





RESEARCH ARTICLE

## Otolith Morphology of Neritic Tuna of the Genus *Auxis* (Teleostei: Scombridae) Reveals Mixed Stocks in the Celebes Sea

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### ABSTRACT

Neritic tunas of the genus *Auxis* have been commercially exploited as early as the 1970s in the Philippines. However, basic biological information on local stocks remains sparse despite being threatened by population declines. This study investigated the otolith shape morphology and phenotypic stocks of bullet tuna (BT, *Auxis rochei*) and frigate tuna (FT, *A. thazard*) within the Celebes Sea (western Celebes Sea, Davao Gulf, Moro Gulf, and Sarangani Bay) using otolith shape analysis. A total of 265 BT and 315 FT otoliths were successfully extracted, and results revealed that as fish grow in body length, otoliths develop more circular and elliptical shapes. All shape morphometric size parameters (otolith length, OL; otolith width, OW; otolith area, OA; and otolith perimeter, OP), alongside shape indices (circularity, CR; ellipticity, EL; rectangularity, RE; form factor, FF; and roundness, RO) showed significant correlations to species' fork length (FL). Applying the "scaling effect" using linear regression minimizes the allometric growth effects on otoliths. While PERMANOVA successfully delineated phenotypic variations within BT ( $F = 13.2$ ;  $p > 0.001$ ) and FT ( $F = 9.24$ ;  $p > 0.001$ ) among sites, the Principal Component Analyses also support that all shape indices (CR, EL, FF, RE, RO) of BT and FT contributed most to the stock differentiation. These results imply that both species have distinct spatial populations, which could be attributed to their life-history traits and long-term habitat exposure. This study provides insights into these species' complex population structures within the Celebes Sea for strengthened site-specific conservation and management measures.

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### 1. INTRODUCTION

Recognizing fish stocks is an important parameter for the applicability of proper management measures (Hüssy et al. 2016), particularly for commercially exploited fish species such as tuna (Kumar et al. 2012; Brophy et al. 2016; Duncan et al. 2018; Muñoz-Lechuga et al. 2023). However, tuna populations in some geographical regions occur in mixed fishery (Brophy et al. 2016; Ollé-Vilanova et al. 2022), and grouping them as one

management unit instead of implementing stock-specific interventions may lead to overexploitation (Utter and Ryman 1993; Brophy et al. 2016; Ollé et al. 2019). This issue is especially concerning for less productive stocks as their biology and genetic diversity could be dramatically altered, reducing their chances to bounce back from intense fishing pressure (MacKenzie et al. 2009; Brophy et al. 2016; Kikuchi et al. 2021b; Moore et al. 2022; Muñoz-Lechuga et al. 2023). To date, delineating fish stocks has remained a great concern for fishery managers (Cadrin et

al. 2014; Kikuchi et al. 2021b; Moore et al. 2022), particularly for pelagic species such as tunas (Relini et al. 2008; Brophy et al. 2016; Muñoz-Lechuga et al. 2023). This management challenge is rooted in the paucity of information on the fish's life history and their population connectivity from other fish stocks separated within large geographical areas (Cabasan et al. 2021; Kikuchi et al. 2021a; Kikuchi et al. 2021b).

Several studies demonstrated the potential of using population genetics (Rosalia et al. 2012; Kumar and Kocour 2015; Pedrosa-Gerasmio et al. 2015), electronic tags (Block et al. 2005; Cadrin et al. 2014; Hare et al. 2021), and otolith chemistry (Feyrer et al. 2007; Rooker et al. 2007; Longmore et al. 2010; Artetxe-Arrate et al. 2021; Maciel et al. 2021) techniques to discriminate fish stocks. Despite these developments, these approaches are time-consuming and expensive (Pawson and Jennings 1996; Campana et al. 2000; Leguá et al. 2013; Hüsey et al. 2016; Deepa et al. 2019), particularly for developing countries like the Philippines, where the management and conservation of marine resources are hampered with limited research capacity and resources (Barber et al. 2014; Digal and Placencia 2017). An alternative and cost-effective method to infer stock identity is by comparing phenotypic characters of a target species population, specifically through otolith shape analysis (Campana and Casselman 1993; Lord et al. 2012; Duncan et al. 2018; Barnuevo et al. 2023). By quantifying the unique morphological characteristics of otoliths, distinct fish populations of Scombrids can be delineated, even among other species that undertake migrations such as the Blue jack mackerel (*Trachurus picturatus*, Moreira et al. 2019), Indian Mackerel (*Rastrelliger kanagurta*, Wujdi et al. 2022), and Little tunny (*Euthynnus alletteratus*, Muñoz-Lechuga et al. 2023). This approach has also effectively segregated phenotypic populations of Opal fish (*Bembrops caudimacula*) in the Indian Ocean (Deepa et al. 2019) and Redtail Scad (*Decapterus kurroides*) in Panay Island, Philippines (Barnuevo et al. 2023).

Sagittal otoliths or sagittae of teleost fishes are commonly used to investigate these phenotypic variations since these are highly stock-specific and metabolically inert (Campana and Casselman 1993; He et al. 2018; Chen et al. 2021; D'Iglio et al. 2021; Wujdi et al. 2022). While evidence suggests that genetics strongly dictate stock-specificity in sagittae (Campana and Casselman 1993; Berg et al. 2018; Morales et al. 2023), other factors such as physiology and environmental conditions also significantly influence its development (Duncan et al. 2018; Moreira

et al. 2019; Hüsey et al. 2020). For example, Hüsey et al. (2020) reviewed the uptake of calcium carbonate (CaCo<sub>3</sub>) and other trace elements (e.g., Sr, Mn, Mg, Cu, Ba) from the environment biomineralized into the otolith's matrix. This process was discussed by Cabasan et al. (2021), who observed that the phenotypic stock differentiation in leopard coral grouper (*Plectropomus leopardus*) between Tawi-Tawi and Quezon Province is likely due to the chemical variation in substrate and water. This result implies that when a population is exposed long-term to a similar environment (Moreira et al. 2019), it can develop a common accretion pattern in its otolith shapes (Hüsey et al. 2016). Thus, it serves as identifiable signatures that set them apart from phenotypic stocks in other habitats (Campana and Casselman 1993; Moreira et al. 2019; Cabasan et al. 2021; Khan et al. 2021; Neves et al. 2021; Barnuevo et al. 2023).

Many species of Scombrids are expected to undertake extensive migration likely due to seasonal food availability and reproductive activities (Dingle and Drake 2007; Mahé et al. 2016; Ollé-Vilanova et al. 2022). However, unlike its large oceanic relatives, the movement patterns of neritic tuna are often associated along the continental shelves (Kumar et al. 2012; Ollé-Vilanova et al. 2022; Muñoz-Lechuga et al. 2023). In the Celebes Sea, evidence suggests that some species of neritic tuna have homogenous genetic population structures, but it remains unclear whether different phenotypic stocks exist in this region (Santos et al. 2010; Pedrosa-Gerasmio et al. 2015). Given this knowledge gap, resolving their complex population structures is important, especially for neritic tuna that often cross different geopolitical regions with varying degrees of fisheries management (Brophy et al. 2016).

Neritic tuna of the genus *Auxis*, particularly bullet tuna (BT; *A. rochei*, Risso, 1810) and frigate tuna (FT; *A. thazard*, Lacepède, 1800), are relatively small, epipelagic migratory species distributed globally across tropical and subtropical coastal habitats (Collette and Nauen 1983; Juan-Jordá et al. 2013; Kodama et al. 2022). In the Philippines, they have been commercially exploited as early as the 1970s (Aprieto 1981), particularly in the southern Philippines, where they support local communities for food security and economic growth (Alcala et al. 2009; Digal and Placencia 2017; Ajik and Tahiluddin 2021; Pechon et al. 2022). One of the largest fish landing sites for tuna in the southern Philippines is the General Santos City Fish Port Complex (GSCFPC) in Region XII (Pechon et al. 2022). Due to its strategic positioning in nearby tuna fishing grounds (Llanto et al. 2016;

USAID Oceans and Fisheries Partnership 2019), six out of seven most extensive tuna processing facilities are established in the city (Hipolito and Vera 2006; Llanto et al. 2016). This makes it one of the highest tuna catch landing contributors across the Philippines [Fisheries Planning and Economics Division – Bureau of Fisheries and Aquatic Resources (FPED-BFAR) 2023]]. From 2013 to 2017, BT and FT productions reached 18,856 metric tons (MT) and 17,662 MT, respectively, out of the total volume of 384,140 MT of tuna landed in GSCFPC (Pechon et al. 2022). Overall, this industry has had significant economic impacts, with an estimated 200,000 workers in General Santos (USAID Oceans and Fisheries Partnership 2017) out of 1.4–1.6 million Filipinos directly working on the tuna value chain nationwide (Llanto et al. 2016; USAID Oceans and Fisheries Partnership 2017).

