

**DNA BARCODING OF THE FAMILY
SCOMBRIDAE, MORPHOMETRICS AND
GENETIC DIVERSITY OF KAWAKAWA
Euthynnus affinis (CANTOR, 1849) FROM
MALAYSIAN WATERS INFERRED BY
MITOCHONDRIAL (MtDNA) GENES**

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by

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LIST OF ABBREVIATIONS

AMOVA	Analysis of molecular variance
BLAST	Basic local alignment search tool
BOLD	Barcode of life database
bp	Base pair
CA	Cluster analysis
COI	Cytochrome oxidase subunit I
CTAB	Cetyl trimethyl ammonium bromide
CVA	Canonical variate analysis
Cyt <i>b</i>	Cytochrome <i>b</i>
DFA	Discriminant function analysis
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
FAO	Food and Agriculture Organization
GM	Geometric morphometric
Hd	Haplotype diversity
ITIS	Integrated taxonomic information system
K2P	Kimura 2-parameter
MgCl ₂	Magnesium chloride
MSN	Minimum spanning network
mt	Metric tons
mt DNA	Mitochondrial DNA
NCBI	National center for biotechnology information
NJ	Neighbor Joining
PCA	Principal component analysis

PCR	Polymerase chain reaction
SPSS	Statistical Package for the Social Sciences
π	Nucleotide diversity

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- Appendix A The measurement data for specimens were collected from four marine regions: the South China Sea, the Straits of Malacca, the Sulu Sea, and the Celebes Sea.
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- Appendix C Selected photos of DNA extraction (gel electrophoresis) from the South China Sea (SCS) and the Strait of Malacca (M).

**PEMBARKODAN FAMILI SCOMBRIDAE, MORFOMETRIK DAN
KEPELBAGAIAN GENETIK KAWAKAWA *Euthynnus affinis* (CANTOR,
1849) DI PERAIRAN MALAYSIA BERDASARKAN GEN DNA
MITOKONDRION (MtDNA)**

ABSTRAK

Kawakawa (*Euthynnus affinis*) adalah tuna epipelagik yang tersebar luas, dianggap antara spesies yang paling signifikan secara ekonomi di perairan subtropika dan tropika rantau Indo-Pasifik. Walau bagaimanapun, kekurangan maklumat secara komprehensif terhadap struktur stok, pengurusan, dan pemuliharaan populasi *E. affinis* di Malaysia menimbulkan kebimbangan tentang potensi tangkapan secara berlebihan dan penyusutan bekalan ikan laut yang penting ini. Bagi memastikan pengurusan spesies yang berkesan dan berjaya, adalah penting untuk mengkaji struktur stoknya. Kajian ini menggunakan kedua-dua pendekatan genetik dan morfometrik untuk menilai pembezaan molekul dan morfometrik spesimen *E. affinis* yang dikumpulkan dari empat kawasan marin: Laut China Selatan (SCS), Selat Melaka (M), Laut Sulu (SS), dan Laut Sulawesi (CS). Dalam langkah pertama kajian ini, analisis multivariat seperti Analisis Komponen Utama (PCA), Analisis Fungsi Diskriminasi (DFA), dan Analisis Kluster (CA) telah dijalankan untuk membezakan sejumlah 398 sampel *E. affinis* berdasarkan 12 ukuran morfometrik. Satu Analisis Komponen Utama (PCA) telah dilakukan dan menghasilkan 12 komponen dengan nilai eigen yang lebih besar daripada 0.3. Komponen pertama (PC1) sahaja menerangkan 81.627% variasi bentuk badan dan dapat membezakan antara sampel-sampel *E. affinis* dengan berkesan. Hasil kajian menunjukkan bahawa sampel-sampel *E. affinis* dari Laut China Selatan (SCS), Selat Melaka (M), Laut Sulu (SS), dan Laut Sulawesi (CS) mempamerkan ciri-ciri

morfologi yang serupa. Berdasarkan jarak Mahalanobis, analisis kluster hierarki menunjukkan bahawa empat kawasan marin utama *E. affinis* dikelompokkan ke dalam tiga kluster yang berbeza. Dalam langkah kajian yang seterusnya, kaedah penandaan bar DNA (DNA barcoding) telah digunakan berdasarkan kawasan standard mitokondria sitokrom oksidase I (COI) (632 bp) untuk mengkaji penandaan bar DNA spesies Scombridae di Malaysia. Analisis mendedahkan purata perbezaan 3X dalam jarak genetik antara dan dalam spesies bagi semua taksa. Analisis pokok Neighbor-Joining (NJ) menunjukkan bahawa semua jenera dikelompokkan mengikut suku masing-masing, iaitu Thunnini, Sardini, Scombrini, dan Scomberomorini. Langkah terakhir kajian ini melibatkan penilaian kepelbagaian genetik dan struktur populasi *E. affinis* di perairan Malaysia menggunakan gen mitokondria sitokrom oksidase I (COI) dan sitokrom b (Cyt *b*). Satu fragmen 632 bp bagi gen COI dan fragmen 522 bp bagi gen Cyt *b* telah disusun, termasuk 372 spesimen *E. affinis* untuk gen COI dan 396 spesimen untuk gen Cyt *b*. Hasil kajian mendedahkan bahawa perbezaan genetik antara empat kawasan *E. affinis* di perairan Malaysia adalah dari tahap rendah hingga tinggi. Purata kepelbagaian haplotip (Hd) dan kepelbagaian nukleotida (π) untuk COI dikira masing-masing sebanyak 0.7577 dan 0.0075. Sebaliknya, purata Hd dan π untuk Cyt *b* didapati masing-masing sebanyak 0.6699 dan 0.0181. Analisis pokok Neighbor-Joining (NJ) dan Rangkaian Minimum Spanning (MSN) mendedahkan bahawa sampel-sampel *E. affinis* yang dikumpulkan dari empat kawasan marin di perairan Malaysia dikelompokkan ke dalam satu klad tunggal. Analisis Varians Molekul (AMOVA) menunjukkan struktur genetik yang signifikan antara empat kawasan marin *E. affinis*, seperti yang ditunjukkan oleh COI: $F_{ST} = 0.5164$ ($P < 0.05$) dan Cyt *b*: $F_{ST} = 0.9218$ ($P < 0.05$). Kajian ini menggunakan gabungan data morfometrik dan

molekul, yang telah terbukti berkesan dalam pengurusan dan pemuliharaan spesies yang penting dari segi komersial di Malaysia.

