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REPRODUCTIVE CHARACTERISTICS OF THE ANEMONE FISHES *AMPHIPRION OCELLARIS* AND *A. PERIDERAION* FROM EASTERN COAST OF PENINSULAR MALAYSIA

I. V. TAN, T. ARAI[*]

Environmental and Life Sciences Programme, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE 1410, Brunei Darussalam

** Corresponding author: takaomi.arai@ubd.edu.bn*

AMPHIPRION
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SPAWNING

ABSTRACT. – In order to understand the reproductive ecology of the anemone fishes, *Amphiprion ocellaris* and *A. perideraion*, the maturation variables gonadosomatic index (GSI) and fecundity were examined throughout the year on the eastern coast of Peninsular Malaysia. Fecundity of *A. ocellaris* and *A. perideraion* ranged from 67 to 1889 and from 120 to 2653, respectively. The monthly GSI fluctuated in both species. The highest GSI values were found in April for both species suggesting that their spawning season peak occurred in this month. The fecundity was lower compared to the other coral reef fish species, which produce tens of thousands to hundreds of thousands eggs. Anemone fishes practice parental care, which might favor lower numbers of eggs compared to other coral reef fishes, which do not provide care and spawn pelagic eggs. This might emphasize the importance of social structure and pairing in regulating gonad maturation and mating success. Anemone fishes are in high demand for marine aquarists and they have become increasingly popular targets for collection. Our findings will be useful for biologists and resources managements in formulating effective strategies for the conservation and aquaculture of these fishes.

INTRODUCTION

The anemone fish (genus *Amphiprion*) is an important fish in the global marine ornamental trade. Many countries in the world are currently involved in marine ornamental trades. Indonesia and Philippines are the main exporters out of 43 supply countries (Wood 2001), and the USA, the European Union and Japan are the main importers involved in the trade (Wood 2001, Wabnitz *et al*. 2003). Among the specimens collected (> 145 000) and traded globally in the period 1997-2002, the false clown anemone fish, *Amphiprion ocellaris*, was the number one species collected in the marine ornamental trade (Wabnitz *et al*. 2003). This suggests that *A. ocellaris* is in great demand in many countries to generate income through the aquarium trade. The population of anemone fishes decreased drastically because most of the marine ornamentals (90 %) are from wild-caught fisheries (Wabnitz *et al*. 2003) as it has a lower cost to traders than breeding the fish (Tlusty 2002). This shows that the majority of traders would not invest in expensive captive-breeding facilities since there are cheap wild-caught specimens available. The 2003 Disney/Pixar motion picture *Finding Nemo* also created an upsurge in harvesting of wild-caught *A. ocellaris* (Militz & Foale 2017). However, overharvesting of marine ornamental species has a negative impact on its population maintenance. The high demand from the aquarium trade and the deterioration of its natural habitat caused by human activities are two leading causes of dramatic decreases in the number of wild anemone fishes (Abol-Munafi *et al*. 2011a). Many studies have reported that the population of anemone fish is decreasing (Wabnitz *et al*. 2003, Shuman *et al*. 2005). In the Philippines, the population of the anemone fishes, together with their host anemones, are significantly lower in exploited area than in protected area (Shuman *et al*. 2005). Since the number of the anemone fishes is decreasing drastically, conservation is needed to maintain its populations. Donaldson (2009) stated that anemone fishes have been categorized as threatened species and recently, the anemone fish such as *A. ocellaris* and *A. percula* are considered for Endangered Species Act listing (Rhyne *et al*. 2017). Anemone fishes highlight the impacts of coral reef degradation, increasing ocean acidification and warming due to climate change. These changes directly or indirectly affect most species in the degraded habitats (Donaldson 2009).

