Project Title:	**The Population Study of *Thunnus tonggol* (Bleeker, 1851) in the Southeast Asian Region**
Responsible Department:	SEAFDEC/MFRDMD
Countries involved:	Brunei Darussalam, Cambodia, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam
Total Project Duration:	Years 2015-2017
Leading Countries:	Malaysia

1.0 Objectives of the project:

This project titled "**The Population Study of *Thunnus tonggol* (Bleeker, 1851) in the Southeast Asian Region**" is being proposed to aim at;

1) To identify the genetic structure of *Thunnus tonggol* (Longtail tuna) in the South China Sea and Andaman Sea waters by using cytochrome b, mitochondrial DNA (mtDNA) marker.

2.0 Introduction:

Tunas are commercially important fishery worldwide. There are at least 13 species of tuna belonging to three genera, out of which genus *Thunnus* has maximum eight species. Based on their availability, they can be characterized as oceanic such as *Thunnus albacares* (yellowfin tuna) or coastal such as *Thunnus tonggol* (longtail tuna). *Thunnus tonggol* is second smallest species of the *Thunnus* genus. It has a narrow coastal distribution in tropical and temperate waters of Indo-Pacific region. *Thunnus tonggol* reaches 145 centimetres (57 in) in length and 35.9 kilograms (79 lb) in weight. Compared to similar-sized tunas, its growth is slower and it lives longer, which may make it vulnerable to overfishing. *Thunnus tonggol* is a commercially important fishery with its great demand in export market.

According to International Union for Conservation of Nature (IUCN), Malaysia and Thailand from the South China Sea are one of the major landing areas of this species besides countries bordering the North Arabian Sea. Longtail tuna is caught mainly by gillnet and in a lesser extent by artisanal purse seiners. This species is listed as Data Deficient by IUCN due to no effort information or stock assessments even the landing is information is increasing from year to year. Therefore more information is needed on the status of this species population, including better catch data and effort information. Management of this species also needs to be included under a fisheries management organization.

Genetic approach to fish stock assessment can be very useful, cost effective and can give high accuracy results. The genetic approach provides information on levels of genetic diversity in fish populations, degree of genetic differentiation among fish population and hence genetic population structure, and level of gene flow among fish populations.

The reasons for the adoption of mitochondrial DNA (mtDNA) as marker of choice are well known. Experimentally, mtDNA is relatively easy to amplify because it appears in multiple copies in the cell. Mitochondrial DNA is maternally inherited that considerably simplifies the representation and analysis of within species variation data. Their gene content is strongly conserved across animals, with very little duplication, no intron, and very short intergenic regions (Gissi et al. 2008). Mitochondrial DNA is highly variable in natural populations because of its elevated mutation rate, which can generate some signal about population history over short time frames. It is for this reason, there appears to be significant variation in mtDNA sequences between species and comparatively small variance within species (Moritz et al., 1987). Mitochondrial DNA is the most convenient and cheapest solution when a new species has to be genetically explored in the wild (Galtier et al, 2009).

A genetic study in coastal tuna *Thunnus tonggol* sampled from across the South China Sea (Pemangkat and Pekalongan, Indonesia and Vung Tau, Vietnam) using mtDNA D-loop region (893 bp) found that the phylogenetic recontruction of genetic relationships revealed high levels of genetic diversity with no clear partitioning between sites. However, there are significant population differentiation (ϕ_{ST}) statistics between the two most geographically distant locations that suggests the presence of at least two genetically differentiated (Pekalongan, Indonesia and Vung Tau, Vietnam), but potentially over-lapping *T. tonggol* stocks (Willette & Leadbitter, unpublished).

Beside that a few studies been conducted on neritic tuna on other neritic tuna species such as *Euthyynus affinis* (kawakawa) by using mitochondrial DNA marker had showed that the samples taken were homologous which this indicating the single population on the selected area. For example, a preliminary study of population structure of kawakawa, *Euthynnus affinis* (Cantor 1849) in 4 location of the straits of Malacca by using 331 bp of (mtDNA) cytochrome b resulted that 99% from the samples taken were homologous which indicated a single population along the straits of Malacca (Masazurah et. al, 2012). Santos et al. 2010, studied genetic population structure using mtDNA control region (D-loop, 300 bp) of five areas across the Philippines and one area at Peninsular, Malaysia also detecting that this species was to be "panmixia" or mixing.

However, population genetic structure has been commonly identified in one species in particular, the skipjack tuna *Katsuwonus pelamis*. Significant divergence has been observed in this species over both vast geographic distances (Japan to India, Menezes et al. 2006). Genetic variation in mtDNA and microsatellite were identified two genetic groups in the northwestern Indian Ocean where the analysis were indicates that the sampled skipjack tuna are likely to represent individuals sourced from discrete breeding grounds that are mixed in feeding grounds in Sri Lanka waters (Dammannagoda et al. 2011). Genetic structure of skipjack tuna *K. pelamis* also studied in Indian region was investigated using mtDNA D-loop region found the occurrence of four genetically differentiated groups of *K. pelamis* across the coastal waters of India (Menezes et al. 2012). These results have direct management implications in recommending that *K. pelamis* be managed as discrete, genetically differentiated stocks.

3.0 Materials and Methods

3.1 Sample collection.

A total number of 50 samples per sampling site of *Thunnus tonggol* will be collected in this study at selected landing port. Fin tissue samples will be preserved in 95% ethanol for DNA extraction. The proposed landing sites are shown in Table 1.

3.2 DNA genomic extraction, DNA Polimerase Chain Reaction (PCR) and DNA sequencing.

DNA genomic will be extracted using (Qiagen Kit). The complete mtDNA cytochrome b region (1138-1141 bp) will be amplify using forward primer, 5' ACC AGG ACT ATT GGC TTG 3' and reverse primer, 5' AGG ATT TTA ACC TCC GAC GTC 3' (Tseng et. al, 2009, 2011). The PCR product will be run in 1.5% agarose gel to confirmed the product size and will be sequence using an automated sequencer.