**DNA BARCODING OF THE FAMILY SCOMBRIDAE, MORPHOMETRICS
AND GENETIC DIVERSITY OF KAWAKAWA *Euthynnus affinis* (CANTOR,
1849) FROM MALAYSIAN WATERS INFERRED BY MITOCHONDRIAL
(MtDNA) GENES**

ABSTRACT

Kawakawa (*Euthynnus affinis*) is a widely distributed epipelagic tuna, considered among the most economically significant species in the subtropical and tropical waters of the Indo-Pacific region. However, the lack of comprehensive information on the stock structure, management, and conservation of *E. affinis* in Malaysian waters raises concerns of potential overfishing and depletion of this important marine fish. To ensure effective and successful species management, it is imperative to study its stock structure. This study employed both genetic and morphometric approaches to assess the molecular and morphometric differentiation of *E. affinis* specimens collected from four marine regions: the South China Sea (SCS), Straits of Malacca (M), Sulu Sea (SS), and Celebes Sea (CS). In the first step of this study, multivariate analyses, such as Principal component analyses (PCA), discriminant function analyses (DFA), and cluster analyses (CA) were conducted to distinguish a total of 398 samples of *E. affinis* based on 12 morphometric measurements. A principal component analysis (PCA) was performed and resulted in 12 components with eigenvalues greater than 0.3. The first component (PC1) alone explained 81.627% of the variation in body shape and effectively distinguished between *E. affinis* samples. The results showed that *E. affinis* samples from the South China Sea (SCS), the Straits of Malacca (M), the Sulu Sea (SS), and the Celebes Sea (CS) exhibited similar morphological characteristics. Based on Mahalanobis distances,

hierarchical cluster analyses revealed that the four main marine regions of *E. affinis* are grouped into three distinct clusters. In the next step of the study, the DNA barcoding method were utilised based on the standard region of the mitochondrial cytochrome oxidase I (COI) (632bp) to examine the DNA barcoding of the Malaysian Scombridae species. The analysis revealed an average of 3X differences in the mean inter and intra-specific genetic distance among all taxa. The Neighbor-Joining (NJ) tree analysis showed that all genera clustered according to their respective tribes, namely Thunnini, Sardini, Scombrini, and Scomberomorini. The final step of this study involved evaluating the genetic diversity and population structure of *E. affinis* in Malaysian waters using mitochondrial DNA cytochrome oxidase I (COI) and cytochrome b (Cyt *b*) genes. A 632 bp fragment for the COI gene and a 522 bp fragment for the Cyt *b* gene were sequenced, including 372 specimens of *E. affinis* for the COI gene and 396 specimens for the Cyt *b* gene. The results revealed that genetic divergence among the four regions of *E. affinis* from Malaysian waters varied from low to high. The average haplotype diversity (Hd) and nucleotide diversity (π) for COI were calculated to be 0.7577 and 0.0075, respectively. On the other hand, the average haplotype diversity (Hd) and nucleotide diversity (π) for Cyt *b* were found to be 0.6699 and 0.0181, respectively. The analysis of the Neighbor-Joining (NJ) tree and the Minimum Spanning Network (MSN) revealed that the samples of *E. affinis* collected from four marine regions in Malaysian waters were clustered into a single clade. Analysis of molecular variance (AMOVA) showed a significant genetic structure among the four marine regions of *E. affinis*, as indicated by the COI: $F_{ST} = 0.5164$ ($P < 0.05$) and Cyt *b*: $F_{ST} = 0.9218$ ($P < 0.05$). The current study utilised a combination of morphometric and molecular data, which has proved to be valuable for the effective

management and conservation of this commercially significant species in Malaysian waters.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Globally, there are 37,109 recognised fish species, encompassing over 11,000 genera and subgenera (Fricke *et al.*, 2025). Fish have existed for a long time, more than 500 million years ago, and have evolved into a wide variety of forms. Their diversity in shape, living environments, behavior and physiology, both today and in the past, makes understanding their evolutionary history and classification quite challenging but also interesting. From the hagfishes and lampreys to sharks, lungfishes, flatfishes, and other types of fish, there are many different adaptations that have allowed them to thrive in practically every aquatic environment on Earth (Hubbs & Nelson., 2016; Wang *et al.*, 2018; Marchetti *et al.*, 2020). In Malaysia, there is a wide variety of marine fish present at major landing areas, with around 200-300 different species. Fish market typically offers 50-100 different types of fish daily. Some fish species may only be available during certain seasons, while others can be found year-round in estuaries, bays, and reefs (Bakar *et al.*, 2018).

Malaysia is a country comprising of two regions: the states of Sabah and Sarawak located in the western portion of Borneo Island, and Peninsular Malaysia, which is located in the south-eastern tip of mainland Asia. Peninsular Malaysia is surrounded by the Strait of Malacca on the west, and the South China Sea on the east. Sabah is enclosed by the South China Sea, the Sulu Sea, and the Celebes Sea, whereas Sarawak is surrounded by the South China Sea. Due to its location and resources, Malaysia has the potential to become a leading fishing country in Southeast Asia, and its marine fishing industry is poised to experience significant growth in the future

(Imtiaz *et al.*, 2016). Fish are not only of economic value, but also provide a valuable source of animal protein for humans and play an important role in biodiversity (Bingpeng *et al.*, 2018; Marchetti *et al.*, 2020). The marine fishing sectors contribute significantly to the Malaysian economy, accounting for 1,483,000 tonnes of marine products worth RM 5.22 billion (USD 1.41 billion) (Bakar *et al.*, 2018). This sector also provides considerable job opportunities and international export opportunities, as well as a protein supply for indigenous rural people. The coastal marine ecosystems that support the fishing industry are some of the most biodiverse areas in the world, particularly in the Coral Triangle region. This area is home to a large number of marine fish species, including an estimated 1400 species, many of which can only be found in this region (Bakar *et al.*, 2018).

