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Connectivity of Corals Reefs Between Marine Park and Non-Marine Park Islands in the Malacca Straits



Prepared by:

**ASSOC. PROF DR. AILEEN TAN SHAU HWAI
School of Biological Sciences,
Universiti Sains Malaysia,
Penang.**

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1.0 Executive summary

In this study, we investigate the genetic population structure and gene flow in the clownfish, *Amphiprion ocellaris*, across the Langkawi and Payar Archipelago in the Straits of Malacca by analysing molecular markers in the mitochondrial control region. The study aims to determine the reefs supplying gametes to the Pulau Payar Marine Park and prioritize the area outside the marine park that is important for the supply of gametes to the marine park.

Considerable genetic structuring was observed throughout the study area. Due to their dependence on the presence of the host anemones in coral reefs, the site-attached behaviour of the adults, demersal eggs and the short larval stage, this species has a low dispersal capacity and hence a strong genetic population structure can be expected. Population of *A. ocellaris* from Payar is genetically distinct from other populations in the study area. However, it is more closely related to the northern Langkawi populations than the southern Langkawi populations. Access to major surface currents seems to facilitate larval dispersal of *A. ocellaris*, indicated by higher connectivity along the passageway. Population genetic analysis also reveals that the populations were most probably separated during sea level low stand in the Quaternary Period.

Among various conservation efforts, the application of marine protected areas (MPA) was shown to be one of the most promising for coral reefs, as these ecosystems are highly complex and the organisms depend on a balanced system. MPAs may not only help to protect marine biodiversity but also to enhance fishing yields in the adjacent areas. Population genetic studies on coral reef organisms may contribute to the conservation efforts by giving information about the connectivity among reefs and the genetic diversity of populations. These different genetic lineages should be managed and protected as separate fishery resources to contribute to the genetic diversity of the area.

2.0 Introduction and objectives

For a sustainable use and the conservation of coral reef resources, various efforts such as quotas and size regulations, licensing, the prohibition of destructive fishing methods, and the setting up of marine protected areas (MPAs) have been implemented (Wood, 2001; Wabnitz *et al.*, 2003). These regulations are important initiatives but are often difficult to manage and control under the local conditions (Wood, 2001). Furthermore, the applied strategies might not be effective for all targeted organisms (Knittweis, 2008) since profound knowledge about the biology of affected organisms is necessary, which to date however is not sufficiently available. Genetic studies can provide information about the genetic diversity of populations, the connectivity among locations, possible source and sink relationships of areas, and dispersal distances of larvae. This information is important for the effective management of marine resources and the conservation of species, e.g. the location and sizing of MPAs or MPA networks.

Thus the objectives of this study are:

1. to analyse the population genetic structure of an organism with low dispersal ability (i.e. *Amphiprion ocellaris*) for the connectivity patterns between the marine park (Payar Marine Park) and non-marine park (Langkawi Islands), and
2. to investigate the possible evolutionary processes leading to the phylogeographic structure of the studied population.

2.1 Justifications

Choice of target species

The targeted species in this study is the false clownfish, *Amphiprion ocellaris*. This species is chosen based on the distribution which is limited by the presence of the host sea anemone. The host depends on light as the major factor that is affecting its distribution (which is similar to corals). Furthermore the adults of clownfish are strongly site attached, thus high geographical structuring can be easily detected in

the population genetics. *Amphiprion ocellaris* is also widely distributed throughout the Indo-Malay Archipelago (Timm and Kochzius, 2008).

Collaboration BOBLME - USM

The conservation of biodiversity and the effective management of Marine Protected Areas is among the key focal areas of the Bay of Bengal Large Marine Ecosystem (BOBLME) Project. This collaborative work is intended to contribute to a better understanding on the coral reef connectivity of reefs located at the Northern Straits of Malacca, and improve the information base for protection of these reefs using an ecosystem approach as a contribution to habitat conservation and sustainable fisheries in the region.

3.0 Research Methodology

3.1 Sample collection

The sites were chosen from the Langkawi group of islands (non-marine park) – Kubang Badak, Pulau Dangli, Pulau Pasir, Pulau Intan and Pulau Singa Besar. For Marine Park, Pulau Lembu was chosen from the Payar group of islands (Figure 1).



Figure 1. Location of study sites.

A total of 59 tissue samples of *Amphiprion ocellaris* were collected in this study. The fishes were caught with aquarium nets. A fin clip from the caudal fin was taken and the fishes were released into their host anemones. The tissue samples were obtained without killing the animals and ensure the survival of the fish. The samples were stored in 96% ethanol.

