RESEARCH ARTICLE

Age Determination and Growth of Frigate Tuna (Auxis thazard, Lacepede, 1800) in the Celebes Sea

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- ABSTRACT -

Frigate tuna (Auxis thazard, Lacepede, 1800) is a commercially valuable food fish thriving in tropical marine waters. In the Philippines, despite its abundance, information on its estimated age and growth patterns is lacking. This study reported the relationship between the size (fork length, FL) and age (otolith growth increment) of frigate tuna using the sagittal otoliths. Sampling was conducted in the Celebes Sea, including the inner bay and gulfs within it (e.g., Moro Gulf, Sarangani Bay, and Davao Gulf) from November 2020 to September 2022. A total of 1,046 frigate tuna samples with sizes ranging from 14.5 cmFL to 44.7 cmFL, caught using ring nets, hand lines, and purse seine nets, were examined. Results of the aggregated fish lengthweight relationship ($R^2 = 0.9819$; a = 0.0068; b = 3.2632) indicated a strong positive allometric growth pattern. Similarly, a strong positive correlation ($R^2 = 0.7130$) between the otolith length (n = 344) and fork length relationship indicates that the otolith increases in length as the fish grows. However, for the successfully aged otolith sections, only individuals collected in the western Celebes Sea (n = 113) demonstrated a good result covering ages ranging from one to four years old. From this information on the age-growth relationship, the von Bertalanffy growth function model generated the following values for L∞ at 62.1 cmFL and K at 1.0016/yr. The latter growth parameter estimates from otolith microstructure is the first record for frigate tuna derived from the age-length data.

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1. INTRODUCTION

Trigate tuna (Auxis thazard, Lacepede, 1800) is ◀ a warm water species among the pelagic neritic tuna that thrives up to 50 m depth (Collette and Nauen 1983; Uchida 1981; Liu 2008; Ajik and Tahiluddin 2021; Zhou et al. 2022). Records of its maximum size in fork length (FL) range from 58.0 cm in Sri Lanka (Collette and Nauen 1983) to 65.0 cm in the eastern tropical Atlantic (Morice 1953). This species is highly migratory, often observed in schools with other scombrids, (Maguire et al. 2006; Gomez 2019) and reported to spawn along its migration route within the Pacific Ocean, which passes through the

Philippines (Ratilla et al. 2016). Pedrosa-Gerasmio et al. (2015) reported that this species has no distinct genetic population in the Southern Philippine Seas, including Palawan, Indonesia, and Malaysia, which reinforces the fact that they are migratory. In the Philippines, it is locally known as "bodboron", "perit," or "mangkoh" and it is one of the most sought-after scombrids due to its lucrative value (Llanto et al. 2018). In 2021, it ranked second and accounted for 20.3% (93,783.72 MT) of the country's total 462,400.9 MT volume production of tuna species (Bureau of Fisheries and Aquatic Resources 2022).

Along the country's vast coastlines, General Santos City is a known fish landing site for frigate tuna

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and its other relatives (Zaragosa et al. 2004; Pechon et al. 2022), primarily due to its proximity to the tuna-fishing grounds in the Celebes Sea, specifically the inner seas (e.g., Sarangani Bay, Davao Gulf, and Moro Gulf) (Figure 1). Furthermore, the city has been dubbed the "Tuna Capital of the Philippines" because of the high volume of daily landed tuna catch (Yu 2010; Macusi et al. 2015; Prieto-Carolino et al. 2021). In 1994, the General Santos City Fish Port Complex (GSCFPC) was built (Israel 2000) and has since supplied fresh tuna for the six processing plants in the city (Nanardx 2016). From 2013 to 2017, the accumulated catch of frigate tuna contributed 4.6% (17,662.0 MT) to the 384,140.0 MT of the total number of all tuna species landed in GSCFPC (Pechon et al. 2022).

However, despite frigate tuna's economic contribution to the Philippines, none so far has attempted to study the relationship between the direct age and growth of frigate tuna. All studies were based on length-frequency analysis (Ingles and Pauly 1984; Armada 2004; Calicdan-Villarao et al. 2017). It has to be recalled that stock assessment and appropriate management schemes are vital keys to the sustainability of a fishery resource. Nowadays, fisheries management stock assessment models heavily depend on the data on size-at-age and growth (Murua et al. 2017), which requires precise age information of the species (Campana 2001; Xu and Xu 2017). Thus, the otolith age-based approach gained popularity because it offers the most accurate, reliable, and easily discernible increments of annual growth (Secor et al. 1992; Campana and Thorrold 2001; Robbins and Choat 2002; Begg et al. 2005; Phelps et al. 2007; Gunn et al. 2008; Choat et al. 2009; Zhiming et al. 2018). Although only a few quantified the age and growth of frigate tuna using the length-frequency approach and counted spines and vertebral increments (Silas et al. 1985; Marriott and Cappo 2000; Ghosh et al. 2010; Khan et al. 2011; 2012; Tao et al. 2012; Calicdan-Villarao et al. 2017; Mudumala et al. 2018; Lelono and Bintoro 2019; Vieira et al. 2022), no present study has investigated counting otolith increments.

Here, we examined the otolith growth increments of frigate tuna individuals within the Celebes Sea. The objectives of this study were to (1) establish a correlation between the fish length-weight and the fish length-otolith length relationships and (2) compare and provide baseline information on its age and growth patterns across study sites. The contribution of this paper is to improve the utility of frigate tuna's stock assessment models and significantly impact the crafting of policies for its fisheries management plans.

