

This is the peer reviewed version of the following article: Wade, N. M., Gabaudan, J. and Glencross, B. D. (2017), A review of carotenoid utilisation and function in crustacean aquaculture. *Rev Aquacult*, 9: 141–156, which has been published in final form at <https://doi.org/10.1111/raq.12109>. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

1 **A Review of Carotenoid Utilisation and Function in Crustacean Aquaculture**

2

3 Nicholas M Wade^{1*} Jacques Gabaudan² and Brett D Glencross¹.

4

5 1. CSIRO Food Futures Flagship, Division of Marine and Atmospheric Research, Ecosciences Precinct, Dutton
6 Park, QLD 4102, Australia.

7 2. DSM Nutritional Products, Aquaculture Centre Asia Pacific, Bangkok, Thailand

8 * corresponding author nick.wade@csiro.au

9 Postal address: GPO Box 2583, Brisbane, QLD 4001, Australia

10 Phone +61 7 3833 5974

11

12 Abstract

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

26

27 Keywords:

28 Shrimp, color, astaxanthin

29

30 Reviewers:

31 Dr Bjorn Bjerken - bjorn.bjerkeng@nofima.no

32 Dr Jane Parisenti - janeparisenti@ibest.com.br

33 Dr. Jaime Eduardo Vernon Carter - jvc@xanum.uam.mx

34 **1 Introduction**

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

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

52 **2 Carotenoids in Crustaceans**

53 *2.1 Tissue Distribution and Carotenoid Types*

54 The majority of crustaceans and crustacean tissues attribute their colouration to
55 the presence of various carotenoids. This topic has largely been covered
56 extensively in the past (Castillo *et al.*, 1982, Lenel *et al.*, 1978) and is not the
57 focus of this review. All wild and cultured crustacean species report the presence
58 of free and esterified forms of various carotenoids, predominantly astaxanthin
59 (Axn) (Castillo *et al.*, 1982, Lenel *et al.*, 1978, Tanaka *et al.*, 1976a). The
60 distribution of these forms of carotenoids also varies with species, life history
61 stages, developmental stage, moult stage and the organ or tissue of the animals
62 (Ribeiro *et al.*, 2001, Lenel *et al.*, 1978, Sachindra *et al.*, 2005, Okada *et al.*, 1994,
63 Pan and Chien, 2000, Dall, 1995, Petit *et al.*, 1998, Pan *et al.*, 1999, Valin *et al.*,
64 1987, Katayama *et al.*, 1971, Petit *et al.*, 1997). The esterification of Axn with
65 specific fatty acids and the presence of carotenoid isomers can significantly

66 increase the complexity of the interaction between the carotenoid and other
67 biological molecules or membranes (Britton, 1995, Goodwin, 1986, Liaaen-
68 Jensen, 1997). The accumulation of certain carotenoids in the tissues of different
69 crustaceans not only indicates that these animals are able to interconvert one
70 carotenoid to another, but also implies that there is a specific function for
71 particular carotenoid in certain tissues.

72

73 2.2 Carotenoid Interconversion and Metabolism

74 Like most animals, crustaceans cannot synthesise carotenoids and must obtain
75 them from their diets (Goodwin, 1952). However, for some time there has been
76 strong evidence that various Decapod crustaceans can convert different dietary
77 carotenoids (including canthaxanthin, lutein or zeaxanthin) into the
78 predominant carotenoid Axn (Castillo and Lenel, 1978, Castillo *et al.*, 1980, Chien
79 and Jeng, 1992, Kour and Subramoniam, 1992, Petit *et al.*, 1991, Yamada *et al.*,
80 1990, Castillo and Negre-Sadargues, 1995, Negre-Sadargues *et al.*, 1993, Mantiri
81 *et al.*, 1995, Vernon-Carter *et al.*, 1996, D' Abrahmo *et al.*, 1983, Tanaka *et al.*,
82 1976b, Tanaka *et al.*, 1976a). Many years ago, the carotenoid metabolic
83 transformative capacity of crustaceans was summarised (Negre-Sadargues,
84 1978, Schiedt *et al.*, 1993, Castillo *et al.*, 1982). The major pathway by which β -
85 carotene is converted to Axn is summarised in Figure 1, although it would appear
86 that crustaceans are able to perform a variety of other carotenoid
87 transformations (Castillo *et al.*, 1982). Crustaceans fall into two broad classes
88 based on their metabolic conversion capacity: those that can convert β -carotene
89 to Axn in their internal organs, such as Penaeid shrimp; or those that can convert
90 β -carotene to Axn in their internal organs but also convert metabolic
91 intermediates in other tissues of their body, such as lobsters and
92 crabs (Katayama *et al.*, 1973). Dietary paprika has been used as a source of
93 carotenoids in *P. monodon* broodstock diets, suggesting that the carotenoids α -
94 carotene, α -cryptoxanthin and capxanthin present in paprika were converted
95 into Axn (Wyban *et al.*, 1997). Similarly, *M. japonicus* has been shown to produce
96 Axn from α -carotene, canthaxanthin, echinenone or zeaxanthin (Chien and Jeng,
97 1992, Tanaka *et al.*, 1976b, Yamada *et al.*, 1990). Carotenoid metabolic capacity is
98 active throughout crustacean early larval and post-larval development (Mantiri

99 *et al.*, 1995, Mantiri *et al.*, 1996, Petit *et al.*, 1991, Berticat *et al.*, 2000), where the
100 carotenoids may be metabolised as a source of retinoids (Dall, 1995). Yet despite
101 the increase in genomic knowledge of crustaceans, including the sequencing of
102 the complete genome of *Daphnia*, there has been very little progress in defining
103 the biochemical pathways responsible carotenoid metabolism in this Class of
104 animals. The variation in different carotenoid types across different
105 developmental, physiological and ecological parameters strongly suggests that
106 crustaceans utilise specific carotenoids for different functions during
107 developmental processes or in response to environmental circumstances.

108

109 2.3 Carotenoid Sources in Crustacean Aquaculture

110 Sources of carotenoids that have been used in crustacean diets include synthetic
111 carotenoids (Castillo and Negre-Sadargues, 1995, Chien and Jeng, 1992, Negre-
112 Sadargues *et al.*, 1993), Antarctic krill (Maoka *et al.*, 1985), brine shrimp (Pan
113 and Chien, 2003), shrimp by-products (Mandeville *et al.*, 1991, Chakrabarti,
114 2002, Meyers and Bligh, 1981), microalgae (Sommer *et al.*, 1991, Supamattaya *et al.*
115 *et al.*, 2005, Armenta-Lopez *et al.*, 2002, Chien and Jeng, 1992), blue green algae
116 (Liao *et al.*, 1993, Okada *et al.*, 1991), and plant extracts (Vernon-Carter *et al.*,
117 1996, D' Abrahmo *et al.*, 1983, Arredondo-Figueroa *et al.*, 2003). More recently,
118 other potential sources of carotenoids for crustacean aquaculture have been
119 investigated, including genetic engineering of higher plants to accumulate high
120 levels of ketocarotenoids such as Axn (Han *et al.*, 2013). Studies assessing the
121 effect of different sources of carotenoids on pigmentation in crustaceans are
122 summarised in Table 1.

123 3 Carotenoid Function in Crustaceans

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

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

142

143 3.1 Carotenoids and Crustacean Colouration

144 The best-established function of carotenoids in crustaceans is pigmentation.
145 Colour plays a major role in consumer acceptability, perceived quality and price
146 paid for commercial crustacean species (Parisenti *et al.*, 2011b, Shahidi *et al.*,
147 1998, Chien and Jeng, 1992, Erickson *et al.*, 2007). Many species of crustacean
148 lose or do not develop pigmentation if not supplied a diet with sufficient
149 carotenoids. Among these included hermit crabs (Castillo and Negre-Sadargues,
150 1995), red king crabs (Daly *et al.*, 2013), crayfish (Sommer *et al.*, 1991), clawed
151 lobsters (Tlusty and Hyland, 2005), spiny lobsters (D' Abrahmo *et al.*, 1983,
152 Barclay *et al.*, 2006), and shrimp (Dall, 1995). In shrimp, poor pigmentation was
153 initially described as a disease status (Howell and Matthews, 1991), although
154 this was subsequently shown to be ameliorated by dietary carotenoid
155 supplementation (Menasveta *et al.*, 1993). Recently, pigmentation in banana
156 shrimp has been shown to be heritable (Nguyen *et al.*, 2014), potentially through
157 improvements in pigment retention. Crustacean colour variations have also been
158 observed that are unrelated to dietary carotenoids. Indeed, much of the colour
159 variation between species is thought to be attributable to differences in the
160 sequence and expression pattern of pigment gene crustacyanin (Wade *et al.*,
161 2009), which will be discussed in more detail in later sections. Rare genetic
162 colour mutations have been observed in clawed lobsters, predominantly
163 Homarid species (Haggin, 2012), but also in prawns and crabs. The spiny lobster

