



UNIVERSITI PUTRA MALAYSIA

**THE SPECIES RICHNESS OF *AMPHIPRION* SPP. (CLOWNFISH) AT
PULAU BIDONG, MALAYSIA**

TULANI UMAYA GUNARATNE

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FPV 2020 99**

**THE SPECIES RICHNESS OF *AMPHIPRION* SPP. (CLOWNFISH) AT PULAU
BIDONG, MALAYSIA**



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FACULTY OF VETERINARY MEDICINE,

UNIVERSITI PUTRA MALAYSIA

SERDANG, SELANGOR

2020/2021

**THE SPECIES RICHNESS OF *AMPHIPRION* SPP. (CLOWNFISH) AT PULAU
BIDONG, MALAYSIA**

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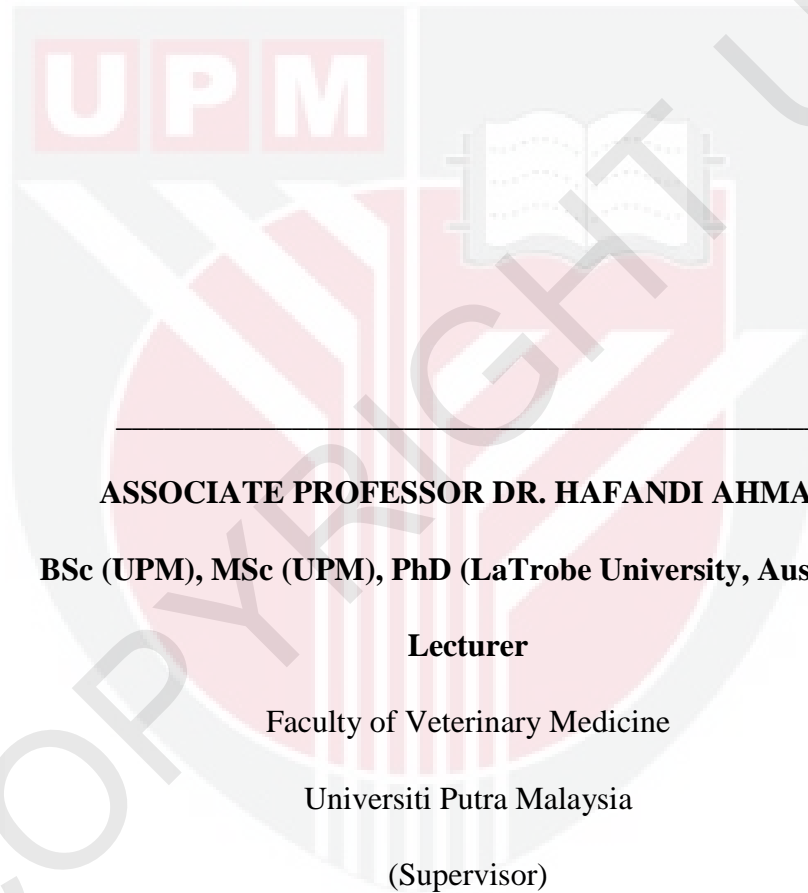
TULANI UMAYA GUNARATNE

**A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia,
Serdang, Selangor Darul Ehsan**

2020/2021

CERTIFICATION

It is hereby certified that we have read the project paper entitled, The Species Richness of *Amphiprion* spp. (Clownfish) at Pulau Bidong, Malaysia, by Tulani Umayra Gunaratne and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD4999 – Final Year Project.



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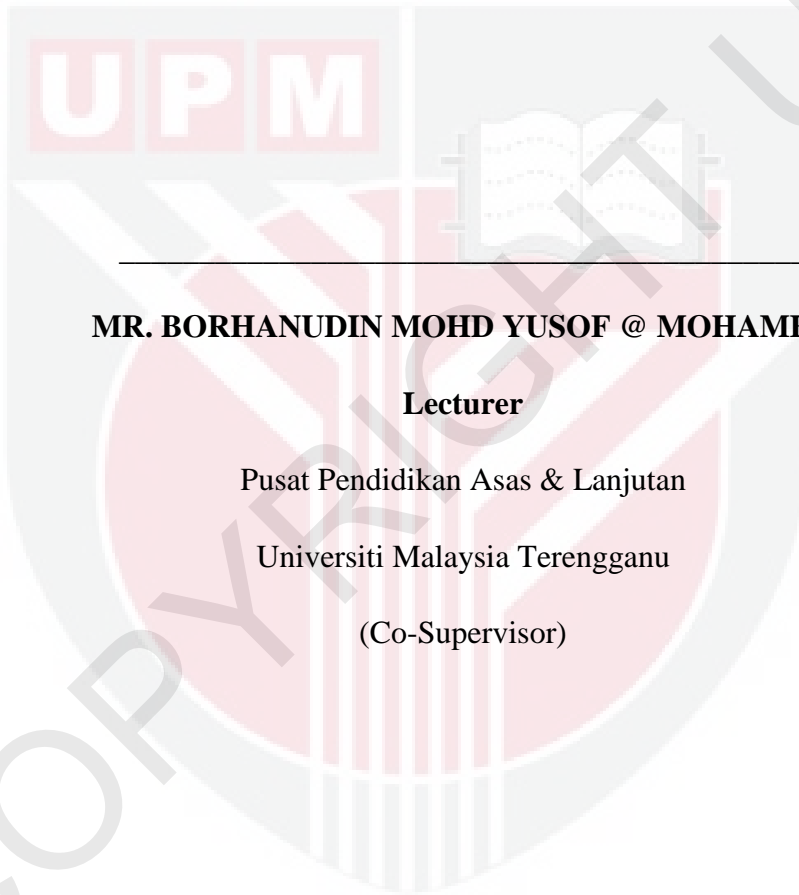
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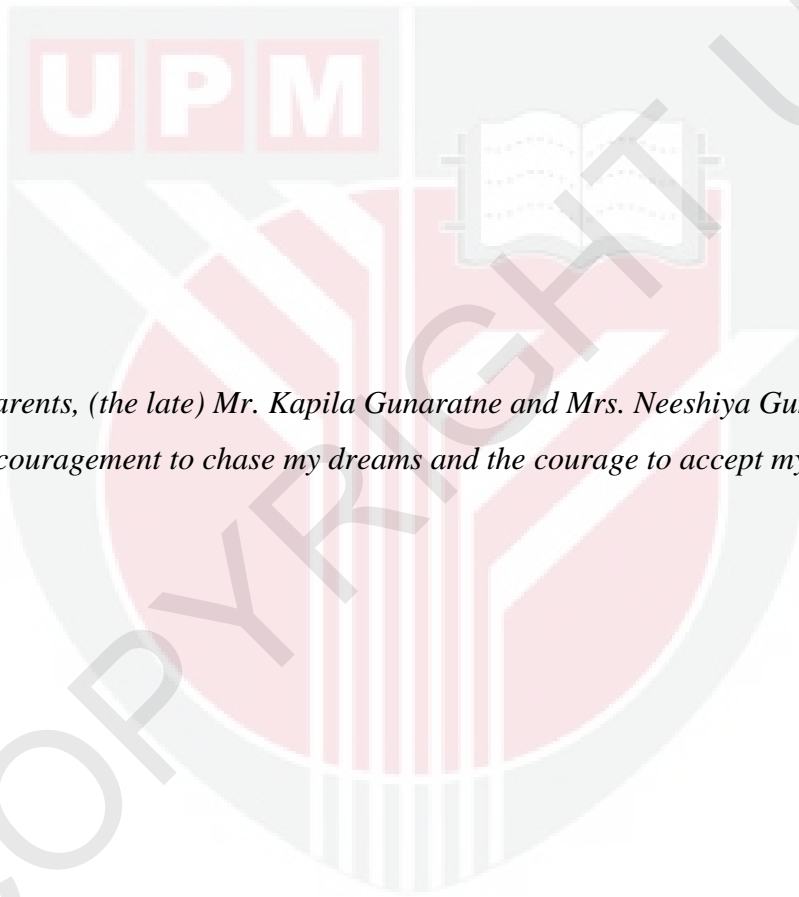
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DEDICATION

