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Effect of Shell Color and Nursery Depth on the Growth of Pearl Oyster Pinctada Maxima in Tekalok West Nusa Tenggara Indonesia

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ABSTRACTS

The correlation between shell color and pearl oyster growth rates during the breeding phase is not well known and is then explored in this study. In addition, the effect of breeding depth on the growth is also assessed. The tests were conducted by monitoring the growth during a nursing season at a local company at Tekalok, East Lombok, Indonesia. The growth rates were measured from length extension, width extension and weight gain of a total of 4,493 seed samples over a three months nursing period. Results showed that average growth of each group of shell color was homogeneous with acceptable variance. The average length extension of all samples was 1.085 cm/month. The shell color significantly correlates with length extension, in which the yellow one being the highest elongation rate. However, no significant difference in width extension and weight gain was found at a specific condition. A slight decline in growth rate was shown at higher depth, but overall variations in growth rates are insignificant. Since the quality of the resulting pearls is better at higher depth, breeding should be done at higher depth, and further growth after nucleus inclusion can be done at lower depth to yield both optimum growth and high-quality pearl.

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1. INTRODUCTION

Pearl oyster *Pinctada maxima* has very high economical value and has long been farmed for pearl agroindustry in Indonesia. Traditionally, pearls were only obtained from nature, but due to scientific advancement, vast majority of pearls today are produced from nursing, nucleus inclusion, then breeding, a technology dominated by Japan-based companies. Pearl oyster of *Pinctada maxima* is the oyster species that produces the best pearl, also known as South Sea Pearls (Septy, 2018). This type of oyster is mostly found in east seas of Indonesia, and sold at high prices of about USD30-100/gram.

Nucleus inclusion stage in *Pinctada maxima* farming allows certain freedom to custom design the expected pearls (McDougall *et al*., 2016). The physiological growth phase of *Pinctada maxima* can be exploited to customize the produced pearls into (more attractive) shapes and colors, suitable for jewelries (rings, braces, necklaces, etc). In addition to the produced pearls, the shells can be further used as a source of sodium carbonate, raw materials for paints, cosmetics, and others. The oyster meat is also rich of proteins (Zheng *et al*., 2009).

A natural pearl formed by *Pinctada maxima* has a very high value. This leads to its excessive exploitation that eventually diminish its wild population (Septy, 2018; Wardana *et al*., 2014). Note that most of wild and mature *Pinctada maxima* oyster do not contain pearl and must be sacrificed. Its undesirable impact prompts the policy maker to enforce companies to cultivate the seed of the pearl oyster in the laboratory-scale, then breed them under their natural habitat. Such kind of farming has only been possible thanks to advancement of nucleus inclusion technology to allow production of semi-natural pearls.

The emergence of effective nucleus inclusion technology leads to rapid development of pearl oyster farming industries that nurse and breed of *Pincatada maxima*. Those industries initially breed the natural seeds produced by wild pearl oyster. Increasing demands of the pearl then leads to excessive exploitation of the natural seeds, exceeding natural supply and thus threatening the wild seeds population (Qingyun *et al*., 2014). The pearl industries require ample seeds for nucleus inclusion process, i.e., a pearl oyster nursing company, in average, requires hundred thousand seeds per year (typically at a size/length of larger than 6 cm), with a market price of USD 0.4/cm. The nucleus inclusion is only effective for the pearls at sizes of 8-10 cm (Septy, 2018). The seeds then must be supplied from lab-based seedlings.

The growth of pearl oyster seeds starts from zygote, followed by larvae, then spat (Gervis & Sims, 1992). It requires at least 40 days to be applicable for sticking on a collector (Mamangkey *et al*., 2009). The spat stick on a collector is then nursed in the seawater until it reaches juvenile stage at a size of $\leq$1 cm. The juvenile of 1-2 cm size is selected to another nursery stage for about 30-40 days (Monteforte *et al*., 2005; Taylor *et al*., 1998). Stickiness of a larvae/spat depends on the condition and the depth of the sea (Liu *et al*., 2006). Further transferring process is done to a bigger block until it reaches a size of 10-12 cm within 12-13 months. The seeds at a size of 10 – 12 cm will then undergo nucleus inclusion operation, followed by breeding for 18 – 20 months (Taylor *et al*., 1998).