3.2 DNA isolation, polymerase chain reaction amplification and DNA sequencing

Genomic DNA was isolated with filter column based extraction kits from QIAGEN™ following the manufacturer's protocol.

A fragment of around 370bp from the mitochondrial control region (CR) was amplified by polymerase chain reaction (PCR) using the primers CR-A (5'-TTCCACCTCTAACTCCCA AAGCTAG-3') and CR-E (5'-CCTGAAGTAGGAACCAGATG-3') following Lee *et al.* (1995). The PCR with a volume of 25µl contained 2.5µl 10 x PCR buffer, 0.075 µmol Mg²⁺, 0.25 µmol dNTP mix, 10 pmol of each primer, 0.5 U Taq polymerase and 10-30 ng genomic DNA of each sample. The temperature profile of the PCR was 95°C for 2 min, followed by 35 cycles of 95°C for 30s, 50°C for 30s and 72°C for 60s. The terminal elongation at 72°C took 2 min. All PCR products were purified with the QIA-quick PCR Purification Kit by QIAGEN™. Sequencing of both strands was conducted with the PCR primers using the BigDye Terminator Cycle Sequencing Kit according to the manufacturer's recommendations and an ABI PRISM 310 and 3100 Automated Sequencer.

3.3 Sequence alignment and analyses

The sequences were edited by viewing the peak of the chromatogram in MEGA 5 and multiple sequence alignments were carried out using Clustal W 1.6 implemented in the same software. The phylogenetic relationships were analysed by using maximum likelihood (ML) algorithms based on the Jukes-Cantor model, which was the best-fitting model selected by the Akaike information criterion (AIC). The statistical confidences were evaluated by 100 bootstrap replicates.

Estimates of evolutionary divergence between sequences of *Amphiprion ocellaris* in this study were determined using the maximum composite likelihood method in MEGA. A composite likelihood is defined as a sum of related log-likelihoods. Since all pair-wise distances in a distance matrix have correlations due to the phylogenetic relationships among the sequences, the sum of their log-likelihoods is a composite likelihood. This model assumes equality of substitution pattern among lineages and of substitution rates among sites. Tajima's D-test for neutrality was also used to test for demographic expansion of *A. ocellaris* populations in this study.

4.0 Findings of study

4.1 Determination on the type and location of coral reef and main components of the reefs systems

Several surveys have been carried out to identify the location of coral reefs around Pulau Langkawi and Pulau Payar. The reef area at Pulau Langkawi can be divided into the northern and southern part (Table 1 and Figure 1). In the north, several coral reef areas have been identified namely Kubang Badak, Pulau Dangli, and Pulau Pasir, whereas in the south, the area that has been surveyed are Pulau Singa Besar and Pulau Intan. As for the marine park, Pulau Lembu has been surveyed in this study,

Table 1. Coral reef areas around Pulau Langkawi and Pulau Payar that have been surveyed in this study.

Location of coral reefs	Type of reefs	Main components of the reef systems
Northern part of Pulau Langkawi (non-marine park) <ul style="list-style-type: none"> • Kubang Badak • Pulau Dangli • Pulau Pasir 	Most are intertidal reef flats that are exposed during the low tide. The reefs can be divided into two zones: upper and lower reef zone.	<ul style="list-style-type: none"> • Live coral cover percentage varies from 0 – 60% • Dominated by Faviidae and Agariciidae • Maximum coral cover can be found at the reef edge
Southern part of Pulau Langkawi (non-marine park) <ul style="list-style-type: none"> • Pulau Singa Besar 	Some reefs at certain part of the islands are exposed during the low tide and others are	<ul style="list-style-type: none"> • Live coral cover percentage varies from 0 – 80% • Dominated by

<ul style="list-style-type: none"> • Pulau Intan 	always submerged in the water (subtidal reef).	Faviidae and Agariciidae
Pulau Payar Group (marine park) <ul style="list-style-type: none"> • Pulau Lembu 	All are subtidal reefs.	<ul style="list-style-type: none"> • Live coral cover percentage varies from 30 – 90% • Dominant coral is Faviidae

4.2 Genetic connectivity and relationship between the selected reefs

A fragment of around 370 base pairs from the mitochondrial control region (CR) was amplified by polymerase chain reaction (PCR). The mitochondrial control region is widely used for population genetic analyses of vertebrates because of its high variability (Lee *et al.*, 1995). A total of 59 tissue samples of *Amphiprion ocellaris* from Pulau Langkawi (which consists of five populations) and Pulau Payar (one population) group of islands were sequenced.

