A Review of Carotenoid Utilisation and Function in Crustacean Aquaculture

Nicholas M Wade[1*] Jacques Gabaudan[2] and Brett D Glencross[1].

1. CSIRO Food Futures Flagship, Division of Marine and Atmospheric Research, Ecosciences Precinct, Dutton Park, QLD 4102, Australia.
2. DSM Nutritional Products, Aquaculture Centre Asia Pacific, Bangkok, Thailand

* corresponding author nick.wade@csiro.au
Postal address: GPO Box 2583, Brisbane, QLD 4001, Australia
Phone +61 7 3833 5974

Abstract

Studies over a number of years have consistently shown that dietary carotenoid supplementation is beneficial for crustacean aquaculture across a range of commercially relevant parameters. Most obvious is the effect on pigmentation, where carotenoid inclusion levels in feeds and duration of feeding diets with carotenoids have been extensively optimised across many species to improve product colour, and subsequently quality and price. However, beneficial effects of carotenoid inclusion have increasingly been demonstrated on other parameters including survival, growth, reproductive capacity, disease resistance and stress resistance. A number of natural and synthetic carotenoid sources have been utilised in crustacean aquaculture. This review focuses on the type, metabolic conversion and function of carotenoids used in crustacean nutrition, and explores the physiological benefits this class of molecules brings to these animals.

Keywords:
Shrimp, color, astaxanthin

Reviewers:
Dr Bjorn Bjerkeng – bjorn.bjerkeng@nofima.no
Dr Jane Parisenti – janeparisenti@ibest.com.br
Dr. Jaime Eduardo Vernon Carter - jvc@xanum.uam.mx

1 Introduction

Carotenoids form the basis of the pigmentation of a wide variety of aquatic organisms (Matsuno, 2001, Britton and Goodwin, 1982, Maoka, 2011), and marine animals extensively utilise a variety of properties that carotenoids possess. Due to their diversity and broad distribution, carotenoid types, structure, metabolism and function have been extensively studied across a wide range of organisms (Britton *et al.*, 2008). Among those organisms studied, crustaceans utilise a range of different carotenoids that vary across species, within individual crustacean tissues or are dependent on various physiological, geographic or ecological parameters (Castillo *et al.*, 1982).

Very little attention has been paid to the specific effects of carotenoid supplementation in crustacean aquaculture, aside from the affect on pigmentation (Bjerkeng, 2008). Up until recently, the physiological effects beyond pigmentation have been inferred from other studies, mostly from fish. The present review summarises the recent progress in the use of carotenoids as a dietary nutrient in crustacean aquaculture, and outlines the effects of this dietary carotenoid supplementation on various aspects specific to crustacean physiology.

2 Carotenoids in Crustaceans

2.1 Tissue Distribution and Carotenoid Types

The majority of crustaceans and crustacean tissues attribute their colouration to the presence of various carotenoids. This topic has largely been covered extensively in the past (Castillo *et al.*, 1982, Lenel *et al.*, 1978) and is not the focus of this review. All wild and cultured crustacean species report the presence of free and esterified forms of various carotenoids, predominantly astaxanthin (Axn) (Castillo *et al.*, 1982, Lenel *et al.*, 1978, Tanaka *et al.*, 1976a). The distribution of these forms of carotenoids also varies with species, life history stages, developmental stage, moult stage and the organ or tissue of the animals (Ribeiro *et al.*, 2001, Lenel *et al.*, 1978, Sachindra *et al.*, 2005, Okada *et al.*, 1994, Pan and Chien, 2000, Dall, 1995, Petit *et al.*, 1998, Pan *et al.*, 1999, Valin *et al.*, 1987, Katayama *et al.*, 1971, Petit *et al.*, 1997). The esterification of Axn with specific fatty acids and the presence of carotenoid isomers can significantly increase the complexity of the interaction between the carotenoid and other biological molecules or membranes (Britton, 1995, Goodwin, 1986, Liaaen-Jensen, 1997). The accumulation of certain carotenoids in the tissues of different crustaceans not only indicates that these animals are able to interconvert one carotenoid to another, but also implies that there is a specific function for particular carotenoid in certain tissues.

2.2 Carotenoid Interconversion and Metabolism

Like most animals, crustaceans cannot synthesise carotenoids and must obtain them from their diets (Goodwin, 1952). However, for some time there has been strong evidence that various Decapod crustaceans can convert different dietary carotenoids (including canthaxanthin, lutein or zeaxanthin) into the predominant carotenoid Axn (Castillo and Lenel, 1978, Castillo *et al.*, 1980, Chien and Jeng, 1992, Kour and Subramoniam, 1992, Petit *et al.*, 1991, Yamada *et al.*, 1990, Castillo and Negre-Sadargues, 1995, Negre-Sadargues *et al.*, 1993, Mantiri *et al.*, 1995, Vernon-Carter *et al.*, 1996, D' Abrahmo *et al.*, 1983, Tanaka *et al.*, 1976b, Tanaka *et al.*, 1976a). Many years ago, the carotenoid metabolic transformative capacity of crustaceans was summarised (Negre-Sadargues, 1978, Schiedt *et al.*, 1993, Castillo *et al.*, 1982). The major pathway by which β-carotene is converted to Axn is summarised in Figure 1, although it would appear that crustaceans are able to perform a variety of other carotenoid transformations (Castillo *et al.*, 1982). Crustaceans fall into two broad classes based on their metabolic conversion capacity: those that can convert β-carotene to Axn in their internal organs, such as Penaeid shrimp; or those that can convert β-carotene to Axn in their internal organs but also convert metabolic intermediates in other tissues of their body, such as lobsters and crabs(Katayama *et al.*, 1973). Dietary paprika has been used as a source of carotenoids in *P. monodon* broodstock diets, suggesting that the carotenoids α-carotene, α-cryptoxanthin and capxanthin present in paprika were converted into Axn (Wyban *et al.*, 1997). Similarly, *M. japonicus* has been shown to produce Axn from α-carotene, canthaxanthin, echinenone or zeaxanthin (Chien and Jeng, 1992, Tanaka *et al.*, 1976b, Yamada *et al.*, 1990). Carotenoid metabolic capacity is active throughout crustacean early larval and post-larval development (Mantiri *et al.*, 1995, Mantiri *et al.*, 1996, Petit *et al.*, 1991, Berticat *et al.*, 2000), where the carotenoids may be metabolised as a source of retinoids (Dall, 1995). Yet despite the increase in genomic knowledge of crustaceans, including the sequencing of the complete genome of Daphnia, there has been very little progress in defining the biochemical pathways responsible carotenoid metabolism in this Class of animals. The variation in different carotenoid types across different developmental, physiological and ecological parameters strongly suggests that crustaceans utilise specific carotenoids for different functions during developmental processes or in response to environmental circumstances.

2.3 Carotenoid Sources in Crustacean Aquaculture

Sources of carotenoids that have been used in crustacean diets include synthetic carotenoids (Castillo and Negre-Sadargues, 1995, Chien and Jeng, 1992, Negre-Sadargues *et al.*, 1993), Antarctic krill (Maoka *et al.*, 1985), brine shrimp (Pan and Chien, 2003), shrimp by-products (Mandeville *et al.*, 1991, Chakrabarti, 2002, Meyers and Bligh, 1981), microalgae (Sommer *et al.*, 1991, Supamattaya *et al.*, 2005, Armenta-Lopez *et al.*, 2002, Chien and Jeng, 1992), blue green algae (Liao *et al.*, 1993, Okada *et al.*, 1991), and plant extracts (Vernon-Carter *et al.*, 1996, D' Abrahmo *et al.*, 1983, Arredondo-Figueroa *et al.*, 2003). More recently, other potential sources of carotenoids for crustacean aquaculture have been investigated, including genetic engineering of higher plants to accumulate high levels of ketocarotenoids such as Axn (Han *et al.*, 2013). Studies assessing the effect of different sources of carotenoids on pigmentation in crustaceans are summarised in Table 1.

3 Carotenoid Function in Crustaceans

Carotenoids are known to be involved in a large number of physiological functions in plants and animals, and these functions are largely based on the structure of the carotenoid (Britton, 2008, Goodwin, 1986). As the major carotenoid in crustacean tissues, Axn provides functions that include pigmentation, photoprotection, antioxidant and a source of provitamin A (Britton, 2008). Benefits to the animal include the enhancement of growth, higher survival, increased stress resistance and improved reproductive potential

(Kumar *et al.*, 2009, Supamattaya *et al.*, 2005, Niu *et al.*, 2014, Paibulkichakul *et al.*, 2008, Linan-Cabello *et al.*, 2002a). An example of these benefits was observed in crayfish exposed to pollution, which had lower levels of vitamins and carotenoids in the hepatopancreas, suggesting these may play a role in tolerating polluted environments (Barim and Karatepe, 2010). The conversion of carotenoids into other biologically active molecules, such as Provitamin A and retinoids has also been implicated (Linan-Cabello *et al.*, 2002a). Since the initial proposals of carotenoid function in crustaceans, there has been substantial progress in gathering scientific evidence to support the range of proposed functions of Axn and its effects on crustacean physiology, which will be discussed in further detail in the following sections.

3.1 Carotenoids and Crustacean Colouration

The best-established function of carotenoids in crustaceans is pigmentation. Colour plays a major role in consumer acceptability, perceived quality and price paid for commercial crustacean species (Parisenti *et al.*, 2011b, Shahidi *et al.*, 1998, Chien and Jeng, 1992, Erickson *et al.*, 2007). Many species of crustacean lose or do not develop pigmentation if not supplied a diet with sufficient carotenoids. Among these included hermit crabs (Castillo and Negre-Sadargues, 1995), red king crabs (Daly *et al.*, 2013), crayfish (Sommer *et al.*, 1991), clawed lobsters (Tlusty and Hyland, 2005), spiny lobsters (D' Abrahmo *et al.*, 1983, Barclay *et al.*, 2006), and shrimp (Dall, 1995). In shrimp, poor pigmentation was initially described as a disease status (Howell and Matthews, 1991), although this was subsequently shown to be ameliorated by dietary carotenoid supplementation (Menasveta *et al.*, 1993). Recently, pigmentation in banana shrimp has been shown to be heritable (Nguyen *et al.*, 2014), potentially through improvements in pigment retention. Crustacean colour variations have also been observed that are unrelated to dietary carotenoids. Indeed, much of the colour variation between species is thought to be attributable to differences in the sequence and expression pattern of pigment gene crustacyanin (Wade *et al.*, 2009), which will be discussed in more detail in later sections. Rare genetic colour mutations have been observed in clawed lobsters, predominantly Homarid species (Haggin, 2012), but also in prawns and crabs. The spiny lobster

Panulirus cygnus undergoes a colour change from deep red to pale pink during a migratory period (Phillips, 1983). This colour change has been attributed to a developmental ontogenic change that provides protective camouflage during migration, as it was not prevented by dietary carotenoid supplementation or triggered by background substrate colour (Wade *et al.*, 2008). In another example of colour variation, seasonal appearance of pink crab disease was shown to be caused by a parasitic infection (Stentiford *et al.*, 2002). Similarly, colour transitions have been observed between juvenile and adult stages of crabs (Krause-Nehring *et al.*, 2010).