2. METHODS

2.1 Sample collection

All frigate tuna samples were collected from different fish landings and wet markets within the

> Celebes Sea (Figure 1), from November 2020 to September 2022. Most of the collected fish samples from GSCFPC came from Moro Gulf and Celebes Sea, while only very few samples were from Davao Gulf. The Sarangani Bay samples were collected from the fish landings and wet markets in Sarangani Kiamba, Province. Additional samples were also collected from the town of Bongao, Tawi-Tawi Province. Strategic sampling was employed to represent, as much as possible

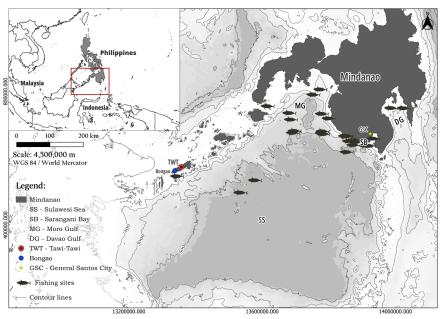


Figure 1. Map showing the study locations within the Southern Philippine Seas composed of Sarangani Bay (SB), Moro Gulf (MG), Davao Gulf (DG), and Celebes Sea (CS). Bathymetry details adapted and modified from Alcala et al. (2008).

the various size classes reported by the Bureau of Fisheries and Aquatic Resources - National Stock Assessment Program (2018). These size classes are as follows: size 1: < 20.0 cmFL; size 2: 20.1–30.0 cmFL; size 3: 30.1-40.0 cmFL; and size 4: > 40.1 cmFL. During the collection process at each landing site, the types of fishing gear used by large private tuna fleets and local fishers were noted. Commercial fishing vessels commonly employ ring nets (locally known as "likum-likum" or "pangulong") and purse seine nets ("pangulong") to catch tuna (Dickson and Natividad 2000; Armada 2004; Macusi et al. 2015; Llanto et al. 2018), whereas local fishers use hand lines ("kawil," "pasol," or "bira-bira"), particularly those that fish in Sarangani Bay (West et al. 2011). Ring nets (known to locals as "kulibo") are commonly employed in the waters of Tawi-Tawi (Ajik and Tahiluddin 2021) or on the western side of the Celebes Sea. All fishing activities that occurred in all areas were found to be closer or within fish aggregating devices (FADs), locally known as "payao" (Barut 2002; Dickson and Natividad 2000; Hipolito and Vera 2006; Macusi et al. 2015).

All collected tuna samples were brought to the Histopathology and Molecular Biology Laboratories at the Regional Science Research Center (RSRC), Mindanao State University, Fatima, General Santos City (MSU-GSC), for further processing. On the other hand, samples from Tawi-Tawi were processed on-site up to otolith extraction at the wet laboratory of the MSU-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO). Each sample was processed as follows: labeling of each specimen, fish morphometrics determination (e.g., individual length and weight) and otolith extraction. For the otolith preparation,

the following steps were followed: image processing, section preparation, and lastly, the annual growth increment readings (Nañola and Fortaleza 2023).

2.2 Fish morphometrics and length-weight relationship

Each tuna sample collected was weighed for its body weight (BW; 0.1 g). The standard fish measurements such as fork length (FL at 0.1 cm; measured from the tip of the tuna's snout to the fork of

the tail), standard length (SL at 0.1 cm; measured from the tip of the tuna's snout to the posterior of the last vertebra), total length (TL at 0.1 cm; measured from the tip of the tuna's snout to the most posterior of the caudal fin), and body girth (at 0.1 cm; measured around the body at its largest point) were also taken. The length-weight relationship was calculated using a power regression function defined by the equation $W = aL^b$ (Ricker, 1975), where 'a' is the proportionality constant or intercept and 'b' is the exponent. The 'b' value indicates whether a somatic growth is allometric (b < or > 3) or isometric (b = 3) (Anibeze 2000; Froese 2006).

2.3 Otolith morphometrics

The pair of sagittal otoliths from each fish individual was carefully extracted from its head through the open-the-hatch technique (Secor et al. 1992). Only one perfectly extracted otolith of each pair (Elumba et al. 2019) was examined under a stereo microscope (Motic SMZ171) and photographed using a mobile phone. The otolith photomicrographs were analyzed through an open-source software (OSS), the ImageJ software v.4.0. The variables measured were as follows: otolith length (OL), otolith width (OW), otolith perimeter (OP), and otolith area (OA). All measurements were recorded in millimeters (0.01 mm) except for OA, which is expressed in mm2. Furthermore, OL is described as the distance from the anterior tip to the posterior tip (Figure 2). Whereas, OW is the distance perpendicular to the length passing through the core (Hunt 1992) (Figure 2). OA was measured based on the perimeter length of the otolith generated from ImageJ software that automatically

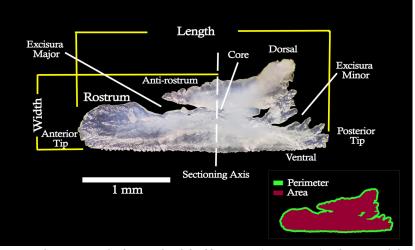


Figure 2. Photomicrograph of a sagittal otolith of frigate tuna (FL = 30.9 cm) with 1 mm scale bar for size reference. Image taken by ACHedoquio.

converts the units into an area (Manginsela et al. 2020; Yedier 2021).