The propagation of *A. ocellaris* in captivity has recently become an important trend due to both commercial purposes and conservation. The reproductive biology of anemone fish is important to facilitate effective management in culturing the species to provide for local and global demands. Extensive studies on reproductive characteristics of many species of anemone fish were found in Guam, the Red Sea, Eniwetok in Marshall Island (*A. melanopus*, *A. bicintus*, *A chrysopterus*, *A. perideraion*, *A. percula* and *A. clarkii*), East coast of Australia (*A. akindynos* and *A. latezonatus*) and Japan (*A. clarkii*). However, limited study exists on reproductive biology of anemone fish in Southeast Asia although it is the biggest exporter (51 % of exports) of marine ornamental species especially in Indonesia and the Philippines (Dominguez & Botella 2014). In the Philippines, only reproductive seasonality and fecundity of *A. clarkii* was examined (Holtswarth *et al.* 2017) whereas only self-recruitment of anemone fishes and the impact of marine ornamental fishery were examined in Indonesia (Madduppa 2012). Most of the studies in the Philippines and Indonesia were related to the socio-economy of marine ornamental fishery and its impact on the population structure of anemone fishes (Maduppa *et al.* 2014), the interaction structure between anemones and anemone fishes (Ricciardi *et al.* 2010), and the population impact of collecting sea anemones and anemone fishes for marine aquarium trade (Shuman *et al.* 2005). In Malaysia, studies have focused on embryonic development (Liew *et al.* 2006), sexual dimorphism on the morphometric characteristics (Abol-Munafi *et al.* 2011b), and gonad histology of the protandrous anemone fish (Abol-Munafi *et al.* 2011a). However, there is limited information on the reproductive characteristics of anemone fishes in Malaysia. The study on the reproductive ecology of anemone fishes in Malaysia is important because it is one of the countries in Southeast Asia included in a biodiversity hotspot. It comprises a high diversity of coral reef fishes such as anemone fishes (Arai 2014). Therefore, Malaysia plays an important role in ornamental fish trade globally with, for example, *A. ocellaris* being mainly exported to the US (Krishnakumar 2017, Rhyne *et al.* 2017). Malaysia is one of the world's largest producers of ornamental fish. Based on the Fisheries Statistics in 2004, the total production of ornamental fish in Malaysia increased of 8.6 % from RM 97.64 million in 2003 to RM 106.03 million in 2004 (Department of Fisheries Malaysia, 2005). However, the aquarium trade in Malaysia is almost exclusively focused on freshwater species. Malaysia has a rich and diverse array of coral fish species that have a high potential to be commercialized. Attempts for the propagation of marine fish species for ornamental purposes are severely limited due to the lack of biological knowledge and technical skills (Abol-Munafi *et al.* 2011a). Therefore, the biological information such as reproduction is important for the effective aquaculture of marine ornamental species. Aquaculture in turn could help to improve livelihood in developing countries which rely heavily on the harvest of marine ornamental species in the wild in Southeast Asia, as well as to prevent over-harvesting of the species in the wild (Reksodihardjo-Lilley & Lilley 2007). It is unfortunate though that, in the present situation with increasing demand, supplies are mostly dependent on wild catches (Cato & Brown 2003, Abol-Munafi *et al.* 2011a). Studies on Malaysian pomacentrids are still lacking (Sin *et al.* 1994), and concerns have been raised about the declining numbers of this species in Malaysian waters due to the possibility of overfishing and deterioration of its natural habitat caused by destructive collection methods (Livengood & Chapman 2007, Abol-Munafi *et al.* 2011a). Therefore, it is important to be able to propagate this species in captivity for both commercial and conservation purposes. Although the anemone fish has been successfully reared in captivity in other countries (Juhl 1992, Moe 1992), the production in Malaysia is still low (Abol-Munafi *et al.* 2011a).

A thorough knowledge of the fecundity of fish is essential for evaluating commercial potentialities, stock study, life history study, particular culture and actual management of the fishery (Rheman *et al.* 2002). Fecundity is an important factor in fishes for determining their reproductive potential. Monthly variations of gonadosomatic index (GSI) provide a reasonable indicator of reproductive seasonality for fish. The seasonal timing of reproduction, spawning time, is often identified from changes in the GSI, which determines the reproductive season (Arruda *et al.* 1993).

In the present study, we examined the fecundity of two species of anemone fishes, the false clown anemone fish, *A. ocellaris* and the pink anemone fish, *A. perideraion*. The monthly variations of GSI in both *A. ocellaris* and *A. perideraion* were also examined to determine the spawning season. The fecundity was also compared to other coral reef fishes to understand the similarities and differences in reproductive characteristics and strategies among coral reef fishes.