3.1.1 Carotenoid Type, Inclusion Levels and Feed Duration

The majority of the focus of dietary carotenoid inclusion has been on the effects on crustacean pigmentation, having been studied over many years across a range of crustacean species. These studies have been summarised in Table 1. In general, pigment development is largely dependent on the amount of carotenoid in the feed and the duration for which it is fed. Dietary Axn concentrations between 50-100 mg/kg fed for one month were sufficient to produce optimal pigmentation in a range of shrimp species (Niu *et al.*, 2012, Niu *et al.*, 2014, Yamada *et al.*, 1990, Petit *et al.*, 1997). However, 80-100 mg/kg dietary Axn supplementation produced a darker external colour more rapidly, although similar pigmentation levels were achieved over a longer duration of feeding at 50 mg/kg (Chien and Jeng, 1992, Tlusty and Hyland, 2005, Barclay *et al.*, 2006). Pigmentation of red king crabs was also significantly improved over a 56 day period when diets were supplemented with 380 mg/kg Axn (Daly *et al.*, 2013), but no lower inclusion levels or shorter feeding periods were tested. There is clear evidence that as dietary carotenoid levels increase, so does the Axn content of the animal, particularly the Axn esters (Yamada *et al.*, 1990, Supamattaya *et al.*, 2005, Boonyaratpalin *et al.*, 2001, Barclay *et al.*, 2006, Kumar *et al.*, 2009, Wade *et al.*, 2008, Wade *et al.*, 2015b). In order to maintain initial carotenoid levels, spiny lobsters required 90 or 120 mg/kg dietary Axn (Barclay *et al.*, 2006). In some cases, the body concentration of carotenoids (mg/kg dry weight) decreased as shrimp grew (Pan *et al.*, 2001, Pan *et al.*, 1999), while in others the carotenoid concentration was maintained as the animals grew (Yamada *et al.*, 1990, Wade *et al.*, 2015b). Accordingly, some studies report that the whole body tissue Axn concentration is an appropriate indicator of body color of shrimp (Menasveta *et al.*, 1993, Negre-Sadargues *et al.*, 2000), while others suggest Axn concentration isn't necessarily reflective of body colour (Tume *et al.*, 2009). Clearly, further work is required to provide some clarity to the objectivity of this method of assessment.

The type of dietary carotenoid also affects the rate at which pigmentation is developed. Shrimp (*P. monodon*) fed dietary Axn at 100 mg/kg showed the highest levels of tissue Axn (16.5 mg/kg body weight) which was 23% and 43% higher than animals fed 100 mg/kg canthaxanthin or β-carotene, respectively (Yamada *et al.*, 1990). Pigmentation of juvenile Kuruma shrimp, *Marsupenaeus japonicus*, was better when animals were fed 100 mg/kg Axn for one month, compared with animals fed 50 mg/kg Axn or 20 – 200 mg/kg β-carotene (Chien and Jeng, 1992). A similar improved carotenoid tissue deposition was also observed in shrimp fed 100 mg/kg Axn, compared with either canthaxanthin or an Axn-canthaxanthin mixture (Negre-Sadargues *et al.*, 1993). For *P. monodon* to achieve a similar colour to that achieved using 50 mg/kg dietary Axn over 4 weeks, β-carotene was required at 125 mg/kg over 7-8 weeks, which was reduced to 5-6 weeks by using 175 mg/kg (Boonyaratpalin *et al.*, 2001). Shrimp fed a diet supplemented with *Artemia* nauplii (which were enriched with 80% canthaxanthin) for 4 weeks had improved deposition of free and esterified Axn compared with those fed a diet supplemented with mauxia shrimp (55% β-carotene) (Pan and Chien, 2003). Dietary supplementation of 200-300 mg/kg of the β-carotene enriched microalgal pigment from *Dunaliella* was required for optimal pigmentation in *Penaeus monodon* (Supamattaya *et al.*, 2005). These observations support that the efficiency with which carotenoid intermediates are converted to Axn depends on their position in the relevant metabolic conversion pathways. Dietary Axn levels greater than 200 mg/kg did not lead to improvements in pigmentation or tissue carotenoid accumulation (Yamada *et al.*, 1990, Merchie *et al.*, 1998), but other potential benefits of these high dietary carotenoid levels were not examined in these studies. Later sections of this review will explore further research in this area.

3.1.2 Chromatophores and Pigmentory Effectors

The colour of crustaceans is present in either the exoskeleton, or in pigment structures within the underlying hypodermal layer known as chromatophores (Rao, 1985). These structures are able to expand and contract, which strongly contributes to the degree of individual colouration, particularly for species with thin opaque shells like shrimp (Fingerman, 1965, Fingerman, 1966). Such physiological colour changes can be rapid, are reversible and often rhythmic in some species of crustaceans. This expansion and contraction is controlled by hormones secreted from glands in the eyestalks of crustaceans: pigment dispersing hormone (PDH) and red pigment concentrating hormone (RPCH), as a response to various physiological cues (Bagnara and Hadley, 1973, Rao, 2001). These cues can span aspects such as background colour, light source and photoperiod (Latscha, 1990, Rao, 1985).

Short-term exposure to black substrates has been shown to improve prawn pigmentation through expansion of hypodermal chromatophores (Parisenti *et al.*, 2011a, Tume *et al.*, 2009, Wade *et al.*, 2015a). An example of the effect that background exposure has on the chromatophores in shrimp epithelial tissue is shown in Figure 2. In addition to expanding and contracting, the chromatophores completely change their pigment content in response to different substrates. In response to dark backgrounds, animals with expanded chromatophores contained high levels of free Axn, while white adapted animals with contracted chromatophores contained high levels of Axn mono-esters (Tume *et al.*, 2009, Wade *et al.*, 2015b). This expansion was also shown to be linked with the accumulation of the colour protein crustacyanin in the hypodermal tissues (Wade *et al.*, 2012), presumably bound to free Axn to create the darker colouration. Tank colour was also shown to affect larval colour, survival and development in crabs (Rabbani and Zeng, 2005). When exposed to constant light, the body color of shrimp (*P. aztecus*) faded and chromatophores lost their diurnal rhythm (Lakshmi *et al.*, 1976). Similarly, the body color of *P. monodon* also became faint when cultured indoors under low light intensity less than 1000 lx (Tseng *et al.*, 1998). However, shrimp (*P. monodon*) subjected to constant light maintained higher carotenoid levels as they grew (Pan *et al.*, 2001). Without addition of Axn in diet, metal halide illumination at 2500 lux resulted in the significant accumulation of Axn in whole body of *L. vannamei* to over 4 mg/kg, compared with animals held in complete darkness at just over 2 mg/kg (You *et al.*, 2006).

Lastly, the colour of *P. monodon* has been observed to become redder when subjected to thermal and hypoxic stress, but this pigment effect was reversible when the stress was removed and hypoosmotic stress had no effect on colour (de la Vega *et al.*, 2007). Hypoxia was shown to increase the levels of CRCN-C1 abundance in the hepatopancreas of *Litopenaeus vannamei* (Jiang *et al.*, 2009), although why this may be occurring is not understood. Other reports of the effect of stress on pigmentation are largely anecdotal, and there is presently very little understanding of why this might be occurring.

3.1.3 Carotenoproteins and Crustacyanin

Carotenoids and associated carotenoprotein complexes have been found in many invertebrate species with tissue distribution ranging from the skin and gonads to the blood, eggs and shell (Zagalsky, 1985, Lakshman and Okoh, 1993, Cheesman *et al.*, 1967, Bhosale and Bernstein, 2007). Carotenoprotein complexes can be divided into two types: lipovitellins and true carotenoproteins. Lipovitellins possess a less stable and non-specific association of the carotenoid with the lipid portion of a lipoprotein and are responsible colouration of such tissues as the blood, epithelium, eggs and ovaries (Zagalsky, 1985, Cheesman *et al.*, 1967). True carotenoproteins display a highly specific and stoichiometric relationship between the carotenoid and a carotenoid binding protein (CBP), and appear to be particularly widespread among the animals in class *Crustacea* as the mechanism of shell colour production (Zagalsky, 1985, Lakshman and Okoh, 1993, Cheesman *et al.*, 1967).

Pigmentation in crustaceans is produced by a combination of the abundance and degree of expansion of different coloured chromatophores, yellow, blue and red (Rao, 1985), although visibility of chromatophores can be influenced by the thickness of the exoskeleton in some species. As noted earlier, dietary Axn supplementation increases the abundance of epithelial Axn, particularly Axn esters (Yamada *et al.*, 1990, Supamattaya *et al.*, 2005, Boonyaratpalin *et al.*, 2001, Barclay *et al.*, 2006, Kumar *et al.*, 2009, Wade *et al.*, 2015b). Similarly, background colour modifies pigment proportions in epithelial tissues, with contracted chromatophores containing high levels of carotenoid esters, and expanded chromatophores containing high levels of free Axn (Tume *et al.*, 2009, Wade *et al.*, 2015b, Wade *et al.*, 2015a) Within the exoskeleton and hypodermal tissue of crustaceans, free Axn is often bound within a multimeric protein complex called crustacyanin (CRCN) (Wald *et al.*, 1948). CRCN is a member of the lipocalin protein family, a functionally diverse group of proteins that bind small hydrophobic molecules such as steroid hormones, carotenoids, odourants and pheromones (Flower, 1996, Flower *et al.*, 2000). The interaction of CRCN and Axn modifies the naturally red carotenoid to blue or any other colour in the visible spectrum, producing the diverse array of colours seen in the exoskeleton of crustaceans (Cianci *et al.*, 2002). During cooking, this interaction is disrupted, releasing the distinct red colouration of cooked seafood. The dimeric β-crustacyanin (β-CRCN) is formed by two types of CRCN subunits (A and C, also called H_1 and H_2) in association with two Axn molecules (Cianci *et al.*, 2002). Eight of these dimers form a larger molecular weight complex known as α-crustacyanin (α-CRCN), which has been extensively studied using crystallographic techniques (reviewed in, (Chayen *et al.*, 2003, Zagalsky, 2003)).

