



## Effect of background colour on the distribution of astaxanthin in black tiger prawn (*Penaeus monodon*): Effective method for improvement of cooked colour

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### ARTICLE INFO

#### Article history:

Received 17 January 2009

Received in revised form 21 July 2009

Accepted 4 August 2009

#### Keywords:

*Penaeus monodon*

Astaxanthin

Chromatophores

Pigmentation

Colour grading

### ABSTRACT

The colouration of prawns is dependent upon the presence of carotenoid pigments (predominantly astaxanthin) and has a significant impact on their market value. In this study we observed that visual appearance of colour in black tiger prawns (*Penaeus monodon*), when assessed using a subjective commercial grading scale, did not always correlate well with total carotenoid content. We also observed that visual appearance was affected by the background colour of the tanks in which the prawns were growing. When prawns were grown in black or white tanks for 28 days, even though they had similar mean total astaxanthin contents (29–33 µg/g prawn tail), those grown in black tanks were much more orange/red when cooked, compared with those from the white tanks. The pigments were mainly located in the cephalothorax, abdominal epidermal layer and abdominal exoskeleton. Light microscopy showed an even distribution of pigment in the epidermal layer of more coloured prawns compared with concentrated pigment areas in the lighter-coloured prawns. Non-esterified astaxanthin was the major carotenoid present in all body locations in prawns from black tanks (50%) with the remainder being made up equally of astaxanthin mono- and di-esters. However, for prawns from white tanks, non-esterified astaxanthin accounted for just 12–13%, with the mono-ester reaching about 60% of the total present. In a separate experiment, we demonstrated that changes in background colour caused a rapid change in visual colour. When prawns were moved from a white to a black tank, there was a significant increase in grade score within 1 h without any change in astaxanthin content. Colour improvement continued to increase over 168 h. Moving prawns from a black to a white tank resulted in a reduction in grade score but the change was much less rapid. This work suggests that, provided prawns have an adequate content of astaxanthin in their diets, it is possible to improve their overall appearance (raw and cooked) by manipulating background colour about the time of harvesting.

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### 1. Introduction

Prawn colour is one of the most important attributes in determining a consumer's decision to purchase prawns and therefore significantly affects their market value. In Australia, cooked black tiger prawns (*Penaeus monodon*) are commonly assessed for colour using a subjective colour grading chart with scores ranging from 1 to 12, reflecting low to high pigmentation (Aqua-Marine Marketing Pty Ltd., Kippa-Ring, Queensland). Prawns having higher grade scores are often valued at AU\$2–4/kg more than lighter-coloured prawns, and often, poorly coloured prawns are unable to be sold at acceptable market prices and are frozen and kept until times of higher demand.

Prawn colour is dependent largely upon the amount of astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) present in the external

tissues; particularly in the exoskeleton and in the epidermal layer between the abdominal muscle and the exoskeleton (Menasveta et al., 1993; Boonyaratpalin et al., 2001). Astaxanthin is commonly present in free and esterified forms (mono- and di-ester) with fatty acids (Okada et al., 1994). These carotenoids may also be present as caroteno-proteins, particularly in the exoskeleton, and when they dissociate from the protein, they change in colour from blue-green to red, as is apparent when prawns are cooked (Britton et al., 1981). Small amounts of other carotenoids including lutein and zeaxanthin have also been reported (Pan et al., 2001; Sachindra et al., 2005).

In addition to pigmentation, carotenoids have also been proposed for a role in a number of important functions in crustaceans such as a source of provitamin A activity (Miki et al., 1982), increased stress tolerance (Torrissen, 1984; Chien et al., 2003) and in various development and differentiation processes, as summarised by Linan-Cabello et al. (2002).

The origin of astaxanthin in prawns, as in other animals, is dietary and in wild prawns much of it originates from conversion from some

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other ingested carotenoids such as zeaxanthin and  $\beta$ -carotene (Katayama et al., 1971). Therefore, the presence of astaxanthin in prawns is dependent upon its availability from feeds in the environment as well as its ability to be absorbed, transported and then deposited in particular anatomical sites. For farmed prawns, addition of synthetic astaxanthin to feeds represents an additional cost and it is often necessary to adjust the amount and duration of supplementation in order to achieve the desired level of pigmentation (Chien and Jeng, 1992). It is often difficult for producers to predict the optimal feed concentration required because of the contribution from algal biota in the ponds, as well as apparent individual genetic colour and banding differences that are visually apparent between prawns.

As part of our study to determine optimal pigmentation of farmed prawns, we measured the total astaxanthin content in a large number of prawns and observed that the amount did not always correlate well with actual colour. We presumed that this discrepancy in appearance may depend on the location of the pigments in the different body regions of the prawn (Sachindra et al., 2005). In addition, it is known that prawns are capable of changing their appearance to blend in with background colour, through movement of pigments in chromatophores in epidermal layers beneath the exoskeleton (Fingerman, 1965; Robison and Charlton, 1973). Morphological and physiological colour changes have been described in crustaceans relating to slow and rapid changes resulting from environmental or hormonal factors respectively (Rao, 1985; Melville-Smith et al., 2003). Such movements of pigments in live prawns may have a direct effect on the colour of cooked prawns. In the work described here, we report on the variation in pigmentation seen in individuals across groups of prawns, the location of pigments in various body components and how they are affected by background lighting.

## 2. Materials and methods

Prawns used in this work were obtained from experimental stocks and from commercial farms. Prawns were not assessed for moulting stage and prawns of various weights were used. However, for specific comparative trials, prawns were of a similar weight.