2.4 Otolith sectioning

As previously described by Wild and Foreman (1980), there are no significant differences in the micro-incremental growth between the left and right otoliths of tropical tuna species. Thus, when available, the left otoliths were prioritized for age estimation. Otherwise, their right counterpart was used. The grind-and-polish method of Robbins and Choat (2002) was followed to section each otolith. A CrystalbondTM 509 was used as a mounting medium while varying sandpaper grits (P800, P1200, and P1500) were used to grind each side of the otolith manually (Adam et al. 1995; Doray et al. 2004). While doing the manual grinding, drops of tap water were added frequently to lessen the friction between the otolith and sandpaper (Charles et al. 2013). At the final grinding stage (after flipping over the otolith), the exposed section of the otolith was lastly polished using a 3-micron lapping film for a smoother finish. During the last step, the sagittal otolith was regularly examined under a light compound microscope (LW Scientific; 100X magnification) to view the annuli and growth micro-increments. Along this final step, regular viewing under the microscope was needed as this is the crucial stage of the grinding process to avoid sanding away the increments caused by over-grinding. It was also observed that there is no standard thinness of the transverse sections, as the appearance of the core and annuli varied among different otolith sizes. Lastly, the burning technique by Thorogood (1987) was applied as needed to enhance the contrast of the dark and light bands, which provides a well-processed

section of good-quality otolith.

2.5 Otolith increment reading

Each dulv prepared transverse section was examined under a compound light microscope (LW Scientific) at 100X and 400X magnification for the increment reading. An annual growth increment of an otolith was identified through alternating opaque and translucent zones (Degens et al. 1969; Kalish et al. 1985; Lou 1992) (Figure 3). A double-blind method developed by Clear et al. (2000) was employed to estimate the age of each otolith section. Due to the more explicit rings observed in the medio-ventral 'long' arms compared to the mediodorsal 'short' arms of the otolith sections, the annuli were quantified from the 'long' arms (Stéquert et al. 1996) (Figure 3). Additionally, photomicrographs of each sample were captured using a similar microscope and mobile phone camera. Compiled pictures of each otolith were digitally enhanced using a Portable Photoshop C6, and counting for annuli rings proceeded.

2.6 Otoith ageing and growth analysis

All age and length data were processed per location of the fishing grounds (i.e., Sarangani Bay, Moro Gulf, Davao Gulf, and at the western part of the Celebes Sea) and per gear type (i.e., purse seine, hand line, and ring net). A separate run of pooled data was also generated. Moreover, a box plot technique was employed for both sets of the data to identify and remove outliers (e.g., peculiar readings from overly ground, wedged, and broken otolith sections) (Elumba et al. 2019) for better visualization of the age composition of the frigate tuna samples. To demonstrate the growth pattern, the von Bertalanffy growth function (VBGF) model with a 95% level of confidence was utilized, as commonly employed for pelagic tuna species (Farley et al. 2006). This model was based on length-at-age data using the non-linear least-squares method, as described by the equation, $L_t = L_{\infty} (1 - e^{-k(t-t)})$, where the L_t represents the length at age t, t signifies the age and to denotes the hypothetical

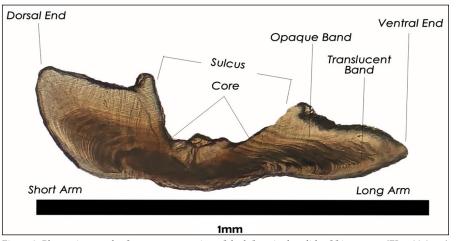


Figure 3. Photomicrograph of a transverse section of the left sagittal otolith of frigate tuna (FL = 39.9 cm) with labeled parts of an otolith section; examined under 100X magnification.

age when the fish has a length of zero. The age at a settlement length of 1.5 cmFL was employed to illustrate the growth curve better (Elumba et al. 2019). The L_{∞} function is the asymptotic length (maximum length), and K (a positive constant) is defined as the resulting growth coefficient of the fish. Both visualizations (box plot and growth curve) were generated using the Paleontological Statistics (PAST) software v.4.03.

3. RESULTS

3.1 Length-weight relationship

A total of 1,046 frigate tuna samples were collected, processed, and analyzed across the sampling locations. The collected samples were dominated by

the areas from the western part of the Celebes Sea (37.0%) and Moro Gulf (34.0%). Very few samples were collected from Sarangani Bay (23.3%), and in Davao Gulf (5.6%). Overall, the recorded body weight ranged from 42.0 g to 1,760.0 g, with a mean value of 307.7 g.

Across all sites, the morphometric parameters observed have the following mean values for SL (23.9 cm), FL (25.5 cm), TL (26.9 cm), and body girth (15.6 cm). The minimum values are as follows: SL (14.0 cm), FL (14.5 cm), TL (15.5 cm), and body girth (6.0 cm). Moreover, the maximum values are as follows: SL (41.8 cm), FL (44.7 cm), TL (46.8 cm), and body girth (34.0 cm) (Table 1a). The minimum and maximum values for SL, FL, and TL per sampling areas are presented in Tables 1b to 1e. Among these sites, the western Celebes Sea (FL: 15.2 to 44.7 cm) has the broadest size

Table 1a. Mean, minimum, and maximum values of the morphometric variables of the *A. thazard* individuals sampled in the Celebes Sea; n = 1,046.