At present, two genes that encode CRCN-A and CRCN-C have been identified across a range of crustaceans (Wade *et al.*, 2009, Ertl *et al.*, 2013, Wang *et al.*, 2007). Their expression is restricted to the outer layer of the hypodermis (Wade *et al.*, 2009, Wang *et al.*, 2007), and the spatial regulation of the *CRCN* genes is thought to define the species-specific shell colors and patterns that different crustaceans display (Wade *et al.*, 2009). In further support of this theory, reconstitution of recombinant CRCN monomers (either A or C) formed complexes with distinct absorption spectra, and the presence of CRCN in various species correlated with the ability to produce certain shell colours (Ferrari *et al.*, 2012).

The development of colour over time in pigment deficient clawed lobsters (*H. americanus*) was dependent on dietary carotenoid concentration, and progressed over three months through either a predominantly red or a predominantly blue phase before achieving a colour considered equivalent to those from the wild (Tlusty and Hyland, 2005). In freshwater shrimp (*M. rosenbergii*), external colour was removed by specific knockdown of a CRCN homolog using RNAi (Yang *et al.*, 2011). In this study, the blue pigment attributed to the Axn-CRCN interaction was removed by decreasing CRCN gene expression, and hence protein abundance, which modified the shrimp colour to red. Although not directly measured, the red colour that remained was likely the underlying red chromatophores containing predominantly Axn esters. This suggests that colour could be preferentially deposited in different chromatophores, although how this might be regulated is not understood.

Exposure to white substrates significantly decreased the amount of CRCN protein in shrimp hypodermal tissue, along with decreased free Axn levels and increased Axn ester levels (Wade *et al.*, 2012). Exposure to black substrates significantly increased the abundance of epithelial CRCN protein (Wade *et al.*, 2012), indicating the presence of this protein was critical to redistributing hypodermal pigments and achieving optimal cooked colour (Wade *et al.*, 2012). However, CRCN gene expression did not vary across the moult cycle or in response to substrate colour (Wade *et al.*, 2012). Albino colour morphs of shrimp (*F. merguiensis*) displayed significantly reduced expression of the CRCN-A and C genes compared with other shrimp, as well as a range of other genes potentially involved in the regulation of crustacean colour (Ertl *et al.*, 2013). However, expression levels of CRCN were not significantly different between light and dark coloured shrimp, and there was no correlation between levels of CRCN gene expression and Axn content (Ertl *et al.*, 2013). Despite extensive knowledge of the mechanism by which CRCN binds Axn to produce crustacean colour, there is very little known about how CRCN gene expression is regulated or how the CRCN protein complexes form or are modified in the crustacean exoskeleton.

3.2 Carotenoids and Growth and Survival

Reports of the effect of dietary carotenoid supplementation on growth and survival in crustaceans have been mixed, with virtually all research having been conducted on shrimp. Some studies reported no significant difference in growth in shrimp that had received dietary carotenoid supplementation (Pan *et al.*, 2001, Negre-Sadargues *et al.*, 1993, Boonyaratpalin *et al.*, 2001). However, an increasing number of studies have shown that either growth or survival, or both, are significantly improved when shrimp are fed a diet that contains carotenoids compared with diets that do not (Niu *et al.*, 2012, Niu *et al.*, 2014, Supamattaya *et al.*, 2005, Yamada *et al.*, 1990, Kumar *et al.*, 2009, Chien and Shiau, 2005, Petit *et al.*, 1997, Darachai *et al.*, 1998, Chien and Jeng, 1992, Flores *et al.*, 2007, Zhang *et al.*, 2013).

Early reports describing the beneficial effects of Axn on shrimp growth were assessed on postlarvae (Darachai *et al.*, 1998, Chien, 1996) with evidence that Axn supplementation shortened the moult frequency (Petit *et al.*, 1997). Larval stages and postlarvae of *P. monodon* showed greater survival and were longer when fed algal Axn (*Haematococcus pluvialis*) supplemented diets (Darachai *et al.*, 1998). Studies on *M. japonicus* juveniles demonstrated that growth performance was similar in shrimp over 8-weeks whether or not 100 mg/kg carotenoid was included (Yamada *et al.*, 1990). However, by the end of 8 weeks animals without dietary carotenoid contained significantly less total carotenoid than those fed 100 mg/kg, and their survival had dropped from 91.3% to 57.1% (Yamada *et al.*, 1990). In a separate experiment by the same authors but using smaller animals, animals that had received 100 mg/kg Axn for 8 weeks had grown significantly better than those that had not been fed Axn, while survival was unaffected (Yamada *et al.*, 1990). Between these two experiments, there was a marked difference in the total carotenoid content prawns at the beginning of the experiment, with poor survival over 8 weeks recorded when initial carotenoid content was low (15.6±0.8 mg/kg). Significant correlations have been observed between tissue carotenoid concentration and survival (Chien and Jeng, 1992) or specific growth rate (You *et al.*, 2006).

Since this initial work, the vast majority of studies have focussed on the giant tiger shrimp, *Penaeus monodon*. Shrimp fed 125-300 mg/kg of algal extract for 8-weeks showed higher weight gain and survival compared with controls (Supamattaya *et al.*, 2005). When fed with 100mg/kg Axn combined with 1% cholesterol for 74 days, shrimp showed higher weight gain and survival compared with those fed diets without carotenoids (Niu *et al.*, 2012), with apparent Axn digestibility of approximately 98%. In a similar study, shrimp fed 100 mg/kg Axn combined with 1% cholesterol also showed significantly higher weight gain and survival (Niu *et al.*, 2014), and showed similarly high (>90%) Axn digestibility. Although less studied, other species have shown a similar response. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6 weeks showed an increased daily growth coefficient and a reduced moult frequency compared with those animals that had not been fed dietary Axn, but survival was unaffected (Flores *et al.*, 2007). Shrimp (*L. vannamei*) fed either 100, 200 or 400 mg/kg Axn for 30 days showed improved weight gain and survival compared with those without dietary carotenoids (Niu *et al.*, 2009). After 56 days, shrimp (*L. vannamei*) fed 125 or 150 mg/kg Axn had higher weight gain than those fed 25, 50, 75 or 100 mg/kg Axn (Zhang *et al.*, 2013), but survival was unaffected. In freshwater *Macrobrachium*, inclusion of 50, 100 or 200 mg/kg Axn improved growth over the reference (Kumar *et al.*, 2009). Shrimp (*M. japonicus*) had improved survival from 37% to over 50% when fed diets containing carotenoids over 9 weeks, (Chien and Shiau, 2005), with a complementary increase in body Axn levels, but no effect on growth. Improved survival, but not growth, was also recorded in red king crab juveniles fed 380 mg/kg Axn for 56 days (Daly *et al.*, 2013).

Combined, these data suggest that survival is not affected when carotenoids are maintained at a certain level, perhaps between 10-15 mg/kg body weight for *P. monodon*, but survival is compromised below that level without carotenoid supplementation. Where tissue carotenoid levels are initially high, perhaps above 20 mg/kg, further carotenoid supplementation allows improved growth. Variability in animal performance in growth trials may be explained by a range of factors, including animal health, quality of feed ingredients, system design and animal husbandry. Detection of growth differences in shrimp fed dietary carotenoids in more recent studies may reflect improvements in trial maintenance and animal husbandry. The study by (Pan *et al.*, 2001) had shown there was no significant increase in survival in animals fed carotenoids compared with those that were not, although overall survival was less than <30% across the experiment, and this low level of survival casts aspersions on the validity of this work. Despite this, it was demonstrated that higher tissue carotenoid levels were correlated with higher survival (Pan *et al.*, 2001). Carotenoid levels in shrimp at the beginning of the study will also be critical, as carotenoid stores in animal tissues may compensate for the lack of dietary carotenoids at least through the initial stages of an experimental growth trial.

3.3 Carotenoids and Tolerance to Disease and Stress

This section will focus on studies where dietary carotenoids have been supplied, then the capacity to tolerate an induced stress has been directly tested under controlled conditions, and the effects on survival or other biochemical parameters assessed. The improved survival described in the previous section was reported after a period of 8-9 weeks of a growth feeding trial in experimental systems using different carotenoids (Axn, β-carotene or canthaxanthin). However, more recent studies have been designed to specifically assess whether responses to acute and chronic stresses, such as hypoxia, salinity or viral infection, are improved after long periods of dietary carotenoid supplementation. Analysis on shrimp (*F. chinensis*) showed that hypoxia alone triggered significant up-regulation of proteins involved in immunity (chymotrypsin and carboxypeptidase), and down regulation of proteins involved in energy production (citrate synthase, ATP synthase), metabolism (transketolase and esterases) and antioxidant capacity (glutathione peroxidase and cMnSOD) (Jiang *et al.*, 2009). Dietary levels of 125 and 150 mg/kg Axn fed to shrimp (*L. vannamei*) for 56 days lowered total antioxidant status, superoxide dismutase (SOD), and catalase activities than those animals fed 25, 50, 65 or 100 mg/kg (Zhang *et al.*, 2013). Carotenoids were found to be less abundant in the digestive gland and ovary of farmed *L. vannamei* compared with wild animals, and levels were concluded to be insufficient to neutralise oxidative stress during ovarian development (Linan-Cabello *et al.*, 2003). Crayfish exposed to pollution had lower levels of vitamins and carotenoids in the hepatopancreas, suggesting these may play a role in tolerating polluted environments (Barim and Karatepe, 2010).