My parents, (the late) Mr. Kapila Gunaratne and Mrs. Neeshiya Gunaratne, for the encouragement to chase my dreams and the courage to accept my limitations.



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My parents, thank you for encouraging me throughout my degree and everything that came before. And for always encouraging me to do my best in everything I do.

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ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek Tahun Akhir.

**KEKAYAAN SPESIS *AMPHIPRION* SPP. (IKAN BADUT) DI PULAU BIDONG,
TERENGGANU MALAYSIA**

oleh

UMAYA GUNARATNE

2021

Supervisor: Assoc. Prof. Dr. Hafandi Ahmad**Co-supervisor: Mr. Borhanudin Mohd. Yusof**

Amphiprioninae (clownfish) adalah salah satu spesies yang paling dikenali di terumbu karang. Kekayaan spesies *Amphiprion* spp. di suatu kawasan sangat bergantung kepada faktor lautan dan bentuk jenis batu karang, iaitu anemon laut. Terdapat variasi dalam ketersediaan spesies *Amphiprion* spp. yang dapat dijumpai secara meluas di terumbu karang perairan tropika dan subtropika cetek. Oleh itu, objektif kajian ini adalah untuk mengenal pasti ketersediaan atau keterdapatan spesies *Amphiprion* spp. (ikan badut) di Pulau Bidong, Terengganu, Malaysia. Kawasan kajian merangkumi tujuh lokasi menyelam di sekitar pulau Bidong, yang terletak di pesisir timur laut Terengganu, di Laut China Selatan. Kamera bawah air digunakan untuk menangkap gambar spesies ikan badut

yang ditemui semasa tinjauan visual menyelam SCUBA. Gambar spesies tersebut dikenal pasti dari sifat morfologi dan habitat luarannya. Kajian ini mengenal pasti dan merekodkan lima jenis spesies ikan badut yang berbeza dari genus *Amphiprion*: *A. frenatus*, *A. perideraion*, *A. ocellaris*, *A. clarkii* dan *A. polymnus*. Penemuan kami juga menunjukkan bahawa hubungan simbiosis yang kuat di antara anemon laut dan ikan badut, yang menyumbang kepada struktur populasi dan kepelbagaian spesies *Amphiprion* di Pulau Bidong. Kajian ini juga memberikan panduan asas untuk penyelidikan di masa hadapan mengenai spesies *Amphiprion* spp. dan faktor habitat lain seperti kualiti anemon dan kualiti persekitaran lautan di sekitar Pulau Bidong; yang akhirnya dapat membantu dalam mengembangkan strategi pemuliharaan yang berjaya untuk "Nemo" yang disayangi semua orang dan juga habitat lautnya.

Kata kunci: *Ikan badut, Amphiprion, ketersediaan spesies, kekayaan spesies, Pulau Bidong*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999-Final Year Project.

**THE SPECIES RICHNESS OF *AMPHIPRION* SPP. (CLOWNFISH) AT PULAU
BIDONG, TERENGGANU MALAYSIA**

by

UMAYA GUNARATNE**2021****Supervisor: Assoc. Prof. Dr. Hafandi Ahmad****Co-supervisor: Mr. Borhanudin Mohd. Yusof**

Amphiprioninae (clownfish) are one of the most widely recognized species found in coral reefs. The species richness of *Amphiprion* spp. in a region depends largely on ocean factors and a special type of coral reef creature, the sea anemone. There is variation in the regional availability of *Amphiprion* spp. species that can be found widely distributed in the coral reefs of shallow tropical and subtropical waters. Therefore, the objective of this study is to identify the species availability of *Amphiprion* spp. (clownfish) at Pulau Bidong, Terengganu, Malaysia. The study areas included seven dive sites around the Bidong island, located off the north-east coast of Terengganu, in the South China Sea. An

underwater camera was used to capture images of clownfish species encountered during the SCUBA diving visual survey. The images of the species were identified by their external morphology and habitat. The study identified and recorded five distinct species of clownfish from the genus *Amphiprion*: *A. frenatus*, *A. perideraion*, *A. ocellaris*, *A. clarkii* and *A. polymnus*. Our findings also demonstrate the strong symbiotic relationship between the sea anemone and clownfish, which contributes to the population structure and *Amphiprion* species diversity at Pulau Bidong. In addition, this study provides a baseline for future research into *Amphiprion* spp. and other habitat factors such as the quality of anemone and the quality of the ocean environment around Bidong Island; which may ultimately aid in the development of successful conservation strategies for everyone's beloved "Nemo" and their marine habitat.

Keywords: *clownfish, Amphiprion, species availability, species richness, Bidong Island*

1.0 INTRODUCTION

Clownfish or Clown Anemonefish are a group of 30 species of marine fish from the family *Pomacentridae*. 29 of the recognized species are of the genus *Amphiprion* and one under the genus *Premnas* (Allen et al., 2008; Allen et al. 2010, Fautin and Allen., 1992). They are found in the warm tropical and subtropical waters of the Western Indian and Pacific oceans (Velasco-Blanco et al., 2019). The species diversity of clownfish in a region depends largely on ocean factors such as water depth, temperature and others that contribute to the availability of a suitable species of host sea anemone (Rolland et al., 2018; Dunn, 1981; Listos et al., 2012).

According to the Fisheries of Terengganu report published in 2011, four members of *Amphiprion* spp. were found around Bidong island: *A. clarkii*, *A. perideraion*, *A. ocellaris* and *A. frenatus* (Matsunuma et al., 2011). Clownfish populations are important indicators of the health of the reef ecosystem and the regional effects of environmental factors such as ocean acidification and climate change. A report by the IUCN Species Survival Commission identified them as one of the ten flagship species to symbolize the effects of climate change (Foden and Stuart, 2009) due to their popularity among hobbyists and the general public.

Therefore, the intention of this study was to produce an updated inventory of species richness of clownfish in the tropical region. This information would support future research on the species and its marine habitat so that suitable management and conservation strategies may be established.

Objective

The objective of this study was to identify the species of *Amphiprion* spp. (clownfish) found in the shallow waters surrounding Pulau Bidong, Malaysia.