The fish of the family Scombridae are well known for their commercial and ecological significance. They can be found in marine waters from tropical to subtropical regions around the world. These fish have long and fusiform body shapes and are typically found in the upper layers of the ocean. Many of them are migratory and their diet mostly consists of crustaceans, mollusks, and cephalopods. Some species of the family have a more limited population range (Collette *et al.*, 2001; Collette, 2003). The tribe Thunnini comprises highly sought-after fish species in the commercial industry and is exported globally, accounting for over 10% of the worldwide marine trade. The consumption of tuna has increased significantly since 1970, with a growth of approximately three million tonnes, which includes raw canned tuna, tuna farms, and tuna loins (Seyhan and Turan, 2016; Amaral *et al.*, 2017). The Pacific Ocean (65%), Indian Ocean (22%), and Atlantic Ocean (12%) are the main fishing areas for tuna, with the majority of the products being distributed in countries such as Taiwan,

Korea, Spain, France, Japan, Indonesia, and the United States (Paquotte, 2003; Seyhan & Turan, 2016; Amaral *et al.*, 2017).

There are six genera of tunas and tuna-like fish, namely *Auxis*, *Euthynnus*, *Katsuwonus*, *Thunnus*, *Sarda*, and *Gymnosarda* (Collette *et al.*, 2001). One of the most important species is Kawakawa, *Euthynnus affinis* (Cantor, 1849), which belongs to the Scombridae family and is found throughout the Indo-Pacific regions's tropical and subtropical waters (Kumar *et al.*, 2012a; Yazawa *et al.*, 2019). It is a small epipelagic, migratory, neritic tuna that has become one of Malaysia's most important commercial tuna species (Masazurah *et al.*, 2012; Yazawa *et al.*, 2015). *Euthynnus affinis*, commonly recognised as the 'little tuna' is a medium-sized tuna transpiring during the near-shore continental reef zones, where water temperatures vary between 18°C and 29°C (Rohit *et al.*, 2012; Ahmed *et al.*, 2015; Syamsuddin *et al.*, 2018). This species is found along the coasts of the Arabian Sea and the Indian Ocean, covering regions from East Africa to the Arab Peninsula, the Indian subcontinent, and Southeast Asia, including places such as Cape St. Francis in South Africa, the Red Sea, the Persian Gulf, and various islands such as Madagascar, Comoros, Mauritius, the Reunion, Seychelles, Lakshadweep, and the Andaman Islands, Sri Lanka, and the Maldives (Ahmed *et al.*, 2015). Additionally, it also ranges northward through mainland China and Taiwan to southern Japan in the northwestern Pacific Ocean (Chiou & Lee 2004; Taghavi *et al.*, 2010).

Knowledge of the stock structure is crucial for developing management plans that will be useful in conserving the biodiversity of species and stocks (Turan *et al.*, 2005), especially for species that are vulnerable to commercial exploitation (Ha *et al.*, 2020). Understanding the stock structure of an examined species is necessary and important for rational and successful management of a fishing resource, as each stock

must be handled separately to maximise productivity (Tzeng, 2004). Ignoring stock structure can lead to a variety of issues, such as genetic diversity loss, modification in biological traits, such as making fish smaller, overfishing less productive stocks, and inaccurate projections of how management practices may impact a stock (Cronin-Fine *et al.*, 2013).

There are a variety of techniques have been proposed by various researchers for stock identification purposes of marine and freshwater fishes, including morphometric technique (Griffiths *et al.*, 2017; El Mghazli *et al.*, 2021; Binashikhubkr *et al.*, 2024), geometric morphometric (Mounir *et al.*, 2019; Mohammadi-sarpiri *et al.*, 2021; Kasinath *et al.*, 2024), meristic (Gain *et al.*, 2017), otolith shape (Wujdi *et al.*, 2022), and DNA-based methods such as mitochondrial genes (Kumar *et al.*, 2012; Kasim *et al.*, 2020; Jamaludin *et al.*, 2022; Binashikhubkr *et al.*, 2024). The mtDNA cytochrome oxidase subunit I (COI) gene is a unique animal barcode region (Hebert *et al.*, 2003). It is the regular DNA barcode region and is highly effective in identifying fish species (Wang *et al.*, 2018). The COI is one of the several conserved protein-encoding genes in the mitochondrial genome, thus, it is used to catalog animal species (Ward *et al.*, 2009). Previous reports on marine fishes have proven that the COI is a helpful gene in species recognition, population genetics, and fisheries management studies (see, e.g. (Bingpeng *et al.*, 2018; Xu *et al.*, 2021; Halim *et al.*, 2022)). Consequently, to give more reliable information about management and assessment of the stock structure, many investigations have been combined morphometric and molecular techniques. For instance, Kerschbaumer *et al.* (2014) applied genetic and morphometric approaches to evaluate the body shape variation of genus *Tropheus* populations collected from the Lake Tanganyika. Similarly, Bakar *et al.* (2018) conducted traditional morphometric and DNA barcoding methods to

recognise bigeye snapper juvenile *Lutjanus* in Malaysian waters. Likewise, Sawalman and Madduppa (2020) and Mohd Yusoff *et al.* (2021) have utilised morphometric and molecular approaches to investigate the stock structure of Yellowstripe Scad *Selaroides leptolepis* and yellowtail stargazer *Uranoscopus cognatus*, respectively. Therefore, to the best of my knowledge, there is no study has been documented on morphometric and molecular investigations of *E. affinis* in Malaysian waters. The outcomes of this study will provide an important baseline information on *E. affinis* and will help fisheries managers to select meticulous and accurate measures for conservation and management of this neritic tuna in Malaysian waters.

1.2 Problem Statement

Fish serve as a vital source of nutrition for billions of people and support millions of livelihoods globally. However, the sustainability of fisheries is increasingly threatened by climate change, habitat degradation, and overexploitation. These factors have altered fish distribution and abundance, leading to resource conflicts and unsustainable exploitation patterns. If current fishing trends persist, it is projected that 80% of global fish stocks will become overfished by the next decade (Burden & Fujita, 2019). Kawakawa *Euthynnus affinis*, a key species in the Indo-Pacific region, has shown rising exploitation levels, as evidenced by multiple studies. Griffiths *et al.* (2020) reported a significant increase in annual catches in the Indian Ocean, from 1,000,000 metric tons in 2010 to 1,250,000 metric tons in 2017. Similarly, Effarina *et al.* (2020) documented an increase in annual landings from 8,000 metric tons in 2015 to 11,000 metric tons in 2019 in the Straits of Malacca. A more recent investigation by Mardlijah *et al.* (2022) found that *E. affinis* in the Java Sea is overfished, recommending a 32% reduction in fishing pressure. Additionally, Abinaya & Sajeewan