MATERIALS AND METHODS

A total of 80 mature female specimens, comprising 40 *A. ocellaris* and 40 *A. perideraion,* were collected during April, June, November 2014 and October 2015 in Bidong Island, Terengganu State, east coast of Peninsular Malaysia, Malaysia (Latitude 5.62°N, Longitude 103.07°E) (Fig. 1). The number of specimens of *A. ocellaris* and *A. perideraion* collected were 8 and 15 specimens in April, 7 and 11 specimens in June, 15 and 6 specimens in November and 10 and 8 specimens in October, respectively. The island has a well-developed coral reef ecosystem comprising a variety of coral and rocky reef associated fishes (Matsunuma *et al.* 2011). Although we tried to sample throughout the whole year, sampling could not be conducted between December and March as we could not access the island due to the northeast monsoon season with rough sea conditions between November and February (Syafrina *et al.* 2015).

All fishes were collected using scoop net by scuba diving. After collecting fishes, they were anesthetized with MS-222, stored in an ice box immediately, brought back to laboratory and kept in the freezer until the fecundity estimations were carried out. Our protocol was in accordance with the guide for animal experimentation of the Universiti Malaysia Terengganu (UMT) and Universiti Brunei Darussalam (UBD), and fish-handling approvals were granted by the animal experiment committees of UMT and UBD.

The total length (TL) (cm), standard length (SL) (cm) and body weight (BW) (g) were measured. To estimate the fecundi-

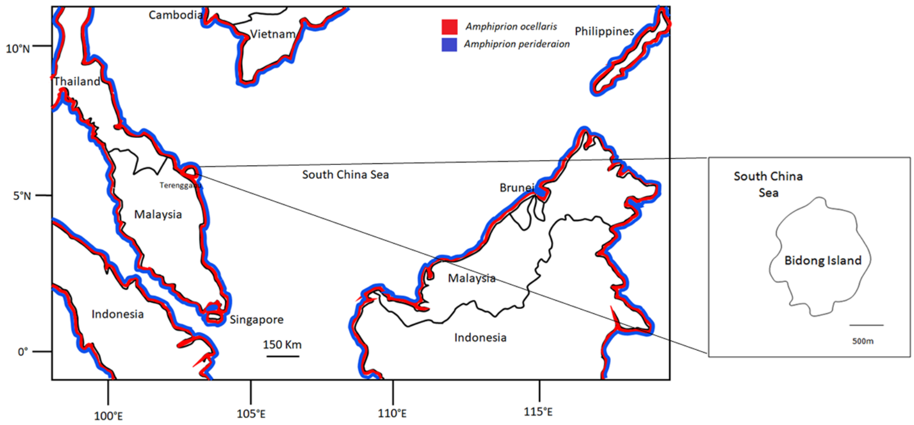

Fig. 1. – Map showing the collection site of the anemone fishes at Bidong Island off the Terengganu State in the east coast of Peninsular Malaysia in Malaysian South China Sea and distribution ranges of *Amphiprion ocellaris* (red lines) and *A. perideraion* (blue line) in Southeast Asia.

ty, the abdomen of each female fish was dissected and the ovary was removed and weighed.

The ovaries of *A. ocellaris* and *A. perideraion* were dissected and then fecundity was estimated using gravimetric method according to Murua *et al*. (2003). Three sub-samples with approximately 0.001 g each were taken out from the front, mid and rear-sections of each ovary and weighed (Murua *et al*. 2003). Each sub-sample (front, mid and rear-sections of each ovary) was placed on slide, installed under the macroscopic staging system and the total number of eggs were counted one by one (Biswas & Ghosh 2015). The GSI of each fish was calculated by the following formula (Murua *et al*. 2003):

GSI = gonad weight/body weight × 100

Simple linear regressions were determined using SPSS statistical analysis software version 22 to determine the correlation significance between the total length and the fecundity and between the body weight and the fecundity. The significances of coefficient of correlation (r) and regression slopes between the total length and the fecundity and between the body weight and the fecundity were determined using t-test (Sokal & Rofhl 1995, Abdul Khadir *et al*. 2017). Differences in TL, BW and fecundity between *A. ocellaris* and *A. perideraion* were examined through a Mann-Whitney *U* test. Monthly differences in GSI levels were examined using ANOVA with SSPS. The Turkey *post hoc* test was used at a 95 % confidence level to provide specific information on which means are significantly different from each other.