### 2.1. Experimental prawns

Experimental prawns (*P. monodon*) were obtained from stocks maintained at CSIRO, Marine and Atmospheric Research (CMAR) laboratories at Cleveland. Until required for experiments, prawns were grown in 2500 L fibreglass tanks. Seawater was pumped through the tanks at  $1.2 \text{ L min}^{-1}$  maintaining water temperatures and salinities at about  $28^\circ\text{C}$  and 32‰ respectively. Photoperiod prior to commencement of the trials was maintained at 12 h light:12 h dark. Prawns were fed a commercial pellet formulation (Lucky Star, Taiwan Hung Kuo Industrial Pty Ltd.) twice each day. The actual content of astaxanthin was determined and was found to be approximately 40 mg astaxanthin per kg feed.

### 2.2. Commercial frozen prawns

Whole frozen, cooked black tiger prawns (weight range approximately 20–50 g) were obtained from two commercial farms in Queensland.

### 2.3. Trial 1 – effect of background colour

*P. monodon* prawns from the same stock were grown together in black-lined tanks within the laboratory and fed a basic commercial prawn feed containing 40 mg astaxanthin/kg feed (feed and other details as stated above). The light intensity (Iso-Tech ILM350, light meter) at a distance of 10 cm above the water surface was about 10 lx. On day 0, half of the prawns ( $n=5$ ) were randomly caught and

transferred to a white-lined tank and were maintained in an environment where the light intensity was of the same illumination regime as before. All prawns continued to be fed the same commercial prawn feed and lighting conditions were maintained constant for each group. On day 28, prawns were removed from both tanks and placed into a slurry of seawater and ice for 5 min until dead and then they were removed and boiled in filtered seawater for 2 min. The mean weight of the prawns after cooking was  $9.27 \pm 0.99$  g. Although the mean weight of the prawns from the black tank was less than that from the white tank ( $8.10 \pm 1.20$  and  $10.43 \pm 1.51$  g respectively) these differences were not significant and reflected the outcome of the random sorting on day 0. Prawns were photographed and then frozen and kept at  $-20^\circ\text{C}$  until required for analysis.

### 2.4. Trial 2 – rate of change of colouration

*P. monodon* prawns of a similar size from the same stock and grown together in a black-lined tank were randomly separated into either black- or white-lined tanks at CMAR, Cleveland for 28 days ( $n=96$ ). During this time, they were each fed identical diets containing 40 mg astaxanthin/kg feed. On the day of commencement of the experiment, at 9 am, prawns were transferred from their tanks and placed in tanks of the opposite colour. Then at each time 0, 1, 3, 6, 12, 24, 72, 168 and 336 h, 6 prawns were removed and were placed in a slurry of seawater and ice for 5 min until dead. They were then removed and boiled in filtered seawater for 2 min and then cooled in ice. Prawns were photographed and frozen and kept at  $-20^\circ\text{C}$  until required for analysis. The lighting routine was continued throughout the duration of the trial which meant that prawns at 12 h had been subjected to about 4 h of darkness.

### 2.5. Subjective assessment of prawn colour

All prawns, commercial and experimental, were assessed for colour by displaying under standardised fluorescent lighting, on a large table in a food processing laboratory set to an air temperature of  $10^\circ\text{C}$ . Subjective assessment of colour was made using the grading score card (1 to 12 for lightest to darkest) for *P. monodon* (Aqua-Marine Marketing Pty Ltd., Kippa-Ring, Queensland). Seven panellists were used for Trial 1 and 14 panellists were used for Trial 2. Although the panellists were untrained for assessing prawn colour they were experienced for various food attributes (including colour) and were given scoring anchor points for extremes.

### 2.6. Relationship between grading score and astaxanthin content

Cooked commercial prawns (mean weight = 34.9 g [range 22 to 51 g],  $n=61$ ) graded for colour by the subjective assessment described in Section 2.5 were used to determine the total astaxanthin content and body distribution in prawns of different grade scores (see Figs. 2 and 3). On arrival at the laboratory the frozen prawns were thawed and in this case, assessed for grade score using a panel of 3 members to reach a consensus on each prawn. Following that, the prawns were arranged in order of grade score and the original assessment was confirmed. Each prawn was then dissected into exoskeleton, epidermal layer, cephalothorax and digestive gland for extraction of astaxanthin.

### 2.7. Carotenoid analysis

Prawns were weighed and then dissected as follows. All procedures, including extractions, were performed under conditions of low-light intensity in a darkened laboratory. In most cases, and unless mentioned otherwise, comparisons between groups of prawns were made on the pigments present in the 'prawn tail', comprising the epidermal layer of the abdominal muscle together with the abdominal