(2023) highlighted an overfishing trend along the southeast coast of India. These reports collectively indicate the urgent need for sustainable management strategies to ensure the long-term viability of *E. affinis* populations. Despite the commercial and ecological importance of *E. affinis*, critical knowledge gaps remain regarding its population structure and stock assessment. As a highly migratory neritic tuna, effective conservation and management efforts require an accurate understanding of its genetic diversity, population connectivity, and morphological variation. However, existing studies have largely focused on catch trends rather than morphometric and genetic analyses. The lack of comprehensive stock structure assessments hinders the implementation of targeted conservation policies. To address these gaps, this study employs morphometric and molecular approaches to assess the morphological differentiation and genetic diversity of *E. affinis* across Malaysian waters. Morphometric analysis enables the detection of phenotypic variations among populations, which may reflect local adaptations, environmental influences, or genetic divergence. Additionally, molecular analyses, particularly DNA barcoding and genetic diversity assessments, provide insights into population connectivity, genetic differentiation, and evolutionary history, which are essential for defining management units. Integrating these methodologies will contribute to a more robust understanding of *E. affinis* stock structure, facilitating informed conservation and management strategies. Without urgent preservation efforts, overfishing may lead to severe ecological imbalances, economic losses, and food security challenges for coastal communities. Therefore, sustainable management of *E. affinis* populations is imperative to ensure the resilience of marine ecosystems and the stability of fisheries-dependent economies.

1.3 Objectives

In this current study, morphometric and molecular approaches were conducted to investigate the stock structure of *E. affinis* collected from four marine regions in Malaysian waters: the Straits of Malacca, the South China Sea, the Sulu Sea, and the Celebes Sea. The information obtained from this present investigation will contribute to the first morphometric and molecular data reports on *E. affinis*. Furthermore, this study will provide insights into the stock structure, genetic diversity, and phylogenetic relationship of *E. affinis* in Malaysian waters.

The specific objectives of this study are as follows:

1. To investigate body shape variations among *E. affinis* specimens in Malaysian waters utilising morphometric approaches.
2. To determine the DNA barcoding of *E. affinis* using the COI gene in Malaysian waters.
3. To determine the genetic diversity of *E. affinis* from Malaysian waters inferred from the COI and Cyt *b* genes.

CHAPTER 2

LITERATURE REVIEW

2.1 Worldwide Fisheries Status, Management, and Conservation

The fisheries and aquaculture industries are growing in social and economic complexity, currently providing employment for 260 million individuals (Mcclanahan *et al.*, 2015). These industries supply an average of 17 kg per person per year of micronutrient-rich animal food globally and generate approximately USD 100 billion annually in global trade. Fish ranks among the most extensively traded food commodities, with 38% of all documented fishery production being traded internationally in 2010 (Mcclanahan *et al.*, 2015). Developing countries contributed slightly over 50% of the world's fishery exports (encompassing both fisheries and aquaculture) by value and 60% by weight, with 67% of these exports destined for developed nations (Mcclanahan *et al.*, 2015). The rise in marine capture fishery yields during the latter half of the 20th century was predominantly driven by the expansion of fishing activities offshore and into tropical regions; however, these yield increases have plateaued over the past two decades (Swartz *et al.*, 2010). There exists a favourable fish trade balance between developing and developed countries, as higher-value species such as Yellowfin tuna (*Thunnus albacares*), Scombridae, and shrimp (Caridae) are exported, while lower-value species like herrings and sardines (Clupeidae) are imported (Mcclanahan *et al.*, 2015; Sumaila *et al.*, 2015).

The rising exploitation of high seas fisheries has raised alarms among scientists, economists, legal experts, governments, and non-governmental organisations. Poor management practices have resulted in the overfishing of numerous economically significant fish stocks (Cullis-Suzuki & Pauly, 2010).

Fisheries targeting these resources often result in by-catch of threatened or vulnerable species and lead to habitat destruction. The current situation of many highly migratory pelagic species, such as tunas and billfishes, is particularly alarming. Over the past fifty years, stocks of tunas and related species have diminished by an average of 60%, with most of these stocks being either fully exploited or overexploited (Sumaila *et al.*, 2015). Deep-sea fishes are also a major concern. The high seas' depths, characterised by limited light and food, host fish that are typically long-lived and slow growing, making them particularly susceptible to overfishing (Norse *et al.*, 2012). Despite technological advancements that enable deep-sea fishing and the accumulation of new knowledge, the vast majority of the deep ocean remains unexplored and poorly understood (Ramirez-Llodra *et al.*, 2010). Additionally, the ecological impacts of deep-sea fishing activities are largely unknown (Sumaila *et al.*, 2015). In general, the preservation and management of marine species are essential for maintaining ocean health and biodiversity. Marine conservation faces numerous challenges such as overfishing, habitat destruction, climate change, and pollution (Rumolo, 2023). Effective strategies include the establishment of Marine Protected Areas (MPAs), the promotion of sustainable fisheries management, and the implementation of habitat restoration initiatives (Hilborn *et al.*, 2020). Addressing transboundary issues requires international collaboration and agreements, while regional fisheries management organisations play a key role in overseeing fish stocks. Furthermore, public awareness and education are vital for increasing consumer demand for sustainable seafood and fostering a conservation mindset (Sequeira *et al.*, 2019). Consequently, comprehensive and collaborative efforts are imperative to ensure the sustainable use of marine resources and the protection of marine biodiversity for future generations.

2.2 Fisheries Trends in Malaysia

Malaysia, surrounded by the sea and enriched with rivers and lakes, boasts vital ecosystems that offer abundant fish and other aquatic resources to its inhabitants. Consequently, fish has become a staple in the Malaysian diet and a primary protein source (Yusoff, 2015). Despite the availability of various other animal protein sources at competitive prices, fish remains a preferred choice among Malaysians, showing little sign of being supplanted. This preference is evident in household spending and consumption patterns, with an average family allocating about 20 percent of their food expenditure to fish (DOF, 2012). The fish consumption index rose from 53.1 kg in 2011 to 61.1 kg in 2020, positioning Malaysia among the world's top fish consumers (DOF, 2012; Yusoff, 2015). While the fish resources in Malaysian waters primarily meet the dietary needs of the population, a small percentage is exported, contributing to income and foreign exchange. The export species mainly include high-value fish such as grouper, snapper, shrimp, and some molluscs (DOF, 2012).

