufacturing is an extensive process comprising milling, mixing, extrusion, pelletizing and drying. Milling has been suggested to exert no significant impact on the stability of astaxanthin (Anderson & Sunderland 2002). In fact, the disintegration or disruption of microalgal cells through milling appears to be the single most important attribute in effective utilization of intracellular astaxanthin (Cuellar-Bermudez *et al.* 2015; Kim *et al.* 2016). Any degradation of astaxanthin during milling is heavily dependent on equipment used, residence time and heat production. Feed mixing is important to ensure uniform distribution of nutrients, which will result in a homogenous nutrient content in each fish pellet as the formulation. However, mixing may incorporate air into the blend causing undesirable oxidation of carotenoids. Using a vacuum mixer is a good way to deal with air exposure, thus eliminating air entry into the mixture. Alternatively, the inclusion of secondary antioxidants (BHT and BHA) has been demonstrated to be efficacious in improving the oxidative stability of dietary carotenoids during feed processing (Schaich 2002; Jintasataporn & Yuangsoi 2012). On the other hand, extrusion is executed in feed processing to improve the digestibility of starch (gelatinization) and protein while minimizing the degradation of food nutrients (Glencross *et al.* 2011, 2012; de Cruz *et al.* 2015). It is also an ideal process to produce either floating or sinking pellet via formula adjustment. Nevertheless, extrusion technology involves high levels of heat, moisture, pressure and mechanical shear that are most likely to influence the stability of carotenoid pigments. The possibility that feed extrusion may stimulate the degradation of astaxanthin has been investigated in several studies. Anderson and Sunderland (2002) discovered that astaxanthin was fairly stable through extrusion with an average retention of 86%. The authors noted the destabilizing effect of increasing dryer processing temperature and extruder discharge moisture on the stability of astaxanthin, while overdrying the final product seemed to exaggerate the destruction of astaxanthin. Retention values for astaxanthin in extruded feed ranging from 86% to 94% were documented previously (Salunkhe *et al.* 1991; Haaland *et al.* 1993). Likewise, Storebakken *et al.* (2004) showed that different extruder temperatures (102, 121 and 137°C) had little influence on the composition of astaxanthin (from red yeast *X. dendrorhous*) during extruded fish feed production with a recovery range of 90–99%. Moreover, carotenoid content in a feed formulation refrigerated at 4°C was also found to be unaffected following pelletization (pelletizing machine without steam) in two separate studies (Gouveia & Empis 2003; Jintasataporn & Yuangsoi 2012). Pelleted diets that are discharged through the die surface of the extrusion system or pelletizer would still contain 30–40% of moisture. A drying process in the vacuum oven is required to evaporate the excess moisture and return the final product back to a shelf-stable moisture condition (<10%) (Hardy & Barrows 2002). Optimal processing temperature (60–80°C) should be applied to reduce the loss of pigment (Haaland *et al.* 1993; Anderson & Sunderland 2002; Jintasataporn & Yuangsoi 2012). If necessary, post-liquid application of fats or oils via coating will avoid the risk of damaging heat-sensitive carotenoids (Boon *et al.* 2010; Dethlefsen *et al.* 2017). The coated feed is subsequently cooled as required. Although the most recent practice is to coat feed pellets with astaxanthin-added fish oil, rapid degradation of pigment on the exposed surface of pellets could significantly reduce the final astaxanthin concentration (Dethlefsen *et al.* 2017). A good storage condition is crucial for the final feed products so as to promote stability and delay the degradation rate of astaxanthin (Niamnuy *et al.* 2008; Martinez-Delgado *et al.* 2017). Vacuum packaging and modified atmosphere packaging (MAP) followed by refrigerated storage have been proven to be excellent for this purpose. MAP involves the removal of air from the packaging chamber and introduction of nitrogen gas prior to sealing (Chouliara *et al.* 2007; Lee 2010; Hur *et al.* 2013). Gouveia and Empis (2003) revealed that the best storage conditions for microalgal dry biomass carotenoids were under vacuum and nitrogen atmosphere in the dark with high retentions (>90%) even after 18 months. In addition, Niamnuy *et al.* (2008) disclosed that storing dried shrimp under vacuum atmosphere at low temperature (4°C) enhanced the retention of astaxanthin. Furthermore, Raposo *et al.* (2012) recorded a low degradation of astaxanthin (10%) in spray-dried *H. pluvialis* biomass when stored under nitrogen at −21°C after 9 weeks. Given the above information, it is important that feed processing conditions should be optimized to prevent astaxanthin loss in feed products.

## Storage stability of astaxanthin

The bioavailability of astaxanthin has suffered a great challenge due to its intrinsic chemical instability which hampered its application as a functional food ingredient. This forces the market to consider new strategies to improve the utilization of astaxanthin in cosmetic, feed, nutraceutical and pharmaceutical industries. Over recent years, numerous studies have been attempted to promote the storage stability of astaxanthin through various approaches. Astaxanthin may be incorporated into a formulation with more fat sources such as edible oils for better stability. Ranga Rao *et al.* (2007) assessed the stability of astaxanthin in various edible oils. The authors indicated that astaxanthin was fairly stable in coconut, gingelly (sesame), groundnut, mustard, olive, rice bran, sunflower and palm oils when stored at room temperature for four straight months. Further, the study revealed that gingelly (sesame), rice bran and palm oils retained 84–90% of astaxanthin when heated at 70°C for eight consecutive hours. The stability of astaxanthin in edible oils is attributed to the presence of compounds such as flavonoids, polyphenols and tocopherols which are known to have stabilizing activity (Ranga Rao *et al.* 2007). On the other hand, emulsion-based delivery systems confer good protective effects particularly at improving the stability of astaxanthin towards physical and chemical changes at different environmental conditions (Clark *et al.* 2000; Wackerbarth *et al.* 2009; Boon *et al.* 2010; McClements & Li 2010; Martinez-Delgado *et al.* 2017). Astaxanthin-enriched caseinate-stabilized emulsions were found to be relatively stable over a range of light exposures, storage temperatures and salt levels (Liu *et al.* 2016a). Ribeiro *et al.* (2005) reported a 70% retention of astaxanthin when loaded into oil/water (O/W) emulsions after 3 weeks. In another study, Anarjan and Tan (2013b) demonstrated that astaxanthin was chemically more stable when its nanodispersions were used in orange juice compared to skimmed milk. Conversely, the bioavailability of astaxanthin was higher when used with protein-based ingredients, especially milk. The greatest stability of astaxanthin oleoresin from *P. rhodozyma* dispersed in aqueous solution of propylene glycol was noted at pH 4 and at low temperatures (Villalobos-Castillejos *et al.* 2013). Moreover, Peng *et al.* (2010) observed improved stability and bioavailability of astaxanthin when encapsulated within liposomes. The stability of astaxanthin-enriched *H. pluvialis* cells (homogenized) was enhanced with only a minimum loss of pigment (8%) after 24 weeks when encapsulated into the rigid polymeric matrix of chitosan and stored under nitrogen atmosphere at −18°C (Kittikaiwan *et al.* 2007). Astaxanthin stability was also well investigated using microencapsulation with polymeric nanospheres, β-cyclodextrin, hydroxypropyl-β-cyclodextrin and sulfobutyl ether β-cyclodextrin as documented by various researchers (Samuel *et al.* 2003; Chen *et al.* 2007; Tachaprutinun *et al.* 2009; Yuan *et al.* 2013).

## Pharmacokinetics of astaxanthin

Since animals are generally incapable of synthesizing carotenoids (e.g. astaxanthin, β-carotene, fucoxanthin and lutein) *de novo* and must therefore depend on dietary sources, the pharmacokinetics of carotenoids have always been of great interest. In layman's terms, pharmacokinetics refers to the fate or movement of externally administered substances (e.g. drugs, hormones, nutrients, pharmaceutical agents and toxins) in a living organism until the point of complete excretion from the body (Ratain & Plunkett 2003; Ruiz-Garcia *et al.* 2008; Di & Kerns 2016; Born *et al.*

2017). In many respects, it can be more precisely envisioned as the actions of the biological system on the administered substance. It is important to note that pharmacokinetics encompasses the mechanisms of absorption, transport or distribution, localization in tissues, metabolism and excretion. Pharmacokinetic properties of the administered substances might be influenced by elements including the site of administration, dose and functioning of body organs (Bryan & Knights 2014; Hinderliter & Saghir 2014). Understanding the pharmacokinetics of astaxanthin is fundamentally important in relation to its beneficial effects on animal health.

The principle mechanisms modulating the pharmacokinetics of astaxanthin in mammalian and nonmammalian systems have not been completely demonstrated. Few scientific literatures to date have reviewed the different steps of absorption, transport and tissue uptake of dietary carotenoids in mammals, including humans (Furr & Clark 1997; Castenmiller & West 1998; Lee *et al.* 1999; During & Harrison 2004; Fernandez-Garcia *et al.* 2012; Nagao 2014). Carotenoid absorption within the mammalian gastrointestinal tract generally involves the following major steps: (i) release of carotenoids from food matrix; (ii) solubilization of carotenoids into mixed lipid micelles; (iii) cellular uptake of carotenoids by intestinal absorptive cells (enterocytes); (iv) incorporation of carotenoids into chylomicrons; (v) secretion of carotenoids and their metabolites associated with chylomicrons into the lymph within the systemic circulation and; (vi) tissue distribution, metabolism and recycling of carotenoids (van het Hof *et al.* 2000; Thyssandier *et al.* 2001; Olson 2004; Harrison 2012). Knowledge with reference to human pharmacokinetics of carotenoids is mostly uncovered from studies conducted with β-carotene (White *et al.* 1994; van Vliet 1996; Yeum & Russell 2002; van Lieshout *et al.* 2003; Gireesh *et al.* 2004; Ho *et al.* 2007, 2010; Shete & Quadro 2013). Available studies on humans elucidated the pharmacokinetics of astaxanthin supplemented in dosages of 40–100 mg as well as its transport and distribution in the plasma by lipoproteins (Osterlie *et al.* 2000; Odeberg *et al.* 2003). In point of fact, observational studies addressing the pharmacokinetics of astaxanthin in aquatic animals have, however, been somewhat scanty. Rajasingh *et al.* (2006) presented a dynamic model to describe and analyse the absorption, transport, deposition, metabolism and excretion of keto-carotenoid astaxanthin in Atlantic salmon *S. salar* at the whole-organism level. The authors postulated that astaxanthin transport and delivery in salmon resemble that of mammals. This model is particularly useful to define a scaffold for modelling carotenoid dynamics in mammalian and nonmammalian systems providing heuristic basis for future experimental researches.

## Metabolic aspects of astaxanthin

Astaxanthin is a hydrophobic xanthophyll which exhibits poor solubility in the aqueous environment of the gastrointestinal tract. Gastrointestinal absorption and metabolism of astaxanthin are known to be strongly influenced by the presence of certain dietary factors which include cholesterol, fatty acids and vitamin E. Astaxanthin is known to be transported alongside these molecules through the intestine and blood. An improvement in astaxanthin absorption and deposition of Atlantic salmon *S. salar* was discovered by increasing dietary cholesterol (Bjerkeng *et al.* 1997; Chimsung *et al.* 2013, 2014), fatty acid (Waagbo *et al.* 1993; Einen & Skrede 1998; Bjerkeng *et al.* 1999b; Hamre *et al.* 2004; Olsen *et al.* 2005) and vitamin E (Christiansen *et al.* 1993; Waagbo *et al.* 1993; Bjerkeng *et al.* 1999a) levels. Studies in rainbow trout *O. mykiss* have provided evidence that absorption and metabolism of astaxanthin were affected appreciably by dietary lipid (Nickell & Bromage 1998; Barbosa *et al.* 1999; Choubert *et al.* 2006). This association has been observed in humans too, where adequate amount of dietary fat is necessary for optimal absorption of carotenoids (Yeum & Russell 2002; Brown *et al.* 2004; Abidov *et al.* 2010; Peng *et al.* 2011).

Most of the available reports describing the metabolism of astaxanthin in aquatic animals were assessed on salmonids. The conspicuous flesh coloration of salmonid fish is dependent upon assimilation of dietary carotenoid pigments. Considerable variations appear to exist in the relative efficiency of different salmonid species to absorb and deposit astaxanthin within the muscle, as documented by many previous researchers (March & MacMillan 1996; Turujman *et al.* 1997; Bjerkeng & Berge 2000; Ytrestoyl *et al.* 2005; Chimsung *et al.* 2014). The apparent digestibility coefficients (ADC) of astaxanthin reported in salmonid fish were 30–90% (Bjerkeng & Berge 2000; Ytrestoyl *et al.* 2005; Page & Davies 2006). Nevertheless, the retention of dietary astaxanthin by the muscle tissues rarely surpasses 18% in rainbow trout (Storebakken & No 1992) and 12% in Atlantic salmon (Torrissen *et al.* 1989; Wathne *et al.* 1998; Bjerkeng & Berge 2000; Ytrestoyl & Bjerkeng 2007b). This is attributable to the poor gastrointestinal absorption that limits the effective utilization of dietary administered astaxanthin. The gut uptake of carotenoids is rather slow compared to other essential micronutrients due to their lipophilic nature (Kiessling *et al.* 1995; Gobantes *et al.* 1997; Aas *et al.* 1999). Maximum plasma astaxanthin concentration was attained after 24 h in rainbow trout when fed a single oral dose containing 500 μg of astaxanthin (March *et al.* 1990). Additionally, Gobantes *et al.* (1997) found maximum concentration of astaxanthin in the blood of rainbow trout approximately 18 h after ingestion of a single dose of 100 μg astaxanthin. Meanwhile, the maximum serum astaxanthin concentration in Atlantic salmon force-fed a single dose of $^{14}$C-astaxanthin was detected after 30 h (Aas *et al.* 1999). These notable differences may well imply that Atlantic salmon are less efficient at laying down pigment in the flesh compared to rainbow trout as assessed by carotenoid retention. Apart from that, March *et al.* (1990) and March and MacMillan (1996) indicated that rapid metabolism of absorbed pigment to colourless derivatives rather than failing absorption was primarily responsible for pigmentation failure in white chinook salmon *Oncorhynchus tshawytscha*. Attempts undertaken to enhance the utilization of astaxanthin in salmonids through intraperitoneal administration recorded higher concentration of pigment in the kidney, liver, muscle, plasma and skin of Atlantic cod *Gadus morhua*, Atlantic salmon and rainbow trout (Maltby *et al.* 2003; Ytrestoyl & Bjerkeng 2007a,b). Plasma and muscle astaxanthin concentrations are reckoned to be good indicators of bioavailability (Choubert *et al.* 1994; Storebakken & Goswami 1996; Kiessling *et al.* 2003, 2006). Much of the earlier works revealed that the degree of flesh pigmentation in Atlantic salmon (Gjerde & Gjedrem 1984), chinook salmon (Withler 1986; McCallum *et al.* 1987) and rainbow trout (Gjerde & Gjedrem 1984; Torrissen & Naevdal 1984; Blanc & Choubert 1985) is genetically determined. For instance, Torrissen and Naevdal (1984) reported marked distinction between the flesh colour of half and full-sibling groups of rainbow trout. In another investigation with chinook salmon, Withler (1986) noticed different levels of carotenoid pigmentation in white-fleshed ($0.24 \pm 0.04$ μg) and red-fleshed ($3.37 \pm 0.14$ μg) fish. Similarly, in a later study, Bjerkeng and Berge (2000) disclosed significantly greater ADC of astaxanthin in white-fleshed Atlantic halibut *Hippoglossus hippoglossus* in comparison with Atlantic salmon that has a higher retention of the pigment. Genetic basis of carotenoid coloration in cichlids has been recently reviewed by Sefc *et al.* (2014). Transporter molecules and enzymes involved in the absorption and metabolism of dietary carotenoids are likely to be genetically controlled, rather than under environmental influence (Yonekura & Nagao 2007; Magalhaes & Seehausen 2010; Hill & Johnson 2012; Takahashi *et al.* 2013; Toews *et al.* 2017). There are also data indicating positive linear correlation between salmonid pigmentation and fish growth rate or body weight (March *et al.* 1990; Bjerkeng *et al.* 1992). The authors confirmed the conclusion reached from earlier observations (McCallum *et al.* 1987; Torrissen 1989) that flesh pigmentation in salmonids only occurs after reaching certain minimum size or body weight. According to Bjerkeng *et al.* (1992) and Baker *et al.* (2002), the deposition of carotenoids in the muscle of Atlantic salmon is concomitant with fish size and slower in young fish. Moreover, Ytrestoyl *et al.* (2005) suggested that both temperature and feed intake affected plasma astaxanthin levels positively and may have therefore influenced the assimilation efficiency of astaxanthin in Atlantic salmon. This is in agreement with the previously reported effects of temperature on the digestibility of nutrients, dry matter, nitrogen (protein) and energy in salmonid fish (Elliot 1976; Nicieza *et al.* 1994; Azevedo *et al.* 1998).

Furthermore, many comparative studies on pigmentation efficacy denoted differences in the utilization of astaxanthin and canthaxanthin among salmonid fish, suggesting species specificity in this context. Astaxanthin tended to be more efficiently absorbed and deposited in the flesh of rainbow trout than canthaxanthin (Torrissen 1986, 1989; Bjerkeng *et al.* 1990; Storebakken & No 1992; Torrissen & Ingebrigtsen 1992; Choubert *et al.* 1994, 2005), whereas the same was not true for Atlantic salmon (Buttle *et al.* 2001; Baker *et al.* 2002; Kiessling *et al.* 2003; Page & Davies 2006; Choubert 2010). Muscle retention of ingested carotenoids in rainbow trout represents 3–18% for astaxanthin and 2–7% for canthaxanthin (Foss *et al.* 1984; Choubert & Storebakken 1989; Hardy *et al.* 1990; Torrissen *et al.* 1990; Choubert *et al.* 1995). In contrast, Atlantic salmon was observed to retain 5.7% and 7.6% of astaxanthin and canthaxanthin, respectively (Buttle *et al.* 2001). Some authors specified that the relative polarity and hydrophobicity of these pigments may potentially influence their degree of deposition in rainbow trout. Astaxanthin has a higher hydrophobicity compared to other carotenoids (β-carotene, β-cryptoxanthin, canthaxanthin, lycopene and zeaxanthin) (Schiedt *et al.* 1985; Buttle *et al.* 2001; Thyssandier *et al.* 2001; Miyazawa *et al.* 2011), which in turn ascribed to better digestibility, greater gastrointestinal absorption and superior bonding affinity in the muscle of rainbow trout (Choubert & Storebakken 1996; Gobantes *et al.* 1997). It is also worth noting that differences in polarity between astaxanthin (more polar) and canthaxanthin (less polar) would cause them to be transported and cleared dissimilarly (Bierer *et al.* 1995; Thyssandier *et al.* 2002; Choubert 2010). Gobantes *et al.* (1997) assessed the kinetics of 100 μg oral dose of either astaxanthin or canthaxanthin in immature rainbow trout based on postprandial serum carotenoid concentration. They claimed that the maximum level of astaxanthin in serum corresponded to 1.6 times higher than that of canthaxanthin and the latter was cleared more rapidly from the plasma of rainbow trout. A previous investigation by Guillou *et al.* (1992) found comparatively higher absorption rates of astaxanthin than canthaxanthin and zeaxanthin in mature female rainbow trout force-fed radiolabelled carotenoids. Accordingly, Choubert *et al.* (2005) studied the bioavailabilities of $^{14}$C-keto-carotenoids in rainbow trout and proved that the maximum blood concentration of $^{14}$C-astaxanthin was greater than $^{14}$C-canthaxanthin. The extent of hepatic carotenoid uptake and metabolism was also indirectly investigated in rainbow trout using an in vitro isolated perfused liver model (Page & Davies 2003). Piscine serum perfusion showed that the cumulative uptake mechanism for both astaxanthin and canthaxanthin was saturable in the carrier models, with astaxanthin uptake saturated relatively faster than for canthaxanthin. Carotenoid absorption rates in the liver are principally limited by the saturation response of lipoprotein uptake mechanism and will be halted when individual lipoprotein capacity for transport is saturated (Page & Davies 2003). This could partially explain the better utilization of astaxanthin in rainbow trout. Conversely, it is well established that the utilization of canthaxanthin is more efficacious in Atlantic salmon compared to astaxanthin. Perhaps, this could well be implicated in the fact that Atlantic salmon possesses lower absorption, rapid clearance rate and active metabolic turnover of astaxanthin (Buttle *et al.* 2001; Baker *et al.* 2002; Choubert 2010). Low intestinal absorptive capacity was postulated to limit astaxanthin uptake in Atlantic salmon (March & MacMillan 1996). Page and Davies (2006) on the other hand observed a higher clearance rate of astaxanthin from blood circulatory system in Atlantic salmon which contributed to lower serum astaxanthin concentration. This finding was partly substantiated by Kiessling *et al.* (2003) that noted a higher serum canthaxanthin concentration in Atlantic salmon when fed equivalent dietary amounts of astaxanthin and canthaxanthin. The authors further asserted the existence of a mutual competition between astaxanthin and canthaxanthin for absorption and blood carrying capacity. Aside from that, Aas *et al.* (1999) evidenced the rapid catabolic transformation of astaxanthin to idoxanthin in Atlantic salmon that had been force-fed single doses of $^{14}$C-astaxanthin. Likewise, Bjerkeng and Berge (2000) acknowledged a similar reductive pathway for astaxanthin metabolism in both Atlantic halibut and Atlantic salmon by detecting the presence of 3′,4′-cis and trans glycolic isomers of idoxanthin (3,3′,4′-trihydroxy-beta,beta-carotene-4′-one) in whole kidney, liver and plasma. Taken together, the assimilation profiles for carotenoids appear to vary within and between salmonid species.

## Uses and benefits of astaxanthin

### Reproductive performance and egg quality

Astaxanthin plays a fundamental role not only in the cultivation but also in the breeding of diverse kinds of aquaculture species. To date, there exist many lines of evidence to suggest that astaxanthin confers a significant impact on reproductive performance, egg production and egg quality of aquatic animals (Vassallo-Agius *et al.* 2001; Ahmadi *et al.* 2006; Paibulkichakul *et al.* 2008; Tizkar *et al.* 2013, 2015; Palma *et al.* 2016). In many aquaculture farms, there have always been attempts to improve egg and larval quality while disregarding sperm quality. As a matter of fact, the optimization of seed production is one of the biggest obstacles limiting aquaculture development. Hence, it is absolutely necessary to understand the dietary requirement of farmed animals as nutrient availability directly influences various aspects of reproductive physiology.