Given the socio-economic relevance of both *A. rochei* and *A. thazard* in the Philippines, this study aimed to (1) describe their otolith shape morphology and morphometry and (2) determine their stock identities in the Celebes Sea using otolith shape analysis. It hypothesizes that phenotypic stocks of *Auxis* spp. exist in this marine ecoregion, which can be differentiated through otolith shape analysis.

As demands for this fishery increase with stocks threatened by overfishing, this study provides insights for future Scombrid research and conservation in the Philippines. Therefore, it should direct fishery managers to formulate stock-specific management measures for the sustainable exploitation of these resources.

## 2. MATERIALS AND METHODS

### 2.1 Study area

The Celebes Sea forms a part to the west of the Pacific Ocean and covers an area of 380,000 km<sup>2</sup> with depths ranging from 4800 to 5400 m at its central basin (Alcala et al. 2009; Asante et al. 2010) (Figure 1). Furthermore, Mindanao Island bordered it to the northeastern edge, the Sulu Sea to the northwest, the Island of Borneo to the west, and Sulawesi Island to the southern end (Asante et al. 2010). This semi-enclosed basin is characterized by shallow underwater sills such as the Sangihe Ridge (~1350 m deep) located east of Mindanao that extends along the Sulawesi (Gordon et al. 2003) and the Sulu Ridge stretching along the western boundary of Zamboanga Peninsula

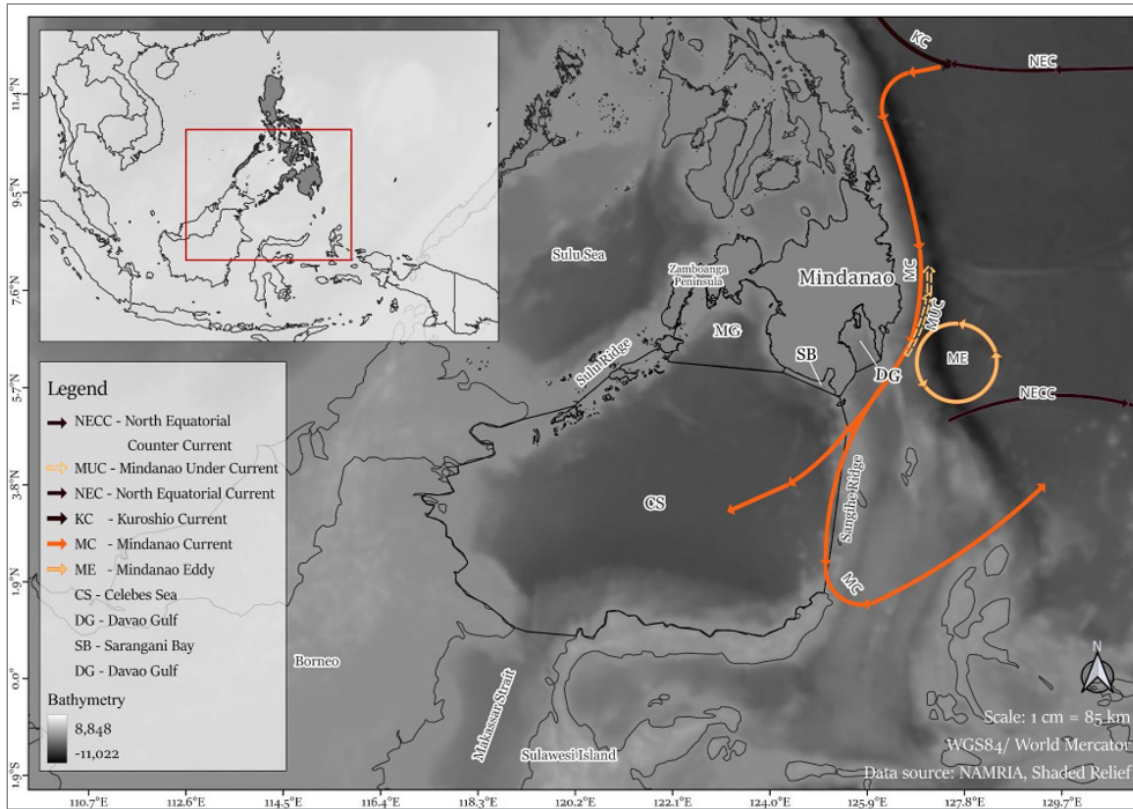


Figure 1. Map of Southeastern Mindanao, Philippines, showing the sampling locations: Celebes Sea, Davao Gulf, Moro Gulf, and Sarangani Bay, including the oceanic currents and underwater topography of the Celebes Sea basin.

in Mindanao to the northeastern edge of Borneo (Murauchi et al. 1973; Takeda et al. 2007). These sills function as submarine topographic barriers by limiting deep-water influx from the neighboring Pacific Ocean and Sulu Sea (Murauchi et al. 1973; Gordon et al. 2003; Takeda et al. 2007). The Celebes Sea also features an oceanic crust with a basalt base encrusted by sediments of fossilized materials from the Eocene (Nichols and Hall 1999). It has several small seamounts scattered across its central basin and a linear trough located underwater at the northern margin of Sulawesi Island (Murauchi et al. 1973).

Sea surface currents originating from the western Pacific Ocean and the Sulu Sea play a crucial role in shaping the water circulation patterns within the Celebes Sea. Among these currents, the Mindanao Current (MC), which bifurcates from the North Equatorial Current (NEC), is a significant contributor to the inter-basin exchange of heat, freshwater, and nutrients, thereby enhancing primary productivity in the Celebes Sea (Lukas et al. 1991; Alcalá et al. 2009; Schönau et al. 2015; Sabater 2021; He et al. 2022). Alongside the MC, the presence of the Mindanao Eddy (ME) and the Mindanao Undercurrent (MUC) further adds complexity to the water mass transport in this basin (Schönau et al. 2015). As the MC enters the Celebes Sea, it connects to the Makassar Strait (Masumoto et al. 2001; Schönau et al. 2015). Additionally, some branches off to form a cyclonic eddy in the western end of the basin (Masumoto et al. 2001; Takeda et al. 2007), while a southward current enters the eastern and central basin, which then flows along with the North Equatorial Counter Current (NECC) (Masumoto et al. 2001). From the southeastern Sulu Ridge, high chlorophyll *a* concentration has been detected likely due to the underwater currents that transport nutrient-rich waters to the surface (Takeda et al. 2007). This upwelling, coupled with the influx of nutrient-rich waters from the Sulu Sea, creates high primary productivity along the southeastern end of the ridge. From the ridge, eastward-flowing surface currents transport these nutrient-dense waters to the central basin of the Celebes Sea (Takeda et al. 2007).

The Coral Triangle, which includes the Celebes Sea, is a global marine biodiversity hotspot renowned for its unique oceanographic characteristics and highly diverse marine species (Muallil et al. 2020). Within this large marine ecoregion, the Celebes Sea forms a dynamic network of interconnected coastal and aquatic habitats that support highly productive fisheries across the region (Miclát et al. 2006; Pedrosa-Gerasmio et al. 2015). Given its ecological significance, this study divides the Celebes Sea into four distinct

sampling sites. These include one bay and two gulfs: the Sarangani Bay, Moro Gulf, Davao Gulf, and the western part of the Celebes Sea. Such partitioning reflects the unique ecological conditions of each site as a result of the complex interactions among the neighboring bodies of water (Pedrosa-Gerasmio et al. 2015; De Vera et al. 2024).

## 2.2 Morphological identification

Prior to the market surveys, researchers underwent training to identify the targeted tuna species based on its morphology, as described by Collette and Aadland (1996), Collette (2001), and Collette et al. (2001). The BT and FT share similar morphological features, and identifying these species in the field may pose difficulties for inexperienced observers, even for fisheries managers (Olle et al. 2019). Based on their works, a few diagnostic keys, such as the corselets, scales, and body shape, can separate these sympatric species. Though both species had short pectoral fins, the fins of the FT reach the vertical line from the anterior end of the scaleless area, while that of the BT does not. Furthermore, FT has a narrow corselet (1–5 scales) and extends beneath the second dorsal fin. At the same time, BT's corselet appears much wider (more than six scales). Lastly, the body shape of FT is rounder and more robust, while a more cylindrical, elongated, or "bullet-shaped" body characterizes BT (Figure 2).