exoskeleton, including uropods and telson. This was done by removing the head and then separating the exoskeleton from the abdominal muscle. The pigmented epidermal layer was separated from the muscle taking care not to include any contents from the gastro-intestinal tract. In all cases, except for investigation of the distribution of pigment (Table 1), the abdominal muscle was not extracted since it did not contribute to prawn pigmentation. Where indicated, carotenoid analyses were also performed on individual dissected components such as abdominal exoskeleton, abdominal epidermal layer, head and digestive gland. Each tissue was weighed, finely chopped and then extracted three times with 20 mL acetone at 2°C, being allowed to stand overnight between each extraction. The prawn residues remaining following this exhaustive extraction were essentially visually devoid of pigmentation. The pooled extracts were adjusted to a total volume of 60 mL and 10 mL H<sub>2</sub>O and 5 mL n-hexane were added, mixed and allowed to phase separate. The upper layer was removed and the lower layer was washed twice with 5 mL H<sub>2</sub>O and 5 mL n-hexane. The combined upper layers containing the pigments were evaporated to dryness under nitrogen and then redissolved in 20 mL n-hexane. The concentration of astaxanthin in the extracts was determined by measuring the absorbance at 477 nm, using a molar extinction coefficient of 2172 in a 1 cm cell. Astaxanthin standard was obtained from Sigma Chemical Co. St Louis, MO, USA and lutein standard from Extrasynthese, Genay, France. From earlier analyses we knew that astaxanthin and its esters accounted for about 95% of the total carotenoids and therefore the lesser ones such as lutein were not specified but would have contributed to the total. All samples were stored in the dark, under nitrogen at -20°C until required for further analysis.

Results for astaxanthin content are expressed as µg/g wet weight of prawn component or as a percentage distribution of pigments within the individual components. For each individual prawn, the total astaxanthin (µg) extracted from each anatomical location was determined and the relative distribution was expressed on a percentage basis.

### 2.8. Separation of astaxanthin and astaxanthin esters by HPLC

Aliquots of carotenoid extracts in n-hexane were transferred to brown sample vials for separation by HPLC on a Waters system including Model 600 pump, Model 717 auto-injector and model 996 photodiode array detector. Astaxanthin was determined at 477 nm. The system was controlled by Waters Empower (2003) software. Separation was achieved using a Luna 5µm C18 (2) 100 Å 250 mm × 4.6 mm column (Phenomenex #00G-4252-E0) fitted with a guard cartridge

**Table 1**  
Effect of background colour on the content and distribution of astaxanthin (including esters) in prawns after 28 days.

| Prawn component           | Weight of components (g) | Black tank (n=5)                             | White tank (n=5)         |
|---------------------------|--------------------------|--|--------------------------|
| Whole prawn               | 9.27 ± 0.995             | Astaxanthin (µg/g prawn tail)<br>33.3 ± 5.73 | 29.1 ± 4.34              |
|                           |                          | Percentage distribution                      |                          |
| Cephalothorax             | 4.14 ± 0.461             | 42.7 ± 0.982 <sup>a</sup>                    | 34.6 ± 1.10 <sup>b</sup> |
| Abdominal epidermal layer | n.m.                     | 31.9 ± 1.58 <sup>a</sup>                     | 39.3 ± 2.27 <sup>b</sup> |
| Abdominal exoskeleton     | 0.98 ± 0.129             | 25.2 ± 0.587                                 | 25.3 ± 1.38              |
| Digestive gland           | n.m.                     | 0.28 ± 0.072                                 | 0.79 ± 0.212             |
| Abdominal muscle          | 3.91 ± 0.518             | n.d.   | n.d.                     |

Means ± SE. Prawn feed contained 40 mg astaxanthin/kg feed. Different superscripts for prawns from black and white tanks indicate a significant difference ( $p \leq 0.05$ ). n.d.: Not determined as pigment not evident. n.m.: Not measurable.

(Phenomenex #AJ0-4287). The gradient solvent system, at a flow rate of 1.5 mL/min, was as follows: solvent A (methanol:H<sub>2</sub>O, 80:20,v/v); solvent B, (ethylacetate) essentially as described by Wade et al. (2005).

### 2.9. Microscopy

Portions of the epidermal layer from the first abdominal segment of individual prawns were carefully removed from the abdominal muscle following removal of the exoskeleton. The sheets of cells were placed on a microscope slide and covered with physiological saline under a cover slip. Chromatophores were viewed using an Olympus microscope, Model BH2 and images were obtained with an attached Nikon Coolpix camera, Model 995, using a magnification of 10×4. Distance between the centres of the chromatophores was measured using a staged micrometer, and the mean distance (±SE) was found to be 380 ± 9.21 µm for prawns weighing between 20 and 30 g.

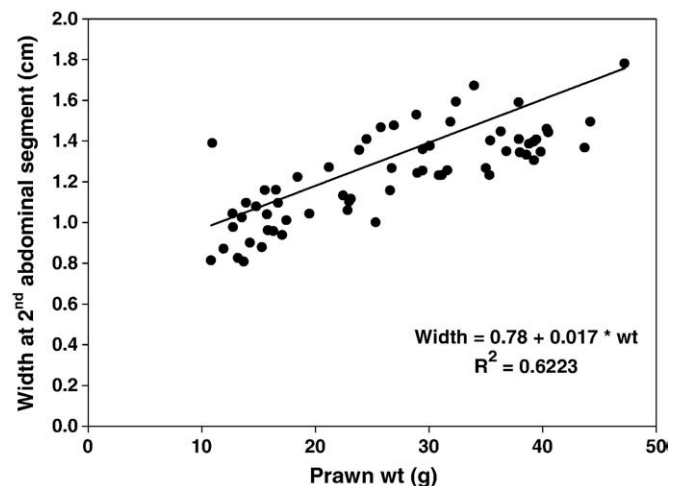
### 2.10. Statistical analysis

A confidence level of 5% was used to compare significant differences between means ( $p \leq 0.05$ ) using Students *t*-test pairwise (Microsoft Excel, XP). For the data presented in Fig. 1 we have examined the relationship between prawn weight and prawn width at the 2nd abdominal segment with linear, quadratic and cubic models. Using the Akaike Information Criterion (AIC) the linear model was chosen as the most appropriate model for the data set as it had the lowest AIC value (Sakamoto et al, 1986).