Morphometric variables/unit	Mean ± SD	Minimum Value	Maximum Value
Standard Length (SL); cm	23.9 ± 2.2	14.0	41.8
Fork Length (FL); cm	25.5 ± 2.3	14.5	44.7
Total Length (TL); cm	26.9 ± 2.5	15.5	46.8
Body girth; cm	15.6 ± 1.9	6.0	34.0
Body Weight (BW); g	307.7 ± 123.2	42.0	1,760.0

Table 1b. Mean, minimum, and maximum values of the morphometric variables of the A. thazard individuals sampled from Sarangani Bay; n = 244.

Morphometric variables/unit	Mean ± SD	Minimum Value	Maximum Value
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Standard Length (SL); cm	22.2 ± 4.0	15.2	39.5
Fork Length (FL); cm	23.4 ± 4.1	15.8	42.0
Total Length (TL); cm	24.5 ± 4.3	16.5	43.5
Body girth; cm	15.3 ± 3.1	6.0	31.3
Body Weight (BW); g	211.6 ± 150.4	50.0	1,510.0

Table 1c. Mean, minimum, and maximum values of the morphometric variables of the *A. thazard* individuals sampled from the Moro Gulf; n = 356.

Morphometric variables/unit	Mean ± SD Minimum Value Maximu		Maximum Value
Standard Length (SL); cm	23.7 ± 3.5	14.0	33.0
Fork Length (FL); cm	25.4 ± 3.6	14.5	35.5
Total Length (TL); cm	26.8 ± 3.9	15.5	37.0
Body girth; cm	15.6 ± 2.2	9.0	21.8
Body Weight (BW); g	280.7 ± 119.3	65.0	758.0

Table 1d. Mean, minimum, and maximum values of the morphometric variables of the A. thazard individuals sampled from the Davao Gulf; n = 59.

Morphometric variables/unit	Mean ± SD	Minimum Value	Maximum Value
Standard Length (SL); cm	22.2 ± 3.0	19.0	38.0
Fork Length (FL); cm	23.9 ± 2.8	20.0	39.5
Total Length (TL); cm	25.3 ± 3.1	22.0	41.0
Body girth; cm	14.1 ± 2.2	12.0	27.1
Body Weight (BW); g	222.1 ± 144.4	120.0	1,200.0

Table 1e. Mean, minimum, and maximum values of the morphometric variables of the *A. thazard* individuals sampled from the western Celebes Sea; n = 387.

Morphometric variables/unit	Mean ± SD	Minimum Value	Maximum Value
Standard Length (SL); cm	23.9 ± 2.2	14.0	41.8
Fork Length (FL); cm	25.5 ± 2.3	14.5	44.7
Total Length (TL); cm	26.9 ± 2.5	15.5	46.8
Body girth; cm	18.2 ± 5.6	7.6	34.0
Body Weight (BW); g	307.7 ± 123.2	42.0	1,756.0

class representation.

Utilizing the entire dataset, the logarithmic regression analysis shows that the FL and BW of the frigate tuna have a strong relationship, as reflected by an R^2 value of 0.9819 and as described by the equation: $BW = 0.0068FL^{3.2632}$ (Figure 4a). The exponent value (b > 3) indicates that the species follows a positive allometric growth, implying that the frigate tuna becomes more robust as it grows. The FL-BW relationships per area are presented in Figures 4b to 4e.

Results reveal apparent variations in the size length distribution of frigate tuna samples per gear type (Figure 5) and sampling location (Figure 6). Among the gear types used, the ring net was observed to represent the broadest size class (15.2 to 44.7 cmFL) with a nice

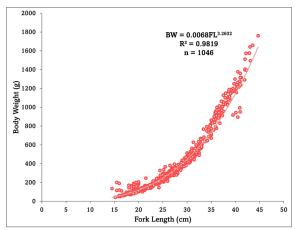


Figure 4a. Pooled data for length-weight relationship (LWR) of frigate tuna individuals sampled across all the sampling sites; n = 1046. Outliers were attributed to empty or full gut.

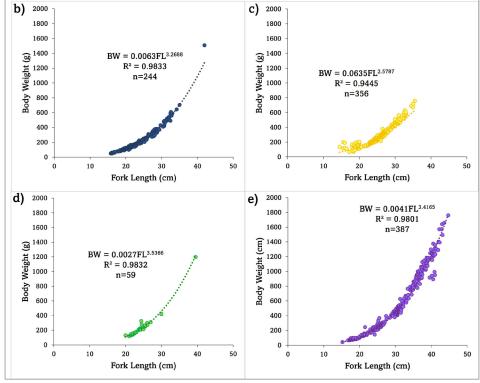


Figure 4b-e. Length-weight relationship (LWR) of frigate tuna individuals sampled across from the different sampling sites; b) Sarangani Bay: n = 244; c) Moro Gulf: n = 356; d) Davao Gulf: n = 59; e) western Celebes Sea: n = 387. Outliers were attributed to empty or full gut.

bell-shaped pattern, dominated by individuals with 23.0 cmFL (Figure 5). On the other hand, those individuals caught by handline with size classes ranging from 15.8 to 42.0 cmFL are skewed to the left, dominated by individuals with a size of 24.0 cmFL (Figure 5). Lastly, the catch of large fishing vessels using purse seine nets that landed their catch at GSCFPC has the same bellshaped pattern as the ring net and handline catches, but unlike the former, it lacks the largest size class individuals (Figure 5). Whereas, per sampling area, the western Celebes Sea had a balanced bell-shaped curve with a peak at a size of 30.0 cmFL (Figure 6). While the rest of the other areas (e.g., Sarangani Bay, Moro Gulf, and Davao Gulf) demonstrate skewed curves leaning to the left, being dominated by small-sized class individuals (Figure 6).

