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

4

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17

18 **Abstract**

19 Cooked prawn colour is known to be a driver of market price and a visual  
20 indicator of product quality for the consumer. Although there is a general  
21 understanding that colour variation exists in farmed prawns, there has been no  
22 attempt to quantify this variation or identify where this variation is most  
23 prevalent. The objectives of this study were threefold: firstly to compare three  
24 different quantitative methods to measure prawn colour or pigmentation, two  
25 different colorimeters and colour quantification from digital images. Secondly, to  
26 quantify the amount of pigmentation variation that exists in farmed prawns  
27 within ponds, across ponds and across farms. Lastly, to assess the effects of ice  
28 storage or freeze-thawing of raw product prior to cooking. Each method was able  
29 to detect quantitative differences in prawn colour, although conversion of image  
30 based quantification of prawn colour from RGB to Lab was unreliable.  
31 Considerable colour variation was observed between prawns from different  
32 ponds and different farms, and this variation potentially affects product value.  
33 Different post-harvest methods prior to cooking were also shown to have a

34 profound detrimental effect on prawn colour. Both long periods of ice storage  
35 and freeze thawing of raw product was detrimental to prawn colour. However,  
36 ice storage immediately after cooking was shown to be beneficial to prawn  
37 colour. Results demonstrated that darker prawn colour was preserved by  
38 holding harvested prawns live in chilled seawater, limiting the time between  
39 harvesting and cooking, and avoiding long periods of ice storage or freeze  
40 thawing of uncooked product.

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43 Keywords:

44 Shrimp, color, astaxanthin

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## 47 **1 Introduction**

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

63

64 Differences in prawn colouration can be potentially due to a range of  
65 factors including carotenoid availability in the diet, background substrate colour,  
66 photoperiod, light intensity, stress, temperature or genetics (Rao, 1985; Latscha  
67 1989). Some of these changes are rapid, reversible, rhythmic and under the  
68 control of eyestalk hormones (Kleinholz, 1961; Rao, 2001), while others are  
69 slower and potentially more permanent, involving modifications of exoskeletal  
70 pigment concentration or composition. The best studied effectors of prawn  
71 pigmentation have been dietary Axn incorporation and exposure to different  
72 coloured substrates. Prawn colouration is dependant largely upon the amount of  
73 Axn present within these tissues, with dietary Axn levels of up to 200 mg/kg  
74 shown to be most effective for optimal colouration in *P. monodon* (Howell and  
75 Matthews 1991; Menasveta et al., 1993; Boonyaratpalin et al., 2001). However,  
76 total prawn Axn content does not correlate well with prawn colour (Tume et al.,  
77 2009). Short-term exposure to black substrates has also been shown to improve  
78 prawn pigmentation through expansion of epithelial chromatophores (Tume et  
79 al., 2009; Parisenti et al., 2011a). An increase in the abundance of epithelial CRCN

80 protein was further demonstrated to be the underlying cause of these pigment  
81 improvements (Wade et al., 2012).

82

83 Cooked prawn colour is commercially scored by subjective comparison of  
84 individuals against either an Australian Tiger Prawn Colour Chart (Aqua Marine  
85 Marketing), or international Salmofan colour scale (DSM Nutritional Products).  
86 Prawn colour has successfully been quantified using colorimeters (Parisenti et al  
87 2011; Wade et al., 2012). These machines quantify colour using the Commission  
88 Internationale de l'Eclairage (CIE) 'Lab' system of colour notation (Publication  
89 CIE No 15, 2004). The absolute colour of a sample is measured on a three  
90 dimensional scale of value, hue and chroma. The value of colour (or lightness  
91 represented by *L*) has a scale of 0 (pure black) to 100 (pure white). The hue has  
92 two components that distinguish opposing colours. The first is '*a*' which  
93 represents the red-green scale, and the other is '*b*' which represents the blue-  
94 yellow scale. Chroma (or saturation) indicates the amount of hue, positive '*a*'  
95 towards red, negative '*a*' towards green and positive '*b*' towards yellow, negative  
96 '*b*' towards blue. Additionally, the use of digital images to quantify colour in live  
97 organisms is common, and has been successfully used to quantify shell pigments  
98 in mangrove crabs (Todd et al., 2011) and clawed lobsters (Tlusty and Hyland  
99 2005).

100 The objectives of this study were firstly to assess three different  
101 quantitative methods to measure prawn colour, two different colorimeters and  
102 colour quantification from digital images, and the ability to compare colour  
103 values from these three methods. Secondly, to use these methods to quantify any  
104 variation that exists between the colour of farmed *Penaeus monodon* from  
105 different ponds, or from different farms. Lastly, to assess how different types of  
106 harvest method, specifically how ice storage and freeze-thawing prior to cooking,  
107 affect the colour of farmed *Penaeus monodon*.

108

109

110

## 111 2 Material and Methods

### 112 2.1 Quantitative and Subjective Measurement of Prawn Colour

113 Prawn colour was quantified using the average colour of the first three  
114 abdominal segments measured using three different methods. The first used a  
115 HunterLab Mini Scan XE colorimeter with a 10 mm aperture and D65  
116 illumination at a 45° angle. The second used a Minolta CR-400 Chroma Meter  
117 with an 8 mm aperture and D65 illumination at a 10° angle. The third method  
118 used digital images taken at a distance of 40 cm using a Canon D-400 (Canon)  
119 fitted with an 18 mm lens, with fixed settings of ISO1600, aperture F22 and  
120 1/100<sup>th</sup> sec shutter speed. Animals were photographed in a 38 x 50 cm light box  
121 illuminated with 2 x 8W 30 cm Fluroglow single reflector full spectrum aquarium  
122 lights (AquaOne). Average RGB values were calculated across a 3600 pixel  
123 square from the first three abdominal segments using ImageJ software  
124 (Schneider et al. 2012). Where necessary, image intensity was adjusted between  
125 photographs using the MacBeth ColorChecker that was positioned in each  
126 photograph (Supplementary Figure 1A). Subjective scoring was performed  
127 against both the Lineal Salmofan (DSM Nutritional Products) and Australian  
128 Tiger Prawn Colour Chart (Aquamarine Marketing) under standardised  
129 illumination by experienced researchers.