RESULTS

TL and BW of *A. ocellaris* ranged from 5.6 to 8.8 cm (mean ± SD: 7.2 ± 0.7 cm) and from 3.7 to 13.4 g (mean ± SD: 8.2 ± 2.4 g), respectively. Those of *A. perideraion* ranged from 6.1 to 9.4 cm (mean ± SD: 7.6 ± 0.8 cm) and from 4.7 to 17.2 g (mean ± SD: 9.8 ± 3.1 g), respectively. There was a significant difference in TL and BW between species (Mann-Whitney *U* test, df = 73-76, $p < 0.05$). The fecundity of *A. ocellaris* and *A. perideraion* varied from 67 to 1889 (mean ± SD; 693 ± 437) and 120 to 2653 (mean ± SD; 1391 ± 707), respectively. The fecundity of *A. perideraion* was significantly higher than that of *A. ocellaris* (Mann-Whitney *U* test, df = 64, $p < 0.0001$) (Fig. 2).

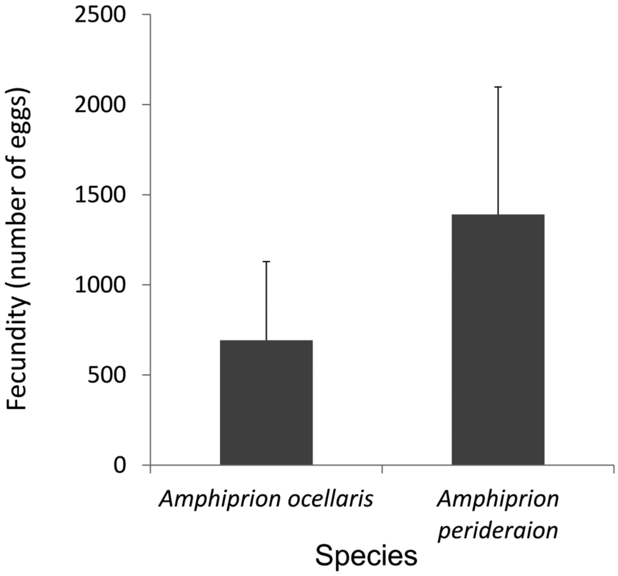

Fig. 2. – Fecundity of *A. ocellaris* and *A. perideraion* in Bidong Island, east coast of Peninsular Malaysia with error bars indicating the standard deviation.

Close linear relationships were found between TL and fecundity (t-test, $r^2 = 0.292$, $p < 0.0001$) (Fig. 3A, B) and between BW and fecundity (t-test, $r^2 = 0.273$, $p = 0.001$) (Fig. 3C, D) in *A. perideraion*, while such relationships were not found in *A. ocellaris* (t-test, $r^2 = 0.0086\text{-}0.0343$, $p = 0.252\text{-}0.570$).

The GSI of *A. ocellaris* ranged from 0.44 to 6.89 (mean ± SD: 2.93 ± 1.73) and the highest mean GSI was found in April 2014. The mean (± SD) GSI in April, June, November 2014 and October 2015 were 4.37 ± 1.93, 1.44 ± 1.02, 2.53 ± 1.53 and 1.28 ± 0.37, respectively (Fig. 4). A significant difference in GSI was found among the four months (ANOVA, df = 39, F = 5.777, $p < 0.001$), with the GSI levels between April and June and October being significantly different ($p < 0.05$), but not between other months ($p > 0.05$).