Given the many physiological functions of carotenoids and astaxanthin, in particular, these pigments are important resources that are limited by dietary availability due to the incapability of higher animals to synthesize them directly. The influence of dietary astaxanthin supplementation on the reproduction and broodstock performance of different aquatic animals has been documented in many past studies (Table 4). Dietary intake of 150 mg astaxanthin $kg^{-1}$ feed (compared to 50 and 100 mg levels) for 150 days significantly promoted the spermatocrit value, sperm concentration, motility, osmolality and fertilization rate of goldfish *Carassius auratus* (Tizkar *et al.* 2015). Pangantihon-Kuhlmann *et al.* (1998) provided valuable insight on the improved fecundity, ovarian development and spawning of giant tiger prawn *Penaeus monodon* broodstock when supplemented with astaxanthin (100 mg $kg^{-1}$ diet) for 61 days. In another related study, the reproductive performance of *P. monodon* broodstock as measured by the number of spermatozoa in male shrimp and amount of eggs in gravid female was greatly enhanced when fed with 500 mg astaxanthin $kg^{-1}$ diet (Paibulkichakul *et al.* 2008). In rainbow trout *O. mykiss*, dietary supplements of astaxanthin are deemed necessary for optimum reproduction (Ahmadi *et al.* 2006). It has been claimed that astaxanthin triggers a speedier oocyte maturation in rainbow trout. The effectiveness of a number of nutrient supplements including astaxanthin, vitamin A and E, and the polyunsaturated fatty acids in augmenting the reproductive physiology in fish have also been reviewed by several authors (Hardy 1985; Bromage 1995; Furuita *et al.* 2000, 2002; Pavlov *et al.* 2004; Wade *et al.* 2015b). Carotenoids particularly astaxanthin are recognized to be involved in the reproductive processes of many organisms due to their accumulation within reproductive organs (Goodwin 1950; Pangantihon-Kuhlmann *et al.* 1998; Wouters *et al.* 2001; Linan-Cabello *et al.* 2002a; Wade *et al.* 2015a). This aspect has been investigated in sexually maturing salmonid fish which noted the predominant redistribution of body carotenoid pool from the flesh into the gonads of female fish and to the skin of male fish (Steven 1949; Crozier 1970; Ando & Hatano 1987; Bjerkeng *et al.* 1992; Synowiecki *et al.* 1993; Hatlen *et al.* 1996; Izquierdo *et al.* 2001; Blount *et al.* 2002; Rajasingh *et al.* 2006; Nie *et al.* 2011). Bjerkeng *et al.* (1999c) postulated that carotenoid transformation and distribution are affected by the steroid sex hormones specifically 17β-estradiol and 11-ketotestosterone. As might be expected, a prominent loss of whole-body carotenoids occurs

**Table 4** Effect of astaxanthin on the reproductive performance of aquatic animals

| Species | Inclusion level/ range (mg kg$^{-1}$) | Source | Response | References |
|---|---|---|---|---|
| Atlantic Cod (*Gadus morhua*) | 73.7 | Synthetic | Improved egg quality and larval production | Sawanboonchun *et al.* (2008) |
| Goldfish (*Carassius auratus*) | 150 | Synthetic | Enhanced egg survival rate and fertilization rate | Tizkar *et al.* (2013) |
| | 150 | Synthetic | Promoted spermatocrit value, sperm concentration, motility, osmolality and fertilization rate | Tizkar *et al.* (2015) |
| Rainbow Trout (*Oncorhynchus mykiss*) | 0.07–92.91 | Synthetic | Improved egg quality, fertilization rate, hatching rate and survival rate | Ahmadi *et al.* (2006) |
| Striped Jack (*Pseudocaranx dentex*) | 10 | Synthetic | Improved egg quality and fertilization rate | Vassallo-Agius *et al.* (2001) |
| Yellowtail (*Seriola quinqueradiata*) | 30 | Synthetic | Improved egg production, egg quality and hatching rate | Verakunpiriya *et al.* (1997) |
| Giant Tiger Prawn (*Penaeus monodon*) | 100 | Synthetic | Improved fecundity, ovarian development and spawning success | Pangantihon-Kuhlmann *et al.* (1998) |
| | 500 | Synthetic | Increased number of spermatozoa in male shrimp and amount of eggs in gravid female | Paibulkichakul *et al.* (2008) |
| Sea Horse (*Hippocampus guttulatus*) | 75–125 | Synthetic | Improved egg quality | Palma *et al.* (2016) |

concurrently with the redistribution process (Crozier 1970; Bjerkeng *et al.* 1992, 1999c; Rajasingh *et al.* 2006). As evidenced by some earlier reports, relatively high contents of unesterified carotenoids were detected in the mature eggs of female fish during oogenesis as carotenoids are mobilized from the flesh or muscle, and liver with subsequent incorporation into the ovary (Steven 1949; Kitahara 1983, 1984; Bjerkeng *et al.* 2000; Svensson *et al.* 2006). Moreover, carotenoid-based ornamentation (e.g. astaxanthin) has been suggested to signal mate quality or sexual selection process, and for this reason, it plays a crucial role in reproduction (Amundsen & Forsgren 2001; Nordeide *et al.* 2006; Svensson *et al.* 2006; Bjerkeng 2008). For example, the conspicuous reddish ornament which is often displayed by female two-spotted gobies *Gobiusculus flavescens* due to their pigmented eggs being visible through the abdominal skin is positively associated with female quality and phenotypic quality traits (Amundsen & Forsgren 2001, 2003; Nordeide *et al.* 2006). In aid to this fact, male gobies may benefit by mating and fertilizing the eggs of ornamented females with excessive amounts of antioxidant carotenoids. A few other investigations on first mate choice revealed male preference for carotenoid-ornamented females in sockeye salmon *O. nerka* (Craig & Foote 2001; Foote *et al.* 2004) as well as two poeciliid fish namely *Priapella olmecae* and *Xiphophorus helleri* (Basolo & Delaney 2001).

The well-established functions of astaxanthin in aquatic animals include, serving as specific precursors to retinoids (provitamin A) and boosting its activity (Torrissen 1990; Linan-Cabello *et al.* 2002a; Moren *et al.* 2002; White *et al.* 2003; Blomhoff & Blomhoff 2006), improving embryonic and larval development (George *et al.* 2001; Pan *et al.* 2001; Chou & Chien 2006; Haga *et al.* 2008; Palma *et al.* 2016), acting as antioxidant (Bell *et al.* 2000; Dufosse *et al.* 2005; McGraw *et al.* 2005), enhancing production of antibodies and proliferation of immune cells (Amar *et al.* 2001, 2004; Magnadottir 2010; Kiron 2012). Astaxanthin has been suggested to be a primary source of retinoids (provitamin A) in eggs and early embryos (Miki 1991; Dall 1995; Dall *et al.* 1995; Linan-Cabello *et al.* 2002b). The particular role of retinoids (provitamin A) in cell signalling during patterning of developing vertebrate embryos has been well defined (Kawakami *et al.* 2005; Duester 2008; Kam *et al.* 2012). Some previous studies have shown experimental evidence regarding the positive effects of retinoids to ovary and larval development of different crustaceans (Dall 1995; Dall *et al.* 1995; Linan-Cabello *et al.* 2002a,b). The significant benefits of astaxanthin is attributable to its potent antioxidant capacity to quench excessive amounts of destructive singlet oxygen and free radicals, thus preventing the peroxidation or oxidative damage of reproductive cells or tissues, and developing eggs (Miki 1991; Mayne 1996; Britton 2008; Palozza *et al.* 2009). Various stressors including exposure to ultraviolet light or chemicals and physiological stress may induce the production of such destructive agents. Blount *et al.* (2002) discovered that male fish actively transport carotenoids linked to lipoproteins into testes to restrain oxidative injury cause by ROS (reactive oxygen species). Oxygen free radicals and peroxides have been demonstrated to lead an oxidative attack on egg biomembranes contributing to quality deterioration and impairment (Bromage & Roberts 1995; Edge *et al.* 1997). It has been presumed that egg colour or carotenoid content provides an indication of egg quality. Pettersson and Lignell

(1999) recorded a considerable offspring mortality in astaxanthin deficient eggs of Baltic salmon *S. salar* afflicted by the M74 syndrome (reproduction disorder). Nutrients (e.g. lipids, vitamin, essential fatty acids and astaxanthin) reserved in egg yolk are crucial determinants of egg quality, which is usually expressed as hatching success, ratio of buoyant eggs and the incidence of morphological abnormalities or impairments in larvae (Lubzens *et al.* 2003; Salze *et al.* 2005). Preparation of high-quality eggs could therefore be essential in aquaculture industry. It should be noted that the accumulation of astaxanthin in reproductive tissues via dietary supplementation provides a significant impact on the reproductive performance characteristics, which include egg quality, egg quantity, hatching success and number of larvae in giant tiger prawn *P. monodon* (Pangantihon-Kuhlmann *et al.* 1998; Paibulkichakul *et al.* 2008), sea horse *Hippocampus guttulatus* (Palma *et al.* 2016), sea urchin *Lytechinus variegatus* (George *et al.* 2001), rainbow trout *O. mykiss* (Craik 1985; Ahmadi *et al.* 2006), striped jack *Pseudocaranx dentex* (Vassallo-Agius *et al.* 2001), yellowtail *Seriola quinqueradiata* (Verakunpiriya *et al.* 1997), red sea bream *Pagrus major* (Watanabe & Miki 1993) and Atlantic cod *G. morhua* (Sawanboonchun *et al.* 2008). Hartmann *et al.* (1947) proposed that astaxanthin acts as a fertilization hormone that stimulates spermatozoa attraction leading to increased fecundity, fertilization rates, promoting gonad development and maturation in fish. Similar observation was also reported in many further studies for fish fed diets containing carotenoids (Hubbs & Stavenhagen 1958; Georgiev 1971; Mikulin & Soin 1975; Craik 1985; Mikulin 2003). Furthermore, the presence of astaxanthin in the eggs of aquatic animals has been described to play beneficial roles in cellular protection from harmful UV light (Torrissen 1990), improved respiratory function (Craik 1985; Mikulin 2000), serving as a source of pigmentation in the embryo (Pan *et al.* 2001) and might be involved in photoreception processes (Ronnestad *et al.* 1998). Astaxanthin content has also been directly linked to the ability of eggs to resist extreme environmental conditions such as increasing ammonia levels, elevated water temperatures and anoxic condition (Eisler 1957; Craik 1985; Torrissen 1990). As detailed above, the availability of supplementary astaxanthin is fundamental for enhancement of reproductive performance and egg quality of aquatic animals.

### Growth performance and survival

In most aquaculture operations, feed represents greater than 60% of a total hatchery management cost depending on production scale and cultivation methods. Therefore, it is absolutely necessary to develop feeds with nutritional ingredients that promote growth and survival of the farmed species as a requisite to minimize production costs. Beneficial role of astaxanthin as a critical nutritive additive essential for excellent growth and survival has been investigated in various aquatic animals. Over the years, available studies have been mixed, with a vast majority of the reports having been conducted on crustaceans. Some reported studies did not observe any noteworthy improvements in growth and survival of several aquatic animals when fed diets incorporated with astaxanthin (Pan *et al.* 2001; Pham *et al.* 2014; Yi *et al.* 2014; Liu *et al.* 2016b). Nevertheless, an increasing number of quantitative research papers revealed significant positive correlations between dietary astaxanthin supplementation and either growth or survival, or both, in different fish and crustaceans (Table 5).

Early studies undertaken during the late 1990s demonstrated the ability of dietary astaxanthin to improve growth and survival in a variety of shrimp species. Administration of supplementary astaxanthin at 100 mg $kg^{-1}$ diet was reported to increase the survival rate of Kuruma prawn *Penaeus japonicus* (91.3%) compared to those fed with a control diet (57.1%) by the end of 8 weeks (Yamada *et al.* 1990). The authors further elucidated that astaxanthin is more effective than β-carotene or canthaxanthin as a pigment source in *P. japonicus*. Petit *et al.* (1997) noticed that feeding astaxanthin-based diet at 60 mg $kg^{-1}$ over 8 weeks modified exuviation frequency and shortened moulting cycle, which ultimately hastened postlarvae growth and development of *P. japonicus*. Chien and Jeng (1992) on the other hand observed significant correlations between specific growth rate or survival and optimal tissue carotenoid concentration of *P. japonicus.* The report also showed that prawn fed with astaxanthin-supplemented diets had a greater survival than those fed with algal meal or β-carotene supplemented diets. In a later study, it was validated that supplementations of natural astaxanthin derived from alga *H. pluvialis* and synthetic astaxanthin in formulated diets at two concentrations, 50 and 100 mg $kg^{-1}$ respectively, resulted in significantly higher survival rate of juvenile *P. japonicus* when fed for 9 weeks (Chien & Shiau 2005). Apart from that, *Penaeus indicus* larvae exhibited markedly greater survival rate (88%) from PZ1 stage until metamorphosis when fed the astaxanthin-enriched nematodes *Panagrellus redivivus* (1.43 μg astaxanthin $g^{-1}$ dry weight of nematode), while neither larval growth nor development was affected (Kumlu *et al.* 1998). The aforementioned study also pointed out that nematode can be effectively utilized as a pigment carrier to penaeid larvae.

Ever since the accomplishment of the initial works, most of the available studies have focused on Pacific white shrimp *Penaeus vannamei* and giant tiger prawn *P. monodon*. Supplementing astaxanthin at 80 mg $kg^{-1}$ diet for 48 days improved daily growth coefficient, survival and moult frequency in juvenile *P. vannamei* acclimated to low

**Table 5** Effect of astaxanthin on the growth and survival of different aquatic animals

| Species | Inclusion level/range | Source | Response | References |
|---|---|---|---|---|
| Giant Freshwater Prawn (*Macrobrachium rosenbergii*) | 50–200 mg kg$^{-1}$ | Synthetic | Boosted weight gain and improved survival | Kumar *et al.* (2009) |
| Giant Tiger Prawn (*Penaeus monodon*) | 100 mg kg$^{-1}$ + cholesterol | Synthetic | Boosted weight gain and improved survival | Niu *et al.* (2012) |
| | 100 mg kg$^{-1}$ + cholesterol | Synthetic | Boosted weight gain and improved survival | Niu *et al.* (2014) |
| | 25–100 mg kg$^{-1}$ | Synthetic | Boosted weight gain and improved growth | Wade *et al.* (2015c) |
| Indian Prawn (*Penaeus indicus*) | 1.43 μg g$^{-1}$ | Synthetic | Improved survival | Kumlu *et al.* (1998) |
| Kuruma Prawn (*Penaeus japonicus*) | 100 mg kg$^{-1}$ | Synthetic | Improved survival | Yamada *et al.* (1990) |
| | 60 mg kg$^{-1}$ | Synthetic | Enhanced growth and moulting frequency | Petit *et al.* (1997) |
| | 50 and 100 mg kg$^{-1}$ | Algal and synthetic | Improved survival | Chien and Shiau (2005) |
| Pacific White Shrimp (*Penaeus vannamei*) | 80 mg kg$^{-1}$ | Synthetic | Enhanced growth, survival and moulting frequency | Flores *et al.* (2007) |
| | 100–400 mg kg$^{-1}$ | Synthetic | Boosted weight gain and improved survival | Niu *et al.* (2009) |
| | 125–150 mg kg$^{-1}$ | Synthetic | Enhanced growth and survival | Zhang *et al.* (2013) |
| Red King Crab (*Paralithodes camtschaticus*) | 380 mg kg$^{-1}$ | Algal | Enhanced growth and survival | Daly *et al.* (2013) |
| Sea Horse (*Hippocampus guttulatus*) | 75–125 mg kg$^{-1}$ | Synthetic | Improved juvenile growth and survival | Palma *et al.* (2016) |
| Atlantic Cod (*Gadus morhua*) | 50–100 mg kg$^{-1}$ | Synthetic | Enhanced growth and survival | Hansen *et al.* (2016) |
| Atlantic Salmon (*Salmo salar*) | 30 mg kg$^{-1}$ | Synthetic | Promoted growth | Torrissen (1984) |
| | 20–40 mg kg$^{-1}$ | Synthetic | Enhanced growth and survival | Christiansen *et al.* (1994) |
| | 6–317 mg kg$^{-1}$ | Synthetic | Enhanced growth and survival | Christiansen *et al.* (1995) |
| | 36–190 mg kg$^{-1}$ | Synthetic | Enhanced growth and survival | Christiansen and Torrissen (1996) |
| Large Yellow Croaker (*Pseudosciaena crocea*) | 0.22–0.89 g kg$^{-1}$ | Algal | Boosted weight gain and improved growth | Li *et al.* (2014) |
| Rainbow Trout (*Oncorhynchus mykiss*) | 12.5–92.9 mg kg$^{-1}$ | Synthetic | Promoted growth | Bazyar Lakeh *et al.* (2010) |
| Red Porgy (*Pagrus pagrus*) | 40 mg kg$^{-1}$ | Synthetic | Promoted growth | Kalinowski *et al.* (2005) |

salinity condition (Flores *et al.* 2007). Niu *et al.* (2009) observed similar improvements in weight gain and survival of *P. vannamei* fed either 100, 200 or 400 mg astaxanthin kg$^{-1}$ diet for 30 days compared to the control diet without astaxanthin inclusion. In another study, larval *P. vannamei* gained considerable body weight during 28th week after feeding on 125 or 150 mg astaxanthin kg$^{-1}$ feed surpassed those fed with 25, 50, 75 or 100 mg astaxanthin kg$^{-1}$ feed comparatively, but survival was not affected (Zhang *et al.* 2013). Both studies concluded that astaxanthin was a necessary dietary component for proper growth and development of larval shrimp. Two recent studies have discovered better weight gain and survival in giant tiger prawn *P. monodon* when fed diet containing astaxanthin (100 mg kg$^{-1}$ feed) and cholesterol (1%) for 74 days, with an apparent high astaxanthin digestibility coefficient (>90%) (Niu *et al.* 2012, 2014). The inclusion of dietary cholesterol was thought to enhance astaxanthin bioavailability, absorption and accumulation in tissue. Likewise, dietary astaxanthin intakes between 25 and 100 mg kg$^{-1}$ diet were found to remarkably boost weight gain and growth of *P. monodon* juveniles without affecting survivability (Wade *et al.* 2015c). In red king crab *Paralithodes camtschaticus*, the survival over 56 days was greatly improved when its diets were enriched with 380 mg astaxanthin kg$^{-1}$ feed (Daly *et al.* 2013). Moreover, Kumar *et al.* (2009) recorded that the growth and survival of subadult giant freshwater prawn *Macrobrachium rosenbergii* receiving 50, 100 or 200 mg astaxanthin kg$^{-1}$ diet throughout a period of 28 days were significantly augmented.

Some of the information on the growth improvement and survival response of fish upon fortification of formulated diets with astaxanthin are also reviewed here. Much of the pioneering researches paid particular attention to Atlantic salmon *S. salar*. Feeding astaxanthin-based diets (30 mg kg$^{-1}$) to *S. salar* fry fostered the most favourable growth during the early start-feeding period of 35 days (Torrissen 1984). This was in accordance with the results obtained by Christiansen and co-workers in separate feeding trials employing various dietary inclusion levels of astaxanthin. Christiansen *et al.* (1994) provided informative fact that 20–40 mg astaxanthin $kg^{-1}$ dry feed exerted a profound effect on the specific growth rate and survivability of fry throughout the first-feeding period of 20 weeks. In addition, Christiansen *et al.* (1995) also put forward the need for optimum dietary astaxanthin concentrations between 6 and 317 mg $kg^{-1}$ dry feed to achieve utmost growth and survival in fry during the start-feeding period of 11 weeks. In contrast, relatively poor growth and low survival rate were noted in groups fed with a control diet without astaxanthin. In another study, *S. salar* juveniles displayed a tendency for higher growth and survival fed with astaxanthin-enriched diets containing either 36 or 190 mg astaxanthin $kg^{-1}$ casein-based purified diets for a 10-week period (Christiansen & Torrissen 1996). Other species of fish have also shown a similar response although less studied. Kalinowski *et al.* (2005) denoted higher growth values in hatchery-reared red porgy *Pagrus pagrus* fed with 40 mg astaxanthin $kg^{-1}$ dry diet after a period of 17 weeks. Meanwhile, rainbow trout *O. mykiss* fry had improved specific growth rates and thermal growth coefficients when supplemented with astaxanthin (12.5–92.9 mg $kg^{-1}$ feed) in an experiment lasting for 45 days, with a complimentary increment in body astaxanthin concentrations (Bazyar Lakeh *et al.* 2010). In large yellow croaker *Pseudosciaena crocea* and Atlantic cod *G. morhua*, a range of astaxanthin levels up to 100 mg $kg^{-1}$ feed was evaluated; large yellow croaker juvenile that received 2.8, 5.6 and 11.2 g *H. pluvialis* $kg^{-1}$ diet resulting in final astaxanthin concentrations of 0.22, 0.45 and 0.89 mg $kg^{-1}$ diet, respectively, gained considerable body weight and higher survival after 66 days (Li *et al.* 2014), while Atlantic cod larvae that accepted 50 and 100 mg astaxanthin $kg^{-1}$ diet at the end of 50-day growth trial attained enhanced growth performance and survival rates (Hansen *et al.* 2016).

These combined evidences consistently suggest the importance of dietary astaxanthin as a nutritional growth factor in stimulating growth and survival of aquatic animals. It is generally accepted that astaxanthin plays a vital role in the intracellular intermediary metabolism of aquatic animals (Segner *et al.* 1989; Kiron, 2012). This would consequently affect the physiological functions and further enhance the nutrient utilization or assimilation of aquatic animals ultimately resulting in intensifying growth performance (Amar *et al.* 2001; Arredondo-Figueroa *et al.* 2003). The effect of astaxanthin on improving survivability of animals appears to be closely linked to its proposed antioxidant property, ensuring optimal cellular functions and conferring physiological improvements in antioxidant capacity under various stressful conditions. Another interesting aspect of astaxanthin is its role in increasing haemolymph ecdysteroid levels via metabolic interactions which is then hydroxylated and transformed into bioactive form of moult-stimulant hormone, thereby having a measurable influence on moulting physiology in crustaceans (Chang 1997; Petit *et al.* 1997; Wade *et al.* 2015b). Thus, it is not surprising that this carotenoid is frequently studied in relation to its effect on growth improvement and survival.

