Coloration Characteristic and Population Genetic Analysis of Wild-Captured Giant Tiger Shrimp (*Penaeus monodon*) from Aceh Timur

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Abstract

Giant tiger shrimp (Penaeus monodon) has become a prime commodity in Indonesia which was produced by aquaculture and capture fisheries activities. Aceh Province, in this case mostly represented by Aceh Timur District, was well-known as the center of wild-captured-adult giant tiger shrimp. Several previous investigations had proved for its high-quality shrimp spawner in producing good eggs in quality and quantity under artificial spawning condition. Two main interesting points of wild giant tiger shrimp from Aceh Timur came from their coloration and population clusters. This report was aimed to provide that information pre-preliminary and highlighted quantitative information of coloration characteristic through RGB (Red Green Blue) and CIE Lab color space data analysis, as well as, 16S rDNA-PCR-RFLP genetic comparison among four population clusters in Aceh Timur Waters. The color analysis resulted in significant differences between wild-captured and pond-cultured giant tiger shrimp which produced R value 0.1524±0.0091 and 0.1268±0.0004, respectively. Total pixel analysis through L a* b* color space has distinguished detailed differentiation between wild-captured and pond-cultured giant tiger shrimp acquired images. It is known that most of the wild-captured image pixels were concentrated in quadrant I (+a, +b) while pond-cultured in quadrant II (-a, +b) and III (-a, -b).Genotyping of represented samples from 4 population clusters, i.e. Aceh Tamiang, Langsa, Peudawa, and Julok produce 2 haplotype composite, AAA and AAB. Among 4 clusters, it was found that Julok has become the only cluster which has a different haplotype composite ratio (1:1) (D 0.0348, V 0,9501) from the others (4:1)(V 0.9504).*

Key words: *Aceh Timur; CIE Lab; PCR-RFLP; Penaeus monodon; RGB.*

Introduction

In aquaculture, Aceh Timur Waters is well-known as a source of high quality of wild-adult giant tiger shrimp (*Penaeus monodon*) for their potential broodstock (Lante *et al.*, 2015). *P. monodon* exploitation in Aceh Timur was held intensively and recorded as prime commodities in this area (Hedianto *et al.*, 2016). Information related to *P. monodon* in Aceh Province had reported previously which is mainly discussing about stock and diversity information (Wardana, 2011; Nawang *et al.*, 2014; Hedianto *et al.*, 2016). Eventhough, comprehensive information related to *P. monodon* characteristic in this area remains limited.

Our investigation conducted in 2015 found interesting findings regarding giant tiger shrimp in Aceh Timur waters. Population distribution of the shrimps was found to be clustered on 4 identified locations (unpublished data). Traditionally, fishermen at several landing centers (Aceh Tamiang, Langsa, Peudawa, and Julok) were capturing tiger shrimps at those areas. Another interesting characteristic of giant tiger shrimps from Aceh Timur was their reddish coloration. This is an attracting visualization compared to other wild-captured tiger shrimps from different locations in Indonesia.

The coloration of shrimps was positively correlated with product quality. This visual appearance indicated high nutrition contents (Boonyaratpalin *et al.*, 2001). Some assessment for coloration quantification was improved in numerous reported investigation, either using standardized and sophisticated instruments colorimetry and spectroscopy (Parisenti *et al*., 2011) or image acquisitions (Wade *et al*., 2014). In Indonesia, information regarding the coloration measurement was here qualitatively. This representation the predominant color based on observer subjectivity visually (Amin *et al.*, 2012; Wade *et al.*, 2014).

Genetic information of black tiger shrimp in Indonesia had been reported previously (Sugama *et al.*, 2002; Walther *et al.*, 2011; Purnamaningrum *et*

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al., 2016). Nevertheless, genetic characterization information for a small region, specifically in Aceh Timur, is limited. Eventhough, this information was essential to provide local genetic characteristic to provide genetic biodiversity resource information (Sherry *et al*., 2001). This investigation reported genetic characteristic of four population cluster considered based on acoustic surveys result through RFLP analysis of 16S rDNA target gene (Lavery *et al.*, 2004). This method gave us more advantages in providing genetic information based on DNA fragmentation post to enzyme digestion. In addition, this method had been proved to provide tiger shrimp genetic characteristic on the previous report (Bouchon *et al.*, 1994; Klinbunga *et al.*, 2001; Prastowo *et al.*, 2009).

