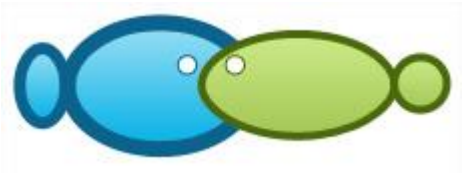

Reproduction performance of maroon clownfish (*Premnas biaculeatus*) in different pairing systems and substrate types

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Abstract. Clownfish is a protandrous hermaphroditic fish, that symbiotes with anemones in nature and spawns as a couple by attaching the eggs on a substrate. These characters become one of the problems in reproduction performance. This study aimed to evaluate the reproduction performance of maroon clownfish *Premnas biaculeatus* in different pairing systems and substrate types. The female fish had 9.87-14.43 cm length and 25.47-73.50 g weight, while the male fish had 5.47-8.37 cm length and 3.40-13.83 g weight. The experiment was designed factorially with pairing systems and substrate types as treatments. Pairing system treatments were composed of natural pairing (P1) and artificial pairing (P2), while substrate type treatments contained anemone (S1), ceramic pot (S2), and PVC pipe (S3). Each treatment was triplicated by distributing the fish couple in an aquarium with flowing-water system, until the reproduction system occurred. The study results showed that natural pairing in three different substrates generated a more adaptive and compatible behavior between male and female fish than artificial pairing. The P1S1 treatment spawned first, after 2.5 months of adaptation in aquarium with the highest number of eggs, fertility rate, number of larvae, and survival rate (2,624.56±720.07 eggs; 98.33±0.36%; 2,541.56±718.4 larvae; 71.06±2.87%, respectively), followed by P1S2 treatment as the second spot ($P<0.05$). In the artificial pairing system, the P2S2 spawned 6.1 months after the fish couple was formed, with the highest hatching rate and longest larval length (98.67±0.22%; 2.61±0.04 mm, respectively) ($P<0.05$). Therefore, spawning with natural and artificial pairing systems can be performed with ceramic substrate to replace anemone, due to producing the best reproduction performance.
Key words: anemones, ceramic, artificial pairing, PVC, reproduction, natural pairing.

Introduction. Maroon clownfish *Premnas biaculeatus* is included in Pomacentridae family. This fish is monophilic, with red maroon color and three white bands on its head, body, and caudal peduncle. Similar to the clownfish, *Amphiprion* included in the same family, *Premnas* genus, and have unique reproduction characteristics, such as social hierarchy, sequential hermaphroditism, and monogamy (Iwata et al 2008; Mitchell 2003). Due to these unique reproduction characteristics, this fish hardly spawns at a mass level and has not been widely cultured. Based on its economical view, this fish is one of the traded commodity exports caught from the nature (Madhu et al 2012). If wild-catching is allowed continuously without any conservation and culture efforts, the biodiversity balance will be threatened.

The reproduction system with social hierarchy is closely affected by several factors, namely individual status (Buston 2003a), broodstock treatment, and substrate usage (Arunde et al 2016). Strong social hierarchy with a dominant group can aggressively control status, size, sex, and non-dominant reproduction status (Hobbs et al 2004; Buston 2003a). According to Mitchell (2003), the social hierarchy position is influenced by the body size, whereas fish are grouped in three types, namely female fish as the largest size (α), male fish as the second largest size (β), and non-breeder as

undifferentiated fish (γ). The mated male and female fish can conduct the reproduction mechanism.

The broodstock treatment in culture condition is highly concerned by the reproduction success. Pairing system can affect the broodstock couple capability to adapt and perform a spawning process. In nature, broodstock chooses its pair by itself, while in culture conditions it should be paired and adapted to form a couple that can spawn. Buston (2003b) stated that for the broodstock it is difficult to choose its pair, when paired according to the hierarchical structure, by placing giant female fish and small male fish by 5 in an aquarium. This situation is caused by its aggressivity level, which persists a long time and affects the reproduction process. According to Thuong et al (2017), *Amphiprion ocellaris* broodstock has been paired after 14 days, then continued by couple adaptation. Anil et al (2012) also found that four couples of *Amphiprion nigripes* were paired after a month, and initial spawning occurred on the 74th day after the matched pair formation in the tank. Balamurugan et al (2018) performed an interspecific hybridization between *Amphiprion percula* and *A. ocellaris*, which spawned a week after the 4 months pairing, with a spawning frequency of three times a month. Thereby, functional and productive male and female broodstock can reproduce in couple, after pairing, once at the sexual maturity.

In addition to pairing system, clownfish reproductivity is also highly determined by substrates, as a residence or an egg placement location. This species displays a demersal spawning activity in nature on a solid substrate and with sea anemones as symbiotes. The anemone and clownfish relationship live in mutualist symbiosis to prevent predation (Litsios et al 2012) and protect the eggs, as female fish place the eggs on a hard substrate near the anemones. Anemone type is varied, depending on the interaction of clownfish (Litsios et al 2014). In nature, this fish uses anemones as a protector and a habitat substrate, however, artificial substrate can also be used for culture condition, such as ceramic pot and PVC pipe, which can be easily moved, before the larvae hatch. So far, there have been no scientific reports regarding the evaluation of pairing system and substrate type manipulations in *P. biaculeatus* culture-scaled spawning. Therefore, this study aimed to evaluate the reproduction performance of *P. biaculeatus* through the manipulation of different pairing systems and substrate types. This study is expected as a database for a controlled, effective, efficient, and sustainable clownfish breeding and growing-out developments.

Material and Method

Materials and tools. The experimental female fish had 9.87-14.43 cm length and 25.47-73.50 g weight, while male fish had 5.47-8.37 cm length and 3.40-13.83 g weight. Substrate types were composed of anemone *Heteractis magnifica*, ceramic pots of 15 cm height and 10 cm diameter, PVC pipes of 15 cm length and 4 inch diameter. Pellet feed (Otohime, Japan) served at feeding the broodstock, while live feed types, namely *Nanochloropsis oculatta*, rotifers, *Artemia* sp. served at feeding the larvae. The tools consisted of 40x40x30 cm^3 aquaria equipped with aeration, Nikon Coolpix Camera, caliper, scales, computer-connected microscope, thermometer, pH meter, DO meter, refractometer, and spectrophotometer.

Broodstock pairing system. Each broodstock couple was reared in an aquarium with water-flowing system for 15 months (April 2021 to July 2022). In the natural pairing system, the broodstocks were paired naturally and reared in aquaria filled with different substrates. In the artificial pairing system, the male broodstock from natural pairs were removed and replaced with the male broodstock from the culture product in aquaria filled with different substrate types. After the adaptation and artificial pairing processes for 14 to 30 days, the broodstock specimens were reared until spawning and egg-hatching. If the broodstock couple was unmatched, it was replaced with a new one. Feeding was performed until apparent satiation three times a day (08.00, 12.00, and 16.00 GMT+7). Syphonization was performed every day and water quality control was performed gradually.