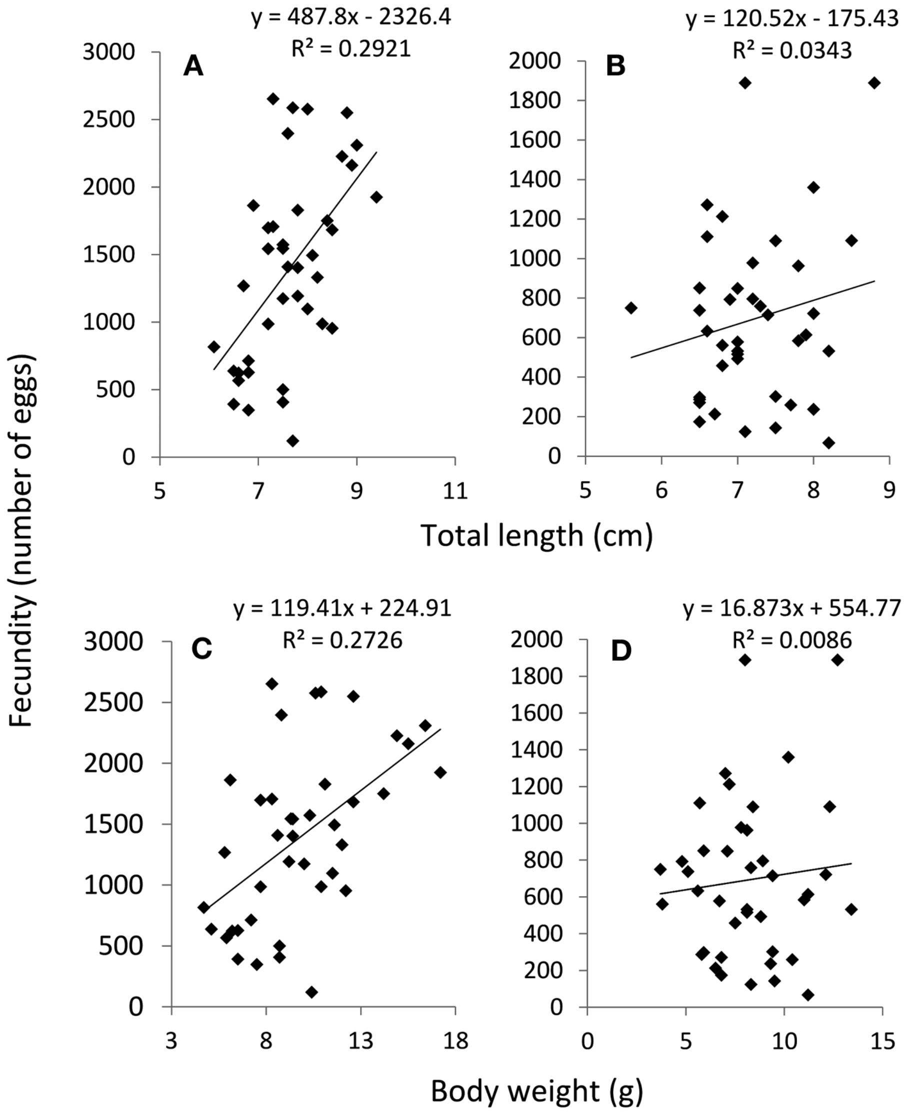

Fig. 3. – Relationships between the total length (TL) (**A**) and body weight (BW) (**c**) and fecundity of *A. perideraion* and between TL (**B**) and body weight BW (**D**) and fecundity of *A. ocellaris* in Bidong Island, east coast of Peninsular Malaysia.

The GSI of *A. perideraion* ranged from 0.53 to 7.54 (mean ± SD: 2.87 ± 1.94), and the highest mean GSI was also found in April 2014. The mean (± SD) GSI found in April, June, November 2014 and October 2015 were 3.87 ± 1.85, 3.63 ± 1.83, 1.21 ± 0.43 and 1.2 ± 0.8, respectively. A significant difference in GSI was found among the 4 months (ANOVA, df = 39, F = 4.827, $p < 0.005$), with the GSI levels between April and October and November being significantly different ($p < 0.05$), but not between other months ($p > 0.05$).