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

173

174 3.1.1 Carotenoid Type, Inclusion Levels and Feed Duration

175 The majority of the focus of dietary carotenoid inclusion has been on the effects
176 on crustacean pigmentation, having been studied over many years across a range
177 of crustacean species. These studies have been summarised in Table 1. In
178 general, pigment development is largely dependent on the amount of carotenoid
179 in the feed and the duration for which it is fed. Dietary Axn concentrations
180 between 50-100 mg/kg fed for one month were sufficient to produce optimal
181 pigmentation in a range of shrimp species (Niu *et al.*, 2012, Niu *et al.*, 2014,
182 Yamada *et al.*, 1990, Petit *et al.*, 1997). However, 80-100 mg/kg dietary Axn
183 supplementation produced a darker external colour more rapidly, although
184 similar pigmentation levels were achieved over a longer duration of feeding at 50
185 mg/kg (Chien and Jeng, 1992, Tlustý and Hyland, 2005, Barclay *et al.*, 2006).
186 Pigmentation of red king crabs was also significantly improved over a 56 day
187 period when diets were supplemented with 380 mg/kg Axn (Daly *et al.*, 2013),
188 but no lower inclusion levels or shorter feeding periods were tested. There is
189 clear evidence that as dietary carotenoid levels increase, so does the Axn content
190 of the animal, particularly the Axn esters (Yamada *et al.*, 1990, Supamattaya *et*
191 *al.*, 2005, Boonyaratpalin *et al.*, 2001, Barclay *et al.*, 2006, Kumar *et al.*, 2009,
192 Wade *et al.*, 2008, Wade *et al.*, 2015b). In order to maintain initial carotenoid
193 levels, spiny lobsters required 90 or 120 mg/kg dietary Axn (Barclay *et al.*,
194 2006). In some cases, the body concentration of carotenoids (mg/kg dry weight)
195 decreased as shrimp grew (Pan *et al.*, 2001, Pan *et al.*, 1999), while in others the
196 carotenoid concentration was maintained as the animals grew (Yamada *et al.*,

197 1990, Wade *et al.*, 2015b). Accordingly, some studies report that the whole body
198 tissue Axn concentration is an appropriate indicator of body color of shrimp
199 (Menasveta *et al.*, 1993, Negre-Sadargues *et al.*, 2000), while others suggest Axn
200 concentration isn't necessarily reflective of body colour (Tume *et al.*, 2009).
201 Clearly, further work is required to provide some clarity to the objectivity of this
202 method of assessment.

203 The type of dietary carotenoid also affects the rate at which pigmentation is
204 developed. Shrimp (*P. monodon*) fed dietary Axn at 100 mg/kg showed the
205 highest levels of tissue Axn (16.5 mg/kg body weight) which was 23% and 43%
206 higher than animals fed 100 mg/kg canthaxanthin or β -carotene, respectively
207 (Yamada *et al.*, 1990). Pigmentation of juvenile Kuruma shrimp, *Marsupenaeus*
208 *japonicus*, was better when animals were fed 100 mg/kg Axn for one month,
209 compared with animals fed 50 mg/kg Axn or 20 – 200 mg/kg β -carotene (Chien
210 and Jeng, 1992). A similar improved carotenoid tissue deposition was also
211 observed in shrimp fed 100 mg/kg Axn, compared with either canthaxanthin or
212 an Axn-canthaxanthin mixture (Negre-Sadargues *et al.*, 1993). For *P. monodon* to
213 achieve a similar colour to that achieved using 50 mg/kg dietary Axn over 4
214 weeks, β -carotene was required at 125 mg/kg over 7-8 weeks, which was
215 reduced to 5-6 weeks by using 175 mg/kg (Boonyaratpalin *et al.*, 2001). Shrimp
216 fed a diet supplemented with *Artemia* nauplii (which were enriched with 80%
217 canthaxanthin) for 4 weeks had improved deposition of free and esterified Axn
218 compared with those fed a diet supplemented with mauxia shrimp (55% β -
219 carotene) (Pan and Chien, 2003). Dietary supplementation of 200-300 mg/kg of
220 the β -carotene enriched microalgal pigment from *Dunaliella* was required for
221 optimal pigmentation in *Penaeus monodon* (Supamattaya *et al.*, 2005). These
222 observations support that the efficiency with which carotenoid intermediates are
223 converted to Axn depends on their position in the relevant metabolic conversion
224 pathways. Dietary Axn levels greater than 200 mg/kg did not lead to
225 improvements in pigmentation or tissue carotenoid accumulation (Yamada *et al.*,
226 1990, Merchie *et al.*, 1998), but other potential benefits of these high dietary
227 carotenoid levels were not examined in these studies. Later sections of this
228 review will explore further research in this area.

229

230 3.1.2 Chromatophores and Pigmentary Effectors

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

243 Short-term exposure to black substrates has been shown to improve prawn
244 pigmentation through expansion of hypodermal chromatophores (Parisenti *et al.*,
245 2011a, Tume *et al.*, 2009, Wade *et al.*, 2015a). An example of the effect that
246 background exposure has on the chromatophores in shrimp epithelial tissue is
247 shown in Figure 2. In addition to expanding and contracting, the chromatophores
248 completely change their pigment content in response to different substrates. In
249 response to dark backgrounds, animals with expanded chromatophores
250 contained high levels of free Axn, while white adapted animals with contracted
251 chromatophores contained high levels of Axn mono-esters (Tume *et al.*, 2009,
252 Wade *et al.*, 2015b). This expansion was also shown to be linked with the
253 accumulation of the colour protein crustacyanin in the hypodermal tissues
254 (Wade *et al.*, 2012), presumably bound to free Axn to create the darker
255 colouration. Tank colour was also shown to affect larval colour, survival and
256 development in crabs (Rabbani and Zeng, 2005). When exposed to constant light,
257 the body color of shrimp (*P. aztecus*) faded and chromatophores lost their
258 diurnal rhythm (Lakshmi *et al.*, 1976). Similarly, the body color of *P. monodon*
259 also became faint when cultured indoors under low light intensity less than 1000
260 lx (Tseng *et al.*, 1998). However, shrimp (*P. monodon*) subjected to constant light
261 maintained higher carotenoid levels as they grew (Pan *et al.*, 2001). Without
262 addition of Axn in diet, metal halide illumination at 2500 lux resulted in the

263 significant accumulation of Axn in whole body of *L. vannamei* to over 4 mg/kg,
264 compared with animals held in complete darkness at just over 2 mg/kg (You *et*
265 *al.*, 2006).

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

274 3.1.3 Carotenoproteins and Crustacyanin

275 Carotenoids and associated carotenoprotein complexes have been found in many
276 invertebrate species with tissue distribution ranging from the skin and gonads to
277 the blood, eggs and shell (Zagalsky, 1985, Lakshman and Okoh, 1993, Cheesman
278 *et al.*, 1967, Bhosale and Bernstein, 2007). Carotenoprotein complexes can be
279 divided into two types: lipovitellins and true carotenoproteins. Lipovitellins
280 possess a less stable and non-specific association of the carotenoid with the lipid
281 portion of a lipoprotein and are responsible colouration of such tissues as the
282 blood, epithelium, eggs and ovaries (Zagalsky, 1985, Cheesman *et al.*, 1967). True
283 carotenoproteins display a highly specific and stoichiometric relationship
284 between the carotenoid and a carotenoid binding protein (CBP), and appear to
285 be particularly widespread among the animals in class *Crustacea* as the
286 mechanism of shell colour production (Zagalsky, 1985, Lakshman and Okoh,
287 1993, Cheesman *et al.*, 1967).

288 Pigmentation in crustaceans is produced by a combination of the abundance and
289 degree of expansion of different coloured chromatophores, yellow, blue and red
290 (Rao, 1985), although visibility of chromatophores can be influenced by the
291 thickness of the exoskeleton in some species. As noted earlier, dietary Axn
292 supplementation increases the abundance of epithelial Axn, particularly Axn
293 esters (Yamada *et al.*, 1990, Supamattaya *et al.*, 2005, Boonyaratpalin *et al.*, 2001,
294 Barclay *et al.*, 2006, Kumar *et al.*, 2009, Wade *et al.*, 2015b). Similarly,
295 background colour modifies pigment proportions in epithelial tissues, with

296 contracted chromatophores containing high levels of carotenoid esters, and
297 expanded chromatophores containing high levels of free Axn (Tume *et al.*, 2009,
298 Wade *et al.*, 2015b, Wade *et al.*, 2015a) Within the exoskeleton and hypodermal
299 tissue of crustaceans, free Axn is often bound within a multimeric protein
300 complex called crustacyanin (CRCN) (Wald *et al.*, 1948). CRCN is a member of the
301 lipocalin protein family, a functionally diverse group of proteins that bind small
302 hydrophobic molecules such as steroid hormones, carotenoids, odourants and
303 pheromones (Flower, 1996, Flower *et al.*, 2000). The interaction of CRCN and
304 Axn modifies the naturally red carotenoid to blue or any other colour in the
305 visible spectrum, producing the diverse array of colours seen in the exoskeleton
306 of crustaceans (Cianci *et al.*, 2002). During cooking, this interaction is disrupted,
307 releasing the distinct red colouration of cooked seafood. The dimeric β -
308 crustacyanin (β -CRCN) is formed by two types of CRCN subunits (A and C, also
309 called H₁ and H₂) in association with two Axn molecules (Cianci *et al.*, 2002).
310 Eight of these dimers form a larger molecular weight complex known as α -
311 crustacyanin (α -CRCN), which has been extensively studied using
312 crystallographic techniques (reviewed in, (Chayen *et al.*, 2003, Zagalsky, 2003)).
313 At present, two genes that encode CRCN-A and CRCN-C have been identified
314 across a range of crustaceans (Wade *et al.*, 2009, Ertl *et al.*, 2013, Wang *et al.*,
315 2007). Their expression is restricted to the outer layer of the hypodermis (Wade
316 *et al.*, 2009, Wang *et al.*, 2007), and the spatial regulation of the *CRCN* genes is
317 thought to define the species-specific shell colors and patterns that different
318 crustaceans display (Wade *et al.*, 2009). In further support of this theory,
319 reconstitution of recombinant CRCN monomers (either A or C) formed
320 complexes with distinct absorption spectra, and the presence of CRCN in various
321 species correlated with the ability to produce certain shell colours (Ferrari *et al.*,
322 2012).