Hypothesis

Based on the Fisheries of Terengganu report by Matsunuma et al. in the year 2011, the hypothesis of this study was that, at least four species of clownfish can be observed around Bidong island, Malaysia.

H1: Four species of Amphiprioninae can be found in the reefs surrounding Pulau Bidong, Malaysia.

H0: Fewer or more than four species of Amphiprioninae can be found in the reefs surrounding Pulau Bidong, Malaysia

2.0 LITERATURE REVIEW

2.1.1 Species

Currently, there are 29 known species under the genus *Amphiprion*: *A. akallopisos*, *A. akindynos*, *A. allardi*, *A. barberi* (Allen et al., 2008), *A. bicinctus*, *A. chagosensia*, *A. chrysogaster*, *A. chrysopterus*, *A. clarkii*, *A. ephippium*, *A. frenatus*, *A. fuscocaudatus*, *A. latezonatus*, *A. latifasciatus*, *A. leucokranos*, *A. mccullochi*, *A.*

melanopus, *A. nigripes*, *A. ocellaris*, *A. omanensis*, *A. pacificus* (Allen et al., 2010), *A. percula*, *A. perideraion*, *A. polymnus*, *A. rubrocinctus*, *A. sandaracinos*, *A. sebae*, *A. thiellei*, and *A. tricinctus*, as well as one under genus *Premnas*: *P. biculeatus* (Fautin and Allen, 1992).

2.1.2 A Clownfish “Family”

Amphiprion spp. live in monogamous breeding pairs or in small groups consisting of one breeding pair and up to four non-breeding subordinates (Buston, 2004), depending on the species, size of the host anemone (Fautin, 1992; Fricke, 1979; Hattori, 1991; Buston, 2003) and sometimes the size of the dominant fish (Mitchell and Dill, 2005). These groups are made up of unrelated individuals (Buston et al., 2007) that live in a strict size hierarchy, in which they queue for breeding positions (Buston, 2004). The dominant female being the largest, followed by the breeding male and then the non-breeders that decrease in size following their social rank. Clownfish are protandrous hermaphrodites, meaning that they are all born biologically male and can change to become a breeding female once they arrive at the dominant position within their social group (Fautin, 1992). If a breeder is removed from the group, the individuals immediately beneath it in rank up and grow to occupy vacancies in the social order, eliminating the need for migration in search of a new mate (Casas et al., 2016).

2.1.3 Physiology and Life Cycle

A breeding pair of Amphiprioninae will spawn throughout the year in the tropics, and seasonally in subtropical and warm temperate regions. They prefer warmer water temperature and a full or near-full moon during which to spawn. The female swims in a slow zig-zagging path, depositing the adhesive eggs on a hard substrate close to their host sea anemone and the male follows close behind, fertilizing them. The benthic, capsule shaped eggs are visible from a distance as a yellow-red mass (Figure 1.2) and are guarded closely by the male. Gradually, the eyes begin to develop (Figure 1.3) and the silvery pupils become clearly visible when the larvae are ready to hatch in 6-7 days (Figure 1.4), usually in the late evening, just after dark (Fautin and Allen, 1992).

The newly hatched transparent fry are planktonic and spend 8-12 days passively drifting with the ocean current until they undergo rapid metamorphosis, and must locate an appropriate host anemone to inhabit (Fautin, 1991). Most clownfish will remain with their new host anemones for life, others such as some juvenile *A. clarkii* will opportunistically occupy smaller “nursery” anemones for a while and then move on to more appropriate hosts by displacing existing occupant clownfish (Fautin, 1991; Ochi, 1986; Huebner et al., 2012) when they are large enough to gain competitive superiority (Fautin and Allen, 1992).

Figure 1.1: Life cycle of *Amphiprion ocellaris*: Image courtesy of American

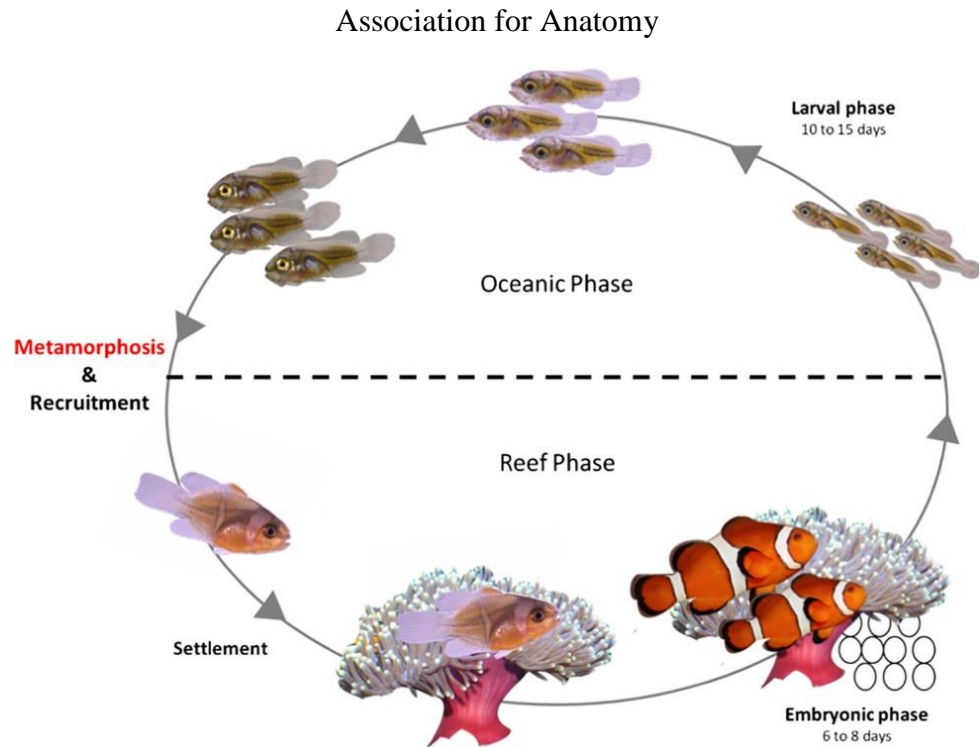


Figure 1.2: Eggs of *Amphiprion ocellaris* in the early stage of development (1-3 days)

– The yolk sac is responsible for the yellow-red colour of the egg mass.