Without supply from laboratory-based nursery, the demand of oyster seed cannot be fulfilled, which leads to excessively exploitation of natural seeds. One of the crucial approaches is by focusing on reproduction of the seed at their developing phase of gamete, including: a) gonad growth; b) spawning and fertilization, and c) further growth phase.

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Among those three, the selection phase plays important role to obtain the ones with fast metamorphosis growth rates (Liu *et al*., 2006; Minaur, 1969). Nonetheless, lab-scale nursery is challenging, costly and must be supported by adequate facilities and technical skills. For instance, the growth rate and metamorphosis time of pearl oyster seeds are dictated by the feed rate, typically of *Tetraselmis sp.* at concentration of 20,000 cells/mL and at a circulation rate of 1.63 L/h. Fortunately, due to substantial skill-transfer to reliable locals, they can perform pearl seeds nursery independently. Considering the economic potential of pearl oyster, local government agencies have also been very active to disseminate the best practice in pearl oyster farming. Therefore, under the current situation, community-based nursery can supply the oyster ready for nucleus inclusion to support the growing pearls farming industries.

The success of nursing breeding is highly affected by local climate of breeding spot (Joubert *et al*., 2014). The results of breeding during years of 1999 – 2002 in Tekalok were very poor because of high rate of mortality. It happened due to floodings and "el-nina", a phenomenon in which seawater temperature decreases beyond normal. El-nina and flooding happened to breeding areas in West Nusa Tenggara Indonesia: Pemenang, Sekotong, Tekalok and Sigui. Due to carried over of soil from flooding to the sea, the seawater became murky/turbid and contained soil particles than covered the shell of the pearl seeds and thus inhibited their growth. Such disastrous event led to huge financial loss on the pearl companies the following years. Moreover, an extreme change in natural conditions and ever decreasing capacity of natural reproduction of pearl oyster results in depletion of pearl population, leading to scarcity of natural pearl (Lind *et al*., 2012; Yukihira *et al*., 1998), and decrease in population size (Lind *et al*., 2009).

The population of wild *Pinctada maxima* pearl oyster becomes very low. Oyster breeding companies were incapable of producing seeds to fulfil their needs, hence the produced pearls do not meet the market demand in term on quantity and quality, let alone to ensure continuity of supply (Septy, 2018).

The key success factors in pearl oyster breeding are on the availability of high quality oyster seeds and their continuous supplies to ensure continuous production (Gervis & Sims, 1992). Pearl oyster breeding industries require large numbers of high quality oyster seeds continuously to produce high quantity and quality of pearls. High quality seeds can be obtained from laboratory-based nursery run by professionals. Improvement of seeds production must be done professionally because in every stage of growth, the mortality of oyster is still high, particularly on larvae and juvenile stages (sizes of less than 5 cm) (Gervis & Sims, 1992). Fertilized ovum's are only about 90 % to form zygote and larvae. Out of those, only about 50 % can grow further to become spat and juvenile, yielding only 25% of the initial ovum (Alagarswami *et al*., 1989). From juvenile in size of 1 cm (after nursery) in the seawater, leaving on only about 10 % to survive. Each breeding period (of 90 – 100 days) only yields about 225.000 seeds of 1 cm size with growth rate of 0.9-1.0 cm/month at a price rate of USD 0.25-0.30 per seed (Septy, 2018).

Pearl oyster habitat lies in plankton-rich regions (Joubert *et al*., 2014). The growth of oyster undergoes complex metamorphosis starting from formation of zygote. It grows much faster during the metamorphosis stage in comparison the rest stages. Morphologically, metamorphosis growth can be seen from growth of the shell (Gervis & Sims, 1992). Conventional wisdom to gauge the quality of oyster seeds of *Pinctada maxima* are through their shell color, weight and size (length and width) (Blay *et al*., 2014; Jerry *et* *al*., 2012). Our preliminary study from seedling survey in Tekalok showed that most of pearl oysters of *Pinctada maxima* in West Nusa Tenggara have shell mantle color of either brown, yellow, black or white. The color variations are the results of genetic and environmental factors (McGinty et al., 2010). However, morphological quality of each color group is still unknown and thus addressed in this study.