3.2 Otolith microstructure

Out of 1,046 frigate tuna samples, only 344 individuals with lengths ranging from 15.5 to 44.7 cmFL with at least one otolith each were utilized for the sagittal otolith microstructure analyses. This provided a success rate of only 32.9%. Despite all efforts in carefully handling them, many were missed out, chipped, or broken during the extraction process because of their size (<3.97 mm), composition, and fragility. Damage also occured during the image processing and mounting preparation in the glass slide. The OL varies between 1.40 mm and 3.97 mm, and the OW ranges from 0.12 to 1.35 mm. Similarly, the OP extends from 5.51 to 19.91 mm, whereas the OA spans from 0.08 to 2.82 mm² (Table 2).

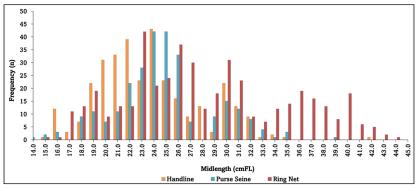


Figure 5. Comparison of the midlength frequency distribution of the frigate tuna caught by the different fishing gears; handline: n = 327; purse seine: n = 271; ring net: n = 448.

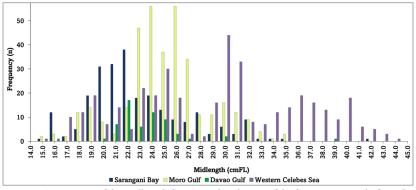


Figure 6. Comparison of the midlength frequency distribution of the frigate tuna caught from the different sampling sites: Sarangani Bay: n = 244; Moro Gulf: n = 356; Davao Gulf: n = 59; western Celebes Sea: n = 387.

Table 2. Mean, minimum, and maximum values of the morphometric data of the left sagittal otoliths of the sampled A. thazard individuals; n = 344.

Morphometrics variables/unit	Mean ± SD	Minimum value	Maximum value
Otolith Length (OL), mm	2.44 ± 0.33	1.40	3.97
Otolith Area (OA), mm ²	1.48 ± 0.31	0.08	2.82
Otolith Width (OW), mm	0.99 ± 0.08	0.12	1.35
Otolith Perimeter (OP), mm	11.52 ± 1.23	5.51	19.91
Body Weight (BW); g	307.7 ± 123.2	42.0	1,756.0

3.3 Fish length and otolith length relationship

The linear relationship between the FL and OL of frigate tuna showed a positive relationship, as indicated by its R² value (0.7130) (Figure 7). This clearly implies that as the tuna grows in length, its otolith also grows.

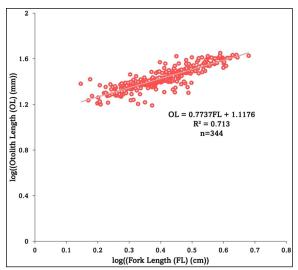


Figure 7. The relationship between fork length and otolith length for frigate tuna, n = 344.

3.4 Growth curve

Of the 344 fish individuals with intact otoliths, only 287 otoliths were successfully sectioned for age determination. Apart from the total number of sectioned otoliths, only 195 sections (66.9%) from individuals with actual sizes ranging between 17.6 and 43.1 cmFL were successfully aged. Other sectioned otoliths were unreadable. Another approach to verify fish age is to provide a demonstration guide of the otolith growth variation at different age classes (Figure 8).

Per sampling area, 113 otoliths were processed for the Western Celebes Sea, 55 for Sarangani Bay, 14 for Moro Gulf and 12 for Davao Gulf (Figure 9). Only the Western Celebes Sea represented the recorded ages from one to four (Vieira et al. 2022), with size classes ranging from 19.0 to 44.0 cmFL (Figure 9). Based on the available data, only the age-length relationship of frigate tuna captured in the western Celebes Sea, which was well represented in terms of size class, was used in the succeeding presentation.

The fitted age-at-length data on the VBGF model (95% confidence level) indicates that as the frigate tuna grows in length, so does its otolith (Figures 10 and 11). Blue lines present the upper and lower limits of the 95% bootstrap confidence, while

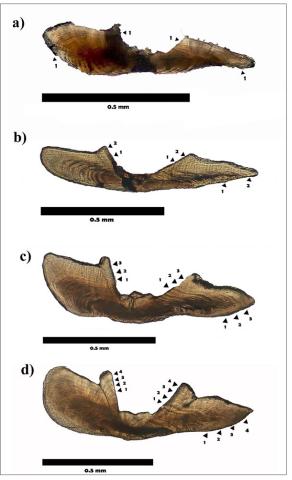


Figure 8. Sample photomicrographs of frigate tuna otolith transverse sections with annuli counts and markings on the opaque zones observed, (a) aged 1-year-old (FL = 19.50 cm, OL = 1.85 mm, OW = 0.82 mm), (b) aged 2 years old (SL = 24.50 cm, OL = 2.53 mm, OW= 1.07 mm), (c) aged 3 years old (FL = 39.90 cm, OL = 3.94 mm, OW = 1.20 mm), and (d) aged 4 years old (FL = 40.90 cm, OL = 4.41mm, OW = 1.33).

the equation L=62.1 gives the actual growth curve (1-1.0016 $e^{(-0.25517x)}$). The growth parameters, asymptotic length (L) and the Brody growth coefficient (K) of the frigate tuna observed were 62.1 cmF L and 1.0016 year-1, respectively. The fastest growth rate observed is between one and three years old and exhibits a less steep growth thereafter (Figure 10).