### Stress tolerance and disease resistance

The emergence of infectious diseases in intensive farming, particularly during the early production stages, represents a major downside or leading threat contributing to significant economic impacts worldwide. High-density aquaculture operations frequently subject animals to various physical stressors which involve grading, transport, handling, vaccination, crowding and confinement or any other forms of physical disturbance that could be extremely stressful and immune-depressive. These negative factors may disrupt the fine balance between aquatic animals and their surrounding environments triggering stress responses (Scholthof 2007). Excessive stress attributes to bodily physiological dysfunction, growth rate reduction, immune suppression, susceptibility towards pathogenic invasions and even mortality (Pillay & Kutty 2005; Ndong *et al.* 2007; Nikoo *et al.* 2010; Noga 2011; Liu *et al.* 2016b). This often invited concern over its management and effective control. Thus, it is of utmost importance in aquaculture research to alleviate adverse conditions that may induce considerable stress and weaken the host organism.

Through the years, substantial research efforts have been directed towards relieving stress and boosting immunity of many crustaceans and fish using astaxanthin in the diet (Table 6). Comparable to growth performance and survival, most existing studies on stress tolerance and disease resistance have been dedicated to prawns and shrimps. An early research revealed that dietary intake between 230 and 810 mg astaxanthin $kg^{-1}$ diet for 4 weeks improved the resistance of postlarvae giant tiger prawn *P. monodon* against salinity shock (Merchie *et al.* 1998). Another study pointed out that astaxanthin (200 mg $kg^{-1}$ diet) was effective in increasing the endurance of *P. monodon* postlarvae to low salinity stress (Darachai *et al.* 1998). Additionally, Chien *et al.* (1999) noticed that dietary inclusion of astaxanthin (360 mg $kg^{-1}$ feed) for 1 week appeared to induce optimal tolerance in the larval stages of *P. monodon* upon exposure to 4 h of low dissolved oxygen level ($<1$ mg $L^{-1}$). The observations made when different stress factors were tested on *P. monodon* juveniles that received astaxanthin (80 mg $kg^{-1}$ diet) over 8 weeks also exhibited enhanced antioxidant defence capability (lower superoxide dismutase SOD), better hepatopancreatic function (lower haemolymph alanine aminotransferase ALT and aspartate aminotransferase AST) and subsequent improvement recovery

**Table 6** Effect of astaxanthin on the stress tolerance and disease resistance of different aquatic animals

| Species | Inclusion level/range | Source | Response | References |
|---|---|---|---|---|
| Giant Freshwater Prawn (*Macrobrachium rosenbergii*) | 0.67 and 1.34 nmol $g^{-1}$ via injection | Synthetic | Improved survival and resistance against *Lactococcus garveae* infection | Angeles *et al.* (2009) |
| Oriental River Prawn (*Macrobrachium nipponense*) | 50–150 mg $kg^{-1}$ | Synthetic | Improved resistance to chemical and physical stress | Tizkar *et al.* (2014) |
| Giant Tiger Prawn (*Penaeus monodon*) | 230–810 mg $kg^{-1}$ | Synthetic | Improved resistance against osmotic stress | Merchie *et al.* (1998) |
| | 200 mg $kg^{-1}$ | Algal and synthetic | Prolonged life and improved endurance to low salinity stress condition | Darachai *et al.* (1998) |
| | 360 mg $kg^{-1}$ | Synthetic | Higher tolerance to low dissolved oxygen | Chien *et al.* (1999) |
| | 80 mg $kg^{-1}$ | Synthetic | Improved resistance against osmotic and thermal stress, greater antioxidant capacity | Chien *et al.* (2003) |
| | 71.5 mg $kg^{-1}$ | Synthetic | Higher resistance to ammonia stress, lower SOD levels, enhanced antioxidant status | Pan *et al.* (2003) |
| | 200–300 mg $kg^{-1}$ *Dunaliella* extract | Synthetic | Higher tolerance to low dissolved oxygen condition, enhanced resistance to WSSV infection | Supamattaya *et al.* (2005) |
| Kuruma Prawn (*Penaeus japonicus*) | 50 and 100 mg $kg^{-1}$ | Algal and synthetic | Higher tolerance to low dissolved oxygen | Chien and Shiau (2005) |
| Pacific White Shrimp (*Penaeus vannamei*) | 80 mg $kg^{-1}$ | Synthetic | Higher tolerance to low salinity stress condition and improved haematological responses | Flores *et al.* (2007) |
| | 100–400 mg $kg^{-1}$ | Synthetic | Higher tolerance to low dissolved oxygen | Niu *et al.* (2009) |
| | 125–150 mg $kg^{-1}$ | Synthetic | Higher tolerance to low dissolved oxygen, enhanced TAS, upregulated catalase, cMnSOD and HIF-1α mRNA expression levels | Zhang *et al.* (2013) |
| | 100 mg $kg^{-1}$ + cholesterol | Synthetic | Enhanced survival during live transportation | Niu *et al.* (2014) |
| | 80 mg $kg^{-1}$ | Algal | Improved immunological parameters, enhanced antioxidant status, promoted resistance to WSSV | Wang *et al.* (2015) |
| Characin (*Hyphessobrycon eques*) | 5–20 mg $kg^{-1}$ | Synthetic | Improved resistance to ammonia stress, enhanced antioxidant status | Pan *et al.* (2011) |
| Yellow Catfish (*Pelteobagrus fulvidraco*) | 80 mg $kg^{-1}$ | Synthetic | Improved resistance to acute crowding stress, improved antioxidant capacity, increased hepatic HSP70 level | Liu *et al.* (2016b) |
| Oscar (*Astronotus ocellatus*) | 200 mg $kg^{-1}$ | Synthetic | Improved immunological parameters, resistance to *Aeromonas hydrophilla* | Alishahi *et al.* (2015) |
| Rainbow Trout (*Oncorhynchus mykiss*) | 100 mg $kg^{-1}$ | Synthetic | Improved resistance to IHNV infection | Amar *et al.* (2012) |

against osmotic and thermal stresses (Chien *et al.* 2003). Similarly, *P. monodon* juveniles fed diet supplemented with 71.5 mg astaxanthin $kg^{-1}$ feed for 8 weeks displayed astounding antioxidant status and higher resistance to different levels of ammonia stress (0.02, 0.2, 2, 20 mg $L^{-1}$) (Pan *et al.* 2003). Lower SOD, ALT and AST in treated prawns reflected improved antioxidant capacity and hepatopancreatic function following biological stress. Both studies suggested that astaxanthin is a critically essential nutrient factor for giant tiger prawn under physiological stress triggered by abiotic changes. None of the abovementioned works have drawn any conclusive results regarding possible enhancement of disease resistance or immunity in the animal subject. Nonetheless, Supamattaya *et al.* (2005) found that *P. monodon* fed 200–300 mg *Dunaliella* extract $kg^{-1}$ diet were more tolerable to hypoxic conditions (0.8–1 mg $L^{-1}$) along with significantly greater resistance to white spot syndrome virus (WSSV), while phenoloxidase activity and total haemocyte count were negatively correlated. The authors also noted highest total carotenoid and astaxanthin contents in prawns fed the *Dunaliella* extract indicating their ability for rapid metabolic conversion of β-carotene to astaxanthin.

Extensive studies on Pacific white shrimp *P. vannamei* and other prawn species also demonstrated similar effects. Supplementing the diet with 80 mg astaxanthin $kg^{-1}$ feed over 6-week trial significantly enhanced hyperosmoregulatory ability and haemolymph concentrations of total haemocyte count, haemocyanin, lactate and glucose of *P. vannamei* juveniles acclimated to low salinity water (3 g $L^{-1}$) (Flores *et al.* 2007). A positive effect of dietary astaxanthin supplementation (100–400 mg $kg^{-1}$ diet) on stress tolerance of *P. vannamei* postlarvae to low dissolved oxygen conditions (0.8–1 mg $L^{-1}$) was also identified by Niu *et al.* (2009). Dietary astaxanthin supplementation between 125 and 150 mg $kg^{-1}$ diet throughout a period of 8 weeks stimulated higher total antioxidant status (TAS) and tolerance of *P. vannamei* which suffered from low dissolved oxygen stress (0.8 mg $L^{-1}$) besides upregulating hypoxia-inducible factor-1α (HIF-1α), cytosolic manganese superoxide dismutase (cMnSOD) and catalase (CAT) mRNA expression levels (Zhang *et al.* 2013). In another related study, Niu *et al.* (2014) validated that dietary combination of astaxanthin (100 mg $kg^{-1}$ feed) and cholesterol (1%) apparently boosted the tolerance of *P. vannamei* juveniles subjected to 36 h of simulated live transportation coupled with augmented expression profiles of hypoxia-inducible factor-1α (HIF-1α) and heat-shock protein 70 (HSP70) mRNAs in hepatopancreas. The authors suggested that astaxanthin was relatively more superior to β-carotene as dietary antioxidant and cholesterol inclusion could positively enhanced tissue astaxanthin retention efficiency in shrimp. More lately, Wang *et al.* (2015) further documented that *P. vannamei* fed with 80 mg $kg^{-1}$ astaxanthin-supplemented diet showed high-level resistance to white spot syndrome virus (WSSV), being associated with remarkable improvement of haemolymph immunological index, including phagocytic activity, total haemocyte count, serum phenoloxidase activity, serum antisuperoxide radical activity, serum bacteriolytic activity and serum antibacterial activity in the feeding period that lasted for 4 weeks. The latter study also denoted that dietary astaxanthin promoted mRNA expression of antioxidant enzyme genes (CAT, cMnSOD and glutathione peroxidase GPx) in the hepatopancreas. On the other hand, the administration of 50 and 100 mg astaxanthin $kg^{-1}$ diet from either algal or synthetic sources resulted in enhanced tolerance of juvenile Kuruma prawn *P. japonicus* to hypoxic conditions (<0.5 mg $L^{-1}$) in a trial lasted for 9 weeks (Chien & Shiau 2005). Two separate studies have reported better stress tolerance and disease resistance of freshwater prawns: (i) in the case of giant freshwater prawn *M. rosenbergii*, systemic astaxanthin injection at either dose of 0.67 or 1.34 nmol $g^{-1}$ through ventral sinus of the cephalothorax has attributed a profound increment in total haemocyte count (THC) and hepatic astaxanthin content leading to improved resistance against *Lactococcus garvieae* infection, although no direct stress test was performed (Angeles *et al.* 2009); (ii) as for oriental river prawn *Macrobrachium nipponense*, endurance to multiple chemical and physical stresses such as deprived oxygen supply (0.5 mg $L^{-1}$), ammonia spike (0.75 mg $L^{-1}$) and thermal shock (0°C) was effectively ameliorated in prawns accepting diets containing different amounts of astaxanthin (50–150 mg $kg^{-1}$ diet) after 10 weeks (Tizkar *et al.* 2014).

Approaches taken to examine the effect of astaxanthin on fish resulted in the same beneficial physiological effects. Providing astaxanthin-supplemented diets (5–20 mg $kg^{-1}$ diet) to characin *Hyphessobrycon eques*, a tropical freshwater tetra generated notable AST, GPx, SOD and TAS activities that contributed to greater resistance against ammonia stress (15 mg $L^{-1}$) after 8 weeks (Pan *et al.* 2011). Moreover, Amar *et al.* (2012) provided insights into the valuable effects of astaxanthin on the modulation of fish health and disease resistance of rainbow trout *O. mykiss*. There has been a marked elevation of serum astaxanthin concentration conferring greater tolerance to infectious hematopoietic virus (IHNV) experimental infection with lowest recorded mortality (22%) in fry that obtained 100 mg astaxanthin $kg^{-1}$ casein-based semipurified diet for 6 weeks. Results evidently highlighted that astaxanthin emerged as the most prominent carotenoid among all the carotenoids tested (β-carotene and canthaxanthin) in terms of fish health improvement. In oscar *Astronotus ocellatus*, mucus and serum lysozyme and bactericidal activity, together with resistance against *Aeromonas hydrophila* infection, were significantly enhanced upon dietary administration of astaxanthin (200 mg $kg^{-1}$ diet for 50 days) (Alishahi *et al.* 2015). Most recently, Liu *et al.* (2016b) investigated the consequences of dietary astaxanthin intake on the stress resistance of yellow catfish *Pelteobagrus fulvidraco*. When offered at 80 mg $kg^{-1}$ feed for 60 days, it improved immune or antioxidative capabilities, hepatic HSP70, hepatic SOD, serum total protein (TP) and tolerance to acute crowding stress. Subsequent challenge with *Proteus mirabilis* signified a significantly enhanced resistance in group fed with supplementary astaxanthin.

Collectively, presented data constantly imply the positive impacts of dietary astaxanthin on antioxidant capacity, stress alleviation, immune response regulation and disease resistance of aquatic animals. Direct experimental evidence clearly exists to prove that astaxanthin supplementation on animals improves a range of factors to enable efficient defence procedure against unfavourable or stressful situations such as disease outbreak, hypoxic condition, ammonia stress, thermal and osmotic fluctuations. The implicated biological functions of astaxanthin can be largely ascribed to its supreme antioxidant properties. This functional pigment is performing a broad protective role in neutralizing or scavenging the excess amounts of destructive singlet oxygen and free radicals generated from acute and long-term chronic stresses which can impair the important parts of the cellular constituents comprising enzyme, membranes and DNA

(Yu 1994; Chew 1995; Chien *et al.* 2003; Palozza *et al.* 2009). The overproduction of reactive oxygen species (ROS) was indicative of the health status of stressed aquatic organisms, deemed as a precursor to the prevalence of disease. Astaxanthin which is considered as one of the principal or dominant carotenoid surpasses the antioxidant benefits of other carotenoids (e.g. canthaxanthin, zeaxanthin, lutein and β-carotene) as well as vitamins C and E (Miki *et al.* 1982; Terao 1989; Jorgensen & Skibsted 1993; Stewart *et al.* 2008; Ranga Rao *et al.* 2010). This carotenoid pigment has been proposed as 'super vitamin E' whereby the antioxidant activities are noted to be approximately 10 times stronger than those of other carotenoids and 500 times greater than α-tocopherol comparatively, while more efficiently assimilated at low-energetic expense (Miki 1991; Petit *et al.*, 1997; Lorenz & Cysewski 2000; Naguib 2000; Goto *et al.* 2001; Chen *et al.* 2003; Dufosse *et al.* 2005; Yamashita 2015). It is scientifically understood that aquatic animals generally exhibit poor ability to biochemically synthesize astaxanthin and must be acquired in the diet. In this respect, the significance of an adequate supplementary astaxanthin in modulating the health of aquaculture organisms is widely acknowledged. This approach could favour the performance of recipient animals by optimizing health and increased protection during periods when they are more susceptible to physiological stress and pathogenic infections. Nutritional status is considered as a major aspect which influences the immune responses, determining the capacity of the animal to resist stress and ward off infections.

## Skin and flesh pigmentation

Perhaps, the greatest potential application of astaxanthin is for aquaculture feed additive to enhance the typical pinkish-red skin or flesh coloration of aquatic animals including salmon, red sea bream (*Chrysophrys major*, *P. major*, red snapper and tai), trout, ornamental fish, crayfish, lobster and shrimp. Skin and muscle pigmentation is due to the absorption and deposition of relatively large amounts of dietary astaxanthin which is frequently administered in their artificial diets as aquatic animals lack the ability to biosynthesize astaxanthin *de novo*. Maintenance of natural pigmentation is of utmost importance from a commercial perspective, being directly associated with the perception and subjective interpretation of consumer as an important quality criterion prior to actual consumption which consequently commands a better demand and product market price. For this reason, colour exerts a strong decisive role when evaluating and determining the quality of the product at the point of sale. The progressive expansion of aquaculture industry has established an insatiable demand for the carotenoid pigment.

Studies assessing the effect of dietary astaxanthin on the skin and flesh pigmentation of aquatic animals are summarized in Table 7. In fact, a great deal of in-depth study has investigated the effective use of dietary astaxanthin in finfish feeds being responsible for the skin and muscle pigmentation of rainbow trout *O. mykiss* (Torrissen 1989; Storebakken & Choubert 1991; No & Storebakken 1992; Sommer *et al.* 1992; Storebakken & No 1992; Choubert *et al.* 1994; Nickell & Bromage 1998; Choubert *et al.* 2006; Ingle de la Mora *et al.* 2006; Page & Davies 2006; Ytrestoyl & Bjerkeng 2007a; Choubert *et al.* 2009; Choubert 2010; Saez *et al.* 2014), red porgy *P. pagrus* (Chatzifotis *et al.* 2005; Kalinowski *et al.* 2005; Tejera *et al.* 2007; Chatzifotis *et al.* 2011), gilthead sea bream *Sparus aurata* (Gomes *et al.* 2002), red sea bream *P. major* (Katayama *et al.* 1965; Tanaka *et al.* 1976; Ibrahim *et al.* 1984; Nakazoe *et al.* 1984; Lin *et al.* 1998), olive flounder *Paralichthys olivaceus* (Pham *et al.* 2014), Atlantic salmon *S. salar* (Torrissen *et al.* 1995; Wathne *et al.* 1998; Bjerkeng & Berge 2000; Buttle *et al.* 2001; Baker *et al.* 2002; Page & Davies 2006; Ytrestoyl & Bjerkeng 2007b; Chimsung *et al.* 2013), Australasian snapper *Pagrus auratus* (Booth *et al.* 2004; Doolan *et al.* 2008a,b), channel catfish *Ictalurus punctatus* (Li *et al.* 2007) and yellow croaker *Larimichthys croceus* (Li *et al.* 2014; Yi *et al.* 2014). Numerous previous investigations reported significant astaxanthin deposition in skin and muscle of farmed rainbow trout resulting in visual enhancement of flesh coloration when fed with increasing dietary astaxanthin concentration (Torrissen 1985; Sommer *et al.* 1991; Choubert & Heinrich 1993; Choubert *et al.* 2009). A minimum level of 10 mg astaxanthin $kg^{-1}$ trout flesh is necessary to impart favourable hue for market requirement (Torrissen 2000). Likewise, flesh pigmentation and skin redness of farmed Atlantic salmon were pronouncedly augmented after successful feeding with astaxanthin pigmented diet suggesting it as an effective pigment source (Wathne *et al.* 1998; Baker *et al.* 2002). In a separate study, Forsberg and Guttormsen (2006) developed a mathematical model which explored relationships between fish size and concentration of dietary astaxanthin requirement on the chemical muscle astaxanthin concentration and visual perception of Atlantic salmon in an integrated manner. This bring forth an immeasurable benefit to farmers as a major tool in optimizing the supplementation of dietary astaxanthin composition to Atlantic salmon that results in the desired product quality accepted by consumers at minimum cost. It was noted that Australasian snapper fed diets without astaxanthin incorporation display reduced colour or lustreless appearance causing consumer demand to edge lower (Doolan *et al.* 2007, 2008b).

**Table 7** Effect of astaxanthin on the skin and flesh pigmentation of aquatic animals

| Species | Inclusion level/range | Source | Response | References |
|---|---|---|---|---|
| Atlantic Salmon (*Salmo salar*) | 2.1–41.4 mg kg$^{-1}$ | Synthetic | Enhanced skin and flesh pigmentation | Wathne *et al.* (1998) |
| | 45 mg kg$^{-1}$ | Synthetic | Elevated levels of astaxanthin in flesh tissue and improved coloration | Baker *et al.* (2002) |
| | 12.5–50 mg kg$^{-1}$ via intraperitoneal injection | Synthetic | Increased astaxanthin concentrations in kidney, liver, muscle, plasma and skin | Ytrestoyl and Bjerkeng (2007b) |
| Australasian Snapper (*Pagrus auratus*) | 36–72 mg kg$^{-1}$ | Synthetic | Enhanced skin and flesh pigmentation | Booth *et al.* (2004) |
| | 30–60 mg kg$^{-1}$ | Synthetic | Enhanced skin and flesh pigmentation | Doolan *et al.* (2008a) |
| | 13–78 mg kg$^{-1}$ | Synthetic | Enhanced skin and flesh pigmentation | Doolan *et al.* (2008b) |
| False Clownfish (*Amphiprion ocellaris*) | 20–100 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Yasir and Qin (2010) |
| Goldfish (*Carassius auratus*) | 25–100 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Paripatananont *et al.* (1999) |
| Kissing Gourami (*Helostoma temminckii*) | 100 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Kopecky (2013) |
| Koi Carp (*Cyprinus carpio*) | 1500 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Sun *et al.* (2012) |
| | 60–100 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Nguyen *et al.* (2014) |
| Orange Chromide (*Etroplus maculatus*) | 20–100 mg kg$^{-1}$ | Synthetic | Enhanced skin and flesh pigmentation | Manimegalai *et al.* (2010) |
| Rainbow Trout (*Oncorhynchus mykiss*) | 3.4–19.9 mg kg$^{-1}$ | Shrimp waste | Enhanced skin and flesh pigmentation | Torrissen (1985) |
| | 40 mg kg$^{-1}$ | Synthetic | Elevated levels of astaxanthin in flesh tissue and improved coloration | Sommer *et al.* (1991) |
| Rainbow Trout (*Oncorhynchus mykiss*) | 100 mg kg$^{-1}$ | Algal and synthetic | Elevated levels of astaxanthin in flesh tissue and improved coloration | Choubert and Heinrich (1993) |
| | 100–200 mg kg$^{-1}$ | Synthetic | Increased serum astaxanthin concentration, muscle astaxanthin retention and muscle colour | Choubert *et al.* (2009) |
| | 100 mg kg$^{-1}$ | Synthetic | Improved muscle astaxanthin retention and muscle colour | Choubert (2010) |
| Red Flame Dwarf Gourami (*Colisa lalia*) | 100 mg kg$^{-1}$ | Synthetic | Stimulated skin coloration | Baron *et al.* (2008) |
| Red Porgy (*Pagrus pagrus*) | 100 mg kg$^{-1}$ | Algal | Stimulated skin coloration | Chatzifotis *et al.* (2005) |
| | 20–40 mg kg$^{-1}$ | Shrimp waste | Stimulated skin coloration | Kalinowski *et al.* (2005) |
| | 25–50 mg kg$^{-1}$ | Algal and synthetic | Stimulated skin coloration | Tejera *et al.* (2007) |
| | 3300 mg kg$^{-1}$ | Algal | Stimulated skin coloration | Chatzifotis *et al.* (2011) |
| American Lobster (*Homarus americanus*) | 40–100 mg kg$^{-1}$ | Synthetic | Improved shell pigmentation | McKay (1987) |
| | 100–220 μg g$^{-1}$ | Synthetic | Preserved shell pigmentation | Tlusty and Hyland (2005) |
| Giant Tiger Prawn (*Penaeus monodon*) | 50 mg kg$^{-1}$ | Synthetic | Improved shell pigmentation and corrected blue colour syndrome | Menasveta *et al.* (1993) |
| | 25–100 mg kg$^{-1}$ | Synthetic | Improved shell pigmentation | Wade *et al.* (2015a) |
| Kuruma Prawn (*Penaeus japonicus*) | 50–400 mg kg$^{-1}$ | Synthetic | Improved shell pigmentation | Yamada *et al.* (1990) |
| | 500–2000 mg kg$^{-1}$ | Synthetic | Elevated levels of astaxanthin in flesh tissue and improved coloration | Chien and Jeng (1992) |
| | 50–100 mg kg$^{-1}$ | Synthetic | Enhanced flesh and shell pigmentation | Chien and Shiau (2005) |
| Red King Crab (*Paralithodes camtschaticus*) | 380 mg kg$^{-1}$ | Algal | Improved shell pigmentation | Daly *et al.* (2013) |

Apart from that, astaxanthin is also likely an important dietary component to many hatchery-reared valuable crustacean species such as giant tiger prawn *P. monodon* (Menasveta *et al.* 1993; Wade *et al.* 2015a), Kuruma prawn *P. japonicus* (Yamada *et al.* 1990; Chien & Jeng 1992; Liao & Chien 1994; Petit *et al.* 1997; Chien & Shiau 2005), American lobster *Homarus americanus* (D'Abramo *et al.* 1983; Lim *et al.* 1997; Tlusty & Hyland 2005) and red king crab *P. camtschaticus* (Daly *et al.* 2013) for colour improvement through the abundance of epithelial astaxanthin within the exoskeleton and hypodermal tissue. Several crustacean species were observed to eventually lose their natural coloration or not develop pigmentation and are often pale blue in colour instead of bluish-green or brown if not provided with adequate dietary carotenoid intake (Dall 1995; Barclay *et al.* 2006; Daly *et al.* 2013; Wade *et al.* 2015a). For instance, the 'blue colour syndrome' or 'blue disease' is one of the most alarming problems which often affects healthy farmed prawns, especially those intensively cultured, mainly caused by insufficient exogenous source of astaxanthin. Menasveta *et al.* (1993) revealed that prawns suffering from blue disease regained their typical pigmentation after 1 month of feeding a diet containing astaxanthin at 50 ppm inclusion level. Interestingly, the optimum pigmentation in a range of prawn species could be induced via 4 weeks of feeding with 50–100 mg $kg^{-1}$ of astaxanthin in their diet (Yamada *et al.* 1990; Chien & Jeng 1992; Petit *et al.* 1997). In another study, Tlusty and Hyland (2005) indicated that American lobsters require a specific amount of astaxanthin supplementation (100–220 μg $g^{-1}$ diet) to preserve its phenotypic brownish-green hue, whereas insufficiency would result in a pale blue coloration.