Parameters. The observed parameters included: pairing behavior, growth, spawning activity, and reproduction performance. Pairing behaviors comprised aggressive and sub-massive behaviors. Aggressive behavior was composed of threatening, chasing, biting, body-jerking, and attacking, while sub-massive behavior was composed of body-vibrating, bowing, and substrate biting. The pairing behavior was observed for 12 hours. The growth measurement was performed by measuring the total length and body weight at the initial and final rearing time. The total length was measured with a caliper (0.01 cm accuracy). The body weight was measured using a digital scale with 0.01 g accuracy. The spawning activity was observed for 12 hours and was recorded until the final egg-caring. The reproduction performance was observed from the egg to larvae phase. The attached eggs on a substrate were recorded for a minute on the first day to calculate the region area, number of eggs (absolute), and spawned-egg size. The egg color change and embryonal development were observed for 7 days by a computer-connected microscope. The fertility rate (FR) and hatching rate (HR) were calculated based on the number of eggs. The number and size of the larvae were observed on the first day after hatching. The larval development was observed for 7 days by a computer connected microscope. The survival rate (SR) was calculated based on the number of larvae on the 30^{th} day after hatching.

Data analysis. Fish behavior data were analyzed descriptively, while growth, spawning activity, and reproduction performance were analyzed using an analysis of variance, then continued by the Duncan's multiple range test at 95% level, with SPSS 22.0. To select the best treatment a scoring was performed. The smallest total score of all parameters is the best treatment.

Results

Pairing system related behavior. Based on the observational results, combination of pairing system and substrate type, the treatments showed a different pairing behavior. Paired broodstock couples were cooperative and adaptive on three substrate types. This condition was displayed by both broodstock enclosed together. In contrast, the artificial pairing system displayed an aggressive and a sub-massive interaction (Table 1). Aggressive behavior occurred in female broodstock against male broodstock, while male broodstock had more sub-massive behavior against female broodstock. This couple will stay away from each other to avoid threat, chase, attack, and bite. Usually, the male broodstock occupies the corner of the aquarium while the female broodstock swims freely. Visually, the presence of broodstock coupling behavior following the treatment combination is shown in Figure 1.

Table 1

Pairing behavior on each treatment combination

Treatment	*Aggressive behavior*					*Sub-massive behavior*		
	Threatening	*Chasing*	*Body-jerking*	*Attacking*	*Biting*	*Body-vibrating*	*Bowing*	*Substrate-biting*
P1S1	-	-	-	-	-	-	-	-
P1S2	-	-	-	-	-	-	-	-
P1S3	-	-	-	-	-	-	-	-
P2S1	+	+	+	+	+	+	+	+
P2S2	+	+	+	+	+	+	+	+
P2S3	+	+	+	+	+	+	+	+

P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; (-): aggressive and sub-massive behaviors absence; (+): aggressive and sub-massive behavior presence.

Figure 1. Visual display of pairing behavior in each treatment combination. (P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate).

Spawning activity. The study results showed that all broodstock couple in the combination treatments could spawn, except the P2S1 treatment. Based on the observational results, female broodstock required a month to accept the matched male fish as a couple, as they spawned on the 81th day. Therefore, the culture process needs a longer time to spawn, which affects the production success. Before spawning, the broodstock couple performed the spawning activities, i.e. pre-spawning, namely: ovipositor formation in female broodstock, shelter selection and cleaning, spawning, and post-spawning egg parental caring by fin fanning and mouth-cleaning. Spawning activities are presented in Figure 2.

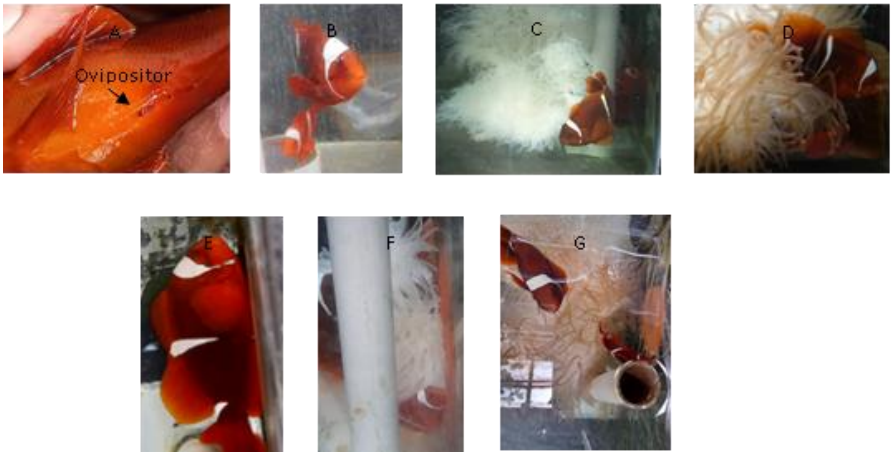

Figure 2. Spawning activity: A: ovipositor formation; B: pairing activity; C: substrate cleaning by male broodstock; D: substrate cleaning by female broodstock; E: egg attachment by female broodstock; F: fertilization by male broodstock; G: egg-caring by male broodstock.

Before spawning, female broodstock has a red protruding ovipositor in the abdomen region, near the genital pore and bulging belly (Figure 2A). Male and female broodstocks perform a pairing activity, selecting and cleaning the egg attachment location for spawning (Figure 2B). Spawning activity is initiated by the female broodstock. The couple performs a side-by-side swimming. Female broodstock leads male broodstock by pushing its body to the side or abdomen of male broodstock, when the spawning occurs. Female broodstock assists the male broodstock once, then only controls regularly its activity. Substrate cleaning is performed by biting the egg-attaching area (Figure 2C). Pre-spawning activity starts from morning until dark, becoming more intensive an hour before spawning. Spawning activity, performed by female broodstock,

occurs as the latter bites the substrate faster with a leaning-body position at 180° for egg-release preparation (Figure 2D). Then, female broodstock will attach its abdomen on the substrate and release eggs with ovipositor. Egg attaching movement is performed sequentially, by adopting a facing-up position on a side, then on the other side, in a continuous manner (Figure 2E).

Eggs are arranged in a lined-up position on the substrate, at a certain distance, without overlapping. This egg attachment is performed by detection of egg or substrate, before the next attachments are carried out. Male broodstock will follow female broodstock from behind to fertilize the eggs, after female broodstock has finished spawning (Figure 2F). Male broodstock also detects the unfertilized eggs with its mouth. Fertilization by male broodstock is performed through a zig-zag movement (Figure 2G). The spawning behavior of the broodstock couple in all treatment combinations had no difference. Eggs were placed appropriately in ceramic and PVC pipe substrate treatments, as well as on the aquarium glass or outlet pipe in the anemone substrate treatment. After spawning, female broodstock will rest, while male broodstock will take care of the eggs. Egg-caring activity contains fin fanning and mouth-cleaning. Quantitatively, spawning activities and egg-caring intensity by broodstock couple is presented in Table 2 and 3.