## 2.3 Sample collection and preparations

Fresh fish samples were sourced from the fishing grounds in the western Celebes Sea, Davao Gulf, Moro Gulf, and Sarangani Bay using a variety of fishing gears covering the period from November 2020 to February 2023. This extensive collection spanned multiple seasons to capture potential fluctuations in intra-seasonal dynamics and variations in tuna size (Muñoz-Lechuga et al. 2023) (Supplementary Table S1). Fish samples from the western Celebes Sea and Moro Gulf were primarily obtained from the GSCFPC, while few samples came from the wet markets in Davao Gulf. Most of the collection came from landing sites in Sarangani Bay, such as Kiamba, Sarangani Province. A few fish samples collected along the Tawi-Tawi Islands in Bongao were also gathered from the local wet markets. Fishing gears were documented at each fish landing site during sample collection whenever possible. Commonly used fishing gears for tuna by commercial vessels are ringnets, purse seine nets, and hand lines (Armada 2004; Macusi et al. 2015), while

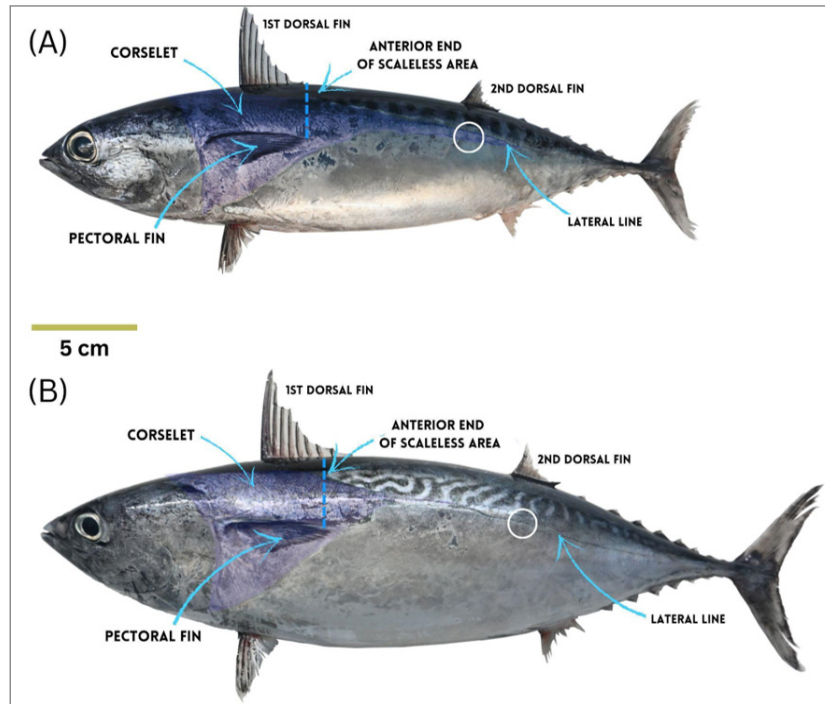


Figure 2. Representative photographs of the two *Auxis* species used in this study: (A) Bullet tuna (*Auxis rochei*, 26.1 cmFL) and (B) Frigate tuna (*A. thazard*, 33.20 cmFL) from the Celebes Sea. Corselet is indicated in blue shade. Photos by Amilussin Ammann.

municipal fishers from Sarangani Bay typically use hand lines for tuna fishing (Well et al. 2011). In Tawi-Tawi, small-pelagic fishing usually employs ring nets (Ajik and Tahiluddin 2021). Fishing activities using these nets have been routinely used alongside Fish Aggregating Devices (FADs) deployed in coastal or offshore fishing grounds (Armada 2004; Macusi et al. 2015). Based on the stock assessment date of 2018 [BFAR-National Stock Assessment Program (BFAR – NSAP) 2018], the fish selection was strategically designed to represent different size classes. These size classes are as follows: (a) BT — size 1 ( $\leq 15.0$  cm FL); size 2 (15.1–25.0 cm FL); size 3 (25.1–35.0 cm FL); and size 4 ( $> 35.1$  cm FL); and (b) FT — size 1 ( $\leq 20.0$  cm FL); size 2 (20.1–30.0 cm FL); size 3 (30.1–40.0 cm FL); and size 4 ( $> 40.1$  cm FL) (BFAR – NSAP 2018; Entia et al. 2024a, 2024b). Unfortunately, no BT samples of size 4 were obtained.

All collected fresh fish samples were brought to the Histopathology and Salokra Laboratories of the Regional Science Research Center (RSRC) of Mindanao State University-General Santos City (MSU-GSC) for further processing. Fish samples were temporarily stored in styrofoam boxes with ice before transporting them to MSU-GSC. Only sagittal otolith samples from Tawi-Tawi were subsequently extracted after field collection at the wet laboratory of MSU -

Tawi-Tawi College of Technology and Oceanography (MSU-TCTO).

Prior to otolith extraction, the following individual fish data were measured and recorded in the field or laboratory, such as fork length (FL, 0.1 cm), total length (TL, 0.1 cm), standard length (SL, 0.1 cm), body girth (0.1 cm), and total weight (TW, 1.0 g) (FISHBIO 2023) per collection site whenever possible. Otolith was subsequently extracted using the open-the-hatch method described by Secor et al. (1992). The left and right sagittal otoliths were extracted cautiously, and any tissue residues attached were manually removed using fine-tipped probes. Otoliths were then washed with tap water in a petri dish, air-dried, and then stored separately in a 1.5-ml clean microcentrifuge tube (Cabasan et al. 2021; Barnuevo et al. 2023). A code was assigned to each extracted left and right sagittal otolith following this coding system: BLT1 S2 CS 9-10-21 L, where “BLT” is the species, “1” is the sample number, “S2” is the fish size class, “CS” is the sampling site, “9-10-21” is the sampling date (mm-dd-yyyy), and “L” is the left or right otolith.

#### 2.4 Otolith imaging

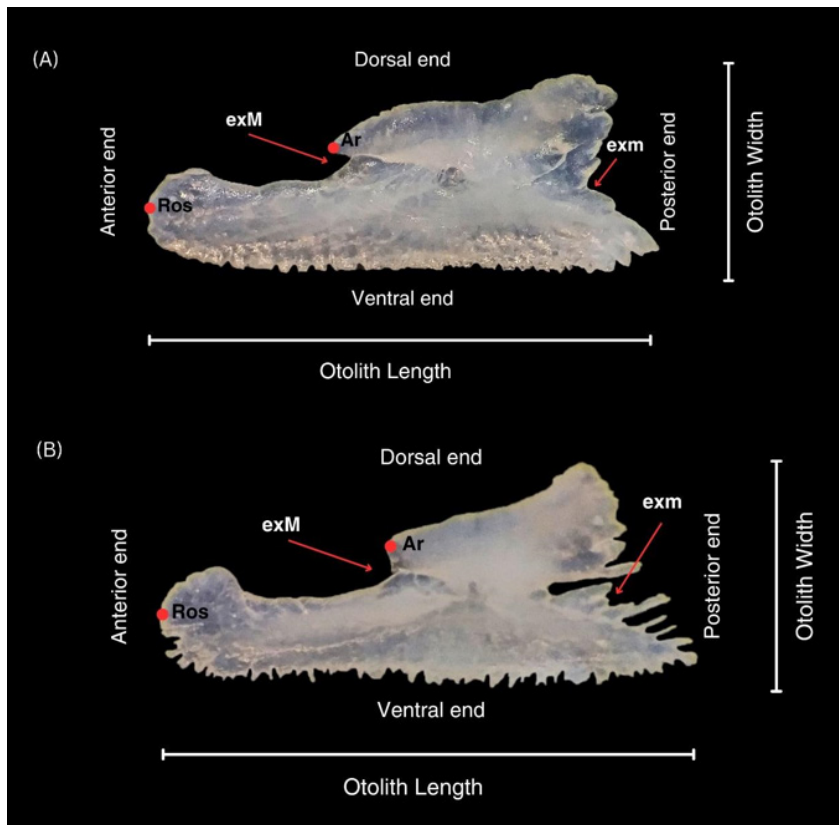
The perfectly extracted sagittal otoliths were placed on a dark background and photographed from

the distal surface facing up under a binocular stereo microscope (Motic SMZ-171) at 50x magnification. Consistently, the otolith sample and the camera were made to be parallel. These images were digitally enhanced, saved in 64-bit BMP format using Adobe Photoshop (CS6 portable ver.), and compiled for further analysis. In Figure 3, otolith shapes for each species were visually inspected using the digital image to describe their general appearance and formation of primary structures in the ventral, posterior, dorsal, and anterior regions (Hecht 1978; Morrow 1979; Smale et al. 1995; Tuset et al. 2006; Tuset et al. 2008;

Rossi-Wongtschowski et al. 2014). The analysis did not include blurred otolith images (Brophy et al. 2016).