130 Validation of the digital image method was performed by quantification of  
131 the MacBeth colour checker, Salmofan and prawn colour chart values measured  
132 from 10 independent photographs (Supplementary Figure 1B, Supplementary  
133 Table 1). Comparison of the three different methods was performed using the  
134 MacBeth color checker, as well as the colour values quantified from the same  
135 randomly selected 45 cooked prawns. Due to the size of the animals, colour  
136 quantification for the 45 animals from digital images was performed across three  
137 photographs containing 15 animals each. RGB values from digital images were  
138 converted to *Lab* values using standard colour conversion algorithms (Nishad  
139 and Chezian, 2013) and validated using measurements of the MacBeth color  
140 checker from 10 independent photographs (Supplementary Figure 1C).

141

142 2.2 *Colour Variation Within and Across Ponds and Across Farms*

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

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

167

168 2.3 *Effect of Harvest Method on Colour*

169 To measure the effect of harvesting prawns live in chilled seawater,  
170 prawns from the same pond were held live in aerated 12°C filtered seawater in  
171 large covered 800 L bins. Twenty animals were collected immediately after  
172 harvesting, individually tagged and colour measured using a HunterLab  
173 Miniscan XE. These same 20 animals were recovered and re-measured at 30 min,  
174 1 hour, 2 hours and 4 hours after the initial measurement. Similarly, to measure

175 the effect of harvesting prawns into an ice slurry, 20 prawns were individually  
176 tagged immediately after harvesting and colour measured using a HunterLab  
177 Miniscan XE. These animals were held in a slurry of ice and filtered seawater and  
178 the colour of each one re-measured every hour over an eight-hour period. The  
179 change in absolute colour over time for both these groups was calculated by  
180 subtracting the average initial *Lab* value from each of the measured *Lab* values of  
181 the 20 prawns at each time point. These individual *delta L*, *delta a* and *delta b*  
182 values were used for comparison over time.

183 To assess the effect of freeze-thawing on uncooked prawn colour, 50  
184 prawns were colour measured raw using a HunterLab Miniscan XE, then frozen  
185 for one day, thawed at room temperature for 1 hour and colour re-measured. To  
186 assess the effect of ice slurry storage on cooked prawn colour, 50 cooked prawns  
187 were colour measured using a HunterLab Miniscan XE, then placed in an ice  
188 slurry for 14 hours, then colour measured again. The change in absolute colour  
189 for these treatments was calculated by subtracting the average initial *Lab* value  
190 from each of the measured *Lab* values of the 50 prawns after being re-measured.  
191 These individual *delta L*, *delta a* and *delta b* values were used for comparison  
192 before and after treatment.

193

#### 194 2.4 Statistical Analysis

195 Where required, statistical significance was assessed by single factor  
196 analysis of variance (ANOVA), followed by Tukey's HSD test allowing 5% error.  
197 F-Test for significant differences in variance between two groups was performed  
198 after Kolmogorov-Smirnov/Lilliefors Test for data normality. All statistical  
199 analyses were performed using StatPlus:Mac 2009 (AnalystSoft Inc, 2009).

200

## 201 **3 Results**

### 202 *3.1 Quantitative Methods for Measuring Prawn Colour*

203 Absolute *Lab* and RGB values from each of the three methods were obtained  
204 from the average of 45 randomly selected animals (Table 1). There was  
205 considerable difference between the average absolute *Lab* values measured with  
206 the different colorimeters. This was expected given the different light incident  
207 angles that the machines have for measurement. We recorded a strong linear  
208 relationship between values of the MacBeth color checker measured with each  
209 colorimeter (Supplementary Figure 1B). Despite this relationship, a simple linear  
210 model was not sufficient to convert *Lab* values from one machine to the *Lab*  
211 values of the other (data not shown). In addition, we also observed a strong  
212 relationship between the *Lab* values of each machine using individual prawns  
213 (Figure 1A).

214 Using digital images and the MacBeth color checker it was possible to reliably  
215 measure colour (Supplementary Figure 1B), and convert RGB values to *Lab*  
216 values (Supplementary Figure 1C). Image quantification could also reliably  
217 reproduce the colour scales of the Lineal Salmofan (DSM Nutritional Products)  
218 and the Australian Tiger Prawn Colour Chart (Aquamarine Marketing), which are  
219 the internationally recognised subjective methods for subjectively grading  
220 prawn colour (Supplementary Table 1). However, using cooked prawns the  
221 conversion of RGB values to *Lab* values did not show any relationship with  
222 measured *Lab* values from colorimeters (Figure 1B).

223

### 224 *3.2 Colour Variation in Farmed Prawns*

225 It was hypothesised that significant variation existed between the colour of  
226 prawns from different ponds. This was found to be true, with farmed prawns  
227 showed considerable variation in colour between ponds when either raw or  
228 cooked. The mean absolute *Lab* values were significantly different between  
229 animals from different ponds, for both their raw colour and their cooked colour  
230 (Table 2). For raw prawns, the *L* values were particularly informative. When  
231 transformed relative to the average of all samples, data showed that raw prawns  
232 from ponds 2 and 3 were significantly higher than those from ponds 1 and 4  
233 (Figure 2A). This result indicated that prawns from ponds 2 and 3 were