Table 2

Spawning activities

Treatment	*Initial spawning time (months)*	*Spawning duration (hours)*	*Spawning frequency (cross)*	*Spawning interval (days)*	*Score total*
P1S1	$2.5\pm2.5^{cd(1)}$	$1.50\pm0.23^{b(3)}$	$12.67\pm4.04^{c(1)}$	$17.68\pm4.06^{b(1)}$	6
P1S2	$6.1\pm4.8^{bc(2)}$	$1.14\pm0.06^{c(1)}$	$13.67\pm5.86^{c(1)}$	$12.94\pm3.51^{b(1)}$	5
P1S3	$14.9\pm0.9^{a(5)}$	$1.91\pm0.12^{a(4)}$	$3.00\pm1.00^{ab(3)}$	$12.33\pm0.58^{b(1)}$	13
P2S1	$nd^{d(6)}$	$nd^{d(5)}$	$nd^{a(4)}$	$nd^{a(2)}$	17
P2S2	$8.4\pm4.1^{b(3)}$	$1.20\pm0.07^{c(1)}$	$7.67\pm5.51^{bc(2)}$	$17.06\pm6.87^{b(1)}$	7
P2S3	$11.7\pm2.6^{ab(4)}$	$1.43\pm0.27^{bc(2)}$	$5.33\pm1.53^{ab(3)}$	$13.33\pm3.06^{b(1)}$	10

P: treatment; P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; nd: no data available; different notations show a significant difference; number behind notation show a score.

Table 3

Egg-caring intensity

Treatment	*Fin fanning by female broodstock (cross/')*	*Mouth-cleaning by female broodstock (cross/')*	*Fin fanning by male broodstock (cross/')*	*Mouth-cleaning by male broodstock (cross/')*	*Score total*
P1S1	$63.33\pm6.51^{c(2)}$	$44.00\pm13.50^{c(1)}$	$85.00\pm6.30^{cd(2)}$	$48.33\pm8.50^{d(1)}$	6
P1S2	$40.00\pm3.00^{b(3)}$	$14.00\pm2.00^{b(2)}$	$57.00\pm2.00^{b(4)}$	$36.67\pm1.20^{c(2)}$	11
P1S3	$35.00\pm3.00^{b(3)}$	$8.00\pm2.00^{ab(3)}$	$42.00\pm2.00^{b(4)}$	$13.00\pm2.00^{b(3)}$	13
P2S1	$nd^{a(4)}$	$nd^{a(4)}$	$nd^{a(5)}$	$nd^{a(4)}$	17
P2S2	$76.67\pm7.51^{d(1)}$	$14.00\pm3.00^{b(2)}$	$94.67\pm9.00^{d(1)}$	$18.00\pm3.00^{b(3)}$	7
P2S3	$39.67\pm3.51^{b(3)}$	$7.67\pm0.60^{ab(3)}$	$73.67\pm19.50^{c(3)}$	$14.00\pm4.00^{b(3)}$	12

P: treatment; P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; nd: no data available; different notations show a significant difference; number behind notation show a score.

Statistical analyses showed a significant effect of the pairing system and substrate type on the spawning activities and egg-caring intensity ($p<0.05$). Based on the Duncan's multiple range test, all treatments obtained a different intensity of the egg-caring and spawning activities. The fastest initial spawning time was found in the P1S1 treatment, the shortest spawning duration and the highest spawning frequency were obtained from the P1S2 treatment, and the highest spawning interval was occurred in the P1S3 treatment. Egg-caring intensity performed by male broodstock was higher than female

broodstock in all treatment combinations. Fin fanning intensity was higher than mouth cleaning. The P2S2 treatment obtained a higher intensity of fin fanning, the P1S1 obtained a higher mouth cleaning intensity in both broodstocks.

Reproduction performance. In general, the absolute number of eggs and the egg size spawned in the natural pairing system (P1) was higher than in the artificial pairing system (P2); the same pattern was observed in the fertilization rate and hatching rate (Table 4). The highest number of absolute eggs was obtained from the P1S1, at 2,624.56 eggs, with fertilization and hatching rates at 93-98%. The longest egg size was found in the P1S2 treatment, while the shortest egg size was found in the P2S3 treatment. Egg color change was similar from the first and sixth day in all treatment combinations, as shown in Figure 3. Egg was colored light orange on the first day, dark orange on the second day, chocolate on the third day, chocolate with black eyes on the fourth day, chocolate with silver eyes on the fifth day, black with silver eyes on the sixth day.

Table 4

Reproduction performance in all treatment combinations

Treatment	*Number of eggs (eggs)*	*Fertilization rate (%)*	*Hatching rate (%)*	*Egg length (mm)*	*Egg wide (mm)*	*Score total*
P1S1	$2,624.56\pm720.07^{d(1)}$	$98.33\pm0.36^{d(1)}$	$96.70\pm0.74^{c(2)}$	$1.40\pm0.14^{c(1)}$	$0.50\pm0.04^{cd(2)}$	7
P1S2	$1,491.02\pm319.93^{c(2)}$	$97.51\pm0.60^{cd(2)}$	$96.82\pm1.02^{c(2)}$	$1.45\pm0.03^{c(1)}$	$0.53\pm0.02^{d(1)}$	8
P1S3	$1,039.91\pm64.98^{bc(3)}$	$96.60\pm0.40^{c(3)}$	$94.10\pm0.45^{b(3)}$	$1.18\pm0.18^{b(2)}$	$0.52\pm0.05^{d(1)}$	12
P2S1	$nd^{a(5)}$	$nd^{a(5)}$	$nd^{a(4)}$	$nd^{a(3)}$	$nd^{a(5)}$	22
P2S2	$1,252.84\pm118.79^{c(2)}$	$96.98\pm0.29^{c(3)}$	$98.67\pm0.22^{d(1)}$	$1.04\pm0.08^{b(2)}$	$0.43\pm0.03^{b(4)}$	12
P2S3	$495.29\pm88.26^{ab(4)}$	$93.61\pm1.33^{b(4)}$	$93.68\pm0.56^{b(3)}$	$1.04\pm0.03^{b(2)}$	$0.45\pm0.04^{bc(3)}$	16

P: treatment; P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; nd: no data available; different notations show a significant difference; number behind notation show a score.

The embryonal development was observed microscopically on the first until the seventh day after fertilization (Table 5). Embryogenesis on the first day presented the rudimentary head and caudal fin candidates at opposite poles (Figure 4). On the third day, there was head and caudal fin movement around the yolk. Eyes were present on the fourth day, while perfect brain was occurred in the fifth day. Head and caudal were separated on the sixth and seventh days, followed by pectoral fins and eyes development.

