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Population Genetic of Kawakawa (*Euthynnus affinis*) in Sumatera Island

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ABSTRACT Over the past five decades, there has been a growing trend in the capture of kawakawa (*Euthynnus affinis*), which, alongside its potential to support small-scale commercial fisheries, highlights its significance.However, there is inadequate data on this species for suitable management and conservation status. This study aimed to investigate the genetic diversity, population structure, and connectivity of the kawakawa in the three major fishing port in Sumatra, namely Belawan , Padang, and Lampung by using DNA mitochondria control region (d-loop) Sequence. In total, 78 sequences of kawakawa with an average size of 303 bp, we found 36 polymorphic sites and 56 haplotypes from all population were identified with DNA compatibility values of 97-100%. We found the genetic diversity values in Sumatera Island were high (h = 0.950; π = 0.034), with the highest genetic diversities value in Padang (h = 0.990; π = 0.082) and the lowest in Belawan (h = 0.929; π = 0.082). AMOVA and F_{st} analyses revealed no differentiation in each population (F_{st} = 0.005). The haplotype distribution and connectivity analyses showed genetic mixing among the three populations. This study showed a single stock at the study sites and suggests management measures at a regional level to maintain the population.

Keywords: Commercial fisheries; population structure; D-loop

INTRODUCTION

Tongkol komo (*Euthynnus affinis*) (Cantor, 1849) is known in international trade as kawakawa or with the FAO code KAW (Hidayat *et al*., 2019). Kawakawa lives in a migratory manner in the epipelagic-neritic zone (Santos *et al*., 2010). This species is widely distributed in tropical and subtropical open waters (oceanic) or near coastlines (neritic) with water temperatures ranging from 18-29 °C (Collette & Nauen, 1983). Kawakawa is known for its epipelagic migratory behavior, where it migrates or swims long distances in search of food and spawning grounds (Menezes, *et al*., 2012). It belongs to the group of small tunas and has distinctive characteristics compared to other tuna species, such as oblique black lines curving above the lateral line and 1-4 black dots near the pectoral fin (Hidayat *et al*., 2018). This fish is an opportunistic predator, feeding on squid, crustaceans, mollusks, and zooplankton (Collette, 2001).

Kawakawa has high economic value in Indonesia (Amri *et al*., 2018). Kawakawa contributes to small-scale commercial fisheries in various countries bordering the Indian Ocean, such as India, Iran, Pakistan, and Sri Lanka. Based on data recorded by the Indian Ocean Tuna Commission (IOTC, 2020) kawakawa caught in the Indian Ocean is exploited using various fishing gear, including i.e gillnets longlines, pole-and-line, purse seine (coastal purse seine, and ring net), and other fishing gear such as baitboats, trawl, lift nets, and driftnets.

The capture trend of kawakawa in Indian Ocean has been increasing since 1950-2019. Indonesia is the top country in kawakawa catch since 2014 with avarege catch each year 40.000 metric ton (IOTC, 2023), followed by India, Iran, and Malaysia (IOTC, 2020). Although the results of stock assessments conducted by IOTC annually in the past five years indicate that the kawakawa fishery is still categorized as sustainable (IOTC, 2020), there is a possibility of the stock becoming overfished and overfishing due to the increasing annual catches in the Indian Ocean. The average catch from 2014 to 2018 was 152.919 tons in the Indian Ocean. This study aims to assess the patterns of genetic population diversity and phylogenetics of several kawakawa populations in the waters of Sumatra Island. Information related to population structure in an exploited species is essential for conservation and sustainable utilization purposes. Genetic information on kawakawa in Indonesia is relatively limited, while in other countries, it is better documented, such as Malaysia, India, the Philippines, Taiwan, and Australia (Chiou & Lee 2004; Robertson *et al*., 2007; Santos *et al*. 2010; Masazurah *et al*., 2012; Kumar *et al*., 2016).