234 significantly lighter than those from ponds 1 and 4. The mean *a* and *b* values of  
235 cooked prawns were most informative, and significant differences were  
236 observed between animals from different ponds (Table 2). When transformed  
237 relative to the average of all animals, higher *a* and *b* values indicated the  
238 presence of more red and yellow hues, respectively, and therefore a darker  
239 coloured prawn. Groups of prawns from different ponds also showed different  
240 amounts of colour variation within each group. The variance of *L* and *a* values of  
241 cooked colour was significantly higher for some ponds than for others (Table 2),  
242 and was reflected by the greater spread of the interquartile range for some  
243 ponds (Figure 2B). This indicated there was a greater amount of individual  
244 colour variation in some ponds compared with others. Interestingly, *b* values did  
245 not show any significant differences in variance between ponds. Some weak  
246 correlations were observed between raw *Lab* values and cooked *Lab* values, the  
247 best of which was a negative correlation between raw *L* value and cooked *a* value  
248 ( $r^2 = 0.161$ ). This indicated that an increase in *L* value of an uncooked prawn  
249 would result in a decrease in the *a* or 'redness' value of the cooked prawn.  
250 When comparing cooked prawn colour across farms, similar variation was  
251 observed. The absolute *Lab* values of cooked prawns was significantly different  
252 between animals from four farms (Figure 3). In some instances, such as farm 1,  
253 lower absolute *a* values were recorded, along with higher *L* values, indicating  
254 that prawns had a lighter colour with less red. Other farms, such as farm 2,  
255 recorded significantly elevated *a* values and slightly reduced *L* values, indicating  
256 darker and deeply red coloured prawns.  
257 Although the methods differed slightly, some comparison was possible between  
258 the absolute *Lab* values recorded for uncooked and cooked *Penaeus monodon* in  
259 this study and the *Lab* values recorded for *Penaeus vannamei* (Parisenti et al  
260 2011b). Raw *L* readings confirmed that *Pmon* ( $L = 16.02$ ) was a much darker  
261 colour than even the most pigmented *Pvan* ( $L = 27.99$ ), and this translated into a  
262 lower cooked colour *L* value (*Pmon*  $L = 40.98$ ; *Pvan*  $L = 61.49$ ). Uncooked *a* and *b*  
263 values showed some small differences, but were all close to zero. However, the  
264 cooked *a* or 'redness' value was markedly higher in *Pmon* ( $a = 38.62$ ) than in  
265 *Pvan* ( $a = 27.25$ ), while *b* values were similar (*Pmon*  $b = 36.04$ ; *Pvan*  $b = 38.34$ ).

266 This supports the notion that uncooked *L* values are the best indicator of cooked  
267 *a* value, and therefore the deep red cooked colour preferred by consumers.

268

### 269 3.3 *Effect of Live Holding in Bins on Colour of Prawns*

270 As a common harvest method, prawns are transferred in large bins from the  
271 harvest pond to the processing shed. It was hypothesised that significant  
272 variation in prawn colour may occur during this process, which may negatively  
273 impact cooked prawn colour. Animals that had been held live in covered and  
274 aerated 800 L bins for different periods of time showed very little change in  
275 cooked colour after up to 4 hours holding prior to cooking (Figure 4A). Only the *b*  
276 value of animals sampled at 30 min and the *L* value of animals sampled at 4  
277 hours were significantly different from the values of animals sampled at other  
278 times. Subjective scores showed that animals retained scores of between 9 and  
279 10 on the Prawn Colour Chart, and 29 on the Salmofan throughout holding (data  
280 not shown). Although prawns have been shown to rapidly respond to the colour  
281 of their surroundings, such as the colour of holding bins (Tume et al., 2009), bins  
282 used in this study had lids that completely blocked the light while animals were  
283 being held. This method was shown to be highly effective at preserving prawn  
284 colour during holding prior to cooking.

285

### 286 3.4 *Effect of Ice Storage or Freezing on Colour of Prawns*

287 Although less common, other commercial harvest methods include direct  
288 immersion of prawns into an ice slurry, or freezing of raw product. It was  
289 hypothesised that these methods were adversely affecting cooked prawn colour.  
290 To assess the effect of ice storage prior to cooking, the same 20 prawns were  
291 colour measured over time during ice storage. We measured a significant  
292 increase in the *L* value after 4 hours while the *a* and *b* values were unaffected  
293 (Figure 4B). In a similar experiment, a group of 50 prawns was measured before  
294 and after 14 hours of ice storage. Animals after this ice storage period showed a  
295 significant increase in their average measured *Lab* values, coupled with a  
296 significant increase in *L* variance (Table 3). The effect of freeze thawing was  
297 assessed using another 50 prawns measured before being frozen and  
298 remeasured once thawed. This treatment also caused a significant increase in

299 each of the measured *Lab* values, and a significant increase in the variance of the  
300 measured *L* values (Table 3).

301 After cooking, prawns are held overnight in large bins containing a salt brine ice  
302 slurry to improve flavour and storage life, but the effect of this treatment on  
303 colouration has not been quantified. To assess the impact of ice-storage after  
304 cooking, the same group of 50 prawns was measured immediately after cooking  
305 and again after 14 hours in an ice slurry. Results showed there was a small but  
306 significant decrease in the measured *L* value of cooked prawns after being held in  
307 an ice slurry, along with a significant increase in the *a* and *b* values (Table 3).  
308 Variance was not significantly changed in any of the *Lab* values after freeze  
309 thawing. This indicated the presence of more red and yellow hues, and  
310 demonstrated this treatment was having a positive effect on prawn colour.

## 311 4 Discussion

312 Our results demonstrate that quantitative differences in individual prawn colour  
313 can be detected by either colorimeter as well as digital images. However, at  
314 present the values measured from prawns using the different techniques cannot  
315 be accurately interconverted. Some improvements in the error rate of  
316 conversion of RGB values to *Lab* values may be possible using neural network  
317 models, instead of linear models such as those used in this study (León et al.,  
318 2004). However, the accuracy of conversion of the MacBeth color checker  
319 suggests that the errors are perhaps not occurring during conversion. It is far  
320 more likely that the inability to convert RGB values from images of prawns to  
321 *Lab* values measured from colorimeters is due to the inconsistency of  
322 measurement with the smaller aperture of the colorimeter. In the past, the use  
323 of colorimeters has been criticised due to the small area represented by the  
324 machine, and that aspects of the overall colour are lost (Mendoza and Aguilera,  
325 2004; Papadakis, et al., 2000). This may be particularly evident with the spatial  
326 variation in colour across prawn segments, and highlights the importance of  
327 establishing a consistent location for colour measurement methods. Given these  
328 difficulties, it is not recommended that conversion of colour values be performed  
329 from images to colorimeters, but data from different colorimeters can potentially  
330 be compared. Although images were not extensively used in this study, they  
331 represent an inexpensive, rapid and accurate method for assessing prawn colour.