### 2.5 Morphometric analyses

A total of 265 and 315 left sagittae of BT and FT, respectively, were extracted and analyzed (Table 1). As no study has investigated any statistical differences between the left and right otoliths of neritic tuna (Muñoz-Lechuga et al. 2023), only the left sagittal otoliths were used for the shape analysis to ensure consistency.



**Figure 3.** Distal view of otolith shape photomicrographs from (A) a 25.5 cmFL bullet tuna (*A. rochei*) and (B) a 27.0 cmFL frigate tuna (*A. thazard*). Ar (anti-rostrum), Ros (rostrum), exM (excisura major), and exm (excisura minor). Photos by AC Hedoquio and CMA De Vera.

**Table 1.** Mean size parameters and shape indices of the left sagittal otoliths of *A. rochei* and *A. thazard* across different sampling sites used in the Linear Regression Analysis, PERMANOVA, and PCA.

	<i>A. rochei</i>				<i>A. thazard</i>			
	Location	N	Mean	SD	Location	N	Mean	SD
<b>Fork Length (FL; mm)</b>	Celebes Sea	99	25.77	1.55	Celebes Sea	145	31.79	6.31
	Moro Gulf	118	24.04	1.17	Moro Gulf	103	24.49	3.48
	Davao Gulf	33	23.29	1.37	Davao Gulf	29	23.9	1.7
	Sarangani Bay	30	23.56	3.07	Sarangani Bay	38	24.2	5.22
	Total	265	23.96	3.21	Total	315	27.76	6.3

**Continuation of Table 1.** Mean size parameters and shape indices of the left sagittal otoliths of *A. rochei* and *A. thazard* across different sampling sites used in the Linear Regression Analysis, PERMANOVA, and PCA.

<b>Otolith Length (OL; mm)</b>	Celebes Sea	99	2.4	0.26	Celebes Sea	145	3.01	0.66
	Moro Gulf	118	2.17	0.24	Moro Gulf	103	2.35	0.37
	Davao Gulf	33	2.09	0.26	Davao Gulf	29	2.22	0.23
	Sarangani Bay	30	2.1	0.3	Sarangani Bay	38	2.32	0.62
	Total	265	2.19	0.11	Total	315	2.64	0.64
<b>Otolith Width (OW; mm)</b>	Celebes Sea	99	0.91	0.08	Celebes Sea	145	1.13	0.16
	Moro Gulf	118	0.86	0.08	Moro Gulf	103	0.99	0.13
	Davao Gulf	33	0.84	0.09	Davao Gulf	29	0.92	0.1
	Sarangani Bay	30	0.86	0.11	Sarangani Bay	38	0.95	0.2
	Total	265	0.86	0.11	Total	315	1.05	0.17
<b>Otolith Perimeter (OP; mm)</b>	Celebes Sea	99	9.83	1.93	Celebes Sea	145	13.57	2.6
	Moro Gulf	118	9.56	1.37	Moro Gulf	103	11.52	2.57
	Davao Gulf	33	9.3	2.1	Davao Gulf	29	10.38	2.15
	Sarangani Bay	30	9.32	1.58	Sarangani Bay	38	11.58	4.68
	Total	265	9.32	0.07	Total	315	12.36	3.11
<b>Circularity (CR)</b>	Celebes Sea	99	69.81	22.78	Celebes Sea	145	96.19	26.57
	Moro Gulf	118	74.96	15.38	Moro Gulf	103	95.4	27.6
	Davao Gulf	33	75.52	21.88	Davao Gulf	29	86.33	26.56
	Sarangani Bay	30	70.88	13.45	Sarangani Bay	38	100.58	42.68
	Total	265	70.88	20.51	Total	315	95.55	29.49
<b>Ellipticity (EL)</b>	Celebes Sea	99	0.45	0.04	Celebes Sea	145	0.45	0.05
	Moro Gulf	118	0.43	0.04	Moro Gulf	103	0.4	0.04
	Davao Gulf	33	0.43	0.03	Davao Gulf	29	0.41	0.04
	Sarangani Bay	30	0.42	0.04	Sarangani Bay	38	0.41	0.05
	Total	265	0.43	0.07	Total	315	0.42	0.05
<b>Form Factor (FF; mm)</b>	Celebes Sea	99	0.2	0.07	Celebes Sea	145	0.14	0.04
	Moro Gulf	118	0.18	0.04	Moro Gulf	103	0.14	0.04
	Davao Gulf	33	0.18	0.04	Davao Gulf	29	0.16	0.04
	Sarangani Bay	30	0.17	0.03	Sarangani Bay	38	0.15	0.05
	Total	265	0.2	0.07	Total	315	0.14	0.04
<b>Rectangularity (RE)</b>	Celebes Sea	99	0.65	0.04	Celebes Sea	145	0.58	0.05
	Moro Gulf	118	0.66	0.04	Moro Gulf	103	0.6	0.06
	Davao Gulf	33	0.66	0.05	Davao Gulf	29	0.62	0.05
	Sarangani Bay	30	0.65	0.04	Sarangani Bay	38	0.59	0.04
	Total	265	0.66	0.04	Total	315	0.59	0.05
<b>Roundness (RO)</b>	Celebes Sea	99	0.32	0.03	Celebes Sea	145	0.29	0.05
	Moro Gulf	118	0.33	0.03	Moro Gulf	103	0.33	0.05
	Davao Gulf	33	0.34	0.04	Davao Gulf	29	0.33	0.03
	Sarangani Bay	30	0.34	0.03	Sarangani Bay	38	0.32	0.04
	Total	265	0.33	0.04	Total	315	0.31	0.05

To identify regional differences, the measured size parameters and shape indices of otolith shapes were used. Due to the minute and fragility of the otoliths, first, size parameters such as otolith length (OL), otolith width (OW), otolith perimeter (OP), and otolith area (OA) were quantified using an open-source software, ImageJ ver. 1.54d (Rasband 2023). Second, otolith shape indices such as Circularity (CR), Ellipticity (EL), Form Factor (FF), Rectangularity (RE), and Roundness (RO) were also calculated following the formula of Osman et al. (2021) using the quantified size parameters.

### 2.6 Data analysis

Relationships of fish size and fork length (FL) between the size parameters and shape indices were evaluated using linear regression analysis in Microsoft Excel version 2312 (Figures 4, 5, 6, and 7) following the protocol of Deepa et al. (2019). Similarly, significant correlations ( $p < 0.05$ ) of size parameters and shape indices with FL were determined using IBM SPSS Statistics version 25 (Deepa et al. 2019). All size parameters and shape indices showed significant correlation with FL (Supplementary Table S2) and were standardized (scaled) using the slope ( $b$ ) from the linear regression models to remove the allometric growth effects (Lleonart et al. 2000; Zischke et al. 2016):

$$M_s = M_o \left( \frac{\bar{x}}{x} \right)^b$$

where,

$M_s$  is the variable corrected for allometry;

$M_o$  is the unscaled otolith size parameter and shape index;

$\bar{x}$  is the mean of fish fork length (FL) for all samples;

$x$  is the fish fork length (FL) of each sample;

$b$  is the slope of the linear regression.

The scaled size parameters and shape indices were processed and tested for the assumption of normality and homoscedasticity using the Shapiro-Wilk and Levene's tests, respectively, in PAST (PAleontological STatistics) software version 4.03 (Zischke et al. 2016; Deepa et al. 2019). Since the dataset violated the assumptions of normality and is heteroscedastic, a non-parametric counterpart of multivariate analysis (One-way PERMANOVA, using Bray-Curtis Similarity Index at a number of 9999 permutations) was performed using the PAST version 4.03 (Deepa et al. 2019). This nonparametric test on

non-normally distributed and non-homogenous datasets offers robust results by reducing inflation of type 1 error (Anderson and Willis 2003). Only CR was log-transformed ( $\log_{10}$ ) because its dataset has relatively larger numeric values compared to other shape indices (Anderson and Willis 2003). A post-hoc test using the sequential Bonferroni correction is then applied to identify inter-sampling site differences.

### 2.7 Principal Component Analysis

Principal Component Analysis (PCA) was applied to the pooled dataset of scaled size parameters and shape indices. This process helps visualize differences in their otolith shapes across sampling sites and identify which variables contribute mainly to these variations (Deepa et al. 2019; Cabasan et al. 2021; Morales et al. 2023). All variables are centered and scaled to interpret the resulting ordination plots (Cabasan et al. 2021). Only correlation with an absolute value higher than 0.3 loadings was accepted in interpreting results. PCA was performed in PAST version 4.03 (Deepa et al. 2019).