Marine fisheries constitute a significant industry within Malaysia's fisheries sector, surpassed only by aquaculture and inland fisheries (DOF, 2019). In 2017, the total fish landings reached 1.46 million metric tonnes, accounting for 8.8 percent of South-East Asia's overall marine fisheries landings (Mohamad *et al.*, 2022). According to DOF 2023, the total tuna landings in Malaysian waters amounted to 69,894 metric tons, with a market value of 122,938,694 USD (Table 2.1). These marine resources are crucial for providing a healthy source of animal protein, minerals, and essential nutrients (Tran *et al.*, 2019). However, they are susceptible to numerous threats, including natural fish mortality and overfishing (Diop *et al.*, 2018), leading to unstable marine landings in Malaysia since 1990 (DOF, 2019). This instability mirrors the global trend of static marine fisheries landings since 1980 (Mohamad *et al.*, 2022). The

situation has worsened due to climate change. Tang (2019) noted that Malaysia's marine, ocean, and coastal resources are particularly vulnerable to climate change. Malaysia reached its peak temperature of 39.3 degrees Celsius in 2016, coinciding with the occurrence of the Super El Nino phenomena. This phenomenon resulted in a severe drought that affected a large area (Mohamad et al., 2022). These events have led to increased sea surface temperatures and acidity, as well as alterations in the ecosystem and biological dynamics (Tang, 2019). Consequently, alterations in the distribution patterns of fisheries have intensified the strain on marine fisheries sustainability (Merino *et al.*, 2012). It is thus crucial to pinpoint the key factors impacting national marine fisheries landings. Should the enduring impacts of climate change remain, fishing efforts will be jeopardised, resulting in a reduction in the abundance of fish stocks (Mohamad *et al.*, 2022). This poses a threat to the long-term viability of the marine fishing industry and, consequently, the overall food security of the nation.

Table 2.1 Tuna landing in Malaysian waters (DOF, 2023).

NO	Type of tuna fish	Metric tons (mt)	Price (USD)
1.	Tuna-like	1,038	1,109,555
2.	Neritic tuna	55,223	103,410,526
3.	Oceanic tuna	13,633	18,418,613
	Total	69,894	122,938,694

Malaysia has implemented several measures to mitigate the impact of fishing activities on marine ecology. These include promoting the use of eco-friendly fishing gear and introducing various regulations, such as prohibitions on certain fishing methods, protections for endangered species, and restrictions on fishing in designated areas. Additionally, Malaysia has enforced vessel operation reporting for deep-sea fisheries and landing surveys for coastal fisheries. These data collection efforts aid in

the management of fishery resources. To reduce by-catch, especially undersized fish and juvenile turtles, the use of Juvenile and Turtle Excluding Devices (JTEDs) is promoted among fishermen. Furthermore, the use of circle hooks is encouraged for longline fishermen (DOF, 2019; Effarina *et al.*, 2020). Overall, maintaining the sustainability of marine fisheries in Malaysia and its surrounding waters is crucial for ensuring both national and international economic thriving and a reliable supply of healthy protein.

2.3 Family Scombridae

The scombrids (Family Scombridae) play a crucial role as both predators and prey within coastal and oceanic marine ecosystems. They support some of the world's most significant fisheries. Understanding their basic biology and life history traits—such as growth, age, and maturity—is essential for sustainable management and for maintaining their critical roles in marine ecosystems (Juan-Jordá *et al.*, 2016; Seyhan & Turan, 2016). Research on these species sheds light on their genetic diversity, migratory patterns, and unique physiological adaptations, which are essential for successful conservation and management, especially in the context of overfishing and habitat depletion (Zeng *et al.*, 2022). Furthermore, Scombridae species are important indicators of environmental changes and play a vital role in human nutrition. Studying their responses to climate change and developing sustainable fishing practices are critical for ensuring their long-term viability and the health of marine ecosystems (Juan-Jordá *et al.*, 2013; Zeng *et al.*, 2022).

The Scombridae family is a group of epipelagic and migratory marine fish characterised by their elongated, fusiform bodies that are moderately compressed. This family includes 15 genera and 55 species (Fricke *et al.*, 2025). The species in this

family are grouped into two sub-families: the Gasterochismatinae, which contains only one species (*Gasterochisma melampus*), and Scombrinae, which contains 54 species and is further divided into four tribes: mackerels (Scombrini), seerfishes or Spanish mackerels (Scomberomorini), bonitos (Sardini), and tunas (Thunnini) (Collette and Nauen 1983; Fricke *et al.*, 2025). Scombrid fishes are economically and environmentally important, and their spread from tropical to subtropical oceans is globally significant (Collette *et al.*, 2001). Froese and Pauly (2013) mentioned that there are 11 genera and 20 species of the family Scombridae in Malaysian waters (Table 2.2). According to the Integrated taxonomic information system (ITIS), the taxonomic hierarchy of Scombridae is as follows:

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Class	Actinopterygii
Subclass	Neopterygii
Order	Perciformes
Suborder	Scombroidei
Family	Scombridae
Subfamily	Gasterochismatinae (Lahille, 1903)
Subfamily	Scombrinae (Bonaparte, 1831)

Table 2.2 List species of Scombridae family in Malaysian waters (Froese and Pauly, 2013).