Similar to growth and survival, the majority of work on tolerance to stress has been performed on shrimp. Early studies showed that larval stages of *P. monodon* supplemented with algal carotenoids were more resistant to low salinity stress than those with synthetic Axn or controls (Darachai *et al.*, 1998). Similarly, survival of *P. monodon* postlarvae during a low salinity stress test exposure to 4 hours of low dissolved oxygen (< 1.0 mg/L) was improved in shrimp (*P. monodon*) fed 360 mg/kg Axn for one week (Chien *et al.*, 1999). In a separate test, these shrimp were also shown to be more tolerant of lower oxygen levels in a lethal oxygen test (Chien *et al.*, 1999). Dietary Axn supplementation at 80 mg/kg enhanced antioxidant capacity in tiger shrimp (*P. monodon*) postlarvae, which resulted in a significant improvement in recovery to both thermal and osmotic stress (Chien *et al.*, 2003). In this study, higher body Axn levels were recorded, total antioxidant status (TAS) was reduced and superoxide dismutase (SOD) levels were reduced. The authors also speculated that hepatopancreas function was improved due to lower levels of aspartate aminotransferase (AST), a blood marker of liver integrity in mammalian systems, being identified in the circulating hemolymph. However, both AST and alanine aminotransferase (ALT) levels were reduced by thermal and osmotic stress, which was opposite to the expected effect of stress. The inclusion of 80 mg/kg in diets for 8-weeks improved shrimp (*P. monodon*) resistance to ammonia stress, and animals showed higher total antioxidant status and lower SOD levels (Pan *et al.*, 2003). AST and ALT levels were lowered by Axn supplementation, and were negatively correlated with TAS. However, aminotransferase levels were not correlated with survival, and may indicate that shrimp mortality was unrelated to hepatopancreas damage. When fed 300 mg/kg of algal carotenoids for 8 weeks, *P. monodon* showed improved tolerance to a nine day period of daily hypoxic stress (<1.0 mg/L) and also higher resistance to WSSV infection (Supamattaya *et al.*, 2005).

Studies in other shrimp also showed similar effects. In *M. japonicus*, inclusion of at least 50 mg/kg dietary Axn, from either synthetic or algal sources, resulted in improved survival to low oxygen stress (Chien and Shiau, 2005). Significantly greater levels of Axn had accumulated during the 9-week feeding trial, along with a reduced oxygen consumption rate, suggesting that Axn may be acting as an intracellular oxygen reserve or as a potent cellular antioxidant. Total carotenoid levels were highest in animals that showed the highest survival, yet total hemocyte count was lower and hemolymph phenoloxidase activity was unchanged. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6-weeks showed significantly higher osmoregulatory capacity than those without dietary Axn after salinity was reduced from 35 to 3 gL^{-1} (Flores *et al.*, 2007). This was coupled with significantly increased levels of hemocytes, hemocyanin and glucose in the hemolymph, and reduced levels of hemolymph lactate (Flores *et al.*, 2007). In a hypoxia stress test, postlarval shrimp (*L. vannamei*) fed either 200 or 400 mg/kg Axn recorded significantly higher survival, but no other physiological parameters were measured (Niu *et al.*, 2009). More recently, freshwater prawns showed a significant increase in phenoloxidase activity and total hemocyte count after 28 days of consuming carotenoid fortified diets (Kumar *et al.*, 2009), although no direct stress test was performed on the animals in this study. Systemic injection of Axn into the same species caused an increase in the total hemocyte count and an increased resistance to bacterial infection, although there was no complementary increase in antioxidant indicators (Angeles *et al.*, 2009). After low dissolved oxygen challenge, shrimp (*L. vannamei*) fed 75-150 mg/kg Axn for 56 days had higher survival than those animals fed 25 or 50 mg/kg Axn, and this was potentially linked with higher expression of hypoxia inducible factor 1 alpha (HIF-1α), cytosolic manganese superoxide dismutase (cMnSOD) and catalase in Axn fed animals (Zhang *et al.*, 2013). After 74 days feeding 100 mg/kg Axn or 250 mg/kg β-carotene, improved growth performance and survival in juvenile *P. monodon* was coupled with lower malondialdehyde levels (an indicator of lipid peroxidation) after a simulated live transport test (Niu *et al.*, 2014). In addition, expression levels of heat shock protein 70 (Hsp-70) were significantly elevated under hypoxia compared with normoxia, and further up-regulated under hypoxic conditions without dietary carotenoids (Niu *et al.*, 2014). Although counter-intuitive, the expression of hypoxia inducible factor 1 alpha (HIF-1α) was decreased under hypoxic condition, but were higher in animals fed β-carotene suggesting that the response to hypoxia had been alleviated (Zhang *et al.*, 2013, Niu *et al.*, 2014).

In summary, data consistently demonstrate that dietary carotenoids increase the total antioxidant capacity in the haemolymph of crustaceans, coupled with decreased activity of other antioxidant enzymes. This may occur through increased Axn levels in the haemolymph and tissues, improved oxygen carrying capacity, decreased oxidation of polyunsaturated fatty acids or cellular proteins or decreased activation of stress response systems. Combined, these data suggest that the stress response is reduced in animals receiving dietary carotenoids which improves survival to that stress, and that Axn is performing a broad protective role against the detrimental effects of oxidative damage in tissues. Similar to growth, many factors can affect survival in experimental systems, which is especially problematic when survival is a key measure of performance against stress. However, clear experimental evidence now exists to show that carotenoid supplementation improves a range of factors to enable crustaceans to tolerate stresses such as disease, hypoxia, temperature and salinity. These effects appear to link the proposed antioxidant function of carotenoids themselves, with physiological improvements in antioxidant capacity in the animals, and improved performance under various stressful conditions. Some inconsistency exists in the physiological responses of animals to dietary carotenoids, which may highlight differences in the way different crustaceans deal with a variety of stressors.

3.4 Carotenoids and Reproductive Performance

Nutrition plays a critical role in the reproductive success of crustaceans, and the accumulation of nutrients in the developing ovaries, particularly lipids and carotenoids, has a direct effect on reproductive measures such as egg number, hatching rate and total nauplii produced (Wouters *et al.*, 2001). Very little progress has been made in understanding the basis by which dietary carotenoids improve crustacean reproduction since it was summarised more than ten years ago (Linan-Cabello *et al.*, 2002a). During early maturation, carotenoids accumulate in the hepatopancreas in both free and esterified form, after which they are transported via the haemolymph to the ovaries during secondary vitellogenesis (Harrison, 1990, Vincent *et al.*, 1988). Carotenoid content and type varies greatly during ovarian development (Dall *et al.*, 1995, Linan-Cabello *et al.*, 2002b, Linan-Cabello *et al.*, 2003, Vincent *et al.*, 1988, Vincent *et al.*, 1989). The darkening that occurs with this accumulation forms the basis of “staging” female ovaries during ovarian maturation (Wouters *et al.*, 2001). Free and esterified Axn is known to accumulate in the hepatopancreas during ovarian maturation, while levels in the integument remain relatively constant (Dall *et al.*, 1995). Captive shrimp contained less carotenoids, particularly in stage IV ovaries, than their wild caught counterparts (Linan-Cabello *et al.*, 2003), strongly suggesting that broodstock nutrition was deficient. Paprika as a source of dietary carotenoids (α-carotene, α-cryptoxanthin and capxanthin) was shown to improve nauplii quality in *P. monodon* broodstock (Wyban *et al.*, 1997), with the assumption that these carotenoids were able to be converted into Axn. Axn supplemented in broodstock diets for *Penaeus monodon* showed improved spawning and fecundity (Pangantihon-Kuhlmann *et al.*, 1998). In the only recent study, high levels of dietary fish oil and Axn have been linked to improved reproductive performance, as measured by egg and spermatozoa number, in *P. monodon* broodstock (Paibulkichakul *et al.*, 2008). As might be expected, increased dietary fish oil led to accumulation of polyunsaturated fatty acids (PUFAs) in hepatopancreas and ovary tissues, particularly 22:6n-3. However, extremely high levels of dietary Axn (300 mg/kg) also led to an accumulation of Axn along with these long chain PUFAs in ovary tissue (Paibulkichakul *et al.*, 2008). Increased focus may be required on the use of carotenoids in conjunction with other nutrients of reproductive significance, such as long chain PUFAs.

The positive effects of Axn can potentially be attributed its extremely high capacity to scavenge oxygen free radicals, and the prevention of peroxidation of PUFAs in tissues and diets (Britton, 2008, Miki, 1991). In various fish species, the accumulation of carotenoids in reproductive tissues through dietary carotenoid supplementation has been shown to improve a number of performance characteristics, such as egg number, egg quality and number of larvae (Bjerkeng, 2008). Oxygen free radicals have been shown to attack biomembrane lipids and proteins, leading to deterioration in egg quality (Bromage and Roberts, 1995). In crustaceans, in conjunction with a depletion of carotenoids in the hepatopancreas and ovary, an elevation of superoxide dismutase (SOD) activity was observed in the haemolymph of captive shrimp compared with wild shrimp (Linan-Cabello *et al.*, 2003). This was suggested to reflect the insufficient scavenger activity to neutralize oxidative stress processes during spawning. Normal developmental and physiological processes, such as ovarian development and reproduction, are also potential sources of oxygen free radicals.

Although not initially identified as necessary for embryonic development, carotenoids are lost from fish and crustacean embryos prior to the first feeding stages (Bjerkeng, 2008, Dall *et al.*, 1995). This implies the carotenoids present in eggs and pre-feeding embryos are metabolised into other colourless molecules, that in turn potentially perform biological functions. Axn has been proposed to be an important source of Provitamin A and retinoids in eggs and early embryos (Dall *et al.*, 1995, Linan-Cabello *et al.*, 2002a, Miki, 1991). Evidence from a number of different crustaceans suggests that the retinols and other retinoid derivatives play a critical role in developmental processes of crustaceans, including ovarian and larval development (Linan-Cabello *et al.*, 2002a). Crustaceans possess a number of retinoids and retinoic acid receptors in crustaceans and the enhancement of the ovarian development in shrimp suggests an important role of these metabolites in shrimp physiology for their successful aquaculture. Carotenoids are the sole source of retinoids in crustaceans, and their role as bioactive molecules may have been largely overlooked (Linan-Cabello *et al.*, 2002a).

4 Conclusion

Carotenoids are considered a semi-essential nutrient that promotes optimal survival and growth at low dietary inclusion levels, approximately 25 mg/kg dietary Axn. Studies demonstrate that some form of dietary carotenoid intake is required in order to maintain carotenoid levels over time as animals grow, whether that intake is from natural pond biota or formulated into feeds. This amount is estimated at 50 mg/kg dietary Axn to maintain between 20-25 mg/kg body weight Axn for juvenile *P. monodon*. Increasingly, evidence suggests that specific carotenoids accumulate in different crustacean tissues over various life history stages. At present this minimum body Axn level is poorly defined, but whole body Axn levels may improve survival and growth across various stages of commercial production.