## 3. RESULTS

### 3.1 Otolith shape morphology and morphometry

Like many species of tuna, the otolith of BT exhibits an oblong shape that is very fragile and thin (Figure 3A). As it matures, its appearance undergoes morphological changes, including the formation of serrations or ridges along the flat ventral end (Supplementary Figure S1). The posterior margin appears irregular and develops multiple cristae-like grooves, including a small notch known as the excisura minor (Figure 3A). Meanwhile, the anterior region, particularly the rostrum, is slightly elongated and slightly rounded fan-shaped, while the anti-rostrum is moderately elongated and has a dull tip. There is also a narrow distance between the rostrum and the anti-rostrum. At maturity, the excisura major is prominent, with the otolith displaying opaque and translucent zones.

The otolith of the FT resembles that of the BT, characterized by its very fragile and thin structure (Figure 3B). It also follows an oblong shape, and its morphology changes as the fish matures (Supplementary Figure S1). During the early developmental phase, the otolith presents a translucent appearance, indicating that the anti-rostrum and posterior tips are still developing. As the fish matures, the otolith thickens and gradually forms into a more



opaque structure with distinct features becoming more pronounced. The dorsal margin appears irregular and moderately sculpted into a posteriorly raised structure. Moreover, the otolith's flat ventral and irregular posterior margins are covered with more distinct serrations or ridges along its edges than BT. The otolith's rostrum is distinctly elongated and slightly rounded, while the anti-rostrum is slightly elongated and has a very prominent narrow tip. At maturity, particularly at size 4, the excisura major located between the rostrum and anti-rostrum is also prominent.

All size parameters of FT are relatively larger than those of BT, especially its CR values (Table 1). However, BT's remaining shape indices, such as EL, FF, RE, and RO, are much larger than FT. All size parameters (OL, OA, OW, and OP) are significantly correlated ( $p < 0.05$ ) to fish length for both species (Supplementary Table S2), which positively increases as fish grows in length (Figures 4 and 5). Meanwhile, regression models for shape indices FF, RE, and RO appear to be negatively correlated with fish length for both species, while CR and EL demonstrated significant positive correlation ( $p < 0.05$ ) with fish length (Figures 6 and 7).

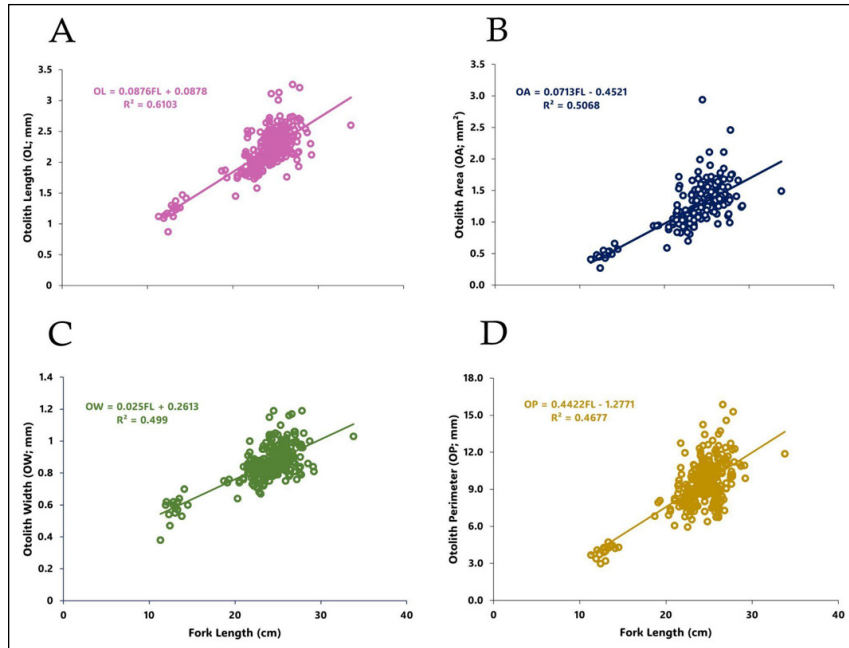


Figure 4. Linear regression of the otolith size parameters of bullet tuna (*A. rochei*) collected from different study areas (CS—Celebes Sea, MG—Moro Gulf, DG—Davao Gulf, SB—Sarangani Bay). (A) Otolith Length (OL; mm), (B) Otolith Area (OA; mm<sup>2</sup>), (C) Otolith Width (OW; mm), (D) Otolith Perimeter (OP; mm) with respect to fork length (FL).

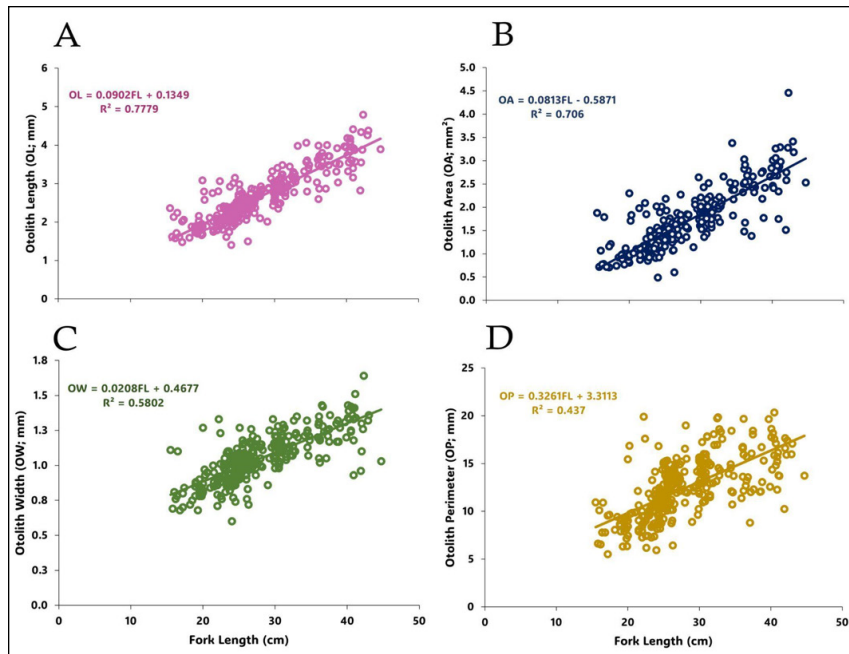
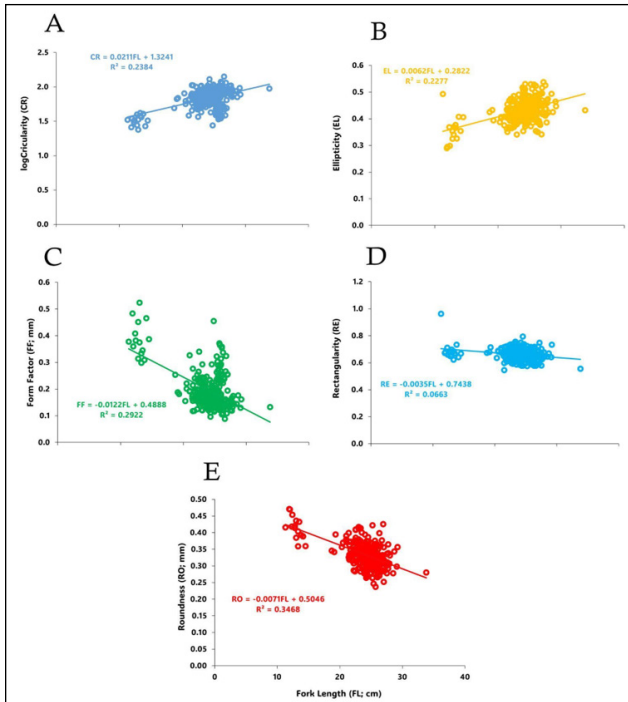
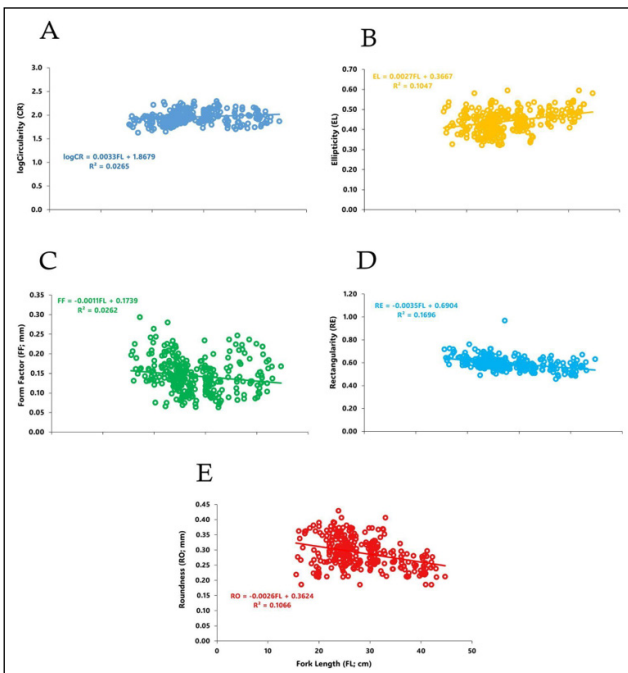


Figure 5. Linear regression of the otolith size parameters of frigate tuna (*A. thazard*) collected from different study areas (CS—Celebes Sea, MG—Moro Gulf, DG—Davao Gulf, SB—Sarangani Bay). (A) Otolith Length (OL; mm), (B) Otolith Area (OA; mm<sup>2</sup>), (C) Otolith Width (OW; mm), (D) Otolith Perimeter (OP; mm) with respect to fork length (FL).



