Preliminary study: paired clownfish (*Amphiprion sp.*) spawning pattern in indoor rearing system

H A Sahusilawane 1,2*, M Z Junior 1, M A Suprayudi 1, D T Soelistyowati 1, I T A Tumbelaka 3 and I Effendi 1

1Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB University), Bogor, Indonesia
2Aquaculture Study Programme, Tual State Fisheries Polytechnic, Southeast Maluku, Indonesia
3Department of Clinic, Reproduction, and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University (IPB University), Bogor, Indonesia

*E-mail: helenafia17@gmail.com

Abstract. *Amphiprion* sp. is known as the most popular tropical marine ornamental fish with unique colours and sequential hermaphroditic, social hierarchy and monogamous characteristics. Information on the spawning pattern of clownfish is very important. The purpose of this study was to evaluate the spawning patterns of *Amphiprion* sp. in indoor rearing system. Observations of spawning patterns were conducted for 14 days in paired of clownfish reared in aquarium sized 40x40x30 cm³ with running water system. The results showed that maturation process in female started with the appearance of swollen abdomen and extended oviduct from the genital hole of female, then parent pair fish together cleaned the egg laying area, and spawned at 13.00-16.00. Spawning occurred on the fourth day to the seventh. The eggs were incubated for one week. During the incubation period, egg colour changed from orange on the first day to silver eye on seventh day, and the eggs hatched at 18.00-20.00 on eighth day. The spawning pattern of clownfish in indoor system is highly determined by time and environmental conditions.

Keywords: clownfish, indoor system, spawning pattern

1. Introduction

Marine ornamental fish is one of the potential fisheries commodities in the domestic and overseas market (Rahmi et al 2017). It is due to the presence of multitudinal colours and beatuiness to attract the interest of aquarists (Ghosh et al 2011) and resulted the large number of consumption fish entrepreneurs turning into ornamental fish businesses (Sihombing et al 2013). However, the ornamental fish trade is still dependent on catches originating from the nature. This causes considerable pressure on the ecosystem such as over-exploitation of natural populations, damage to coral reefs and environmental degradation (Ignatius et al 2001). Therefore, it is necessary to develop marine ornamental fish cultivation to reduce pressure on the ecosystem. In addition, marine ornamental fish has a very significant prospect of
cultivation development especially in hatchery and grow out technologies in meeting availability of sustainable stocks.

One of the largest groups of reef fishes that is great demand and needs to be developed in cultivation systems in clownfish (*Amphiprion* sp.) according to Dhaneesh *et al* (2011). These fish belong to Pomacentridae family, distributed in tropical and sub-tropical seas. The family consisted of 29 genera and 350 species under four sub-families (Allen 1991). There are 29 species of clown fishes with two generic hybrids between Amphiprions and Premnas (*Allen et al* 2008). Clownfish have some of the advantages consist of variations in shapes and colours, unique patterns of behavior, adaptive under certain conditions (*Wabnitz et al* 2003).

The uniqueness of this fish include the symbiotic relationship of mutualism with anemones sea as a host (*Fautin and Allen* 1992), has a reproductive system of sequential hermaphrodites, social hierarchy and monogamy (*Ross 1978*). In addition, these fish are also able to adapt in captivity, and are easy to accept artificial feed (*Ignatius et al* 2001). *Ghosh et al* (2011) stated clown fish that hatch in nature will grow and survive better in captivity and have more attractive prices that occur continuously. In addition to its superiority and uniqueness, there are obstacles due to over fishing activity. This obstacle causes scarcity of seed and female parent in nature. Alternative solution that can be developed are cultivation activities in an effort to fulfill market demand. In Indonesia, several site of clownfish cultivation include Lampung, Lombok, Maluku, and Bali (*Rahmi et al* 2017).