MATERIALS AND METHODS

Materials

Cryogenic vials 2ml (Cat. No: 81-8204-Biologix) used for sample storage and extraction, Blue tips (Cat. No: 20-1000-Biologix) used to take up large amount of solutions up to 1000 µL, white tips (Cat. No: 21-0010-Biologix) used to take up small amount of solutions up to 10 µL, Yellow tips (Cat. No: 21-0200- Biologix) used to take up moderate amount of solutions up to 200 µL, MasterMix (My Taq TM HS Red Mix Bioline) used as pre re-prepared mixture containing all the components needed for the PCR reaction, except for the template DNA, primers, and water, D-loop Primer sequences of kawakawa (GeneBank accession no.NC005318) 5'-CCGGACGTCGGAGGTTAAAAT-3'(forward) and 5'-AGGAACCAAATGCCAGGAATA-3'

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(reverse) (Genetika sciences) play a crucial rose in initiating DNA amplification, sybergreen (Qiagen) used as a fluorescent dye that binds to double-stranded DNA (dsDNA) molecules allowing it to be used as a detection method for monitoring the amplification of the target DNA sequence in real-time, Loadingdye (Qiagen) used to helps increasing density of the sample without mixing with the buffer., Agarose (Cat. No: PC0701-Vivantis) used as a matrix for separating DNA by size through electrophoresis, TAE Buffer 10X (Cat. No: V4271-Promega) used for buffer solution in agarose gel, Thermocycler gradien (Biorad) used to multiple annealing temperatures simultaneously across a range of temperature gradients, 96-well polypropylene deep well plate (S030754935-Corning), The samples used in this study was muscle tissue around the caudal fork excute using strile knife (Marwayana, 2015).

Methods

Study sites

This research was conducted from October 2019 to July 2021. Sample collection took place at several Fish Landing Sites in the waters of Sumatra Island, namely PP Belawan in North Sumatra, TPI Padang in West Sumatra, and TPI Lempasing in Lampung (Figure 1). Molecular analysis was carried out at the Oceanogen Laboratory in Bogor.

Sample collection

A total of 78 samples of muscle tissue around the caudal peduncle (Marwayana, 2015) of kawakawa, each measuring 0.5 cm^3, were taken and preserved in sample bottles containing 96% ethanol. The sample size consisted of 26 individuals from each research location. The technical procedure for muscle tissue sampling was based on a genetic sampling protocol that had been implemented through collaborative cooperation between CSIRO (Australia), AZTI Tecnalia (Spain), IRD (France), and LRPT (Indonesia).

Extraction, Amplification, and DNA Sequencing

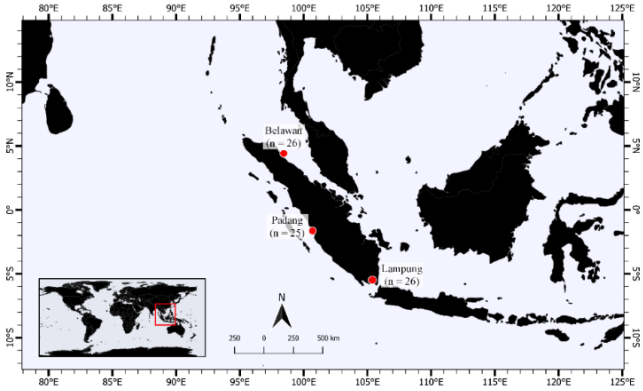

Figure 1. The map shows the locations of kawakawa sample collection at Pelabuhan Perikanan Belawan in North Sumatra, Fish Landing Site Padang in West Sumatra, and Fish Landing Site Lempasing in Lampung.
Description: The letter "n" in the research point indicates the number of samples taken.

The DNA fragments were extracted following the Chelex 10% DNA extraction method (Walsh *et al.*, 2013). The DNA amplification, also known as polymerase chain reaction (PCR), targeted the half portion of the hypervariable region of the mitochondrial control region (mtDNA D-loop) using the first half of the control region (D-loop) forward primer (5' – CCG GAC GTC GG A GGT TAA AAT – 3') and the reverse primer (5' – AGG AAC CAA ATG CCA GGA ATA) (Menezes *et al.*, 2006). Each PCR reaction in one tube had a total volume of 25 µl. The components of each reaction included 3 µl of DNA template, 12.5 µl of MasterMix (My TaqTM HS Red Mix Bioline), 7.5 µl of ddH2O, 1 µl of the forward primer, and 1 µl of the reverse primer.