## 3. Results and discussion

### 3.1. Relationship between grading score and astaxanthin content

Determining the carotenoid content of whole prawns may provide a satisfactory indication of the adequacy of feed carotenoids level for growth or health, but it may not necessarily be the best method of expression for visualisation of prawn colour, as some pigments may be located in regions and organs such as the digestive gland, and therefore not contribute to the appearance. Also we need to contend with the relatively wide ranges in prawn weights that have been encountered during these trials. We argue on the basis of weight and visual area relationships, assessed by body width, that pigment content, expressed per weight of abdominal muscle plus abdominal exoskeleton, provides a satisfactory set of units. To support this, we



**Fig. 1.** Relationship between prawn width at the second abdominal segment and prawn weight.

have found a linear relationship ( $R^2 = 0.623$ ) between the width at the second abdominal segment (and therefore indicative of surface area) and prawn weight (Fig. 1). On this basis we have expressed weight of extracted pigment ( $\mu\text{g}$ ) per wet weight of abdominal muscle plus abdominal exoskeleton.

However, during the course of our work to determine optimal concentrations of pigment in feeds to achieve good colouration, we have measured total astaxanthin content (expressed on a weight basis of the abdominal muscle and abdominal exoskeleton) in a large number of prawns and observed that the amount did not relate well with visual appearance. This was further demonstrated by measuring the content of astaxanthin in prawns that had been subjectively visually graded into various categories using a colour grading chart. Mean grading scores of prawns ranged from 6 to 11. The relationship between grade score and total astaxanthin content is shown in Fig. 2. It can be seen that colour grading score, or visual appearance, is not tightly related to measured astaxanthin content. For example, at a grade score of 9, astaxanthin content ranged from 5 to 25  $\mu\text{g/g}$  prawn tail. Therefore, there must be some other explanation for the discrepancy observed.

3.2. Distribution of pigment within prawns

The distribution of total astaxanthin in components is presented in Fig. 3 for each grade score between 6 and 11 (range of prawns obtained). Essentially, total astaxanthin was distributed almost equally between the abdominal exoskeleton, abdominal epidermal layer and the cephalothorax (epidermal and exoskeleton not separated), with only 1–2% present in the digestive gland. These findings are essentially similar to those observed by Negre-Sandargues et al., 1993 and Paibulkichakul et al., 2008. The distribution of pigment did not effectively change across the grade scores investigated here. It should be noted that in other situations where prawns are about to moult, the percentage of total astaxanthin present in the exoskeleton is markedly reduced as the pigment is mobilised back into the epidermal layer as has been observed in western red lobster (Wade et al., 2005).

3.3. Effect of background colour on distribution of astaxanthin in live prawns

Prawns, previously raised together under identical conditions, changed their appearance after they were separated and then kept in either black or white tanks, even though they were fed the same feed rations. These differences were clearly visible in the uncooked prawns;

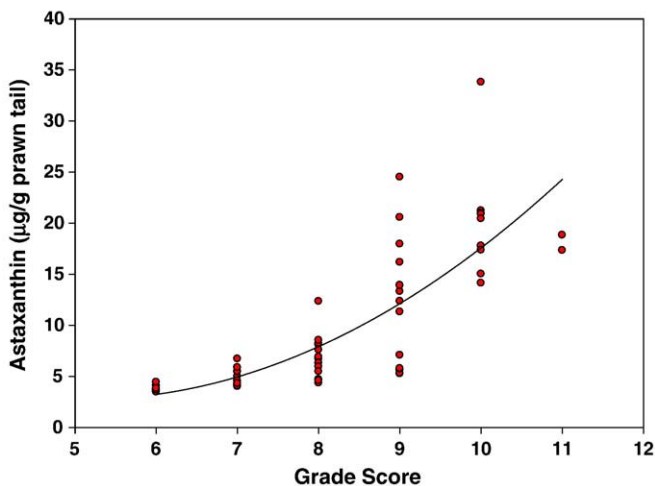


Fig. 2. Influence of total astaxanthin content on subjective grade score of prawns.

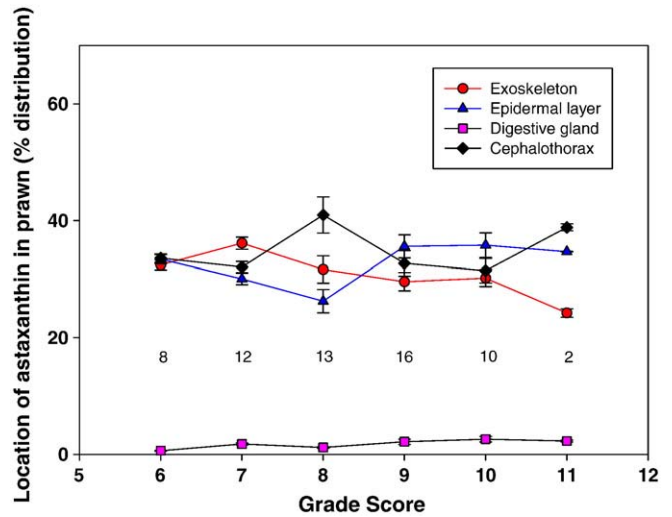
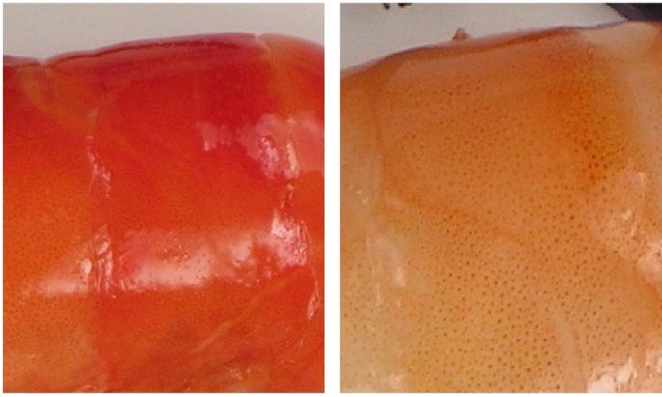