Optimal shrimp pigmentation can be achieved within several weeks by including Axn in the diet at levels of between 50-100 mg/kg, which can be reduced by using higher dietary inclusion levels. At these and even higher inclusion levels, utilisation efficiency of dietary carotenoids is extremely high and often exceeds 90%. In Penaeid shrimp, the amount of carotenoid required to be deposited in the tissues to achieve optimal colour is around 30-50 mg/kg body weight. However, this amount does not result in the same overall colour of different species, i.e. *P. monodon* is darker than *L. vannamei* at the same body Axn level. In other crustaceans, this body Axn level may need to be significantly higher. Background colour and light intensity are highly effective at redistributing carotenoid pigments, both to make shrimp darker or lighter in colour. Optimal pigmentation can lead to substantially higher sale prices, but there can be a preference for either darker or lighter shrimp depending on the target market.

Although presently poorly defined, the carotenoid levels required to elicit the physiological improvements in disease resistance, hypoxia or reproductive performance may be considerably higher than those for pigmentation. These beneficial effects have been demonstrated on various physiological characteristics such as survival, growth and resistance to stress. However, unlike colour, accurate measurement of these effects is often difficult due to a range of external factors. Improvements in research methods and techniques have led to a stronger understanding of the physiological mechanisms underlying carotenoid function in crustaceans. Very little is known about the genetic mechanisms that underlie the absorption, transport, tissue accumulation or metabolic transformations of carotenoids in any animal species. It is reasonable to assume that the accumulation of these carotenoids underpins the physiological changes that lead to improved performance of a variety of commercially relevant traits in aquaculture. More detailed studies are required to define the basis of the benefits of carotenoids in crustacean aquaculture. Although some functions of carotenoids may be preserved, we cannot continue to rely on research from vertebrate systems to draw conclusions on their effect in crustaceans.

5 References

Angeles, I.P., Chien, Y.H. & Tayamen, M.M. (2009) Effects of different dosages of astaxanthin on giant freshwater prawn Macrobrachium rosenbergii (De Man) challenged with *Lactococcus garvieae*. *Aquaculture Research,* **41,** 70-77.

Armenta-Lopez, R., Guerrero, I. & Huerta, S. (2002) Astaxanthin extraction from shrimp waste by lactic fermentation and enzymatic hydrolysis of the carotenoprotein complex. *J. Food Sci.,* **67,** 1002-1006.

Arredondo-Figueroa, J.L., Pedro-Islas, R., Ponce-Palafox, J.T. & Vernon-Carter, E.J. (2003) Pigmentation Of Pacific White Shrimp (*Litopenaeus vannamei,* Boone 1931) With Esterified And Saponified Carotenoids From Red Chili (*Capsicum annuum*) In Comparison To Astaxanthin. *Revista Mexicana de Ingenieria Quimica,* **2,** 101-108.

Bagnara, J.T. & Hadley, M.E. (1973) *Chromatophores and color change : the comparative physiology of animal pigmentation,* Prentice-Hall, Englewood Cliffs, N.J :.

Barclay, M.C., Irvin, S.J., Williams, K.C. & Smith, D.M. (2006) Comparison of diets for the tropical spiny lobster Panulirus ornatus: astaxanthin-supplemented feeds and mussel flesh. *Aquaculture Nutrition,* **12,** 117-125.

Barim, O. & Karatepe, M. (2010) The effects of pollution on the vitamins A, E, C, beta-carotene contents and oxidative stress of the freshwater crayfish, Astacus leptodactylus. *Ecotoxicology and environmental safety,* **73,** 138-142.

Berticat, O., Negre-Sadargues, G. & Castillo, R. (2000) The metabolism of astaxanthin during the embryonic development of the crayfish *Astacus leptodactylus* Eschscholtz (Crustacea, Astacidea). *Comp Biochem Physiol,* **127B,** 309-318.

Bhosale, P. & Bernstein, P.S. (2007) Vertebrate and invertebrate carotenoid-binding proteins. *Archives of Biochemistry and Biophysics,* **458,** 121-127.

Bjerkeng, B. (2008) Carotenoids in Aquaculture: Fish and Crustaceans In *Carotenoids. Volume 4: natural functions* (Britton, G.*, et al.* eds.), pp. 237-254. Birkhauser Verlag AG, Basel.

Boonyaratpalin, M., Thongrod, S., Supamattaya, K., Britton, G. & Schlipalius, L.E. (2001) Effects of ß-carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. *Aquaculture Research,* **32,** 182-190.

Britton, G. (1995) Structure and properties of carotenoids in relation to function. *Faseb J,* **9,** 1551-1558.

Britton, G. (2008) Functions of intact carotenoids In *Carotenoids. Volume 4: natural functions* (Britton, G*., et al.* eds.), pp. 189-212. Birkhauser Verlag AG, Basel.

Britton, G. & Goodwin, T.W. (1982) *Carotenoid Chemistry and Biochemistry,* Pergamon Press, Oxford.

Britton, G., Liaaen-Jensen, S. & Pfander, H. (2008) *Carotenoids Volume 4: natural functions,* Birkhauser Verlag AG, Basel.

Bromage, N.R. & Roberts, J.R. (1995) *Broodstock management and egg and larval quality.,* Wiley, Oxford, UK.

Castillo, R. & Lenel, R. (1978) Determination and Metabolism of Carotenoid Pigments in Hermit Crab *Clibanarius erythropus* Latreille (1818) [Syn *C.misanthropus* Heller (1863)]. *Comp Biochem Physiol,* **59B,** 67-73.

Castillo, R., Lenel, R. & Negre-Sadargues, G. (1980) Determination, Tissue Distribution and Metabolism of Carotenoid Pigments in the Hermit Crab *Pagurus prideauxi* Leach (1815) Decapoda, Anomoura. *Comp Biochem Physiol,* **66B,** 483-489.

Castillo, R. & Negre-Sadargues, G. (1995) Effect of Different Dietary Carotenoids on the Pigmented Pattern of the Hermit Crab *Clibanarius erythropus* Latreille (Crustacea, Decapoda). *Comp Biochem Physiol,* **111A,** 533-538.

Castillo, R., Negre-Sadargues, G. & Lenel, R. (1982) General Survey of the Carotenoids in Crustacea In *Carotenoid Chemistry and Biochemistry*, pp. 211-224. Pergamon Press, Oxford.

Chakrabarti, R. (2002) Carotenoprotein from tropical brown shrimp shell waste by enzymatic process. *Food Biotechnology,* **16,** 81-90.

Chayen, N.E., Cianci, M., Grossmann, J.G., Habash, J., Helliwell, J.R., Nneji, G.A., Raftery, J., Rizkallah, P.J. & Zagalsky, P.F. (2003) Unravelling the structural chemistry of the colouration mechanism in lobster shell. *Acta crystallographica. Section D, Biological crystallography,* **59,** 2072-2082.

Cheesman, D.F., Lee, W.L. & Zagalsky, P.F. (1967) Carotenoproteins in Invertebrates. *Biological Reviews of the Cambridge Philosophical Society,* **42,** 131-137.

Chien, Y.-H. & Jeng, S.C. (1992) Pigmentation of kuruma prawn, *Penaeus japonicus* Bate, by various pigment sources and levels and feeding regimes. *Aquaculture,* **102,** 333-346.

Chien, Y.-H., Pan, C.-H. & Hunter, B. (2003) The resistance to physical stresses by *Penaeus monodon* juveniles fed diets supplemented with astaxanthin. *Aquaculture,* **216,** 177-191.

Chien, Y.H. (1996) Biological effects of astaxanthin in shrimp, a review In *3rd Annual Roche Aquaculture Centre Conference on Nutrition and Disease*Bangkok, Thailand.

Chien, Y.H., Chen, I.M., Pan, C.H. & 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, Y.H. & Shiau, W.C. (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.

Cianci, M., Rizkallah, P.J., Olczak, A., Raftery, J., Chayen, N.E., Zagalsky, P.F. & Helliwell, J.R. (2002) The molecular basis of the coloration mechanism in lobster shell: beta-crustacyanin at 3.2-A resolution. *Proceedings of the National Academy of Sciences of the United States of America,* **99,** 9795-9800.

D' Abrahmo, L.R., Baum, N.A., Bordner, C.E. & Conklin, D.E. (1983) Carotenoids as a source of pigmentation in juvenile lobsters fed a purified diet. *Can J Fish Aquat Sci,* **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.M. & Moore, L.E. (1995) Carotenoids in the Tiger Prawn *Penaeus esculentus* During Ovarian Maturation. *Mar Biol,* **123,** 435-441.

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

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 *Advances in Shrimp Biotechnology, Proceedings to the Special Session on Shrimp Biotechnology, 5th Asian Fisheries Forum* (Flegel, T.W. ed. BIOTEC, Chiengmai, Thailand.

de la Vega, E., Hall, M.R., Wilson, K.J., Reverter, A., Woods, R.G. & Degnan, B.M. (2007) Stress-induced gene expression profiling in the black tiger shrimp *Penaeus monodon*. *Physiological genomics,* **31,** 126-138.

Erickson, M.C., Bulgarelil, M.A., Resurreccion, A.V.A., Vendetti, R.A. & Gates, K.A. (2007) Consumer Differentiation, Acceptance, and Demographic Patterns to Consumption of Six Varieties of Shrimp. *Journal of Aquatic Food Product Technology,* **15,** 35-51.

Ertl, N.G., Elizur, A., Brooks, P., Kuballa, A.V., Anderson, T.A. & Knibb, W.R. (2013) Molecular Characterisation of Colour Formation in the Prawn *Fenneropenaeus merguiensis*. *Plos One,* **8,** e56920.

Ferrari, M., Folli, C., Pincolini, E., McClintock, T.S., Rossle, M., Berni, R. & Cianci, M. (2012) Structural characterization of recombinant crustacyanin subunits from the lobster Homarus americanus. *Acta Crystallogr Sect F Struct Biol Cryst Commun,* **68,** 846-853.

Fingerman, M. (1965) Neurosecretory Control of Pigmentary Effectors in Crustaceans. *American Zoologist,* **5,** 675-703.

Fingerman, M. (1966) Neurosecretory Control of Pigmentary Effectors in Crustaceans. *American Zoologist,* **6,** 169-185.

Flores, M., Diaz, F., Medina, R., Re, A.D. & 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.

Flower, D.R. (1996) The Lipocalin Protein Family: structure and function. *Biochem J,* **318,** 1-14.

Flower, D.R., North, A.C. & Sansom, C.E. (2000) The lipocalin protein family: structural and sequence overview. *Biochim Biophys Acta,* **1482,** 9-24.