Tribes	Name of species	Common name	Distribution
Scombrini	1. <i>Rastrelliger brachysoma</i> (Bleeker, 1851)	Short mackerel /Kembong/ Kembong pelaling /Kembong perempuan	Pacific Ocean, Andaman Sea to Thailand, Indonesia, Papua New Guinea, Philippines, Solomon Island and Fiji
	2. <i>Rastrelliger faughni</i> (Matsui, 1967)	Island mackerel / Kembong / Kembong Lampai / Mabong / Rumah	Indo-west Pacific
	3. <i>Rastrelliger kanagurta</i> (Cuvier, 1816)	Indian mackerel/ Kembong / Kembong Borek / Kembong Jantan	Indo-Pacific, Eastern Pacific
	4. <i>Scomber australasicus</i> (Cuvier, 1832)	Blue mackerel / Aya / Tenggiri Biru	Indo-west Pacific
Sardini	5. <i>Gymnosarda unicolor</i> (Ruppel, 1836)	Dogtooth tuna / Tuna / Tuna Tenggiri	Indo-Pacific
	6. <i>Sarda orientalis</i> (Temminck & Schlegel, 1844)	Striped bonito / Aya / Aya bonito / Kayu / Tenggiri	Southwest Pacific
Thunnini	7. <i>Auxis roche</i> (Risso, 1810)	Bullet tuna / Aya / Aya peluru / Aya Selaseh / Kayu / Tongkol	Atlantic, Indian and Pacific Ocean
	8. <i>Auxis thazard</i> (Lacepede, 1800)	Frigate tuna / Aya Kurik / Aya Selaseh / Bakulan / Tongkol kurik / Selaseh	Atlantic, Indian and Pacific Ocean
	9. <i>Euthynnus affinis</i> (Cantor, 1849)	Kawakawa / Aya / Aya Kurik / Tongkol kurik / Kayu	Indo-west Pacific
	10. <i>Katsuwonus pelamis</i> (Linnaeus, 1758)	Skipjack tuna / Oceanic bonito /Aya / Aya jalur / Aya jepun / Tongkol	Cosmopolitan in tropical and warm-temperate waters
	11. <i>Thunnus alalunga</i> (Bonnaterre, 1788)	Albacore / Aya / Kayu / Tongkol	Cosmopolitan in tropical and temperate waters of all oceans

Table 2.2 (Continued)

Tribes	Name of species	Common name	Distribution
	12. <i>Thunnus Albacares</i> (Bonnaterre, 1788)	Yellowfin tuna / Aya / Aya tuna / Tongkol	Tropical and subtropical seas, but absent from the Mediterranean Sea
	13. <i>Thunnus obesus</i> (Lowe, 1839)	Bigeye tuna / Aya / Aya Hitam / Kayu / Tongkol	Atlantic, Indian and Pacific
	14. <i>Thunnus tonggol</i> (Bleeker, 1851)	Longtail tuna / Aya Hitam	Indo-west Pacific
	15. <i>Acanthocybium solandri</i> (Cuvier, 1832)	Wahoo / Tenggiri wahoo / Barracuda	Atlantic, Indian and Pacific Oceans
	16. <i>Grammatorcynus bilineatus</i> (Ruppell, 1836)	Double-lined mackerel / Aya	Indian Ocean, Western Pacific
	17. <i>Scomberomorus commerson</i> (Lacepede, 1800)	Narrow-barred Spanish mackerel / Tenggiri	Indo-west Pacific
Scomberomorini	18. <i>Scomberomorus guttatus</i> (Bloch & Schneider, 1801)	Indo-Pacific king mackerel / Tenggiri	Indo-west Pacific
	19. <i>Scomberomorus koreanus</i> (Kishinouye, 1915)	Korean seerfish / Tenggiri	Indo-west Pacific
	20. <i>Scomberomorus lineolatus</i> (Cuvier, 1829)	Streaked seerfish / Tenggiri	Indo-west Pacific

2.3.1 Tribe Thunnini

The tribe Thunnini belongs to the Scombridae family, which includes the most economically important species, contains 13 species in four genera: *Thunnus* (7 species), *Katsuwonus* (1 species), *Euthynnus* (3 species), and *Auxis* (2 species) (Collette & Nauen, 1983). Tunas are known as the "travelers" of the world's oceans due to their highly migratory nature. They can be found throughout the world's oceans but are typically found in tropical and temperate waters between 45 degrees north and 45 degrees south of the Equator. As one of the most valuable fish in the world, tuna

has been a valuable resource for humans for thousands of years. According to archaeological data, tuna has been collected by humans for at least 42,000 years (Kumar & Kocour, 2015).

Euthynnus Lütken, 1882; *Auxis* Cuvier, 1829; *Thunnus* South, 1845, and *Katsuwonus* Kishinouye, 1923 are four genera belonging to the Thunnini tribe that have been discovered in Malaysian waters (Collette *et al.*, 2001). The *Auxis* genus is after *Allothunnus* the next primitive genus (Collette *et al.*, 2001). The *Auxis thazard* and *Auxis rochei* are two of the four species of that genus found in Malaysia. Among the three species of the genus *Euthynnus*, only *E. affinis* has been reported in Malaysia. The genus *Katsuwonus* is a monotypic genus that is closely similar to *Thunnus* and *Euthynnus* (Collette *et al.*, 2001). Its single member, *Katsuwonus pelamis* also lives in Malaysian waters. The genus *Thunnus* within the Scombridae family is the most advanced, with seven distinct species. Of these, four can be found in the local waters of Malaysia: *T. albacares*, *T. obesus*, *T. alalunga*, and *T. tonggol* (Froese & Pauly, 2013).

2.3.1(a) Genus *Euthynnus* Lütken, 1882

The genus *Euthynnus*, derived from the Greek word "eu" meaning good and "thynnos" meaning tuna, encompasses three distinct species: *Euthynnus affinis*, *E. alletteratus*, and *E. lineatus*. *Euthynnus affinis* can be found in the warm seas of the Indo-West Pacific, including oceanic islands and archipelagos. In contrast, *E. alletteratus* is restricted to the Atlantic Ocean and *E. lineatus* is found in the Pacific Ocean (Kumar & Kunal, 2013; Sulistyaningsih *et al.*, 2014).

2.3.1(a)(i) Diagnostic Morphological Characteristics of Genus *Euthynnus*

A median fish with a strong, elongated, and fusiform body shape, featuring 37 to 39 vertebrae. It has small, conical teeth in a single row, numbering 25 to 35 on each side of the lower jaw, and also has palatine teeth (Collette & Nauen, 1983). The number of gill rakers is 29 to 45 on the first arch, and the gill teeth are 28 to 32 on the posterior surface of the first-gill arch. The fish has two dorsal fins, with 10 to 15 spines in the first dorsal fin. Both fins are divided at a small intersection. The second dorsal fin is much shorter than the first dorsal fin and is followed by 8 to 10 finlets. The pectoral fins are short, with 25 to 29 rays, and the anal fins have 11 to 15 rays, followed by 6 to 8 finlets. The body is naked, except for the corselet and sideline. The caudal peduncle is very slender, located between the small keels of the caudal fin, with a prominent side intrapelvic keel. The color of the fish is dark blue and iridescent green in one species, with a complicated striped design that does not extend beyond the center of the first dorsal fin. The bottom and abdomen are silvery blue and white, without dark longitudinal stripes. Many characteristic dark spots are present among the pectoral and pelvic fins, though they may not always be very visible (Collette & Nauen, 1983).