In this study, we assessed the impact of the shell color and nursery depth on the growth of the oyster seed. The seed quality was assessed in term of length and width extension and weight gain over 3 months of nursery period. The test was conducted by monitoring a cycle of nursery and oyster breeding on one of the pearl oyster farming company located in Tekalok, East Lombok, Indonesia. Finally, we evaluate the effect of shell color on the quality of the produced pearls and compose a recommendation on the basic procedure for nursing and breeding of *Pinctada maxima* pearl oyster.

2. MATERIALS AND METHODS

The research was conducted using pearl oyster of *Pinctada maxima* in a pearl oyster breeding company of PT Bumi (East Lombok, Indonesia). Two parameters were evaluated, namely shell color and the breeding depth from sea level. For study on the effect of shell color, a total number of *Pinctada maxima* seeds was 4493. They were in four different colors: brown (1635), yellow (1058), black (1375) and white (430). The seeds were grown at a depth of 10 m from the sea level. The effect of breeding depth was evaluated at 5, 10, 15, 20, and 25 m from the sea level. Seeds with different colors were applied when using each depth of 12.

The growths of seeds were monitored by measuring the weight (gr, using OHAUS Precision Standard) and the size (width and length, cm) before and after 3 months of the breeding. A magnifying lens was also used for helping measurement processes when necessary. The dimensions of the oyster were measured as in **Figure 1 (a)**. The oyster is enclosed inside a pair of concave shells. The shape, size and the color of the shells depend on the species. The morphology and characteristics of the shells that define the anterior and posterior of the oyster are shown in **Figure 1 (b).** (Septy, 2018). All data were statitically analysed using SPSS software.

In addition to the growth during the breeding phase, qualitative evaluation on the quality of resulting pearls and techno-economical assessment were also conducted at the end of the breeding process.

3. RESULTS AND DISCUSSION

3.1. Effect of shell color

The growth rates (weight gain, length extension, width extension) of *P. maxima* with different colors are depicted in **Figure 2**. The brown, yellow, black and white shell colors have length extension, width extension and weight gain (all presented as average ±variant) of 3.231±0.283 cm, 3.114± 0.275cm and 0.564±0.010 gr; 3.326± 0.403cm, 3.135± 0.347cm and 0.557±0.012; 3.245± 0.303 cm, 3.120± 0.336 cm and 0.565± 0.054; 3.217± 0.328 cm, 3.101±0.276 cm and 0.575± 0.013 gr. Such growth rates are considered acceptable for typical cultivation, as reported elsewhere (Liang *et al*., 2016).

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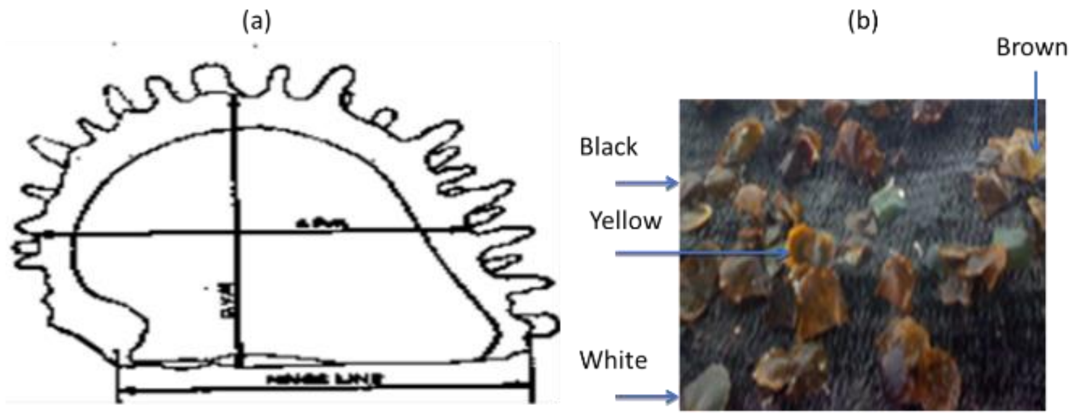

Figure 1. (a) Morphology and characteristics of oyster shells that define the anterior and the posterior of Pinctada sp. AMP: anteroposterior measuremt, DVM: dorsoventral measurement. (b) A picture showing the mounting of pearl oysters with different colors mounted on a similar net.