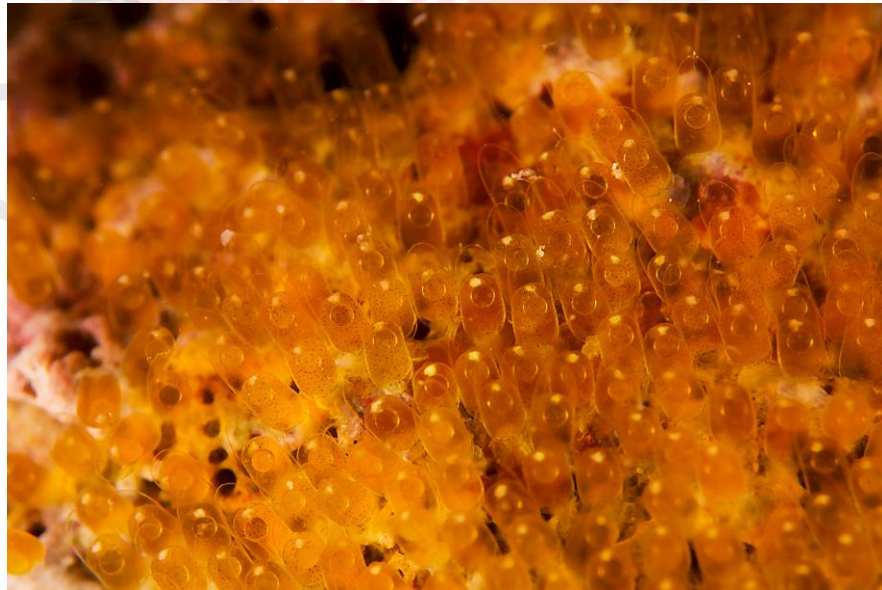
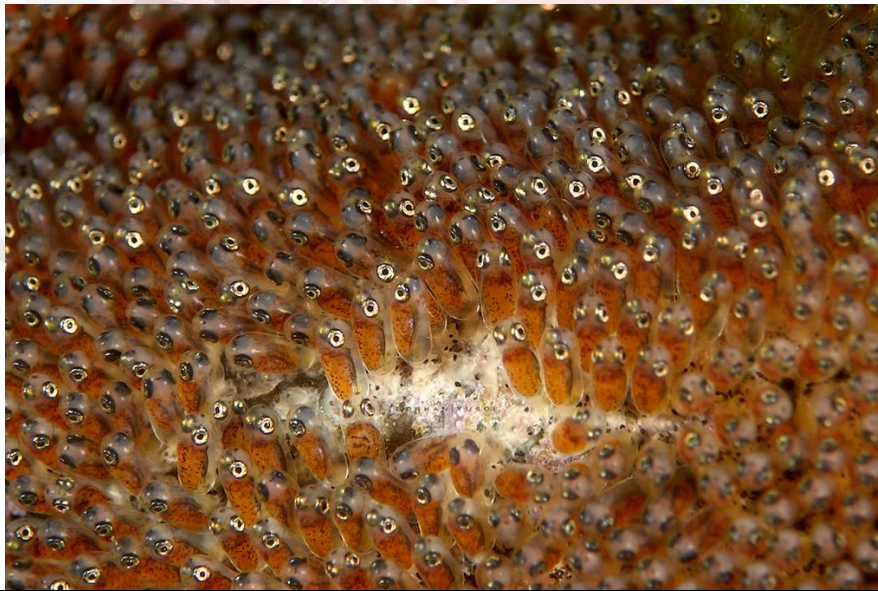


Figure 1.3: Eggs of *A. perideraion* at 4-6 days after spawning; the eyes are visible at this stage.



Figure 1.4: *Amphiprion ocellaris* eggs, approximately 7-10 days post-spawning. Larval eyes are already well developed and silvery pupils are clearly visible and ready to hatch.



2.1.4 Habitat

The above-mentioned species live in obligatory symbiosis with a venomous invertebrate host: the sea anemone (da Silva & Nedosyko, 2016). Although there are almost 1000 known anemone species, only ten species, belonging to genera *Actiniidae*, *Stichodactylidae* and *Thalassianthidae*, are recognized as suitable hosts for clownfish (Fautin & Allen, 1993). They are usually seen in shallow waters at 1-12m deep, although some species have been encountered at depths of up to 50m, usually in coral reefs but also in sea grass beds and the sandy bottom beyond the reef (Maison & Graham 2016; Bridge et al., 2012).

Some species of clownfish are host generalists; known to interact with multiple species of sea anemone, often across multiple genera. Whereas others are associated with a smaller range of anemone species and are known as specialist clownfish (Listos et al., 2012). These relationships are a strong determinant of the geographical distribution of the various species, as clownfish are only known to exist in environments suitable for their preferred hosts (Elliott and Mariscal 2001, Maison & Graham, 2016).

2.1.5 Clownfish and Sea Anemone

The fascination with the unlikely relationship between sea anemone and clownfish has led to the consideration of multiple hypotheses to explain how the fish is able to survive in such a hostile environment. Anemones are covered in microscopic stinging capsules known as nematocysts that produce toxins that are have hemolytic,

immunomodulating, neurotoxic and cardiotoxic properties. However, clownfish swim comfortably among the anemone tentacles and are seemingly unaffected by the toxic nematocysts (da Silva & Nedosyko, 2016).

While it was previously believed that the clownfish immunity to the anemone venom was definitively either innate (Lubbock, 1980,1981) or acquired (Mariscal, 1969; Allen, 1972; Miyagawa 1989), the current consensus is that both innate and acquired mechanisms play a role in protecting the clownfish from the anemone venom, depending on the species as well as the species pairs involved (Fautin, 1991). Clownfish produce an inert mucus that is three to four times thicker than that of other damselfish and lacks stimulatory substances to avoid stimulation of nematocyst discharge (Elliot and Mariscal, 1997).

The mucus coat on some *A. clarkii* have been found to contain antigens of their anemone host that were not found on those individuals not associated with a host, thus preventing the anemone from recognizing the fish as foreign and inhibiting nematocyst discharge (da Silva & Nedosyko, 2016). These antigens are thought to be acquired by a process of acclimation, during which the fish adapts to the host via a stereotyped series of movements, during which it gradually acquires the host antigens (Fautin and Allen, 1992). In this way, host generalists such as *A. clarkii* are able to visually locate and habituate to virtually any host anemone (Fautin, 1991).

Host specialists that are only known to associate with one or two anemone species are not able to adapt as readily to unfamiliar anemone species. Specialist pairs are believed

to have evolved together (Fautin 1991), thus affording a relationship that relies on chemical cues for location and adaptation (Lubbock, 1980) which does not require acclimation behaviour as sometimes seen in generalists, but limits host availability for specialist clownfish species. These specialists are usually competitively superior to other species for their hosts since they lack alternatives (Fautin, 1991).

After hatching, juvenile clownfish use their sense of smell to recognize chemical signals produced by their hosts (Da Silva & Nedosyko, 2016; Murata et al., 1986) and in the absence of a preferred host species, clownfish are able to form relationships with species of anemone that they are not usually found to be associated with. However, the choice of host can have an effect on clownfish fitness and behaviour (Da Silva & Nedosyko, 2016). A preference for a host anemone is controlled by three factors; an innate host preference, competitive superiority between fish and ecological coincidence (Fautin and Allen, 1992). As *Amphiprion* species expand their geographical range, their host specificity decreases (Palaniappan et al., 2003).

In return for the protection from predators and parasites, as well as nourishment and reproductive advantages provided by the anemone, the clownfish is fiercely territorial and provides its host with defense against predators (Godwin and Fautin, 1992), oxygenation (Szczebak et al., 2013) and nutritious ammonia waste (Roopin et al., 2008). Anemones living in symbiosis with clownfish have improved survivability, growth and reproduction (Holbrook and Schmitt 2005; da Silva & Nedosyko, 2016).