3.3 Data analysis

Sequences will be aligned using Clustal X (Thompson et al., 1997) and estimates of haplotype diversity, nucleotide diversity and divergence will be calculated using MEGA 5.0 (Tamura et al., 2011) and Arlequin 3.5 (Excoffier & Lischer, 2011) based on Kimura 2P distance measures. Phylogenetic trees will be constructed using MEGA 5.0 to visualize the relationships among observed mtDNA variants. AMOVA (10,000 replicates) (Excoffier et al., 1992) and Pairwise F_{ST} tests (10,000 replicates) (Slatkin, 1991) implemented in the population genetics package Arlequin 3.5 will be used to examine the genetic structure among the surveyed populations. Both nucleotide (Kimura 2P) and conventional F_{ST} distance measures will be used to calculate within and among population diversity. The GenBank database (National Center for Biotechnology Information, USA: NCBI Homepage http://www.ncbi.nlm.nih.gov) will be searched for similar sequences.

Table 1: Propose landing site for samples collection (if possible).

Country		Sampling Site/s	Country & Sampling Site Code	No. of Sample
Andaman Sea Sub-Region				
➢ Indonesia	1.	Banda Aceh (will be confirmed)	INBA	50
	2.	Belawan (will be confirmed)	INBW	50
➢ Malaysia	3.	Kuala Perlis	MYKP	50
➢ Myanmar	4.	Myeik	MMMK	50
➢ Thailand	5.	Ranong	THRG	50
	6.	Phuket	THPT	50
South China Sea and Gulf of Thailand Sub-Region				
➢ Brunei	1.	Brunei Darussalam	BRBD	50
➢ Cambodia	2.	Sihanokville – GN (will be confirmed)	CBSV	50
	3.	Koh Kung – GN (will be confirmed)	CBKK	50
	4.	Kampot – GN (will be confirmed)	CBKT	50
➢ Indonesia	5.	Pemangkat	INPT	50
➢ Malaysia	6.	Tok Bali	MYTB	50
	7.	Kota Kinabalu	MYKK	50
	8.	Tawau	MYTU	50
➢ the Philippines	9.	Masinloc (Zambales) – PS	PHMC	50
	10.	Sta. Cruz (Zambales) – PS	PHSC	50
	11.	Puerto Princesa (Palawan) – PS and RN	PHPP	50
	12.	Antique - GN, PS and RN	PHAT	50
	13.	General Santos City – PS and RN	PHGS	50
➢ Thailand	14.	Trat	THTR	50
	15.	Songkhla	THSK	50
	16.	Pattani	THPT	50
➢ Viet Nam	17.	Nghe An – GN and PS (will be confirmed)	VTNA	50
	18.	Danang – GN and PS (will be confirmed)	VTDG	50
	19.	Vung Tau - GN	VTVT	50
	20.	Tien Giang - PS (will be confirmed)	VTTG	50
	21.	Kien Giang (GoT) – PS (will be confirmed)	VTKG	50

Note: Budget proposed base on 20 sampling sites only.

4.0 Schedule and timeline:

Year		2015						2016												2017					
Quarter		Q3			Q4			Q1			Q2			Q3			Q4			Q1			Q2		
Month		7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
1.	Literature review and project preparation																								
2.	Sampling																								
3.	Laboratory work																								
4.	Data analysis																								
5.	Prepare report																								

5.0 Budget schedule:

No	Particulars	Unit	Qty	Unit Cost (USD)	Total Cost (USD)
1.	One Contract staff	month	24	520	12,480
2.	Sampling	unit	1000		15,600
3.	Chemicals, extraction and PCR and sequencing	/samples	1000	35	35,000
4.	Workshop prepare final report				3,000
5.	Misc. materials				1,500
				Total	**67,580**

Note: This budget only for samples collection and DNA analysis for 20 sampling sites with 50 samples for each sampling sites.

Expected Output

This study will be identified the stock structure of Longtail tuna in the Southeast Asian region. The stock structure of this species is very essential to this species resources management. This will help determine to what extent, if any, the population of Longtail tuna from this area is connected to population elsewhere in the world.

Reference

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Proposed budget sampling tuna

The South China Sea	No. of sites	No of samples	x 8 USD	Chemicals and etc.	Shipping	DSA (DSAxPxD)	Accommodation (Hotel*P*N*site)	Transportation	Total in USD
Brunei	1	50	400	40	70	210	0	50	770
Cambodia	2	50	400	80	140	420	400	100	1540
Indonesia	1	50	400	40	70	210	200	50	970
Malaysia	3	50	400	120	210	630	600	150	2110
Philippines	3	50	400	120	210	630	600	150	2110
Thailand	3	50	400	120	210	630	600	150	2110
Viet Nam	2	50	400	80	140	420	400	100	1540
Sub Total	15	350	2800	600	1050	3150	2800	750	11150
Andaman Sea									
Indonesia	1	50	400	40	70	210	200	50	970
Malaysia	1	50	400	40	70	210	200	50	970
Myanmar	1	50	400	40	70	210	200	50	970
Thailand	2	50	400	80	140	420	400	100	1540
Sub Total	5	200	1600	200	350	1050	1000	250	4450
								TOTAL	**15600**

No	Particulars	Qty	Unit Cost (USD)	Total Cost (USD)
1	One Contract staff	24	520	12,480
2	Chemicals, extraction and PCR and sequencing	1000	35	35,000
3	Workshop prepare final report			3,000
4	Misc. materials and op. expenses			1,500
		Total		51,980