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Quantitative methods to measure pigmentation variation in farmed Giant Tiger Prawns, *Penaeus monodon*, and the effects of different harvest methods on cooked colour

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ABSTRACT

Cooked prawn colour is known to be a driver of market price and a visual indicator of product quality for the consumer. Although there is a general understanding that colour variation exists in farmed prawns, there has been no attempt to quantify this variation or identify where this variation is most prevalent. The objectives of this study were threefold: firstly to compare three different quantitative methods to measure prawn colour or pigmentation, two different colorimeters and colour quantification from digital images. Secondly, to quantify the amount of pigmentation variation that exists in farmed prawns within ponds, across ponds and across farms. Lastly, to assess the effects of ice storage or freeze-thawing of raw product prior to cooking. Each method was able to detect quantitative differences in prawn colour, although conversion of image based quantification of prawn colour from RGB to *Lab* was unreliable. Considerable colour variation was observed between prawns from different ponds and different farms, and this variation potentially affects product value. Different post-harvest methods prior to cooking were also shown to have a profound detrimental effect on prawn colour. Both long periods of ice storage and freeze thawing of raw product were detrimental to prawn colour. However, ice storage immediately after cooking was shown to be beneficial to prawn colour. Results demonstrated that darker prawn colour was preserved by holding harvested prawns alive in chilled seawater, limiting the time between harvesting and cooking, and avoiding long periods of ice storage or freeze thawing of uncooked product.

1. Introduction

Most prawns have thin opaque shells, and colour is present in the hypodermal layer in pigment structures, known as chromatophores (Rao, 1985). These structures are known to expand and contract which strongly contributes to the degree of individual colouration (Fingerman, 1965), particularly in response to the colour of the substrate the animal is exposed to. The colour itself is due to the presence of the carotenoid astaxanthin (Axn) in the hypodermal tissue and in the exoskeleton (Katayama et al., 1971). Like all crustaceans, pigmentation in the Black Tiger Prawn, *Penaeus monodon*, is known to be produced by the interaction between Axn and a protein called crustacyanin (CRCN) (Zagalsky, 1985). This interaction turns the colour of Axn from red to blue, but when prawns are cooked this interaction is disrupted, releasing the red colour once again and providing the distinct red colouration of cooked crustaceans. This colour has been shown to be a strong element in consumer preference and acceptance (Erickson et al., 2007; Parisenti et al., 2011a), with consistently dark red coloured animals attracting premium prices.

Differences in prawn colouration can be potentially due to a range of factors including carotenoid availability in the diet, background substrate colour, photoperiod, light intensity, stress, temperature or genetics (Latscha, 1989; Rao, 1985). Some of these changes are rapid, reversible, rhythmic and under the control of eyestalk hormones (Kleinholz, 1961; Rao, 2001), while others are slower and potentially more permanent, involving modifications of exoskeletal pigment concentration or composition. The best studied effectors of prawn pigmentation have been dietary Axn incorporation and exposure to different coloured substrates. Prawn colouration is dependent largely upon the amount of Axn present within these tissues, with dietary Axn levels of up to 200 mg/kg shown to be most effective for optimal colouration in *P. monodon* (Boonyaratpalin et al., 2001; Howell and Matthews, 1991; Menasveta et al., 1993). However, total prawn Axn content does not correlate well with prawn colour (Tume et al., 2009). Short-term exposure to black substrates has also been shown to improve prawn pigmentation through expansion of epithelial chromatophores (Parisenti et al.,

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2011b; Tume et al., 2009). An increase in the abundance of epithelial CRCN protein was further demonstrated to be the underlying cause of these pigment improvements (Wade et al., 2012).

The attribute of prawn colour is frequently used as an assessment tool to measure change over time. Cooked prawn colour is commercially scored by subjective comparison of individuals against either an Australian Tiger Prawn Colour Chart (Aqua Marine Marketing), or an international SalmoFan colour scale (DSM Nutritional Products). It is well understood that these subjective methods are particularly susceptible to measurement bias, and comparison across experiments is often difficult. Colorimeters have been developed to quantify the absolute colour of a sample measured on a three dimensional scale of value, hue and chroma, using the Commission Internationale de l'Eclairage (CIE) '*Lab*' system of colour notation (Publication CIE No 15., 2004). The value of colour (or lightness represented by '*L*') has a scale of 0 (pure black) to 100 (pure white). The hue has two components that distinguish opposing colours. The first is '*a*' which represents the red-green scale, and the other is '*b*' which represents the blue-yellow scale. Chroma (or saturation) indicates the amount of hue, positive '*a*' towards red, negative '*a*' towards green and positive '*b*' towards yellow, negative '*b*' towards blue. Although using this standardised colour notation system, different colorimeters have different physical parameters by which that colour is measured, including light source, reflectance geometry, and aperture size.