Table 5

Clownfish embryonal development

Day	*Characteristics*
1	The cytoplasm of the fertilized egg is clear on the first day, with a different yolk size, compared to the droplets scattering it. Embryogenesis begins with the rudimentary head and caudal fin at opposite poles
2	The anterior end of the spinal cord has enlarged to form head, colorless eye sockets begin to form on both sides of the head, oil droplets decrease. When the caudal fin extends past the yolk, melanophores begin to appear in the yolk
3	The embryo presents a clearly visible head, mytosomes, and caudal fin. The yolk size decreases, while melanophore increases. Head starts to become segmented with enlarged and slightly dark eye sockets. Caudal fin begins to move around the yolk
4	Clearly-visible eyes; brain begins to develop; the caudal fin moves frequently; the yolk size keeps decreasing. Caudal fin and melanophore begin to separate with clear blood circulation. Rudimentary intestinal cavity begins to appear adjacent to the yolk sac
5	Visible rudimentary gill arches behind the eyes. Only a few of oil globules present in the yolk mass, while yolk decreases; visible perfect brain
6	Head and caudal fin are clearly separated from the yolk; visible development of pectoral fins with very clear intestinal cavity. Eyes are round and black. The head barely makes up one third of the egg capsule
7	The yolk sac becomes very small and is covered by the embryonic abdomen. Melanophores are distributed throughout the body. The fins and eyes are well-developed

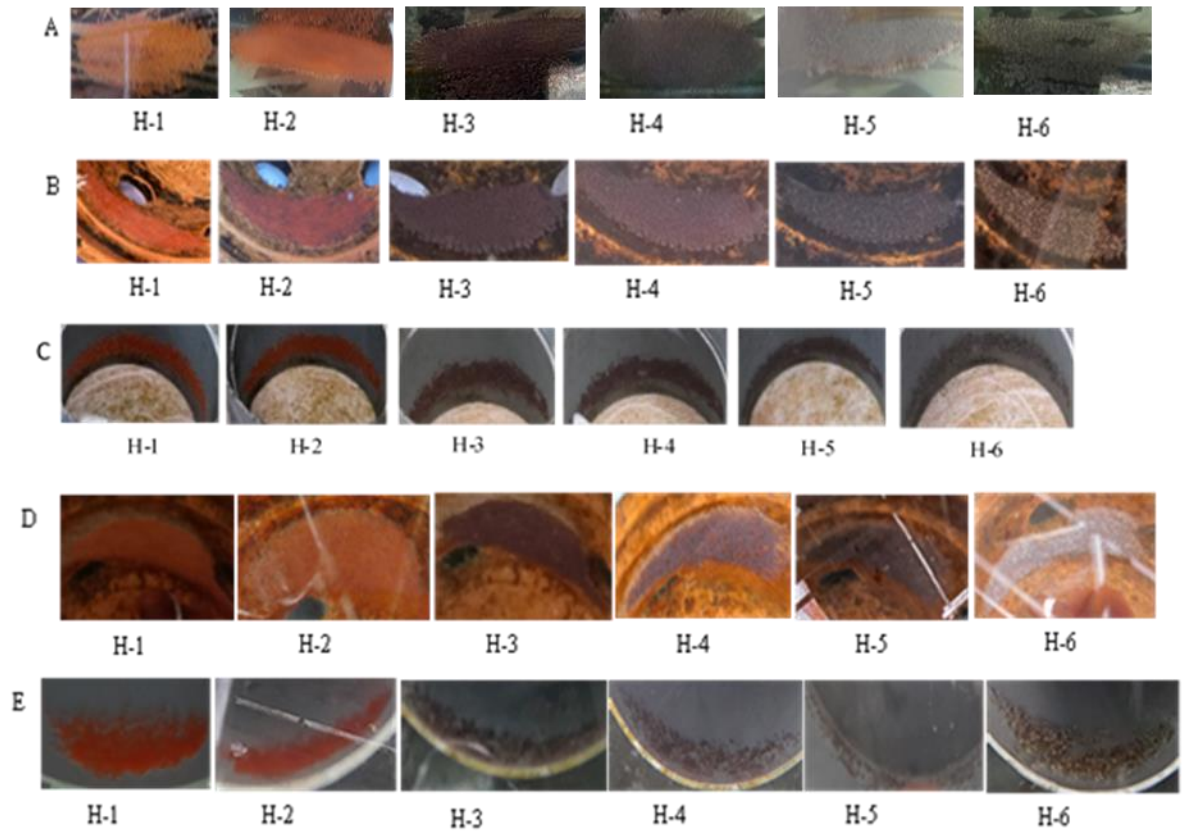

Figure 3. Egg color change in all treatment combinations. A: natural pairing system with anemone substrate; B: natural pairing system with ceramic pot substrate; C: natural pairing system with PVC pipe substrate; D: artificial pairing system with ceramic pot substrate; E: artificial pairing system with PVC pipe substrate; H-1: first day; H-2: second day; H-3: third day; H-4: fourth day; H-5: fifth day; H-6: sixth day.

The highest number and survival rate of the 30-day-old larvae was found in the P1S1 treatment, while the lowest values were found in the P2S3 treatment. Larvae had 1.85-2.61 mm length and 0.41-0.54 mm width. The Duncan's multiple range test indicated that the longest larvae length was in the P2S2 treatment, while the widest larval width was in the P1S2 treatment (Table 6).

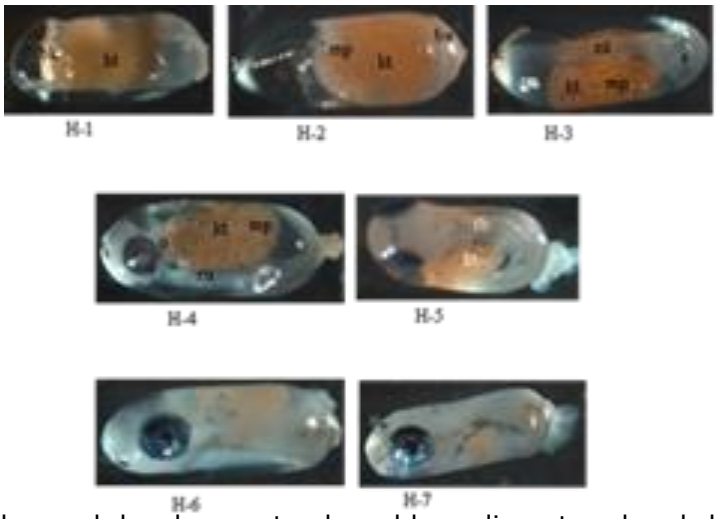

Figure 4. The embryonal development, where bk: rudimentary head; kt: yolk sac; be: rudimentary caudal fin; k: head; rm: eye cavity; mp: melanophore; nt: notochord; e: caudal fin; o: brain; ru: intestinal cavity; li: gill arch; tb: vertebrae; H1-H7: first to seventh day.

Table 6

Larval performance and survival rate in each treatment combination

Treatment	*Number of larvae (larvae)*	*First day length (mm)*	*First day width (mm)*	*Survival rate (%)*	*Score total*
P1S1	2,541.56±718.40$^{d(1)}$	2.37±0.24$^{c(1)}$	0.50±0.06$^{b(1)}$	71.06±2.87$^{e(1)}$	4
P1S2	1,445.00±318.21$^{c(2)}$	2.58±0.07$^{c(1)}$	0.54±0.01$^{b(1)}$	53.13±1.93$^{cd(3)}$	7
P1S3	978.56±62.01$^{bc(3)}$	1.85±0.25$^{b(2)}$	0.41±0.14$^{b(1)}$	44.95±1.51$^{bc(4)}$	10
P2S1	nd$^{a(5)}$	nd$^{a(3)}$	nd$^{a(2)}$	nd$^{a(6)}$	16
P2S2	1,236.00±114.48$^{c(2)}$	2.61±0.04$^{c(1)}$	0.52±0.06$^{b(1)}$	61.10±11.28$^{d(2)}$	6
P2S3	464.11±84.00$^{ab(4)}$	2.06±0.07$^{b(2)}$	0.46±0.03$^{b(1)}$	40.74±2.53$^{b(5)}$	12

P: treatment; P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; nd: no data available; different notations show a significant difference; number behind notation show a score.