2.1.6 Threats to Clownfish

While none of the *Amphiprion* species are currently at risk of extinction (IUCN, 2020), regional populations may be at risk due to various natural and anthropogenic alterations to their habitat. With many species being host specialists, threats to sea anemones will invariably alter the population dynamics of the clownfish that depend on them. Ocean acidification and temperature fluctuations are thought to be largely responsible for anemone bleaching (Albright, 2018). Clownfish are less likely to select a bleached anemone as their host, however they may do so in the absence of a suitable alternative and their physical and reproductive fitness may be compromised (Scott & Dixon, 2016).

Furthermore, both clownfish and their hosts are popular commodities in the marine aquarium trade (Murray & Watson, 2014) and anemonefish are used extensively in laboratory studies as they are relatively easier to raise in captivity than other damselfish (Kumar et al., 2010). Neither are well suited to captive culture and thus must be collected from natural reefs. There is little reliable information on the magnitude of regional exports of these species and the impact of trade on wild populations are unclear; however recent reports indicate that rates of exploitation may be on the decline (Maison & Graham, 2016).

Pollution originating from land sources are a growing threat. Runoff from human settlements lead to sediment buildup and nutrient enrichment of offshore reefs. This is a key issue in anemone survival and larval duration of Amphiprionae; many of which are non-migratory and live in shallow waters, close to shore (Maison & Graham, 2016).

2.1.7 Clownfish in Malaysia

A study by Palaniappan et al. in 2003 used underwater visual census and encountered eight species of clownfish in marine parks surrounding Payar Island, Tinggi Island, Redang Island, and Tanjung Tuan in Malaysia: *A. alkallapisos*, *A. clarkii*, *A. ephippium*, *A. frenatus*, *A. melanopus*, *A. ocellaris*, *A. perideraion*, and *A. sandaracinos*. These were associated with four of ten known species of anemone known to host clownfish: *Heteractis magnifica*, *H. crispera*, *Stichodactyla mertensii* and *Entacmaea quadricolor*.

While there is no published data on clownfish found specifically around Bidong Island, four species of clownfish have previously been photographed in that region: *A. clarkii*, *A. frenatus*, *A. ocellaris* and *A. perideraion* (Matsunuma et al., 2011).

The disappearance of Amphiprion species from Tanjung Tuan in Melaka due to exploitation, as referenced in the paper by Palaniappan et al. is an important local example of the importance of clownfish conservation.

3.0 MATERIALS AND METHODS

3.1 Experimental Design

Data was collected in collaboration with researchers at Universiti Malaysia Terengganu (UMT). Field observations were made at seven randomly chosen dive sites around Bidong Island: Pantai Pasir Cina (UMT House Reef), Pantai Pasir Pengkalan,

Pantai Pasir Tenggara, Pantai Teluk Belanga, Christmas Garden, Batu Menangis, and Batu Payung.

Visual census using random paths was carried out at all seven sites within the limits of safe SCUBA diving. Photographs of clownfish encountered were taken using a Nikon D7200 camera, 60mm lens, with Sea & Sea Housing, and Inon Z240 strobe flash.

The images were later compared to descriptive keys as published by Matsunuma et al. (2011) and Fautin and Allen (1992) for species identification.

3.2 Sampling Location - Pulau Bidong

Bidong island is an uninhabited island located 18 nautical miles off the northeast coast of Terengganu, and 8 nautical miles south of Redang island in the South China Sea (Lat. 5.62°, Long. 103.07°). It is surrounded by sandy beaches and fringing reefs with up to 26 commercially recognized dive sites. A research station under Universiti Malaysia Terengganu (UMT) has been established on the left side of the island, at Pantai Pasir Cina. Bidong island is growing in popularity as a tourism destination due to its historical significance as a Vietnamese settlement in 1978 and recent establishment of Asia's first underwater gallery.

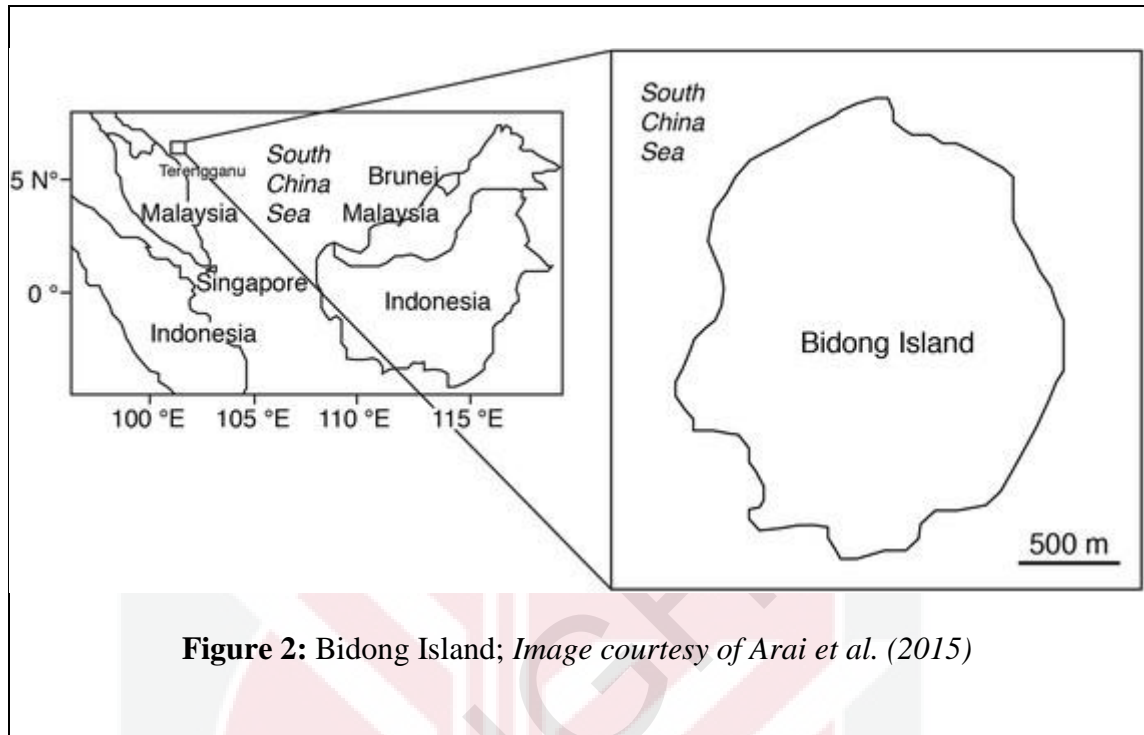


Figure 2: Bidong Island; *Image courtesy of Arai et al. (2015)*

4.0 RESULTS

Five morphologically distinct species of clownfish were encountered cumulatively across the seven dive sites explored in this study: *Amphiprion frenatus*, *Amphiprion ocellaris*, *Amphiprion perideraion*, *Amphiprion clarkii* and *Amphiprion polymnus*.

Amphiprion frenatus, commonly known as the Tomato Clownfish is seen here with its host *Entacmaea quadricolor*. The male is smaller and lacks the black pigmentation (left; Figure 3.1) whereas the larger female is black colored over most of its body (right; Figure 3.1, Figure 3.2). This species was encountered at Christmas Garden and Pantai Pasir Cina at depths of 4-8m.



