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Population structure of longtail tuna (*Thunnus tonggol*) within and across Indonesia's fisheries management areas (FMAs) and neighboring countries based on mitochondrial control region

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ABSTRACT

Longtail tuna (*Thunnus tonggol*), one of the neritic tuna species commonly found in the coastal areas of the Indo-Pacific region, is known to be in an overfishing state in certain areas, including in Indonesia. Understanding the condition of the *T. tonggol* population within Indonesia is very important in managing its fisheries policy. This research aims to understand the population genetic structure of *T. tonggol* across Indonesia's Fisheries Management Areas (FMAs), as well as to investigate the phylogeography of *T. tonggol* within the Indo-Pacific region. A total of 586 samples were collected from 18 locations within Indonesia's six FMAs, and two locations from Malaysian waters. A 520 bp portion of mtDNA control region was amplified and sequenced. Data analysis was conducted using 586 total sequences generated from this study, and 336 sequences retrieved from GenBank, as well as provided by the author of previous study. Population structure analysis indicated a panmictic population of *T. tonggol* within six Indonesian FMAs and within the neighboring countries (Malaysia, Andaman Sea, Vietnam, Philippines, and India), with an indication of population expansion. These data do, however, reveal two potentially distinctive clades, one showing an affinity among samples from certain parts of Indonesia (East Aceh, West Aceh, Medan - FMA571 & FMA572) and India. Based on this finding, the *T. tonggo*l fisheries should be managed as a single management unit both within Indonesia's FMA and within neighboring countries, while also emphasizing localized genetic diversity to protect the sustainability of the species and its fishery.

1. Introduction

Tunas and tuna-like species can be categorized into three groups, i.e. oceanic tuna, neritic tuna, and tuna-like species (Collette et al., 2019). Oceanic tuna are known as the most economically valuable fishes, but neritic tunas are also important for fisheries commodities in providing food for domestic consumption, and bringing high economic revenues for many countries, particularly in Southeast Asia (Hidayat and Noegroho, 2018; SEAFDEC, 2022). *Thunnus tonggol* (Bleeker 1851), longtail tuna, is a neritic species that is commonly found in the coastal areas of the Indo-Pacific region between 47°N and 33°S (Froese and Pauly, 2011). In different parts of the Indo-Pacific region, this species is referred to by many different names, including *tongkol abu – abu*, *tongkol hitam*, or *tongkol aya* (Restianingsih and Hidayat, 2018; Kasim et al.,

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2020). Although described as the second smallest *Thunnus* species, it is the largest growing species among neritic tuna. Longtail tuna has not been considered for industrial fisheries due to several reasons, including its distribution being restricted to coastal areas, its small size (often <60 cm fork length), and its behavior of not forming large, dense schools that would be conducive for capture in commercial quantities (Yesaki, 1994).

Despite not being suited for industrial fishing, *T. tonggol* is exploited as part of artisanal and small-scale commercial fisheries (Collette, 2001; Griffiths, 2010), and is also considered an important recreational fish (Griffiths et al., 2019). The report of the global capture of longtail tuna was 355,595.987 tons in 2021 (FAO, 2024). Meanwhile, current reports on Indonesia fisheries from 2017 to 2021 indicated an increasing catch rate for *T. tonggol* within Indonesian waters - with a production of 106.300 tons in 2017, and increasing in production to 208.179 tons in 2021 (FAO, 2024). This declining condition in Indonesia is also reflected in FAO data with the catch of *T. tonggol* decreasing from 291.264 tonnes to 237.124 tonnes from 2007 to 2016 from (FAO, 2018). Several stock assessment studies have been conducted to understand this situation, which has led to the conclusion that the *T. tonggol* stock has been subjected to overfishing, especially in the Indo-Pacific region (Abdussamad et al., 2012; Martin, Fu, 2017; Darvishi et al., 2018; Restianingsih and Hidayat, 2018).

Understanding the *T. tonggol* population within Indonesia is very important, especially because Indonesian fisheries are managed by the different Fisheries Management Areas (FMA)-also known as "*Wilayah Pengelolaan Perikanan*" (WPP). Indonesia fisheries management is divided into 11 areas, with each area having its own equally complex levels of governance, decentralization and traditional fishing tenure rights. Various data have been collected to analyze the management of Indonesian fisheries, including landing data collection, onboard observer programs, logbooks, e-logbooks, catch per unit effort (CPUE) standardization, regional fisheries management units, vessel tracking, and even genetic assessments (Jaya et al., 2022). Nevertheless, significant information is still needed, especially the basic population parameters, such as the number and distribution of stock, as well as population genetic diversity, which is essential to support resource recovery and to aid in delineating and monitoring populations for fishery management (Roldan et al., 2000; Kunal et al., 2014).