The PCR profile consisted of the following steps: 90 °C for 5 minutes (denaturation), 50 °C for 1 minute (annealing), 72 °C for 1 minute (extension), and 72 °C for 5 minutes (final extension). The PCR process was repeated for 35 cycles (Menezes *et al.*, 2006). The resulting PCR products (amplikon) were visualized using agarose gel electrophoresis with 1.5% agarose gel and ethidium bromide. Electrophoresis was carried out for 15 minutes at 200 volts and 400 mA current. The presence of DNA bands was observed using a UV transilluminator. Positive DNA results were sent for sequencing using the Sanger sequencing method (Sanger *et al.*, 1977) with BigDye Terminator v3.1 and the Applied Biosystems 3730 protocol by 1st BASE Malaysia.

Data analysis

A total of 77 mitochondrial DNA D-loop fragments were analyzed using the ClustalW (2.1) model (Larkin *et al.*, 2007) and species confirmation was conducted using the Basic Local Alignment Tools (BLAST) database available in the MEGA X program (Molecular Evolutionary Genetics Analysis) (Kumar *et al.*, 2018). The phylogenetic tree was constructed using the Neighbor-joining method with the Maximum Composite Likelihood model and 1000x bootstraps. Genetic diversity parameters, including the number of haplotypes (Hn), haplotype diversity (h), and nucleotide diversity (π), were analyzed using the DnaSP (DNA Sequence Polymorphism) 6.0 software (Rozas *et al.*, 2017) to estimate the number of polymorphic sites and the number of unique haplotypes (nh) (Menezes, *et al.*, 2012).

Genetic diversity analysis, haplotype diversity (h), and nucleotide diversity (Masatoshi, 1973), as well as population structure, were measured as the basis for assessing the level of genetic diversity in the mitochondrial DNA control region sequences. Population genetic structure was evaluated using the Arlequin 3.5.2.2 program (Excoffier & Lischer, 2010). The analysis of differences in genetic distances between populations was performed using the fixation index (Fst) (Excoffier *et al.*, 1992). Genetic distances within and between populations were analyzed based on distance parameters (Nei, 1973). The distribution of haplotypes and the tree among sequences was visualized using the Network 10 application (https://www.fluxus-engineering.com) (Bandelt *et al.*, 1999)

RESULTS AND DISCUSSION

Figure 2. Personal documentation of photos of kawakawata samples that were landed at TPI Belawan.

The query-based confirmation findings encompass the level of similarity (Hosein *et al*., 2017). The 78 samples were identified as kawakawa, except for one sample that was identified as Auxis thazard (frigate tuna). (PDG15). This misidentification occurred due to visual errors during the sample search process in the field. Each individual kawakawa was analyzed using phylogenetic tree reconstruction, with frigate tuna/*Katsuwonus pelamis* as the outgroup (Figure 3), revealing 9 different clades. The genetic analysis of the 77 D-loop gene sequences showed a fragment DNA length of 303 base pairs (bp) from three sampling locations (Belawan, Padang, and Lampung).