Statistical analysis results show that only length extension that has significant different (with 95% confidence level) among the four tested shell colors, in which F_{calc} (7.476) > F_{table} (2.607) with P_{value} (0.000). The pearl oyster of yellow shell showed the highest extension of length, followed by black, brown and white. On the other hand, there is no significant difference on the width extension among the four shell colors: F_{calc} (0.480) < F_{table} (2.608) with P_{value} of 0.696>0.5, in which the yellow one is the widest followed by black, brown and white. Like width extension, no significant different on weight gain was observer: F_{calc} (1.436) < F_{table} (2.608) with P_{value} of 0.230 > 0.05, in which the white one was the heaviest, followed the black, brown and the yellow ones. It is worth noting that the length growth rate of 1.085 cm/month, width growth rate of 1.040 cm/month and weight growth rate of 0.188 gram/month are all within the acceptable range. It suggests that the location is appropriate for breading of *Pinctada maxima*.

3.2. Effect of breeding depth.

Figure 3 shows the effect of breeding depth on the growth of *Pinctada maxima*. The maximum extension of length and width was found at the depth of 15 m, but the maximum weight gain was found for the depth of 10 m. However, the differences in growth on group-to-group basis are not significant. The F_{cal}s are less than F_{crit}s for all parameters. The F_{cal}/F_{crit} for length, width and weight are 1.701/2.540, 1.237/2.540 and 1.947/2.540 respectively. However, overall trend shows a slight decline in growth at deeper breeding depth within the tested range (5-25 m), as also reported by others (Liang *et al*., 2016; McDougall *et al*., 2016). During a breeding phase, it is essential to employ an optimum depth if applicable. In another case, the effect of breeding depth maybe varies for another location and breeding session. The availability of food is highly essential in ensuring adequate growth during the breeding phase when nutrients availability is important (Joubert *et al*., 2014). At lower depth, availability of planktonic food is more abundant because of easy penetration of sun light for photosynthesis of planktonic organisms.

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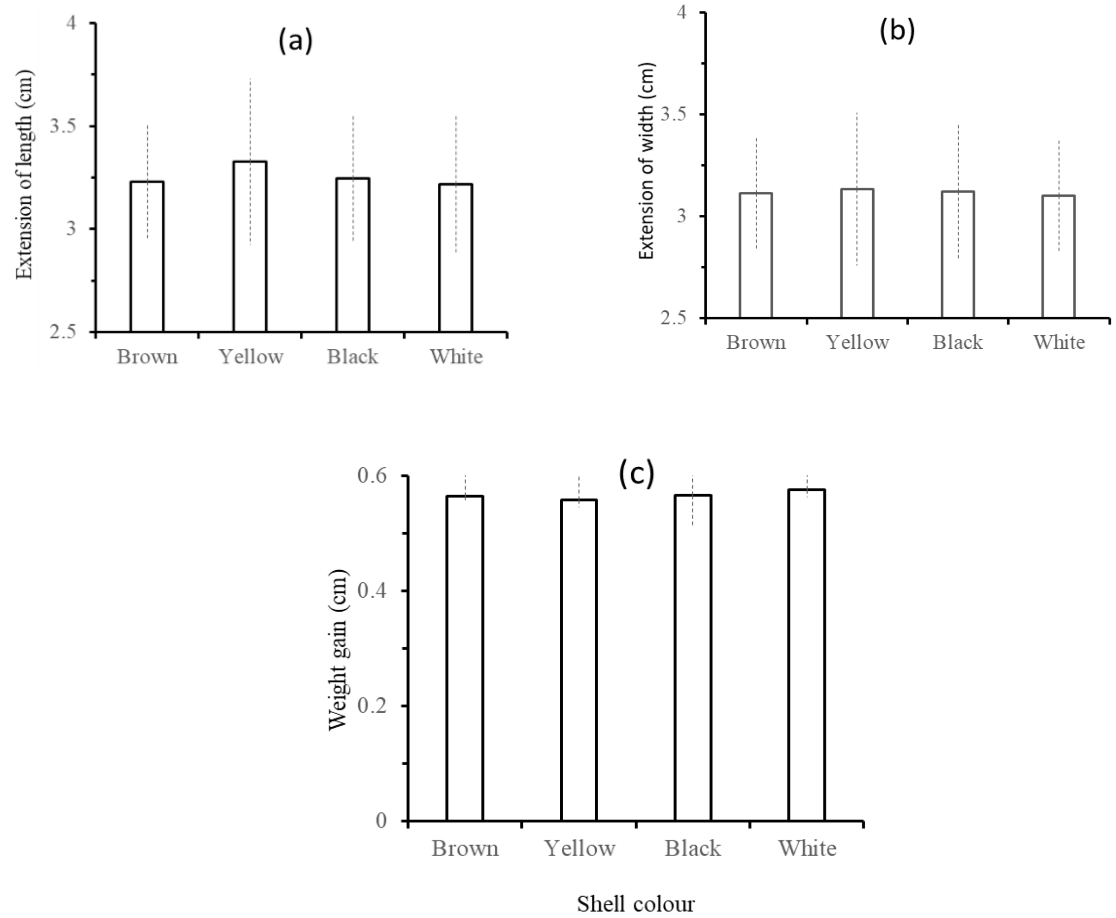