**Figure 6.** Linear regression of the otolith shape indices of bullet tuna (*A. rochei*) collected from different study areas (CS—Celebes Sea, MG—Moro Gulf, DG—Davao Gulf, SB—Sarangani Bay). (A) log-transformed Circularity (CR), (B) Ellipticity (EL), (C) Form Factor (FF; mm), (D) Rectangularity (RE), and (E) Roundness (RO) with respect to fork length (FL).



**Figure 7.** Linear regression of otolith shape indices of frigate tuna (*A. thazard*) collected from different study areas (CS—Celebes Sea, MG—Moro Gulf, DG—Davao Gulf, SB—Sarangani Bay). (A) log-transformed Circularity (CR), (B) Ellipticity (EL), (C) Form Factor (FF; mm), (D) Rectangularity (RE), and (E) Roundness (RO) with respect to fork length (FL).

### 3.2 Principal Component Analysis

Principal components and variance proportions of size parameters and shape indices of BT and FT were presented in Tables 2 and 3. For BT, the total proportion of variation captured by PCA is 77.27%, where PC 1 explained 52.11% of the model, and PC2 captured 25.16% of the variation (Figure 8A). PC 1 captured the variation related to RE ( $r^2 = 0.44$ ), RO ( $r^2 = 0.44$ ), FF ( $r^2 = 0.42$ ), LogCR ( $r^2 = 0.41$ ), and EL ( $r^2 = 0.38$ ). Meanwhile, PC2 captured the variation in OL ( $r^2 = 0.57$ ) followed by OA ( $r^2 = 0.50$ ), OP ( $r^2 = 0.47$ ), and OW ( $r^2 = 0.43$ ) (Table 3). The shape indices in BT positively correlate as they appear relatively close to each other (less than a 90-degree angle) between Axes 1 and 4 of the planes (Figure 8A).

PCA results of FT revealed that PC 1 and PC 2 captured 82.51% of the variation in the dataset (Figure 8B). The proportion of variation captured by PC 1 is 52.10%, where RE ( $r^2 = 0.44$ ) contributed the most to this variability, followed by LogCR ( $r^2 = 0.43$ ), RO ( $r^2 = 0.42$ ), EL ( $r^2 = 0.40$ ), OW ( $r^2 = 0.35$ ), and FF ( $r^2 = 0.31$ ). In PC 2, the proportion of variance is 30.41%, with variables OA ( $r^2 = 0.53$ ), OL ( $r^2 = 0.51$ ), OP ( $r^2 = 0.48$ ), and FF ( $r^2 = -0.31$ ) being the most influential variables in defining this principal component (Table 3). Overall, among the size parameters and shape indices evaluated, LogCR, EL, FF, RE, and RO were the most important variables differentiating the stocks of BT and FT across all sampling sites, as shown by PC 1 (Figure 8B).

### 3.3 PERMANOVA

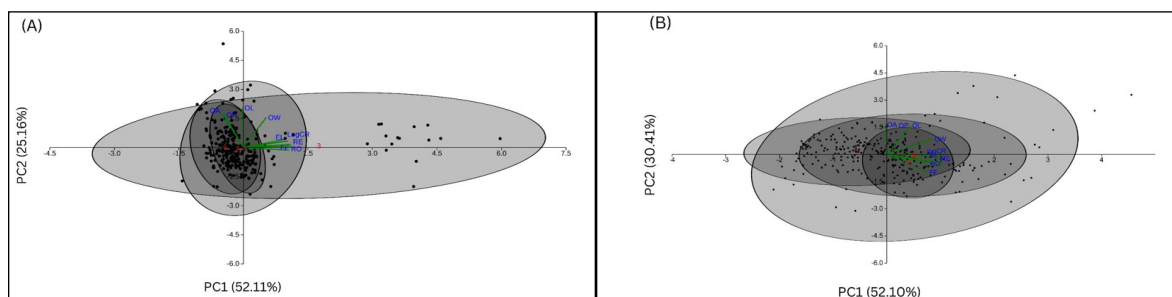
Size parameters and shape indices for each species revealed significant differences ( $p < 0.05$ ) across the geographical areas (Table 4). Upon pairwise comparisons using the sequential Bonferroni correction, specific geographical areas with significant phenotypic differences were identified (Supplementary Table S3). Applying the sequential Bonferroni correction to  $p$ -values revealed that western Celebes Sea ( $p = 0.039$ ) and Moro Gulf ( $p = 0.80$ ) stocks are non-significant with that of Sarangani Bay. The remaining geographical areas showed significant differences with each other.

**Table 2.** The proportion of variance in the first five axes of the PCA.

Principal Components	Species			
	<i>A. rochei</i>		<i>A. thazard</i>	
	Eigenvalue	% variance	Eigenvalue	% variance
1	4.68966	52.107	4.68884	52.098
2	2.26423	25.158	2.73685	30.409
3	0.835843	9.2871	0.819656	9.1073
4	0.622845	6.9205	0.537255	5.9695
5	0.296519	3.2947	0.161468	1.7941

**Table 3.** Correlation coefficient values between principal components and otolith morphometric variables, with correlations exceeding an absolute value of 0.3, are highlighted in bold.

Species	Variables	PC 1	PC 2	PC 3	PC 4	PC 5
<i>A. rochei</i>	LogCR	0.41286	0.09388	0.22104	0.30657	0.13097
	EL	0.37821	0.1139	0.41566	-0.34299	0.43602
	FF	0.42246	-0.0375	-0.26813	-0.30671	-0.23084
	RE	0.44453	0.047221	0.11574	-0.01364	0.13749
	RO	0.44022	0.020573	-0.11243	0.23083	-0.14289
	OA	-0.20029	0.49832	-0.33314	-0.22407	0.61364
	OP	-0.17687	0.46721	0.47141	0.48803	-0.0705
	OL	-0.00933	0.56684	0.18647	-0.48031	-0.56032
	OW	0.21246	0.43156	-0.55898	0.34894	-0.06813
<i>A. thazard</i>	LogCR	0.43222	-0.027551	-0.37207	-0.00012729	-0.16122
	EL	0.40172	-0.031483	-0.046365	0.66252	-0.057217
	FF	0.31356	-0.31397	0.54868	0.0068577	-0.2044
	RE	0.43537	-0.15294	0.049873	0.033265	0.5037
	RO	0.41869	-0.18372	0.011457	-0.34756	0.31994
	OA	-0.012329	0.52787	0.48952	-0.082616	0.46057
	OP	0.17355	0.48206	-0.49454	-0.039028	0.19937
	OL	0.18373	0.50724	0.25763	0.35056	-0.2733
	OW	0.353	0.27242	0.071989	-0.55486	-0.4963



**Figure 8.** Differentiation of otolith shape morphometry of (A) *A. rochei* and (B) *A. thazard* across the study sites using Principal Component Analysis. Codes: 1—Celebes Sea, 2—Moro Gulf, 3—Davao Gulf, and 4—Sarangani Bay.

Contrary to the FT population, Sarangani Bay, Davao Gulf, and Moro Gulf demonstrated significant phenotypic differences ( $p < 0.05$ ) when each geographical area was compared to the western Celebes Sea. Moreover, the stock from Moro Gulf demonstrated a significant phenotypic difference

when compared with the stock from Davao Gulf ( $p < 0.05$ ) but non-significant with Sarangani Bay ( $p = 0.35$ ). Lastly, the fish population from Davao Gulf showed no significant phenotypic difference ( $p = 0.34$ ) from the stock from Sarangani Bay.