From this study, the population structuring was further confirmed between the populations of *A. ocellaris*. The phylogenetic tree showed genetic differentiations between these populations with three clades (or groupings) was observed (Figure 2).

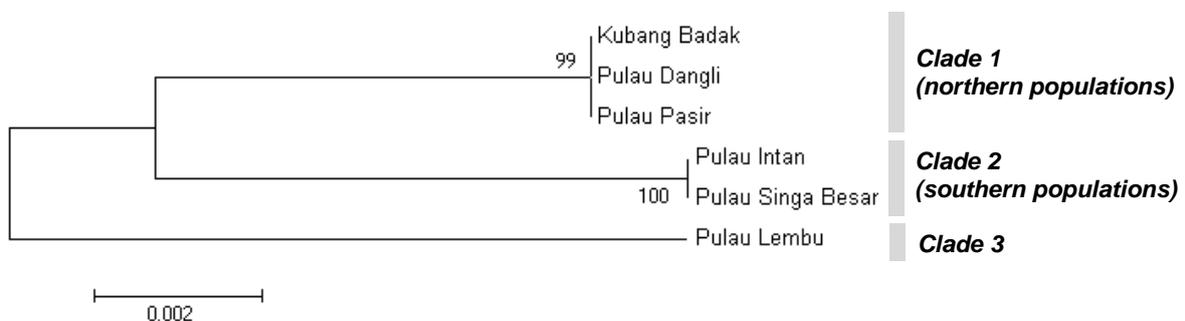


Figure 2. Molecular phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model.

A. ocellaris population from north of Langkawi seems to be more closely related to the population from south of Langkawi, whereas population from Pulau Lembu (Payar Group) is isolated from the other two populations and forming its own clade. The estimates of evolutionary divergence (genetic distance) between sequences further supported this finding (Table 2).

Table 2. Estimates of evolutionary divergence between sequences of *Amphiprion ocellaris* in this study.

	Kubang Badak	P. Dangli	P. Pasir	P. Intan	P. Singa Besar
P. Dangli	0.0001				
P. Pasir	0.0002	0.0000			
P. Intan	0.0115	0.0118	0.0118		
P. Singa Besar	0.0119	0.0122	0.0122	0.0001	
P. Lembu	0.0149	0.0147	0.0147	0.0161	0.0161

The highest genetic divergence (lower levels of gene flow) was observed between the populations of Pulau Lembu and the southern populations of *A. ocellaris* from Pulau Intan and Pulau Singa Besar (1.61%), followed by the northern populations (1.47-1.49%) of Pulau Dangli, Pulau Pasir and Kubang Badak. These findings showed that Pulau Lembu is more closely related to the northern populations than the southern populations of *A. ocellaris*. The genetic divergence between northern and southern Langkawi populations was recorded in the range of 1.15 to 1.22%. No genetic divergence was recorded within populations in similar geographical area such as Pulau Dangli and Pulau Pasir, and the least was recorded elsewhere (0.01-0.02%). Although the values are small, due to the comparison of a species group within a small geographical area (less than 100km), they do clearly indicate the genetic connectivity between populations of *A. ocellaris* in this area. Other studies on *A. ocellaris* revealed genetic distances more than 8% with about 800km separation between populations (Timm and Kochzius, 2008). Populations from Pulau Intan and Pulau Singa Besar was expected to

be more closely related to the population from Pulau Lembu, because both islands are located at the south of Pulau Langkawi. However that is not the case in this study. Langkawi populations are more closely related to each other compared to the distant population found at Pulau Lembu. Overall, these can be summarized in Figure 3.