Moreover, astaxanthin has also been extensively utilized worldwide as an excellent pigment source or natural colorant for a variety of ornamental fish. Most of the fish species lose their natural skin colorations under captivity or rearing conditions. Accurate replication and preservation of the natural luminous colour of captivated fish is one of the biggest problems facing today's tropical ornamental fish industry. The beautiful display of vibrant colours is arguably among the most distinctive characteristics of aquatic creatures. Naturally, ornamental fish acquire carotenoids to achieve skin pigmentation from feeding upon certain algae or red yeast, corals, crustaceans, zooplankton or insects that have amassed the pigments within the food chain. Colour is the single most important quality criterion that dictates the price and marketability of various fresh and marine ornamental fish traded across the globe. Ako and Tamaru (1999) documented that the pigmentations of various commercially important ornamental fish species particularly cichlids, danios, koi, goldfish, gouramis and tetras were profoundly improved when fed with diet containing 30 ppm of astaxanthin. Trials were also conducted by Kopecky (2013) to investigate skin colour enhancement in kissing gourami *Helostoma temminckii* by feeding diets supplemented with astaxanthin (100 mg $kg^{-1}$ diet). It was recorded that noticeable colour change first occurred in kissing gourami during the 4th week of feeding and intensified immensely on the 10th week. Thus, the pigmentation of gourami fish was significantly affected by dietary astaxanthin intake. A recently published study showed that the koi carp (*Cyprinus carpio*) juveniles fed the diet containing 100 ppm astaxanthin exhibited more intense coloration than those fed with 60 ppm astaxanthin (Nguyen *et al.* 2014). Aside from that, Paripatananont *et al.* (1999) postulated that dietary astaxanthin concentration within the range of 36–37 mg $kg^{-1}$ diet was deemed as effective doses to escalate the colour intensity of goldfish (*C. auratus*). In the same way, Yasir and Qin (2010) have proved in their study that the incorporation of 100 ppm astaxanthin into the diet of false clownfish *Amphiprion ocellaris* has the potential to bring about significant enhancement on the red hue by the end of 5th week. On the other hand, Manimegalai *et al.* (2010) provided experimental evidence that dietary astaxanthin content of 20–100 mg $kg^{-1}$ was far more superior than β-carotene in developing skin pigmentation of orange chromide *Etroplus maculatus*. Baron *et al.* (2008) also concluded that the overall brightness, saturation and reddish appearance of red flame dwarf gourami *Colisa lalia* were markedly augmented when supplemented with 100 ppm of synthetic astaxanthin (Lucanthin® Pink) for 12 weeks. Additionally, luminous coloration derived from astaxanthin supplementation could serve as a cue of individual fish health due to its potent antioxidant properties and can play pivotal roles in promoting the immune systems of fish (Kodric-Brown 1985; Ho *et al.* 2013). Thereby, the interplay between astaxanthin-based ornamentation and immune defence can lead to the use of the ornaments for honest signalling of individual health, that is, more colourful fish being healthier. This aspect has been demonstrated at least in cichlids *Pundamilia nyererei*, whereby males with greater red score had reduced parasite loads and occupied bigger territories than the comparatively duller males (Maan *et al.* 2006). Hence, astaxanthin is considered as a crucial component widely presented in the diets of ornamental fish to improve the relative intensity of skin colour.

## Immune-relevant gene expression

Valuable clues concerning gene function are obtainable through measurement of gene expression levels via real-time RT–PCR. Accurate quantification of gene expression enables the detection of an alteration in the immune-relevant gene expression levels in response to specific biological stimuli such as dietary administration of immunostimulants (e.g. astaxanthin, β-carotene and vitamin C) to promote immunity and health of aquatic animals. The last decade has witnessed a spurt in research towards the enhancement of disease resistance in animal host. As such, the screening of immune-relevant functional genes is of great significance in the identification of the molecular mechanisms of immunity in aquatic organisms and developing effective ways for immunogenicity enhancement of cultured animals to diseases (Zheng *et al.* 2006; Fraga *et al.* 2008; Zhu *et al.* 2013). In fact, a great deal of potential immune-related genes for both adaptive and innate immunity has been characterized from various fish species which are acquirable from the public genomic databases.

To date, no prospective studies have ever been initiated concerning the role of carotenoids on the immune-related gene expression in aquatic animals, with only strong evidence linking to other popular additives or active ingredients (e.g. algal biomass, probiotics and prebiotics). Cerezuela and co-workers examined the effects of three orally administered microalgae (*Nannochloropsis gaditana*, *Phaeodactylum tricornutum* and *Tetraselmis chuii*) on different immune-associated genes in gilthead sea bream *S. aurata* (Cerezuela *et al.* 2012a,b). Fish that received *N. gaditana* and *T. chuii* at 100 g $kg^{-1}$ feed, respectively, exhibited a significantly upregulated expression levels of β-defensin (β-def), colony-stimulating factor receptor-1 (CSR-1R) and major histocompatibility complex class IIα (MHCIIα) immuno-related genes in head kidney and gut following 2 and 4 weeks of treatment, respectively. In another related study on *S. aurata* from the same research group, supplementing probiotic (*Bacillus subtilis* at $10^7$ cfu $g^{-1}$ feed) and microalgae meals (*P. tricornutum* and *T. chuii* at 100 mg $kg^{-1}$ feed, respectively) either singly or in combination were also noted to provoke higher transcriptions of β-def gene in head kidney after 4 weeks of feeding trial (Cerezuela *et al.* 2012a,b). On the other hand, Nootash *et al.* (2013) provided robust evidence that green tea (*Camellia sinensis*) administered at 100 mg $kg^{-1}$ feed for 5 weeks effectively upregulated interleukin 1-β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) transcription levels in the kidney, liver and spleen of rainbow trout *O. mykiss*. More recently, enrichment of *S. aurata* diet with probiotic *Shewanella putrefaciens* (Pdp11, $10^9$ cfu $g^{-1}$) remarkably elevated cyclooxygenase 2 (cox2), hepcidin (hamp) and β-def gene expression in the skin following 2 weeks of feeding trial (Cerezuela *et al.* 2016). Although the precise mechanisms responsible for the functional ingredients such as microalgae meals and green tea evaluated in previous studies on immune-relevant gene expression remain to be explored more, it might be related to carotenoid components contained in the substances. Nevertheless, further in-depth studies are necessary to unravel the biological implications of carotenoids on the immune-related gene expression of aquatic animals, similar to those attempted earlier.

## Safety of astaxanthin

Astaxanthin is a naturally occurring xanthophyll, most well-known for its commercial utilization as a feed additive in the aquaculture industry. There has been an explosive demand globally for natural astaxanthin in human health applications. In fact, this pigment has a long history of use for human consumption, being originally isolated and identified from lobster *Astacus gammarus* in 1937 (Kuhn & Soerensen 1938). Focus on the significant metabolic functions of astaxanthin, particularly its powerful antioxidant property, has generally led to its characterization as being a valuable nutritive component.

Notwithstanding that the beneficial effects of astaxanthin have been immensely investigated on aquatic animals, its adverse effects and potential toxicity have not been systematically established or reported. Experimental evidence from feeding trials reviewed in previous chapters has not indicated any notable negative impacts of dietary astaxanthin intake on animal health during any of these studies regardless of the source whether natural or synthetic. Supplementation of astaxanthin up to 317 mg $kg^{-1}$ feed did not result in any evidence of adverse effects on Atlantic salmon *S. salar* fry after 11 weeks (Christiansen *et al.* 1995); likewise, administration of up to 810 mg astaxanthin $kg^{-1}$ diet for 4 weeks had no effects on the postlarvae of giant tiger prawn *P. monodon* (Merchie *et al.* 1998). These results agree with the observations in giant freshwater prawn *M. rosenbergii* (Angeles *et al.* 2009; Kumar *et al.* 2009), kuruma prawn *P. japonicus* (Chien & Shiau 2005), pacific white shrimp *P. vannamei* (Niu *et al.* 2009; Zhang *et al.* 2013), Atlantic cod *G. morhua* (Sawanboonchun *et al.* 2008; Hansen *et al.* 2016), rainbow trout *Oncorhynchus mykiss* (Bazyar Lakeh *et al.* 2010; Rahman *et al.* 2016) and red porgy *P. pagrus* (Kalinowski *et al.* 2005), fed diets supplemented with astaxanthin. In a previous study, Brizio *et al.* (2013) verified the absence of negative effects of astaxanthin-enriched diet on *O. mykiss*. In point of fact, the authors also highlighted that astaxanthin residues in trout fillets could represent an important source of astaxanthin for increasing human health, alternative to the use of synthetic astaxanthin from dietary supplements. According to a recent scientific opinion on the safety and efficacy of synthetic astaxanthin (Carophyll® Pink), dietary level of 908 mg astaxanthin $kg^{-1}$ diet was well tolerated by salmonids and ornamental fish (EFSA, 2014). Meanwhile, synthetic astaxanthin is tolerated in crustaceans up to 400 mg $kg^{-1}$ complete feed. The FEEDAP Panel also further asserted that the use of astaxanthin up to the authorized maximum dietary level (100 mg $kg^{-1}$ complete feed) for salmon, trout and crustaceans does not pose any significant concerns on consumer health and safety. Moreover, it has also been concluded thus far by the FEEDAP Panel that the notified use of synthetic astaxanthin at the maximum permitted dietary level is highly unlikely to constitute any unintended or harmful ecological risks. Taking into account former assessment of astaxanthin dimethyldisuccinate (Carophyll® Stay-Pink), the FEEDAP Panel has established a similar maximum dietary limit (100 mg $kg^{-1}$ fish feed) for this colorant to be incorporated as a feed additive to salmon and trout (EFSA, 2007b). Astaxanthin dimethyldisuccinate is a derivative from esterification of astaxanthin that serves as a substitutive product of other astaxanthin sources. Gavagely administered astaxanthin dimethyldisuccinate is hydrolysed and de-esterified to free astaxanthin in the gastrointestinal tract of fish, then absorbed, distributed and metabolized in the same manner as free astaxanthin. With regard to toxicity, several acute and subchronic studies were tested on rats using either synthetic (Buesen *et al.* 2015) or natural astaxanthin (Stewart *et al.* 2008; Katsumata *et al.* 2014). All these studies consistently reported the absence of significant toxicity, mortality, clinical signs and any behavioural alterations in the healthy test subjects when different sources of astaxanthin were supplemented up to a daily dose of at least 500 mg $kg^{-1}$ body weight $day^{-1}$. Apart from that, in a few reported human clinical studies, no harmful effects were observed upon gavage administration of dosages up to 40 mg astaxanthin $day^{-1}$ for 2 months (equivalent to 0.67 mg $kg^{-1}$ body weight $day^{-1}$ for a 60 kg healthy human) (Andersen *et al.* 2007; Kupcinskas *et al.* 2008; Kim *et al.* 2011). Based on a study conducted on 33 healthy volunteers, Aquasearch Inc. (1999) disclosed no toxicity or ill effects attributable to the daily ingestion of either 3.85 mg (low dose) or 19.25 mg (high dose) of algal astaxanthin derived from *H. pluvialis* for 29 consecutive days as analysed by clinical and medical parameters. In another related study, Spiller and Dewell (2003) revealed that healthy adults could safely consume 6 mg astaxanthin $day^{-1}$ derived from a *H. pluvialis* algal extract without any physiological effects on blood pressure or serum safety markers. The effects of astaxanthin on human blood rheology were investigated by measuring whole blood transit time in 20 healthy adult males with a single blind method (Miyawaki *et al.* 2008). Continuous ingestion of astaxanthin (6 mg $day^{-1}$) for a ten-day period improved blood rheology as evidenced by a shortening of blood transit time. Furthermore, escalating concentrations of astaxanthin (0.3–100 μM) were assessed *in vitro* with blood taken from 8 aspirin-treated and 12 aspirin-naive volunteers having multiple risk factors for cardiovascular disease (Serebruany *et al.* 2010). The authors concluded that even supratherapeutic concentration of astaxanthin (100 μM) *in vitro* had no profound effects on coagulation, platelet and fibrinolytic indices in either aspirin-treated or aspirin-naive subjects. These findings strongly support the safety profile of astaxanthin for future human clinical trials. No observable serious side effects of astaxanthin have been recorded thus far in any of the published human clinical studies when administered to humans, and there is evidence of a suppression in biomarkers of inflammation and oxidative stress (Spiller & Dewell 2003; Kim & Chyun 2004; Iwabayashi *et al.* 2009; Satoh *et al.* 2009; Park *et al.* 2010; Fassett & Coombes 2011; Yamashita 2015). A growing body of research suggests that dietary astaxanthin supplementation could exert protective actions against atherosclerotic cardiovascular disease (CVD) via its potential as a therapeutic agent to ameliorate endothelial inflammation, oxidative stress, neutrophil functions, flexibility of red blood cell membranes, lipid and glucose metabolism (Macedo *et al.* 2010; Riccioni *et al.* 2012; Kishimoto *et al.* 2016). Accumulating evidence from available data over the yesteryears have unanimously suggested that astaxanthin is relatively safe to be incorporated into the feed of various animals and human subjects as a nutritional supplement or additive without any identifiable negative consequences.

## Conclusion

The global aquafeed industry has evolved dramatically over the last few decades, where the overall aim is to improve formulated feed quality with valuable ingredients that fulfil the physiological requirements of farmed animals. Continuous efforts along the optimizations of feed processing conditions and storage stability have effectively enhanced the utilization of nutritional components in animal feeds. Future aquatic feeds are expected to ensure the dual benefits of prime health and superior growth. Astaxanthin is one of the most essential carotenoids in nature that gained substantial interests from nutritionists and food scientists alike due to its broad health implications on aquatic organisms. Astaxanthin is a xanthophyll carotenoid contained in microorganisms, specifically *H. pluvialis* and *X. dendrorhous*. This particular pigment is responsible for the attractive appearance of most aquatic animal products, especially colour, which contributes an important characteristic quality criterion for marketing, price and consumer demands of aquaculture products. Numerous peer-reviewed scientific publications that appeared over a number of years have consistently provide us with a comprehensive understanding on the diverse roles of astaxanthin in targeting several animal health conditions across a range of commercially relevant parameters. These include various improvements in growth performance, survival, reproductive physiology, stress tolerance, disease resistance and immune-relevant gene expression. This conclusion is supported by the powerful bioactive antioxidant property of astaxanthin and its beneficial role in protecting the cells, tissues and organs from oxidative damage. Recent perspectives and approaches to the understanding of biological processes via the integration of gene expression, direct molecular interactions and cellular environment using high-throughput computational and experimental methods would pave the way towards fascinating insights into the crucial role of astaxanthin on the immune system of aquatic animals. Extensive and rigorous research study is still needed to validate the much broader functions of astaxanthin to more target animal species.

## Acknowledgements

This review was supported by Putra Grant, Universiti Putra Malaysia (GP-IPS/2014/9433901) and HICoE (Higher Institution Centre of Excellence) grant from the Ministry of Higher Education Malaysia.

## References

Aas GH, Bjerkeng B, Storebakken T, Ruyter B (1999) Blood appearance, metabolic transformation and plasma transport proteins of 14C-astaxanthin in Atlantic salmon (*Salmo salar* L.). *Fish Physiology and Biochemistry* **21**: 325–334.

Abidov M, Ramazanov Z, Seifulla R, Grachev S (2010) The effects of Xanthigen in the weight management of obese premenopausal women with non-alcoholic live disease and normal liver fat. *Diabetes, Obesity and Metabolism* **12**: 72–81.

Aflalo C, Meshulam Y, Zarka A, Boussiba S (2007) On the relative efficiency of two- vs. one-stage production of astaxanthin by the green alga *Haematococcus pluvialis*. *Biotechnology and Bioengineering* **98**: 300–305.

Ahmadi MR, Bazyar AA, Safi S, Ytrestoyl T, Bjerkeng B (2006) Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). *Journal of Applied Ichthyology* **22**: 388–394.

Ako H, Tamaru CS (1999) Are feeds for food fish practical for aquarium fish? *International Aquafeed* **2**: 30–36.

Alishahi M, Karamifar M, Mesbah M (2015) Effects of astaxanthin and *Dunaliella salina* on skin carotenoids, growth performance and immune response of *Atronotus ocellatus*. *Aquaculture International* **23**: 1239–1248.

Amar EC, Kiron V, Satoh S, Watanabe T (2001) Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* **32**: 162–173.

Amar EC, Kiron V, Satoh S, Watanabe T (2004) Enhancement of innate immunity in rainbow trout *Oncorhynchus mykiss* (Walbaum) associated with dietary intake of carotenoids from natural products. *Fish and Shellfish Immunology* **16**: 527–537.

Amar EC, Kiron V, Akutsu T, Satoh S, Watanabe T (2012) Resistance of rainbow trout *Oncorhynchus mykiss* to infectious hematopoietic necrosis virus (IHNV) experimental infection following ingestion of natural and synthetic carotenoids. *Aquaculture* **330–333**: 148–155.

Ambati RR, Phang SM, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial application-a review. *Marine Drugs* **12**: 128–152.

Amundsen T, Forsgren E (2001) Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 13155–13160.

Amundsen T, Forsgren E (2003) Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology* **54**: 55–64.

Anarjan N, Tan CP (2013a) Effects of storage temperature, atmosphere and light on chemical stability of astaxanthin nanodispersions. *Journal of the American Oil Chemists' Society* **90**: 1223–1227.

Anarjan N, Tan CP (2013b) Chemical stability of astaxanthin nanodispersions in orange juice and skimmed milk as model food systems. *Food Chemistry* **139**: 527–531.

Andersen L, Holck S, Kupcinskas L, Kiudelis G, Jonaitis L, Janciauskas D *et al.* (2007) Gastric inflammatory markers and interleukins in patients with functional dyspepsia treated with astaxanthin. *FEMS Immunology and Medical Microbiology* **50**: 244–248.

Anderson JS, Sunderland R (2002) Effect of extruder moisture and dryer processing temperature on vitamin C and E and astaxanthin stability. *Aquaculture* **207**: 137–149.

Ando S, Hatano M (1987) Metabolic pathways of carotenoids in chum salmon *Oncorhynchus keta* during spawning migration. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **87**: 411–416.

Angeles IP, Chien YH, Tayamen MM (2009) Effects of different dosages of astaxanthin on giant freshwater prawn *Macrobrachium rosenbergii* (De Man) challenged with *Lactococcus garvieae*. *Aquaculture Research* **41**: 70–77.

Aquasearch Inc. (1999) *Haematococcus pluvialis* and astaxanthin safety for human consumption. Technical Report TR 3005.001.

Armenta RE, Guerrero-Legarreta I (2009) Stability studies on astaxanthin extracted from fermented shrimp byproducts. *Journal of Agricultural and Food Chemistry* **57**: 6095–6100.

Arredondo-Figueroa JL, Pedroza-Islas R, Ponce JT, Vernon-Carter EJ (2003) Pigmentation of Pacific white shrimp (*Litopenaeus vannamei*, Boone 1931) with esterified and saponified carotenoids from chilli *Capsicum annum* in comparison to astaxanthin. *Revista Mexicana De Ingenieria Qumica* **3**: 101–108.

Asker D, Awad T, Beppu T, Ueda K (2012) A novel radio-tolerant astaxanthin-producing bacterium reveals a new astaxanthin derivative: astaxanthin dirhamnoside. *Methods in Molecular Biology* **892**: 61–97.

Azevedo PA, Cho CY, Leeson S, Bureau DP (1998) Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resources* **11**: 227–238.

Baker RT, Pfeiffer AM, Schoner FJ, Smith-Lemmon L (2002) Pigmenting efficacy of astaxanthin and canthaxanthin in freshwater-reared Atlantic salmon, *Salmo salar*. *Animal Feed Science and Technology* **99**: 97–106.

Banerjee K, Ghosh R, Homechaudhuri S, Mitra A (2009) Biochemical composition of marine macroalgae from Gangetic delta at the apex of Bay of Bengal. *African Journal Basic Applied Sciences* **1**: 96–104.

Barbosa MJ, Morais R, Choubert G (1999) Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **176**: 331–341.