Fig. 3. Distribution of total astaxanthin (mean  $\pm$  SE,  $n$  = number of prawns indicated for each grade score) in various locations in prawns as affected by a subjective grade score.

those taken from the black tanks being dark green/brown compared with lighter pigmentation of those from the white tanks. The total astaxanthin content (Table 1) of prawns raised in black or white tanks for 28 days was not significantly different (33.3  $\mu\text{g/g}$  prawn tail for black tank compared with 29.1  $\mu\text{g/g}$  prawn tail for white tank,  $p \geq 0.05$ ) and would not be expected to result in any noticeable colour difference. However, there were marked differences in their cooked colour (Fig. 4), with prawns from black tanks being much more orange/red compared with those maintained in white tanks. Close inspection of the exoskeleton/epidermal layer (Fig. 5) revealed that the highly-coloured prawns had pigment more uniformly distributed compared with the lighter prawns which showed dense concentrations of pigments in small spots (chromatophores).



Fig. 4. Cooked prawns after growing in black tank (left) or white tank (right) for 28 days.



**Fig. 5.** Photograph showing the first abdominal segment of prawns grown in black (left) or white (right) tanks for 28 days. Densely packed chromatophores can be seen on the right compared with dispersed pigment from the prawns grown in dark tanks.

The distribution of astaxanthin in different body components was also determined in prawns after 28 days under each of the background colour conditions (Table 1). It is apparent that prawns raised in the black tanks had a greater proportion ( $p \leq 0.05$ ) of total

astaxanthin in the cephalothorax (42.7%) compared with those in the white tank (34.6%), even though the abdominal epidermal layer was higher ( $p \leq 0.05$ ) in those from the white tank (39.3% compared with 31.9%).

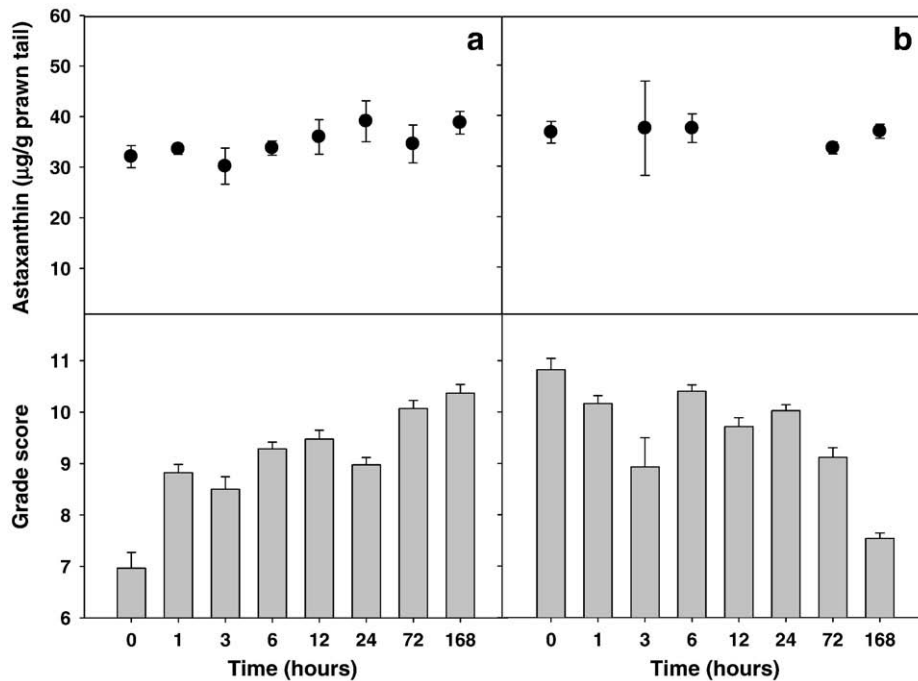
The major carotenoid pigments in prawns contributing to their colour are astaxanthin (non-esterified) and astaxanthin esters (mono- or di-esters), together with smaller amounts of other carotenoids such as lutein and  $\beta$ -carotene (Okada et al., 1994; Boonyaratpalin et al., 2001). We investigated the distribution of these pigments in three different locations, abdominal epidermal layer, abdominal exoskeleton and cephalothorax, for prawns kept in black or white tanks for 28 days (Table 2). Essentially, we found that irrespective of body location, the percentage distribution of individual pigments was very different depending on whether the prawns had been kept in black or white tanks. For prawns grown in black tanks, astaxanthin, in the non-esterified form, accounted for about 50% of the total carotenoids. The remainder largely comprised about equal proportions of astaxanthin mono-esters and astaxanthin di-esters (about 20% each), together with a small amount of lutein (5–7%). However, for prawns kept in white tanks, the distribution was very different. Non-esterified astaxanthin was very much reduced, representing only about 12% of the total carotenoid. Conversely, astaxanthin mono-esters increased from about 20% to 60% of the total. The proportion of astaxanthin di-esters did not change appreciably.