323 The development of colour over time in pigment deficient clawed lobsters
324 (*H. americanus*) was dependent on dietary carotenoid concentration, and
325 progressed over three months through either a predominantly red or a
326 predominantly blue phase before achieving a colour considered equivalent to
327 those from the wild (Tlustý and Hyland, 2005). In freshwater shrimp
328 (*M. rosenbergii*), external colour was removed by specific knockdown of a CRCN

329 homolog using RNAi (Yang *et al.*, 2011). In this study, the blue pigment attributed
330 to the Axn-CRCN interaction was removed by decreasing CRCN gene expression,
331 and hence protein abundance, which modified the shrimp colour to red.
332 Although not directly measured, the red colour that remained was likely the
333 underlying red chromatophores containing predominantly Axn esters. This
334 suggests that colour could be preferentially deposited in different
335 chromatophores, although how this might be regulated is not understood.
336 Exposure to white substrates significantly decreased the amount of CRCN
337 protein in shrimp hypodermal tissue, along with decreased free Axn levels and
338 increased Axn ester levels (Wade *et al.*, 2012). Exposure to black substrates
339 significantly increased the abundance of epithelial CRCN protein (Wade *et al.*,
340 2012), indicating the presence of this protein was critical to redistributing
341 hypodermal pigments and achieving optimal cooked colour (Wade *et al.*, 2012).
342 However, CRCN gene expression did not vary across the moult cycle or in
343 response to substrate colour (Wade *et al.*, 2012). Albino colour morphs of shrimp
344 (*F. merguensis*) displayed significantly reduced expression of the CRCN-A and C
345 genes compared with other shrimp, as well as a range of other genes potentially
346 involved in the regulation of crustacean colour (Ertl *et al.*, 2013). However,
347 expression levels of CRCN were not significantly different between light and dark
348 coloured shrimp, and there was no correlation between levels of CRCN gene
349 expression and Axn content (Ertl *et al.*, 2013). Despite extensive knowledge of
350 the mechanism by which CRCN binds Axn to produce crustacean colour, there is
351 very little known about how CRCN gene expression is regulated or how the CRCN
352 protein complexes form or are modified in the crustacean exoskeleton.

353

354 3.2 Carotenoids and Growth and Survival

355 Reports of the effect of dietary carotenoid supplementation on growth and
356 survival in crustaceans have been mixed, with virtually all research having been
357 conducted on shrimp. Some studies reported no significant difference in growth
358 in shrimp that had received dietary carotenoid supplementation (Pan *et al.*,
359 2001, Negre-Sadargues *et al.*, 1993, Boonyaratpalin *et al.*, 2001). However, an
360 increasing number of studies have shown that either growth or survival, or both,
361 are significantly improved when shrimp are fed a diet that contains carotenoids

362 compared with diets that do not (Niu *et al.*, 2012, Niu *et al.*, 2014, Supamattaya *et*
363 *al.*, 2005, Yamada *et al.*, 1990, Kumar *et al.*, 2009, Chien and Shiau, 2005, Petit *et*
364 *al.*, 1997, Darachai *et al.*, 1998, Chien and Jeng, 1992, Flores *et al.*, 2007, Zhang *et*
365 *al.*, 2013).

366 Early reports describing the beneficial effects of Axn on shrimp growth were
367 assessed on postlarvae (Darachai *et al.*, 1998, Chien, 1996) with evidence that
368 Axn supplementation shortened the moult frequency (Petit *et al.*, 1997). Larval
369 stages and postlarvae of *P. monodon* showed greater survival and were longer
370 when fed algal Axn (*Haematococcus pluvialis*) supplemented diets (Darachai *et*
371 *al.*, 1998). Studies on *M. japonicus* juveniles demonstrated that growth
372 performance was similar in shrimp over 8-weeks whether or not 100 mg/kg
373 carotenoid was included (Yamada *et al.*, 1990). However, by the end of 8 weeks
374 animals without dietary carotenoid contained significantly less total carotenoid
375 than those fed 100 mg/kg, and their survival had dropped from 91.3% to 57.1%
376 (Yamada *et al.*, 1990). In a separate experiment by the same authors but using
377 smaller animals, animals that had received 100 mg/kg Axn for 8 weeks had
378 grown significantly better than those that had not been fed Axn, while survival
379 was unaffected (Yamada *et al.*, 1990). Between these two experiments, there was
380 a marked difference in the total carotenoid content prawns at the beginning of
381 the experiment, with poor survival over 8 weeks recorded when initial
382 carotenoid content was low (15.6 ± 0.8 mg/kg). Significant correlations have been
383 observed between tissue carotenoid concentration and survival (Chien and Jeng,
384 1992) or specific growth rate (You *et al.*, 2006).

385 Since this initial work, the vast majority of studies have focussed on the giant
386 tiger shrimp, *Penaeus monodon*. Shrimp fed 125-300 mg/kg of algal extract for 8-
387 weeks showed higher weight gain and survival compared with controls
388 (Supamattaya *et al.*, 2005). When fed with 100mg/kg Axn combined with 1%
389 cholesterol for 74 days, shrimp showed higher weight gain and survival
390 compared with those fed diets without carotenoids (Niu *et al.*, 2012), with
391 apparent Axn digestibility of approximately 98%. In a similar study, shrimp fed
392 100 mg/kg Axn combined with 1% cholesterol also showed significantly higher
393 weight gain and survival (Niu *et al.*, 2014), and showed similarly high (>90%)
394 Axn digestibility. Although less studied, other species have shown a similar

395 response. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6 weeks
396 showed an increased daily growth coefficient and a reduced moult frequency
397 compared with those animals that had not been fed dietary Axn, but survival was
398 unaffected (Flores *et al.*, 2007). Shrimp (*L. vannamei*) fed either 100, 200 or 400
399 mg/kg Axn for 30 days showed improved weight gain and survival compared
400 with those without dietary carotenoids (Niu *et al.*, 2009). After 56 days, shrimp
401 (*L. vannamei*) fed 125 or 150 mg/kg Axn had higher weight gain than those fed
402 25, 50, 75 or 100 mg/kg Axn (Zhang *et al.*, 2013), but survival was unaffected. In
403 freshwater *Macrobrachium*, inclusion of 50, 100 or 200 mg/kg Axn improved
404 growth over the reference (Kumar *et al.*, 2009). Shrimp (*M. japonicus*) had
405 improved survival from 37% to over 50% when fed diets containing carotenoids
406 over 9 weeks, (Chien and Shiau, 2005), with a complementary increase in body
407 Axn levels, but no effect on growth. Improved survival, but not growth, was also
408 recorded in red king crab juveniles fed 380 mg/kg Axn for 56 days (Daly *et al.*,
409 2013).

410 Combined, these data suggest that survival is not affected when carotenoids are
411 maintained at a certain level, perhaps between 10-15 mg/kg body weight for
412 *P. monodon*, but survival is compromised below that level without carotenoid
413 supplementation. Where tissue carotenoid levels are initially high, perhaps
414 above 20 mg/kg, further carotenoid supplementation allows improved growth.
415 Variability in animal performance in growth trials may be explained by a range
416 of factors, including animal health, quality of feed ingredients, system design and
417 animal husbandry. Detection of growth differences in shrimp fed dietary
418 carotenoids in more recent studies may reflect improvements in trial
419 maintenance and animal husbandry. The study by (Pan *et al.*, 2001) had shown
420 there was no significant increase in survival in animals fed carotenoids
421 compared with those that were not, although overall survival was less than
422 <30% across the experiment, and this low level of survival casts aspersions on
423 the validity of this work. Despite this, it was demonstrated that higher tissue
424 carotenoid levels were correlated with higher survival (Pan *et al.*, 2001).
425 Carotenoid levels in shrimp at the beginning of the study will also be critical, as
426 carotenoid stores in animal tissues may compensate for the lack of dietary
427 carotenoids at least through the initial stages of an experimental growth trial.

428

429 3.3 Carotenoids and Tolerance to Disease and Stress

430 This section will focus on studies where dietary carotenoids have been supplied,
431 then the capacity to tolerate an induced stress has been directly tested under
432 controlled conditions, and the effects on survival or other biochemical
433 parameters assessed. The improved survival described in the previous section
434 was reported after a period of 8-9 weeks of a growth feeding trial in
435 experimental systems using different carotenoids (Axn, β -carotene or
436 canthaxanthin). However, more recent studies have been designed to specifically
437 assess whether responses to acute and chronic stresses, such as hypoxia, salinity
438 or viral infection, are improved after long periods of dietary carotenoid
439 supplementation. Analysis on shrimp (*F. chinensis*) showed that hypoxia alone
440 triggered significant up-regulation of proteins involved in immunity
441 (chymotrypsin and carboxypeptidase), and down regulation of proteins involved
442 in energy production (citrate synthase, ATP synthase), metabolism
443 (transketolase and esterases) and antioxidant capacity (glutathione peroxidase
444 and cMnSOD) (Jiang *et al.*, 2009). Dietary levels of 125 and 150 mg/kg Axn fed to
445 shrimp (*L. vannamei*) for 56 days lowered total antioxidant status, superoxide
446 dismutase (SOD), and catalase activities than those animals fed 25, 50, 65 or 100
447 mg/kg (Zhang *et al.*, 2013). Carotenoids were found to be less abundant in the
448 digestive gland and ovary of farmed *L. vannamei* compared with wild animals,
449 and levels were concluded to be insufficient to neutralise oxidative stress during
450 ovarian development (Linan-Cabello *et al.*, 2003). Crayfish exposed to pollution
451 had lower levels of vitamins and carotenoids in the hepatopancreas, suggesting
452 these may play a role in tolerating polluted environments (Barim and Karatepe,
453 2010).