**Table 4.** Results from the PERMANOVA for the two *Auxis* spp. with Bray-Curtis Similarity Index at 9999 permutations.

Species	Total sum of squares	Within-group sum of squares	F	p-value
<i>A. rochei</i>	1.476	1.291	13.2	<b>0.0001</b>
<i>A. thazard</i>	2.407	2.21	9.238	<b>0.0001</b>

## 4. DISCUSSION

### 4.1 Growth pattern of otolith shape

Based on the linear regression models for both *Auxis* spp., multiple shape indices and size parameters revealed significant correlations with fish length. This implies that otolith shape development is linked to fish growth (Campana and Casselman 1993; Tuset et al. 2003; Tuset et al. 2008; Bani et al. 2013; Leguá et al. 2013). Specifically, the growth pattern of otolith for both species is strongly associated with an increase in CR and EL as well as OA, OL, OW, and OP, as inferred from the statistically significant linear relationship with FL ( $p < 0.05$ ) (Supplementary Table S2). This suggests that the growth increment of the otolith (Tuset et al. 2003) is not even throughout but consistently shows an increase for the parameters OA, OL, OW, and OP, including the overall shape, such as CR and EL. In contrast, the shape indices FF, RE, and RO almost remained the same (Figures 6 and 7). These results strongly indicate that otoliths of the *Auxis* spp. develop more circular and elliptical shapes as they grow in body length.

The relatively large mean values of all otolith size parameters in FT compared to BT indicate an association with the somatic growth in fish. Specifically, the FT otolith appears more circular than the BT, as inferred from its large CR mean value. Similar to other teleost fishes, Bani et al. (2013) observed that CR is one of the most efficient variables that explained the otolith differences between the sympatric Caspian goby (*Neogobius caspius*) and Caspian bighead goby (*Ponticola gorlap*) in the Caspian Sea, which is characterized by a more circular shape due to its relatively large observed CR value.

Otolith shapes are stock and species-specific (Campana and Casselman 1993; Neves et al. 2011; Avigliano et al. 2018; Barnuevo et al. 2023; Park et al. 2023). Even sympatric congeners are expected to be distinct (e.g., Bani et al. 2013; Zischke et al. 2016; Avigliano et al. 2018). One example of its stock specificity is by visually comparing the similar but structurally distinct otolith morphologies of BT and FT of the Philippines from the *Auxis* spp. that inhabit the waters of Taiwan (see Tsai 2010a, 2010b). These variations may be due to past population

expansions that separated these stocks by various phylogeographic barriers. Because of these barriers, it resulted in the unique genetic make-up of each species and population, which currently plays most of these phenotypic differences (Pedrosa-Gerasmio et al. 2015; Ollé-Vilanova et al. 2022; Muñoz-Lechuga et al. 2023). As a result, these differences may significantly influence tunas' morphology, distribution, life history, and population dynamics (Uchida 1981; Sharp 2001).

### 4.2 Life-history traits influence differences in phenotypic stocks

In this work, otolith morphometric analysis successfully differentiated the phenotypic stocks of the *Auxis* spp. within the Celebes Sea. This result is consistent with the estimated pseudo-F-values for the PERMANOVA test used in similar studies on otolith morphometric analysis to distinguish phenotypic stocks of Redtail Scad (*D. kurroides*, Barnuevo et al. 2023), Opal fishes (*B. caudimacula*, Deepa et al. 2019), and Little tunny (*E. alletteratus*, Muñoz-Lechuga et al. 2023). Moreover, pairwise comparisons further revealed that BT and FT stocks from Sarangani Bay are connected and phenotypically similar to the stock from Moro Gulf. This observation is also true for the FT stock of Sarangani Bay, which showed connectivity with Davao Gulf. Interestingly, the western Celebes Sea stock appears connected to Sarangani Bay. These results imply that mixed stocks of BT and FT exist within the Celebes Sea.

Tuna are generally assumed to have homogenous population structures within and across large ocean basins, likely due to their migratory behavior (Kumar et al. 2012). For example, Santos et al. (2010) and Pedrosa-Gerasmio et al. (2015) found that Eastern little tuna (*E. affinis*; ELT) and FT within the Celebes Sea have homogenous genetic population structures, respectively. However, evidence suggests that aside from genetics, life history traits and the environmental parameters where fish inhabit may create demographic population subdivisions among neritic tuna species (Kumar et al. 2012). Furthermore, these population subdivisions may vary in mortality rates, larvae recruitment, and fish growth (Neves et al. 2021). In this study, pairwise comparisons revealed that the group of FT from the western Celebes Sea is

significantly different with Davao Gulf and Moro Gulf, implying separate phenotypic stocks likely due to FT samples collected along the southern margin of the Tawi-Tawi Islands near the Sulu Sea.

The pairwise comparison results of BT and FT from Sarangani Bay sharing the same stocks with Moro Gulf indicate population connectivity. This connectivity can be explained by the fact that neritic tuna spawn in coastal areas (Kodama et al. 2022; Gonzalo et al. 2023) and undertake short migration distances along continental shelves (Kumar et al. 2012, 2015). The present study also collected tuna samples with smaller FL in Sarangani Bay. This may imply that the bay is a nursery ground for neritic tuna species. Alvarez and colleagues (2015) suggest that larval retention in bays is relatively high due to the interactions between geostrophic velocities and their topographic features. These interactions often create suitable environmental conditions for fish growth, survival, and reproduction (Block et al. 2005; Kumar et al. 2012; Brophy et al. 2016; Wells et al. 2020; Ollé-Vilanova et al. 2022). This phenomenon was observed in the study of Servidad-Bacordo et al. (2012), where some of the *Auxis* spp. egg larvae were present in nearby coastal areas of the Celebes Sea near Sarangani Bay and Davao Gulf. Therefore, this may explain why BT stock from Sarangani Bay is phenotypically similar to the Celebes Sea and Moro Gulf.

Overall, these life-history traits, such as coastal distribution, limited migration distance, and homing behavior, were observed in ELT in Malaysia (Binashikhbubkr et al. 2023), Indian mackerel (*R. kanagurta*) in Indonesia (Wujdi et al. 2022), and FT in the Indian Ocean (Kumar et al. 2012). Such traits are significantly different from their large, highly migratory Scombrid counterparts, such as the Yellowfin tuna (*Thunnus albacares*), Bigeye tuna (*T. obesus*), Atlantic bluefin tuna (*T. thynnus*), and Skipjack tuna (*Katsuwonus pelamis*), which generally undergo oceanic migration, occupy extensive reproductive areas, and have high larval dispersal and population connectivity (Block et al. 2005; Maguire et al. 2006).

#### 4.3. Long-term habitat exposure and phenotypic variations in otoliths

Evidence also strongly supports the impact of the local environment on phenotypic differences between stocks in various fish taxa (Neves et al. 2021). This variation in the shape morphology may be due to the prolonged exposure to local habitats that offer

unique profiles of water current, temperature, water chemistry, underwater topography, monsoon season, and prey availability (Moreira et al. 2019; Neves et al. 2021). This process is assumed to create demographic isolation of stocks due to phenotypic plasticity in fish (Campana and Casselman 1993; Ferguson et al. 2011; Chen et al. 2021; Kikuchi et al. 2021a).

For example, studies showed that oceanic currents create barriers to the dispersal or transport of fish larvae (Cowen and Sponaugle 2009; Jackson et al. 2014). In the western Pacific Ocean near the Davao Gulf, the geostrophic velocities caused by the complex interactions among the southward-flowing Mindanao Current, the anti-clockwise direction of the Mindanao Undercurrent, the Mindanao Eddy (Jackson et al. 2014; Schonau et al. 2015), and the Sangihe Ridge (Gordon et al. 2003) form strong vertical gradients in the water column. As a result, these interactions may form barriers or extend the distribution of larvae relative to the direction of these ocean currents (Cowen and Sponaugle 2009; Jackson et al. 2014; Damatac and Santos 2016).

In this study, distinct oceanographic conditions of each sampling site may have influenced the shape morphology variations among stocks. One factor that contributes to this differentiation is upwelling (Neves et al. 2011; Neves et al. 2021). Upwelling can impact the incorporation rates of trace elements in otoliths, likely due to variations in the chemical composition of the water and the seabed's geochemistry (Neves et al. 2011; Cabasan et al. 2021). These trace elements are usually absorbed in the otolith's matrix through water intake or prey consumption (Hüssy et al. 2020; Nazir and Khan 2021). For instance, Asante et al. (2010) observed high amounts of Sr in the tissues of small pelagic fishes in the Celebes Sea, alongside Zinc (Zn), Mn, Mg, and Cu. Although these trace elements are observed to occur in fish tissues (Asante et al. 2010), it is possible that these elements may accumulate in fish otoliths (Hüssy et al. 2020). This may be true in most tuna species since they are considered top predators of the marine food web. They consume prey at high rates to compensate for the high energetic costs of migration, reproduction, and prey hunting (Kojadinovic et al. 2007). Therefore, they are predicted to forage around upwelling zones since highly productive areas attract abundant prey items (Kumar et al. 2012; Arrizabalaga et al. 2015). In the Celebes Sea, upwellings commonly occur near its underwater sills (e.g., Sulu Ridge and Sangihe Ridge) (Takeda et al. 2007). These underwater sills function as topographic barriers against incoming

underwater currents from the Sulu Sea and the western Pacific Ocean – where less dense, cold water of the Celebes Sea is pushed upward to the surface, creating upwelling areas (Takeda et al. 2007).