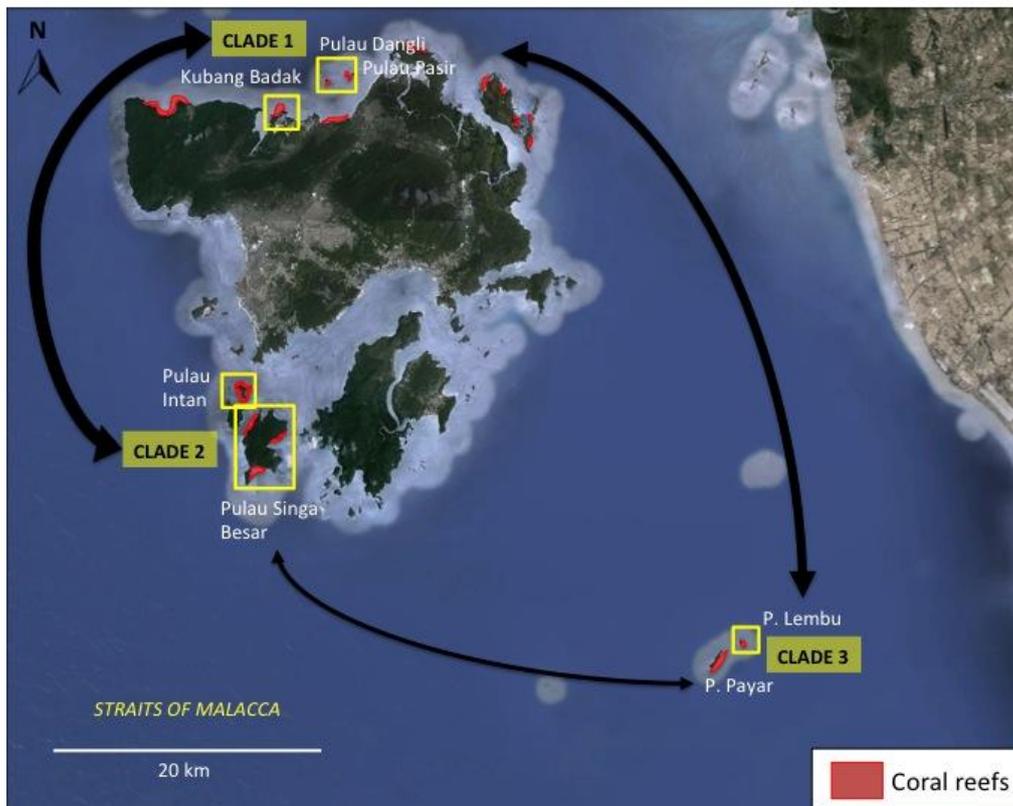


Figure 3. Summary of intensity of gene flow between clades of *Amphiprion ocellaris* in the study area indicated by the thickness of the black arrow. The thicker the arrow, the more closely related the populations of *A. ocellaris* between the clades.

4.3 Phylogeographic structure of *Amphiprion ocellaris*

In other study of anemonefish species (e.g. *A. polymnus*), populations with high proportions of self-recruitment have been observed (Jones *et al.*, 2005). Anemone fishes are strongly site-attached, which leads to a strong genetic population structure on even much smaller scales, which is also facilitated by complex geography (Hall, 1996) and current patterns (Wyrcki, 1961) in the region. The current patterns in the Straits of Malacca are highly influenced by the northeast (NE) and southwest (SW) monsoon, which occurs in the months of December through February, and June through September, respectively (Wyrcki, 1961; Rizal *et al.*, 2010). During the NE monsoon, the currents move northwest (towards Indonesian waters and Andaman Sea), and move southeast during the SW monsoon as shown in Figure 4 (modified from Wyrcki, 1961).

Breeding in *Amphiprion ocellaris* may occur all year round and spawning is concentrated around the full moon. According to Thresher (1984), possible reasons for this include stronger water currents for larval distribution, greater food supplies due to invertebrate spawning at the same time, and overall increased visibility. After the incubation period, the planktonic larval stage lasts from 8-12 days and ends when the juvenile fish returns to the bottom and attempt to find an anemone to inhabit (Fautin and Allen, 1992). The present study has shown that gene flow, and therefore larval dispersal, was restricted on a small spatial scale. The dispersal distance in the studied species is probably only within an order of a few tens of kilometres. Larval dispersal of marine organisms can vary greatly, and was estimated to be in the order of 25-150km, but it was suggested that a single-generation dispersal of only a few tens of kilometres might be common in many marine organisms (Timm and Kochzius, 2008). For such organisms, marine protected areas (MPAs) should be sized to ensure effectively self-seeding, because exchange with populations several hundreds of kilometres away is ecologically rare (Palumbi, 2003).