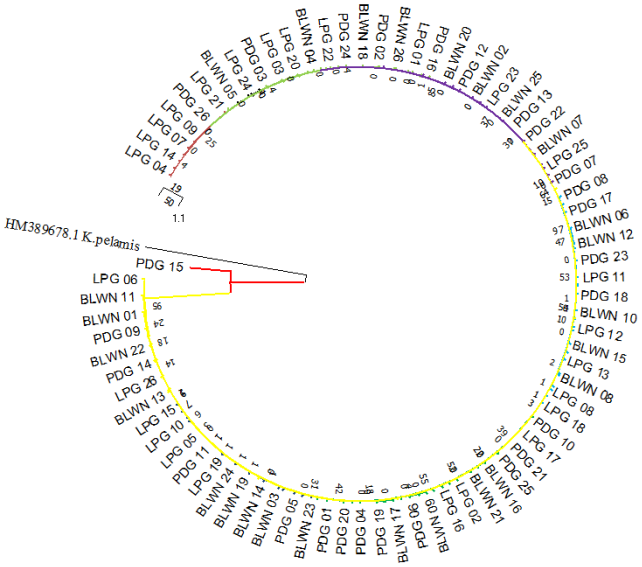

Figure 3. The phylogenetic tree reconstruction was performed using the Maximum Composite Likelihood method with 1000x bootstraps on 77 kawakawa fish samples, with *Katsuwonus pelamis* as the outgroup.

Table 2. Genetic diversity of kawakawa fish, number of samples (n), number of haplotypes (Hn), genetic diversity (h), and nucleotide diversity (π) at 3 research locations.

Locations	n	Genetic diversity		
		H_n	h	
Belawan	26	15	0.929±0.031	0.082±0.049
Padang	25	19	0.990±0.029	0.082±0.049
Lampung	26	22	0.963±0.027	0.080±0.048
All locations	77	46	0.950±0.016	0.034±0.020

The genetic diversity values of kawakawa populations based on haplotype (h) and nucleotide (π) diversity are uniform and high (Table 2). The genetic diversity of kawakawa populations in Lampung (h = 0.963; π = 0.080) has the highest values, followed by Padang population (h = 0.990; π = 0.082), and Belawan population (h = 0.929; π = 0.082). Overall, the genetic diversity value of kawakawa populations in the waters of Sumatra is h = 0.950; π = 0.034. Research conducted in India by Kumar *et al*. (2012) on kawakawa fish showed lower genetic and nucleotide diversity values compared to those in Indonesia, which are 0.040-0.262 and 0.080-0.697, respectively.

Differences in genetic diversity values may be attributed to several factors such as the number of samples (Masatoshi, 1987), spawning patterns, random drift leading to genetic drift, distribution, and migration (Hoban *et al*., 2021). Furthermore, Madduppa *et al*. (2021) explained that the genetic diversity value of a population is influenced by the number of haplotypes (Hn) present. A higher number of haplotypes correlates with higher genetic diversity. High haplotype diversity combined with low nucleotide diversity suggests a large population size that has recently expanded, allowing the preservation of new alleles within the population but not enough time for additional nucleotide changes to accumulate across haplotypes (Chen *et al*., 2014; Delrieu-Trottin *et al*., 2017; Kasim *et al*., 2020).

The migration of kawakawa fish also contributes to the high genetic diversity value by allowing encounters with other populations and increasing the likelihood of interbreeding between different populations. High genetic diversity values can play a role in reducing the risk of inbreeding in a population (Frankham, 2005)and providing opportunities for organisms living in their natural habitat to adapt to potential environmental changes (Wernberg *et al*., 2018).

Population structure

The results of the AMOVA analysis for populations in Sumatra are presented in Table 4, and the interpopulation F_{st} values in Table 3 indicate no significant differences among the kawakawa populations in the waters of Sumatra, with F_{st} values ranging from 0.001 to 0.004. Fakhri *et al*. (2015) stated that F_{st} values ≥0 and <0.5 indicate no

Table 3. The results of AMOVA for degrees of freedom (df), diversity (%), F_{st} values, and significance level (p) within and among kawakawa populations in Sumatra.

Variance	db	Diversity (%)	F_{st}	P-value
Indonesia				
Inter population	2	0.49	0.005	0.294 ± 0.013
Intra population	74	99.51		
Total	76			

significant differences, while F_{st} values >0.5 and ≤1 are considered to have significant differences. These results suggest that there are no subpopulations within the kawakawa population in the waters of Sumatra.