Colour has successfully been quantified in prawns using colorimeters (Parisenti et al., 2011b; Wade et al., 2012), but while using standardised D65 illumination these different types of colorimeters use a different light reflectance angle to quantify colour. Additionally, the use of digital images to quantify colour in live organisms is common, and has been successfully used to quantify shell pigments in mangrove crabs (Todd et al., 2011) and clawed lobsters (Tlusty and Hyland, 2005). However, there is currently no standard methodology accepted as the method of choice for conducting such assessments. Researchers use the tools they have at hand and there is no knowledge of whether results arising from different methodologies can be directly compared from one study to another. The objectives of this study were firstly to assess three different quantitative methods to measure prawn colour, two different colorimeters and colour quantification from digital images, and define the ability to compare colour values from these three methods. Secondly, to use these methods to quantify whether any significant variation exists between the colour of farmed *P. monodon* from different ponds, or from different farms. Lastly, to assess how different types of harvest method, specifically how ice storage and freeze-thawing prior to cooking, affect the colour of farmed *P. monodon*.

2. Material and methods

2.1. Quantitative and subjective measurement of prawn colour

Prawn colour was quantified using the average colour of the first three abdominal segments measured using three different methods. The first used a HunterLab MiniScan XE colorimeter with a 10 mm aperture and D65 illumination at a 45° angle as used in Wade et al. (2012). The second used a Minolta CR-400 Chroma Meter with an 8 mm aperture and D65 illumination at a 10° angle as used in Parisenti et al. (2011b). The third method used digital images taken at a distance of 40 cm using a Canon D-400 (Canon) fitted with an 18 mm lens, with fixed settings of ISO1600, aperture F22 and 1/100th of a second shutter speed. Animals were photographed in a 38 × 50 cm light box illuminated with two 30 cm FluroGlow single reflector full spectrum 8W aquarium lights (AquaOne). Average RGB values were calculated across a 3600 pixel square from the first three abdominal segments using ImageJ software (Schneider et al., 2012). Where necessary, image intensity was adjusted between photographs using the MacBeth ColorChecker that was positioned in each photograph (Supplementary Fig. 1A). Subjective scoring was performed against both the Lineal Salmofan (DSM Nutritional Products) and Australian Tiger Prawn Colour Chart (Aquamarine Marketing) under standardised illumination by experienced researchers.

Validation of the digital image method was performed by quantification of the MacBeth ColorChecker, SalmoFan and Prawn Colour Chart values measured from 10 independent photographs (Supplementary Fig. 1B). Comparison of the three different methods was performed using the MacBeth ColorChecker, as well as the colour values quantified from the same randomly selected 45 cooked prawns. Due to the size of the animals, colour quantification for the 45 animals from digital images was performed across three photographs containing 15 animals each. RGB values from digital images were converted to *Lab* values using standard colour conversion algorithms (Nishad and Chezian, 2013) and validated using measurements of the MacBeth ColorChecker from 10 independent photographs (Supplementary Fig. 1C).

2.2. Colour variation within and across ponds and across farms

Prawn colour variation was assessed from different ponds from the one farm using a HunterLab MiniScan XE colorimeter. Fifty prawns were selected at random from holding bins immediately after harvesting from different ponds. The average *Lab* reading from the first 3 abdominal segments was used as the measure of colour for individual prawns. Individuals were tagged, colour measured raw, then cooked in commercial salt brine boilers and re-measured on the cooked prawns. All animals were from domesticated stocks of the same genetic origin, and fed the same commercial diet according to an optimal pigmentation regime that incorporated 50 ppm astaxanthin for at least 4 weeks before harvest and sampling. To assess colour variation between groups, each individual *L*, *a* and *b* colour value was standardised by subtracting the mean value of the entire group. These individual *delta L*, *delta a* and *delta b* values were used to assess the mean and variance for each group of animals. This transformation also allowed effective comparison of measurements performed using different colorimeters despite their difference in absolute colour value.

Comparison of prawn colour was performed from four different farms using a Minolta CR-400 Chroma Meter. A random sample of 40 cooked animals and measured, having been harvested from a mixture of different ponds and processed at the separate farms on the day of sampling. The average *Lab* reading from the first 3 abdominal segments was used as the measure of colour for individual prawns. Similar to above, to assess colour variation between groups each individual *L*, *a* and *b* colour value was standardised by subtracting the mean value of the entire group. These individual *delta L*, *delta a* and *delta b* values were used to assess the mean and variance for each group of animals.

2.3. Effect of harvest method on colour

To measure the effect of harvesting live prawns in chilled seawater, prawns from the same pond were held alive in aerated 12 °C filtered seawater in large covered 800 L bins. Twenty animals were collected immediately after harvesting, individually tagged and colour measured a using a HunterLab MiniScan XE. These same 20 animals were recovered and re-measured at 30 min, 1 h, 2 h and 4 h after the initial measurement. Similarly, to measure the effect of harvesting prawns into an ice slurry, 20 prawns were individually tagged immediately after harvesting and colour measured using a HunterLab MiniScan XE. These animals were held in a slurry of ice and filtered seawater and the colour of each one re-measured every hour over an eight-hour period. The change in absolute colour over time for both these groups was calculated by subtracting the average initial *Lab* value from each of the measured *Lab* values of the 20 prawns at each time point. These individual *delta L*, *delta a* and *delta b* values were used for comparison over time.

To assess the effect of freeze-thawing on uncooked prawn colour, 50 prawns were colour measured raw using a HunterLab MiniScan XE,