A newly-hatched larvae has a transparent body, giant eyes, visible mouth, and small yolk sac. On the body, along the line near the vertebrae and intestinal region, there is a little melanophore with elongated dorsal, caudal, and anal fins. Larvae can swim freely, with slow movements. The yolk decreases on the second day after hatching and the digestive system starts to develop with the head and abdomen pigmentation, ventral fin, dorsal, and caudal fin development. After the yolk absorption ends, melanophores intensify along the vertebral line or around the intestine region. On the third to seventh days, the larvae metamorphoses perfectly with shrunk yolk, transparent dorsal, abdomen, pelvic, and caudal fin, and developed fin rays. Dark chocolate pigmentation is present on the lateral side and head region (Figure 5). A vertical white-band, as a Pomacentridae distinctive character, firstly emerged on the head region, in the 12th day, then on the body, in the 13th day. Both bands are enlarged on the 16th day. Moreover, the third white band occurs on the caudal peduncle in the 22th day.

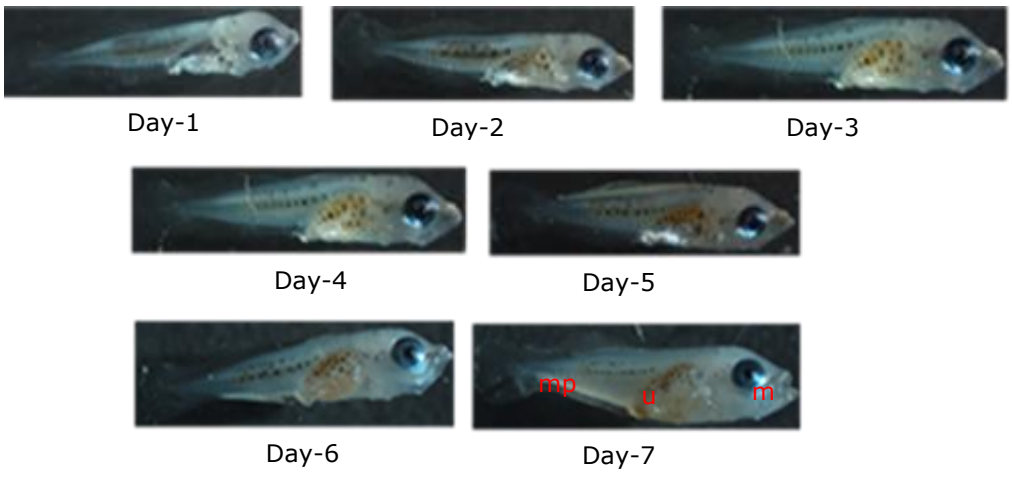

Figure 5. Day 1 to Day 7 larval development: m-eye; mp-melanophore; u-intestine.

Growth. The female and male fish experienced length and weight increase during the rearing. Length, weight, and specific growth rate of female and broodstocks are presented in Table 7 and 8. Based on the statistical analyses, pairing systems and substrate types showed a significantly different effect on the length and weight of all broodstocks (besides the female and male fish difference in the specific weight growth) ($P<0.05$). Based on the Duncan's multiple range test, the P1S1 and P1S3 treatments obtained the highest length and weight growth in female fish, compared to the male fish. The highest weight specific growth rate in female and male broodstocks was found in the P2S3 treatment. Moreover, the highest specific length growth rate in female broodstock was found in the P1S2 treatment, while in male broodstock was found in the P2S1 treatment.

Table 7

Female broodstock growth in each treatment combination

Treatment	*Female broodstock*						*Score total*
	Initial total length (cm)	*Initial weight (g)*	*Final total length (cm)*	*Final weight (g)*	*Daily length growth (%)*	*Daily weight growth (%)*	
P1S1	13.90±0.98$^{b(1)}$	72.43±13.71$^{b(1)}$	14.40±0.98$^{ab(2)}$	91.17±15.83$^{b(1)}$	0.01±0.001$^{a(3)}$	0.06±0.01$^{a(1)}$	9
P1S2	11.80±1.30$^{ab(2)}$	42.47±12.85$^{a(2)}$	13.77±0.49$^{ab(2)}$	68.20±12.39$^{ab(2)}$	0.04±0.02$^{b(1)}$	0.13±0.08$^{a(1)}$	10
P1S3	14.23±0.65$^{b(1)}$	73.50±12.58$^{b(1)}$	15.37±0.50$^{b(1)}$	88.17±9.06$^{b(1)}$	0.02±0.01$^{ab(2)}$	0.05±0.04$^{a(1)}$	7
P2S1	9.87±1.06$^{a(3)}$	25.47±6.09$^{a(2)}$	11.57±2.24$^{a(3)}$	47.20±22.72$^{a(3)}$	0.04±0.02$^{ab(2)}$	0.15±0.07$^{a(1)}$	14
P2S2	10.20±2.07$^{a(3)}$	30.20±20.66$^{a(2)}$	11.30±2.01$^{a(3)}$	38.93±19.41$^{a(3)}$	0.02±0.01$^{ab(2)}$	0.09±0.06$^{a(1)}$	14
P2S3	10.93±1.98$^{a(3)}$	31.80±14.08$^{a(2)}$	12.57±2.31$^{ab(2)}$	56.80±26.04$^{ab(2)}$	0.03±0.02$^{ab(2)}$	0.15±0.06$^{a(1)}$	12

P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; different notations show a significant difference; number behind notation show a score.

Table 8

Male broodstock growth in each treatment combination

Treatment	*Male broodstock*						*Score total*
	Initial total length (cm)	*Initial weight (g)*	*Final total length (cm)*	*Final weight (g)*	*Daily length growth (%)*	*Daily weight growth (%)*	
P1S1	8.37±0.99$^{c(1)}$	13.17±5.05$^{bc(2)}$	9.17±1.25$^{b(1)}$	14.43±4.65$^{ab(2)}$	0.02±0.01$^{a(1)}$	0.03±0.02$^{a(3)}$	10
P1S2	7.73±0.75$^{bc(2)}$	10.47±4.01$^{a-c(3)}$	8.60±1.08$^{ab(2)}$	11.27±4.01$^{ab(2)}$	0.03±0.01$^{a(1)}$	0.02±0.01$^{a(3)}$	13
P1S3	8.23±0.95$^{c(1)}$	13.83±5.30$^{c(1)}$	9.20±1.25$^{b(1)}$	16.47±6.02$^{b(1)}$	0.03±0.01$^{a(1)}$	0.05±0.01$^{ab(2)}$	7
P2S1	5.47±0.57$^{a(4)}$	3.40±1.04$^{a(5)}$	6.83±1.00$^{a(3)}$	6.23±3.62$^{a(3)}$	0.06±0.02$^{a(1)}$	0.15±0.07$^{b(1)}$	17
P2S2	6.33±0.90$^{ab(3)}$	5.83±2.90$^{ab(4)}$	7.27±0.70$^{ab(2)}$	7.63±2.54$^{a(3)}$	0.04±0.01$^{a(1)}$	0.09±0.06$^{ab(2)}$	15
P2S3	6.37±1.46$^{ab(3)}$	6.13±4.16$^{ab(4)}$	7.50±1.47$^{ab(2)}$	8.27±3.71$^{a(3)}$	0.05±0.04$^{a(1)}$	0.10±0.11$^{ab(2)}$	15

P1: natural pairing; P2: artificial pairing; S1: anemone substrate; S2: ceramic pot substrate; S3: PVC pipe substrate; different notations show a significant difference; number behind notation show a score.