Maluku as one of the areas where clownfish is cultivated has a cultivation system with cultivation technology that is beginning to develop. Such technology uses recirculation systems and the provision of natural and artificial feed in various phases of fish development. So far the study of the reproductive system in clown fish in Maluku is still limited, whereas of the clown fish is unique (*Dhaneesh et al* 2012). The uniqueness of the reproductive system is due to the hermaphrodite protandry characteristic, born as male individuals and then undergoes differentiation and sex reversal into adult female gonads (*Buston 2003*). In a clownfish population, a social hierarchy will be formed including undifferentiated individuals, male and female parent that already have a functional gonads (*Mitchel 2003*). The existence of parent pair in a population gives rise to monogamous traits (*Fricke and Fricke 1977*). These three unique characteristics cause no mass spawning that occurs in indoor systems. Thus, spawning activity in clown fish needs to be investigated because it is the initial step toward their breeding (*Falk-Petersen 2005*). Spawning aspects in captivity are influenced by environmental factors such as water quality and nutrition (*Righton et al* 2010). Reproductive activities include frequency and time of spawning, incubation periods and seasonal variation in egg production (*Madhu and Madhu 2007*, *Madhu et al* 2012). Naturally, females lay eggs on a hard substrate near the host anemone (*Fautin and Allen 1992*). This study was aimed to evaluate the pattern and behavior of spawning in *Amphiprion* sp. reared in an indoor systems.

2. Materials and methods

This preliminary study was conducted at the Marine of Development and Culture Center Ambon for two weeks. This study used observation and interview methods. Observation was carried out by observing the behavior of clownfish mate pairs while interviews involved some laboratory staff and technician as source of information.

2.1. Materials

The materials used in this study were three pairs of clownfish broodstock that had spawned before, carpet anemone (*Stichodactyla haddoni*), and PVC pipe with a diameter 4 of inches and a length of 15 cm.
2.2. **Methods**

2.2.1. **Broodstock management.** The male broodstock was 7 cm in size and female broodstock was 9 cm in size and had been spawned, were reared in a 40x40x30 cm aquarium provided with running water system and aeration. Pairing process of broodstock was conducted gradually. Pairs of clownfish need days to get used to living, without anemone to avoid solitary trait. If the male and female of the pair reject each other, then a new pair was introduced. Pair change was done by placing both parents into freshwater simultaneously for five minute to relax. Furthermore, the new pairs were put into the aquarium and the female parent was performed osmoregulation process to reduce her fierce nature. After the two parents attracted to each other, the anemone was placed into the aquarium as a host. Feed given in the form of Otohime and blood worm twice a day (8:00 and 16:00). Siphoning media was done once a day after feeding. Water quality was measured every two days including water temperatures (26°C-29°C), salinity (33-34 ppm). Rearing was carried out on photoperiod condition with 12 h light and 12 h dark.

2.2.2. **Spawning behavior.** Observations of spawning activities was carried out for one week on three pairs of broodstock in a separate aquarium and documented with a Nikon COOLPIX L840 digital camera. Observations of spawning activities and behavior included maintenance and cleaning of substrate, spawning, egg production, and egg care activities, fertilization, and hatching. The eggs spawned and attached to the substrate were documented and recorded for 1 minute on the first and second day (Buston and Elith 2011). The area where the egg attached was calculated using ImageJ software (Holtswarth et al 2017). The number of eggs was calculated in 1 cm² area where the egg attached, then calculating the number of eggs was multiplied by the area occupied by the eggs (Dhaneesh et al 2011). Developed broodstocks were tested for their efficiency of hatching rate (HR) using the formula (Gunasekara et al 1996):

\[
HR = \frac{\text{No of eggs hatched}}{\text{No of total eggs fertilized}} \times 100
\]  

2.2.3. **Statistical analysis.** Recording spawning behavior description, substrate cleaning activity, egg number, egg care, egg colour change, and egg incubation period were analyzed descriptively.

3. **Results and discussion**

3.1. **Female gonadal mature and prepare of spawning**

Based on the results of observations, broodstock pairs that had been well adapted showed more aggressive and cooperative (figure 1a). An anemone was occupied by broodstock pairs as territorial areas. Fautin and Allen (1992) reported that before spawning the male swims up and down in front of the female or laterally covers the female.