This indication of a single stock is consistent with the findings of Kumar *et al.* (2012[a]) and Kumar *et al.* (2012[b]), who revealed a single stock of kawakawa population throughout the waters of India. The close geographic proximity allows kawakawa populations to mix during migration, increasing the likelihood of interpopulation reproductive processes in that area. These findings provide important initial evidence that kawakawa in the waters of Sumatra is still genetically connected. Similar results have been reported in studies of kawakawa population structure in Taiwan by Chiou & Lee (2004) and Filipina by Santos *et al.* (2010).

The high mobility of kawakawa during migration among these three populations can lead to gene flow. Kawakawa is widely distributed in open tropical and subtropical waters (oceanic) or near coastlines (neritic) with water temperatures ranging from 18-29°C (Collette & Nauen, 1983). As a member of the genus Euthynnus, which is widely distributed in tropical and subtropical waters, kawakawa is commonly found in the Indian Ocean, Central Pacific, and Western Pacific (Ollé *et al.*, 2021).

Table 4. The F_{st} values using the pairwise distance method (lower diagonal) and the significance level (p) values (upper diagonal) for kawakawa populations in Belawan, Padang, and Aceh are as follows.

Location	Belawan	Lampung	Padang
Belawan	-	0.315	0.459
Lampung	0.003	-	0.279
Padang	0.001	0.004	-

Population connectivity

The analysis of the distribution of 56 haplotypes from a total of 77 kawakawa samples shows connections among each kawakawa haplotype (Figure 4). The haplotypes are distributed among all individuals, making it impossible to group (clade) them based on different geographical locations. There are several connected haplotypes and others that are separate. The highest connectivity among kawakawa haplotypes is observed in haplotype 4 in Figure 4, consisting of 12 samples, indicating the mixing of individuals from all three research populations. Haplotype 4 comprises 5 individuals from the Belawan population (42.66%), 4 individuals from the Lampung population (33.33%), and 3 individuals from the Padang population (25%). Haplotype 3, on the other hand, consists of 6 samples with 4 individuals from the Belawan population and one individual each from the

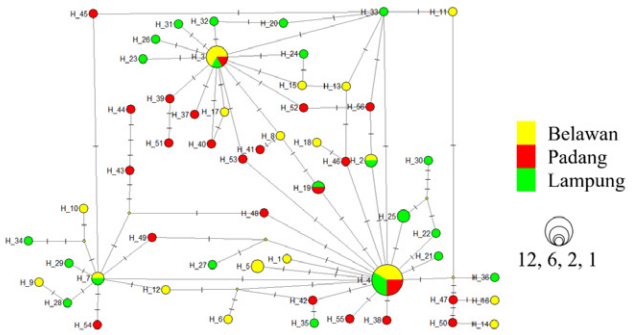

Figure 4. Haplotype connectivity of kawakawa in the Sumatra waters.

Lampung and Padang populations.

Figure 5 illustrates the distribution of 55 haplotypes across all research locations. The Belawan population is dominated by haplotypes 3 (19%) and 4 (15%), while Padang and Lampung are dominated by haplotype 4 (12%) with only a few haplotypes 3 (4%). Haplotype 1 is found only in the Belawan population. The distribution of these haplotypes can be influenced by several factors. Firstly, it is related to the migratory nature of kawakawa species, as kawakawa is one of the pelagic fish species that migrates across oceans. The dispersal of pelagic fish larvae is influenced by ocean currents during their release into the water column (Adams *et al.*, 2019). Haplotype and nucleotide diversity are essential indicators of genetic variation, and the variation within groups is influenced by the magnitude of these indicators (Zhang *et al.*, 2020). Kawakawa is known for its epipelagic migratory characteristics, migrating or swimming long distances in search of food and spawning (Kumar *et al.*, 2012).

The values of genetic diversity parameters reveal that kawakawa in Sumatra, Indonesia form a mixed population pattern, as evidenced by the wide species distribution range and low, non-significant F_{st} values across all investigated populations. Genetic analysis of kawakawa samples from three locations indicates the presence of a single stock between Belawan, Padang, and Lampung. The observed population diversity of kawakawa in the waters of Sumatra highlights the importance of developing better participatory management strategies among institutions in Indonesia, especially in Sumatra and at the regional level. A significant amount of work is needed to manage fisheries resources to preserve and restore genetic stocks for sustainable harvesting. Molecular analysis, combined with other methods, can provide a valid assessment for effective harvesting strategies (Toro *et al.*, 2003).