Barclay MC, Irvin SJ, Williams KC, Smith DM (2006) Comparison of diets for the tropical spiny lobster *Panulirus ornatus*: astaxanthin-supplemented feeds and mussel flesh. *Aquaculture Nutrition* **12**: 117–125.

Baron M, Davies S, Alexander L, Snellgrove D, Sloman K (2008) The effect of dietary pigments on the coloration and behaviour of flame-red dwarf gourami, *Colisa lalia*. *Animal Behaviour* **75**: 1041–1051.

Basolo AL, Delaney KJ (2001) Male biases for male characteristics in females *in Priapella olmecae* and *Xiphophorus helleri* (Family Poeciliidae). *Ethology* **107**: 431–438.

Bazyar Lakeh AA, Ahmadi MR, Safi S, Ytrestoyl T, Bjerkeng B (2010) Growth performance, mortality and carotenoid pigmentation of fry offspring as affected by dietary supplementation of astaxanthin to female rainbow trout (*Oncorhynchus mykiss*) broodstock. *Journal of Applied Ichthyology* **26**: 35–39.

Begum H, Yusoff FM, Banerjee S, Khatoon H, Shariff M (2016) Availability and utilization of pigments from microalgae. *Critical Review in Food Science and Nutrition* **56**: 2209–2222.

Bell JG, McEvoy J, Tocher DR, Sargent JR (2000) Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *American Society for Nutritional Sciences* **43**: 1800–1807.

Bierer TL, Merchen NR, Erdman JW (1995) Comparative absorption and transport of five common carotenoids in preruminant calves. *Journal of Nutrition* **125**: 1569–1577.

Bjerkeng B (2008) Carotenoids in aquaculture: fish and crustaceans. In: Britton G, Liaaen-Jensen S, Pfander H (eds) *Carotenoids. Volume 4: Natural Functions*, pp. 237 254. Birkhauser Verlag AG, Basel.

Bjerkeng B, Berge GM (2000) Apparent digestibility coefficients and accumulation of astaxanthin E/Z isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **127**: 423–432.

Bjerkeng B, Storebakken T, Liaaen-Jensen S (1990) Response to carotenoids by rainbow trout in the sea: resorption and metabolism of dietary astaxanthin and canthaxanthin. *Aquaculture* **91**: 153–162.

Bjerkeng B, Storebakken T, Liaaen-Jensen S (1992) Pigmentation of rainbow trout from start feeding to sexual maturation. *Aquaculture* **108**: 333–346.

Bjerkeng B, Refstie S, Fjalestad KT, Storebakken T, Rodbotten M, Roem AJ (1997) Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet. *Aquaculture* **157**: 297–309.

Bjerkeng B, Hamre K, Hatlen B, Wathne E (1999a) Astaxanthin deposition in fillets of Atlantic salmon *Salmo salar* L. fed two dietary levels of astaxanthin in combination with three levels of α-tocopheryl acetate. *Aquaculture Research* **30**: 637–646.

Bjerkeng B, Hatlen B, Wathne E (1999b) Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* **180**: 307–319.

Bjerkeng B, Johnsen K, Mayer I, Storebakken T, Nilssen K (1999c) Influence of 11-ketotestosterone, 17β-estradiol and 3,5,3′-triiodo-thyronine on distribution and metabolism of carotenoids in Arctic charr, *Salvelinus alpinus*, L. *Fish Physiology and Biochemistry* **21**: 353–364.

Bjerkeng B, Hatlen B, Jobling M (2000) Astaxanthin and its metabolites idoxanthin and crustaxanthin in flesh, skin, and gonads of sexually immature and maturing Arctic charr (*Salvelinus alpinus* L.). *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **125**: 395–404.

Blanc JM, Choubert G (1985) Genetic variation of flesh colour in canthaxanthin-fed rainbow trout. *Genetics Selection Evolution* **17**: 243–250.

Blomhoff R, Blomhoff HK (2006) Overview of retinoid metabolism and function. *Journal of Neurobiology* **66**: 606–630.

Blount JD, Houston DC, Moller AP (2002) Why egg yolk is yellow. *Trends in Ecology and Evolution* **15**: 47–49.

Boon CS, McClements DJ, Weiss J, Decker EA (2010) Factors influencing the chemical stability of carotenoids in foods. *Critical Reviews in Food Science and Nutrition* **50**: 515–532.

Booth MA, Warner-Smith RJ, Allan GL, Glencross BD (2004) Effects of dietary astaxanthin source and light manipulation on the skin colour of Australian snapper *Pagrus auratus* (Bloch and Schneider, 1801). *Aquaculture Research* **35**: 458–464.

Born T, Desmarchelier C, Dragsted LO, Nielsen CS, Stahl W, Ruhl R *et al.* (2017) Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. *Molecular Nutrition Food Research* https://doi.org/10.1002/mnfr.201600685.

Borowitzka MA (2013) High-value products from microalgae-their development and commercialisation. *Journal of Applied Phycology* **25**: 743–756.

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. *Physiologia Plantarum* **108**: 111–117.

Boussiba S, Bing W, Yuan JP, Zarka A, Chen F (1999) Changes in pigment profile in the green algae *Haematococcus pluvialis* exposed to environmental stress. *Biotechnology Letters* **21**: 601–604.

Britton G (1995) Structure and properties of carotenoids in relation to function. *FASEB Journal* **9**: 1551–1558.

Britton G (2008) Functions of intact carotenoids. In: Britton G, Liaaen-Jensen S, Pfander H (eds) *Carotenoids. Volume 4: Natural Functions*, pp. 189–212. Birkhauser Verlag AG, Basel.

Brizio P, Benedetto A, Righetti M, Prearo M, Gasco L, Squadrone S *et al.* (2013) Astaxanthin and canthaxanthin (xanthophyll) as supplements in rainbow trout diet: in vivo assessment of residual levels and contributions to human health. *Journal of Agricultural and Food Chemistry* **61**: 10954–10959.

Bromage NR (1995) Broodstock management and seed quality-general considerations. In: Bromage NR, Roberts RJ (eds) *Broodstock Management and Egg and Larval Quality*, pp. 1–24. Blackwell, London.

Bromage NR, Roberts JR (1995) *Broodstock Management and Egg and Larval Quality*. Blackwell Scientific Publications, Oxford.

Brown MJ, Ferruzzi MG, Nguyen ML, Cooper DA, Eldridge AL, Schwartz SJ *et al.* (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *The American Journal of Clinical Nutrition* **80**: 396–403.

de Bruijn WJC, Weesepoel Y, Vincken JP, Gruppen H (2016) Fatty acids attached to all-trans-astaxanthin alter its cis-trans equilibrium, and consequently its stability, upon light-accelerated autoxidation. *Food Chemistry* **194**: 1108–1115.

Bryan B, Knights K (2014) *Pharmacology for Health Professionals*, 4th edn. Elsevier, Mosby, NSW.

Buesen R, Schulte S, Strauss V, Treumann S, Becker M, Groters S *et al.* (2015) Safety assessment of [3S,3S']-astaxanthin-subchronic toxicity study in rats. *Food and Chemical Toxicology* **81**: 129–136.

Bustos-Garza C, Yanez-Fernandez J, Barragan-Huerta BE (2013) Thermal and pH stability of spray-dried encapsulated astaxanthin oleoresin from *Haematococcus pluvialis* using several encapsulation wall materials. *Food Research International* **54**: 641–649.

Buttle LG, Crampton VO, Williams PD (2001) The effect of feed pigment type on flesh pigment deposition and colour in farmed Atlantic salmon, *Salmo salar* L. *Aquaculture Research* **32**: 103–111.

Castenmiller JJM, West CE (1998) Bioavailability and bioconversion of carotenoids. *Annual Review of Nutrition* **18**: 19–38.

Cerezuela R, Guardiola FA, Meseguer J, Esteban MA (2012a) Enrichment of gilthead sea bream (*Sparus aurata* L.) diet with microalgae: effects of the immune system. *Fish Physiology and Biochemistry* **38**: 1729–1739.

Cerezuela R, Guardiola FA, Gonzalez P, Meseguer J, Esteban MA (2012b) Effects of dietary *Bacillus subtilis*, *Tetraselmis chuii* and *Phaeodactylum tricornutum*, singularly or in combination, on the immune response and disease resistance of sea bream (*Sparus aurata* L.). *Fish and Shellfish Immunology* **33**: 342–349.

Cerezuela R, Guardiola FA, Cuesta A, Esteban MA (2016) Enrichment of gilthead seabream (*Sparus aurata* L.) diet with palm fruit extracts and probiotics: effect on skin mucosal immunity. *Fish and Shellfish Immunology* **49**: 100–109.

Chang ES (1997) Chemistry of crustacean hormones that regulate growth and reproduction. In: Fingerman M, Nagabhushanam R, Thompson MF (eds) *Recent Advances in Marine Biotechnology*, pp. 163–178. Science Publishers, Mishawaka, IN.

Chatzifotis S, Pavlidis M, Jimeno CD, Vardanis G, Sterioti A, Divanach P (2005) The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). *Aquaculture Research* **36**: 1517–1525.

Chatzifotis S, Juan I, Kyriazi P, Divanach P, Pavlidis M (2011) Dietary carotenoids and skin melanin content influence the coloration of farmed red porgy (*Pagrus pagrus*). *Aquaculture Nutrition* **17**: 90–100.

Chekanov K, Lobakova E, Selyakh I, Semenova L, Sidorov R, Solovchenko A (2014) Accumulation of astaxanthin by a new *Haematococcus pluvialis* strain BM1 from the White Sea coastal rocks (Russia). *Marine Drugs* **12**: 4504–4520.

Chen Y, Li D, Lu W, Xing J, Hui B, Han Y (2003) Screening and characterization of astaxanthin-hyperproducing mutants of *Haematococcus pluvialis*. *Biotechnology Letters* **25**: 527–529.

Chen X, Chen R, Guo Z, Li C, Li P (2007) The preparation and stability of the inclusion complex of astaxanthin with β-cyclodextrin. *Food Chemistry* **101**: 1580–1584.

Chew BP (1995) Antioxidant vitamins affect food animal immunity and health. *Journal of Nutrition* **125**: 1804–1808.

Chien YH, Jeng SC (1992) Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. *Aquaculture* **102**: 333–346.

Chien YH, Shiau WC (2005) The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth and low dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus* Bate. *Journal of Experimental Marine Biology and Ecology* **318**: 201–211.

Chien YH, Chen IM, Pan CH, Kurmaly K (1999) Oxygen depletion stress on mortality and lethal course of juvenile tiger prawn *Penaeus monodon* fed high level of dietary astaxanthin. *Journal of the Fisheries Society of Taiwan* **26**: 85–93.

Chien YH, Pan CH, Hunter B (2003) The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture* **216**: 177–191.

Chimsung N, Lall SP, Tantikitti C, Verlhac-Trichet V, Milley JE (2013) Effects of dietary cholesterol on astaxanthin transport in plasma of Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **165**: 73–81.

Chimsung N, Tantikitti C, Milley JE, Verlhac-Trichet V, Lall SP (2014) Effects of various dietary factors on astaxanthin absorption in Atlantic salmon (*Salmo salar*). *Aquaculture Research* **45**: 1611–1620.

Choi SK, Park YS, Choi DK, Chang HI (2008) Effects of astaxanthin on the production of NO and the expression of COX-2 and iNOS in LPS-stimulated BV2 microglial cells. *Journal of Microbiology and Biotechnology* **18**: 1990–1996.

Chou YH, Chien YH (2006) Effects of astaxanthin and vitamin E supplement in Japanese sea bass (*Lateolabrax japonicus*) broodstock diet on their spawning performance and egg quality. *Journal of the Fisheries Society of Taiwan* **33**: 157–169.

Choubert G (2010) Response of rainbow trout (*Oncorhynchus mykiss*) to varying dietary astaxanthin/canthaxanthin ratio: colour and carotenoid retention of the muscle. *Aquaculture Nutrition* **16**: 528–535.

Choubert G, Heinrich O (1993) Carotenoid pigments of the green alga *Haematococcus pluvialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. *Aquaculture* **112**: 217–226.

Choubert G, Storebakken T (1989) Dose response to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoid concentrations. *Aquaculture* **81**: 69–77.

Choubert G, Storebakken T (1996) Digestibility of astaxanthin and canthaxanthin in rainbow trout as affected by dietary concentration. *Annales de Zootechnie* **45**: 445–453.

Choubert G, Gomez R, Milicua JCG (1994) Response of serum carotenoid levels to dietary astaxanthin and canthaxanthin in immature rainbow trout *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology. A, Molecular and Integrated Physiology* **109**: 1001–1006.

Choubert G, Milicua JCG, Gomez R, Sance S, Petit H, Negre-Sadargues G *et al.* (1995) Utilization of carotenoids from various sources by rainbow trout: muscle colour, carotenoid digestibility and retention. *Aquaculture International* **3**: 205–216.

Choubert G, Cravedi J, Laurentie M (2005) Pharmacokinetics and availabilities of 14C-keto-carotenoids, astaxanthin and canthaxanthin, in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture Research* **36**: 1526–1534.

Choubert G, Mendes-Pinto MM, Morais R (2006) Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: effect of dietary astaxanthin and lipid sources. *Aquaculture* **257**: 429–436.

Choubert G, Cravedi J, Laurentie M (2009) Effect of alternate distribution of astaxanthin on rainbow trout (*Oncorhynchus mykiss*) muscle pigmentation. *Aquaculture* **286**: 100–104.

Chouliara E, Karatapanis A, Savvaidis IN, Kontominas MG (2007) Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 40°C. *Food Microbiology* **24**: 607–617.

Christiansen R, Torrissen OJ (1996) Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. Juveniles. *Aquaculture Nutrition* **2**: 55–62.

Christiansen R, Waagbb R, Torrissen OJ (1993) Effects of polyunsaturated fatty acids and vitamin E on flesh pigmentation in Atlantic salmon (*Salmo salar*). In: Kaushik SJ, Luquet P (eds) *Fish Nutrition in Practice*, pp. 339–343. INRA Editions, Versailles.

Christiansen R, Lie O, Torrissen OJ (1994) Effect of astaxanthin and vitamin A on growth and survival during first feeding of Atlantic salmon, *Salmo salar* L. *Aquaculture Research* **25**: 903–914.

Christiansen R, Lie O, Torrissen OJ (1995) Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First-feeding fry. *Aquaculture Nutrition* **1**: 189–198.

Clark RM, Yao L, She L, Furr HC (2000) A comparison of lycopene and astaxanthin absorption from corn oil and olive oil emulsions. *Lipids* **35**: 803–806.

Coral-Hinostroza GN, Bjerkeng B (2002) Astaxanthin from the red crab langostilla (*Pleuroncodes planipes*): optical R/S isomers and fatty acid moieties of astaxanthin esters. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **133**: 437–444.

Craig JK, Foote CJ (2001) Countergradient variation and secondary sexual color: phenotypic convergence promotes genetic divergence in carotenoid use between sympatric anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). *Evolution* **55**: 380–391.

Craik JCA (1985) Egg quality and egg pigment content in salmonid fishes. *Aquaculture* **47**: 61–88.

Crozier GF (1970) Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). *Journal of the Fisheries Research Board of Canada* **27**: 973–975.

de Cruz CR, Kamarudin MS, Cr S, Ramezani-Fard E (2015) Effects of extruder die temperature on the physical properties of extruded fish pellets containing taro and broken rice starch. *Animal Feed Science and Technology* **199**: 137–145.

Cuellar-Bermudez SP, Aguilar-Hernandez I, Cardenas-Chavez DL, Ornelas-Soto N, Romero-Ogawa MA, Parra-Saldivar R (2015) Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins. *Microbial Biotechnology* **8**: 190–209.

Cysewski GR, Lorenz RT (2004) Industrial production of microalgal cell mass and secondary products-species of high potential *Haematococcus*. In: Richmond A (ed.) *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, pp. 281–288. Blackwell Science, Oxford.

D'Abramo LR, Baum NA, Bordner CE, Conklin DE (1983) Carotenoids as a source of pigmentation in juvenile lobsters fed a purified diet. *Canadian Journal of Fisheries and Aquatic Sciences* **40**: 699–704.

Dall W (1995) Carotenoids versus retinoids (vitamin A) as essential growth factors in penaeid prawns (*Penaeus semisulcatus*). *Marine Biology* **124**: 209–213.

Dall W, Smith D, Moore L (1995) Carotenoids in the tiger prawn *Penaeus esculentus* during ovarian maturation. *Marine Biology* **123**: 435–441.

Daly B, Swingle J, Eckert G (2013) Dietary astaxanthin supplementation for hatchery-cultured red king crab, *Paralithodes camtschaticus*, juveniles. *Aquaculture Nutrition* **19**: 312–320.

Damiani MC, Leonardi PI, Pieroni OI, Caceres EJ (2006) Ultrastructure of the cyst wall of *Haematococcus pluvialis* (Chlorophyceae): wall development and behaviour during cyst germination. *Phycologia* **45**: 616–623.

Darachai J, Piyatiratitivorakul S, Kittakoop P, Nitithamyong C, Menasveta P (1998) Effects of astaxanthin on larval growth and survival of the giant tiger prawn, *Penaeus monodon*. In: Flegel TW (ed.) *Advances in Shrimp Biotechnology, Proceedings to the Special Session on Shrimp Biotechnology*, pp. 117–121. BIOTEC, Chiengmai, Thailand.

Denery JR, Dragull K, Tang CS, Li QX (2004) Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum*. *Analytica Chimica Acta* **501**: 175–181.

Dethlefsen MW, Hjermitslev NH, Frosch S (2017) Effect of storage on oxidative quality and stability of extruded astaxanthin-coated fish feed pellets. *Animal Feed Science and Technology* **221**: 157–166.

Dhankar J, Kadian S, Sharma A (2012) Astaxanthin: a potential carotenoid. *IJPSR* **3**: 1246–1259.

Di L, Kerns EH (2016) Pharmacokinetics. In: Di L, Kerns EH (eds) *Drug-Like Properties: Concepts, Structure, Design and Methods*, pp. 267–281. Academic Press, Burlington, MA.

Dong S, Huang Y, Zhang R, Wang S, Liu Y (2014) Four different methods comparison for extraction of astaxanthin from green alga *Haematococcus pluvialis*. *Scientific World Journal* **2014**: 1–7.

Doolan BJ, Booth MA, Jones PL, Allan GL (2007) Effect of cage colour and light environment on the skin colour of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801). *Aquaculture Research* **38**: 1395–1403.

Doolan BJ, Allan GL, Booth MA, Jones PL (2008a) Effect of carotenoids and background colour on the skin pigmentation of Australian snapper *Pagrus auratus* (Bloch and Schneider, 1801). *Aquaculture Research* **39**: 1423–1433.

Doolan BJ, Booth MA, Allan GL, Jones PL (2008b) Effects of dietary astaxanthin concentrations and feeding period on the skin pigmentation of Australian snapper *Pagrus auratus* (Bloch and Schneider, 1801). *Aquaculture Research* **40**: 60–68.

Doucha J, Livansky K (2008) Influence of processing parameters on disintegration of *Chlorella* cells in various types of homogenizers. *Applied Microbiology and Biotechnology* **81**: 431–440.

Duester G (2008) Retinoic acid synthesis and signalling during early organogenesis. *Cell* **134**: 921–931.

Dufosse L, Galaupa P, Yaronb A, Arad SM, Blanc P, Chidambara Murthy KN *et al.* (2005) Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? *Trends in Food Science and Technology* **16**: 389–406.

During A, Harrison E (2004) Intestinal absorption and metabolism of carotenoids: insights from cell culture. *Archives of Biochemistry and Biophysics* **430**: 77–88.

Dursun D, Dalgic AC (2016) Optimization of astaxanthin pigment bioprocessing by four different yeast using wheat wastes. *Biocatalysis and Agricultural Biotechnology* **7**: 1–6.

Dutta D, Chaudhuri UR, Chakraborty R (2005) Structure, health benefits, antioxidant property and processing and storage of carotenoids. *African Journal of Biotechnology* **13**: 1510–1520.

Edge R, McGarvey DJ, Truscott TG (1997) The carotenoids as anti-oxidants – a review. *Journal of Photochemistry and Photobiology. B, Biology* **41**: 189–200.

EFSA (2005) Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the European commission on the safety of use of colouring agents in animal human nutrition. *EFSA Journal* **291**: 1–40.

EFSA (2007a) Safety and efficacy of panaferd-AX (red carotenoid-rich bacterium *Paracoccus carotinifaciens*) as feed additive for salmon and trout. *EFSA Journal* **546**: 1–30.

EFSA (2007b) Scientific opinion on the safety and efficacy of CAROPHYLL® Stay-Pink (astaxanthin dimethyldisuccinate) as feed additive for salmon and trout. *EFSA Journal* **574**: 1–25.

EFSA (2014) Scientific opinion on the safety and efficacy of astaxanthin (CAROPHYLL® Pink 10% CWS) for salmonids and ornamental fish. *EFSA Journal* **12**: 1–35.

Einen O, Skrede G (1998) Quality characteristics in raw and smoked fillets of Atlantic salmon, *Salmo salar*, fed high-energy diets. *Aquaculture Nutrition* **4**: 99–108.

Eisler R (1957) Some effects of artificial light on salmon eggs and larvae. *Transactions of the American Fisheries Society* **87**: 151–162.

Elliot JM (1976) Energy losses in the waste products of brown trout (*Salmo trutta* L.). *Journal of Animal Ecology* **45**: 561–580.

Eonseon J, Polle JW, Lee HK, Hyun SM, Chang MJ (2003) Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application. *Microbial Biotechnology* **13**: 165–174.

Fassett RG, Coombes JS (2011) Astaxanthin: a potential therapeutic agent in cardiovascular disease. *Marine Drugs* **9**: 447–465.

Fernandez-Garcia E, Carvajal-Lerida I, Jaren-Galan M, Garrido-Fernandez J, Perez-Galvez A, Hornero-Mendez D (2012) Carotenoids bioavailability from foods: from plant pigments to efficient biological activities. *Food Research International* **46**: 438–450.

Flores M, Diaz F, Medina R, Re AD, Licea A (2007) Physiological, metabolic and haematological responses in white shrimp *Litopenaeus vannamei* (Boone) juveniles fed diets supplemented with astaxanthin acclimated to low-salinity water. *Aquaculture Research* **38**: 740–747.

Foo SC, Yusoff FM, Ismail M, Basri M, Chan KW, Khong MH *et al.* (2015a) Production of fucoxanthin rich-fraction (FxRF) from a diatom, *Chaetoceros calcitrans* (Paulsen) Takano 1968. *Algal Research* **12**: 26–32.