332

333 This study quantified the variation that existed in farmed prawns, and  
334 demonstrated that there are significant colour differences both between farms  
335 and more interestingly between ponds at the same farm. Some of the observed  
336 variation may be due to a range of farm specific conditions, such as different  
337 pigmentation regimes in feeds, lined or earthen ponds or different pond algal  
338 densities. Carotenoid inputs from pelleted feeds were consistent across ponds  
339 measured (50 mg/kg), although differences in the total amount of feed intake for  
340 different ponds cannot be accounted for. Although not measured specifically in  
341 this study, a large amount of variation has been shown to exist in the  
342 phytoplankton, algal and bacterial populations of different prawn ponds  
343 (Burford 1997, Xiong et al., 2014). While the diversity of species was similar

344 across ponds, the abundance of species varied markedly and rapidly, often  
345 relative to the amounts nutrients available in the water (Burford 1997, Xiong et  
346 al., 2014). Potential effects of this pond to pond variation on pigmentation  
347 include different levels of cyanobacteria capable of producing carotenoids that in  
348 turn affect carotenoid intake. In addition, variation in pond dynamics can  
349 potentially affect two other known effectors of crustacean colour: light intensity  
350 (Pan et al., 2001) and background substrate colour (Tume et al., 2012). *Penaeus*  
351 *mondon* postlarvae cultured under constant light conditions recorded a higher  
352 total Axn concentration than those in constant darkness, and this effect was  
353 attributed to increased production and accumulation of Axn in algae within the  
354 tank that was in turn ingested by the animals (Pan et al., 2001). Prawn colour  
355 was also shown to be rapidly darkened by exposure to dark coloured  
356 background substrates, but there was no change in Axn concentration (Tume et  
357 al., 2009). Potential variation in the colour of pond substrates could not be  
358 quantified in this study, but this may influence the colour of the final cooked  
359 product. Other reports of the effect of harvest stress on pigmentation are largely  
360 anecdotal, with no scientific methods employed to specifically investigate any  
361 potential effect. Given the colour variation measured from individual ponds from  
362 one farm, the quantified colour variation across farms was more likely due to the  
363 variation produced by the conditions within a particular pond at the time of  
364 harvesting. Identifying the true source of the measured variation in prawn colour  
365 was beyond the scope of this project, and would require a much more detailed  
366 study with few additional benefits to the current study.

367 Although it was not possible to predict the precise effect on cooked colour from  
368 the measured raw *Lab* values, it was possible to infer the effect from on cooked  
369 colour from the negative correlation recorded earlier between raw *L* value and  
370 cooked *a* value. This demonstrated that prawns that recorded a higher raw *L*  
371 values were not only lighter in colour before cooking, but would record lower *a*  
372 values when cooked and were therefore less pigmented. By measuring the same  
373 prawns at different times and through different treatments, this study eliminated  
374 the variability that had been recorded between individual prawns. Results  
375 showed that uncooked prawns that were either held on ice for periods longer  
376 than 4 hours or frozen and then thawed became significantly paler in colour. The

377 effect of freeze thawing raw product was a similar magnitude to that seen over 8  
378 hours of ice storage, and would result in a less pigmented product, a lower colour  
379 grade score and corresponding lower price. Very few studies have been done in  
380 this area. Flavour has been enhanced in *Macrobrachium rosenbergii* by post-  
381 harvest salt acclimation (Schilling et al., 2013), but any potential effects of ice  
382 storage on colour were not assessed. Despite improving flavour, this study  
383 shows that perceived quality may be adversely affected due to such pre-cooking  
384 treatments. This finding may also be relevant for the holding of prawns during  
385 wild fisheries operations. Although impractical to immediately cook prawns at  
386 time of harvest, the method by which they are stored on board the trawler may  
387 significantly impact product quality. Prior to the development of accurate and  
388 unbiased methods in this study, and the ability to correlate raw prawn colour  
389 with cooked colour, the effects of different harvest methods could not be  
390 quantified.

391 Once cooked, prawns are often preserved in salted ice slurry overnight to  
392 improve flavour and shelf-life in storage. However, the effect of this treatment on  
393 colour has not been quantified in the past. This study demonstrated that post-  
394 cooking storage in an ice slurry was having minimal, if not a slightly beneficial,  
395 effect on prawn colour. Cooking time has been shown to affect the appearance of  
396 dark spots during frozen storage of *Penaeus vannamei* (Manheem et al., 2013),  
397 but the effect on absolute colour was not assessed. The ability to preserve this  
398 cooked colour during frozen storage under commercial conditions is currently  
399 under further investigation.

400 The focus of this study was to identify and quantify whether any variation  
401 existed in pigmentation under different commercial conditions and from  
402 commercial different farms. What is evident is that the differences in perceived  
403 quality, and therefore price, are affected by conditions during commercial grow-  
404 out and harvesting. Based on the results of this study, it is recommended that  
405 prawns be held live during harvest prior to cooking and processed as quickly as  
406 possible after harvest. Salt brining post cooking is beneficial to both colour and  
407 flavour. The information from these studies provides industry a sound basis for  
408 product handling decisions during processing of prawns to retain maximum red  
409 colouration of product.

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416

417

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498 **7 Figure Legends**

499

500 **Figure 1. Comparison of quantitative methods of measuring prawn colour.**

501 The colour of the same 45 cooked prawns was quantified using two different  
502 colorimeters and also digital images. Comparison of the absolute *Lab* values  
503 taken using a Minolta CR-400 Chroma Meter and a HunterLab Miniscan XE  
504 colorimeter (A). Comparison of the absolute *Lab* values taken using a HunterLab  
505 Miniscan XE colorimeter and the absolute RGB values quantified from digital  
506 images that had been converted to *Lab* values using standard algorithms (B).

507

508 **Figure 2. Colour Variation in Farmed Prawns Across Ponds.**

509 The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values  
510 from fifty prawns from seven different ponds when uncooked (A) and cooked  
511 (B). The delta *Lab* values were calculated as the difference in the value of each  
512 individual from the average of all the animals across the 7 ponds. Significant  
513 differences in mean and variance between groups are shown in Table 2.

514

515 **Figure 3. Colour Variation in Farmed Prawns Across Farms.**

516 The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values  
517 from forty cooked prawns from a further four different farms measured using a  
518 Minolta Chroma Meter. The delta *Lab* values were calculated as the difference in  
519 the value of each individual from the average of all the animals across the four  
520 farms.