2.3.1(a)(ii) Habitat and Distribution of Genus *Euthynnus*

The genus *Euthynnus*, found primarily in neritic waters, is rarely found in temperatures below 20-23°C. The warmest water temperatures trigger spawning. They typically school with other animals, but not as developed as the *Auxis* genus. They also have a tendency to scatter during certain times of the year (Kumar *et al.*, 2019a). The three *Euthynnus* species have complementary geographical distributions, with limited overlap in tropical and subtropical coastal waters. These species are highly adaptable

predators that feed on fish, crustaceans, calves, and tunicates. They compete for food with other marine animals, such as dolphins and cetaceans, and are also preyed upon by larger marlins, sharks, and yellowfin tuna (Collette & Nauen, 1983).

2.3.1(a)(iii) *Euthynnus affinis* Cantor, 1849

The original name of *Euthynnus affinis*, created by Cantor in 1849, was *Thynnus affinis*, and the type locality for this species is the Sea of Penang, Malaysia. *Euthynnus affinis* is primarily found in the western Pacific and Indian Ocean, on continental shelves and islands. It is a highly migratory species that congregates in large groups, often alongside other scombrid species. While sexually mature fish can be found year-round, spawning patterns vary by region. For example, in Indonesia, spawning occurs from August to October, while in Philippine waters, it occurs from March to May. In the waters off the coast of East Africa, spawning occurs from January to July, during the transition from the northwest monsoon to the southeast monsoon (Syamsuddin *et al.*, 2018). *Euthynnus affinis* is known to migrate between feeding and spawning regions (Chiou & Lee, 2004; Kumar *et al.*, 2012). In many countries bordering the Central, Western Pacific Ocean, South-western, and East and West Indian Oceans, *Euthynnus affinis* is a significant contributor to commercial and artisanal fisheries (Ardelia *et al.*, 2017; Kumar & Kocour, 2015). It is distributed along the Asian continent from Malaysia northeastward through Mainland China, Taiwan, and southern Japan (Masazurah *et al.*, 2012). *Euthynnus affinis* primarily lives in neritic waters, where fishing pressure is typically high due to its commercial significance. Efforts to cultivate and crossbreed this species have been studied, similar to many other species (Matsumoto *et al.*, 2015).

2.3.1(a)(iv) Characteristics, Habitat, Distribution, and Size of *Euthynnus affinis*

Euthynnus affinis possesses 29 to 33 gill rakers on the first arch, 28 or 29 gill teeth, and lacks vomerine teeth. It has 13 or 14 anal fin rays and 39 vertebrae. The 33rd and 34th vertebrae have bony caudal keels, with no traces of vertebral protuberances. Its coloration includes broken oblique lines on the dorsal side (Figure 2.2). This epipelagic and neritic species inhabits warm waters in the Indo-West Pacific region, including ocean islands and archipelagos. A few stray specimens have also been found in the eastern tropical Pacific. The maximum length of the fork is approximately 100 cm, with common lengths of 60 cm and a weight of around 13.6 kg (Collette & Nauen, 1983).

2.3.1(a)(v) Classification of *Euthynnus affinis*

Euthynnus affinis was classified according to Fricke *et al.* (2025)

Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Gnathostomat
Class	Osteichthyes
Subclass	Actinopterygii
Order	Perciformes
Suborder	Scombroidei
Family	Scombridae
Subfamily	Scombrinae
Tribe	Thunnini
Genus	<i>Euthynnus</i>
Species	<i>Euthynnus affinis</i> (Cantor, 1849)

2.3.1(a)(vi) Population Dynamics, Biological Characteristics and Morphometrics Studies on Genus *Euthynnus* and Other Tuna Species

Earlier studies have been carried out on the population dynamics, biological characteristics, and morphometrics of genus *Euthynnus*. One early study, by Williamson (1970), focused on the biology of *E. affinis* in Hong Kong. Other studies have looked at factors such as age, growth, reproduction, mortality, movement patterns, and stock assessment (see e.g. Silas *et al.*, 1985; James *et al.*, 1993; Khan, 2004; Rohit *et al.*, 2012; Johnson & Tamatamah, 2013; Nissar *et al.*, 2015; Ardelia *et al.*, 2016; Faizal *et al.*, 2019; Griffiths, 2020; Hashemi *et al.*, 2021) (Table 2.2). Likewise, Taghavi Motlagh *et al.* (2010) estimated the population parameters for the assessment of *E. affinis* stock in the Coastal Waters of the Persian Gulf and Sea of Oman (Hormozgan Province). Kaymaram and Darvishi (2012) studied the growth and mortality parameters of *E. affinis* in the northern part of the Persian Gulf and Oman Sea. In contrast, some studies have estimated the length-frequency distribution and population parameters of *E. affinis* (Chodrija *et al.*, 2013; Sulistyaningsih *et al.*, 2014; Kumar *et al.*, 2019; Asrial *et al.*, 2021). Haputhantri and Bandaranayake (2013) examined the length-weight and length-length relationships of *E. affinis* landings in Sri Lanka. Hajjej *et al.* (2013) have conducted a discriminant function analysis to discriminate morphological differences of *E. alletteratus* in Tunisian waters and Eastern Atlantic. The findings outlined the presence of four morphologically separated groups of *E. alletteratus*. Karakulak *et al.* (2016) explored the morphometric differentiation between two juvenile tuna species, *T. thynnus* and *E. alletteratus* in Turkish waters. Their results revealed a strong correlation with head length, pectoral fin length, first predorsal length, and second dorsal fin height between *T. thynnus* and *E. alletteratus*. In Australian waters, Griffiths *et al.* (2017) investigated the morphometric relationships (fork length–total length and length-weight) among four

Scombridae fish species (*Euthynnus affinis*, *Thunnus tonggol*, *Rastrelliger kanagurta*, and *Cybiosarda elegans*). Their results demonstrated that there are highly significant relationships between fork length–total length and length-weight among the four Scombridae fish species. Overall, a review of previous studies revealed a lack of research utilising morphometrics techniques to study *E. affinis* in Malaysian waters.