Figure 3.1: A pair of *Amphiprion frenatus*



Figure 3.2: *Amphiprion frenatus* with host *Entacmaea quadricolor*

Amphiprion ocellaris or False Percula Clownfish with *Stichodactyla gigantea* (Figure 4.1) and *Heteractis crispa* (Figure 4.2) hosts. Encountered at a range of depths at all dive sites studied.



Figure 4.1: *Amphiprion ocellaris* with host *Heteractis magnifica*



Figure 4.2: *Amphiprion ocellaris* with host *Stichodactyla gigantea*

Amphiprion perideraion (Figure 5.1 and 5.2) or Pink Skunk Clownfish with host *Heteractis magnifica*. Encountered at a range of depths at all dive sites studied.



Figure 5.1: *Amphiprion perideraion*



Figure 5.2: *Amphiprion perideraion*

Amphiprion clarkii or Clark's Anemonefish (Figure 6.1) with *Entacmaea quadricolor* host (Figure 6.2). Encountered at a range of depths at all dive sites studied.



Figure 6.1: *Amphiprion clarkii*



Figure 6.2: *Amphiprion clarkii* with its host Bubble-tip Anemone (*Entacmaea quadricolor*)

Amphiprion polymnus or Panda or Saddleback clownfish with *Stichodactyla haddoni* host (Figure 7.1 and 7.2). Encountered at depths of up to 14m at Pantai Pasir Cina and Teluk Belanga on the sandy bottom, away from the true reef. There is no previously published record of the existence of this species around Bidong Island.



Figure 7.1: *Amphiprion polymnus* with its host Haddon's sea anemone (*Stichodactyla haddoni*)



Figure 7.2: *Amphiprion polymnus* seeks shelter on its host when threatened

All species of clownfish lay their eggs on the surface of hard substrate near the host anemone of the breeding pair. *A. polymnus* pairs found associated with *S. haddoni* on the sandy bottom are known to lay their eggs on hard surfaces such as drift wood, dead corals, rocks, trash cans and other solid materials, that they may drag towards the anemone and position below the oral disc beside the anemone column (Fautin and Allen, 1992).

Figure 7.3 shows *Amphiprion polymnus* (Saddleback Clownfish) nursing its eggs, laid on a piece of dead coral. Males spend more time guarding the eggs, although the females are known to help after they spend the first few days feeding to replenish the energy lost in producing and laying the eggs. By hovering close to the eggs and fanning their fins, they promote oxygen circulation for the growing larvae (Fautin and Allen, 1992).



Figure 7.3: A Saddleback clownfish nursing its eggs, laid on dead coral

5.0 DISCUSSION

5.1 Geographic Distribution

In nature, clownfish are not known to exist without their anemone hosts. The availability of clownfish in a region is strongly associated with the distribution of their preferred host species (Allen, 1980, Palaniappan et al., 2003), which are restricted by environment factors such as water temperature (Dunn, 1981). Both are well adapted to thrive in the warm waters of the West Indo-Pacific region (Velasco-Blanco et al., 2019; Fautin, 1992). As with most other marine species, *Amphiprion* spp. are believed to derive from the Indo-Malayan region (Ekman, 1953; Palaniappan et al., 2003) where the rapid decline in water levels during the Quaternary Period, caused dramatic alterations to the Sunda shelves that resulted in the evolution of ideal physical conditions for shallow water

anemones and clownfish (Woodland, 1983; Palaniappan et al., 2003). Gradually, warm water currents allowed range expansion westwards (Palaniappan et al., 2003) but long-distance distribution is limited by the relatively short pelagic duration of anemonefish larvae (Fautin, 1992) and adverse hydrographic factors that are detrimental to larval survivability (Ortega-García, 2014). A study by Jones et al., (2005) found that the larvae rarely drifted more than 100m away from their hatch site, and this showed significant self-recruitment, which is an important consideration when designing conservation strategies for these species.

5.2 Marine Population Assessment

An increasing demand for coral reef fish for trade and research purposes (Labrosse, 2002), as well as the growing popularity of reefs for recreational tourism activities (Cinner and Pollnac, 2004) has put a strain on the populations of their inhabitants. Thus, information on marine populations are collected using various capture, mixed and non-capture methods to monitor and manage existing resources (Labrosse, 2002).

In our study, we faced certain time and resource constrains and chose the non-capture method of visual census using random paths that allowed us to collect qualitative data on species richness over a large area within a shorter time period, without the need for a large amount of equipment or personnel.

Further studies to augment our findings with data on the relative abundance of the five Amphiprioninae identified in this study, may use other survey methods such as belt or line transects as described by Labrosse, (2002).

5.3 Identifying Clownfish

All 29 species of *Amphiprion* are morphologically distinct. The principal features for identification are the colours and patterns of the fish. The correct identification of juveniles using this method is less reliable as species differences in immature animals are less apparent and they may change colours and lose or gain the characteristic white stripes as they grow. Certain “species pairs” such as *Amphiprion ocellaris* and *Amphiprion percula*, have similar colouration that make them difficult to differentiate. However, these are widely separated by the geographic range; *A. ocellaris* is found in the Andaman Sea, Indo-Malayan Archipelago, Philippines, northwestern Australia and along the coasts of Southeast Asia northwards to the Ryukyu Islands, whereas *A. percula* can be found along the reefs of Northern Queensland and Melanesia (Fautin and Allen, 1992). Thus, species accounts on regional availability and global distribution would also aid in an identification.

Individual species accounts can also provide information on the sea anemone hosts that each clownfish is known to associate with. Although juveniles have been known to occupy non-preferred anemone species (Heubner et al., 2012), their choice of host is usually a reliable endorsement of their species identity.

Other morphological characteristics that can be used for identification are the body proportions, head scalation and tooth shape (Fautin and Allen, 1992) that would require capture methods of sample collection such as traps, baits and poisons (Labrosse, 2002) and are of more value in the identification of preserved laboratory specimens (Fautin and Allen, 1992).

5.3.1 *Amphiprion clarkii*

The *Amphiprion clarkii* (Clark's anemonefish) are the most widely distributed species of all Amphiprioninae, with individuals showing geographic variation in their morphological pattern. Usually, they are black with varying amounts of orange on their fins, head and ventrum. However, individuals associated with certain anemone species such as *S. mertensii* are melanistic and are entirely black with an orange to orange-red snout and three white bars. Adults have white bars on the head, body and tail-base and juveniles have less black pigmentation and have only two bars on their head and body (Fautin and Allen, 1992).

Amphiprion clarkii are extreme host generalists and are found in association with all anemone species known to host or "house" clownfish (Fautin and Allen, 1992; Ollerton et al., 2007).

5.3.2 *Amphiprion ocellaris*

Amphiprion ocellaris is bright orange with three white bars on the head, body and tail-base, each with a thin black margin. The body bar has a forward projecting bulge

(Fautin and Allen, 1992). They are almost identical to the True Percula Clownfish (*Amphiprion percula*) in appearance, but they can be differentiated by counting the spines on their first dorsal fin. *A. ocellaris* and *A. percula* have 11 (sometimes 10) and 10 (sometimes 9) dorsal spines respectively. Another distinction is the black margins on the white bars which are thicker on *A. percula* than *A. ocellaris* (Maison and Graham, 2016), but this is less reliable than enumeration of the dorsal spines due to the individual variation in the thickness of the black outlines and possibility of melanistic expression in the clownfish.