454

455 Similar to growth and survival, the majority of work on tolerance to stress has
456 been performed on shrimp. Early studies showed that larval stages of
457 *P. monodon* supplemented with algal carotenoids were more resistant to low
458 salinity stress than those with synthetic Axn or controls (Darachai *et al.*, 1998).
459 Similarly, survival of *P. monodon* postlarvae during a low salinity stress test
460 exposure to 4 hours of low dissolved oxygen (< 1.0 mg/L) was improved in

461 shrimp (*P. monodon*) fed 360 mg/kg Axn for one week (Chien *et al.*, 1999). In a
462 separate test, these shrimp were also shown to be more tolerant of lower oxygen
463 levels in a lethal oxygen test (Chien *et al.*, 1999). Dietary Axn supplementation at
464 80 mg/kg enhanced antioxidant capacity in tiger shrimp (*P. monodon*)
465 postlarvae, which resulted in a significant improvement in recovery to both
466 thermal and osmotic stress (Chien *et al.*, 2003). In this study, higher body Axn
467 levels were recorded, total antioxidant status (TAS) was reduced and superoxide
468 dismutase (SOD) levels were reduced. The authors also speculated that
469 hepatopancreas function was improved due to lower levels of aspartate
470 aminotransferase (AST), a blood marker of liver integrity in mammalian systems,
471 being identified in the circulating hemolymph. However, both AST and alanine
472 aminotransferase (ALT) levels were reduced by thermal and osmotic stress,
473 which was opposite to the expected effect of stress. The inclusion of 80 mg/kg in
474 diets for 8-weeks improved shrimp (*P. monodon*) resistance to ammonia stress,
475 and animals showed higher total antioxidant status and lower SOD levels (Pan *et*
476 *al.*, 2003). AST and ALT levels were lowered by Axn supplementation, and were
477 negatively correlated with TAS. However, aminotransferase levels were not
478 correlated with survival, and may indicate that shrimp mortality was unrelated
479 to hepatopancreas damage. When fed 300 mg/kg of algal carotenoids for 8
480 weeks, *P. monodon* showed improved tolerance to a nine day period of daily
481 hypoxic stress (<1.0 mg/L) and also higher resistance to WSSV infection
482 (Supamattaya *et al.*, 2005).

483 Studies in other shrimp also showed similar effects. In *M. japonicus*, inclusion of
484 at least 50 mg/kg dietary Axn, from either synthetic or algal sources, resulted in
485 improved survival to low oxygen stress (Chien and Shiau, 2005). Significantly
486 greater levels of Axn had accumulated during the 9-week feeding trial, along with
487 a reduced oxygen consumption rate, suggesting that Axn may be acting as an
488 intracellular oxygen reserve or as a potent cellular antioxidant. Total carotenoid
489 levels were highest in animals that showed the highest survival, yet total
490 hemocyte count was lower and hemolymph phenoloxidase activity was
491 unchanged. Post-larval shrimp (*L. vannamei*) fed 80 mg/kg Axn for 6-weeks
492 showed significantly higher osmoregulatory capacity than those without dietary
493 Axn after salinity was reduced from 35 to 3 gL⁻¹ (Flores *et al.*, 2007). This was

494 coupled with significantly increased levels of hemocytes, hemocyanin and
495 glucose in the hemolymph, and reduced levels of hemolymph lactate (Flores *et al.*,
496 *et al.*, 2007). In a hypoxia stress test, postlarval shrimp (*L. vannamei*) fed either 200
497 or 400 mg/kg Axn recorded significantly higher survival, but no other
498 physiological parameters were measured (Niu *et al.*, 2009). More recently,
499 freshwater prawns showed a significant increase in phenoloxidase activity and
500 total hemocyte count after 28 days of consuming carotenoid fortified diets
501 (Kumar *et al.*, 2009), although no direct stress test was performed on the animals
502 in this study. Systemic injection of Axn into the same species caused an increase
503 in the total hemocyte count and an increased resistance to bacterial infection,
504 although there was no complementary increase in antioxidant indicators
505 (Angeles *et al.*, 2009). After low dissolved oxygen challenge, shrimp
506 (*L. vannamei*) fed 75-150 mg/kg Axn for 56 days had higher survival than those
507 animals fed 25 or 50 mg/kg Axn, and this was potentially linked with higher
508 expression of hypoxia inducible factor 1 alpha (HIF-1 α), cytosolic manganese
509 superoxide dismutase (cMnSOD) and catalase in Axn fed animals (Zhang *et al.*,
510 2013). After 74 days feeding 100 mg/kg Axn or 250 mg/kg β -carotene, improved
511 growth performance and survival in juvenile *P. monodon* was coupled with lower
512 malondialdehyde levels (an indicator of lipid peroxidation) after a simulated live
513 transport test (Niu *et al.*, 2014). In addition, expression levels of heat shock
514 protein 70 (Hsp-70) were significantly elevated under hypoxia compared with
515 normoxia, and further up-regulated under hypoxic conditions without dietary
516 carotenoids (Niu *et al.*, 2014). Although counter-intuitive, the expression of
517 hypoxia inducible factor 1 alpha (HIF-1 α) was decreased under hypoxic
518 condition, but were higher in animals fed β -carotene suggesting that the
519 response to hypoxia had been alleviated (Zhang *et al.*, 2013, Niu *et al.*, 2014).

520 In summary, data consistently demonstrate that dietary carotenoids increase the
521 total antioxidant capacity in the haemolymph of crustaceans, coupled with
522 decreased activity of other antioxidant enzymes. This may occur through
523 increased Axn levels in the haemolymph and tissues, improved oxygen carrying
524 capacity, decreased oxidation of polyunsaturated fatty acids or cellular proteins
525 or decreased activation of stress response systems. Combined, these data suggest
526 that the stress response is reduced in animals receiving dietary carotenoids

527 which improves survival to that stress, and that Axn is performing a broad
528 protective role against the detrimental effects of oxidative damage in tissues.
529 Similar to growth, many factors can affect survival in experimental systems,
530 which is especially problematic when survival is a key measure of performance
531 against stress. However, clear experimental evidence now exists to show that
532 carotenoid supplementation improves a range of factors to enable crustaceans to
533 tolerate stresses such as disease, hypoxia, temperature and salinity. These effects
534 appear to link the proposed antioxidant function of carotenoids themselves, with
535 physiological improvements in antioxidant capacity in the animals, and
536 improved performance under various stressful conditions. Some inconsistency
537 exists in the physiological responses of animals to dietary carotenoids, which
538 may highlight differences in the way different crustaceans deal with a variety of
539 stressors.

540

541 3.4 Carotenoids and Reproductive Performance

542 Nutrition plays a critical role in the reproductive success of crustaceans, and the
543 accumulation of nutrients in the developing ovaries, particularly lipids and
544 carotenoids, has a direct effect on reproductive measures such as egg number,
545 hatching rate and total nauplii produced (Wouters *et al.*, 2001). Very little
546 progress has been made in understanding the basis by which dietary carotenoids
547 improve crustacean reproduction since it was summarised more than ten years
548 ago (Linan-Cabello *et al.*, 2002a). During early maturation, carotenoids
549 accumulate in the hepatopancreas in both free and esterified form, after which
550 they are transported via the haemolymph to the ovaries during secondary
551 vitellogenesis (Harrison, 1990, Vincent *et al.*, 1988). Carotenoid content and type
552 varies greatly during ovarian development (Dall *et al.*, 1995, Linan-Cabello *et al.*,
553 2002b, Linan-Cabello *et al.*, 2003, Vincent *et al.*, 1988, Vincent *et al.*, 1989). The
554 darkening that occurs with this accumulation forms the basis of “staging” female
555 ovaries during ovarian maturation (Wouters *et al.*, 2001). Free and esterified Axn
556 is known to accumulate in the hepatopancreas during ovarian maturation, while
557 levels in the integument remain relatively constant (Dall *et al.*, 1995). Captive
558 shrimp contained less carotenoids, particularly in stage IV ovaries, than their
559 wild caught counterparts (Linan-Cabello *et al.*, 2003), strongly suggesting that

560 broodstock nutrition was deficient. Paprika as a source of dietary carotenoids (α -
561 carotene, α -cryptoxanthin and capxanthin) was shown to improve nauplii quality
562 in *P. monodon* broodstock (Wyban *et al.*, 1997), with the assumption that these
563 carotenoids were able to be converted into Axn. Axn supplemented in
564 broodstock diets for *Penaeus monodon* showed improved spawning and
565 fecundity (Pangantihon-Kuhlmann *et al.*, 1998). In the only recent study, high
566 levels of dietary fish oil and Axn have been linked to improved reproductive
567 performance, as measured by egg and spermatozoa number, in *P. monodon*
568 broodstock (Paibulkichakul *et al.*, 2008). As might be expected, increased dietary
569 fish oil led to accumulation of polyunsaturated fatty acids (PUFAs) in
570 hepatopancreas and ovary tissues, particularly 22:6n-3. However, extremely
571 high levels of dietary Axn (300 mg/kg) also led to an accumulation of Axn along
572 with these long chain PUFAs in ovary tissue (Paibulkichakul *et al.*, 2008).
573 Increased focus may be required on the use of carotenoids in conjunction with
574 other nutrients of reproductive significance, such as long chain PUFAs.

575 The positive effects of Axn can potentially be attributed its extremely high
576 capacity to scavenge oxygen free radicals, and the prevention of peroxidation of
577 PUFAs in tissues and diets (Britton, 2008, Miki, 1991). In various fish species, the
578 accumulation of carotenoids in reproductive tissues through dietary carotenoid
579 supplementation has been shown to improve a number of performance
580 characteristics, such as egg number, egg quality and number of larvae (Bjerkeng,
581 2008). Oxygen free radicals have been shown to attack biomembrane lipids and
582 proteins, leading to deterioration in egg quality (Bromage and Roberts, 1995). In
583 crustaceans, in conjunction with a depletion of carotenoids in the
584 hepatopancreas and ovary, an elevation of superoxide dismutase (SOD) activity
585 was observed in the haemolymph of captive shrimp compared with wild shrimp
586 (Linan-Cabello *et al.*, 2003). This was suggested to reflect the insufficient
587 scavenger activity to neutralize oxidative stress processes during spawning.
588 Normal developmental and physiological processes, such as ovarian
589 development and reproduction, are also potential sources of oxygen free
590 radicals.