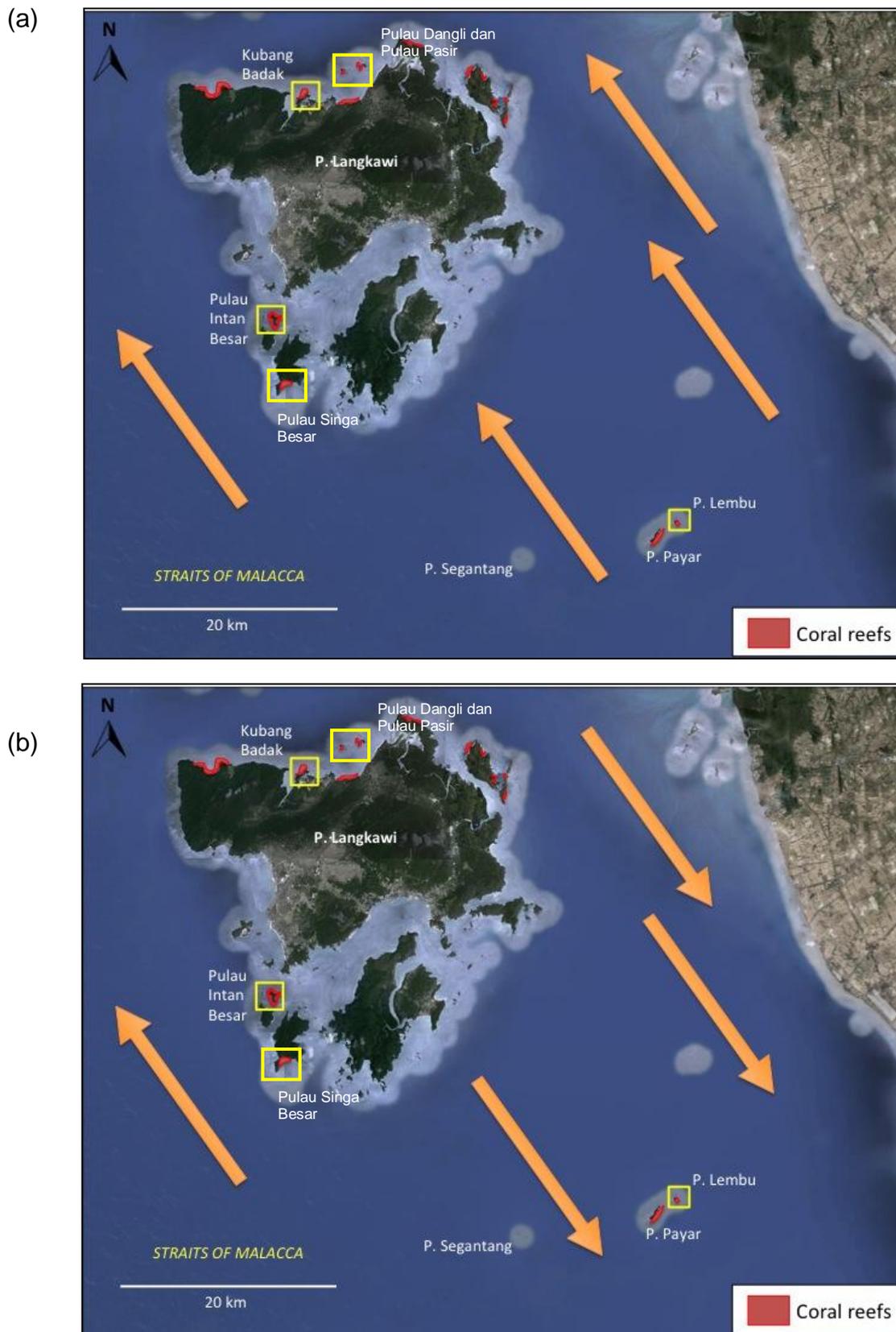


Figure 4. The surface current pattern at the study area during (a) NE (February) and (b) SW monsoon (August) (modified from Wyrтки, 1961).

It was also shown in this study, populations with access to major currents in the Straits of Malacca showed higher dispersal distances along such passageways, which explains the relatedness of the populations of northern Langkawi (Clade 1) with population of Pulau Lembu (Clade 3), and populations of northern Langkawi (Clade 1) with population of southern Langkawi (Clade 2). Figure 5 showed the clades and the accessibility to the major currents in the Straits of Malacca. Example shown during the strongest current movement recorded in the Straits of Malacca that is during the northeast monsoon.