2.3.1(a)(vii) Genetic Studies of Genus *Euthynnus* and Other Tuna Species

Several prior investigations have been conducted on the *Euthynnus affinis* specifically for genetic research. The first study on this species, conducted by Chiou and Lee (2004) found no genetic variation among samples collected from Taiwan and the northern Philippines. Santos *et al.* (2010), who investigated the population structure of *E. affinis* in Peninsular Malaysia (Pangkor Island) and Philippines using a mitochondrial DNA control region (D-loop) marker highlighted that the population of *E. affinis* was a single stock. Moreover, other studies have reported that the genetic structure of *E. affinis* was also a single stock (Kumar *et al.*, 2012a,b; Masazurah *et al.*, 2012; Johnson *et al.*, 2016) (see Table 2.2). Mudumala *et al.* (2011) used the mitochondrial cytochrome c oxidase I (COI) gene to analyse genetic differences among *T. tonggol*, *E. affinis*, *A. thazard*, and *A. rochei* collected from the North-Western Indian. Kumar and Kunal (2013) used the mitochondrial COI gene to examine historic demography and phylogenetic relationship of *Euthynnus* species: *E. affinis*, *E. alletteratus*, and *E. lineatus* in the Indian Ocean. Their results demonstrated that *E. affinis* is more closely related to *E. lineatus* than *E. alletteratus* while *E. alletteratus* is the most ancestral species. Kumar *et al.* (2014) utilised the mitochondrial DNA D-loop region to estimate the DNA sequence variation and phylogenetic relationship among five tuna species (*A. thazard*, *E. affinis*, *K. pelamis*, *T. tonggol*, and *T.*

albacares). Their results showed that the intra-specific sequence variation in studied species was low, ranging from 0.027 to 0.080 by using Kimura's two-parameter distance, whereas values of interspecific variation ranged from 0.049 to 0.491. On the other hand, the phylogenetic analysis also suggests that *E. affinis* is more closely related to *E. lineatus* than *E. alletteratus*. Xu *et al.* (2016) used PCR-RFLP analysis of mitochondrial DNA fragments to identify tuna species (Thunnini tribe). The Mitogenome sequence of *E. affinis* was determined by using next-generation sequencing technology in the Subi Reef of the South China Sea (Li *et al.*, 2016). Other studies have determined the whole mitochondrial genome of *A. thazard* and *A. rochei* (Xu *et al.*, 2019a; 2019b). A mitochondrial control region (D-loop) has been employed to determine the genetic diversity of longtail tuna *T. tonggol* from Malaysian and Indonesian waters. Their results revealed that the population structure of longtail tuna was a single stock (Kasim *et al.*, 2020; Astarini *et al.*, 2021). Generally, previous studies on the genetic diversity and structure of the genus *Euthynnus* have shown that there is a single genetic stock of *E. affinis* across various regions worldwide.

Table 2.3 Population dynamics, biological, morphometrics, and genetic studies of tuna species in the Indo-Pacific region.

Region	Sample size	Methodology	Conclusion of the study	Reference
Taiwan	50	Sequencing of mtDNA D-loop region	Taiwanese and the Philippine kawakawa (<i>E. affinis</i>) belong to the same genetic stock	Chiou and Lee (2004)
Philippines & Peninsular Malaysia (Pangkor Island, Penang)	45	Sequencing of mtDNA D-loop region	Kawakawa is panmixing in Southeast Asia	Santos <i>et al.</i> (2010)
North Indian Ocean	400	PCR-RFLP and sequencing of mtDNA D-loop region	Single genetic stock of kawakawa in coastal waters of India	Kumar <i>et al.</i> (2012a,b)
Tanzania coastal waters	46	Mitochondrial DNA Control Region	Single genetic structure of the Kawakawa in the northern Tanzania coastal waters	Johnson <i>et al.</i> (2016)
Straits of Malacca	113	Mitochondrial DNA (mtDNA) cytochrome b (cyt b) gene	Single genetic structure of kawakawa along the straits of Malacca.	Masazurah <i>et al.</i> (2012)
North Indian Ocean	315	Sequencing of mtDNA D-loop region	Four genetic stocks of skipjack tuna in coastal waters of India	Menezes <i>et al.</i> (2012)
North Indian Ocean	324	Sequencing of mtDNA D-loop region	Three genetic stocks of yellowfin tuna in coastal waters of India	Kunal <i>et al.</i> (2013)
Indian Ocean	35	Mitochondrial COI gene	Phylogenetic analysis suggests that Kawakawa is more closely related to <i>E. lineatus</i> than <i>E. alletteratus</i>	Kumar and Kunal (2013)

Table 2.3 (Continued)

Region	Sample size	Methodology	Conclusion of the study	Reference
Coastal Waters of the Persian Gulf and Sea of Oman (Hormozgan Province).	9988	Estimated the population parameters and conducted an assessment of the Kawakawa stock	Kawakawa stock was calculated to be over 0.5, it is strongly recommended to minimize the fishing activity in this area.	Taghavi Motlagh <i>et al.</i> (2010)
Persian Gulf and Oman Sea.	—	Growth and mortality parameters of kawakawa and population dynamics.	Kawakawa grows very fast in the first two years.	Kaymaram and Darvishi (2012)
Arabian Sea	25	Reproductive biology of Kawakawa	The peak of spawning season of Kawakawa occurred during December– March.	Nissar <i>et al.</i> (2015)
Northwest Sumatera	4,225	Population Parameters of Kawakawa	Kawakawa was fully exploited in the Indian Ocean.	Sulistyaningsih <i>et al.</i> (2014b)
Java Sea Waters	—	Population parameters of Kawakawa	Kawakawa was fully exploited in the area sampling.	Chodrijah <i>et al.</i> (2013)
Southwestern Bay of Bengal	—	Population parameters	Possibility of enhancing the production of kawakawa from the capture fisheries.	Kumar <i>et al.</i> (2019)
Maharashtra waters	—	Age and growth, mortality and stock of kawakawa	Kawakawa was fully exploited.	Khan (2004)
Sri Lanka	100	Population parameters of Kawakawa	All relationships were significant at 0.01 between length measurements and weight measurements.	Haputhantri and Bandaranayake (2013)
Sunda Strait	—	Reproduction biology of Kawakawa	Males and females showed positive allometric growth patterns.	Ardelia <i>et al.</i> (2016)