Foo SC, Yusoff FM, Ismail M, Basri M, Khong MH, Chan KW *et al.* (2015b) Efficient solvent extraction of antioxidant-rich extract from a tropical diatom, *Chaetoceros calcitrans* (Paulsen) Takano 1968. *Asian Pacific Journal of Tropical Biomedicine* **5**: 834–840.

Foo SC, Yusoff FM, Ismail M, Basri M, Yau SK, Khong MH *et al.* (2017a) Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. *Journal of Biotechnology* **10**: 175–183.

Foo SC, Yusoff FM, Ismail M, Basri M, Yau SK, Khong MH *et al.* (2017b) HPLC fucoxanthin profiles of a microalga, a macroalga and a pure fucoxanthin standard. *Data in Brief* **10**: 583–586.

Foote CJ, Brown GS, Hawryshyn CW (2004) Female colour and male choice in sockeye salmon: implications for the phenotypic convergence of anadromous and nonanadromous morphs. *Animal Behaviour* **67**: 69–83.

Forsberg OI, Guttormsen AG (2006) A pigmentation model for farmed Atlantic salmon: nonlinear regression analysis of published experimental data. *Aquaculture* **253**: 415–420.

Foss P, Storebakken T, Schiedt K, Liaaen-Jensen S, Austreng E, Streiff K (1984) Pigmentation of rainbow trout with individual optical isomers of astaxanthin in comparison with canthaxanthin. *Aquaculture* **41**: 213–226.

Fraga D, Meulia T, Fenster S (2008) Real-time PCR. *Current Protocols Essential Laboratory Techniques* **10.1**: 10.3.1–10.3.34.

de la Fuente JL, Rodriguez-Saiz M, Schleissner C, Diez B, Peiro E, Barredo JL (2010) High-titer production of astaxanthin by the semi-industrial fermentation of *Xanthophyllomyces dendrorhous*. *Journal of Biotechnology* **148**: 144–146.

Fujii K (2012) Process integration of supercritical carbon dioxide extraction and acid treatment for astaxanthin extraction from a vegetative microalga. *Food and Bioproducts Processing* **90**: 762–766.

Furr HC, Clark RM (1997) Intestinal absorption and tissue distribution of carotenoids. *The Journal of Nutritional Biochemistry* **8**: 364–377.

Furuita H, Tanaka H, Yamamoto T, Shiraishi M, Takeuchi T (2000) Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* **187**: 387–398.

Furuita H, Tanaka H, Yamamoto T, Suzuki N, Takeuchi T (2002) Effects of high levels of n-3 HUFA in broodstock diet on egg quality and egg fatty acid composition of Japanese flounder, *Paralichthys olivaceus*. *Aquaculture* **210**: 323–333.

George SB, Lawrence JM, Lawrence AL, Smiley J, Plank L (2001) Carotenoids in the adult diet enhance egg and juvenile production in the sea urchin *Lytechinus variegatus*. *Aquaculture* **199**: 353–369.

Georgiev GS (1971) Carotenoids and vitamin A content in *Salmo irideus* eggs and their significance in the initial periods of the embryogenesis. *Folia Balcania* **2**: 1–11.

Gireesh T, Nair PP, Sudhakaran PR (2004) Studies on the bioavailability of the provitamin A carotenoid, β-carotene, using human exfoliated colonic epithelial cells. *British Journal of Nutrition* **92**: 241–245.

Gjerde B, Gjedrem T (1984) Estimates of phenotypic and genetic parameters for carcass traits in Atlantic salmon and rainbow trout. *Aquaculture* **36**: 97–110.

Glencross B, Hawkins W, Evans D, Rutherford N, McCafferty P, Dods K *et al.* (2011) A comparison of the effect of diet extrusion or screw-press pelleting on the digestibility of grain protein products when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **312**: 154–161.

Glencross B, Blyth D, Tabrett S, Bourne N, Irvin S, Anderson M *et al.* (2012) An assessment of cereal grains and other starch sources in diets for barramundi (*Lates calcarifer*)-implications for nutritional and functional qualities of extruded feeds. *Aquaculture Nutrition* **18**: 388–399.

Gobantes I, Choubert G, Laurentie M, Milicua JCG, Gomez R (1997) Astaxanthin and canthaxanthin kinetics after ingestion of individual doses by immature rainbow trout *Oncorhynchus mykiss*. *Journal of Agricultural and Food Chemistry* **45**: 454–458.

Goh LP, Loh SP, Yusoff FM, Perumal K (2009) Bioaccessibility of carotenoids and tocopherols in marine microalgae, *Nannochloropsis* sp. and *Chaetoceros* sp. *Malaysian Journal of Nutrition* **15**: 77–86.

Gomes E, Dias J, Silva P, Valente L, Empis J, Gouveia L *et al.* (2002) Utilization of natural and synthetic sources of carotenoids in the skin pigmentation of gilthead seabream (*Sparus aurata*). *European Food Research and Technology* **214**: 287–293.

Goodwin TW (1950) Carotenoids and reproduction. *Biological Reviews* **25**: 391–413.

Goto S, Kogure K, Abe K, Kimata Y, Kitahama K, Yamashita E *et al.* (2001) Efficient radical trapping at the surface and inside the phospholipid membrane is responsible for highly potent antiperoxidative activity of the carotenoid astaxanthin. *Biochimica et Biophysica Acta* **1512**: 251–258.

Gouveia L, Empis J (2003) Relative stabilities of microalgal carotenoids in microalgal extracts, biomass and fish feed: effect of storage conditions. *Innovative Food Science and Emerging Technologies* **4**: 227–233.

Grung M, Metzger P, Liaaen-Jensen S (1994) Algal carotenoids 53: secondary carotenoids of algae 4. Secondary carotenoids in the green alga *Botryococcus braunii*, race L, new strain. *Biochemical Systematic and Ecology* **22**: 25–29.

Guerin M, Huntley M, Olaizola M (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. *Trends in Biotechnology* **21**: 210–216.

Guerra BA, Otton R (2011) Impact of the carotenoid astaxanthin on phagocytic capacity and ROS/RNS production of human neutrophils treated with free fatty acids and high glucose. *International Immunopharmacology* **11**: 2220–2226.

Guerra BA, Bolin AP, Otton R (2012) Carbonyl stress and a combination of astaxanthin/vitamin C induce biochemical changes in human neutrophils. *Toxicology in Vitro* **26**: 1181–1190.

Guillou A, Choubert G, de la Noue J (1992) Absorption and blood clearance of labelled carotenoids [$^{14}$C] astaxanthin, [$^{3}$H] canthaxanthin and [$^{3}$H] zeaxanthin in mature female rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology. A, Molecular and Integrated Physiology* **103**: 301–306.

Gunerken E, D'Hondt E, Eppink M, Elst K, Wijffels R (2016) Influence of nitrogen depletion in the growth of *N. oleoabundans* on the release of cellular components after beadmilling. *Bioresource Technology* **214**: 89–95.

Haaland H, Ladstein K, Rosenlund G, Oliveira MA (1993) Stability of vitamins and pigment in extruded fish feed. In: Reinertsen H, Dahle LA, Jorgensen L, Tvinnereim K (eds) *Fish Farming Technology*, pp. 419–477. Balkema, Rotterdam, the Netherlands.

Haga S, Uji S, Suzuki T (2008) Evaluation of the effects of retinoids and carotenoids on egg quality using a microinjection system. *Aquaculture* **282**: 111–116.

Hamre K, Christiansen R, Waagbo R, Maage A, Torseinsen BE, Lygren B *et al.* (2004) Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar* L.): effects on growth performance and fillet quality. *Aquaculture Nutrition* **10**: 113–123.

Han D, Li Y, Hu Q (2013) Astaxanthin in microalgae: pathway, functions and biotechnological implications. *Algae* **28**: 131–147.

Hansen OJ, Puvanendran V, Bangera R (2016) Broodstock diet with water and astaxanthin improve condition and egg output of brood fish and larval survival in Atlantic cod, *Gadus morhua* L. *Aquaculture Research* **47**: 819–829.

Haque F, Dutta A, Thimmanagari M, Chiang Y (2016) Intensified green production of astaxanthin from *Haematococcus pluvialis*. *Food and Bioproducts Processing* **99**: 1–11.

Hara K, Morita T, Mochizuki M, Yamamoto K, Ogino C, Araki M *et al.* (2014) Development of a multi-gene expression system in *Xanthophyllomyces dendrorhous*. *Microbial Cell Factories* **13**: 1–7.

Hardy R (1985) Salmonid broodstock nutrition. In: Iwamoto R, Sower S (eds) *Salmonid Reproduction*, pp. 98 108. Washington Sea Grant Programme, University of Washington, Seattle.

Hardy RW, Barrows FT (2002) Diet formulation and manufacture. In: Halver JE, Hardy RW (eds) *Fish Nutrition*, pp. 506 601. Academic Press, San Diego, CA.

Hardy RW, Torrissen OJ, Scott TM (1990) Absorption and distribution of $^{14}$C-labeled canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **87**: 331–340.

Harrison EH (2012) Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *BBA* **1821**: 70–77.

Hartmann M, Medem F, Kuhn R, Bielig H (1947) Untersuchungen uber die Befruchtungsstoffe der Regenbogenforelle. *Zeitschrift fur Naturforsch B* **2**: 330–349.

Hatlen B, Arnesen A, Jobling M (1996) Muscle carotenoid concentrations in sexually maturing and immature Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture Nutrition* **2**: 207–212.

Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea F (2006) Astaxanthin: a review of its chemistry and applications. *Critical Reviews in Food Science and Nutrition* **46**: 185–196.

Hill GE, Johnson JD (2012) The vitamin A-redox hypothesis: a biochemical basis for honest signaling via carotenoid pigmentation. *The American Naturalist* **180**: E127–E150.

Hinderliter P, Saghir SA (2014) Pharmacokinetics. *Reference Module in Biomedical Sciences* **1**: 849–855.

Ho CC, De Moura FF, Kim SH, Clifford AJ (2007) Excentral cleavage of β-carotene in vivo in a healthy man. *The American Journal of Clinical Nutrition* **85**: 770–777.

Ho CC, De Moura FF, Kim SH, Burri BJ, Clifford AJ (2010) A minute dose of $^{14}$C-β-carotene is absorbed and converted to retinoids in humans. *Journal of Nutrition* **139**: 1480–1486.

Ho A, Orlando Bertran NM, Lin J (2013) Dietary esterified astaxanthin concentration effect on dermal coloration and chromatophore physiology in spinecheek anemonefish, *Premnas biaculeatus*. *Journal of the World Aquaculture Society* **44**: 76–85.

van het Hof KH, West CE, Weststrate JA, Hautvast JG (2000) Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition* **130**: 503–506.

Hubbs C, Stavenhagen L (1958) Effects of maternal carotenoid deficiency on the viability of darter (Osteichthyes) offspring. *Physiological Zoology* **31**: 280–283.

Hur SJ, Jin SK, Park JH, Jung SW, Lyu HJ (2013) Effect of modified atmosphere packaging and vacuum packaging on quality characteristics of low grade beef during cold storage. *Asian-Australas Journal of Animal Sciences* **26**: 1781–1789.

Hussein G, Sankawa U, Goto H, Matsumoto K, Watanabe H (2006) Astaxanthin, a carotenoid with potential in human health and nutrition. *Journal of Natural Products* **69**: 443–449.

Ibrahim A, Shimizu C, Kono M (1984) Pigmentation of cultured red sea bream, *Chrysophrys major*, using astaxanthin from Antarctic krill, *Euphausia superba*, and a mysid, *Neomysis* sp. *Aquaculture* **38**: 45–57.

Ingle de la Mora G, Arredondo-Figuero JL, Ponce-Palafox JT, Barriga-Soca I, Vernon-Carter JE (2006) Comparison of red chilli (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhynchus mykiss*) fillet pigmentation. *Aquaculture* **258**: 487–495.

Iwabayashi M, Fujioka N, Nomoto K, Miyazaki R, Takahashi H, Hibino S *et al.* (2009) Efficacy and safety of eight-week treatment with astaxanthin in individuals screened for increased oxidative stress burden. *Journal of Anti-Aging Medicine* **6**: 15–21.

Izquierdo M, Fernandez-Palacios H, Tacon A (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture* **197**: 25–42.

Jahanshahi M, Sun Y, Santos E, Pacek AW, Franco TT, Nienow A *et al.* (2002) Operational intensification by direct product sequestration from cell disruptates: application of a pellicular adsorbent in a mechanically integrated disruption-fluidised bed adsorption process. *Biotechnology and Bioengineering* **80**: 201–212.

Jintasataporn O, Yuangsoi B (2012) Stability of carotenoid diets during feed processing and under different storage conditions. *Molecules* **17**: 5651–5660.

Johnson EA, An GH (1991) Astaxanthin from microbial sources. *Critical Reviews in Biotechnology* **11**: 297–326.

Johnson E, An G (2008) Astaxanthin from microbial sources. *Critical Reviews in Biotechnology* **11**: 297–326.

Jorgensen K, Skibsted L (1993) Carotenoid scavenging radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **196**: 423–429.

Kaczor A, Baranska M (2011) Structural changes of carotenoid astaxanthin in a single algal cell monitored in situ by Raman spectroscopy. *Analytical Chemistry* **83**: 7763–7770.

Kalinowski CT, Robaina LE, Fernandez-Palacios H, Schuchardt D, Izquierdo MS (2005) Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. *Aquaculture* **244**: 223–231.

Kam KT, Deng Y, Chen Y, Zhao H (2012) Retinoic acid synthesis and functions in early embryonic development. *Cell and Bioscience* **2**: 1–14.

Kang CD, Sim SJ (2008) Direct extraction of astaxanthin from *Haematococcus* culture using vegetable oils. *Biotechnology Letters* **30**: 441–444.

Katayama T, Ikeda N, Harada K (1965) Carotenoids in sea breams, *Chrysophrys major Temmick* and *Schlegel*. *Bulletin of the Japanese Society of Scientific Fisheries* **31**: 947–952.

Katsumata T, Ishibashi T, Kyle D (2014) A sub-chronic toxicity evaluation of a natural astaxanthin-rich carotenoid extract of *Paracoccus carotinifaciens* in rats. *Toxicology Reports* **1**: 582–588.

Kawakami Y, Raya A, Raya R, Rodriguez-Esteban C, Belmonte J (2005) Retinoic acid signalling links left-right asymmetric patterning and bilaterally symmetric somitogenesis in the zebrafish embryo. *Nature* **435**: 165–171.

Kidd P (2011) Astaxanthin, cell membrane nutrient with diverse clinical benefits and anti-aging potential. *Alternative Medicine Review* **16**: 355–364.

Kiessling A, Dosanjh B, Higgs D, Deacon G, Rowshandeli N (1995) Dorsal aorta cannulation: a method to monitor changes in blood levels of astaxanthin in voluntary feeding Atlantic salmon *Salmo salar* L. *Aquaculture Nutrition* **1**: 43–50.

Kiessling A, Olsen RE, Buttle L (2003) Given the same dietary carotenoid inclusion, Atlantic salmon, *Salmo salar* (L.) display higher blood levels of canthaxanthin than astaxanthin. *Aquaculture Nutrition* **9**: 253–261.

Kiessling A, Dosanjh B, Koppe W, Higgs D (2006) Relationship between blood and muscle levels of astaxanthin in dorsal aorta cannulated Atlantic salmon. *Aquaculture* **254**: 653–657.

Kim YK, Chyun JH (2004) The effects of astaxanthin supplements on lipid peroxidation and antioxidant status in postmenopausal women. *Journal of Nutritional Science* **7**: 41–46.

Kim JH, Kang SW, Kim SW, Chang HI (2005) High-level production of astaxanthin by *Xanthophyllomyces dendrorhous* mutant JH1 using statistical experimental designs. *Bioscience, Biotechnology and Biochemistry* **69**: 1743–1748.

Kim ZH, Kim SH, Lee HS, Lee CG (2006) Enhanced production of astaxanthin by flashing light using *Haematococcus pluvialis*. *Enzyme and Microbial Technology* **39**: 414–419.

Kim JH, Chang MJ, Choi HD, Youn YK, Kim JT, Oh JM *et al.* (2011) Protective effects of *Haematococcus* astaxanthin on oxidative stress in healthy smokers. *Journal of Medicinal Food* **14**: 1469–1475.

Kim D, Vijayan D, Ramasamy P, Oh Y (2016) Cell-wall disruption and lipid/astaxanthin extraction from microalgae: *Chlorella* and *Haematococcus*. *Bioresource Technology* **199**: 300–310.

Kiron V (2012) Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology* **173**: 111–133.

Kishimoto Y, Tani M, Uto-Kondo H, Lizuka M, Saita E, Sone H *et al.* (2010) Astaxanthin suppresses scavenger receptor expression and matrix metalloproteinase activity in macrophages. *European Journal of Nutrition* **49**: 119–126.

Kishimoto Y, Yoshida H, Kondo K (2016) Potential anti-atherosclerotic properties of astaxanthin. *Marine Drugs* **14**: 1–13.

Kitahara T (1983) Behavior of carotenoids in the chum salmon migration. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **76**: 97–101.

Kitahara T (1984) Behavior of carotenoids in the chum salmon (*Oncorhynchus keta*) during development. *Bulletin of the Japanese Society for the Science of Fish* **50**: 531–536.

Kittikaiwan P, Powthongsook S, Pavasant P, Shotipruk A (2007) Encapsulation of *Haematococcus pluvialis* using chitosan for astaxanthin stability enhancement. *Carbohydrate Polymers* **70**: 378–385.

Kobayashi M, Kurimura Y, Sakamoto Y, Tsuji Y (1997) Selective extraction of astaxanthin and chlorophyll from the green alga *Haematococcus pluvialis*. *Biotechnology Techniques* **11**: 657–660.

Kodric-Brown A (1985) Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* **17**: 199–205.

Koller M, Muhr A, Braunegg G (2014) Microalgae as versatile cellular factories for valued products. *Algal Research* **6**: 52–63.

Kopecky J (2013) The effect of astaxanthin and β-carotene on the colour of kissing gourami (*Helostoma temminckii*). *Acta Fytotechnica et Zootechnica* **16**: 65–68.

Kuhn R, Soerensen NA (1938) The coloring matters of the lobster (*Astacus gammarus* L). *Zeitschrift Fur Angewandte Chemie* **51**: 465–466.

Kumar V, Pillai BR, Sahoo PK, Mohanty J, Mohanty S (2009) Effect of dietary astaxanthin on growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man). *Asian Fisheries Science Journal* **22**: 61–69.

Kumlu M, Fletcher DJ, Fisher CM (1998) Larval pigmentation, survival and growth of *Penaeus indicus* fed the nematode *Panagrellus redivivus* enriched with astaxanthin and various lipids. *Aquaculture Nutrition* **4**: 193–200.

Kupcinskas L, Lafolie P, Lignell A, Kiudelis G, Jonaitis L, Adamonis K *et al.* (2008) Efficacy of the natural antioxidant astaxanthin in the treatment of functional dyspepsia in patients with or without *Helicobacter pylori* infection: a prospective, randomized, double blind, and placebo-controlled study. *Phytomedicine* **15**: 391–399.

Lee KT (2010) Quality and safety aspects of meat products as affected by various physical manipulations of packaging materials. *Meat Science* **86**: 138–150.

Lee YK, Ding SY (1994) Cell cycle and accumulation of astaxanthin in *Haematococcus lacustris* (Chlorophyta). *Journal of Phycology* **30**: 445–449.

Lee CM, Boileau AC, Boileau T, Williams AW, Swanson KS, Heintz KA *et al.* (1999) Reviews of animal models in carotenoid research. *The Journal of Nutrition* **129**: 2271–2277.

Lee SJ, Bai SK, Lee KS, Namkoong S, Na HJ, Ha KS *et al.* (2003) Astaxanthin inhibits nitric oxide production and inflammatory gene expression by suppressing I(kappa)B kinase-dependent NF-kappaB activation. *Molecules and Cells* **31**: 97–105.

Lemoine Y, Schoefs B (2010) Secondary ketocarotenoid astaxanthin biosynthesis in algae: a multifunctional response to stress. *Photosynthesis Research* **106**: 155–177.

Li MH, Robison EH, Oberle DF (2007) Effects of various dietary carotenoid pigments on fillet appearance and pigment absorption in channel catfish, *Ictalurus punctatus*. *Journal of the World Aquaculture Society* **38**: 557–563.

Li J, Zhu D, Niu J, Shen S, Wang G (2011) An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnology Advances* **29**: 568–574.

Li M, Wu W, Zhou P, Xie F, Zhou Q, Mai K (2014) Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. *Aquaculture* **434**: 227–232.

Liao IC, Chien YH (1994) Culture of kuruma prawn (*Penaeus japonicus*) in Asia. *World Aquaculture* **25**: 18–33.

van Lieshout M, West CE, van Breemen RB (2003) Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly β-carotene, in humans: a review. *The American Journal of Clinical Nutrition* **77**: 12–28.

Lim BK, Sakurai N, Sugihara T, Kittaka J (1997) Survival and growth of the American lobster (*Homarus americanus*) fed formulated feeds. *Bulletin of Marine Science* **61**: 159–163.

Lin MQ, Ushio H, Ohshima T, Yamanaka H, Koizumi C (1998) Skin color control of the red sea bream (*Pagrus major*). *Lebensmittel-Wissenschaft und-Technologie* **31**: 27–32.

Lin S, Chen Y, Chen R, Chen L, Ho H, Tsung Y *et al.* (2016) Improving the stability of astaxanthin by microencapsulation in calcium alginate beads. *PLoS One* **11**: e0153685.

Linan-Cabello MA, Paniagua-Michel J, Hopkins PM (2002a) Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition* **8**: 299–309.

Linan-Cabello MA, Paniagua-Michel J, Zenteno-Savin T (2002b) Carotenoids and retinoids like regulators of oxidative stress during the gonadic maturation of *Litopenaus vannamei*. In: Biennial X, Pasquier C (eds) *Meeting of the Society for Free Radical Research International*, pp. 607 610. Monduzzi Editore International Proceedings Division, Salzburg.

Liu YS, Wu JY, Ho KP (2006) Characterization of oxygen transfer conditions and their effects on *Phaffia rhodozyma* growth and carotenoid production in shake-flask cultures. *Biochemical Engineering Journal* **27**: 331–335.

Liu X, McClements DJ, Cao Y, Xiao H (2016a) Chemical and physical stability of astaxanthin-enriched emulsion-based delivery systems. *Food Biophysics* **11**: 302–310.