In the wild, *Amphiprion ocellaris* are known to associate with three species of host anemones such as *Heteractis magnifica*, *Stichodactyla mertensii*, and *Stichodactyla gigantea* (Fautin and Allen, 1992).

5.3.3 *Amphiprion perideraion*

The Pink Skunk Clownfish can be readily identified by their pink to pink-orange colouration and pale fins. They grow to around 10cm in length and have one narrow white bar on the head and another white dorsal stripe. This species is known to associate with several hosts such as *Heteractis magnifica*, *Heteractis crispa*, *Stichodactyla gigantea* and *Macroactyla doreensis* (Fautin and Allen, 1992).

5.3.4 *Amphiprion frenatus*

Amphiprion frenatus are commonly known as Tomato Clownfish and show strong sexual dimorphism. Females are significantly larger than males and can grow to be up to 14cm in length. They have more black pigmentation on their sides and are bright red on

the snout, ventrum and fins. Males are smaller and red overall with no black colouration. Adults of both sexes have one white bar on the head with narrow black margins and can be differentiated from juveniles who have two to three white bars that disappear as they grow (Fautin and Allen, 1992).

Amphiprion frenatus is usually only found in association with *Entacmaea quadricolor* anemones and is considered a strict host specialist. Of the five species encountered at Bidong Island, *A. frenatus*, especially the females were the most aggressive and were seen to fiercely protect their territory.

5.3.5 *Amphiprion polymnus*

Amphiprion polymnus or Panda or Saddleback clownfish are brown to orange colour with one complete white bar on the head. The body bar appears as a saddle shape that slants slightly backwards, extending to the dorsal fin. The caudal fin is dark brown with broad whitish margins. The exact pattern and colouration shows geographical variation (Fautin and Allen, 1992). Usually, the species found on sandy area, and they lay the eggs on the hard surface (e.g.: drifted woods, dead corals, rocks, trash can and other solid materials) (Astakhov, 2001).

They are moderate host specialists and are seen associated with *Stichodactyla haddoni*, *Heteractis crispa* (Fautin and Allen, 1992), and in rare cases *Macroactyla dorensis* and *Entacmaea quadricolor* (Astakhov, 2001). Individuals associated with *Heteractis crispa* anemones are usually melanistic with black pigmentation all over the

body except for the white bars, snout and tail. Another study by Miltz et al., (2016) reported that dominant female *Amphiprion polymnus* in Papua New Guinea were more likely to be melanistic than lower ranking fish (Miltz et al., 2016).

6.0 CONCLUSION

In this preliminary study to identify the species richness of *Amphiprion* spp. corroborates the findings published in Fisheries of Terengganu by Matsunuma et al., (2011) that report the presence of *Amphiprion clarkii*, *Amphiprion frenatus*, *Amphiprion ocellaris* and *Amphiprion perideraion* in the shallow waters surrounding Bidong Island, Terengganu, Malaysia. Furthermore, one species, *Amphiprion polymnus*, previously unreported at Bidong Island was encountered at two of the seven dive sites that were included in this study. Thus, five distinct species of Amphiprioninae were identified in Bidong Island, Terengganu Malaysia.

7.0 RECOMMENDATION

According to Fautin and Allen in 1992, majority of the research on *Amphiprion* species is focused on *Amphiprion bicinctus*, *Amphiprion chrysopterus*, *Amphiprion clarkii*, *Amphiprion melanopus*, *Amphiprion ocellaris*, *Amphiprion perideraion* and *Amphiprion tricinctus*. While since then, extensive research has been conducted on the remaining species, the presence of less abundant varieties such as *Amphiprion polymnus*

at Bidong Island is a rare opportunity for research into the individual species profile of *Amphiprion polymnus* and their habitat. Furthermore, the identification of the available species sets a baseline for future studies on the species diversity, population dynamics and regional characteristics of Amphiprioninae in this area, which will provide better understanding of the population numbers and how natural environmental changes and anthropogenic influence such as the increasing human activity for both recreational and academic activity affects clownfish in the area. Furthermore, the location of Bidong Island away from the mainland is ideal for investigations of larval distribution and the effects of topographical influences on the development of morphological or behavioural characteristics unique to the Bidong sub-population of clownfish.

8.0 REFERENCES

- Albright, R. (2018). Ocean acidification and coral bleaching. In *Coral Bleaching* (pp. 295-323). Springer, Cham.
- Allen, G. R. (1972). *The anemonefishes: their classification and biology*. Neptune City: TFH Publications.
- Allen, G. R., Drew, J. A., & Kaufman, L. (2008). *Amphiprion barberi*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa.
- Allen, G. R., Drew, J., & Fenner, D. (2010). *Amphiprion pacificus*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, Samoa, and Wallis Island. *Aquaculture*, 16, 129-138.
- Arai, T., Amalina, R., & Bachok, Z. (2015). Variation in fatty acid composition of the bigeye snapper *Lutjanus lutjanus* collected in coral reef habitats of the Malaysian South China Sea. *Journal of Biological Research-Thessaloniki*, 22(1), 5.
- Bridge, T., Scott, A., & Steinberg, D. (2012). Abundance and diversity of anemonefishes and their host sea anemones at two mesophotic sites on the Great Barrier Reef, Australia. *Coral Reefs*, 31(4), 1057-1062.
- Buston, P. (2003). Size and growth modification in clownfish. *Nature*, 424(6945), 145-146.
- Buston, P. M. (2004). Territory inheritance in clownfish. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(suppl_4), S252-S254.
- Buston, P. M., Bogdanowicz, S. M., Wong, A., & Harrison, R. G. (2007). Are clownfish groups composed of close relatives? An analysis of microsatellite DNA variation in *Amphiprion percula*. *Molecular Ecology*, 16(17), 3671-3678.
- Casas, L., Saborido-Rey, F., Ryu, T., Michell, C., Ravasi, T., & Irigoien, X. (2016). Sex change in clownfish: molecular insights from transcriptome analysis. *Scientific reports*, 6, 35461.
- Cinner, J. E., & Pollnac, R. B. (2004). Poverty, perceptions and planning: why socioeconomics matter in the management of Mexican reefs. *Ocean & Coastal Management*, 47(9-10), 479-493.
- da Silva, K. B., & Nedosyko, A. (2016). Sea anemones and anemonefish: a match made in heaven. In *The Cnidaria, past, present and future* (pp. 425-438). Springer, Cham.