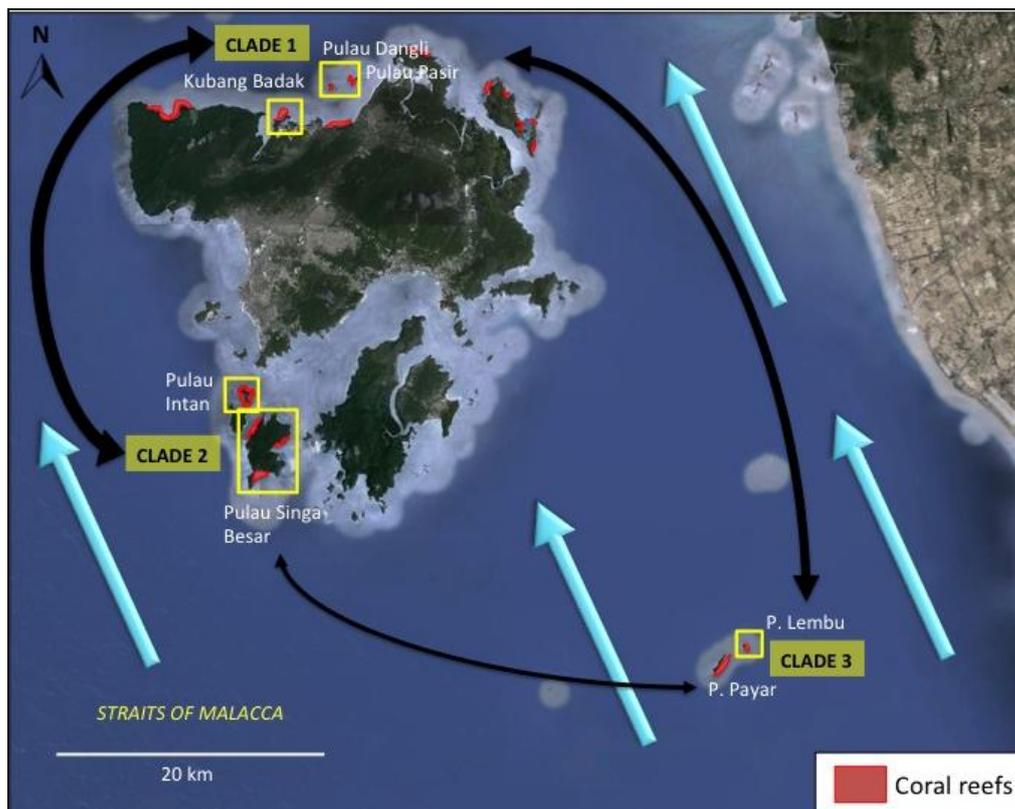


Figure 5. The populations of *Amphiprion ocellaris* and the accessibility to the major currents (blue arrow) in the Straits of Malacca. Thickness of the black arrows showed the relative genetic connectivity between populations, with thicker arrow denotes higher genetic connectivity between populations.

Another explanation for the phylogeographic population structuring found in this study is shown by the Tajima's D-test for neutrality of 0.596333 ($P < 0.05$). Tajima's D-test will reveal the sequences that do not fit the neutral theory model of equilibrium between mutation and genetic drift. A positive Tajima's D signifies low levels of both low and high frequency polymorphisms, indicating a decrease in population size and/or balancing selection. Therefore, it explains the isolation of Pulau Lembu population from other populations, whereby the population might have experienced the isolation of genetic lineages during the glacial periods. The fall in sea levels during periods of glacial maxima in the Quaternary Period would result in the entire Sunda shelf becoming terrestrial, thus limiting the distribution of *A. ocellaris*. The ebb and flow of sea levels over the Sunda shelf would likely result in corresponding retreats and re-dispersals of *A. ocellaris* populations. At approximately 7000 years ago, the sea level had risen and started to link the area between Langkawi and Payar (Figure 6). Nelson et al. (2000) hypothesized that the rim sites may have "seeded" the suitable sites for *A. ocellaris* on the Sunda shelf as sea levels rose during glacial retreat. Clearly the dispersal capacity of this strongly localised species is low, due to its biology (site-attached adults; demersal eggs; short larval stage; Fautin and Allen, 1994). Therefore, the imprint of isolation during sea level low stands is still present in the genetic population structure of *A. ocellaris*.