The female was performed gonadal maturation with a characters i.e., a swollen abdomen and extended oviducts and appear coulored milky red before spawning (figure 1e, 1f). Similar to Liew et al (2010) which mentioned that in the genital hole near the caudal fin at the ventral body part of the female will show a cone-shaped small tube known as ovipositor. Ovipositor is coloured milky red with black spots on the tip and will be changed to light pink. Bailey and Sainford (2003) reported that mature female had large body size, rounder, and coloured light pink abdomen.

Toward spawning, the broodstock pairs working together to clean the substrate or wall area of the shelter actively. Cleaning was conducted by the mouth and fin for 4 to 5 h with the purpose to obtain of rough substrate surface for egg attachment (figure 1b, 1c, 1d). Average substrate cleaning activity by the male took place 14 times/minute with the mouth and the female 10 times/minute. According to Liew et al
(2010), cleaning of substrate for eggs attachment by male can occur as much as 114 times for 4 to 5 hours with mouth and 26 times with caudal fin. In contrary, the female was doing cleaning with mouth and caudal fin about 39 times. Next, shelter area attachment of the eggs is oval or round shape and can be located on inner or outer wall of the shelter. Anil et al (2012) mentioned that pairs started clearing substrate for attachment of eggs at two days before spawning.

3.2. Spawning
The results of observation showed that the female observed swim slowly and clearing the area for attachment of 236 eggs. The female approached and attached the genital hole on wall shelter and released eggs at 13.00-15.00 (figure 1g). Some of researches reported about spawning time from Amphiprion sp. In the morning hours, spawning occurred on A. chrysogaster (Gopakumar et al 1999), A. sebae (Ignatius et al 2001), A. ocellaris (Anil et al 2010), A. nigripes (Anil et al 2012). It was also reported that the eggs were attached on the cleared substrate and sperms was released at the site and happened during 20 to 40 minute.

In addition, some of clownfish was spawned on the morning (08.00-09.00 a.m) such as A. melanopus and A. nigripes while on the afternoon amongst A. clarkii, A. percula, and A. ocellaris. The spawning mechanism undergoes 50 to 60 minute for completing fertilization (Siva and Haq 2017). Balamurugan et al (2018) also reported that clown anemofish pairs was spawned from 08.00 to 10.00 a.m and orange clownfish pairs from 09.00 a.m to 14.00 p.m. Eggs attachment and arrangement were conducted regularly so that it does not overlap each other on the shelter wall. According to Litsios et al (2012), clown fish placed one layer of eggs on harsh surface of substrate. Next, the male secretes sperm to fertilize of the egg (figure 1h) until the end time of spawning. The new egg is capsule shaped and orange coloured. Usually, attachment of eggs just for one area but if the broodstock feel disturbed, the broodstock move and attach the eggs on another safety area. Attachment of the eggs on two area causes difficulty on caring their eggs.

Furthermore, the eggs were cared by the broodstock during incubation time especially by the male. Unlike male, the female did not always care their eggs, but oversee around area attachment of eggs especially from another disturbance. This in accordance with Dhaneeesh et al (2009) report that the male have more role play in caring of the eggs. The same broodstock can be spawned again seven to ten times after hatching period. This case depend on nutrition and environmental condition. Balamurugan et al (2018) stated that frequency of spawning on Amphiprion occurs as many as three time/month.

3.3. Egg determination
Based on the results, the average number of eggs from a pair for each spawning was 236 eggs. Mazzoni et al (2019) mentioned that the number of Amphiprion eggs produced were minimum of 300 in each 15 days. Difference of the eggs number were caused by filtration system and food diet. Recirculation systems with stable parameters resulted three times/month in spawning frequency. In other hand, some factors which affect egg number such as genetic variation, food supply, and photoperiod. Those three factors affect nutrition utilization and sustainability for gonadal development, mature eggs, healthy status of broodstock. Therefore, improvement of nutrition broodstock can increase the quality of eggs and sperm, and also seed production (Izquierdo et al 2001). Dhaneeesh et al (2009) also explain that number of eggs was determined by the size of the female. At first small number of eggs were produced yet will be increased at the end of spawning.