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522 **Figure 4. Colour change in uncooked prawns over time.**

523 Prawns harvested from the same pond were held in large 800 L aerated bins that contained  
524 seawater at 12°C. Twenty animals were taken at random immediately after  
525 harvesting and after being held in the bins for different lengths of time. Animals  
526 were cooked and then quantitatively colour measured using a HunterLab  
527 colorimeter. Results are shown as delta *Lab*, which is the difference in absolute  
528 *Lab* colour value at each time point relative to the initial sample. \* denote  
529 significant ( $P < 0.05$ ) differences in *Lab* value from the initial measurement.

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531 **Supplementary Figure 1. Validation of Colour Quantification from Digital**

532 **Images.** Photos were taken under standardized light and camera settings, and

533 each included the Prawn Colour Chart, Salmofan and MacBeth colour checker

534 array as colour references. Quantification of prawn colour of each individual was

535 performed using an average of 3 equally sized squares located on the first three

536 abdominal segments as shown by the numbers on one of the animals (A).

537 Correlation between the measured *Lab* values from the Hunterlab Miniscan XE

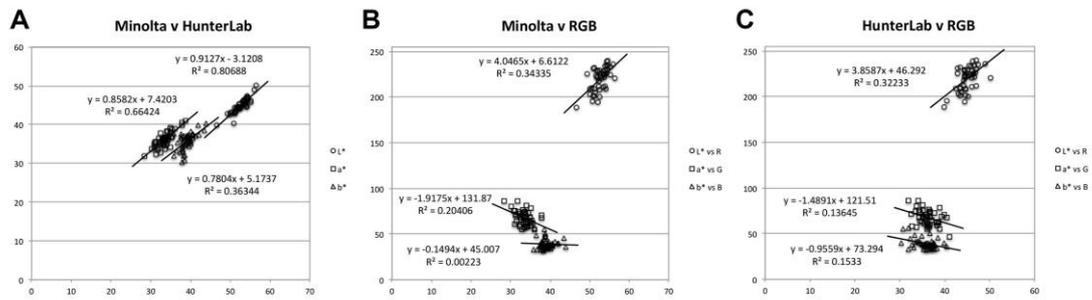
538 and the Minolta CR-400 chroma meter (B). Correlation between expected *Lab*

539 values from the MacBeth colour checker and the converted *Lab* values from the

540 average measured RGB values of the same squares quantified from ten digital

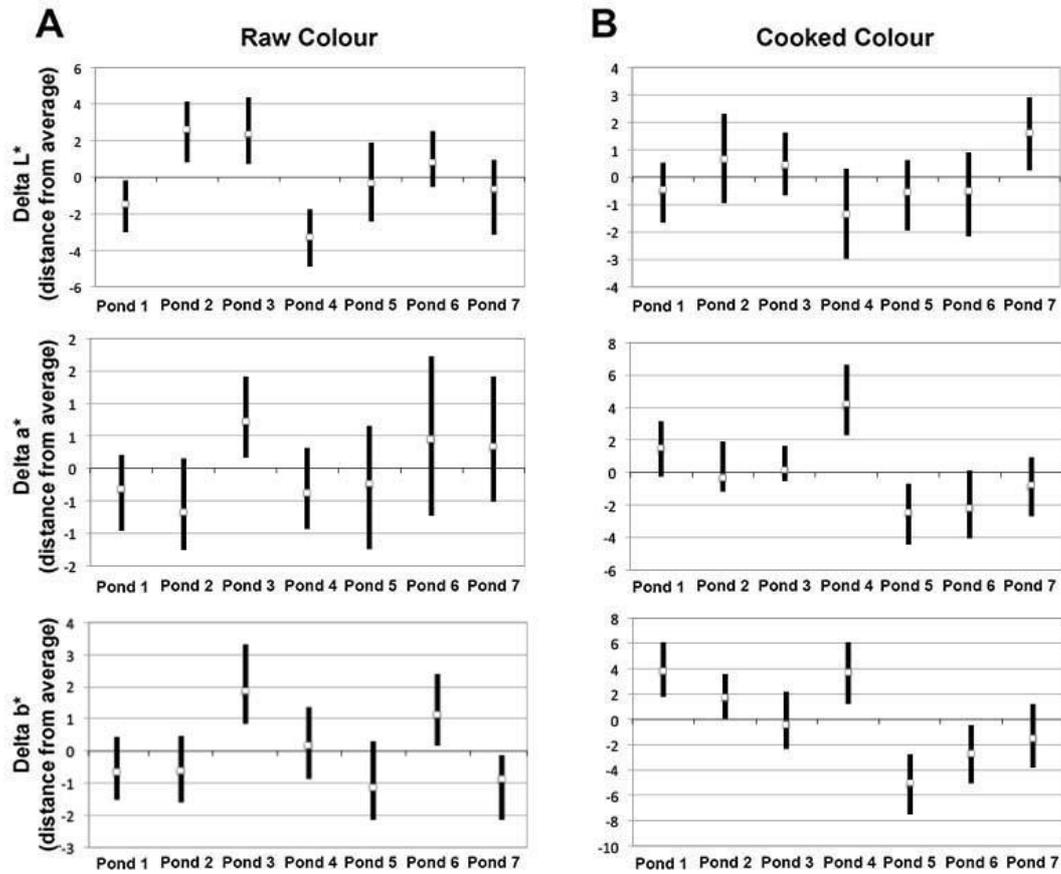
541 photographs (C).