Figure 2. Effect of shell color on (a) length extension, (b) width extension and (c) weight gain of *Pinctada maxima* after a complete cycle of three months breeding.

These findings suggest that availability of feed (planktonic organisms) for *Pinctada maxima* growth in the breeding location is slightly decrease, despite of less significance, over the sea depth. Typically, the seeding stage is conducted at a depth of 2-3 m for the side of less than 5 cm length. Further breeding is typically conducted at depths of more than 5 m. In addition to the sea depth as depicted in **Figure 3**, the coastal conditions and session also affect the growth rates (Joubert *et al*., 2014), and thus important to be addressed in the following study. As reported earlier, the growth rate of pearl oyster is largely affected by environment, especially the availability of plankton, temperature and current (Liu *et al*., 2006; Septy, 2018; Wardana *et al*., 2014; Zheng *et al*., 2009).

3.3. Pearl quality and practical implication

Pearl oyster of *Pinctada maxima* has cultivation potential in Indonesia. Therefore, attempts on optimization of its breeding process have been conducted. Adequate and continuous breeding is paramount important to meet the ever-increasing market demand. Overall results show that neither breading depth, nor the shell color significantly affects the growth of *Pinctada maxima* during the breeding phase. All dominant varieties of seed thus can be breaded at practically feasible depth. This finding can justify of community based breeding to support the local companies. Such approach can stimulate cooperation between community and companies, nurse continuity of seeds supplies and support economy growth.