Eggs incubation period occured for seven days according to Wilkerson (2001), Dhaneeesh et al (2012), Ghani et al (2015). Eggs incubation period ranged between six to eight days. It is depend on variation of species and environmental condition. In addition, Bailey and Sainford (2003) stated that eggs incubation process occurs seven to ten days and affected by variation of fish species and water temperature as explained by Madhu dan Madhu (2007). This means that different species of fish have
different incubation periods. This is regulated by genetic factors internally related to reproductive physiological mechanisms. It was also explained that at high water temperatures, the frequency of spawning will increase.

During incubation period, the broodstock pairs will be take care of the egg together (parental care). This can be proved by broodstock activity which fanning of eggs with pectoral and caudal fin and mouthing of water. Similar to Ghosh et al (2012) mentioned that the eggs reared by male with a way fanning and mouthing until hatching. Yamamoto (1975) stated that development of fertilization eggs is affected by oxygen content in water. Furthermore, oxygen content on eggs very determined by oxygen content at broodstock.

![Figure 1. Spawning pattern of *Amphiprion* sp. (a) the broodstock pairs; (b) substrate cleaning by male; (c) substrate cleaning by the broodstock pairs; (d) substrate cleaning by female; (e) maturity female; (f) ovopositor on female; (g) eggs incubation; (h) fertilization; (i) care of eggs by the broodstock pairs; (j) care of eggs by male; (k) care of eggs by female.](image-url)
Average activity of male in carrying the eggs occurred as many as 10 times/minute with mouth, 26 times/minute with pectoral fin and caudal fin of 5 times/minute. Meanwhile, the female cares as many as 2 time/minute with mouth and 4 times/minute with caudal fin. Another hand, Dhaneesh et al (2009) explain that the role of broodstock in fanning with using pectoral fin was to regulate temperature in attachment area, to prevent eggs from broken. In addition, if the incubation period extended, it will increase fanning frequency (Anil et al 2012). Otherwise, the broodstock will discard the sterile eggs, dead eggs, and particle or dirt that attached on eggs with mouth. Fertile eggs will change into black colour whilst the sterile change into white. According to Siva and Haq (2017) which explain that sterile eggs, dead eggs, and dust particles were removed by mouthing process. 

Before hatching, the egg changed the colour in seven days. The observation results showed the change of colour from orange to brown-black with gold colour on the pupil (figure 2a-2g). This is in accordance with Buston and Elith (2011), Balamurugan et al (2013) which explained that the colour changed sequentially from orange in the first day, brown in the second and third day, brown with black eye on the fourth day, brown with silver eye on the fifth day, eye with pupil on the sixth day, and gold eye pupil on the seventh day.

On seventh day, the eggs hatched into larvae with transparent colour and has gold colour eyes. Eggs were hatched in tank with 2 tonnes capacity filled with 50 L of phytoplankton at 06.00 pm. Eggs on the shelter coincide with broodstock were moved into tank using plastic basket that equipped with bouy. Broodstock were moved in order to increase the hatching rate. Hatching occurred at 18.00-20.00. After the eggs hatched, broodstock were moved again into the aquarium to avoid canabalism trait. Hatching was high if supported with handling technique, water temperature, and aeration control.

Based on observation and calculation, the average of hatching rate (HR) was 99%. This is proved with the eggs attached on shelter. Anil et al (2012) mention that hatching percentage varied between 94 to 95 %. During the eggs caring pairs of broodstock removed dead eggs.

**Figure 2.** Change of eggs colour. (a) orange in the first day, (b) & (c) brown in the second and third days, (d) brown with black eye on the fourth day, (e) brown with silver eye on the fifth day, (f) eye with pupil on the sixth day, (g) ready eggs to hatch on the seventh day.
4. Conclusion

The reproduction activities started from pairing, gonadal maturing, substrate cleaning, spawning, fertilization, eggs incubation, changes of eggs colour, and hatching. The spawning pattern of clownfish in indoor system is highly determined by time and environmental conditions.