591 Although not initially identified as necessary for embryonic development,
592 carotenoids are lost from fish and crustacean embryos prior to the first feeding

593 stages (Bjerkeng, 2008, Dall *et al.*, 1995). This implies the carotenoids present in
594 eggs and pre-feeding embryos are metabolised into other colourless molecules,
595 that in turn potentially perform biological functions. Axn has been proposed to
596 be an important source of Provitamin A and retinoids in eggs and early embryos
597 (Dall *et al.*, 1995, Linan-Cabello *et al.*, 2002a, Miki, 1991). Evidence from a
598 number of different crustaceans suggests that the retinols and other retinoid
599 derivatives play a critical role in developmental processes of crustaceans,
600 including ovarian and larval development (Linan-Cabello *et al.*, 2002a).
601 Crustaceans possess a number of retinoids and retinoic acid receptors in
602 crustaceans and the enhancement of the ovarian development in shrimp
603 suggests an important role of these metabolites in shrimp physiology for their
604 successful aquaculture. Carotenoids are the sole source of retinoids in
605 crustaceans, and their role as bioactive molecules may have been largely
606 overlooked (Linan-Cabello *et al.*, 2002a).

607

608

609 **4 Conclusion**

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

621 Optimal shrimp pigmentation can be achieved within several weeks by including
622 Axn in the diet at levels of between 50-100 mg/kg, which can be reduced by
623 using higher dietary inclusion levels. At these and even higher inclusion levels,
624 utilisation efficiency of dietary carotenoids is extremely high and often exceeds
625 90%. In Penaeid shrimp, the amount of carotenoid required to be deposited in
626 the tissues to achieve optimal colour is around 30-50 mg/kg body weight.
627 However, this amount does not result in the same overall colour of different
628 species, i.e. *P. monodon* is darker than *L. vannamei* at the same body Axn level. In
629 other crustaceans, this body Axn level may need to be significantly higher.
630 Background colour and light intensity are highly effective at redistributing
631 carotenoid pigments, both to make shrimp darker or lighter in colour. Optimal
632 pigmentation can lead to substantially higher sale prices, but there can be a
633 preference for either darker or lighter shrimp depending on the target market.

634 Although presently poorly defined, the carotenoid levels required to elicit the
635 physiological improvements in disease resistance, hypoxia or reproductive
636 performance may be considerably higher than those for pigmentation. These
637 beneficial effects have been demonstrated on various physiological
638 characteristics such as survival, growth and resistance to stress. However, unlike
639 colour, accurate measurement of these effects is often difficult due to a range of
640 external factors. Improvements in research methods and techniques have led to

641 a stronger understanding of the physiological mechanisms underlying
642 carotenoid function in crustaceans. Very little is known about the genetic
643 mechanisms that underlie the absorption, transport, tissue accumulation or
644 metabolic transformations of carotenoids in any animal species. It is reasonable
645 to assume that the accumulation of these carotenoids underpins the
646 physiological changes that lead to improved performance of a variety of
647 commercially relevant traits in aquaculture. More detailed studies are required
648 to define the basis of the benefits of carotenoids in crustacean aquaculture.
649 Although some functions of carotenoids may be preserved, we cannot continue
650 to rely on research from vertebrate systems to draw conclusions on their effect
651 in crustaceans.

652 **5 References**

- 653 Angeles, I.P., Chien, Y.H. & Tayamen, M.M. (2009) Effects of different dosages of
654 astaxanthin on giant freshwater prawn *Macrobrachium rosenbergii* (De Man)
655 challenged with *Lactococcus garvieae*. *Aquaculture Research*, **41**, 70-77.
- 656 Armenta-Lopez, R., Guerrero, I. & Huerta, S. (2002) Astaxanthin extraction from
657 shrimp waste by lactic fermentation and enzymatic hydrolysis of the
658 carotenoprotein complex. *J. Food Sci.*, **67**, 1002-1006.
- 659 Arredondo-Figueroa, J.L., Pedro-Islas, R., Ponce-Palafox, J.T. & Vernon-Carter, E.J.
660 (2003) Pigmentation Of Pacific White Shrimp (*Litopenaeus vannamei*, Boone
661 1931) With Esterified And Saponified Carotenoids From Red Chili (*Capsicum*
662 *annuum*) In Comparison To Astaxanthin. *Revista Mexicana de Ingenieria*
663 *Quimica*, **2**, 101-108.
- 664 Bagnara, J.T. & Hadley, M.E. (1973) *Chromatophores and color change : the*
665 *comparative physiology of animal pigmentation*, Prentice-Hall, Englewood
666 Cliffs, N.J. :
- 667 Barclay, M.C., Irvin, S.J., Williams, K.C. & Smith, D.M. (2006) Comparison of diets
668 for the tropical spiny lobster *Panulirus ornatus*: astaxanthin-supplemented
669 feeds and mussel flesh. *Aquaculture Nutrition*, **12**, 117-125.
- 670 Barim, O. & Karatepe, M. (2010) The effects of pollution on the vitamins A, E, C,
671 beta-carotene contents and oxidative stress of the freshwater crayfish,
672 *Astacus leptodactylus*. *Ecotoxicology and environmental safety*, **73**, 138-142.
- 673 Berticat, O., Negre-Sadargues, G. & Castillo, R. (2000) The metabolism of
674 astaxanthin during the embryonic development of the crayfish *Astacus*
675 *leptodactylus* Eschscholtz (Crustacea, Astacidea). *Comp Biochem Physiol*,
676 **127B**, 309-318.
- 677 Bhosale, P. & Bernstein, P.S. (2007) Vertebrate and invertebrate carotenoid-
678 binding proteins. *Archives of Biochemistry and Biophysics*, **458**, 121-127.
- 679 Bjerkgeng, B. (2008) Carotenoids in Aquaculture: Fish and Crustaceans In
680 *Carotenoids. Volume 4: natural functions* (Britton, G., et al. eds.), pp. 237-254.
681 Birkhauser Verlag AG, Basel.
- 682 Boonyaratpalin, M., Thongrod, S., Supamattaya, K., Britton, G. & Schlipalius, L.E.
683 (2001) Effects of β -carotene source, *Dunaliella salina*, and astaxanthin on

684 pigmentation, growth, survival and health of *Penaeus monodon*. *Aquaculture*
685 *Research*, **32**, 182-190.

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

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

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

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

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

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

700 Castillo, R., Lenel, R. & Negre-Sadargues, G. (1980) Determination, Tissue
701 Distribution and Metabolism of Carotenoid Pigments in the Hermit Crab
702 *Pagurus prideauxi* Leach (1815) Decapoda, Anomoura. *Comp Biochem Physiol*,
703 **66B**, 483-489.

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

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

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

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

- 716 Cheesman, D.F., Lee, W.L. & Zagalsky, P.F. (1967) Carotenoproteins in
717 Invertebrates. *Biological Reviews of the Cambridge Philosophical Society*, **42**,
718 131-137.
- 719 Chien, Y.-H. & Jeng, S.C. (1992) Pigmentation of kuruma prawn, *Penaeus*
720 *japonicus* Bate, by various pigment sources and levels and feeding regimes.
721 *Aquaculture*, **102**, 333-346.
- 722 Chien, Y.-H., Pan, C.-H. & Hunter, B. (2003) The resistance to physical stresses by
723 *Penaeus monodon* juveniles fed diets supplemented with astaxanthin.
724 *Aquaculture*, **216**, 177-191.
- 725 Chien, Y.H. (1996) Biological effects of astaxanthin in shrimp, a review In *3rd*
726 *Annual Roche Aquaculture Centre Conference on Nutrition and Disease* Bangkok,
727 Thailand.
- 728 Chien, Y.H., Chen, I.M., Pan, C.H. & Kurmaly, K. (1999) Oxygen depletion stress on
729 mortality and lethal course of juvenile tiger prawn *Penaeus monodon* fed high
730 level of dietary astaxanthin. *Journal of the Fisheries Society of Taiwan*, **26**, 85-
731 93.
- 732 Chien, Y.H. & Shiau, W.C. (2005) The effects of dietary supplementation of algae
733 and synthetic astaxanthin on body astaxanthin, survival, growth, and low
734 dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus*
735 Bate. *Journal of Experimental Marine Biology and Ecology*, **318**, 201-211.
- 736 Cianci, M., Rizkallah, P.J., Olczak, A., Raftery, J., Chayen, N.E., Zagalsky, P.F. &
737 Helliwell, J.R. (2002) The molecular basis of the coloration mechanism in
738 lobster shell: beta-crustacyanin at 3.2-A resolution. *Proceedings of the*
739 *National Academy of Sciences of the United States of America*, **99**, 9795-9800.
- 740 D'Abrahmo, L.R., Baum, N.A., Bordner, C.E. & Conklin, D.E. (1983) Carotenoids as
741 a source of pigmentation in juvenile lobsters fed a purified diet. *Can J Fish*
742 *Aquat Sci*, **40**, 699-704.
- 743 Dall, W. (1995) Carotenoids versus retinoids (Vitamin A) as essential growth
744 factors in penaeid prawns (*Penaeus semisulcatus*). *Marine Biology*, **124**, 209-
745 213.
- 746 Dall, W., Smith, D.M. & Moore, L.E. (1995) Carotenoids in the Tiger Prawn
747 *Penaeus esculentus* During Ovarian Maturation. *Mar Biol*, **123**, 435-441.