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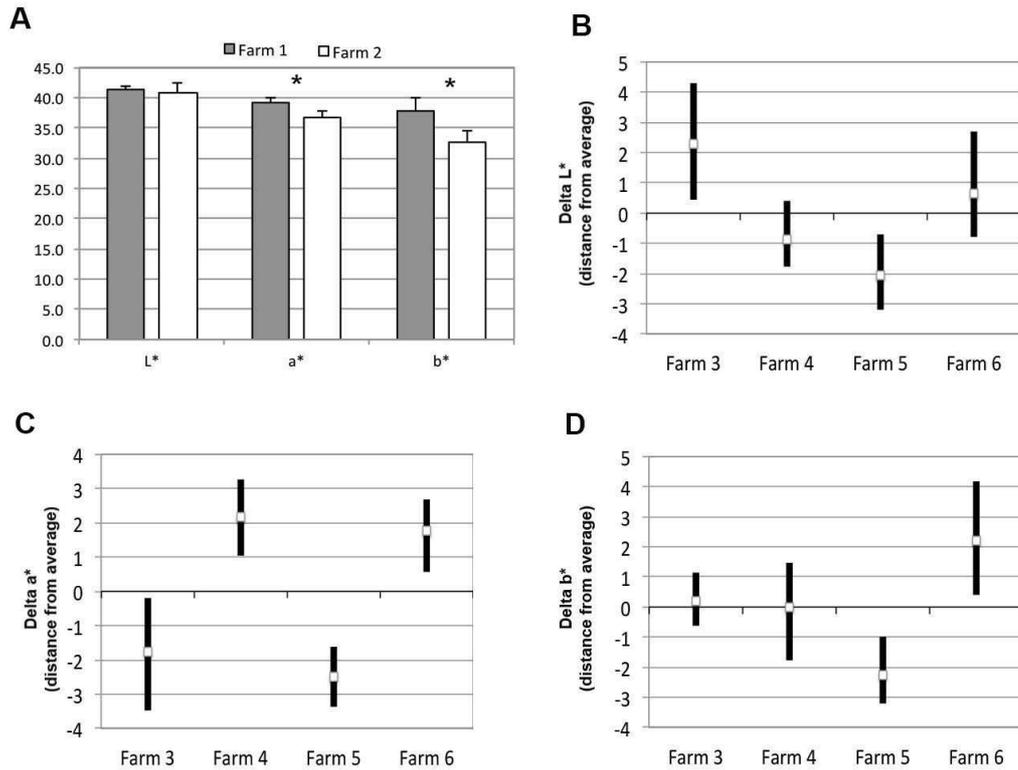
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**Figure 1. Comparison of quantitative methods of measuring prawn colour.** The colour of the same 45 cooked prawns was quantified using 2 different colorimeters and also digital images. **A.** Comparison of the absolute *Lab* values taken using a Minolta CR-400 Chroma Meter and a HunterLab Miniscan XE colorimeter. **B.** Comparison of the absolute *Lab* values taken using a Minolta CR-400 and the absolute RGB values quantified from digital images. **C.** Comparison of the absolute *Lab* values taken using a HunterLab Miniscan XE colorimeter and the absolute RGB values quantified from digital images.



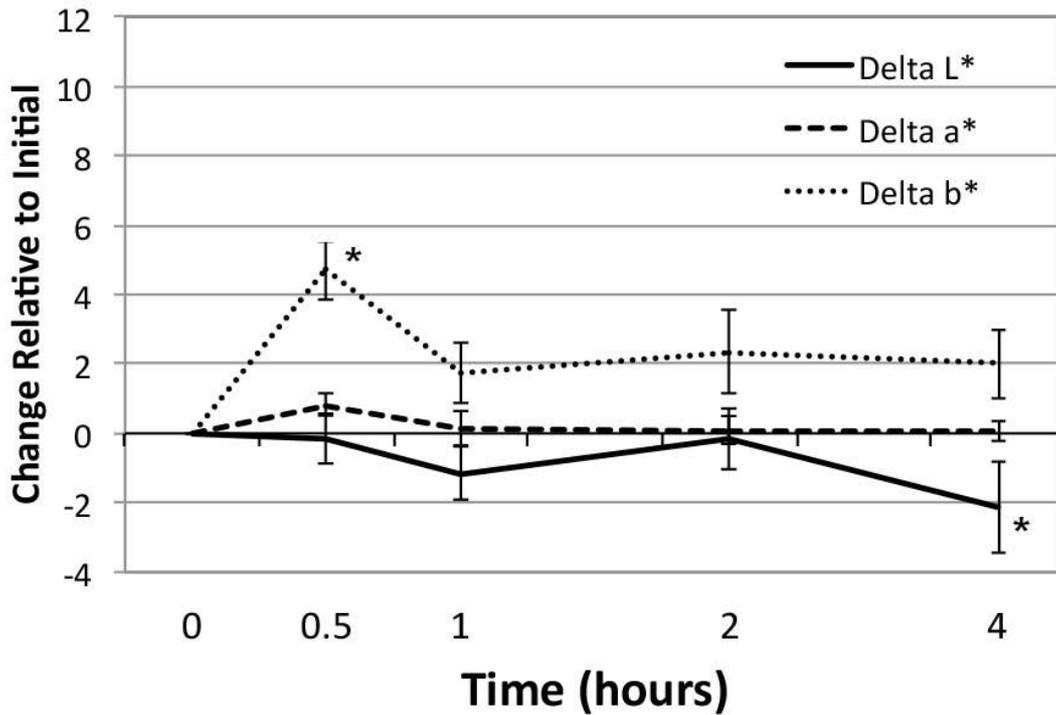
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**Figure 2. Colour Variation in Farmed Prawns Across Ponds.** The median (square) and Q1-Q3 interquartile range (bars) distribution of delta *Lab* values from fifty uncooked prawns from 7 different ponds when uncooked (A) and cooked (B). The delta *Lab* values were calculated as the difference in the value of each individual from the average of all the animals across the 7 ponds.