**Table 2**

Distribution (%) of carotenoids in different anatomical regions of prawns as affected by background colour; black or white tanks (means  $\pm$  SE,  $n = 5$ ).

|                         | Epidermal layer              |                              | Exoskeleton                  |                              | Cephalothorax                |                              |
|-------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                         | Black                        | White                        | Black                        | White                        | Black                        | White                        |
| Astaxanthin             | 51.9 $\pm$ 5.79 <sup>a</sup> | 12.8 $\pm$ 1.69 <sup>b</sup> | 57.3 $\pm$ 5.99 <sup>a</sup> | 12.4 $\pm$ 0.71 <sup>b</sup> | 49.2 $\pm$ 6.34 <sup>a</sup> | 13.0 $\pm$ 0.91 <sup>b</sup> |
| Astaxanthin mono-esters | 20.8 $\pm$ 7.55 <sup>a</sup> | 66.2 $\pm$ 1.53 <sup>b</sup> | 17.7 $\pm$ 7.90 <sup>a</sup> | 58.6 $\pm$ 1.68 <sup>b</sup> | 21.4 $\pm$ 8.48 <sup>a</sup> | 56.2 $\pm$ 1.80 <sup>b</sup> |
| Astaxanthin di-esters   | 22.5 $\pm$ 1.84              | 19.0 $\pm$ 3.31              | 17.5 $\pm$ 2.39 <sup>a</sup> | 27.1 $\pm$ 1.31 <sup>b</sup> | 21.9 $\pm$ 4.05              | 29.0 $\pm$ 1.93              |
| Lutein                  | 4.73 $\pm$ 0.69 <sup>a</sup> | 1.97 $\pm$ 0.22 <sup>b</sup> | 7.53 $\pm$ 1.05 <sup>a</sup> | 1.89 $\pm$ 0.09 <sup>b</sup> | 7.51 $\pm$ 1.00 <sup>a</sup> | 1.86 $\pm$ 0.07 <sup>b</sup> |

Different superscripts for each anatomical location indicate a significant difference ( $p \leq 0.05$ ) between prawns in black and white tanks.



**Fig. 6.** Effect of moving prawns from a light to dark environment (a) or dark to light environment (b) on subjective grade score and total astaxanthin content (mean  $\pm$  SE,  $n = 5$ ) at the times indicated.

This work therefore suggests that the form in which astaxanthin is present in the peripheral tissues of the prawn is dependent upon the background light conditions. Thus, it is proposed that in a darkened environment, a large proportion of astaxanthin mono-esters are hydrolysed, whereas under light conditions, the free astaxanthin is esterified with one fatty acid forming the mono-ester. The specific fatty acids involved in this process are of particular interest and are currently being investigated.

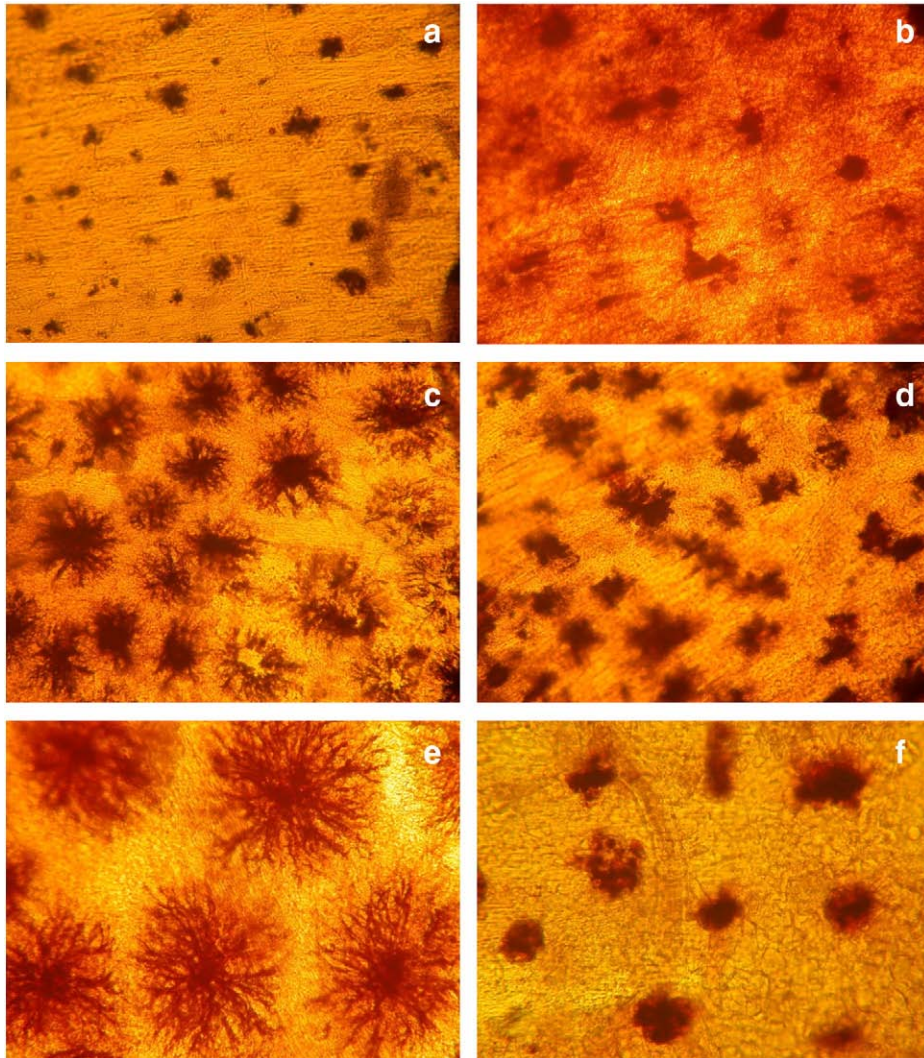
Whilst our findings relate mainly to background colour, they do appear to differ from those reported by You et al. (2006) who investigated the effects of different light sources and types of illumination on prawn colour and growth. Using *Litopenaeus vannamei*, they observed that body colour was greatest in those prawns subjected to the highest level of light intensity and suggested that high astaxanthin was a requirement under these conditions to minimise potential damage from ultraviolet light. The reason for this increased pigmentation is unclear but might relate to the greater growth of phytoplankton, bacteria etc in the water, and therefore greater availability of astaxanthin precursors, under the brighter light conditions (Tseng et al., 1998). Alternatively, the efficiency of the uptake and deposition process may be higher in prawns grown under brighter conditions.