Materials and Methods

Image acquisition and processing

Fresh giant tiger shrimp from captured (wild-type) and cultured (pond-type) were used for image analysis (n_{wild}= 10,n_{pond}= 10, CL= 4–7.2 cm). Image samples were acquired using 12 MP resolution camera under daylight illuminance and clear white background. In order to minimize light bias, image acquisition was conducted in short time duration (n= 10). Furthermore, preprocessing image data were employed by involving pixel normalization (1). Prior to normalization process, controlled image reduction was applied in order to suit data dimension for the software maximum data range. The original images, which were having 2200×1400 pixel dimension (containing more than 3 million pixels), reduced to 400×400 pixels (containing more than 160.000 pixels) and enhanced from 72 dpi to 96 dpi resolution. This was essential to conduct in order to maintain data quality during reduction processes. Thus, image normalization was conducted using equation (Vezhnevets *et al.*, 2003). Normalization step produce white color subtraction and turn the background of original in to black color (R = G = B = 0). This process revealed stronger object color and by this, the *L* value (corresponding to light/ brightness/illuminance related factors) become neglected in further analysis processes. Classification of dominance color on each pixel was divided into three segmented images using Kmeans equation (Likas *et al.*, 2003).

Segmentation results were then re-calculated to gather average R, G, and B value from images sample, respectively. Calculation of color area from each segmented image were conducting by RGB to binary image conversion (colored pixel= 1, black pixel= 0) using available syntax in Matlab [im2bw]. Identification of pixel color distribution an analysis using CIE Lab color space was employed. As previously described, the *L** value was no longer use since it has been neglected during image normalization. The *a** and *b** values were used to inform detailed color position of images pixel. A normalized RGB image was converted to Lab image using [srgb2lab] syntax. To provide higher resolution of the segmented image, 6 segmentation were classified using nearest neighbor equation (Duin *et al*., 2000). One Way Anova analysis facilitated by Origin 7 (OriginLab, US) was used to determine the difference between coloration characteristic of wild-type and cultured-type shrimp.

DNA extraction, amplification, and digestion

Freshly landed *P. monodon* (n_{total}= 40, CL= 3.2–7.3 cm) purchased from fishermen located in Aceh Tamiang, Langsa, Peudawa, and Julok. These fishermen operate their trammel net in different locations closing to population cluster desired in this investigation. Fresh tissue was collected and preserved in 96% ethanol, then transported to the laboratory and put the samples in the fridge.DNA extraction conducted using chelex 10% in TE buffer (pH 8,0) with 25 mg tissue sample, approximately. Extracted genomic DNA was purified using QIAcolumn purification kit based on manufacturer protocol. This is essential to prevent the presence of inhibitors.

Gene segment was amplified using PCR (Polymerase Chain Reaction). Amplification used based on Klinbunga *et al*. (2001), 16SrDNA F : 5'-CGC CTG TTT AAC AAA AAC AT -3' and 16 SrDNA R : 5'- CCG GTC TGA ACT CAG ATC ATG T -3'. Amplification was conducted under pre-denaturation 95°C for 2 minutes, followed by 29 cycles consisting of denaturation 93°C for 30 seconds; annealing 50°C for the 30s ; and extension 72°C for 45s and a final extension of 72°C for 5 minutes. The amplicons were tested electrophoresis using 1,5 % agarose gel in 1X TBE (Tris-borate-EDTA) buffer. The amplicons proceeded to digested using Alu I, HaeIII, EcoRI, HinfI, MboI, and DdeI. Restriction product was visualized using 2% agarose gel electrophoresis in 2X loading dye. The results were scored and analyzed using TFPGA (Miller, 1997).

Results and Discussion

Coloration characteristic

In this investigation, image processing using K-means and nearest neighbor algorithm have successfully segmented the objects. RGB-based segmented image produces 3 image segment which was visualized strong red, light red, and black object

cluster for wild-type *P. monodon* image and strong green, light red, and black for cultured-type *P. monodon* image (Figure 1.). Color quantification facilitated by RGB color space analysis classified 3 main colors which were forming single pixel coloration by the ratio of R (red), G (green), and B (blue) value. Theoretically, absolute red color were consisted by R= 1, G= 0, B= 0, so for others. Extracted RGB value from normalized image have shown the varied value of R, G, and B. For the wild-type the R, G, and B value are 0.1524, 0.1024 and 0.0922, respectively. For the cultured type, the R, G and B value are 0.1268, 0.1131, and 0.0973 respectively. These values represent quantitatively green coloration in cultured-type tiger shrimps (Figure 2.). The Strong red coloration of wild-type tiger shrimps achieved the highest value (0.1524± 0.0091). Color variation between wild-type and cultured-type of giant tiger shrimp were significantly different. One Way ANOVA analysis show P value for comparison of R, G, B from both sample type were 0,00023 (R), 0,000057 (G), 0,0068 (B) ($P<0.05$).