Water quality. The average water quality parameters were determined during the experimental period, as in Table 9. From the measurement results, water quality in rearing media was still close to the standard range for clownfish rearing.

Table 9

Water quality during experimental period

Parameter	*Value*	*Quality standards*
Temperature (ºC)	26.8-30	28-30
pH	7.8-8.02	7-8.5
DO (mg L^{-1})	4.0-4.24	>5
Salinity (ppt)	30.00-32.89	33-34
Nitrite (mg L^{-1})	0.009-0.04	-
Nitrate (mg L^{-1})	<0.050	0.008
Ammonia (mg L^{-1})	0.39-1.21	0.3

Source: Minister of the Environment 2004.

The pairing system success in clownfish was evaluated based on the broodstock couple adaptation and matching, which can continuously perform the spawning process. The adaptation and matching capabilities present several behaviors, namely aggressive, sub-massive, and cooperative (Sahusilawane et al 2020; Fitzpatrick et al 2006). Broodstock couples from nature, that chose their own couple, had more adaptive and cooperative behaviors in an aquarium with anemone substrate, following the natural conditions. The artificial pairing female and male broodstocks present more aggressive and sub-massive behaviors in all substrate types. Sub-massive behavior in male fish is part of the adaptive behavior to be accepted on the female fish territorial area and to prevent a continuous aggressivity. Aggressive behavior in female fish as a dominant individual in hierarchical group will not occur continuously, if the male fish remains in the lowest hierarchy and involves in peace and non-opposite cooperations (Buston & Balshine 2007; Wong et al 2007; Buston & Cant 2006; Buston 2004). The aggressivity of female fish towards male fish will stop when both are adaptive and matched to perform the spawning process. Conversely, the aggressive behavior of female fish will increase by injuring the male fish to death, if both fish are unmatched or not mutually adaptive. According to Hobbs et al (2004) and Buston (2003a), the behavior of species living in social groups has a strong social hierarchy, namely members of the dominant group aggressively manage the status, size, sex, and reproductive status of the hierarchical group below them (non-dominant).

After adaptation, the broodstock couple will spawn, depending on the gonadal maturation. Before spawning, female broodstock presents a gonadal maturation sign, namely a red cone-shaped protruding ovipositor formation along the oviduct (Sahusilawane et al 2020; Liew et al 2010). The fastest initial spawning time and the highest spawning frequency in culture condition was found in the natural pairing couple with anemone substrate. The fastest spawning was found in the matched-pair couple with pipe substrate. Ghosh et al (2012) mentioned that different spawning time and number of eggs were determined by various factors, such as the female broodstock body size and the broodstock capability to begin the reproduction cycle.

In addition, in this study, the event of a broodstock couple which did not spawn (P2S1) was caused by the smaller average size and body weight than in other broodstock couples, which prevent the female and/or male to reach their broodstock reproductive maturation. This condition was in line with Buston & Ellith (2011), Mitchell (2003), who stated that one of the determining factors in clownfish *A. percula* reproduction success was the mass change and standard length of the female broodstock. Madhu et al (2006) also mentioned that the male and female broodstock *P. biaculeatus* sizes were of 55-60 mm and 120-140 mm, respectively. Roy et al (2014) also reported that a female broodstock fish with 50.62 g weight and 11.4 cm length, and a male broodstock fish with 11.63 g weight and 8.0 cm length have performed spawning process as a couple. Moreover, this couple was formed from the artificial pairing, thus more energy was

released for adaptation and protection than for the reproduction activity. This condition was il line with Madhu et al (2020), who stated that clownfish is a monogamous fish that will require time to adapt with a new pair, if the previous pair is removed, thus avoiding the aggressivity of the fish pair or other subordinates, which is detrimental to the reproductive activities. Other factors that need to be considered in clownfish broodstock spawning success are age and fertility of the couple (Rattanayuvakorn et al 2005). In this study, female broodstock was collected from nature, so the age and fertility rate were unknown.

After the eggs were fertilized, the broodstock couple took care of the eggs until hatching, by keeping, fanning, cleaning with mouth, caudal fin and pectoral fins, and by the dead eggs removal. Ghosh et al (2012) stated that egg-eating behavior could be caused by less supportive environmental condition, physical disruption, or other biological damages. Egg-caring activity was more performed by male broodstock (Olivotto et al 2017; Madhu et al 2012). Fanning was performed to increase the oxygen supply for eggs (Sahusilawane et al 2020). Olivotto et al (2011) also stated that broodstock fanning depended on the water circulation in the rearing tank. When the air supply in the water is appropriate, the male broodstock deploys a lower effort in its activity to fan the embryo. Initially, female broodstock was inactive in the egg-caring. This condition was caused as female broodstock was more focused on nutrients preservation for the oogenesis (Olivotto et al 2017; Madhu et al 2012).

Spawning success is affected by the substrate use. Broodstock couple reared in anemone substrate could spawn faster. Anemone and clownfish symbiosis supports the broodstock's fitness increase by providing advantages, such as: protection against predators (Arvedlund et al 2000), additional nutrients from the anemone tentacles and egg protection (Saenz-Agudelo et al 2011). Mitchell (2003) mentioned that various spawning durations depend on the host movement. Biological characteristics of anemones (size, shape and toxicity) influence the habitat characteristics of clownfish (Chausson et al 2018).

Ceramic pot substrate impacts on bacterial adhesion related to porosity, specific surface area, hydrophobic/hydrophilic characteristics, substrate material and substrate topography (Colombo et al 2013; Colombo et al 2010; Luyten et al 2010). Ceramic has a steady macro- and microstructure in contact with water, even on the the long-term (Wesseling et al 2015). PVC pipe has a disadvantage: it is a non-porous material, which requires sufficient aeration to help egg-caring activity. In this study, eggs were plastered with fungi and bacteria, so the eggs became white and were unable to develop and eventually they died and unhatched. The eggs death rate reached 100%, before hatching (for the first spawning). According to Wilkerson & Frakes (1998), insufficient egg aeration causes dirt and fungi bulking.

The egg incubation period until hatching was averagely occurred in 7 days. The egg hatched on evening (19.00 or 20.00 GMT+7) for 15-20 minutes at 26-30°C. This condition was similar to Gopakumar & Johnson (2014) and Madhu et al (2020), who reported that clownfish egg-hatching was occurred 1-2 hours after dark and required 15-20 minutes to hatch at 27-29°C. The average number of eggs in the P1S1 treatment was affected by female broodstock size and anemone substrate. In many commercial fish species, broodstock size is considered in the selection, due to its influence on the fecundity level and egg size (Kamler 2005; Kolm 2002). In Banggai cardinalfish *Pterapogon kaudernii*, a giant fish size increased the eggs production (Kolm 2002), in an ideal environment for the sexual maturation and the natural reproduction (Mylonas et al 2010).