In a controlled environmental study with juvenile rock lobsters (*Jasus edwardsii*), Stuart et al. (1996) were unable to demonstrate any

significant effects of substrate background colour (either black rocks or white gravel) on exoskeletal colour. This study observed a one-year period of growth under the specified conditions and concluded that any anecdotal differences reported by commercial lobster fishers probably was the result of genetic differences.

#### 3.4. Rate of colour change

In the previous trial, the prawns were maintained under the specified background colour conditions for 28 days before being evaluated. The following trial was undertaken to determine just how rapidly the colour changed since this could significantly affect the commercial application of the process. In this trial, prawns were maintained under the specified background conditions for 28 days prior to changed conditions at time 0 h. At this time, prawns from the white tank had similar ( $p \geq 0.05$ ) total astaxanthin contents to those from the black tank ( $32.1 \pm 2.20 \mu\text{g/g}$  prawn tail and  $36.7 \pm 2.16 \mu\text{g/g}$  prawn tail respectively). In order to obtain more accurate data (through increased numbers) on the astaxanthin content of the prawns from these two tanks, all prawns used over 168 h were analysed. Overall values were  $35.1 \pm 1.096 \mu\text{g/g}$  prawn tail for prawns moved into the white tanks and  $36.7 \pm 1.108 \mu\text{g/g}$  prawn tail for those moved into the black tanks (see Fig. 6). Although the total pigment



**Fig. 7.** Light microscopy of separated epidermal layer taken from the first abdominal segment of prawns showing chromatophores at various times after transfer between tanks (white to black; a, 0 h, c, 3 h, e, 7 days and black to white; b, 0 h, d, 3 h and f, 7 days). Overall magnification varied between individual images however, mean distance between centres of chromatophores was approximately 380  $\mu\text{m}$ .

content of the two groups of prawns was similar at 0h, there were large visual differences in the colour of the raw and cooked prawns. When graded by the panellists, cooked prawns from the white tank were significantly ( $p \leq 0.001$ ) lighter in colour, having a mean grade score of about  $7.0 \pm 0.306$ , compared with  $10.8 \pm 0.225$  for those from the black tank (Fig. 6).

However, within 1h of transferring live prawns from a white to black tank, there was a large increase in pigmentation, and when the prawns were cooked, the red colour of the prawns was assessed by the panellists to be a grade score of  $8.8 \pm 0.147$ . This was different from the prawns removed from the white tank at time 0 ( $p = 0.0148$ ). With further time, this trend continued, increasing to about grade score 10 at 72 and 168h after transfer. The transfer of prawns from black to white tanks also resulted in a change in colour, with the prawns appearing lighter in colour, but the differences during the first 1h ( $p = 0.189$ ) and 12h ( $p = 0.024$ ) were not as dramatic as was observed with the move from white to black tanks. However, by 168h, the grade score had reduced from about 11 to about 7.5, indicating a similar magnitude change to that seen when background changes were made in the other direction.

These findings indicate that colour changed quite rapidly and is likely to result from the changes in the chromatophores as indicated above (Fig. 5). It would be expected that colour changes should occur quite rapidly as prawns need to adapt to different environments through a camouflage process (Fingerman, 1965). Light microscopy of dissected epidermal layers taken from the first abdominal segment of prawns indicated that indeed, gross morphological changes occurred within a short time of prawns being moved from one environment to another (Fig. 7). Investigation of the epidermal layer from prawns maintained in a white tank until time 0h (Fig. 7a) showed small, densely pigmented chromatophores. When similar epidermal layers were viewed from prawns that had been moved to black tanks and left for 3h or 7 days (Fig. 7c and e, respectively), there had been a dispersion of pigment throughout the chromatophores, such that by day 7, pigment was over the majority of the surface, located in stellate chromatophores.

The sequence in Fig. 7 (b, d and f) shows the reverse occurring when prawns were moved from black to white tanks. Dispersed pigment (Fig. 7b) was slightly more concentrated at 3h (Fig. 7d) and tightly concentrated after 7 days (Fig. 7f).

We have shown that the degree of esterification of astaxanthin appears to be dependent upon the background colour and it is possible that it is the changed chemical form that determines its location in the chromatophores.