4. DISCUSSION

4.1 Length-weight relationship and somatic growth pattern

Based on the pooled dataset, this study demonstrated that frigate tuna observed positive allometric growth as ind icated by the exponent value (b>3) obtained via the power regression analysis

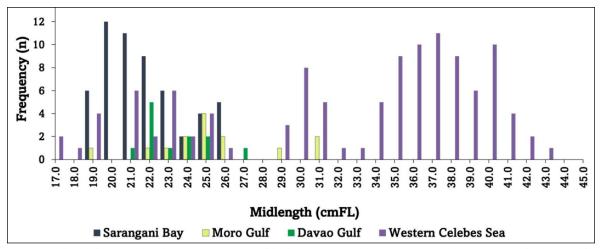


Figure 9. Midlength frequency distribution (FL range, cm) of the frigate tuna samples per sampling area that were successfully assessed for ageing analysis. Sarangani Bay (n = 55); Moro Gulf (n = 14); Davao Gulf (n = 12); and western Celebes Sea (n = 113).

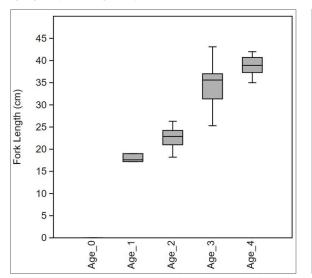
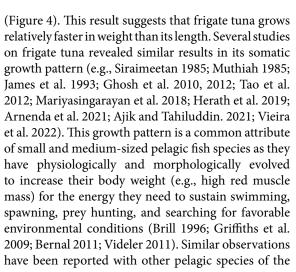


Figure 10. Box plot of age estimates from frigate tuna from the western Celebes Sea, with age at length at settlement (0 cm at 1.5 cm FL), n = 113.



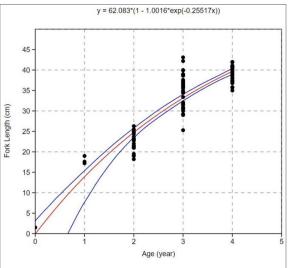


Figure 11. Length-at-age data of frigate tuna caught in the western Celebes Sea fitted in a von Bertalanffy growth function (VBGF) model at a 95% level of confidence, n = 113.

same body shape as the frigate tuna, such as anchovies (Plounevez and Champalbert 1999), sardines (Ganias et al. 2007; Ganias 2009; Queiros et al. 2019), herrings (Ivlev 1960), mackerels (Graham et al. 1983), round scad (Sululu et al. 2022), and many others.

The estimated asymptotic length using agegrowth data in this study at 62.1 cmFL was not far from what has been calculated for the country (Moro Gulf) at 63.0 cmFL by Ingles and Pauly (1984) using length-frequency data through ELEFAN II (Gayanilo et al. 2005). This study recorded only a maximum size of 44.7 cmFL in the Western Celebes Sea, whereas Calicdan-Villarao et al. (2017) recorded 55.0 cmFL from the Babuyan Channel in the northernmost part of the country. In other parts of frigate tuna's known distribution, Collette and Nauen (1983) reported in

their catalog a maximum length of this species at 58.0 cmFL collected off Sri Lanka and 51.0 cmFL from the Indian Ocean. Morice (1953) also documented a much higher maximum length at 65.0 cmFL from the eastern tropical Atlantic. In India, individuals were reported to have lengths greater than 50.0 cmFL/TL (Ghosh et al. 2012; Abdussamad et al. 2013; Hameed et al. 2021).

Despite several attempts, as mentioned, this study only acquired the largest individual, measuring 44.7 cmFL from the Western Celebes Sea (Figure 5). A majority (26.9%; n=281) of the frigate tuna samples have sizes ranging only from 23.5 to 26.5 cmFL (Figure 5). This size range fell over the recorded length at first maturity (L__) values - 21.5-38.6 cmFL of the frigate tuna in the Philippines (Froese and Pauly 2000). The observed larger samples (38.5-44.7 cmFL, 4.7%; n=49) collected from the wet markets in Tawi-Tawi further strengthen the observation that the Western Celebes Sea is a breeding ground for frigate tuna, along with its other neritic tuna relatives as previously reported by others (Mamalangkap et al. 2018; Ajik and Tahiluddin 2021). On the other hand, the absence of large individuals would mean that they do not stay long in a given area as they are migratory species (Maguire et al. 2006; Gomez 2019), experiencing heavy fishing pressure (Floyd and Pauly 1984; Juan-Jordá et al. 2011; Calicdan-Villarao et al. 2017) or die naturally for being a short-lived species (5 years old) (Tao et al. 2012).

Sampling tools used could also influence the availability of adults. Pechon et al. (2022) assessed the catch of tuna species at GSCFPC and discovered a similar observation which is that ring nets provided a wider length range of frigate tuna compared to those caught by purse seine nets. But so far, the size classes reported, regardless of fishing gears, are still within the range of 15.5-44.7 cmFL, with a mean of 25.5 cmFL that covers the sexually matured frigate tuna, as reported by Froese and Pauly (2000).