The number of eggs and egg size range produced from this study was almost similar to Roy et al (2014) and Madhu et al (2007), as *P. biaculeatus* produced a various number of eggs, of 150-2,148 eggs per spawning, in a 15-20 days interval; thus the spawning could be performed twice per month. Roy et al (2014) found that the average of egg length was at 1.705±0.02 mm. Different egg size was affected by species variations, fecundity, incubation time duration, and sequential spawning from the same couple. The embryonal development in all treatments showed no significant difference. On the first day, the initial fertilized eggs were colored light orange with yolk and giant oil

globules on the dorsal region and clear perivitelline region. On the seventh day, embryos were colored black with silver eyes across the whole egg capsule. During the dark period, eggs were hatched and the larvae broke the egg capsule by firstly emerging its caudal fin. The egg color change in this study was similar to Madhu et al (2020).

The average length of newly-hatched larvae in this study was 1.85-2.61 mm, while Madhu et al (2006) reported that the newly-hatched larvae length was 2.5-3.6 mm. On the seventh day, larvae were perfectly metamorphosized. Larval development was fully supported by the live feed supply, namely phytoplankton, from the 1st to 15th day after hatching. Another live feed species, such as rotifer *B. rotundiformis* was supplied to the larvae from the 2nd to 10th day, then newly-hatched nauplii *Artemia*were supplied from the 11th day until the juvenile phase. The emergence of white bands on the larvae body in this study was also reported by Roy et al (2014), where two vertical white bands emerged on the 12th day. In this study, the first vertical white band (head region) emerged on the 12th day, followed by the second white band (body region) on the 13th day. The two white strips were enlarged and much clearer on the 15th and 16th days. The third soft white band (caudal fin region) emerged on the 22nd day, then enlarged on the 24th day, along with the fish growth. Madhu et al (2012) stated that the three white bands started to emerge on the 23rd day after hatching, then became more distinctive and enlarged on the 45th day. The average survival rate of larvae for 30 days, in all treatments, was of 55.45%. This survival rate level was higher than in Roy et al (2014), at <6%, ranging in the survival rate reported by Madhu et al (2012), namely 30.2–78.9%.

Growth is one of the indicators for reproduction success. Madhu et al (2006) stated that a proper female broodstock size was of 12-14 cm, while the male broodstock size was of 5.5-7 cm. These proportions were similar to the sizes of the broodstock used in this study. In the artificial pairing, the couple spent most of their energy to adapt to each other, while in natural pairing more energy was available for reproduction. In addition, natural feeding habits changed, due to the pellet feeding during the rearing, which caused the slow response of the fish to the feed, which affected the growth. Hamzah (2017) stated that female clownfish obtained the highest growth rate when fed with blood cockle and pellet mixture, while the lowest growth rate was found in fish fed with only commercial pellets. Male fish will restrict the feeding supply to prevent a conflict with the dominant individuals and eviction (Wong et al 2008), according to the hierarchical position (Iwata et al 2008). Moreover, male broodstock spends most of its energy for substrate cleaning and egg-caring activities during the spawning preparation and spawning process. Gopakumar & Ignatius (2006) stated that male broodstock spent averagely 30-60% of its time to take care of the eggs and reduce the feeding pattern. Dhaneesh et al (2011) also added that anemone fish required high energy for re-maturation, continuous spawning, and intensive parental-care during the spawning period.

Anemone substrate placed in the rearing aquarium provided more comfort than ceramic pot and PVC pipe, but this study showed that fish could live with other substrates, namely ceramic pot and PVC pipe. According to Lubis et al (2013), clownfish *A. ocellaris* that lives in aquarium no longer needs anemones to survive, due to their replacement with artificial anemones, as aquarium ornaments. Therefore, live anemone exploitation as ornaments can be limited for the species conservation.

Conclusions. Natural pairing system with anemone substrate obtains a higher reproduction success than the artificial pairing system. In an artificial pairing system, ceramic pot substrate produces the best reproduction performance. Ceramic pot substrate can be used as an anemone replacement, both in natural and artificial pairing systems.

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References

Anil M. K., Santhosh B., Prasad B. O., George R. M., 2012 Broodstock development and breeding of black-finned anemone fish *Amphiprion nigripes* Regan, 1908 under captive condition. Indian Journal of Fisheries 59(1):77-82.

Arunde E., Sinjal H. J., Monijung R. D., 2016 [The effect of different substrats on fertility and survival rate of catfish larva, *Clarias* sp]. Jurnal Budidaya Perairan 4(1):715. [In Indonesian].

Arvedlund M., Bundgaard I., Nielsen L. E., 2000 Host imprinting in anemonefishes (Pisces: Pomacentridae): does it dictate spawning site preferences? Environmental Biology of Fishes 58:203-213.

Balamurugan J., Kathiresan K., Meenakumari B., 2018 Interspecific hybridization and reproductive biology of marine clownfishes, the orange clownfish *Amphiprion percula* and clown anemonefish *A. ocellaris*. North American Journal of Aquaculture, pp. 1-9.

Buston P. M., 2003a Social hierarchies, size and growth modification in clownfish. Nature 424(6945):145-146.

Buston P. M., 2003b Forcible eviction and prevention of recruitment in the clown anemonefish. Behavioral Ecology 14(4):576-582.

Buston P. M., 2004 Does the presence of non-breeders enhance the fitness of breeders: An experimental analysis in the clown anemonefish *Amphiprion percula*. Behavioral Ecology and Sociobiology 57:23-31.

Buston P. M., Balshine S., 2007 Cooperating in the face of uncertainty: A consistent framework for understanding the evolution of cooperation. Behavioural Processes 76:152-159.

Buston P. M., Cant M. A., 2006 A new perspective on size hierarchies in nature: patterns, causes, and consequences. Oecologia 149:362–372.

Buston P. M., Elith J., 2011 Determinants of reproductive success in dominant of clownfish: a boosted regression. Journal of Animal Ecology 80:528-538.

Chausson J., Srinivasan M., Jones G. P., 2018 Host anemone size as a determinant of social group size and structure in the orange clownfish (*Amphiprion percula*). PeerJ 1-21.

Colombo P., Mera G., Riedel R., Sorarù G. D., 2010 Polymer-derived-ceramics: 40 years of research and innovation in advanced ceramics. Journal of the American Ceramic Society 93:1805-37.

Colombo P., Bernardo E., Parcianello G., 2013 Multifunctional advanced ceramics from preceramic polymers and nano-sized active fillers. Journal of the European Ceramic Society 33:453-469.

Dhaneesh K. V., Kumar T. T. A., Vinoth R., Shunmugaraj T., 2011 Influence of brooder diet and seasonal temperature on reproductive efficiency of clownfish *Amphiprion sebae* in captivity. Recent Research in Science and Technology 3(2):95-99.

Fitzpatrick J. L., Desjardins J. K., Stiver K. A., Montgomerie R., Balshine S., 2006 Male reproductive suppression in the cooperatively breeding fish *Neolamprologus pulcher*. Behavioral Ecology 17(1):25-33.