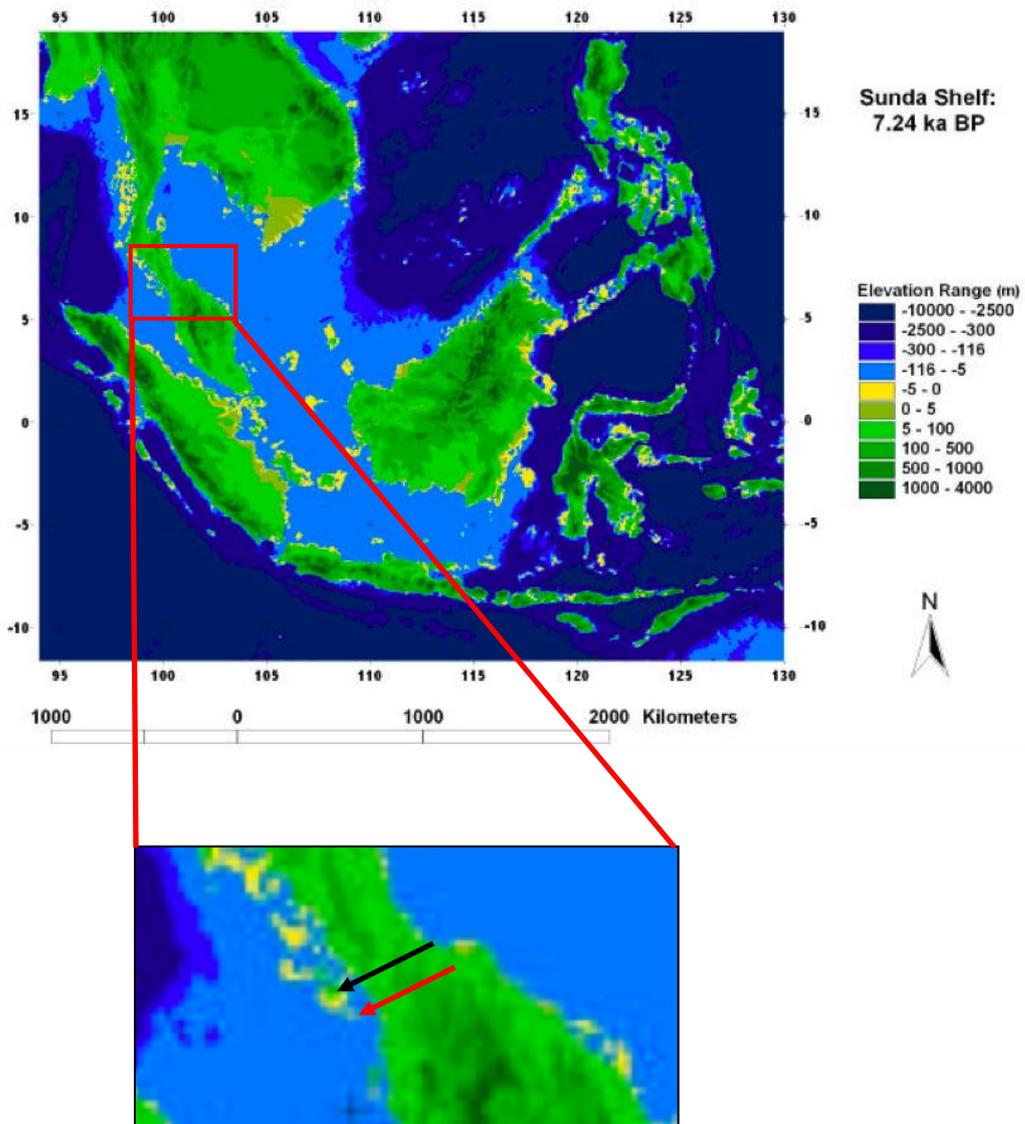


Figure 6. The sea level transgression on the Sunda Shelf approximately 7000 years before present (modified from Sathiamurthy and Voris, 2006). Black arrow denotes the location of Langkawi, whereas red arrow denotes Payar area on the map. Southern part of Langkawi was still exposed (yellow area) during the transgression period, which is in contrast to the inundated area of the northern part of Langkawi.

5.0 Conclusion and recommendations

In summary, gene flow among *Amphiprion ocellaris* populations is possible if the reef systems are strongly connected by ocean currents. Longer distances between MPAs can be tolerated if higher connectivity is measured but spacing should be closer and sizes of reserves are large enough in areas further away from the major currents. Different genetic lineages should be protected as separate “conservation unit”, providing “genetic stocks” for the biodiversity. However, the genetic structuring of the present *A. ocellaris* population in this study is also predominantly influenced by past dispersal events, particularly events that are associated with past glaciations during the Quaternary Period. In this study, Pulau Payar Marine Park may be constituted as a “conservation unit” based on the genetic differences with other populations in the area. The northern Langkawi populations should also be protected to maintain the genetic contribution to the distinct and unique genetic pool in the Payar Marine Park.