Liu F, Shi H, Guo Q, Yu Y, Wang A, Lv F *et al.* (2016b) Effects of astaxanthin and emodin on the growth, stress resistance and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). *Fish and Shellfish Immunology* **51**: 125–135.

Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. *Trends in Biotechnology* **18**: 160–167.

Lubzens E, Lissauer L, Levavi-Sivan B, Avarre J, Sammar M (2003) Carotenoid and retinoid transport to fish oocytes and eggs: what is the role of retinol binding protein? *Molecular Aspects of Medicine* **24**: 441–457.

Maan ME, Van Der Spoel M, Jimenez P, Van Alphen JM, Seehausen O (2006) Fitness correlates of male coloration in a Lake Victoria cichlid fish. *Behavioral Ecology* **17**: 691–699.

Macdermid JC, Vincent JI, Gan BS, Grewal R (2012) A blinded placebo-controlled randomized trial on the use of astaxanthin as an adjunct to splinting in the treatment of carpal tunnel syndrome. *Hand* **7**: 1–9.

Macedo RC, Bolin AP, Marin DP, Otton R (2010) Astaxanthin addition improves human neutrophils function: in vitro study. *European Journal of Nutrition* **49**: 447–457.

Machmudah S, Shotipruk A, Goto M, Sasaki M, Hirose T (2006) Extraction of astaxanthin from *Haematococcus pluvialis* using supercritical $CO_2$ and ethanol as entrainer. *Industrial and Engineering Chemistry Research* **45**: 3652–3657.

Magalhaes IS, Seehausen O (2010) Genetics of male nuptial colour divergence between sympatric sister species of a Lake Victoria cichlid fish. *Journal of Evolutionary Biology* **23**: 914–924.

Magnadottir B (2010) Immunological control of fish diseases. *Marine Biotechnology* **12**: 361–379.

Maltby JB, Albright LJ, Kennedy CJ, Higgs DA (2003) Effect of route of administration and carrier on bioavailability and kinetics of astaxanthin in Atlantic salmon *Salmo salar* L. *Aquaculture Research* **34**: 829–838.

Manimegalai M, Bupesh G, Mirunalini M, Vasanth S, Karthikeyini S, Subramanian P (2010) Colour enhancement study on *Etroplus maculatus* using astaxanthin and β-carotene. *International Journal of Environmental Science* **1**: 403–416.

Maoka T, Etoh H (2010) Some biological functions of carotenoids in Japanese Food. In: Shi J, Ho CT, Shahidi F (eds) *Functional Foods of the East*, pp. 85–97. CRC Press, Boca Raton, FL.

March BE, MacMillan C (1996) Muscle pigmentation and plasma concentrations of astaxanthin in rainbow trout, chinook salmon, and Atlantic salmon in response to different dietary levels of astaxanthin. *The Progressive Fish-Culturist* **58**: 178–186.

March BE, Hajen WE, Deacon G, MacMillan C, Walsh MG (1990) Intestinal absorption of astaxanthin, plasma astaxanthin concentration, body weight, and metabolic rate as determinants of flesh pigmentation in salmonid fish. *Aquaculture* **90**: 313–322.

Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnology Advances* **31**: 1532–1542.

Martin HD, Jager C, Ruck C, Schmidt M (1999) Anti- and prooxidant properties of carotenoids. *Journal fur Praktische Chemie* **341**: 302–308.

Martinez-Delgado A, Khandual S, Villanueva-Rodriguez S (2017) Chemical stability of astaxanthin integrated into a food matrix: effects of food processing and methods for preservation. *Food Chemistry* **225**: 23–30.

Masaki H (2010) Role of antioxidants in the skin: anti-aging effects. *Journal of Dermatological Science* **58**: 85–90.

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Review* **14**: 217–232.

Mayne ST (1996) β-carotene, carotenoids, and disease prevention in humans. *FASEB Journal* **10**: 690–701.

McCallum IM, Cheng KM, March BE (1987) Carotenoid pigmentation in two strains of chinook salmon (*Oncorhynchus tshawytscha*) and their crosses. *Aquaculture* **67**: 291–300.

McClements DJ, Li Y (2010) Structured emulsion-based delivery systems: controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science* **159**: 213–228.

McGraw KJ, Adkins-Regan E, Parker RS (2005) Maternally derived carotenoid pigments affect offspring survival, sex ratio and sexual attractiveness in a colorful songbird. *Naturwissenschaften* **92**: 375–380.

McKay C (1987) The effectiveness of a dietary astaxanthin supplement in respect to the pigmentation and growth response in the American lobster, *Homarus americanus*. *Crustacean Nutrition Newsletter* **4**: 5–6.

Menasveta P, Worawattanamateekul W, Latscha T, Clark J (1993) Correction of black tiger prawn (*Penaeus monodon* Fabricius) coloration by astaxanthin. *Aquaculture Engineering* **12**: 203–213.

Mendes-Pinto MM, Raposo MFJ, Bowen J, Young AJ, Morais R (2001) Evaluation of different cell disruption processes on encysted cells of *Haematococcus pluvialis*: effects on astaxanthin recovery and implications for bio-availability. *Journal of Applied Phycology* **13**: 19–24.

Mercer P, Armenta RE (2011) Developments in oil extraction from microalgae. *European Journal of Lipid Science and Technology* **113**: 539–547.

Merchie G, Kontara E, Lavens P, Robles R, Kurmaly K, Sorgeloos P (1998) Effect of vitamin C and astaxanthin on stress and disease resistance of postlarval tiger shrimp, *Penaeus monodon* (Fabricius). *Aquaculture Research* **29**: 579–585.

Miki W (1991) Biological functions and activities of animal carotenoids. *Pure and Applied Chemistry* **63**: 141–146.

Miki W, Yamaguchi K, Konosu S (1982) Comparison of carotenoids in the ovaries of marine fish and shellfish. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **71**: 7–11.

Mikulin AE (2000) *Functional Role of Pigments and Pigmentation in Fish Ontogeny*. VNIRO, Moscow.

Mikulin AY (2003) *The Influence of Carotenoids Contained in the Eggs Upon the Offspring Quality at Artificial Fish Breeding*. International Symposium, Coldwater Aquaculture, Russia.

Mikulin AY, Soin SG (1975) The functional significance of carotenoids in the embryonic development of teleosts. *Journal of Ichthyology* **15**: 749–759.

Milledge JJ (2011) Commercial application of microalgae other than as biofuels: a brief review. *Reviews in Environmental Science and Biotechnology* **10**: 31–41.

Miyawaki H, Takahashi J, Tsukahara H, Takehara I (2008) Effects of astaxanthin on human blood rheology. *Journal of Clinical Biochemistry Nutrition* **43**: 69–74.

Miyazawa T, Nakagawa K, Kimura F, Satoh A, Miyazawa T (2011) Plasma carotenoid concentrations before and after supplementation with astaxanthin in middle-aged and senior subjects. *Bioscience, Biotechnology and Biochemistry* **75**: 1856–1858.

Molina-Grima E, Belarbi EH, Acien-Fernandez FG, Medina AR, Christi Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances* **20**: 491–515.

Moren M, Næss T, Hamre K (2002) Conversion of β-carotene, canthaxanthin and astaxanthin to vitamin A in Atlantic halibut (*Hippoglossus hippoglossus* L.) juveniles. *Fish Physiology and Biochemistry* **27**: 71–80.

Moriel DG, Chociai MB, Machado IMP, Fontana JD, Bonfim TMB (2005) Effect of feeding methods on the astaxanthin production by *Phaffia rhodozyma* in fed-batch process. *Brazilian Archives of Biology and Technology* **48**: 397–401.

Nagao A (2014) Bioavailability of dietary carotenoids: intestinal absorption and metabolism. *Japan Agricultural Research Quarterly* **48**: 385–391.

Naguib YM (2000) Antioxidant activities of astaxanthin and related carotenoids. *Journal of Agriculture and Food Chemistry* **48**: 1150–1154.

Nakano T, Tosa ME, Takeuchi MJ (1995) Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin. *Journal of Agricultural and Food Chemistry* **43**: 1570–1573.

Nakazoe J, Ishii S, Kamimoto H, Takeuchi M (1984) Effects of supplemental carotenoid pigments on the carotenoid accumulation in young red sea bream *Chrysophrys major*. *Bulletin of Tokai Regional Fisheries Research Laboratory* **113**: 29–41.

Ndong D, Chen Y, Lin Y, Vaseeharan B, Chen J (2007) The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures. *Fish and Shellfish Immunology* **22**: 686–694.

Nguyen K (2013) Astaxanthin: a comparative case of synthetic vs. natural production. *Chemical and Biomolecular Engineering Publishing and Other Works* **1**: 1–11.

Nguyen NV, Khanh TV, Hai PD (2014) Study on development of formulated feed for improving growth and pigmentation of koi carp (*Cyprinus carpio* L., 1758) juveniles. *Journal of Life Sciences* **8**: 433–441.

Ni H, Chen QH, He GQ, Wu GB, Yang YF (2008) Optimization of acidic extraction of astaxanthin from *Phaffia rhodozyma*. *Journal of Zhejiang University Science B* **9**: 51–59.

Niamnuy C, Devahastin S, Soponronnarit S, Raghavan GV (2008) Kinetics of astaxanthin degradation and color changes of dried shrimp during storage. *Journal of Food Engineering* **87**: 591–600.

Nicieza AG, Reiriz L, Brana F (1994) Variation in digestive performance between geographically disjunct populations of Atlantic salmon: counter-gradient in passage time and digestion rate. *Oecologia* **99**: 243–251.

Nickell DC, Bromage NR (1998) The effect of dietary lipid level on variation of flesh pigmentation in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **161**: 237–251.

Nie X, Zie J, Haubner N, Tallmark B, Snoeijs P (2011) Why Baltic herring and sprat are weak conduits for astaxanthin from zooplankton to piscivorous fish. *Limnology and Oceanography* **56**: 1155–1167.

Nikoo M, Falahatkar B, Alekhorshid M, Haghi BN, Asadollah A, Dangsareki MZ *et al.* (2010) Physiological stress responses in kutum *Rutilus frisii* kutum subjected to captivity. *International Aquatic Research* **2**: 55–60.

Nir Y, Spiller G, Multz C (2002) Effect of an astaxanthin containing product on rheumatoid arthritis. *Journal of the American College of Nutrition* **21**: 490–492.

Niu J, Tian LX, Liu YJ, Yang HJ, Ye CX, Gao W (2009) Effect of dietary astaxanthin on growth, survival, and stress tolerance of postlarval shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society* **40**: 795–802.

Niu J, Li CH, Liu YJ, Tian LX, Chen X, Huang Z (2012) Dietary values of astaxanthin and canthaxanthin in *Penaeus monodon* in the presence and absence of cholesterol supplementation: effect on growth, nutrient digestibility and tissue carotenoid composition. *British Journal of Nutrition* **108**: 80–91.

Niu J, Wen H, Li CH, Liu YJ, Tian LX, Chen X (2014) Comparison effect of dietary astaxanthin and β-carotene in the presence and absence of cholesterol supplementation on growth performance, antioxidant capacity and gene expression of *Penaeus monodon* under normoxia and hypoxia condition. *Aquaculture* **422–423**: 8–17.

No H, Storebakken T (1992) Pigmentation of rainbow trout with astaxanthin and canthaxanthin in freshwater and saltwater. *Aquaculture* **101**: 123–134.

Nobre B, Marcelo F, Passos R, Beiro L, Palavra A, Gouveia L (2006) Supercritical carbon dioxide extraction of astaxanthin and other carotenoids from the microalga *Haematococcus pluvialis*. *European Food Research and Technology* **223**: 787–790.

Noga JE (2011) *Fish Disease: Diagnosis and Treatment*, 2nd edn. Willey-Blackwell Publishing Limited, Ames, IA.

Nootash S, Sheikzadeh N, Baradaran B, Oushani A, Moghadam M, Nofouzi K *et al.* (2013) Green tea (*Camellia sinensis*) administration induces expression of immune relevant genes and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology* **35**: 1916–1923.

Nordeide JT, Rudolfsen G, Egeland ES (2006) Ornaments or offspring? Female sticklebacks (*Gasterosteus aculeatus* L.) trade off carotenoids between spines and eggs. *Journal of Evolutionary Biology* **19**: 431–439.

O'Connor I, O'Brien N (1998) Modulation of UVA light-induced oxidative stress by β-carotene, lutein and astaxanthin in cultured fibroblasts. *Journal of Dermatological Science* **16**: 226–230.

Odeberg JM, Lignell A, Pettersson A, Hoglund P (2003) Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. *European Journal of Pharmaceutical Sciences* **19**: 299–304.

Olsen RE, Kiessling A, Milley JE, Ross NW, Lall SP (2005) Effect of lipid source and bile salts in diet of Atlantic salmon, *Salmo salar* L., on astaxanthin blood levels. *Aquaculture* **250**: 804–812.

Olson JA (2004) Carotenoids: absorption, transport, and metabolism of carotenoids in humans. *Pure and Applied Chemistry* **66**: 1011–1016.

Orosa M, Torres E, Fidalgo P, Abalde J (2000) Production and analysis of secondary carotenoids in green algae. *Journal of Applied Phycology* **12**: 553–556.

Orosa M, Valero JF, Abalde J (2001) Comparison of the accumulation of astaxanthin in *Haematococcus pluvialis* and other green microalgae under N-starvation and high light conditions. *Biotechnology Letters* **23**: 1079–1085.

Osterlie M, Bjerkeng B, Liaaen-Jensen S (1999) Accumulation of astaxanthin all-E, 9Z and 13Z geometrical isomers and 3 and 3′ RS optical isomers in rainbow trout (*Oncorhynchus mykiss*) is selective. *Journal of Nutrition* **129**: 391–398.

Osterlie M, Bjerkeng B, Liaaen-Jensen S (2000) Plasma appearance and distribution of astaxanthin E/Z isomers in plasma lipoproteins of after single dose administration of astaxanthin. *Journal of Nutritional Biochemistry* **11**: 482–490.

Page GI, Davies SJ (2003) Hepatic carotenoid uptake in rainbow trout (*Oncorhynchus mykiss*) using an isolated organ perfusion model. *Aquaculture* **225**: 405–419.

Page GI, Davies SJ (2006) Tissue astaxanthin and canthaxanthin distribution in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Comparative Biochemistry and Physiology. A, Molecular and Integrated Physiology* **143**: 125–132.

Paibulkichakul C, Piyatiratitivorakul S, Sorgeloos P, Menasveta P (2008) Improved maturation of pond-reared, black tiger shrimp (*Penaeus monodon*) using fish oil and astaxanthin feed supplements. *Aquaculture* **282**: 83–89.

Palma J, Andrade J, Bureau D (2016) The impact of dietary supplementation with astaxanthin on egg quality and growth of long snout seahorse (*Hippocampus guttulatus*) juveniles. *Aquaculture Nutrition* **23**: 304–312.

Palozza P, Torelli C, Boninsegna A, Simone R, Catalano A, Mele M *et al.* (2009) Growth-inhibitory effects of the astaxanthin-rich alga *Haematococcus pluvialis* in human colon cancer cells. *Cancer Letters* **283**: 108–117.

Pan CH, Chien YH, Cheng JH (2001) Effects of light regime, algae in the water, and dietary astaxanthin on pigmentation, growth, and survival of black tiger prawn *Penaeus monodon* post-larvae. *Zoological Studies* **40**: 371–382.

Pan CH, Chien YH, Hunter B (2003) The resistance to ammonia stress of *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin. *Journal of Experimental Marine Biology and Ecology* **297**: 107–118.

Pan CH, Chien YH, Wang YJ (2011) Antioxidant defence to ammonia stress of characins (*Hyphessobrycon eques* Steindachner) fed diets supplemented with carotenoids. *Aquaculture Nutrition* **17**: 258–266.

Pangantihon-Kuhlmann MP, Millamena O, Chern Y (1998) Effect of dietary astaxanthin and vitamin A on the reproductive performance of *Penaeus monodon* broodstock. *Aquatic Living Resources* **11**: 403–409.

Panis G, Carreon J (2016) Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: a microalgae process model and a techno-economic assessment all through production line. *Algal Research* **18**: 175–190.

Paripatananont T, Tangtrongpairoj J, Sailasuta A, Chansue N (1999) Effect of astaxanthin on the pigmentation of goldfish *Carassius auratus*. *Journal of the World Aquaculture Society* **30**: 454–460.

Park JS, Chyun JH, Kim YK, Line LL, Chew BP (2010) Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. *Nutrition and Metabolism* **7**: 1–10.

Pashkow FJ, Watumull DG, Campbell CL (2008) Astaxanthin: a novel potential treatment for oxidative stress and inflammation in cardiovascular disease. *American Journal of Cardiology* **101**: 58–68.

Pavlov D, Kjorsvik E, Refsti T, Anderson O (2004) Brood stock and egg production. In: Moksness E, Kjorsvik E, Olsen Y (eds) *Culture of Cold-Water Marine Fish*, pp. 129 203. Blackwell, Oxford.

Peng J, Xiang WZ, Tang QM, Sun N (2008) Comparative analysis of astaxanthin and its esters in the mutant E1 of *Haematococcus pluvialis* and other green algae by HPLC with a C30 column. *Science China Life Sciences* **51**: 1108–1115.

Peng CH, Chang CH, Peng RY, Chyau CC (2010) Improved membrane transport of astaxanthin by liposomal encapsulation. *European Journal of Pharmaceutics and Biopharmaceutics 75: 154* **16**: 1.

Peng J, Yuan JP, Wu CF, Wang JH (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Marine Drugs* **9**: 1806–1828.

Perez-Lopez P, Gonzalez-Garcia S, Jeffryes C, Agathos SN, McHugh E, Walsh D *et al.* (2014) Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. *Journal of Cleaner Production* **64**: 332–344.

Petit H, Negre-Sadargues G, Castillo R, Trilles JP (1997) The effects of dietary astaxanthin on growth and moulting cycle of postlarval stages of the prawn, *Penaeus japonicus* (Crustacea, Decapoda). *Comparative Biochemistry and Physiology. A, Physiology* **117**: 539–544.

Pettersson A, Lignell A (1999) Astaxanthin deficiency in eggs and fry of Baltic salmon (*Salmo salar*) with the M74 syndrome. *Ambio* **28**: 43–47.

Pham MA, Byun H, Kim K, Lee S (2014) Effects of dietary carotenoid source and level on growth, skin pigmentation, antioxidant activity and chemical composition of juvenile olive flounder *Paralichthys olivaceus*. *Aquaculture* **431**: 65–72.

Pillay T, Kutty M (2005) *Aquaculture Principles and Practices*, 2nd edn. Willey- Blackwell Publishing Limited, Daryaganz, India.

Postma PR, Miron TL, Olivieri G, Barbosa MJ, Wijffels RH, Eppink MH (2015) Mild disintegration of the green microalgae *Chlorella vulgaris* using bead milling. *Bioresource Technology* **184**: 297–304.

Postma PR, Suarez-Garcia E, Safi C, Yonathan K, Olivieri G, Barbosa MJ *et al.* (2017) Energy efficient bead milling of microalgae: effect of bead size on disintegration and release of proteins and carbohydrates. *Bioresource Technology* **224**: 670–679.

Qin S, Liu GX, Hu ZY (2008) The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (Chlorophyceae). *Process Biochemistry* **43**: 795–802.

Rahman M, Khosravi S, Chang KH, Lee S (2016) Effects of dietary inclusion of astaxanthin on growth, muscle pigmentation and antioxidant capacity of juvenile rainbow trout (*Oncorhynchus mykiss*). *Preventive Nutrition and Food Science* **21**: 281–288.

Rajasingh H, Oyehaug L, Vage D, Omholt S (2006) Carotenoids dynamics in Atlantic salmon. *BMC Biology* **4**: 1–15.

Raman R, Mohamad SE (2012) Astaxanthin production by freshwater microalgae *Chlorella sorokiniana* and marine microalgae *Tetraselmis* sp. *Pakistan Journal of Biological Sciences* **15**: 1182–1186.

Ranga Rao AR, Sarada R, Ravishankar GA (2007) Stabilization of astaxanthin in edible oils and its use as an antioxidant. *Journal of the Science of Food and Agriculture* **87**: 957–965.

Ranga Rao AR, Raghunath Reddy RL, Sarada R, Baskaran V, Ravishankar GA (2010) Characterization of microalgal carotenoids by mass spectrometry and their bioavailability and antioxidant properties in rat model. *Journal of Agricultural and Food Chemistry* **58**: 8553–8559.

Raposo MF, Morais AM, Morais RM (2012) Effects of spray-drying and storage on astaxanthin content of *Haematococcus pluvialis* biomass. *World Journal of Microbiology and Biotechnology* **28**: 1253–1257.

Ratain MJ, Plunkett WK (2003) Principles of pharmacokinetics. In: Kufe DW, Pollock RE, Weichselbaum RR, Bast RC, Gansler TS, Holland JF, Frei E (eds) *Holland-Frei Cancer Medicine*, 2400 pp. BC Decker Publications, Hamilton.

Razon LF, Tan RR (2011) Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*. *Applied Energy* **88**: 3507–3514.

Ribeiro HS, Rico LG, Badolato GG, Schubert H (2005) Production of o/w emulsions containing astaxanthin by repeated premix membrane emulsification. *Journal of Food Science* **70**: E117–E123.

Riccioni G, Speranza L, Pesce M, Cusenza S, D'Orazio N, Glade M (2012) Novel phytonutrient contributors to antioxidant protection against cardiovascular disease. *Nutrition* **28**: 605–610.

Rodriguez-Saiz M, de la Fuente JL, Barredo JL (2010) *Xanthophyllomyces dendrorhous* for the industrial production of astaxanthin. *Applied Microbiology and Biotechnology* **88**: 645–658.

Ronnestad I, Helland S, Lie O (1998) Feeding Artemia to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods. *Aquaculture* **165**: 159–164.

Ruen-ngam D, Shotipruk A, Pavasant P (2010) Comparison of extraction methods for recovery of astaxanthin from *Haematococcus pluvialis*. *Separation Science and Technology* **46**: 64–70.

Rufer C, Moeseneder J, Briviba K, Rechkemmer G, Bub A (2006) Bioavailability of astaxanthin stereoisomers from wild (*Oncorhynchus* spp.) and aquacultured (*Salmo salar*) salmon in healthy men: a randomised, double-blind study. *British Journal of Nutrition* **99**: 1048–1054.

Ruiz-Garcia A, Bermejo M, Moss A, Casabo VG (2008) Pharmacokinetics in drug discovery. *Journal of Pharmaceutical Sciences* **97**: 654–690.

Saez PJ, Abdel-Aal EM, Bureau DP (2014) Feeding increasing level of corn gluten meal induces suboptimal muscle pigmentation of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Resources* **47**: 1972–1983.