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

751 Darachai, J., Piyatiratitivorakul, S., Kittakoop, P., Nitithamyong, C. & Menasveta, P.
752 (1998) Effects of Astaxanthin on Larval Growth and Survival of the Giant Tiger
753 Prawn, *Penaeus monodon* In *Advances in Shrimp Biotechnology, Proceedings to*
754 *the Special Session on Shrimp Biotechnology, 5th Asian Fisheries Forum* (Flegel,
755 T.W. ed. BIOTEC, Chiangmai, Thailand.

756 de la Vega, E., Hall, M.R., Wilson, K.J., Reverter, A., Woods, R.G. & Degnan, B.M.
757 (2007) Stress-induced gene expression profiling in the black tiger shrimp
758 *Penaeus monodon*. *Physiological genomics*, **31**, 126-138.

759 Erickson, M.C., Bulgarelil, M.A., Resurreccion, A.V.A., Vendetti, R.A. & Gates, K.A.
760 (2007) Consumer Differentiation, Acceptance, and Demographic Patterns to
761 Consumption of Six Varieties of Shrimp. *Journal of Aquatic Food Product*
762 *Technology*, **15**, 35-51.

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

766 Ferrari, M., Folli, C., Pincolini, E., McClintock, T.S., Rossle, M., Berni, R. & Cianci, M.
767 (2012) Structural characterization of recombinant crustacyanin subunits from
768 the lobster *Homarus americanus*. *Acta Crystallogr Sect F Struct Biol Cryst*
769 *Commun*, **68**, 846-853.

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

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

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

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

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

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

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

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

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

790 Harrison, K.E. (1990) The role of nutrition in maturation, reproduction and
791 embryonic development of decapod
792 crustaceans: a review. *Journal of Shellfish Research*, **9**, 1-28.

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

796 Jiang, H., Li, F., Xie, Y., Huang, B., Zhang, J., Zhang, J., Zhang, C., Li, S. & Xiang, J.
797 (2009) Comparative proteomic profiles of the hepatopancreas in
798 *Fenneropenaeus chinensis* response to hypoxic stress. *PROTEOMICS*, **9**, 3353-
799 3367.

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

803 Katayama, T., Kunisaki, Y., Shimaya, M., Simpson, K.L. & Chichester, C.O. (1973)
804 The biosynthesis of astaxanthin-XIV. The conversion of labelled β -carotene-
805 15, 15'-3H₂ into astaxanthin in the crab, *Portunus trituberculatus*. *Comp*
806 *Biochem Phys B*, **46**, 269-272.

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

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

813 Kumar, V., Pillai, B.R., Sahoo, P.K., Mohanty, J. & Mohanty, S. (2009) Effect of
814 dietary astaxanthin on growth and immune response of Giant freshwater
815 prawn *Macrobrachium rosenbergii* (de man). *Asian Fisheries Science*, **22**, 61-
816 69.

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

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

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

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

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

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

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

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

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

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

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

845 European Lobster *Homarus gammarus* (Linne, 1758). *Comp Biochem Physiol*,
846 **111B**, 553-558.

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

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

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

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

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

859 Merchie, G., Kontara, E., Lavens, P., Robles, R., Kurmaly, K. & Sorgeloos, P. (1998)
860 Effect of vitamin C and astaxanthin on stress and disease resistance of
861 postlarval tiger shrimp, *Penaeus monodon* (Fabricius). *Aquaculture Research*,
862 **29**, 579-585.

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

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

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

869 Negre-Sadargues, G., Castillo, R., Petit, H., Sance, S., Martinez, R.G., Milicua, J.,
870 Choubert, G. & Trilles, J. (1993) Utilization of synthetic carotenoids by the
871 prawn *Penaeus japonicus* reared under laboratory conditions. *Aquaculture*,
872 **110**, 151-159.

873 Negre-Sadargues, G., Castillo, R. & Segonzac, M. (2000) Carotenoid pigments and
874 trophic behaviour of deep-sea shrimps (Crustacea, decapoda, alvinocarididae)
875 from a hydrothermal area of the mid-atlantic ridge. *Comp Biochem Physiol*,
876 **127A**, 293-300.

- 877 Nguyen, N., Quinn, J., Powell, D., Elizur, A., Thoa, N., Nocillado, J., Lamont, R.,
878 Remilton, C. & Knibb, W. (2014) Heritability for body colour and its genetic
879 association with morphometric traits in Banana shrimp (*Fenneropenaeus*
880 *merguiensis*). *BMC Genet*, **15**, 132.
- 881 Niu, J., Li, C.-H., Liu, Y.-J., Tian, L.-X., Chen, X., Huang, Z. & Lin, H.-Z. (2012) Dietary
882 values of astaxanthin and canthaxanthin in *Penaeus monodon* in the presence
883 and absence of cholesterol supplementation: effect on growth, nutrient
884 digestibility and tissue carotenoid composition. *British Journal of Nutrition*,
885 **108**, 80-91.
- 886 Niu, J., Tian, L.X., Liu, Y.J., Yang, H.J., Ye, C.X., Gao, W. & Mai, K.S. (2009) Effect of
887 Dietary Astaxanthin on Growth, Survival, and Stress Tolerance of Postlarval
888 Shrimp, *Litopenaeus vannamei*. *Journal of the World Aquaculture Society*, **40**,
889 795-802.
- 890 Niu, J., Wen, H., Li, C.-H., Liu, Y.-J., Tian, L.-X., Chen, X., Huang, Z. & Lin, H.-Z. (2014)
891 Comparison effect of dietary astaxanthin and β -carotene in the presence and
892 absence of cholesterol supplementation on growth performance, antioxidant
893 capacity and gene expression of *Penaeus monodon* under normoxia and
894 hypoxia condition. *Aquaculture*, **422-423**, 8-17.
- 895 Okada, S., Liao, W.L., Mori, T., Yamaguchi, K. & Watanabe, T. (1991) Pigmentation
896 of Cultured Striped Jack Reared on Diets Supplemented with the Blue-Green-
897 Alga *Spirulina maxima*. *Nippon Suisan Gakkaishi*, **57**, 1403-1406.
- 898 Okada, S., Nur-E-Borhan, S.A. & Yamaguchi, K. (1994) Carotenoid Composition in
899 the Exoskeleton of Commercial Black Tiger Prawns. *Fish. Sci.*, **60**, 213-215.
- 900 Paibulkichakul, C., Piyatiratitivorakul, S., Sorgeloos, P. & Menasveta, P. (2008)
901 Improved maturation of pond-reared, black tiger shrimp (*Penaeus monodon*)
902 using fish oil and astaxanthin feed supplements. *Aquaculture*, **282**, 83-89.
- 903 Pan, C.H. & Chien, Y.H. (2000) Astaxanthin distribution in juvenile *Penaeus*
904 *monodon* at various molting stages. *Journal of the Fisheries Society of Taiwan*,
905 **27**, 33-43.
- 906 Pan, C.H., Chien, Y.H. & Cheng, J.H. (1999) Carotenoid content in various tissues of
907 cultured *Penaeus monodon* by their sizes, sexes and molting stages. *Journal of*
908 *the Fisheries Society of Taiwan*, **26**, 51-57.

- 909 Pan, C.H., Chien, Y.H. & Hunter, B. (2003) The resistance to ammonia stress of
910 *Penaeus monodon* Fabricius juvenile fed diets supplemented with astaxanthin.
911 *Journal of Experimental Marine Biology and Ecology*, **297**, 107-118.
- 912 Pan, C.H. & Chien, Y.H. (2003) Concentration and composition of astaxanthin in
913 black tiger prawn *Penaeus monodon* postlarvae fed *Artemia* sp nauplii or
914 mauxia shrimp *Acetes intermedius*. *Journal of the World Aquaculture Society*,
915 **34**, 57-65.
- 916 Pan, C.H., Chien, Y.H. & Cheng, J.H. (2001) Effects of light regime, algae in the
917 water, and dietary astaxanthin on pigmentation, growth, and survival of black
918 tiger prawn *Penaeus monodon* post-larvae. *Zool. Stud.*, **40**, 371-382.
- 919 Pangantihon-Kuhlmann, M.P., Millamena, O. & Chern, Y. (1998) Effect of dietary
920 astaxanthin and vitamin A on the reproductive performance of *Penaeus*
921 *monodon* broodstock. *Aquatic Living Resources*, **11**, 403-409.
- 922 Parisenti, J., Beirao, L.H., Mourino, J.L., Vieira, F., Buglione, C.C. & Maraschim, M.
923 (2011a) Effect of Background Color on Shrimp Pigmentation. *Biol Inst Pesca*,
924 **37**, 177-182.
- 925 Parisenti, J., Beirão, L.H., Tramonte, V.L.C.G., Ourique, F., da Silveira Brito, C.C. &
926 Moreira, C.C. (2011b) Preference ranking of colour in raw and cooked
927 shrimps. *International Journal of Food Science & Technology*, **46**, 2558-2561.
- 928 Petit, H., Negre-Sadargues, G., Castillo, R. & Trilles, J.P. (1997) The effects of
929 dietary astaxanthin on growth and moulting cycle of postlarval stages of the
930 prawn, *Penaeus japonicus* (Crustacea, Decapoda). *Comparative Biochemistry*
931 *and Physiology a-Physiology*, **117**, 539-544.
- 932 Petit, H., Negre-Sadargues, G., Castillo, R., Valin, S. & Trilles, J.P. (1998) The effects
933 of dietary astaxanthin on the carotenoid pattern of the prawn *Penaeus*
934 *japonicus* during postlarval development. *Comp Biochem Physiol*, **119A**, 523-
935 527.
- 936 Petit, H., Sance, S., Negre-Sadargues, G., Castillo, R. & Trilles, J.P. (1991) Ontogeny
937 of Carotenoid Metabolism in the Prawn *Penaeus japonicus* Bate (1888)
938 (Crustacea, Penaeidea) - a Qualitative Approach. *Comp Biochem Physiol*, **99B**,
939 667-671.
- 940 Phillips, B.F. (1983) Migrations of Pre-Adult Western Rock Lobsters, *Panulirus*
941 *cygnus*, in Western-Australia. *Marine Biology*, **76**, 311-318.