The implication of this study for conservation may be explained by the basis of the species chosen in this study – the short larval duration of *A. ocellaris* (within a week, similar to majority of coral larvae) and also the specificity to the host anemone (which is dependent on light for distribution), which may reveal one of the “worst case scenarios” (lowest dispersal ability) for the connectivity of communities between reefs. However, this does not mean total exclusion of the habitat forming species such as scleractinian corals which form much of the three dimensional structure of the reef. Overall, **it is important to determine the objectives of the MPA if they are to maximise biodiversity and conserve ecosystem health, or to enhance production of extractive activities (e.g. fisheries), or both.**

6.0 Acknowledgements

The study was supported by The Bay of Bengal Large Marine Ecosystem (BOBLME) who shares the common interest in conserving fisheries resources and marine biodiversity, among others in the Straits of Malacca and also through effectively managed marine protected areas. This includes promoting multi-national approaches to manage and address issues affecting transboundary coastal or marine ecosystems within the broader BOBLME region, and obtaining consensus on approaches to the establishment and management of marine protected areas and fish refugia for sustainable fish management and biodiversity conservation objectives.

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7.0 References

- Faizan, M. (2014). Study on the impact of anthropogenic pressure on coral reefs around Cape Rachado, Malacca and recommendations to improve its management. Doctoral dissertation, University of Malaya.
- Fautin, D., & Allen, G. (1992). Field Guide to Anemonefishes and their Host Sea Anemones. Perth: Western Australian Museum. Fautin, D. G., & Allen, G. R. (1994). Anemonenfische und ihre Wirte. Tetra-Verlag.
- Jones, G. P., Planes, S., & Thorrold, S. R. (2005). Coral reef fish larvae settle close to home. *Current Biology*, 15(14), 1314-1318.
- Knittweis, L., Krämer, W.E., Timm, J., Kochzius, M. (2008). Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts. *Conservation Genetics*, doi: 10.1007/s10592-008-9566-5.
- Lee, W. J., Conroy, J., Howell, W. H., & Kocher, T. D. (1995). Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, 41(1), 54-66.

- Nelson, J. S., Hoddell, R. J., Chou, L. M., Chan, W. K., & Phang, V. P. E. (2000). Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. *Marine Biology*, 137(4), 727-736.
- Palaniappan, S. S., Azizah, M. S., & Yusuf, Y. (2003). Notes on the Occurrence of *Amphiprion*. *Asian Fisheries Science*, 16, 235-240.
- Palumbi, S. R. (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, 13(sp1), 146-158.
- Rizal, S., Setiawan, I., Iskandar, T., Ilhamsyah, Y., Wahid, M. A., & Musman, M. (2010). Currents simulation in the Malacca Straits by using three-dimensional numerical model. *Sains Malaysiana*, 39(4), 519-524.
- Sathiamurthy, E. V. H. K., & Voris, H. K. (2006). Maps of Holocene sea level transgression and submerged lakes on the Sunda Shelf. *The Natural History Journal of Chulalongkorn University*, Supplement, 2, 1-43.
- Thresher, R. E. (1984). *Reproduction in reef fishes*. TFH publications.
- Timm, J. (2008). *Molecular ecology and evolution of anemone fishes (Amphiprion spp.) in the Indo-Malay Archipelago*. Doctoral dissertation, University of Bremen.
- Timm, J., & Kochzius, M. (2008). Geological history and oceanography of the Indo - Malay Archipelago shape the genetic population structure in the false clown anemone fish (*Amphiprion ocellaris*). *Molecular Ecology*, 17(18), 3999-4014.
- Wabnitz, C., Taylor, M., Green, E., Razak, T. (2003). *From Ocean to Aquarium*. UNEP-WCMC, Cambridge, UK.
- Wood, E. (2001). *Collection of Coral Reef Fish for aquaria: Global Trade, Conservation Issues and Management Strategies*. Marine Conservation Society, Ross-on-Wye, UK.
- Wyrtki K (1961) *Physical Oceanography of the Southeast Asian Waters*. NAGA Report Vol. 2, The University of California, Scripps Institution of Oceanography, La Jolla, California.