The segmented images produced by K-means classification were calculated to measure the pixel area of each segment. Generally, Kmeans classifications were not identified green coloration on wild-captured shrimps. Strong red and light red becoming dominant color among all wild-type samples and covered 53.55±8.43 % for strong red coloration and 30.97±8.87 % for light red coloration (all red coloration 84.52%, approximately) (Table 1.). Pond-cultured shrimps were dominantly covered 64.32±4.50% by green coloration, small-light reddish smear coloration was found in 17.80±0.67 % coverage pixel area. Dark black coloration was found in both samples type which was mainly located in the abdominal segment. This coloration covered either wild-type or pond-type in equal proportion (15.46±4.98% and 17.87±5.14%, respectively).

According to this finding, wild-captured tiger shrimps from Aceh Timur covered by red coloration from the carapace, pereopods, and pleopods to the most of the abdominal segments (Figure 1.). Among all observed samples, wild-type shrimps have similar coloration characteristic. Nevertheless, pond-cultured shrimps have light red coloration on their pleopods and pereopods. RGB color space, in general, considered as an effective parameter to observe color range from an object, but, more comprehensive observation for the non-absolute RGB color (Mendoza *et al.*, 2006). CIE Lab color space has been used for numerous applications regarding to aquatic organism and its derivation products color analysis (Misimi *et al*., 2007; Sreenath *et al*., 2008; Maury-Ramirez *et al*., 2013). These wide range applications were triggered by its ability to provide single-pixel color distribution visualized in Cartesian coordinate systems (Leon *et al.*, 2006). Since human visual color opinion becomes unreliable, CIE Lab had been used as color space analysis for color standardization (Connolly and Fleiss, 1997).

CIE *Lab* color analysis proved detailed single-pixel coloration between both samples type. Wild-type shrimps pixel color concentrated in quadrant I which represent orange-red coloration characteristic (+*a*, +*b*) (Figure 3.). Highest Pixel densities were observed in range (a10, b5) to (a40, b35). In contrast, pixel coloration of pond-type shrimps distributed on wider area range that covering quadrant I, II, and III (anti-clockwise quadrant order). Nevertheless, reduced pixel (see Figure 3. on black marked area) showed pixel densities were concentrated in greenish (-*a*,-*b*), and yellowish-green (-*a*,+*b*) area.

Crustacean coloration was mainly affected by the presence of astaxanthin content in hypodermal and exoskeleton (Lopez *et al.*, 2002). In a natural condition, astaxanthin was found as a pigment-protein complex, crustacyanin (Krawczyk and Britton, 2001). This complex form affects to wavelength shifting which turns original red coloration of astaxanthin to greenish-blue color effect (Parisenti *et al*., 2011; Wade *et al*., 2014). Denaturation of the pigment-protein complex would release free-formed astaxanthin and reveal the original red color of astaxanthin (Pacheco *et al*., 2009; Jantakoson *et al.*, 2012).

Wild-captured tiger shrimps from Aceh Timur Waters naturally has belonging red coloration. The coloration possibly caused by several factors, i.e. depth pressure and feeding behavior (Parisenti *et al*., 2011; Jantakoson *et al.,* 2012). Our previous investigation found that most of the wild-type shrimps, which was captured using trammel net in 50 m depth, were used small crustacean as their main diet (Anonymous, 2016). It was related to the several investigations that proved that astaxanthin synthesis was greatly affected by the carotenoid-rich diet (Boonyaratpalin *et al.,* 2001; Amin *et al*., 2012). Besides that, an ecological assessment of toxic materials was essential to conduct. Martinez *et al*. (2014) reported that Cu contamination significantly related to the strong red coloration in shrimps.

Genetic population characteristic

Amplification conducted by targeting 16SrDNA gene resulted amplicon 560 bp in size, approximately. Then, this PCR product was digested