DISCUSSION

This is the first study on the fecundity in tropical anemone fishes in Malaysia. Although the fecundity of *A. perideraion* was significantly higher than that of *A. ocellaris* in the present study, this fecundity overlapped with other anemone fishes such as *A. clarkii* (1000-5400; Bell 1976, Ochi 1985, 1989), *A. akindynos* (700-5025; Richardson *et al.* 1997) and *A. latezonatus* (800-3870; Richardson *et al.* 1997). The fecundity of *A. perideraion* was in the range of 300 to 700 in Eniwetok and Guam (Allen 1972, Ross 1978). The fecundity of *A. ocellaris* overlapped with the laboratory condition (400-800) (Ajith Kumar & Balasubramanian 2009), although there was no data found for the wild condition. Fecundity is a highly variable trait, which can differ among populations but also within one population between consecutive years (Duponchelle *et al.* 2000). According to the energetic cost of gamete production, food is probably one of the most important environmental factors involved in the regulation of fecundity (Wooton & Evans 1976). Anemone fishes live in an obligate association with anemones, a small and discrete habitat where fish must derive all critical resources. Larger anemones are likely to provide more shelter, as well as an increased area for reproduction and laying eggs (Chausson *et al.* 2018). Therefore, such an

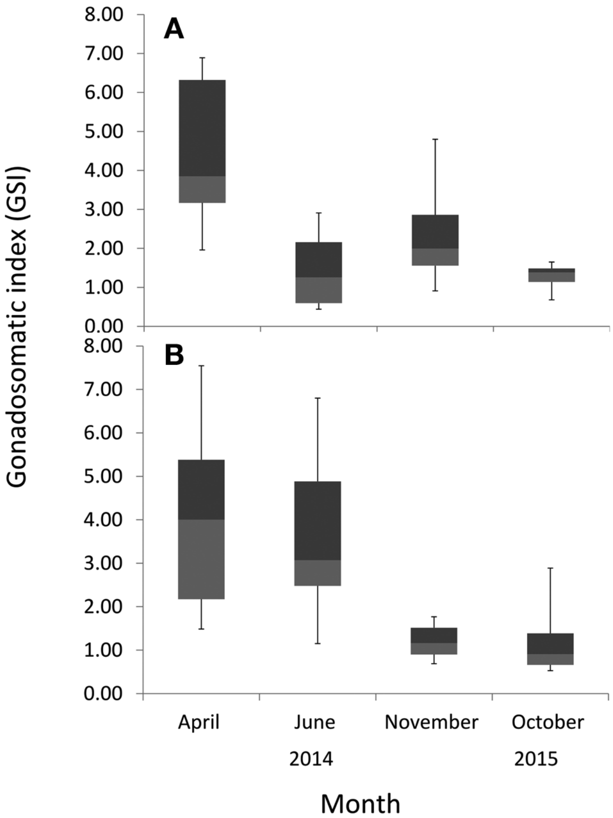

Fig. 4. – Monthly fluctuations of gonadosomatic index (GSI) in *A. ocellaris* (**A**) and *A. perideraion* (**B**) collected in Bidong Island, east coast of Peninsular Malaysia during April, June, November 2014 and October 2015 (the bottom error bar indicates the minimum GSI, the top error bar indicates the maximum GSI, the lower column indicates the lower quartile range, the middle column indicates the median of GSI and the upper column indicates the upper quartile).

individual-specific habitat might vary in fecundity in the present study.

The fecundity of *A. perideraion* was significantly higher than that of *A. ocellaris* in the present study. The two species were collected from the same coral reef habitats and their habitats overlapped. The body size of *A. perideraion* was significantly higher than that of *A. ocellaris*. It was reported that larger fish had greater fecundity (Duarte & Alcaraz 1989). Therefore, the higher fecundity found in *A. perideraion* might be due to the greater body sizes at maturations in the present study.