In recent studies conducted on *T. tonggol*, population genetics has been chosen as one of the reliable tools to understand its stock delineation and population dynamics (Hauser and Seeb, 2008). Highly polymorphic D-loop region of mitochondrial DNA (mtDNA) has been widely employed in population studies of various tuna species (Kumar and Kocour, 2015; Kunal et al., 2013; Menezes et al., 2012; Pertiwi et al., 2017). Prior studies on *T. tonggol* genetic stock population in the Indo-Pacific region have been done within India (Kunal et al., 2014), Malaysia (Kasim et al., 2020), South China Sea; including a part of Indonesia, Vietnam, and Philippines (Willette et al., 2016). Similar studies have also been done in Indonesia (Astarini et al., 2021; Malik et al., 2020; Ningsih et al., 2021), although limited to certain areas, i.e. Bali, Banyuwangi (Ningsih et al., 2021), Batam, Surabaya (Astarini et al., 2021), Semarang, Banjarmasin, Jakarta (Malik et al., 2020), revealing a population of high genetic diversity and no significant structure. Although a lack of population structure has been indicated within various study locations, it has also been shown that there could be significant partitioning across the wider region (Kasim et al., 2020).

Indonesia's water lies between the Indo-Pacific regions and acts as the hub between different oceanic basins, and the fisheries that Indonesia's water supports are managed with different factors in each of its FMAs. Therefore, this study aims to understand the population genetics of *T. tonggol* within Indonesian FMAs, as well as investigate the phylogeography of *T. tonggol* within the Indo-Pacific region, with the addition of sequence data from Vietnam and the Philippines (provided by the author of Willete et al., 2016), as well as data from India, Andaman Sea, Malaysia (all haplotype sequences retrieved from GenBank). This study contains information on *T. tonggol* populations from locations that have not been studied in the previous research in Indonesia, as well as the population's condition across FMAs. The output from this study should be beneficial for the conservation and fisheries management of this species within Indonesia and nearby countries.

2. Materials and Methods

2.1. Sample collections

A total of 586 longtail tuna samples were collected from port and fish markets around Indonesia (563 samples) and Malaysia (23 samples). Highlighting the Indonesian Fisheries Management Areas (FMA), sampling sites are shown in Fig. 1, with the number of samples from each FMA reported in Table 1. The number of samples collected within areas varied, depending on the availability of samples during collection efforts (January 2018-June 2019). Samples were collected in the form of fin clips and preserved in 96 % ethanol in 2.5 ml vials, before being transported to the laboratory for DNA extraction (Knebelsberger and Stoger, 2012). Interviews with local fishermen were conducted to confirm the catch location and determine the FMAs of the specimens collected, which were up to 50 miles offshore of each site.

2.2. Molecular analysis

Molecular analysis for samples collected around Indonesia was carried out at the Yayasan Biodiversitas Indonesia (Bionesia) Laboratory, Bali, Indonesia; meanwhile, samples collected from Malaysia were analyzed at the Faculty of Resources Science and Technology Laboratory, University Malaysia Sarawak. Genomic DNA was extracted using 10 % chelex extraction (Walsh et al., 1991). A portion of mitochondrial DNA (mtDNA) fragment of the control region (d-loop) locus was amplified using Polymerase Chain Reaction (PCR) methods, with the forward primer (CRK: 5′- AGC TCA GCG CCA GAG CGC CGG TCT TGT AAA - 3′) and reverse primer (CRE: 5'-CCT GAA GTA GGA ACC AGA TG - 3′) (Lee et al., 1995).

The PCR protocol and thermo-cycling profile were carried out with some modifications following the methods in Allen et al. (2017). Each PCR reaction was 25 μL in volume, consisting of a reagent solution containing 12.5 μL ddH2O, 2.5 μL 10x PCR buffer (PE-II), 2.5 μL dNTP, 2.0 μL $MgCl_2$ and 1.25 μL primer CRK - CRE and 0.125 μL of PE Amplitaq, and 3 μL of DNA template. Each microtube was vortexed for 30 seconds to homogenize the mixture. PCR thermo-cycles were as follows: initial denaturation at 94°C for 10 s, 38 cycles of 94°C for 15 s, 50°C for 30 s, 72°C for 45 s, with final extension of 72°C for 5 min. PCR products were visualized using 1 % gel agarose stained by Biotium® gel red stain. Successfully amplified products were sent to a DNA sequencing facility in Jakarta, Indonesia for sequencing using Big Dye Chain Termination process.

2.3. Data analysis

Sequences were edited and aligned using the CLUSTAL-W algorithm in MEGA X (Kumar et al., 2018). In order to confirm the samples as *T. tonggol*, sequence data were compared with NCBI (The National Center for Biotechnology Information; https://www.ncbi.nlm.nih.gov) data using BLAST (Basic Local Alignment Search Tools; https://blast.ncbi.nlm.nih.gov/). Then, genetic distance between samples was computed by p-distance model with Transitions + Transversions substitutions (Strimmer et al., 2009). A phylogenetic tree was constructed using Maximum Likelihood with 1000 bootstrap replicates in IQ-Tree version 2 (Minh et al., 2020). The tree was constructed using the TIM3+F+R4 model – the best nucleotide substitution model with the lowest BIC score (Bayesian Information Criterion) according to IQ-Tree analysis program (Minh et al., 2020). Additional sequence of *T. albacares* (KP299023.1) from GenBank was used as an outgroup. To confirm that