The local geophysical makeup of the Celebes Sea basin may have contributed to the accretion of otoliths in fish. Generally, the Celebes Sea basin is encrusted by sediments from volcanoes, biological materials, and eroded mafic and ultramafic rocks (Murauchi et al. 1973; Nichols and Hall 1999; Wong 2005). Nichols and Hall (1999) discovered that the seafloor's basement is composed of basalt, while the upper portion is layered with sediment materials derived from local and regional land erosion, carbonates, and volcanoes. Davao Gulf's seafloor, however, is covered with black volcanic sedimentary deposits from Mount Apo and potentially from the Sangihe Ridge (Wong 2005). Meanwhile, the southern margin of Tawi-Tawi is characterized by basic and ultrabasic igneous rocks, which are then layered by sediment deposits from eroded marine clastics (Murauchi et al. 1973). Its remaining islands are made of limestone deposits from dead corals, volcanic basalts, environmental sediments, and metamorphic rocks deposited during the pre-Jurassic era (Murauchi et al. 1973). In Sarangani Bay, Krause (1966) suggested that the bathymetric features of the bay facilitated the transport of sedimentary materials deposited in the central basin of the Celebes Sea through underwater currents. Krause (1966) also discussed that Rio Grande de Mindanao — the largest river basin on Mindanao Island — contributed much of the sedimentary deposits in Illana Bay, located within the Moro Gulf. Overall, these geophysical processes impact the chemical composition of substrate in each local habitat (Cabasan et al. 2021), shaping the amount of trace elements in the water column such as Sr, Silicon (Si), Aluminum (Al), Iron (Fe), Mg, Mn, and CaCO<sub>3</sub>, and thus influence otolith shape variation among stocks (Hüseyin et al. 2020; Nazir and Khan 2021).

#### 4.3. Conservation implications

As early as 1996, species of the genus *Auxis* have been predicted to be exploited beyond their sustainable threshold (Collette and Aadland 1996). This conservation concern has now been reported in various geographical areas across the world, such as the (a) BT in the Mediterranean Sea, where no management systems exist due to the paucity of data on their biology and population structures (Ollé-Vilanova et al. 2022); (b) fisheries of FT and BT in the Babuyan

Channel in northern Philippines (Calicdan-Villarao et al. 2017); and (c) FT in the Indian Ocean (Ghosh et al. 2012). In the GSCFPC, Emperua et al. (2018) provided direct evidence of the declining catch volume of small pelagic fish, including the Scombrids, which dropped by 6% from 2008 (92%) to 2012 (86%). Similarly, FT's national catch volume production has declined by 24.91% from 111,916.27 MT in 2018 to 84,012.67 MT in 2022 (FPED-BFAR 2023). These declines are likely attributed to increased resource exploitation due to the growing market demands, habitat degradation, illegal fishing, and poor enforcement of fishing regulations (Pechon et al. 2022).

Although these decrease in fish catch is a result of a combination of factors, it is particularly concerning in the Philippines, considering that limited studies on the stock structure of neritic tunas are available and are geographically biased toward the Celebes Sea (e.g., ELT, Santos et al. 2010; FT, Pedrosa-Gerasmio et al. 2015). Such a lack of research effort on their population structures could mean that a unique genetic or phenotypic lineage may become overfished, potentially leading to declines in population abundance (Juan-Jordá et al. 2015; McCauley et al. 2015). These changes in population abundance could significantly impact trophic interactions in the local marine ecosystems since these Scombrids are vectors of trophic energy transfer (Estes et al. 2016; Varela et al. 2024). To prevent increased exploitation of tuna stocks to unsustainable levels (Barut and Santos 2000), enhancing knowledge acquisition on its population structure is imperative to develop effective management strategies for improved outcomes and thus prevent population collapse (Aprieto 1981; MacKenzie et al. 2009; Gadut et al. 2021).

Otolith shape analysis can be a promising tool for developing countries with limited technical expertise and resources (Darwall and Allison 2002; Headley 2020). To date, otolith shape analysis is used by fishery managers not only to taxonomically classify sympatric fish species (e.g., Bani et al. 2013; Zischke et al. 2016; Avigliano et al. 2018; Neves et al. 2021; Morales et al. 2023) but also delineate different phenotypic stocks across vast oceanic spaces. Only Muñoz-Lechuga and colleagues (2023) applied the otolith shape analysis to delineate phenotypic stocks for neritic tuna species. However, no study has used this method on tuna and tuna-like species in the Celebes Sea. Despite this, the wide applicability of otolith shape analysis reinforces this technique's significance in informing strategies in fisheries management (Brophy et al. 2016; Neves et al. 2021; Muñoz-Lechuga

et al. 2023) and should warrant an increase in research allocation for these highly exploited species.

## 5. CONCLUSION AND RECOMMENDATIONS

Through otolith shape analysis, this study found that *A. rochei* and *A. thazard* occur in mixed stocks in the Celebes Sea. Moreover, this work is the first to investigate the differences in otolith shape morphology and phenotypic stock variation of these species in the Philippines. Despite this contribution, this research shares a few limitations that should motivate future research on neritic tuna and its closely related species. First, the unequal distribution of sample size per size class may have contributed to some biases in the analysis. This may be true for *A. rochei* since no size 4 samples were included in the study. To reduce this inter-sample bias, equal size class representation and maintaining a balanced sample size among the sampling sites are needed for a more robust interpretation of the results. Second, since the sampling was conducted for over three years, seasonal dynamics of population structures due to interannual variability could also influence the result. Third, sex differences may also impact otolith shape morphology, and should be examined further since the reproductive biology of the *Auxis* spp. in the southern Philippines has recently been elucidated (Entia et al. 2024a, 2024b). Specifically, studies comparing the biological and reproductive characteristics of these distinct spatial populations within the Celebes Sea must be carried out, such as their spawning periods, otolith-based aging, and larval dispersal modeling.

While genetic analysis has proven to be the most reliable technique to delineate fish stocks, it must be combined with otolith shape analysis and chemical fingerprinting using trace elements (Neves et al. 2011; Neves et al. 2021). Such an integrated approach will significantly improve the understanding of the complex population structures in the Celebes Sea. Despite this, the information from this report should help improve stock assessments and sustainable fishery management for these highly exploited commercial species with poorly understood life history and population structure in the country.

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## SUPPLEMENTARY MATERIAL

Below is the link to the electronic supplementary material. Supplementary file

## AUTHOR CONTRIBUTIONS

**Tampoy DA:** Investigation, Methodology, Writing - Original draft, Data curation, Software, Visualization. **De Vera CMA:** Investigation, Methodology, Software, Data curation, Visualization, Editing - Original draft preparation. **Templado JBJ:** Methodology, Investigation, Data Curation. **Ledda IVS:** Methodology, Investigation, Data Curation. **Hedoquio AC:** Visualization, Investigation, Data Curation. **Abella RP:** Methodology, Investigation, Data Curation. **Matunog MWM:** Methodology, Investigation, Data Curation. **Cuanan AM:** Visualization, Investigation, Data Curation. **Amoncio**

**RADA:** Visualization, Investigation, Data Curation.  
**Ortiz AT:** Investigation, Methodology, Visualization  
**Guevarra EP:** Project Administration, Supervision, Conceptualization, Funding Acquisition. **Nañola CL:** Conceptualization, Methodology, Software, Writing-Reviewing and Editing, Data Validation.

#### CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest with respect to the research, authorship, and/or publication of this paper.

#### DATA AVAILABILITY

The datasets used or generated in this study are available from the corresponding authors upon reasonable request.

#### ETHICS STATEMENT

This research guarantees strict compliance with institutional and national restrictions on animal use as subjects. The study collected fish of commercial value. Permits to conduct the study in the Sarangani Bay Protected Seascape (SBPS) were obtained from the Protected Area Management Board of the SBPS (PAMB-SBPS) through the PAMB-SBPS Resolution No. 2020-041. Moreover, the fish were already dead when collected at fish landing ports or fisherfolk, which exempted this research from the Department of Agriculture's Admin Order No 21 Series of 1999 and the Republic Act No. 8485 or the Animal Welfare Act of 1998.

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