This is also the first study reporting on the spawning season of anemone fishes in Malaysia. The highest GSI in *A. ocellaris* and *A. perideraion* were observed in April and then decreased in the subsequent months. Sampling could not be conducted from December to March due to the northeast monsoon season. The northeast monsoon season occurs from November to February (Syafrina *et al*. 2015). However, matured ovaries were found in both species throughout the study period. Furthermore, anemone fishes occurring in tropical waters spawn throughout the year (Allen 1972, Ross 1978). These results suggest that both species spawn throughout the year. This result is similar to previous studies such as on *A. ocellaris* in Indonesia (Madduppa 2012), *A. melonopus* in Guam, *A. chrysopterus* and *A. perideraion* in Eniwetok Atoll, Marshall Island in tropical waters (Allen 1972, Ross 1978). However, our results were in contrast with previous findings from Moyer (1980) and Lamb (2006). The spawning season of *A. perideraion* only occurred from April to August, and in February (Lamb 2006). Furthermore, the spawning season of *A. clarkii* was highly seasonal with spawning season from May to October in Miyake-Jima Island, Japan (Moyer 1980). Such differences in spawning season might be due to the different environmental habitats between tropical and temperate area. The constant warm water temperature throughout the year is found in Malaysia (25 °C to 31 °C) (World Sea Temperature 2018) and this temperature range is common in tropical region. Such environmental condition might induce monthly spawning in anemone fishes from tropical waters. Year-round spawning appears to be an indication of the stability of environmental conditions both in captivity (hatcheries) and in the wild within tropical latitudes (Gordon & Bok 2001). In contrast, the low water temperatures and more unstable environmental conditions of temperate regions result in spawning occurring only during the summer months (Richardson *et al*. 1997).

The fecundity of *A. ocellaris* and *A. perideraion* was lower than those of other coral reef fish species which spawned ten thousands to hundred thousands of eggs. *Amphiprion ocellaris* (Agustin 2016) and *A. perideraion* (Rainer 2016) are demersal spawners which also tend to spawn large-sized eggs in small numbers. Demersal spawners increase their reproductive success by producing large in size but small numbers of eggs to increase larval survival (Duarte & Alcaraz 1989). In addition, most reef fish species produce pelagic eggs and larvae (Sadovy 1996). The larvae of many reef fish species have been collected in open water, some at great distances from the shore (Clarke 1991, Brogan 1994). There might be advantages to offshore dispersal, in contrast there is also the problem of return transport once pelagic young reach the settlement stage. Although a variety of hydrographic mechanisms may aid the return of settlers (Kingsford *et al*. 1991, Brogan 1994), considerable variation in their availability and effectiveness should be expected. The relative benefit (or detriment) of such a distribution can be described in terms of mortality rates before and after larvae become competent to settle (Brogan 1994). For example, the eggs and young larvae of pelagic spawners drift passively with the currents. Older larvae may be capable swimmers, but by then substantial drift away from reefs may have already occurred (Brogan 1994). In contrast, non-pelagic spawners (live-bearers, brooders, and demersal spawners) keep their embryos on the reef. Such differences in reproductive characteristics and larval life history might induce differences in the number of spawned eggs. Fish which produce small pelagic eggs tend to spawn many eggs and those fish are pelagic spawners in contrast to demersal spawners. This might be due to the fact that pelagic eggs are subjected to temporal variability of oceanic circulation and patchy nature of the marine environment (Duarte & Alcaraz 1989). Furthermore, the anemone fishes such as *A. ocellaris* and *A. perideraion* practice parental care after they laid their eggs close to their sea anemone (Perrone & Zaret 1979), and fishes with parental care tend to produce lower number of eggs (McElroy *et al*. 2016). Therefore, the fecundity of the anemone fishes might be lower than those of other coral reef fishes. In the present study, we could reveal the reproductive strategies in coral reef fishes by means of their fecundities.

This study has provided a more detailed understanding of the fundamental reproduction characteristics of *Amphiprion ocellaris* and *A. perideraion* in Malaysian waters. Fecundity showed a positive correlation with TL and BW in *A. perideraion*, indicating that body size is an important indicator of the female reproductive output. Both species might spawn throughout the year. The fecundity of *A. ocellaris* and *A. perideraion* were lower than those of other coral reef fish species. This suggests the importance of social structure and pairing in regulating gonad maturation and mating success. Anemone fishes are in high demand for marine aquarists and they have become increasingly popular targets for collection. Reef destruction and degradation due to human activities remain the greatest threat for maintaining their populations as the present study suggested their lower fecundity among coral reef fish species. Our findings will be useful for biologists and resource managers in formulating effective strategies for the conservation and aquaculture of *A. ocellaris* and *A. perideraion* in Malaysia.

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