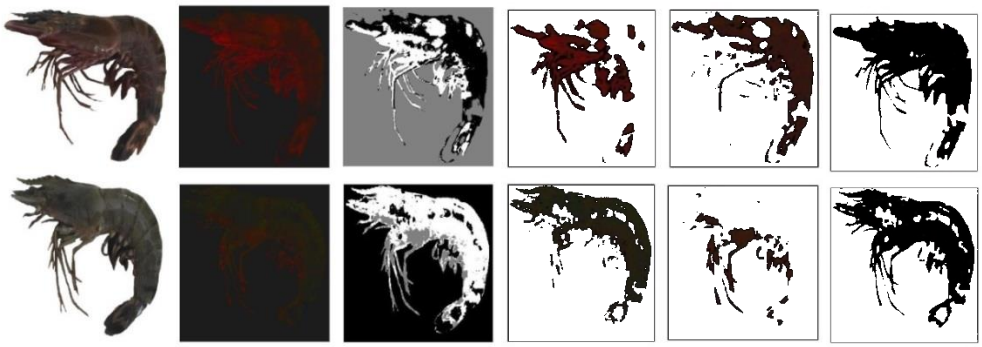

Figure 1. Giant tiger shrimp image processing step, from left to right: original image (upper image: wild shrimp, bottom image: pond shrimp), normalized image, total segmented image, segmented image 1, segmented image 2, and segmented image 3.

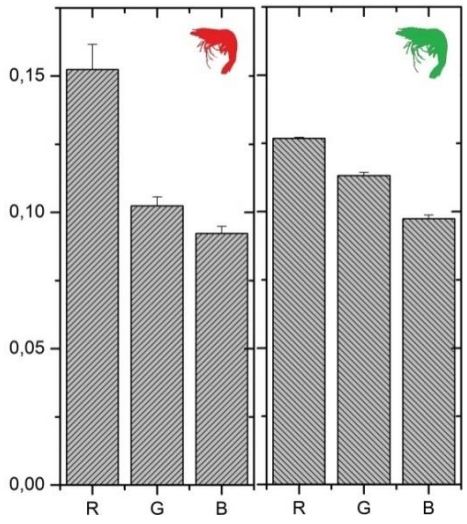

Figure 2. RGB average value from the image of Giant tiger shrimp from wild (red) and cultured pond (green).

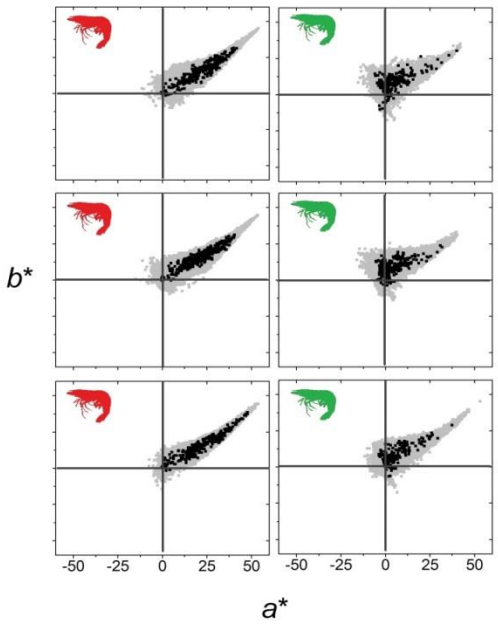

Figure 3. *a** and *b** color space coordinate position of representative image samples of Giant tiger shrimp from wild (red) and pond (green). Pixel coordinate area is marked in gray represented total pixel color (n = 160.000) while black marked area represented reduced pixel area (n = 1000).

using 6 restriction enzymes, *i.e*., Alu I, HaeIII, EcoRI, HinfI, MboI, and DdeI. Consistent restrictions were found in HaeIII, HinfI, and MboI.HaeIII cutting position was 5'..GG↓CC..3', HinfI cutting position was 5'..G↓ANTC..3', and MboI cutting position was 5'..↓GATC..3'. According to digestion product produced by those 3 enzymes, two alleles were identified as AAA and AAB (Table 2.). This result indicated low haplotype variance presence among population clusters.

Composite haplotype AAA was known as dominated haplotype in the observed giant tiger shrimp from all investigated locations. 80% haplotype composite in Aceh Tamiang, Langsa, and Peudawa, as well as 50% in Julok, were dominated by AAA. These findings show that AAA haplotype shrimps are distributed in all observed samples. Giant tiger shrimp heterozygosity in Aceh Timur Water was considered as low heterozygosity especially in Aceh Tamiang, Langsa, and Peudawa (0.1067). Giant tiger shrimp heterozygosity from Julok was slightly greater than those others three locations (0.1667). Thus, genetic variances show insignificant variation among the population (0.9501-0.9504) (Table 3.). Low heterozygosity indicated close genetic distance among the observed population (Table 4.). Based on 16SrDNA gene, digested by HaeIII, HinfI, and MboI, Tiger shrimp from Aceh Tamiang, Langsa, and Peudawa show similar genetic character (100%). In Julok, 3,48 % differences in genetic character was found (Figure 4.)