Sajilata MG, Singhal RS, Kamat MY (2008) The carotenoid pigment zeaxanthin-a review. *Comprehensive Reviews in Food Science and Food Safety* **7**: 28–49.

Salunkhe DK, Bolin HR, Reddy NR (1991) *Storage, Processing and Nutritional Quality of Fruits and Vegetables*, pp. 115 145. CRC Press, Boca Raton, FL.

Salze G, Tocher DR, Roy WJ, Robertson DA (2005) Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research* **36**: 1488–1499.

Samuel FL, Sean O, Gerold LM (2003) Improved aqueous solubility of crystalline astaxanthin (3,3-dihydroxy-β, β'-carotene-4,4-dione) by Captisol (sulfobutyl ether β-cyclodextrin). *Journal of Pharmaceutical Sciences* **92**: 922–926.

Sanchez-Camargo A, Meireles M, Ferreira A, Saito E, Cabral A (2012) Extraction of ὼ-3 fatty acids and astaxanthin from Brazilian redspotted shrimp waste using supercritical $CO_2$+ethanol mixtures. *The Journal of Supercritical Fluids* **61**: 71–77.

Sarada R, Vidhyavathi R, Usha D, Ravishankar GA (2006) An efficient method for extraction of astaxanthin from green alga *Haematococcus pluvialis*. *Journal of Agricultural and Food Chemistry* **54**: 7585–7588.

Sato K, Naoki S, Shiho S, Yoshinori Y, Takashi Y, Tamio M (1998) Identification of principal pigments in *Haematococcus* algae color. *Journal of the Food Hygienic Society of Japan* **39**: 368–374.

Satoh A, Tsuji S, Okada Y, Murakami N, Urami M, Nakagawa K *et al.* (2009) Preliminary clinical evaluation of toxicity and efficacy of a new astaxanthin-rich *Haematococcus pluvialis* extract. *Journal of Clinical Biochemistry and Nutrition* **44**: 280–284.

Sawanboonchun J, Roy W, Robertson D, Bell J (2008) The impact of dietary supplementation with astaxanthin on egg quality in Atlantic cod broodstock (*Gadus morhua* L.). *Aquaculture* **283**: 97–101.

Schaich KM (2002) Free radical generation during extrusion: a critical contributor to texturization. *ACS Symposium Series* **807**: 35–48.

Schiedt K, Leuenberger FJ, Vecchi M, Glinz E (1985) Absorption, retention and metabolic transformations of carotenoids in rainbow trout, salmon and chicken. *Pure and Applied Chemistry* **57**: 685–692.

Scholthof KG (2007) The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology* **5**: 152–156.

Schwenzfeier A, Wierenga PA, Gruppen H (2011) Isolation and characterization of soluble protein from the green microalgae *Tetraselmis* sp. *Bioresource Technology* **102**: 9121–9127.

Sefc KM, Brown AC, Clotfelter ED (2014) Carotenoid-based coloration in cichlid fishes. *Comparative Biochemistry and Physiology. B, Molecular and Integrative Physiology* **173**: 42–51.

Segner H, Arend P, Von Poeppinghaussen K, Schmidt H (1989) The effect of feeding astaxanthin to *Oreochromis niloticus* and *Colisa labiosa* on the histology of the liver. *Aquaculture* **79**: 381–390.

Serebruany V, Malinin A, Goodin T, Pashkow F (2010) The in vitro effects of Xancor, a synthetic astaxanthine derivative, on hemostatic biomarkers in aspirin-naive and aspirin-treated subjects with multiple risk factors for vascular disease. *American Journal of Therapeutics* **17**: 125–132.

Shah M, Liang Y, Cheng J, Daroch M (2016) Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. *Frontiers in Plant Science* **7**: 1–28.

Shang M, Ding W, Zhao Y, Xu J, Zhao P, Li T *et al.* (2016) Enhanced astaxanthin production from *Haematococcus pluvialis* using butylated hydroxyanisole. *Journal of Biotechnology* **236**: 199–207.

Shete V, Quadro L (2013) Mammalian metabolism of β-carotene: gaps in knowledge. *Nutrients* **5**: 4849–4868.

Smith T (1998) Carotenoids and cancer: prevention and potential therapy. *British Journal of Biomedical Science* **55**: 268–275.

Solovchenko AE (2015) Recent breakthroughs in the biology of astaxanthin accumulation by microalgal cell. *Photosynthesis Research* **125**: 437–449.

Sommer TR, Potts WT, Morrissy NM (1991) Utilization of microalgal astaxanthin by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **94**: 79–88.

Sommer TR, D'Souza FM, Morrissy NM (1992) Pigmentation of adult rainbow trout, *Oncorhynchus mykiss*, using the green alga *Haematococcus pluvialis*. *Aquaculture* **106**: 63–74.

Spiller GA, Dewell A (2003) Safety of an astaxanthin-rich *Haematococcus pluvialis* algal extract: a randomized clinical trial. *Journal of Medicinal Food* **6**: 51–56.

Steven DM (1949) Studies on animal carotenoids: II. Carotenoids in the reproductive cycle of the brown trout. *Journal of Experimental Biology* **26**: 295–303.

Stewart J, Lignell A, Pettersson A, Elfving E, Soni M (2008) Safety assessment of astaxanthin-rich microalgae biomass: acute and subchronic toxicity studies in rats. *Food and Chemical Toxicology* **46**: 3030–3036.

Storebakken T, Choubert G (1991) Flesh pigmentation of rainbow trout fed astaxanthin or canthaxanthin at different feeding rates in freshwater and saltwater. *Aquaculture* **95**: 289–295.

Storebakken T, Goswami UC (1996) Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, *Salmo salar*. *Aquaculture* **146**: 147–153.

Storebakken T, No H (1992) Pigmentation of rainbow trout. *Aquaculture* **100**: 209–229.

Storebakken T, Sorensen M, Bjerkeng B, Hiu S (2004) Utilization of astaxanthin from red yeast, *Xanthophyllomyces dendrorhous*, in rainbow trout, *Oncorhynchus mykiss*: effects of enzymatic cell wall disruption and feed extrusion temperature. *Aquaculture* **236**: 391–403.

Suganuma K, Nakajima H, Ohtsuki M, Imokawa G (2010) Astaxanthin attenuates the UVA-induced up-regulation of matrix-metalloproteinase-1 and skin fibroblast elastase in human dermal fibroblasts. *Journal of Dermatological Science* **58**: 136–142.

Sun X, Chang Y, Ye Y, Ma Z, Liang Y, Li T *et al.* (2012) The effects of dietary pigments on the coloration of Japanese ornamental carp (koi, *Cyprinus carpio* L.). *Aquaculture* **342–343**: 62–68.

Supamattaya K, Kiriratnikom S, Boonyaratpalin M, Borowitzka L (2005) Effect of a *Dunaliella* extract on growth performance, health condition, immune response and disease resistance in black tiger shrimp (*Penaeus monodon*). *Aquaculture* **248**: 207–216.

Svensson PA, Pelabon C, Blount JD, Surai PF, Amundsen T (2006) Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens*, Gobiidae)? *Functional Ecology* **20**: 689–698.

Synowiecki J, Shahidi F, Penney RW (1993) Nutrient composition of meat and uptake of carotenoids by Arctic char (*Salvelinus alpinus*). *Journal of Aquatic Food Product Technology* **2**: 37–58.

Tachaprutinun A, Udomsup T, Luadthong C, Wanichwecharungruang S (2009) Preventing the thermal degradation of astaxanthin through nanoencapsulation. *International Journal of Pharmaceutics* **374**: 119–124.

Takahashi T, Sota T, Hori M (2013) Genetic basis of male colour dimorphism in a Lake Tanganyika cichlid fish. *Molecular Ecology* **22**: 3049–3060.

Tanaka Y, Katayama T, Simpson KL, Chichester CO (1976) The biosynthesis of astaxanthin-XX. The carotenoids in marine red fish and the metabolism of the carotenoids in sea bream, *Chrysophrys major Temminck* and *Schlegel*. *Bulletin of the Japanese Society of Scientific Fisheries* **42**: 1177–1182.

Tanaka T, Shnimizu M, Moriwaki H (2012) Cancer chemoprevention by carotenoids. *Molecules* **17**: 3202–3242.

Taucher J, Baer S, Schwerna P, Hofmann D, Hummer M, Buchholz R *et al.* (2016) Cell disruption and pressurized liquid extraction of carotenoids from microalgae. *Journal of Thermodynamics and Catalysis* **7**: 1–7.

Tejera N, Cejas J, Rodriguez C, Bjerkeng B, Jerez S, Bolanos A *et al.* (2007) Pigmentation, carotenoids, lipid peroxides and lipid composition of skin of red porgy (*Pagrus pagrus*) fed diets supplemented with different astaxanthin sources. *Aquaculture* **270**: 218–230.

Terao J (1989) Antioxidant activity of β-carotene-related carotenoids in solution. *Lipids* **24**: 659–661.

Thana P, Machmudah S, Goto M, Sasaki M, Pavasant P, Shotipruk A (2008) Response surface methodology to supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis*. *Bioresource Technology* **99**: 3110–3115.

Thyssandier V, Lyan B, Borel P (2001) Main factors governing the transfer of carotenoids from emulsion lipid droplets to micelles. *Biochimica et Biophysica Acta* **1533**: 285–292.

Thyssandier V, Choubert G, Grolier P, Borel P (2002) Carotenoids, mostly the xanthophylls, exchange between plasma lipoproteins. *International Journal for Vitamin and Nutrition Research* **72**: 300–308.

Tizkar B, Soudagar M, Bahmani M, Hosseini S, Chamani M (2013) The effects of dietary supplementation of astaxanthin and β-carotene on the reproductive performance and egg quality of female goldfish (*Carassius auratus*). *Caspian Journal of Environmental Sciences* **11**: 217–231.

Tizkar B, Seidavi A, Ponce-Palafox J, Pourashoori P (2014) The effect of astaxanthin on resistance of juvenile prawns *Macrobrachium nipponense* (Decapoda: Palaemonidae) to physical and chemical stress. *Revista de Biologia Tropical* **62**: 1331–1341.

Tizkar B, Kazemi R, Alipour A, Seidavi A, Naseralavi G, Ponce-Palafox J (2015) Effects of dietary supplementation with astaxanthin and β-carotene on the semen quality of goldfish (*Carassius auratus*). *Theriogenology* **84**: 1111–1117.

Tlusty M, Hyland C (2005) Astaxanthin deposition in the cuticle of juvenile American lobster (*Homarus americanus*): implications for phenotypic and genotypic coloration. *Marine Biology* **147**: 113–119.

Toews D, Hofmeister NR, Taylor SA (2017) The evolutions and genetics of carotenoid processing in animals. *Trends in Genetics* **33**: 171–182.

Tominaga K, Hongo N, Karato M, Yamashita E (2012) Cosmetic benefits of astaxanthin on human subjects. *Acta Biochimica Polonica* **59**: 43–47.

Torrissen OJ (1984) Pigmentation of salmonids-effect of carotenoids in eggs and start-feeding diet on survival and growth rate. *Aquaculture* **43**: 185–193.

Torrissen OJ (1985) Pigmentation of salmonids: factors affecting carotenoid deposition in rainbow trout (*Salmo gairdneri*). *Aquaculture* **46**: 133–142.

Torrissen OJ (1986) Pigmentation of salmonids-a comparison of astaxanthin and canthaxanthin as pigment sources for rainbow trout. *Aquaculture* **53**: 271–278.

Torrissen OJ (1989) Pigmentation of salmonids: Interactions of astaxanthin and canthaxanthin on pigment deposition in rainbow trout. *Aquaculture* **79**: 363–374.

Torrissen OJ (1990) Biological activities of carotenoids in fishes. The current status of fish nutrients in aquaculture. In: Takeda M, Watanabe T (eds) *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, pp. 387–399. Tokyo University of Fisheries, Tokyo.

Torrissen OJ (2000) Dietary delivery of carotenoids. In: Decker E, Faustman C, Lopez-Bote CJ (eds) *Antioxidants in Muscle Food*, pp. 289–313. John Wiley and Sons Inc., New York, NY.

Torrissen OJ, Ingebrigtsen K (1992) Tissue distribution of 14C-astaxanthin in the Atlantic salmon (*Salmo salar*). *Aquaculture* **108**: 381–386.

Torrissen OJ, Naevdal G (1984) Pigmentation of salmonids-genetic variation in carotenoid deposition in rainbow trout. *Aquaculture* **38**: 59–66.

Torrissen OJ, Hardy RW, Shearer KD (1989) Pigmentation of salmonids-carotenoid deposition and metabolism. *Critical Reviews in Aquatic Sciences* **1**: 209–225.

Torrissen OJ, Hardy RW, Shearer KD, Scott TM, Stone FE (1990) Effects of dietary canthaxanthin level and lipid level on apparent digestibility coefficients for canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **88**: 351–362.

Torrissen O, Christiansen R, Struksnaes G, Estermann R (1995) Astaxanthin deposition in the flesh of Atlantic Salmon, *Salmo salar* L., in relation to dietary astaxanthin concentration and feeding period. *Aquaculture Nutrition* **1**: 77–84.

Turujman SA, Wamer WG, Wei RR, Albert RH (1997) Rapid liquid chromatographic method to distinguish wild salmon from aquacultured salmon fed synthetic astaxanthin. *Journal of AOAC International* **80**: 622–632.

Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A (2010) Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy* **2**: 127–142.

Vassallo-Agius R, Watanabe T, Imaizumi H, Yamazaki T, Satoh S, Kiron V (2001) Effects of dry pellets containing astaxanthin and squid meal on the spawning performance of striped jack *Pseudocaranx dentex*. *Fisheries Science* **67**: 667–674.

Verakunpiriya V, Mushiake K, Kawano K, Watanabe T (1997) Supplemental effect of astaxanthin in broodstock diets on the quality of yellowtail eggs. *Fisheries Science* **63**: 816–823.

Villalobos-Castillejos F, Cerezal-Mezquita P, Jesus HD, Lourdes M, Barragan-Huerta BE (2013) Production and stability of water-dispersible astaxanthin oleoresin from *Phaffia rhodozyma*. *International Journal of Food Science and Technology* **48**: 1243–1251.

van Vliet T (1996) Absorption of β-carotene and other carotenoids in humans and animal models. *European Journal of Clinical Nutrition* **50**: 32–37.

Waagbo R, Sandnes K, Torrissen OJ, Sandvin A, Lie O (1993) Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed three levels of N-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chemistry* **46**: 361–366.

Wackerbarth H, Stoll T, Gebken S, Pelters C, Bindrich U (2009) Carotenoid protein interaction as an approach for the formulation of functional food emulsions. *Food Research International* **42**: 1254–1258.

Wade N, Budd A, Irvin S, Glencross B (2015a) The combined effects of diet, environment and genetics on pigmentation in the Giant Tiger Prawn, *Penaeus monodon*. *Aquaculture* **449**: 78–86.

Wade NW, Gabaudan J, Glencross BD (2015b) A review of carotenoid utilisation and function in crustacean aquaculture. *Reviews in Aquaculture* 1–16.

Wade NM, Cheers S, Bourne N, Irvin S, Blyth D, Glencross BD (2015c) Dietary astaxanthin levels affect colour, growth, carotenoid digestibility and the accumulation of specific carotenoid esters in the Giant Tiger Shrimp, *Penaeus monodon*. *Aquaculture Research* **48**: 395–406.

Wang Y, Peng J (2008) Growth associated biosynthesis of astaxanthin in heterotrophic *Chlorella zofingiensis* (Chlorophyta). *World Journal of Microbiology and Biotechnology* **24**: 1915–1922.

Wang L, Yang B, Yan B, Yao X (2012) Supercritical fluid extraction of astaxanthin from *Haematococcus pluvialis* and its antioxidant potential in sunflower oil. *Innovative Food Science and Emerging Technologies* **13**: 120–127.

Wang H, Dai A, Liu F, Guan Y (2015) Effects of dietary astaxanthin on the immune response, resistance to white spot syndrome virus and transcription of antioxidant enzyme genes in Pacific white shrimp *Litopenaeus vannamei*. *Iranian Journal of Fisheries Sciences* **14**: 699–718.

Wang N, Guan B, Kong Q, Sun H, Geng Z, Duan L (2016) Enhancement of astaxanthin production from *Haematococcus pluvialis* mutants by three-stage mutagenesis breeding. *Journal of Biotechnology* **236**: 71–77.

Watanabe T, Miki W (1993) Astaxanthin: an effective dietary component for red sea bream broodstock. In: Kaushik SJ, Luquet P (eds) *Fish Nutrition in Practice*, pp. 27–36. INRA, Paris.

Wathne E, Bjerkeng B, Storebakken T, Vassvik V, Odland AB (1998) Pigmentation of Atlantic salmon (*Salmo salar*) fed astaxanthin in all meals or in alternating meals. *Aquaculture* **159**: 217–231.

Wayama M, Ota S, Matsuura H, Nango N, Hirata A, Kawano S (2013) Three dimensional ultrastructural study of oil and astaxanthin accumulation during encystment in the green alga *Haematococcus pluvialis*. *PLoS One* **8**: e53618.

White WS, Stacewicz-Sapuntzakis M, Erdman JW, Bowen PE (1994) Pharmacokinetics of β-carotene and canthaxanthin after ingestion of individual and combined doses by human subjects. *Journal of the American College of Nutrition* **13**: 665–671.

White DA, Moody AJ, Serwata RD, Bowen J, Soutar C, Young AJ *et al.* (2003) The degree of carotenoid esterification influences the absorption of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Nutrition* **9**: 247–251.

Withler RE (1986) Genetic variation in carotenoid pigment deposition in the red-fleshed and white-fleshed chinook salmon (*Oncorhynchus tshawytscha*) of the Quesnel River, British Columbia. *Canadian Journal of Genetic Cytology* **28**: 587–594.

Wouters R, Lavens P, Nieto J, Sorgeloos P (2001) Penaeid shrimp broodstock nutrition: an updated review on research and development. *Aquaculture* **202**: 1–21.

Xiao A, Ni H, Cai H, Li L, Su W, Yang Q (2009) An improved process for cell disruption and astaxanthin extraction from *Phaffia rhodozyma*. *World Journal of Microbiology and Biotechnology* **25**: 2029–2034.

Yamada S, Tanaka Y, Sameshima M, Ito Y (1990) Pigmentation of prawn (*Penaeus japonicus*) with carotenoids. 1. Effect of dietary astaxanthin, β-carotene and canthaxanthin on pigmentation. *Aquaculture* **87**: 323–330.

Yamaoka Y (2008) Microorganism and production of carotenoid compounds. *U.S. Patent* 20040253724 A1.

Yamashita E (2013) Astaxanthin as a medical food. *FFHD* **3**: 254–258.

Yamashita E (2015) Let astaxanthin be thy medicine. *PharmaNutrition* **3**: 115–122.

Yasir I, Qin J (2010) Effect of dietary carotenoids on skin colour and pigments of false clownfish, *Amphiprion ocellaris*, Cuvier. *Journal of the World Aquaculture Society* **41**: 308–318.

Yeum KJ, Russell RM (2002) Carotenoid bioavailability and bioconversion. *Annual Review of Nutrition* **22**: 483–504.

Yi X, Xu W, Zhou H, Zhang Y, Luo Y, Zhang W *et al.* (2014) Effects of dietary astaxanthin and xanthophylls on the growth and skin pigmentation of large yellow croaker *Larimichthys croceus*. *Aquaculture* **433**: 377–383.

Yokoyama A, Adachi K, Shizuri Y (1995) New carotenoid glucosides, astaxanthin glucoside and adonixanthin glucoside, isolated from the astaxanthin producing marine bacterium, *Agrobacterium aurantiacum*. *Journal of Natural Products* **58**: 1929–1933.

Yonekura L, Nagao A (2007) Intestinal absorption of dietary carotenoids. *Molecular Nutrition and Food Research* **51**: 107–115.

Ytrestoyl T, Bjerkeng B (2007a) Intraperitoneal and oral administration of astaxanthin in rainbow trout (*Oncorhynchus mykiss*)-plasma uptake and tissue distribution of geometrical E/Z isomers. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **147**: 250–259.

Ytrestoyl T, Bjerkeng B (2007b) Dose response in uptake and deposition of intraperitoneally administered astaxanthin in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.). *Aquaculture* **263**: 179–191.

Ytrestoyl T, Struksnæs G, Koppe W, Bjerkeng B (2005) Effects of temperature and feed intake on astaxanthin digestibility and metabolism in Atlantic salmon, *Salmo salar*. *Comparative Biochemistry and Physiology. B, Biochemistry and Molecular Biology* **142**: 445–455.

Yu BP (1994) Cellular defenses against damage from reactive oxygen species. *Physiological Reviews* **74**: 139–162.

Yuan JP, Chen F, Liu X, Li XZ (2002) Carotenoid composition in the green microalgae *Chlorococcum*. *Food Chemistry* **76**: 319–325.

Yuan J, Peng J, Yin K, Wang J (2011) Potential health-promoting effects of astaxanthin: a high value carotenoid mostly from microalgae. *Molecular Nutrition and Food Research* **55**: 150–165.

Yuan C, Du L, Jin Z, Xu X (2013) Storage stability and antioxidant activity of complex of astaxanthin with hydroxypropyl-β-cyclodextrin. *Carbohydrate Polymers* **91**: 385–389.

Zhang DH, Ng ML, Phang SM (1997) Composition and accumulation of secondary carotenoids in *Chlorococcum* sp. *Journal of Applied Phycology* **9**: 147–155.

Zhang J, Liu YJ, Tian LX, Yang HJ, Liang GY, Yue YR (2013) Effects of dietary astaxanthin on growth, antioxidant capacity and gene expression in Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition* **19**: 917–927.

Zhang W, Wang J, Wang J, Liu T (2014) Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. *Bioresource Technology* **158**: 329–335.

Zheng W, Liu G, Ao J, Chen X (2006) Expression analysis of immune relevant genes in the spleen of large yellow croaker (*Pseudosciaena crocea*) stimulated with poly I: C. *Fish and Shellfish Immunology* **21**: 414–430.

Zhu L, Nie L, Zhu G, Xiang L, Shao J (2013) Advances in research of fish immune-relevant genes: a comparative overview of innate and adaptive immunity in teleosts. *Developmental and Comparative Immunology* **39**: 39–62.

Zou TB, Jia Q, Li HW, Wang CX, Wu HF (2013) Response surface methodology for ultrasound-assisted extraction of astaxanthin from *Haematococcus pluvialis*. *Marine Drugs* **11**: 1644–1655.