- Dunn, D. F. (1981). The clownfish sea anemones: Stichodactylidae (Coelenterata: Actiniaria) and other sea anemones symbiotic with pomacentrid fishes. *Transactions of the American Philosophical Society*, 71(1), 3-115.
- Ekman S., (1953). Zoogeography of the sea. Sidwick & Jackson, London.
- Elliott, J. K., & Mariscal, R. N. (1997). Acclimation or innate protection of anemonefishes from sea anemones? *Copeia*, 284-289.
- Elliott, J. K., & Mariscal, R. N. (2001). Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. *Marine Biology*, 138(1), 23-
- Fautin, D. G. (1991). Review article The Anemonefish Symbiosis: What is Known and What is Not. Symbiosis.
- Fautin, D. G., Allen, G. R. (1992). Field guide to anemonefishes and their host sea anemones.
- Foden, W., & Stuart, S. N. (2009). Species and climate change: more than just the Polar Bear. Gland, Switzerland, IUCN Species Survival Commission.
- Fricke, H. W. (1979). Mating system, resource defence and sex change in the anemonefish *Amphiprion akallopisos*. *Zeitschrift für Tierpsychologie*, 50(3), 313-326.
- Godwin, J., & Fautin, D. G. (1992). Defense of host actinians by anemonefishes. *Copeia*, 1992(3), 902-908.
- Goemans, B. "Anemonefishes". Retrieved 20 September 2020.
- Hattori, A. (1991). Socially controlled growth and size-dependent sex change in the anemonefish *Amphiprion frenatus* in Okinawa, Japan. *Japanese Journal of Ichthyology*, 38(2), 165-177.
- Holbrook, S. J., & Schmitt, R. J. (2005). Growth, reproduction and survival of a tropical sea anemone (Actiniaria): benefits of hosting anemonefish. *Coral reefs*, 24(1), 67-73.
- Huebner, L. K., Dailey, B., Titus, B. M., Khalaf, M., & Chadwick, N. E. (2012). Host preference and habitat segregation among Red Sea anemonefish: effects of sea anemone traits and fish life stages. *Marine Ecology Progress Series*, 464, 1-15.
- Jones, G. P., Planes, S., & Thorrold, S. R. (2005). Coral reef fish larvae settle close to home. *Current Biology*, 15(14), 1314-1318.
- Kumar, T. T., Setu, S. K., Murugesan, P., & Balasubramanian, T. (2010). Studies on captive breeding and larval rearing of clown fish [a 1], *Amphiprion sebae* (Bleeker, 1853) using estuarine water.

- Litsios, G., Sims, C. A., Wüest, 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(1), 212.
- Maison, K. A., & Graham, K. S. (2015). Status Review Report: Orange Clownfish (*Amphiprion percula*). *Report to Natural Marine Fisheries Service, Office of Protected Resources*, (April), 1–67. <https://doi.org/10.7289/V5J10152>
- Mariscal, R. N. (1969). The protection of the anemone fish, *Amphiprion xanthurus*, from the sea anemone, *Stoichactis kenti*. *Experientia*, 25(10), 1114-1114.
- Mariscal, R. N. (1970). The nature of the symbiosis between Indo-Pacific anemone fishes and sea anemones. *Marine Biology*, 6(1), 58-65.
- Mariscal, R. N., Fautin, D. G., & Allen, G. R. (1993). Field Guide to Anemonefishes and Their Host Sea Anemones. *Copeia*, 1993(3), 899. <https://doi.org/10.2307/1447266>
- Matsunuma, M., Motomura, H., Matsuura, K., Shazili, N. A. M., & Ambak, M. A. (2011). *Fishes of Terengganu East coast of Malay Peninsula, Malaysia. East*. Retrieved from http://www.museum.kagoshima-u.ac.jp/staff/motomura/TFG_lowres.pdf
- Militz, T. A., McCormick, M. I., Schoeman, D. S., Kinch, J., & Southgate, P. C. (2016). Frequency and distribution of melanistic morphs in coexisting population of nine clownfish species in Papua New Guinea. *Marine biology*, 163(10), 200.
- Mitchell, J. S., & Dill, L. M. (2005). Why is group size correlated with the size of the host sea anemone in the false clown anemonefish? *Canadian Journal of Zoology*, 83(2), 372-376.
- Miyagawa, K. (1989). Experimental analysis of the symbiosis between anemonefish and sea anemones. *Ethology*, 80(1-4), 19-46.
- Murata, M., Miyagawa-Kohshima, K., Nakanishi, K., & Naya, Y. (1986). Characterization of compounds that induce symbiosis between sea anemone and anemone fish. *Science*, 234(4776), 585-587.
- Murray, J. M., & Watson, G. J. (2014). A critical assessment of marine aquarist biodiversity data and commercial aquaculture: identifying gaps in culture initiatives to inform local fisheries managers. *PLoS One*, 9(9), e105982.
- Ochi, H. (1986). Growth of the anemonefish *Amphiprion clarkii* in temperate waters, with special reference to the influence of settling time on the growth of 0-year olds. *Marine Biology*, 92(2), 223-229.
- Ortega-García, K. (2014). Clownfish: commercial interest and culture.

- Palaniappan, S. S., Azizah, M. N. S., & Yusuf, Y. (2003). Notes on the Occurrence of Amphiprion (Perciformes: Pomacentridae) in Peninsular Malaysia. *Asian Fisheries Science*, 16, 235–240.
- Palaniappan, S. S., Azizah, S., & Yusuf, Y. (2003). Notes on the Occurrence of Amphiprion (Perciformes: Pomacentridae) in Peninsular Malaysia.
- Rolland, J., Silvestro, D., Litsios, G., Faye, L., & Salamin, N. (2018). Clownfishes evolution below and above the species level. *Proceedings of the Royal Society B: Biological Sciences*, 285(1873). <https://doi.org/10.1098/rspb.2017.1796>
- Roopin, M., Henry, R. P., & Chadwick, N. E. (2008). Nutrient transfer in a marine mutualism: patterns of ammonia excretion by anemonefish and uptake by giant sea anemones. *Marine Biology*, 154(3), 547-556.
- Roux, N., Lami, R., Salis, P., Magré, K., Romans, P., Masanet, P., ... Laudet, V. (2019). Sea anemone and clownfish microbiota diversity and variation during the initial steps of symbiosis. *Scientific Reports*, 9(1), 1–13. <https://doi.org/10.1038/s41598-019-55756-w>
- Scott, A., & Dixon, D. L. (2016). Reef fishes can recognize bleached habitat during settlement: sea anemone bleaching alters anemonefish host selection. *Proceedings of the Royal Society B: Biological Sciences*, 283(1831), 20152694.
- Szczebak, J. T., Henry, R. P., Al-Horani, F. A., & Chadwick, N. E. (2013). Anemonefish oxygenate their anemone hosts at night. *Journal of Experimental Biology*, 216(6), 970-976.
- Velasco-Blanco, G., Re, A. D., Díaz, F., Ibarra-Castro, L., la Parra, M. I. A. de, Rodríguez-Ibarra, L. E., & Rosas, C. (2019). Thermal preference, tolerance, and thermal aerobic scope in clownfish *Amphiprion ocellaris* (Cuvier, 1830) predict its aquaculture potential across tropical regions. *International Aquatic Research*, 11(2), 187–197. <https://doi.org/10.1007/s40071-019-0228-7>
- Woodland, D. J. (1983). Zoogeography of the Siganidae (Pisces): an interpretation of distribution and richness patterns. *Bulletin of marine science*, 33(3), 713-717.