An acoustic survey conducted in 2015 gave interesting result regarding to the position of giant tiger shrimp population cluster in Aceh Timur waters (Anonymous, 2016). It was identified that in coastal-offshore of Aceh Timur waters, the tiger shrimps biomass clustered in Aceh Tamiang, Langsa, Peudawa, and Julok (Anonymous, 2016). This report was considered as the Sampling location in the current investigation. In population genetic study, the represented locations were essential to provide representative population genetic data (Petit *et al.*, 1998). One of the advantages of using genetic population analysis, the ability to provide the current condition of inter and/or intrapopulation variation. This information gave us new perception to describe environmental pressure which may be caused by nature or anthropogenic factors (Wilson and Clarke, 1996). Giant tiger shrimp capture rate in Aceh Timur water was considered as overexploited (Hedianto *et al.*, 2016). This condition was possibly triggered to decrease the genetic variation of giant tiger shrimp. Several previous investigations reported significant factors influencing low genetic variance, e.g. geographical barrier, inbreeding, and overfishing (Christiansen and Reyer, 2011; Pinsky and Palumbi, 2014; Willoughby *et al*., 2015)

16SrDNA gene was shown low variation among all samples (Figure 4). Short genetic distance among population clusters may considerably to conclude as single stock population. These results are possible to use in conservation strategy policy. Larger population comparison studies were

Table 1. Pixel area detection using the image of Giant tiger shrimp from wild and pond.

Sample	Strong red area pixel	Red area pixel	Black area pixel
Wild	53.55±8.43 %	30.97±8.87 %	15.46±4.98 %
Sample	Red area pixel	Green area pixel	Black area pixel
Pond	17.80 ±0.67 %	64.32±4.50 %	17.87±5.14 %

Table 2. Haplotype distribution of Giant tiger shrimp from each sampled location

Haplotype Composite	Haplotype Frequencies (%)			
	Aceh Tamiang	Langsa	Peudawa	Julok
AAA	1.6	1.6	1.6	1
AAB	0.4	2	2	1
n-Allele	2	2	2	2

Table 3. Heterozygosity analysis and genetic variation result of Giant tiger shrimp from sampled location

Population	Observed Heterozygosity	Expected Heterozygosity	Genetic variance
Aceh Tamiang	0.1067	0.1123	0.9501
Langsa	0.1067	0.1123	0.9501
Peudawa	0.1067	0.1123	0.9501
Julok	0.1667	0,1754	0.9504

Table 4. Genetic distance result among Giant tiger shrimp population

Population	Aceh Tamiang	Julok	Peudawa	Langsa
Aceh Tamiang				
Julok	0.0348			
Peudawa	0.0000	0.0348		
Langsa	0.0000	0.0348	0.0000	

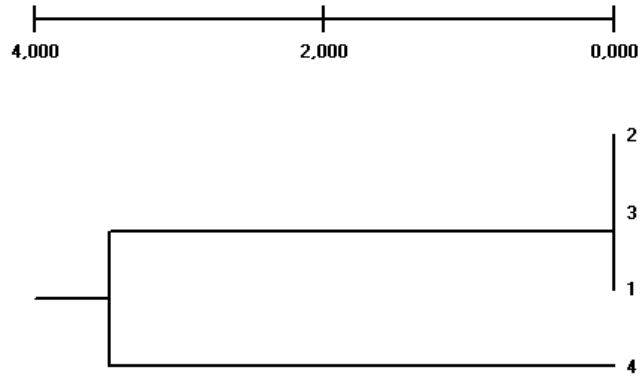

Figure 4. Genetic distance dendrogram of Giant tiger shrimp based on UPGMA cluster.

conducted previously by Sugama *et al*. (2002). *P. monodon* tissue sample was studied from Aceh, Cilacap, Madura, Tarakan, Bali, Dompu, and Sulawesi Selatan. Six polymorphic locus show genetic distance in range 0.2-4.7%. Based on this investigation, it is clear that there is a low *P. monodon* genetic variation observed. In contrast, a significant variation (24%) *of P. monodon* collected from Malaysia and Australia using 16S rDNA marker was known. Thus, Indo-pacific *P. monodon* was estimated to have a high variety of genetic characteristic by geographic position.

Conclusion

Coloration characteristic of giant tiger shrimp from Aceh Timur quantified by RGB and CIE Lab color space had shown significant differences between wild and pond type. RGB and Lab color space analysis showed that strong red and light red coloration were found consistently among all wild-type samples pixel. Genotyping through 16SrDNA PCR-RFLP analysis remains showing a capability to characterize the genetic population of giant tiger shrimp in smaller water area. Compared to larger geographic distance study, in this study, we found that small-barrier less aquatic ecosystem was still had 3.48% genetic differences potency.

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