Goodwin, T.W. (1952) *The Comparative Biochemistry of Carotenoids,* Chapman and Hall Ltd, London.

Goodwin, T.W. (1986) Metabolism, nutrition, and function of carotenoids. *Annu Rev Nutr,* **6,** 273-297.

Haggin, P. (2012) Lobster: Now Available in Multiple Colors In *Time Magazine*. Time Magazine.

Han, D.X., Li, Y.T. & Hu, Q. (2013) Astaxanthin in microalgae: pathways, functions and biotechnological implications. *Algae,* **28,** 131-147.

Harrison, K.E. (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod

crustaceans: a review. *Journal of Shellfish Research,* **9,** 1-28.

Howell, B.K. & Matthews, A.D. (1991) The carotenoids of wild and blue disease affected farmed tiger shrimp (*Penaeus monodon*, Fabricus). *Comparative biochemistry and physiology. B, Comparative biochemistry,* **98,** 375-379.

Jiang, H., Li, F., Xie, Y., Huang, B., Zhang, J., Zhang, J., Zhang, C., Li, S. & Xiang, J. (2009) Comparative proteomic profiles of the hepatopancreas in *Fenneropenaeus chinensis* response to hypoxic stress. *PROTEOMICS,* **9,** 3353-3367.

Katayama, T., Hirata, K. & Chichester, C.O. (1971) The biosynthesis of astaxanthin-IV. The carotenoids in the prawn, *Penaeus japonicus* Bate (Part I). *Bull. Jpn. Soc. Sci. Fish.,* **37,** 614-620.

Katayama, T., Kunisaki, Y., Shimaya, M., Simpson, K.L. & Chichester, C.O. (1973) The biosynthesis of astaxanthin-XIV. The conversion of labelled β-carotene-15, 15′-3H2 into astaxanthin in the crab, *Portunus trituberculatus*. *Comp Biochem Phys B,* **46,** 269-272.

Kour, V.R.D. & Subramoniam, T. (1992) Carotenoid Metabolism during Embryonic Development of a Marine Crab, *Emerita asiatica* (Milne-Edwards). *Invertebrate Reproduction & Development,* **21,** 99-106.

Krause-Nehring, J., Matthias Starck, J. & Palmer, A.R. (2010) Juvenile colour polymorphism in the red rock crab, *Cancer productus*: patterns, causes, and possible adaptive significance. *Zoology (Jena, Germany),* **113,** 131-139.

Kumar, V., Pillai, B.R., Sahoo, P.K., 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,* **22,** 61-69.

Lakshman, M.R. & Okoh, C. (1993) Carotenoid-protein complexes. *Methods Enzymol,* **214,** 74-86.

Lakshmi, G.J., Venkataramiah, A. & Gunter, G. (1976) Effects of salinity and photoperiod on the burying behavior of brown shrimp *Penaeus aztecus* Ives. *Aquaculture,* **8,** 327-336.

Latscha, T. (1990) *Carotenoids: Their Nature and Significance in Animal Feeds,* F. Hoffman-La Roche, Animal Nutrition and Health, Basel, Switzerland.

Lenel, R., Negre-Sadargues, G. & Castillo, R. (1978) Carotenoid Pigments in Crustacea. *Arch Zool Exp Gen,* **119,** 297-334.

Liaaen-Jensen, S. (1997) Stereochemical aspects of carotenoids. *Pure and Applied Chemistry,* **69,** 2027-2038.

Liao, W.L., Nureborhan, S.A., Okada, S., Matsui, T. & Yamaguchi, K. (1993) Pigmentation of Cultured Black Tiger Prawn by Feeding with a Spirulina-Supplemented Diet. *Nippon Suisan Gakkaishi,* **59,** 165-169.

Linan-Cabello, M.A., Paniagua-Michel, J. & Hopkins, P.M. (2002a) Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition,* **8,** 299-309.

Linan-Cabello, M.A., Paniagua-Michel, J. & Zenteno-Savin, T. (2002b) Carotenoids and retinoids like regulators of oxidative stress during the gonadic maturation of *Litopenaus vannamei*. *Xi Biennial Meeting of the Society for Free Radical Research International***,** 607-610.

Linan-Cabello, M.A., Paniagua-Michel, J. & Zenteno-Savin, T. (2003) Carotenoids and retinal levels in captive and wild shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition,* **9,** 383-389.

Mandeville, S., Yaylayan, V., Simpson, B. & Ramaswamy, H. (1991) Isolation and Identification of Carotenoid Pigments, Lipids and Flavor Active Components from Raw Commercial Shrimp Waste. *Food Biotechnology,* **5,** 185-195.

Mantiri, D.M.H., Negre-Sadargues, G., Castillo, R. & Trilles, J.P. (1995) Evolution of Carotenoid Metabolic Capabilities During the Early Development of the

European Lobster *Homarus gammarus* (Linne, 1758). *Comp Biochem Physiol,* **111B,** 553-558.

Mantiri, D.M.H., Negre-Sadargues, G., Charmantier, G., Trilles, J.P., Milicua, J.C.G. & Castillo, R. (1996) Nature and metabolism of carotenoid pigments during the embryogenesis of the European lobster *Homarus gammarus* (Linne, 1758). *Comp Biochem Physiol,* **115A,** 237-241.

Maoka, T. (2011) Carotenoids in Marine Animals. *Marine Drugs,* **9,** 278-293.

Maoka, T., Katsuyama, M., Kaneko, N. & Matsuno, T. (1985) Stereochemical Investigation of Carotenoids in the Antarctic Krill *Euphausia superba*. *Bulletin of the Japanese Society of Scientific Fisheries,* **51,** 1671-1673.

Matsuno, T. (2001) Aquatic animal carotenoids. *Fisheries Science,* **67,** 771-783.

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

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.

Meyers, S.P. & Bligh, D. (1981) Characterization of astaxanthin pigments from heat-processed crawfish waste. *J Agric Food Chem,* **29,** 505-508.

Miki, W. (1991) Biological Functions and Activities of Animal Carotenoids. *Pure and Applied Chemistry,* **63,** 141-146.

Negre-Sadargues, G. (1978) Metabolic Transformations of Carotenoid Pigments in Crustacea Literature Survey. *Ann Biol-Paris,* **17,** 415-454.

Negre-Sadargues, G., Castillo, R., Petit, H., Sance, S., Martinez, R.G., Milicua, J., Choubert, G. & Trilles, J. (1993) Utilization of synthetic carotenoids by the prawn *Penaeus japonicus* reared under laboratory conditions. *Aquaculture,* **110,** 151-159.

Negre-Sadargues, G., Castillo, R. & Segonzac, M. (2000) Carotenoid pigments and trophic behaviour of deep-sea shrimps (Crustacea, decapoda, alvinocarididae) from a hydrothermal area of the mid-atlantic ridge. *Comp Biochem Physiol,* **127A,** 293-300.

Nguyen, N., Quinn, J., Powell, D., Elizur, A., Thoa, N., Nocillado, J., Lamont, R., Remilton, C. & Knibb, W. (2014) Heritability for body colour and its genetic association with morphometric traits in Banana shrimp (*Fenneropenaeus merguiensis*). *BMC Genet,* **15,** 132.

Niu, J., Li, C.-H., Liu, Y.-J., Tian, L.-X., Chen, X., Huang, Z. & Lin, H.-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., Tian, L.X., Liu, Y.J., Yang, H.J., Ye, C.X., Gao, W. & Mai, K.S. (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., Wen, H., Li, C.-H., Liu, Y.-J., Tian, L.-X., Chen, X., Huang, Z. & Lin, H.-Z. (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.

Okada, S., Liao, W.L., Mori, T., Yamaguchi, K. & Watanabe, T. (1991) Pigmentation of Cultured Striped Jack Reared on Diets Supplemented with the Blue-Green-Alga *Spirulina maxima*. *Nippon Suisan Gakkaishi,* **57,** 1403-1406.

Okada, S., Nur-E-Borhan, S.A. & Yamaguchi, K. (1994) Carotenoid Composition in the Exoskeleton of Commercial Black Tiger Prawns. *Fish. Sci.,* **60,** 213-215.

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.

Pan, C.H. & Chien, Y.H. (2000) Astaxanthin distribution in juvenile *Penaeus monodon* at various molting stages. *Journal of the Fisheries Society of Taiwan,* **27,** 33-43.

Pan, C.H., Chien, Y.H. & Cheng, J.H. (1999) Carotenoid content in various tissues of cultured *Penaeus monodon* by their sizes, sexes and molting stages. *Journal of the Fisheries Society of Taiwan,* **26,** 51-57.

Pan, C.H., Chien, Y.H. & 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, C.H. & Chien, Y.H. (2003) Concentration and composition of astaxanthin in black tiger prawn *Penaeus monodon* postlarvae fed Artemia sp nauplii or mauxia shrimp Acetes intermedius. *Journal of the World Aquaculture Society,* **34,** 57-65.

Pan, C.H., Chien, Y.H. & Cheng, J.H. (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. *Zool. Stud.,* **40,** 371-382.

Pangantihon-Kuhlmann, M.P., 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.

Parisenti, J., Beirao, L.H., Mourino, J.L., Vieira, F., Buglione, C.C. & Maraschim, M. (2011a) Effect of Background Color on Shrimp Pigmentation. *Biol Inst Pesca,* **37,** 177-182.

Parisenti, J., Beirão, L.H., Tramonte, V.L.C.G., Ourique, F., da Silveira Brito, C.C. & Moreira, C.C. (2011b) Preference ranking of colour in raw and cooked shrimps. *International Journal of Food Science & Technology,* **46,** 2558-2561.

Petit, H., Negre-Sadargues, G., Castillo, R. & Trilles, J.P. (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.

Petit, H., Negre-Sadargues, G., Castillo, R., Valin, S. & Trilles, J.P. (1998) The effects of dietary astaxanthin on the carotenoid pattern of the prawn *Penaeus japonicus* during postlarval development. *Comp Biochem Physiol,* **119A,** 523-527.

Petit, H., Sance, S., Negre-Sadargues, G., Castillo, R. & Trilles, J.P. (1991) Ontogeny of Carotenoid Metabolism in the Prawn *Penaeus japonicus Bate* (1888) (Crustacea, Penaeidea) - a Qualitative Approach. *Comp Biochem Physiol,* **99B,** 667-671.

Phillips, B.F. (1983) Migrations of Pre-Adult Western Rock Lobsters, *Panulirus cygnus*, in Western-Australia. *Marine Biology,* **76,** 311-318.