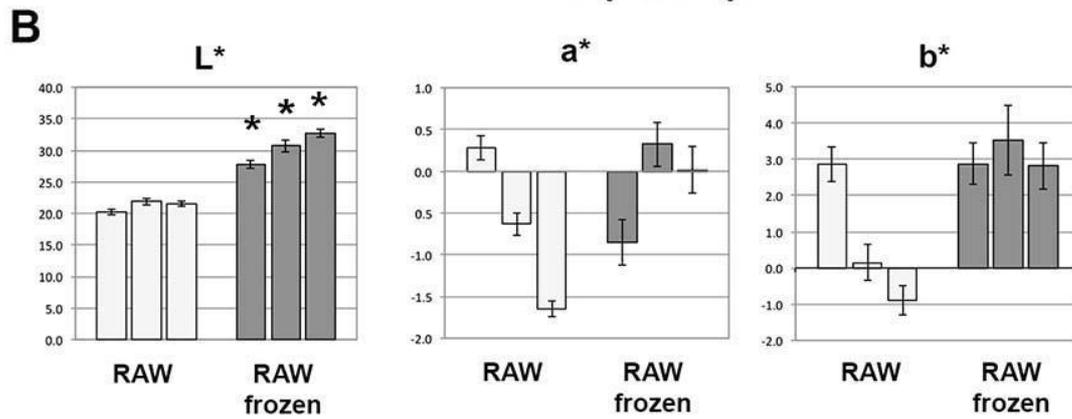
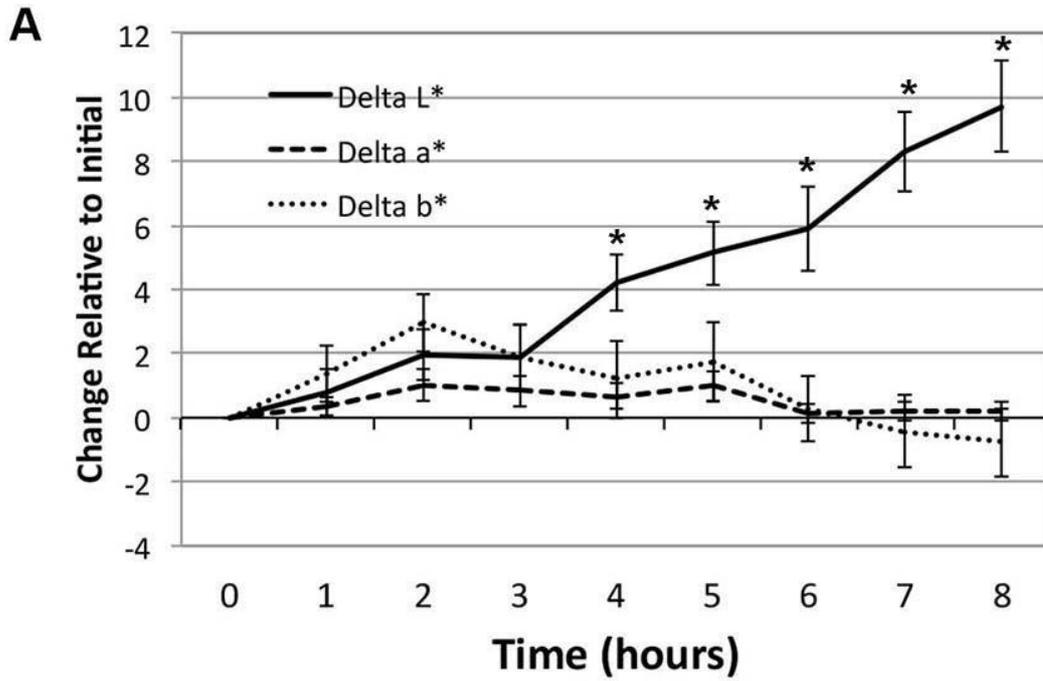
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**Figure 3. Colour Variation in Farmed Prawns Across Farms.** **A.** The absolute Lab values for farms 1 and 2 were taken from 3 sets of 50 animals and measured using a HunterLab colorimeter. **B-D.** The median (square) and Q1-Q3 interquartile range (bars) distribution of delta Lab values from forty cooked prawns from 6 different farms measured using a Minolta Chroma Meter. The delta Lab values were calculated as the difference in the value of each individual from the average of all the animals across the 4 farms. \* denote significant ( $P < 0.05$ ) differences in Lab value between farms.



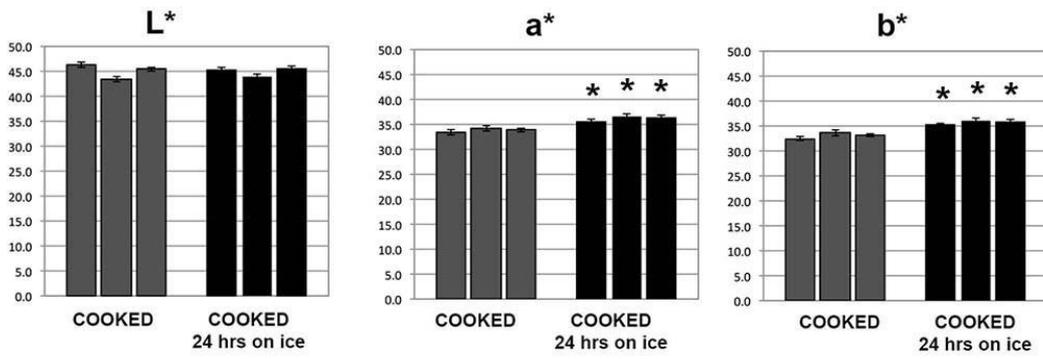
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**Figure 4. Colour change in uncooked prawns held live in chilled seawater.** Prawns harvested from the same pond were held in large 800L aerated bins that contained seawater at 12°C. Twenty animals were taken at random immediately after harvesting and after being held in the bins for different lengths of time. Animals were cooked and then quantitatively colour measured using a HunterLab colorimeter. Results are shown as delta *Lab*, which is the difference in absolute *Lab* colour value at each time point relative to the initial sample. \* denote significant ( $P < 0.05$ ) differences in *Lab* value from the initial measurement.



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**Figure 5. Colour change in uncooked prawns stored on ice or frozen.** **A.** The average *Lab* values were recorded at one hour intervals over 8 hours for twenty uncooked prawns that were stored in an ice slurry. **B.** Each bar represents the average *Lab* colour readings taken from 15 uncooked individuals across the first three prawn abdominal segments. The same animals were measured before and after being frozen and then thawed. \* denote significant ( $P < 0.05$ ) differences in *Lab* value from the initial measurement.



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**Figure 6. Colour change in cooked prawns after 24 hours ice storage.** Each bar represents the average Lab colour readings taken from 15 cooked individuals across the first three prawn abdominal segments. The same animals were measured before and after 14 hours of storage in an ice slurry. \* denote significant ( $P < 0.05$ ) differences in Lab value from the initial measurement.

593 Table 1. Mean *Lab* values and variance of colour quantified from farmed Giant  
 594 Tiger Prawns *Penaeus monodon*.