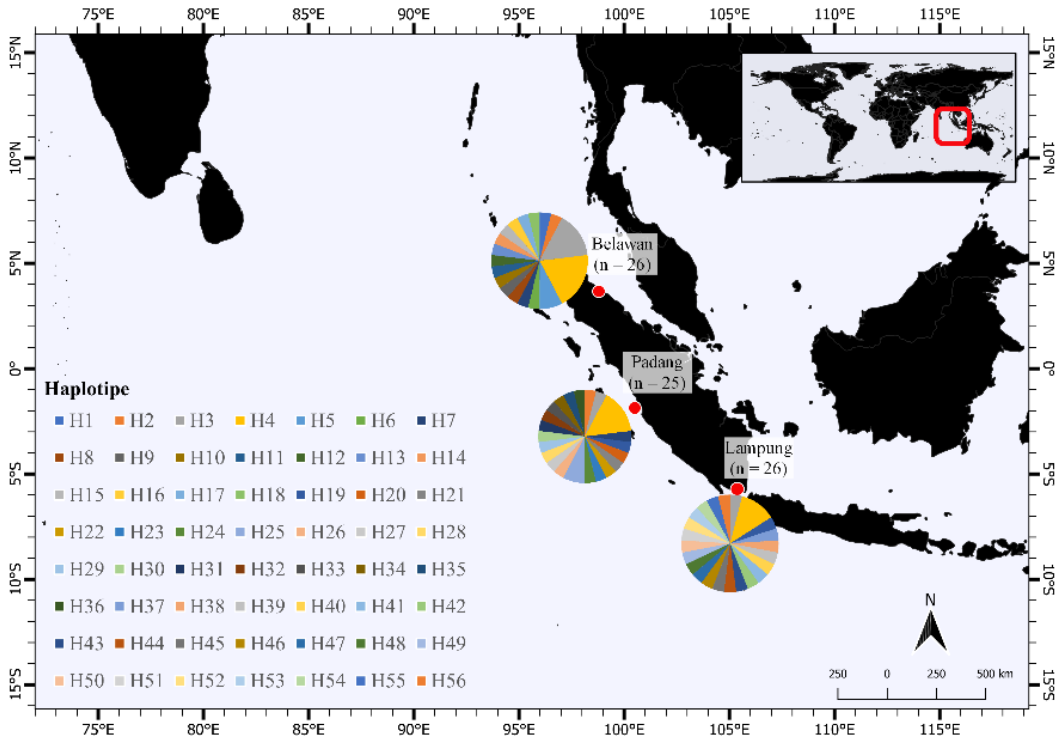

Figure 5. Map of the distribution of kawakawa population haplotypes in the waters of Sumatra Island.

CONCLUSION AND RECOMMENDATION

Conclusion

The analysis of genetic diversity and population structure of kawakawa fish in the waters of Sumatra, Indonesia, does not show any detected population structure based on haplotype composition, indicating genetic connectivity and relatedness. Stock-based management and collaborative cross-regional management are needed to support tuna fish management efforts in Indonesia.

Recomendation

Future research on the population genetics of kawakawa (*Euthynnus affinis*) in Sumatera should expand the sample size and geographic coverage, incorporate additional genetic markers like SNPs and mitochondrial DNA, and include temporal sampling to assess genetic changes over time. Collaborating with other research groups and integrating genetic data into conservation and management plans are essential for the sustainable management of kawakawa populations

AUTHORS' CONTRIBUTION

RRZ is doing research idea, write manuscript and translation, data & sample collection, data analysis; HHM is doing guidance contribution, data analysis; NPZ is guidance contribution; BS is doing guidance contribution; LMIS is doing data analysis.

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