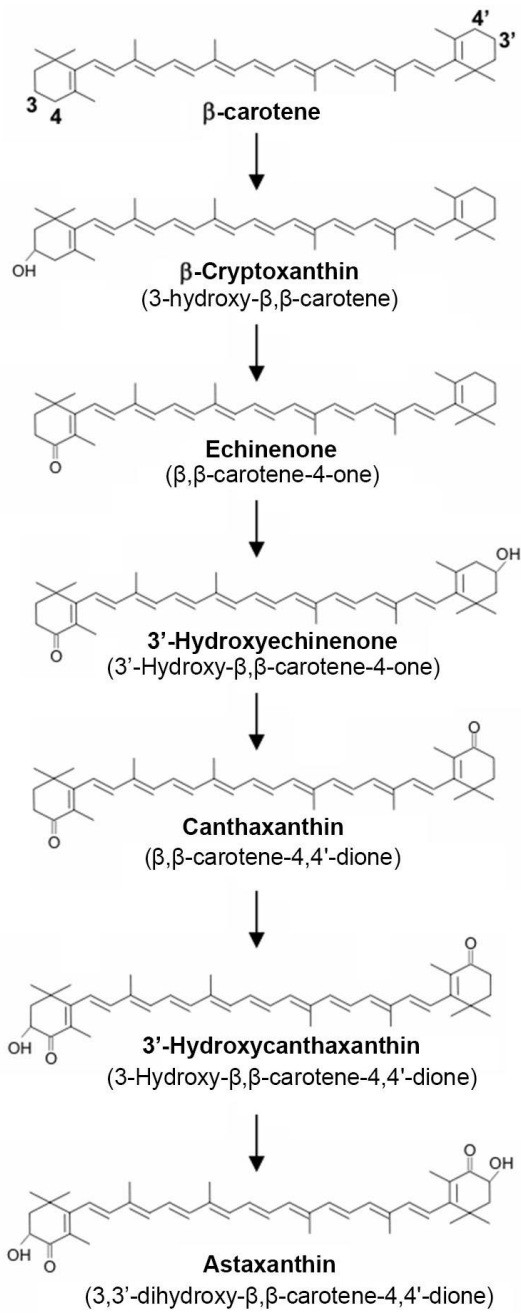
- 942 Rabbani, A. & Zeng, C. (2005) Effects of tank colour on larval survival and
943 development of mud crab *Scylla serrata* (Forskal). *Aquaculture Research*, **36**,
944 1112-1119.
- 945 Rao, K.R. (1985) Pigmentary Effectors In *Integuments, Pigments and Hormonal*
946 *Processes* (Bliss, D.E. & Mantel, L.H. eds.), Vol. 9, pp. 395-462. Academic Press,
947 New York.
- 948 Rao, K.R. (2001) Crustacean Pigmentary-Effector Hormones: Chemistry and
949 Functions of RPCH, PDH, and Related Peptides. *Integrative and Comparative*
950 *Biology*, **41**, 364-379.
- 951 Ribeiro, E.A., Genofre, G.C. & McNamara, J.C. (2001) Identification and
952 quantification of carotenoid pigments during the embryonic development of
953 the freshwater shrimp *Macrobrachium olfersii* (Crustacea, Decapoda). *Mar.*
954 *Freshw. Behav. Physiol.*, **34**, 105-116.
- 955 Sachindra, N.M., Bhaskar, N. & Mahendrakar, N.S. (2005) Carotenoids in different
956 body components of Indian shrimps. *J. Sci. Food Agric.*, **85**, 167-172.
- 957 Schiedt, K., Bischof, S. & Glinz, E. (1993) Metabolism of Carotenoids and *In vivo*
958 Racemization of (3s,3's)-Astaxanthin in the Crustacean *Penaeus*. *Methods in*
959 *Enzymology*, **214**, 148-168.
- 960 Shahidi, F., Metusalach & Brown, J.A. (1998) Carotenoid pigments in seafoods
961 and aquaculture. *Crit Rev Food Sci Nutr*, **38**, 1-67.
- 962 Sommer, T.R., Morrissey, N.M. & Potts, W., T. (1991) Growth and Pigmentation of
963 Marron (*Cherax tenuimanus*) Fed a Reference Ration Supplemented with the
964 Microalga *Dunaliella-Salina*. *Aquaculture*, **99**, 285-295.
- 965 Stentiford, G.D., Green, M., Bateman, K., Small, H.J., Neil, D.M. & Feist, S.W. (2002)
966 Infection by a Hematodinium-like parasitic dinoflagellate causes Pink Crab
967 Disease (PCD) in the edible crab *Cancer pagurus*. *Journal of Invertebrate*
968 *Pathology*, **79**, 179-191.
- 969 Supamattaya, K., Kiriratnikom, S., Boonyaratpalin, M. & Borowitzka, L. (2005)
970 Effect of a *Dunaliella* extract on growth performance, health condition,
971 immune response and disease resistance in black tiger shrimp (*Penaeus*
972 *monodon*). *Aquaculture*, **248**, 207-216.

- 973 Tanaka, Y., Matsuguchi, H., Katayama, T., Simpson, K.L. & Chichester, C.O. (1976a)
974 The biosynthesis of astaxanthin- XVI. The carotenoids in Crustacea. *Comp*
975 *Biochem Physiol*, **54B**, 391-393.
- 976 Tanaka, Y., Matsuguchi, H., Katayama, T., Simpson, K.L. & Chichester, C.O. (1976b)
977 The biosynthesis of astaxanthin- XVIII. The metabolism of the carotenoids in
978 the prawn, *Penaeus japonicus* Bate. *Bull Jap Soc Scient Fish*, **42**, 197-202.
- 979 Tlustý, M. & Hyland, C. (2005) Astaxanthin deposition in the cuticle of juvenile
980 American lobster (*Homarus americanus*): implications for phenotypic and
981 genotypic coloration. *Marine Biology*, **147**, 113-119.
- 982 Tseng, K.F., Su, H.M. & Su, M.S. (1998) Culture of *Penaeus monodon* in a
983 recirculating system. *Aquacultural Engineering*, **17**, 138-147.
- 984 Tume, R.K., Sikes, A.L., Tabrett, S. & Smith, D.M. (2009) Effect of background
985 colour on the distribution of astaxanthin in black tiger prawn (*Penaeus*
986 *monodon*): Effective method for improvement of cooked colour. *Aquaculture*,
987 **296**, 129-135.
- 988 Valin, A., Castillo, R., Negre-Sadargues, G. & Lenel, R. (1987) Quantitative Aspects
989 of Carotenoid Pigment Circadian Variations in the Crayfish *Astacus*
990 *leptodactylus*. *Biochem Syst Ecol*, **15**, 607-610.
- 991 Vernon-Carter, E.J., Ponce-Palafox, J.T. & Pedroza-Islas, R. (1996) Pigmentation of
992 Pacific white shrimp (*Penaeus vannamei*) using Aztec marigold (*Tagetes*
993 *erecta*) extracts as the carotenoid source. *Archivos latinoamericanos de*
994 *nutrición*, **46**, 243-246.
- 995 Vincent, M., Ramos, L. & Oliva, L. (1988) Qualitative and quantitative variations
996 in carotenoid pigments in the ovary and hepatopancreas of *Penaeus schmitti*
997 during ovarian maturation. *Arch Int Physiol Biochim*, **96**, 155-164.
- 998 Vincent, M., Ramos, L. & Oliva, M. (1989) Variation in the composition of fatty
999 acids of zeaxanthin and astaxanthin monoesters in the ovary and
1000 hepatopancreas of *Penaeus schmitti* during ovogenesis. *Arch Int Physiol*
1001 *Biochim*, **97**, 71-78.
- 1002 Wade, N.M., Anderson, M., Sellars, M.J., Tume, R.K., Preston, N.P. & Glencross, B.D.
1003 (2012) Mechanisms of colour adaptation in the prawn *Penaeus monodon*.
1004 *Journal of Experimental Biology*, **215**, 343-350.