Acknowledgments

We are grateful to laboratory ornamental fish, Marine of Development and Culture Center Ambon. Thanks for BUDI Scholarship 2017 from Ministry of Finance, Republic of Indonesia.

References

Allen G R 1991 *Damselfishes of the World* (Germany: Mergus Publishers Mello)
Allen G R, Drew J and Kaufman L 2008 *Amphiprion barberi*, a new species of anemone fish (Pomacentridae) from Fiji, Tonga and Samoa *Aqu. Int. J. Ichthyol.* 14 105-114
Anil M K, Santhos B, Jasmine S, Reenamole S, Unnikrishnan C and Anukumar A 2010 Techniques for mass production of two species of clown fish: Clown anemone fish *Amphiprion ocellaris* Cuvier, 1830 and Spinecheek anemonefish *Premnas biaculeatus* (Bloch, 1790) *Proc. National Seminar on Technology and Trade Prospects in Ornamental Aquaculture* (Fisheries Research and Extension Center, Tamil Nadu Veterinary and Animal Sciences University) ed S Felix (India: Chennai) pp 96-102
Anil M K, Santhosh B, Prasad B O and George R M 2012 Broodstock development and breeding of black-finned anemone fish *Amphiprion nigripes* Regan, 1908 under captive conditions *Indian J. Fish.* 59 77-82
Bailey M and Sainford G 2003 *Aquarium Fish: a Comprehensive and Authoritative Guide to Tropical Freshwater, Brackish and Marine Fishes* (New York: Hernnes Hourse Anness Publishing Inc.) p 128
Balamurugan R J, Ajith Kumar T T and Balasubramanian T 2013 Influence of host anemone (*Stichodactyla haddoni*, Saville-Kent, 1893) locomotion on its resident anemone fish reproduction *Anim. Reprod. Sci.* 140 103-107
Balamurugan J, Kathiresan K and Meenakumari B 2018 Interspecific hybridization and reproductive biology of marine clownfishes, the orange clownfish *Amphiprion percula* and clown anemonefish *A. ocellaris*. *N. Am. J. Aquacult.* 1-9
Buston P 2003 Social hierarchies, size and growth modification in clownfish *Nature* 424 145-146
Buston P M and Elith J 2011 Determinants of reproductive success in dominant pairs of clownfish: a boosted regression tree analysis *J. Anim. Ecol.* 80 528-538
Dhaneeesh K V, Kumar T T A and Shunmugara T 2009 Embryonic development of percula clownfish, *Amphiprion percula* (Lacepede, 1802) *J. Sci. Res.* 4 84-89
Dhaneeesh K V, Kumar T T A, Vinoth R and Shunmugaraj T 2011 Influence of brooder diet and seasonal temperature on reproductive efficiency of clownfish *Amphiprion sebae* in captivity *Rec. Res. Sci. Techno.* 3 95-99
Dhaneeesh K V, Kumar T T A, Ghosh S and Balasubramanian T 2012 Breeding and mass scale rearing of clownfish *Amphirion percula*: feeding and rearing in brackishwater *Chin. J. Oceanol. Limn.* 30 528-534
Falk-Petersen I B 2005 Comparative organ differentiation during early life stages of marine fish *Fish Shellfish Immnun.* 19 397-412
Fautin D G and Allen E R 1992 *Field Guide to Anemonfishes and Their Host Sea Anemones* (Perth: Western: Australian Museum)
Fricke H W and Fricke S 1977 Monogamy and sex change by aggressive dominance in coral reef fish *NPG.* 266 830-832
Ghani A, Hariyano, Tahang H, and Basir E A 2015 Produksi ikan hias nemo hybrid varian black photon skala rumah tangga JTBL 5 1-8