4.2 Age and growth

This study reports the first examined relationship between the size (length) and age (otolith growth increment) of frigate tuna using sagittal otoliths with a computed L of 62.1 cmFL (Figure 11). Of all the frigate tuna otolith sections, only 39.4% (n=113) of the successfully sectioned otolith samples from the western part of the Celebes Sea were used for age determination. Despite this limitation, age-length data showed a strong relationship (Figure 11) after

applying the box plot tool that discriminates outliers (Elumba et al. 2019).

Several studies on selected tuna species utilizing otolith as a tool in age estimation also noted similar difficulties in reading its growth increments. For example, Chang et al. (2019) observed irregular discernable rings in the otoliths of Pacific bluefin tuna from Taiwan. In addition, Rodriguez-Marin et al. (2007), Farley et al. (2013), and Andrews et al. (2020) also struggled to determine the first annuli of the bigeye, bluefin, and yellowfin tuna, respectively. Similarly, these difficulties in viewing recognizable growth rings and identifying the first annual increment in the frigate tuna otolith sections were observed in the present study. The quality of how each otolith was sectioned, including the factors mentioned above, was the main reason why only 66.9% of 287 intact otoliths were successfully aged.

Additionally, the value of each otolith morphometric parameter observed in this study supports the growing evidence of how minuscule the frigate tuna's otolith structure is (Table 2). This underpins that pelagic fishes typically have small otoliths (Robbins and Choat 2002). Given the fragility and minuscule size of the frigate tuna otolith, using it as a tool for age and growth estimation poses more challenges in addressing the knowledge gaps on this species' life history, population structure and dynamics, biology, and ecology.

Regardless of this impediment, the otolith increments observed for frigate tuna in this study revealed an age range from one to four years old, which is within the same age bracket as that of studies that utilized dorsal fin spine in South Brazil (Vieira et al. 2022). Similarly, for its closest relative, Auxis thazard thazard, Tao et al. (2012) also documented an age range from 0 to 5 years using the vertebrae annual growth increments of the individuals caught off Taiwan Strait (Table 3). Thus, this shows that the ageing analysis using otoliths of frigate tuna concurs with other studies utilizing different techniques, as mentioned (Table 3).

Undeniably, there is difficulty in ageing fishes with short lifespans (< 5 years), such as the *A. thazard*. However, a very distinct pattern was observed through diligence and patience in examining hundreds of sectioned otoliths (Figure 8). It was observed that the otolith not only grows in length and overall size but also, its transverse section develops into a distinct pattern (Figure 8). Specifically, the sulcal groove becomes more pronounced as the frigate tuna ages. Age 1 is characterized by a blunt protrusion at both the

Table 3. Summary of global studies that examined the age range and estimated the growth parameters [L_ - asymptotic length; K (year¹) - Brody's growth coefficient, and T_{max} - maximum age] of frigate tuna using various methods (LF- Length Frequency, Spine, Vertebra, and Otolith). Size range recorded in Fork Length (FL).

Reference	Size Range in FL (cm)	Tool	Country	Age Range	L_{∞} (cm)	K (year-1)	T _{max}
Pillai and Ganga (2008)	-	LF	India	-	54.0	0.90	4
Abdussamad et al. (2005)	18.0 - 30.0	LF	India	-	52.9	0.07/mo *	3
James et al. (1993)	-	LF	India	-	56.0	0.77	-S
V (2002)		LF	- 1		Female: 51.2	1.30	3
Kasim (2002)	-		India	-	Male: 49.0	1.30	3
Ghosh et al. (2010)	20.0 - 47.9	LF	India	-	46.6	0.93	2
Ghosh et al. (2012)	18.0 - 55.9	LF	India	-	58.0	1.20	-
Calicdan-Villarao et al. (2017)	16.0 - 55.0#	LF	Philippines	-	41.0	0.60	-
Abdussamad et al. (2013)	18.0 - 56.0	LF	India		58.0	1.20	2
Lelono and Bintoro (2019)	-	LF	Indonesia	-	35.4	0.58	4.8
Silas et al. (1985)	-	LF	India	-	63.0	0.49	4
Zapadaeva (2021)	-	LF	Russia	-	48.6	0.48	-
Vieira et al. (2022)	Female: 26.9 - 49.4		D 1	0 – 4 yrs. old	Female: 47.7	0.47	4
	Male: 26.5 – 45.2	Spine	Spine Brazil	0 – 4 yrs. old	Male: 49.8	0.35	4
Armada (2004)	-	LF	Philippines (Davao Gulf)	-	40.5	0.85	-
Ingles and Pauly (1984)	-	LF	Philippines (Moro Gulf)	-	63.5	0.72	-
Tao et al. (2012)	19.8 – 45.6	Vertebra	Taiwan	0 – 5 yrs. old	48.2	0.52	5
This Study	17.2 - 43.1	Otolith	Philippines (western Celebes Sea)	1 – 4 yrs. old	62.1	1.00	4

^{* -} k (month-1)

outer canal of the sulcus in the ventral and dorsal arms of the otolith. At age 2, this structure starts to appear as a small bump, and at ages 3 and 4, these structures become more prominent. Without sophisticated machines, this pattern can also be used for age verification. It can be concluded that the higher the mentioned bump, the older the frigate tuna individual is like spines and vertebrae, otoliths can also be a helpful tool in estimating the growth of frigate tuna. Various studies also supported that otolith ageing offers an accurate evaluation of the life history and age of fish as compared with other hard structures (i.e., vertebrae, scales, and spines) (Marriott and Cappo 2000; Khan et al. 2011; Ma et al. 2017). Moreover, Neilson and Campana (2008) used the radiocarbon method to evaluate otolith ageing. Its reliability has been attested before (Rodriguez-Marin et al. 2007). However, this process is expensive and not readily available in regions where frigate tuna abounds.