Rabbani, A. & Zeng, C. (2005) Effects of tank colour on larval survival and development of mud crab *Scylla serrata* (Forskal). *Aquaculture Research,* **36,** 1112-1119.

Rao, K.R. (1985) Pigmentary Effectors In *Integuments, Pigments and Hormonal Processes* (Bliss, D.E. & Mantel, L.H. eds.), Vol. 9, pp. 395-462. Academic Press, New York.

Rao, K.R. (2001) Crustacean Pigmentary-Effector Hormones: Chemistry and Functions of RPCH, PDH, and Related Peptides. *Integrative and Comparative Biology,* **41,** 364-379.

Ribeiro, E.A., Genofre, G.C. & McNamara, J.C. (2001) Identification and quantification of carotenoid pigments during the embryonic development of the freshwater shrimp *Macrobrachium olfersii* (Crustacea, Decapoda). *Mar. Freshw. Behav. Physiol.,* **34,** 105-116.

Sachindra, N.M., Bhaskar, N. & Mahendrakar, N.S. (2005) Carotenoids in different body components of Indian shrimps. *J. Sci. Food Agric.,* **85,** 167-172.

Schiedt, K., Bischof, S. & Glinz, E. (1993) Metabolism of Carotenoids and *In vivo* Racemization of (3s,3's)-Astaxanthin in the Crustacean *Penaeus*. *Methods in Enzymology,* **214,** 148-168.

Shahidi, F., Metusalach & Brown, J.A. (1998) Carotenoid pigments in seafoods and aquaculture. *Crit Rev Food Sci Nutr,* **38,** 1-67.

Sommer, T.R., Morrissey, N.M. & Potts, W., T. (1991) Growth and Pigmentation of Marron (*Cherax tenuimanus*) Fed a Reference Ration Supplemented with the Microalga Dunaliella-Salina. *Aquaculture,* **99,** 285-295.

Stentiford, G.D., Green, M., Bateman, K., Small, H.J., Neil, D.M. & Feist, S.W. (2002) Infection by a Hematodinium-like parasitic dinoflagellate causes Pink Crab Disease (PCD) in the edible crab *Cancer pagurus*. *Journal of Invertebrate Pathology,* **79,** 179-191.

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.

Tanaka, Y., Matsuguchi, H., Katayama, T., Simpson, K.L. & Chichester, C.O. (1976a) The biosynthesis of astaxanthin- XVI. The carotenoids in Crustacea. *Comp Biochem Physiol,* **54B,** 391-393.

Tanaka, Y., Matsuguchi, H., Katayama, T., Simpson, K.L. & Chichester, C.O. (1976b) The biosynthesis of astaxanthin- XVIII. The metabolism of the carotenoids in the prawn, *Penaeus japonicus* Bate. *Bull Jap Soc Scient Fish,* **42,** 197-202.

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.

Tseng, K.F., Su, H.M. & Su, M.S. (1998) Culture of Penaeus monodon in a recirculating system. *Aquacultural Engineering,* **17,** 138-147.

Tume, R.K., Sikes, A.L., Tabrett, S. & Smith, D.M. (2009) Effect of background colour on the distribution of astaxanthin in black tiger prawn (*Penaeus monodon*): Effective method for improvement of cooked colour. *Aquaculture,* **296,** 129-135.

Valin, A., Castillo, R., Negre-Sadargues, G. & Lenel, R. (1987) Quantitative Aspects of Carotenoid Pigment Circadian Variations in the Crayfish *Astacus leptodactylus*. *Biochem Syst Ecol,* **15,** 607-610.

Vernon-Carter, E.J., Ponce-Palafox, J.T. & Pedroza-Islas, R. (1996) Pigmentation of Pacific white shrimp (*Penaeus vannamei*) using Aztec marigold (*Tagetes erecta*) extracts as the carotenoid source. *Archivos latinoamericanos de nutrición,* **46,** 243-246.

Vincent, M., Ramos, L. & Oliva, L. (1988) Qualitative and quantitative variations in carotenoid pigments in the ovary and hepatopancreas of *Penaeus schmitti* during ovarian maturation. *Arch Int Physiol Biochim,* **96,** 155-164.

Vincent, M., Ramos, L. & Oliva, M. (1989) Variation in the composition of fatty acids of zeaxanthin and astaxanthin monoesters in the ovary and hepatopancreas of *Penaeus schmitti* during ovogenesis. *Arch Int Physiol Biochim,* **97,** 71-78.

Wade, N.M., Anderson, M., Sellars, M.J., Tume, R.K., Preston, N.P. & Glencross, B.D. (2012) Mechanisms of colour adaptation in the prawn *Penaeus monodon*. *Journal of Experimental Biology,* **215,** 343-350.

Wade, N.M., Budd, A., Irvin, S. & Glencross, B.D. (2015a) The Combined Effects of Diet, Environment and Genetics on Pigmentation in the Giant Tiger Prawn, *Penaeus monodon*. *Aquaculture,* **submitted**.

Wade, N.M., Cheers, S., Bourne, N., Irvin, S., Blyth, D. & Glencross, B.D. (2015b) Dietary astaxanthin levels affect growth, carotenoid digestibility and the deposition of specific carotenoid esters in the Giant Tiger Prawn, *Penaeus monodon*. *Aquaculture Nutrition,* **submitted**.

Wade, N.M., Melville-Smith, R., Degnan, B.M. & Hall, M.R. (2008) Control of shell colour changes in the lobster, *Panulirus cygnus*. *Journal of Experimental Biology,* **211,** 1512-1519.

Wade, N.M., Tollenaere, A., Hall, M.R. & Degnan, B.M. (2009) Evolution of a novel carotenoid-binding protein responsible for crustacean shell color. *Molecular Biology and Evolution,* **26,** 1851-1864.

Wald, G., Nathanson, N., Jencks, W.P. & Tarr, E. (1948) Crustacyanin, the blue carotenoprotein of the lobster shell. *Biological Bulletin,* **95,** 249-250.

Wang, M.-R., Zhu, X.-J., Yang, J.-S., Dai, Z.-M., Mahmood, K., Yang, F. & Yang, W.-J. (2007) Prawn lipocalin: characteristics and expressional pattern in subepidermal adipose tissue during reproductive molting cycle. *Comparative Biochemistry and Physiology, Part B,* **147,** 222-229.

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

Wyban, W., Martinez, G. & Sweeney, J. (1997) Adding paprika to *Penaeus vannamei* maturation diet improves nauplii quality. In *World Aquaculture*.

Yamada, S., Tanaka, Y., Sameshima, M. & Ito, Y. (1990) Pigmentation of Prawn (*Penaeus japonicus*) with Carotenoids .1. Effect of Dietary Astaxanthin, Beta-Carotene and Canthaxanthin on Pigmentation. *Aquaculture,* **87,** 323-330.

Yang, F., Wang, M.-R., Ma, Y.-G., Ma, W.-M. & Yang, W.-J. (2011) Prawn lipocalin: characterization of a color shift induced by gene knockdown and ligand binding assay. *Journal of experimental zoology. Part A, Ecological genetics and physiology*.

You, K., Yang, H.S., Liu, Y., Liu, S.L., Zhou, Y. & Zhang, T. (2006) Effects of different light sources and illumination methods on growth and body color of shrimp *Litopenaeus vannamei*. *Aquaculture,* **252,** 557-565.

Zagalsky, P.F. (1985) Invertebrate carotenoproteins. *Methods Enzymol,* **111,** 216-247.

Zagalsky, P.F. (2003) beta-Crustacyanin, the blue-purple carotenoprotein of lobster carapace: consideration of the bathochromic shift of the protein-bound astaxanthin. *Acta crystallographica. Section D, Biological crystallography,* **59,** 1529-1531.

Zhang, J., Liu, Y.J., Tian, L.X., Yang, H.J., Liang, G.Y., Yue, Y.R. & Xu, D.H. (2013) Effects of dietary astaxanthin on growth, antioxidant capacity and gene expression in Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition,* **19,** 917-927.

Figure 1. Schematic diagram of the major conversion pathway of β-carotene to astaxanthin in crustacean tissues.

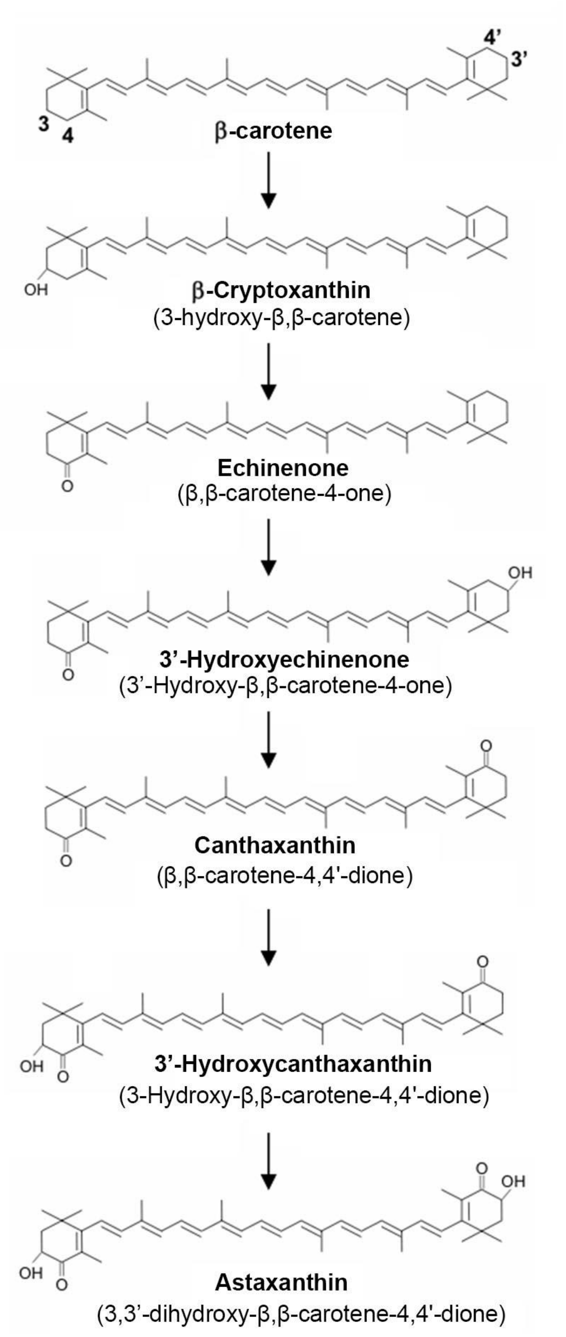

Figure 2 The response of crustacean abdominal epithelial chromatophores when exposed to black (A and C) or white (B and D) coloured substrates.