- 1005 Wade, N.M., Budd, A., Irvin, S. & Glencross, B.D. (2015a) The Combined Effects of
1006 Diet, Environment and Genetics on Pigmentation in the Giant Tiger Prawn,
1007 *Penaeus monodon*. *Aquaculture*, **submitted**.
- 1008 Wade, N.M., Cheers, S., Bourne, N., Irvin, S., Blyth, D. & Glencross, B.D. (2015b)
1009 Dietary astaxanthin levels affect growth, carotenoid digestibility and the
1010 deposition of specific carotenoid esters in the Giant Tiger Prawn, *Penaeus*
1011 *monodon*. *Aquaculture Nutrition*, **submitted**.
- 1012 Wade, N.M., Melville-Smith, R., Degnan, B.M. & Hall, M.R. (2008) Control of shell
1013 colour changes in the lobster, *Panulirus cygnus*. *Journal of Experimental*
1014 *Biology*, **211**, 1512-1519.
- 1015 Wade, N.M., Tollenaere, A., Hall, M.R. & Degnan, B.M. (2009) Evolution of a novel
1016 carotenoid-binding protein responsible for crustacean shell color. *Molecular*
1017 *Biology and Evolution*, **26**, 1851-1864.
- 1018 Wald, G., Nathanson, N., Jencks, W.P. & Tarr, E. (1948) Crustacyanin, the blue
1019 carotenoprotein of the lobster shell. *Biological Bulletin*, **95**, 249-250.
- 1020 Wang, M.-R., Zhu, X.-J., Yang, J.-S., Dai, Z.-M., Mahmood, K., Yang, F. & Yang, W.-J.
1021 (2007) Prawn lipocalin: characteristics and expressional pattern in
1022 subepidermal adipose tissue during reproductive molting cycle. *Comparative*
1023 *Biochemistry and Physiology, Part B*, **147**, 222-229.
- 1024 Wouters, R., Lavens, P., Nieto, J. & Sorgeloos, P. (2001) Penaeid shrimp
1025 broodstock nutrition: an updated review on research and development.
1026 *Aquaculture*, **202**, 1-21.
- 1027 Wyban, W., Martinez, G. & Sweeney, J. (1997) Adding paprika to *Penaeus*
1028 *vannamei* maturation diet improves nauplii quality. In *World Aquaculture*.
- 1029 Yamada, S., Tanaka, Y., Sameshima, M. & Ito, Y. (1990) Pigmentation of Prawn
1030 (*Penaeus japonicus*) with Carotenoids .1. Effect of Dietary Astaxanthin, Beta-
1031 Carotene and Canthaxanthin on Pigmentation. *Aquaculture*, **87**, 323-330.
- 1032 Yang, F., Wang, M.-R., Ma, Y.-G., Ma, W.-M. & Yang, W.-J. (2011) Prawn lipocalin:
1033 characterization of a color shift induced by gene knockdown and ligand
1034 binding assay. *Journal of experimental zoology. Part A, Ecological genetics and*
1035 *physiology*.

- 1036 You, K., Yang, H.S., Liu, Y., Liu, S.L., Zhou, Y. & Zhang, T. (2006) Effects of different
1037 light sources and illumination methods on growth and body color of shrimp
1038 *Litopenaeus vannamei*. *Aquaculture*, **252**, 557-565.
- 1039 Zagalsky, P.F. (1985) Invertebrate carotenoproteins. *Methods Enzymol*, **111**, 216-
1040 247.
- 1041 Zagalsky, P.F. (2003) beta-Crustacyanin, the blue-purple carotenoprotein of
1042 lobster carapace: consideration of the bathochromic shift of the protein-
1043 bound astaxanthin. *Acta crystallographica. Section D, Biological*
1044 *crystallography*, **59**, 1529-1531.
- 1045 Zhang, J., Liu, Y.J., Tian, L.X., Yang, H.J., Liang, G.Y., Yue, Y.R. & Xu, D.H. (2013)
1046 Effects of dietary astaxanthin on growth, antioxidant capacity and gene
1047 expression in Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*
1048 *Nutrition*, **19**, 917-927.
- 1049
1050
1051

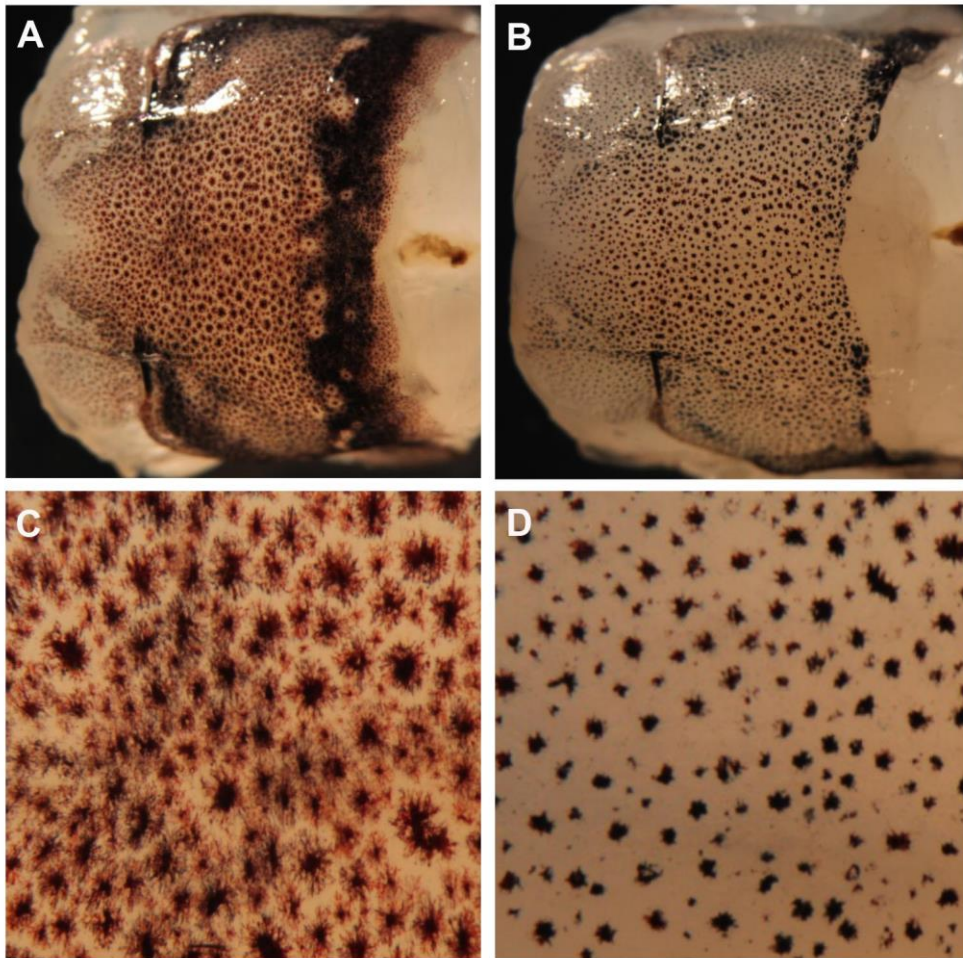
1052 Figure 1. Schematic diagram of the major conversion pathway of β -carotene to
1053 astaxanthin in crustacean tissues.



1054

1055

1056 Figure 2 The response of crustacean abdominal epithelial chromatophores when
1057 exposed to black (A and C) or white (B and D) coloured substrates.



1058

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

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

Kuruma Shrimp (<i>Marsupenaeus japonicus</i>)				
(Chien and Jeng, 1992)	50 – 200 mg/kg	Astaxanthin / β -carotene	Synthetic / algal	100 mg/kg Astaxanthin
(Negre-Sadargues <i>et al.</i> , 1993)	100 mg/kg	Astaxanthin/ Canthaxanthin	Synthetic	50 mg AX + 50 mg CX
(Petit <i>et al.</i> , 1997)	0 – 220 mg/kg	Astaxanthin/ Canthaxanthin	Synthetic / <i>Artemia</i>	60 mg/kg Astaxanthin
(Chien and Shiau, 2005)	0 – 100 mg/kg	Astaxanthin	Synthetic / algal	100 mg/kg
Giant Freshwater Prawn <i>Macrobrachium rosenbergii</i>)				
(Kumar <i>et al.</i> , 2009)	0 – 200 mg/kg	Astaxanthin	Synthetic	200 mg/kg
Hermit Crab (<i>Clibanarius erythropus</i>)				
(Castillo and Negre-Sadargues, 1995)	200 mg/kg	Astaxanthin / β -carotene / Canthaxanthin	Synthetic	200 mg/kg Astaxanthin
Red King Crab (<i>Paralithodes camtschaticus</i>)				
(Daly <i>et al.</i> , 2013)	0 – 380 mg/kg	Astaxanthin	Synthetic / algal	380 mg/kg
American Clawed Lobster (<i>Homarus americanus</i>)				
(Tlusty and Hyland, 2005)	0 – 220 mg/kg	Astaxanthin	Synthetic	220 mg/kg
Tropical Spiny Crayfish (<i>Panulirus ornatus</i>)				
(Barclay <i>et al.</i> , 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
<i>Growth and Survival</i>			
(Yamada <i>et al.</i> , 1990)	100 mg/kg Axn	<i>M. japonicus</i>	Improved survival or growth
(Darachai <i>et al.</i> , 1998)	various	<i>P. monodon</i>	Improved post-larval survival
(Chien and Shiau, 2005)	50-100 mg/kg	<i>M. japonicus</i>	Improved survival
(Supamattaya <i>et al.</i> , 2005)	300 mg/kg β -carotene	<i>P. monodon</i>	Greater weight gain and improved survival
(Flores <i>et al.</i> , 2007)	80 mg/kg Axn	<i>L. vannamei</i>	Improved growth and moult frequency
(Kumar <i>et al.</i> , 2009)	50-200 mg/kg Axn	<i>M. rosenbergii</i>	Greater weight gain and improved survival
(Niu <i>et al.</i> , 2009)	100-400 mg/kg Axn	<i>L. vannamei</i>	Greater weight gain and improved survival
(Niu <i>et al.</i> , 2012)	100 mg/kg Axn + cholesterol	<i>P. monodon</i>	Greater weight gain and improved survival
(Daly <i>et al.</i> , 2013)	380 mg/kg	<i>Paralithodes camtschaticus</i>	Improved survival
(Zhang <i>et al.</i> , 2013)	125-150 mg/kg Axn	<i>L. vannamei</i>	Improved growth
(Niu <i>et al.</i> , 2014)	100 mg/kg Axn + cholesterol	<i>P. monodon</i>	Greater weight gain and improved survival
<i>Tolerance to Disease and Stress</i>			
(Darachai <i>et al.</i> , 1998)	various	<i>P. monodon</i>	Improved tolerance to low salinity
(Chien <i>et al.</i> , 1999)	360 mg/kg Axn	<i>P. monodon</i>	Improved survival to low dissolved oxygen
(Chien <i>et al.</i> , 2003)	80 mg/kg Axn	<i>P. monodon</i>	Improved recovery from thermal and osmotic stress, enhanced anti-oxidant capacity.

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