It was further observed in this investigation that individuals with lengths of 18.2-26.3 cmFL were found to be at least two years old (Figure 10). This finding supports what Tao et al. (2012) reported for A. thazard thazard. Additionally, the oldest specimens of frigate tuna obtained are four years old (Figure 10), indicating that this species may be captured before reaching their asymptotic length (Figure 11). As explained earlier, sizes greater than 44.7 cmFL were not sampled in the four areas examined (Table 3).

4.3 Calculated growth (VBGF curve)

The species' highest calculated growth parameters using LF data was around 63.0 cmFL from the Indian Ocean and the Moro Gulf of the Philippines (Ingles and Pauly 1984; Silas et al. 1985; Table 3). Using age-length estimates, this study yielded a maximum size estimate of 62.1 cmFL. While others estimated

^{# -} total length

L values were 49.8 cmFL for male specimens and 47.7 cmFL for female individuals (Vieira et al. 2022) and 48.2 cmFL (Tao et al. 2012) for both sexes. The observed discrepancies in growth, as previously mentioned, can be attributed to various factors, such as environmental conditions (Murua et al. 2017) and food availability (Brett 1979; Wootton 1999; Desai and Singh 2009) that can retard or increase their growth length but not their age. This was observed in Figure 4e, wherein some individuals had longer lengths buts weighed less or vice versa (Figure 4c).

Similarly, the growth coefficient (K) varies across sites or habitats. This study appeared to have a higher value (K=1.0016 yr-1) than what other researchers have found (Table 3) but lower than what others have reported, from K=1.20-1.30 yr⁻¹ (Table 3). The calculated L_. (62.1 cmFL) value for frigate tuna conforms to the previous report by Silas et al. (1985) in India and by Ingles and Pauly (1984) in Moro Gulf, who recorded maximum lengths of 63.0 cmFL and 63.5 cmFL respectively. Further, it is larger than the largest frigate tuna individual recorded by Calicdan-Villarao (2017) at 55.0 cmFL in the Babuyan Channel, north of the Philippines. In contrast, it is smaller than Morice (1953) reported at 65.0 cmFL from the eastern tropical Atlantic. Such large sizes of frigate tuna from these areas can be attributed to other factors probably not examined in this study.

5. CONCLUSION AND RECOMMENDATION

This study was the first report to examine the relationship between the size (length) and age of frigate tuna, Auxis thazard, based on sagittal otolith growth increments in the Western Celebes Sea. It also highlights a very distinct pattern of otolith calcification for this species. First, a thicker deposition occurs in the dorsal and ventral regions of the otolith, resulting in a deeper sulcal groove. Second, protrusions/bumps at the outer canal of the sulcus in both regions also become prominent as the frigate tuna ages. These observations provide secondary information to double-check the age estimates. Although limited otolith samples were successfully aged, application of the box plot technique eliminated under- and overestimation of its age. Embedding the fragile otolith using an epoxy mold is highly recommended to make it durable for sectioning procedures. The manual grinding using sandpaper cannot be employed once the otolith is embedded in an epoxy mold. Thus, a high-precision otolith grinder can then be utilized in the sectioning process. Furthermore, a good quality stereo microscope with a high-resolution camera is highly recommended for such a study. Finally, extra care must be performed throughout the handling of the otolith, from extraction to resin embedding, to avoid spoilage. If affordable, radiocarbon dating is highly recommended for an accurate age determination. The limited studies on frigate tuna can be attributed to their migratory behavior. All ages or various size classes cannot be observed in a single body of water alone. Sampling must be conducted within its range of distribution. Moreover, it is highly encouraged that future research endeavors should look into the impacts of the varying environmental conditions on their age, growth, and reproduction as they move from one area to another.

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ETHICS STATEMENT

The researchers were assured to observe strict compliance with all the institutional and national restrictions regarding the use of animals as subjects. This meant that they were only permitted to use commercially and locally harvested fish in the research.

AUTHOR CONTRIBUTIONS

De Vera CMA: Investigation, Methodology, Software, Data curation, Visualization, Writing Original draft preparation. Abella RP.: Methodology, Investigation, Data Curation. Hedoquio AC: Visualization, Investigation, Data Curation. Tampov DA: Editing-Original draft, Investigation, Data Curation. Templado JBJ: Methodology, Investigation, Data Curation. Matunog MWM: Methodology, Investigation, Data Curation. Cuanan AM: Visualization, Investigation, Data Curation. Edna P. Guevarra EP: Project Administration, Supervision, Conceptualization, Funding Acquisition. Nañola CL: Conceptualization, Methodology, Software, Writing -Reviewing and Editing, and Data Validation.

CONFLICTS OF INTEREST

As the authors of this study, we assert that we have no knowledge of any conflicting personal or financial interests that could have influenced the research we conducted. Therefore, we can confidently state that there is no conflict of interest present in our work.

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