	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6	Pond 7
Mean							
L*	40.51 <sup>a</sup>	41.64 <sup>b</sup>	41.57 <sup>c</sup>	39.63 <sup>a</sup>	40.44 <sup>a</sup>	40.49 <sup>a</sup>	42.62 <sup>d</sup>
a*	40.10 <sup>a</sup>	38.32 <sup>bcg</sup>	38.74 <sup>bcg</sup>	42.80 <sup>d</sup>	36.13 <sup>ef</sup>	36.42 <sup>ef</sup>	37.84 <sup>g</sup>
b*	40.00 <sup>ad</sup>	37.72 <sup>b</sup>	35.68 <sup>cg</sup>	40.29 <sup>ad</sup>	30.88 <sup>e</sup>	33.18 <sup>fg</sup>	34.55 <sup>cfg</sup>
Variance							
L*	2.34 <sup>a</sup>	5.40 <sup>b</sup>	2.34 <sup>c</sup>	4.81 <sup>d</sup>	4.58 <sup>cd</sup>	8.35 <sup>ce</sup>	5.22 <sup>bcde</sup>
a*	6.13 <sup>a</sup>	10.87 <sup>b</sup>	6.00 <sup>ac</sup>	8.34 <sup>ab</sup>	10.41 <sup>b</sup>	10.11 <sup>b</sup>	7.18 <sup>abc</sup>
b*	10.68	9.08	9.35	13.41	11.48	11.69	9.65

595 Superscripts denote significant ( $P < 0.05$ ) differences between measured values.  
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**Supplementary Table 1. Validation of image based quantification.** Absolute RGB values for each MacBeth Colorchecker square compared with the values quantified from 10 independent photos. The combined RGB values produced the corresponding colours as shown in the table.

MacBeth ColorChecker	Expected Values			Measured Values			
	R	G	B	R	G	B	
dark skin	115	82	68	92 ± 1.33	58 ± 1.31	51 ± 1.39	
light skin	194	150	130	177 ± 1.29	123 ± 1.49	110 ± 1.29	
blue sky	98	122	157	87 ± 1.33	105 ± 1.41	160 ± 1.70	
foliage	87	108	67	83 ± 2.31	101 ± 1.34	75 ± 2.23	
blue flower	133	128	177	134 ± 1.85	125 ± 1.92	182 ± 1.27	
bluish green	103	189	170	91 ± 2.98	176 ± 1.60	186 ± 1.68	
orange	214	126	44	181 ± 1.35	81 ± 2.47	38 ± 2.89	
purplish blue	80	91	166	70 ± 1.93	71 ± 1.75	141 ± 1.28	
moderate red	193	90	99	186 ± 1.60	73 ± 1.72	80 ± 1.50	
purple	94	60	108	88 ± 0.85	56 ± 0.56	107 ± 0.96	
yellow green	157	188	64	141 ± 0.95	182 ± 1.74	71 ± 2.21	
orange yellow	224	163	46	225 ± 1.74	146 ± 1.46	59 ± 1.50	
blue	56	61	150	42 ± 1.76	49 ± 1.53	120 ± 1.45	
green	70	148	73	40 ± 1.30	122 ± 1.50	74 ± 1.64	
red	175	54	60	183 ± 1.39	54 ± 1.99	53 ± 1.75	
yellow	231	199	31	221 ± 1.91	187 ± 1.87	53 ± 2.51	
magenta	187	86	149	199 ± 1.08	85 ± 1.87	142 ± 1.74	
cyan	8	133	161	36 ± 1.96	114 ± 1.67	173 ± 1.84	
white	243	243	242	238 ± 1.58	239 ± 1.78	230 ± 1.96	
neutral 8	200	200	200	204 ± 1.64	208 ± 2.20	200 ± 1.89	
neutral 6.5	160	160	160	159 ± 1.51	161 ± 1.92	161 ± 1.88	
neutral 5	122	122	121	125 ± 1.59	123 ± 2.03	125 ± 2.00	
neutral 3.5	85	85	85	89 ± 3.02	83 ± 3.48	92 ± 3.49	
black	52	52	52	51 ± 2.80	48 ± 3.21	51 ± 3.34	

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604 **Supplementary Table 2. Quantification using digital images of colour grade charts used for**  
 605 **subjective prawn colour grade scoring.** The average RGB colour of an equal sized square was  
 606 quantified across 10 individual photos. The combined RGB values produced the corresponding  
 607 colours as shown in the table.

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Salmofan	Average			
	R	G	B	
20	251 ± 0.79	148 ± 3.45	83 ± 2.77	
21	254 ± 0.28	132 ± 2.45	76 ± 1.94	
22	254 ± 0.34	118 ± 2.19	62 ± 1.56	
23	251 ± 0.89	105 ± 2.00	57 ± 1.33	
24	250 ± 0.93	92 ± 2.23	48 ± 1.46	
25	252 ± 0.72	84 ± 1.99	41 ± 1.29	
26	249 ± 0.95	75 ± 1.42	34 ± 1.08	
27	252 ± 0.66	70 ± 1.80	28 ± 1.07	
28	245 ± 1.79	58 ± 2.01	23 ± 1.02	
29	247 ± 1.73	53 ± 2.20	21 ± 1.20	
30	239 ± 2.43	45 ± 2.10	21 ± 1.47	
31	235 ± 2.49	42 ± 1.65	20 ± 1.41	
32	233 ± 2.27	43 ± 1.58	23 ± 1.33	
33	227 ± 2.59	37 ± 1.73	18 ± 1.40	
34	195 ± 2.74	26 ± 1.88	18 ± 1.77	
Prawn Colour Chart				
	R	G	B	
PCC 7	244 ± 1.93	118 ± 1.64	51 ± 1.61	
PCC 8	245 ± 1.56	113 ± 3.19	60 ± 2.51	
PCC 9	245 ± 1.46	99 ± 2.57	58 ± 2.00	
PCC 10	246 ± 1.30	78 ± 1.81	45 ± 1.33	
PCC 11	244 ± 1.65	66 ± 2.48	44 ± 1.98	
PCC 12	242 ± 1.92	58 ± 1.73	44 ± 1.46	