Ghosh S., Kumar T. T. A., Gunasundari S., Balasubramanian T., 2012 Reproductive biology of *Amphiprion nigripes* (Regan, 1908) at Lakshadweep archipelago: Implication for specific reef fish conservation in Asia. Scientific Reports 1(11):1-5.

Gopakumar G., Ignatius B., 2006 A critique towards the development of a marine ornamental industry in India. Proceeding International Symposium on Improved sustainability of fish production system and appropriate technologies for utilization, Cochin, pp. 606-614.

Gopakumar G., Johnson B., 2014 An overview of marine ornamental fisheries in India. Proceeding International Seminar on "Ornamental Fish Breeding, Farming and Trade", Cochin, pp. 54-62.

Hamzah Z. B., 2017 Controlling and influencing the growth and breeding success on different gender of common clownfish and tomato clownfish using feed as key factor from within. Proceeding 1st International Malaysia-Indonesia-Thailand Symposium on Innovation and Creativity (iMIT-SIC), Arau, pp. 251-255.
Hobbs J. P. A., Munday P. L., Jones G. P., 2004 Social induction of maturation and sex determination in a coral reef fish. Proceedings of the Royal Society B: Biological Sciences, London, pp. 2109-2114.
Iwata E., Nagai Y., Hyoudou M., Sasaki H., 2008 Social environment and sex differentiation in the false clown anemonefish, *Amphiprion ocellaris*. Zoological Science 25:123-128.
Kamler E., 2005 Parent–egg–progeny relationships in teleost fishes: an energetics perspective. Reviews in Fish Biology and Fisheries 15:399-421.
Kolm N., 2002 Male size determines reproductive output in a paternal mouthbrooding fish. Animal Behaviour 63:727-733.
Liew H. J., Ambak M. A., Abol-Munafi A. B., 2010 False clownfish breeding, behavioral, embryonic and larval development, rearing and management of clownfish under captivity. Lambert Academic Publishing, Germany, 74 p.
Litsios G., Sims C. A., Wuest R. O., Pearman P. B., Zimmermann N. E., Salamin N., 2012 Mutualism with sea anemones triggered the adaptive radiation of clownfishes. BMC Evolutionary Biology 12(212):1-15.
Litsios G., Kostikova A., Salamin N., 2014 Host specialist clownfishes are environmental niche generalists. Proceedings of the Royal Society B: Biological Sciences, London, 281(1795):1-7.
Lubis M. Z., Pujiyati S., Mujahid M., 2013 [The influence anemone (*Heteractis magnifica*) againts vitality fish clown (*Amphiprion ocellaris*) to minimize the use of living coral in the artificial sea aquarium]. Jurnal Teknologi Perikanan dan Kelautan 4(2):1-6. [In Indonesian].
Luyten J., Mullens S., Thijs I., 2010 Designing with pores–synthesis and applications. Kona Powder and Particle Journal 28:131-142.
Madhu K., Madhu R., Gopakumar G., Sasidharan C. S., Venugopalan K. M., 2006 Breeding, larval rearing and seed production of maroon clown *Premnas biaculeatus* under captive conditions. Marine Fisheries Information Service Technical and Extension Series 190:1-5.
Madhu K., Madhu R., Rajagopalan M., Sasldharan C. S., Venugopalan K. M., 2007 Success in captive breeding of maroon clown *Premnas biaculeatus* (Bloch, 1790). Fishing Chimes 27(2):30.
Madhu K., Madhu R., Retheesh, 2012 Broodstock development, breeding, embryonic development and larviculture of spine-cheek anemonefish, *Premnas biaculeatus* (Bloch, 1790). Indian Journal of Fisheries 59(1):65-75.
Madhu K., Madhu R., Mohandas M. P., Vijayan M. T., Baby S. T., 2020 Marine ornamental fishes and their breeding. CMFRI initiatives 130-149.
Mitchell J. S., 2003 Social corralates of reproductive success in false clown anemonefish; subordinate group members do not pay-to-stay. Evolutionary Ecology Research 5:89-104.
Mylonas C. C., Fostier A., Zanuy S., 2010 Broodstock management and hormonal manipulations of fish reproduction. General and Comparative Endocrinology 165:516-534.
Olivotto I., Planas M., Simoes N., Holt G. J., Avella M. A., Calado R., 2011 Advances in breeding and rearing marine ornamentals. Journal of the World Aquaculture Society 42(2):135-166.
Olivotto I., Leu M. Y., Blazquez M., 2017 Life cycles in marine ornamental species–fishes as a case study patterns of sexuality in fish. In: Marine ornamental species aquaculture. Calado R., Olivotto I., Oliver M. P., Holt G. J. (eds), John Wiley & Sons Ltd, pp. 23–49.
Rattanayuvakorn S., Mungkornkarn P., Thongpan A., Chatchavalvanich K., 2005 Embryonic development of saddleback anemonefish *Amphiprion polymnus*, Linnaeus (1758). Kasetsart Journal: Natural Science 39:455-463.

Roy D. S., Ray B. C., Sarma K., George G., 2014 Captive breeding and larval rearing of maroon spiny damsel fish, *Premnas biaculeatus* (Bloch, 1790). Journal of the Andaman Science Association 19(1):78-87.
Saenz-Agudelo P., Jones G. P., Thorrold S. R., Planes S., 2011 Detrimental effects of host anemone bleaching on anemonefish populations. Coral Reefs 30:497–506.
Sahusilawane H. A., Junior M. Z., Suprayudi M. A., Soelistyowati D. T., Tumbelaka L. I. T. A., Effendi I., 2020 Preliminary study: paired clownfish (*Amphiprion* sp.) spawning pattern in indoor rearing system. IOP Conference Series: Earth and Environmental Science 404(012034):1-8.
Thuong N. P., Sung Y. Y., Ambak M. A., Abol-Munafi A. B., 2017 The hormone 17β-estradiol promotes feminization of juveniles protandrous hermaphrodite false clownfish (*Amphiprion ocellaris*). Marine Freshwater Behaviour and Physiology 1-10.
Wesseling W., Wittka S., Kroll S., Soltmann C., Kegler P., Kunzmann A., Riss H. W., Lohmeyer M., 2015 Functionalised ceramic spawning tiles with probiotic *Peudoalteromonas* biofilms designed for clownfish aquaculture. Aquaculture 446:57-66.
Wilkerson J. W., Frakes T. A., 1998 Clownfishes: A guide to their captive care breeding and natural history. TFH publications, New York, 240 p.
Wong M. Y. L., Buston P. M., Munday P. L., Jones G. P., 2007 The threat of punishment enforces peacefull cooperations and stabilizes queues in a coral-reef fish. Proceedings of the Royal Society B: Biological Science, London, 274(1613):1093-1099.
Wong M. Y. L., Munday P. L., Buston P. M., Jones G. P., 2008 Monogamy when there is potential for polygyny: tests of multiple hypotheses in a group-living fish. Behavioral Ecology 19(2):353–361.
*** Minister of the Environment, 2004 [Decree of the State Minister for the Environment number 51 of 2004 concerning sea water quality standards]. Jakarta, Indonesia, 8 p. [In Indonesian].

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