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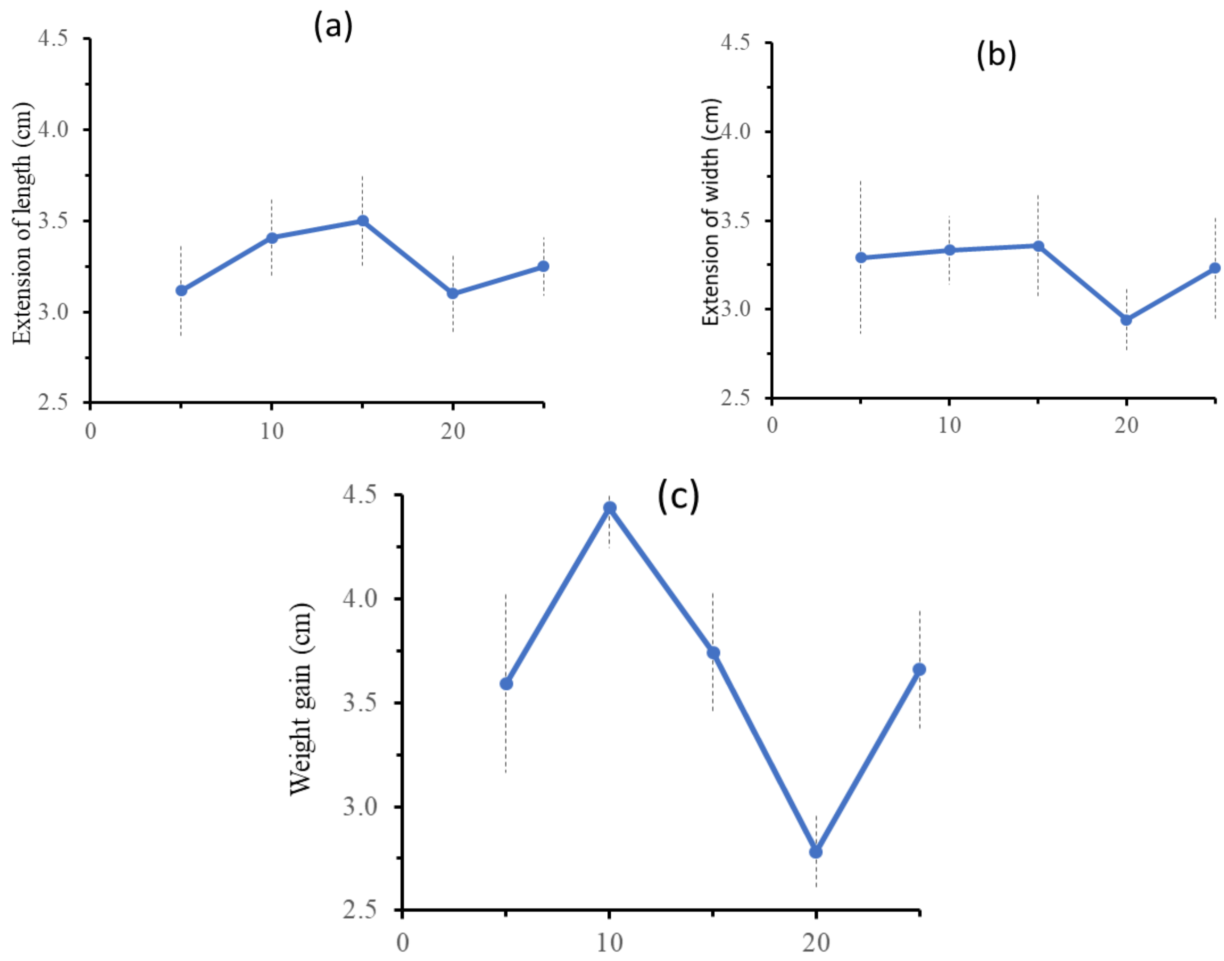

Figure 3. Effect of breeding depth on (a) length extension, (b) width extension and (c) weight gain of Pinctada maxima.

Based on visual observation on the produced pearls, the depth is found to affect the pearl quality. Both the shell and the mantle colors can be used as indicators to predict the color of the resulting pearl, especially from the color of the *seibo* as donor for nucleus inclusion as explained elsewhere (Septy, 2018). Therefore, the shell colors can be used as parameter for determining the final color of the produced pearls (Acosta-Salmón, 2004; Ky *et al*., 2013; Tayale *et al*., 2012). The deeper the location, the better the pearl quality shown by its shiny nature (Tranter, 1958). The shell is thicker and heavier. Therefore, optimization on breeding depth can be done by selecting low depths during juvenile cultivation and breeding, but later transferred to deeper location to achieve high quality pearls. Each shell color results in homogeneous pearls color.

4. CONCLUSION

Assessments on the effect of shell colors of brown, yellow, black and white for a total of 4493 seeds found that shell colors do not really affect both the width extension and weight gain during the breeding process. However, seeds with yellow shell were significantly longer than the rests but most likely have thinner morphology. Moreover, despite generally showing a trend of declining of

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growth rate as function of breading depth, group-to-group assessment shows insignificant differences. However, qualitative observation show that the resulting pearls quality is better for breeding at higher depths. Therefore, we recommend multi-stage breeding, being at lower depth initially until nucleus inclusion, followed by breeding at higher depth to achieve desirable growth and high-quality pearls. Since shell color does not really affect the growth and pearls quality, it is recommended to develop cooperation for *Pinctada maxima* breeding between local community on the company until reaching nucleus inclusion phase to develop local economy and mutual partnership.

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6. AUTHORS' NOTE

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free of plagiarism.

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