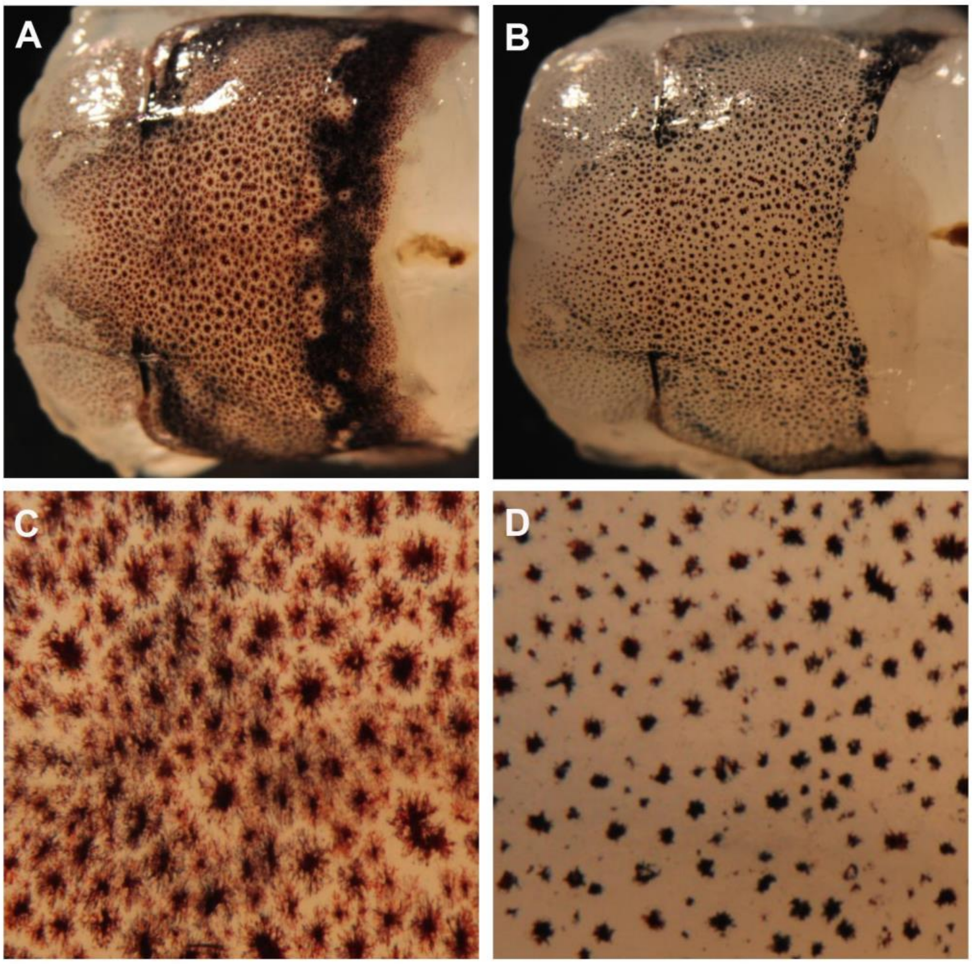

Table 1. Summary of carotenoid research in crustacean diets that improves pigmentation.

Reference	Inclusion range	Carotenoid	Source	Optimal Pigmentation
Giant Tiger Prawn (*Penaeus monodon*)				
(Yamada *et al.*, 1990)	0 – 400 mg/kg	Astaxanthin / β-carotene / Canthaxanthin	Synthetic	200 mg/kg Astaxanthin
(Liao *et al.*, 1993)	3%	β-carotene / Zeaxanthin	Spirulina / Krill Oil	3% Spirulina
(Menasveta *et al.*, 1993)	0 – 50 mg/kg	Astaxanthin	Synthetic	50 mg/kg
(Merchie *et al.*, 1998)	230 – 810 mg/kg	Astaxanthin	Synthetic	inconclusive
(Boonyaratpalin *et al.*, 2001)	125 – 175 mg/kg	β-carotene	Algal	125 mg/kg
(Supamattaya *et al.*, 2005)	125 – 300 mg/kg	β-carotene	Algal	200 – 300 mg/kg
(Niu *et al.*, 2012)	70 – 200 mg/kg	Astaxanthin / Canthaxanthin	Synthetic	100 mg/kg Astaxanthin + cholesterol
(Niu *et al.*, 2014)	100 – 250 mg/kg	Astaxanthin / β-carotene	Synthetic	100 mg/kg Astaxanthin + cholesterol
Pacific White Shrimp (*Litopenaeus vannamei*)				
(Vernon-Carter *et al.*, 1996)		Astaxanthin / Lutein	Synthetic / Marigold	Marigold
(Arredondo-Figueroa *et al.*, 2003)	200-250 mg/kg	Capsanthin	*Capsicum annuum*	
(Niu *et al.*, 2009)	0 – 400 mg/kg	Astaxanthin	Synthetic	100 - 200 mg/kg
(Ju *et al.*, 2011)	25 – 150 mg/kg	Astaxanthin	Algal and Synthetic	75 – 100 mg/kg

Kuruma Shrimp (*Marsupenaeus japonicus*)				
(Chien and Jeng, 1992)	50 – 200 mg/kg	Astaxanthin / β-carotene	Synthetic / algal	100 mg/kg Astaxanthin
(Negre-Sadargues *et al.*, 1993)	100 mg/kg	Astaxanthin/ Canthaxanthin	Synthetic	50 mg AX + 50 mg CX
(Petit *et al.*, 1997)	0 – 220 mg/kg	Astaxanthin/ Canthaxanthin	Synthetic / *Artemia*	60 mg/kg Astaxanthin
(Chien and Shiau, 2005)	0 – 100 mg/kg	Astaxanthin	Synthetic / algal	100 mg/kg
Giant Freshwater Prawn *Macrobrachium rosenbergii*)				
(Kumar *et al.*, 2009)	0 – 200 mg/kg	Astaxanthin	Synthetic	200 mg/kg
Hermit Crab (*Clibanarius erythropus*)				
(Castillo and Negre-Sadargues, 1995)	200 mg/kg	Astaxanthin / β-carotene / Canthaxanthin	Synthetic	200 mg/kg Astaxanthin
Red King Crab (*Paralithodes camtschaticus*)				
(Daly *et al.*, 2013)	0 – 380 mg/kg	Astaxanthin	Synthetic / algal	380 mg/kg
American Clawed Lobster (*Homarus americanus*)				
(Tlusty and Hyland, 2005)	0 – 220 mg/kg	Astaxanthin	Synthetic	220 mg/kg
Tropical Spiny Crayfish (*Panulirus ornatus*)				
(Barclay *et al.*, 2006)	30 – 120 mg/kg	Astaxanthin	Synthetic	120 mg/kg

Table 2. Summary of carotenoid research in crustacean diets that improves physiological performance.

Reference	Inclusion level	Species	Response
Growth and Survival			
(Yamada *et al.*, 1990)	100 mg/kg Axn	*M. japonicus*	Improved survival or growth
(Darachai *et al.*, 1998)	various	*P. monodon*	Improved post-larval survival
(Chien and Shiau, 2005)	50-100 mg/kg	*M. japonicus*	Improved survival
(Supamattaya *et al.*, 2005)	300 mg/kg β-carotene	*P. monodon*	Greater weight gain and improved survival
(Flores *et al.*, 2007)	80 mg/kg Axn	*L. vannamei*	Improved growth and moult frequency
(Kumar *et al.*, 2009)	50-200 mg/kg Axn	*M. rosenbergii*	Greater weight gain and improved survival
(Niu *et al.*, 2009)	100-400 mg/kg Axn	*L. vannamei*	Greater weight gain and improved survival
(Niu *et al.*, 2012)	100 mg/kg Axn + cholesterol	*P. monodon*	Greater weight gain and improved survival
(Daly *et al.*, 2013)	380 mg/kg	*Paralithodes camtschaticus*	Improved survival
(Zhang *et al.*, 2013)	125-150 mg/kg Axn	*L. vannamei*	Improved growth
(Niu *et al.*, 2014)	100 mg/kg Axn + cholesterol	*P. monodon*	Greater weight gain and improved survival
Tolerance to Disease and Stress			
(Darachai *et al.*, 1998)	various	*P. monodon*	Improved tolerance to low salinity
(Chien *et al.*, 1999)	360 mg/kg Axn	*P. monodon*	Improved survival to low dissolved oxygen
(Chien *et al.*, 2003)	80 mg/kg Axn	*P. monodon*	Improved recovery from thermal and osmotic stress, enhanced anti-oxidant capacity.

(Pan *et al.*, 2003)	80 mg/kg Axn	*P. monodon*	Improved resistance to ammonia stress, higher anti-oxidant status, lower SOD levels.
(Chien and Shiau, 2005)	50 mg/kg Axn	*M. japonicus*	Improved survival to low oxygen
(Supamattaya *et al.*, 2005)	300 mg/kg Axn	*P. monodon*	Improved survival to daily hypoxia stress, increased resistance to WSSV infection
(Flores *et al.*, 2007)	0-150 mg/kg Axn	*L. vannamei*	Improved tolerance to low salinity
(Niu *et al.*, 2009)	200-400 mg/kg Axn	*L. vannamei*	Improved survival to daily hypoxia stress
(Angeles *et al.*, 2009)	1.34 nmol g^{-1} Axn injected	*M. rosenbergii*	Improved survival to bacterial infection
(Zhang *et al.*, 2013)	75-150 mg/kg Axn	*L. vannamei*	Improved survival to hypoxia stress, increased HIF-1α, cMnSOD and catalase expression.
(Niu *et al.*, 2014)	100 mg/kg Axn 250 mg/kg β-carotene	*L. vannamei*	Improved survival in live transport test, reduced malondialdehyde and HSP-70 levels
Reproductive Performance			
(Wyban *et al.*, 1997)	Various	*L. vannamei*	Improved nauplii quality
(Pangantihon-Kuhlmann *et al.*, 1998)	100 mg/kg Axn	*P. monodon*	Improved spawning and fecundity
(Paibulkichakul *et al.*, 2008)	50-300 mg/kg Axn	*P. monodon*	Increased number of eggs and spermatozoa, accumulation of Axn in ovary tissue