#### 4. Conclusions

Pigmentation is an important commercial trait for the marketing of prawns. Although natural and artificial pigments are an essential component of feed formulations and adjusted to optimise prawn colour, large variations in colouration have been observed. We conclude from this work that at least part of the reason for this variation is the ability of prawns to undergo camouflage, and to conceal or exhibit the astaxanthin pigment through movement within the chromatophores in their epidermal layers. We propose that in light surroundings, pigment is concentrated, and prawns appear lighter than when prawns are in darker environments, where pigment is more dispersed. Importantly, it is proposed that the chemical form of astaxanthin changes as it moves within the epidermal chromatophores, becoming esterified with fatty acids or alternatively, hydrolysed to free astaxanthin, as it moves from the concentrated to the dispersed state (respectively). Given that prawns darken in colour quite rapidly when subjected to black or darkened background colours, this approach provides the prawn farming industry with a

simple means to increase prawn pigmentation and reduce variability without additional feed costs. Thus, consideration of background colour conditions can have a significant impact on market value of prawns.

#### Acknowledgements

This work is a component of CSIRO's, Food Futures Flagship. We are grateful to Ms Jan Wakeling for her initial observations relating to live prawn colour in various experimental tanks. We wish to acknowledge the technical support of Ms Janet Stark, Ms Joanne Mountford and Mr Shane Beilken. Thanks also to Mr Simon Irvin for his assistance with the live prawn experiments.

#### References

- Boonyaratpalin, M., Thongrod, S., Supamattaya, K., Britton, G., Schlipalius, L.E., 2001. Effects of  $\beta$ -carotene source, *Dunaliella salina*, and astaxanthin on pigmentation, growth, survival and health of *Penaeus monodon*. *Aquaculture Research* 32 (Suppl. 1), 182–190.
- Britton, G., Armit, G.M., Lau, S.Y.M., Petal, A.K., Shone, C.C., 1981. Carotenoproteins. In: Britton, G., Goodwin, T.W. (Eds.), *Carotenoid Chemistry & Biochemistry*. Pergamon Press, Oxford, pp. 237–251.
- 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.
- Fingerman, M., 1965. Chromatophores. *Physiol. Rev.* 45, 296–339.
- Katayama, T., Hirata, K., Chichester, C.O., 1971. The biosynthesis of astaxanthin IV. The carotenoids in the prawn *Penaeus japonicus*. *Bull. Jpn Soc. Sci. Fish* 37, 614–620.
- Linan-Cabello, M.A., Paniagua-Michel, J., Hopkins, P.M., 2002. Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition* 8, 299–309.
- Melville-Smith, R., Cheng, Y.W., Thompson, A.W., 2003. Factors affecting colour change in 'white' western rock lobsters, *Panulirus cygnus*. *Journal of Experimental Marine Biology and Ecology* 291, 111–129.
- Menasveta, P., Worawattanamateekul, W., Latscha, T., Clark, J.S., 1993. Correction of black tiger prawn (*Penaeus monodon* Fabricius) coloration by astaxanthin. *Aquacultural Engineering* 12, 203–213.
- Miki, W., Yamaguchi, K., Konosu, S., 1982. Comparison of carotenoids in the ovaries of marine fish and shellfish. *Comparative Biochemistry and Physiology* 71B, 7–11.
- Negre-Sandargues, G., Castillo, R., Petit, H., Sance, S., Gomez-Martinez, R., Milicua, J.-C.G., Choubert, G., Trilles, J.-P., 1993. Utilization of synthetic carotenoids by the prawn *Penaeus japonicus* reared under laboratory conditions. *Aquaculture* 110, 151–159.
- Okada, S., Nur-E-Borhan, S.H., Yamaguchi, K., 1994. Carotenoid composition in the exoskeleton of commercial black tiger prawns. *Fisheries Science* 60, 213–215.
- Paibullkichakul, 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., 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. *Zoological Studies* 40, 371–382.
- Rao, K.R., 1985. Pigmentary effectors, in integuments, pigments and hormonal processes. In: Bliss, D.E., Mantel, L.H. (Eds.), *The Biology of Crustacea*, Vol. 9. Academic Press, New York, pp. 395–462.
- Robison Jr, W.G., Charlton, J.S., 1973. Microtubules, microfilaments, and pigment movement in the chromatophores of *Palaemonetes vulgaris* (Crustacea). *J. Experimental Zoology* 186, 279–304.
- Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike Information Criterion Statistics. In: D. Reidel Publishing Company.
- Sachindra, N.M., Bhaskar, N., Mahendrakar, N.S., 2005. Carotenoids in different body components of Indian shrimps. *J. Science Food Agriculture* 85, 167–172.
- Stuart, T., MacMillan, D.L., Thomas, M., 1996. The effect of background colour on the colour of developing juvenile rock lobsters, *Jasus edwardsii* (Crustacea: Decapoda). *Marine and Freshwater Behaviour Physiology* 27, 269–273.
- Torrissen, O., 1984. Pigmentation of salmonids – effect of carotenoids in eggs and start-feeding diet on survival and growth rate. *Aquaculture* 43, 185–193.
- Tseng, K.-F., Su, H.-M., Su, M.-S., 1998. Culture of *Penaeus monodon* in a recirculating system. *Aquacultural Engineering* 17, 138–147.
- Wade, N., Goulter, K.C., Wilson, K.J., Hall, I.M.R., Degnan, B.M., 2005. Esterified astaxanthin levels in lobster epithelia correlate with shell colour intensity: potential role in crustacean shell colour formation. *Comparative Biochemistry and Physiology*, Part B 141, 307–313.
- You, K., Yang, H., Liu, Y., Liu, S., 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.