Ghosh S, Kumar T T A, Nanthinidevi K and Balasubramanian T 2011 Hatchery production of clark’s clownfish, Amphiliprion clarkii (Bennett, 1830) using brackish water IPCBEE 22 51-56

Ghosh S, Kumar T T A, Gunasundari S and Balasubramanian 2012 Reproductive biology of Amphiliprion nigripes (Regan, 1908) at Lakshadweep archipelago: Implication for specific reef fish conservation in Asia Sci. Rep. 1 2-5

Gopakumar G, George R M and Jasmine S 1999 Breeding and larval rearing of the clownfish Amphiliprion chrysogether Mar. Fish. Infor. Serv. 161 8-11

Gunasekara R M, Shim K F and Lam T J 1996 Effect protein level on spawning performance and acid amino composition of eggs of Nile tilapia, Oreochromis niloticus Aquacult. 146 121-134

Holtswarth J N, San Jose S B, Montes Jr H R, Morley J W and Pinsky M L 2017 The reproductive seasonality and fecundity of yellowtail clownfish (Amphiliprion clarkii) in the Philippines Bull. Mar. Sci. 93 997-1007

Ignatius B, Rathore G, Jagadis I, Kandasami D and Victor A C C 2001 Spawning and larval rearing technique for tropical clown fish Amphiliprion sebae under captive condition J. Aqua. Trop. 16 241-249

Izquierdo M S, Fernandez-Palacios H and Tacon A G J 2001 Effect of broodstock nutrition on reproduction performance of fish Aquacult. 197 25-42

Liew H J, Ambak M A and Abol-Munafi A B 2010 Breeding, Behavioral, Embryonic and Larval Development, Rearing and Management of clownfish Under Captivity (Germany: Lambert Academic)

Litsios G, Sims C A, Wüst R O, Pearman P B, Zimmermann N E and Salamin N 2012 Mutualism with sea anemones triggered the adaptive radiation of clownfishes BMC. Evol. Biol. 12 1-15

Madhu K and Madhu R 2007 Influence of lunar rhythm on spawning of clown anemo fish Amphiliprion percula under captive condition in Andaman and Nicobar islands J. Mar. Biol. Assoc. India. 49 58-64

Madhu R, Madhu K and Retheesh T 2012 Life history pathways in false clown Amphiliprion ocellaris Cuvier, 1830: A journey from egg to adult under captive condition JMBAI. 54 77-90

Mazzoni T S, Junior H R, Viadanna R R and da Silva G C 2019 Clown fishes breeding in captivity using low cost resources and water recycling World J. Aquacult. Res. Dev. 1 023-026

Mitchell J S 2003 Social correlates of reproductive success in false clown anemonefish; subordinate group members do not pay-to-stay Evol. Ecol. Res. 5 89-104

Rahmi, Ramses and Pramuanggit P N 2017 Pemberian pakan pelet dan cacing sutera pada pemeliharaan benih ikan hias nemo Simbiosa. 6 40-47

Righton D A, Anderson K H and Neat F 2010 Thermal niche of Atlantic Gadus morhua: limits, tolerance and optimar Mar. Ecol. Prog. Ser. 420 1-13

Ross R M 1978 Reproductive behavior of the anemone fish Amophiliprion melanopus on Guam Copeia. 1978 103-107

Sihombing F, Artini N W and Dewi R K 2013 Kontribusi pendapatan nelayan ikan hias terhadap pendapatan total rumah tangga di desa Serangan JAA. 2 178-190

Siva M U and Haq M A B 2017 Embryonic development of anemone fishes in captivity J. Oceanogr. Mar. Sci. 8 1-13

Wabnitz C, Taylor M, Green E and Razak T 2003 From Ocean to Aquarium (Cambridge UK: UNEP-WCMC)

Wilkerson J D 2001 Clownfishes: a Guide to Their Captive Care, Breeding and Natural History Microsom (USA: Ltd USA) p 240

Yamamoto T 1975 Stage in The Development available source: http://www.bio11.bio.nagoya-u.ac.jp:8000/Stages.html.