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Research Article

# Length-based fishery status of frigate tuna (Auxis thazard) and bullet tuna (A. rochei) in the northern Oman Sea

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#### **Keywords**

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#### Article info

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#### Abstract

In the present study, the population characteristics of Auxis thazard and A. rochei were evaluated by sampling at three fish landing sites in the Oman Sea including Beris, Pozm, and Konarak from March 2020 to March 2023. The biometric analysis was performed on 2400 fish specimens. Growth and mortality indices were calculated, including infinite length (L∞=61 and 45 cm), growth coefficient (K=0.40 and 0.46 (yr<sup>-1</sup>)), growth performance index ( $\Phi$ =3.18 and 2.93), natural mortality (M=0.77 and  $0.68(yr^{-1})$ ), fishing mortality (F=0.25 and 0.10 (yr<sup>-1</sup>)), total mortality (Z=1.02 $\pm$ 0.09 and 0.78 $\pm$ 0.09 (yr<sup>-1</sup>)), and exploitation coefficient (E=0.25 and 0.13 (yr<sup>-1</sup>)), as well as t<sub>0</sub> (-0.33 and -0.32 yr<sup>-1</sup>). Relative production per recruitment, relative biomass per recruitment, and exploitation rate of this species were Y / Rp = 0.04,  $B/Rp=0.23(yr^{-1})$ , and U=0.2 and  $0.09(yr^{-1})$ , respectively. The present study showed that the Frigate tuna (FRT) and Bullet tuna (BLT) had desirable stock conditions, and therefore, specific measures should not be taken to reduce catch and fishing efforts. The ratio of spawning potential based on length (LBSPR) of A. thazard and A. rochei was 0.39 (0.35-0.44) and 0.66 (0.59-0.77), respectively. The present study (ratio of LBSPR index and the value of fishing, total mortality and exploitation coefficient) showed that the Frigate tuna and Bullet tuna stock have not reached overfished status. The insights garnered from this research are poised to aid in the management and enduring exploitation of the fish stocks. The findings of this study can assist in the management and sustainable harvest of these tuna species.

#### Introduction

In 2024, global capture fisheries production reached approximately million tons, with 82% coming from seawater and 18% from inland waters. The Indian Ocean contributed over 12 million tons to this total, with around 6 million tons caught in its western region. A key concern sustainable fishing and ocean development is the balance between stocks at Biologically Sustainable Levels (BSLs) and those at Biologically Unsustainable Levels (BULs), defined based on the biomass amount of the species and their fishing pressure. In 1974, about 10% of stocks were classified as BULs, which increased to roughly 37% by 2022. Conversely, stocks at BSLs decreased from 90% in 1974 to about 63% in 2022. The Mediterranean Sea, Black Sea, Southeast Pacific Ocean, and Southwest Atlantic Ocean have the highest concentrations of BULs (FAO, 2024).

Tuna is part of the Perciformes order and the Scombridae family, which includes 52 species across 15 genera (Collette and Nauen, 1983; Froese and Pauly, 2024). The Frigate tuna (*Auxis thazard*, species code: FRI) and Bullet tuna (*Auxis rochei*, species code: BLT) are small fish belonging to the Scombridae family, found in tropical and subtropical oceans around the globe. They typically inhabit open surface waters, reaching depths of up to 50 meters (Agustina *et al.*, 2023; Froese and Pauly, 2024).

The Bullet tuna is identifiable by its blue-black back adorned with zig-zag dark patterns and a silver belly. This species is relatively slender, growing to a maximum length of about 50 centimeters, and is known for its quick growth, short lifespan, and migratory habits (Ollé-Vilanova et al., 2022). Bullet tuna is important to marine ecosystems and is sought after in both commercial and recreational fishing. As predators, they feed on small fish, squid, planktonic crustaceans, and stomatopod larvae. While they are considered a minor commercial species, they are primarily caught in coastal areas and around islands (IOTC, 2017; IOTC, 2019a). Their spawning season varies by ocean, usually occurring closer to shore than other tuna species, and at sea surface temperatures of 24°C or higher. In terms of conservation status, the Bullet tuna is currently classified as Least Concern by the IUCN (Froese and Pauly, 2024).

The maximum recorded fork length (FL) for frigate tuna in the eastern Atlantic Ocean is 65 cm (Cayré et al., 1993). This species is a coastal species, classified as both epipelagic and neritic, and is found globally in tropical and subtropical oceans (Collette and Nauen, 1983). It exhibits localized migratory patterns, primarily inhabiting oceanic islands and continental shelves, and is known for its strong schooling behavior (Pillai and Ganga, 1985; Lu et al.,1991; Abdussamad et al., 2005; Ghosh et al., 2010; Ghosh et al., 2012; Tao et al., 2012; Calicdan-Villarao et al., 2017; Mudumala et al., 2018; Lelono and Bintoro, 2019; Darvishi et al., 2020; Zapadaeva, 2021; Vieira et al., 2022). This fish is typically found in shallow waters and is commonly captured using set nets. It can also be caught by small-scale fisheries employing surface gear, such as fishing lines, nets, and traps (Collette and Nauen, 1983).

This research examined the demographic traits of *Auxis thazard* and *Auxis rochei* in the northern Oman Sea. The main goal was to gather essential information for the proper identification and management of this fish stock's exploitation.

This study also sought to deepen knowledg e about the biological and population traits of these species, especially given their sig nificant catch increase in recent years.

#### Materials and methods

The population characteristics of *Auxis* thazard and *Auxis* rochei were assessed

through sampling at three fish landing sites in the Oman Sea, specifically in the southern waters of Iran. The ports included in the study were Beris (60° 10′E, 28° 52′N), Pozm (60° 28′E, 25° 14′N) and Konarak (60° 25′E, 25° 59′N). Sampling sites were chosen in the ports of Beris, Pozm, and Konarak considering commercial fishing conditions. Gill nets with a mesh size of about 140 mm were used in the Oman Sea (Iranian southern waters) (Fig. 1).



Figure 1: The map illustrates the locations of A. thazard and A. rochei sampling sites in the Oman Sea.

#### Length frequency distribution

Monthly sampling was conducted at the fish landing sites of Beris, Pozm, and Konarak. From March 2020 to March 2023, random samples were taken from the commercial catches at these ports. A total of 2411 fish underwent biometric analysis, specifically measuring their fork length using a ruler with a precision of 1 cm.

## Growth assay

L∞ was estimated via the Froese and Binohlan equation, given by

 $\log L\infty = 0.044 + 0.9841* \log(Lmax)$  (Froese and Binohlan, 2000). The growth rate was assessed using the ELEFAN method, employing an optimization model in RStudio (Version 2024.04.2) and the TropFishR package (Mildenberger *et al.*, 2017). Pauly's experimental formula was used to calculate the optimal value of t0, expressed as Log(-t0) = -0.3922-0.2752  $Log L\infty - 1.038$  Log K (Froese and Binohlan, 2000). Growth indices, such as infinite length (L $\infty$ ) and growth factor (K),

were compared using the equation  $\Phi = Log(K) + 2Log(L\infty)$ .

## Mortality estimate

The Pauly equation (Sparre and Venema, 1998) was used to determine natural mortality (M), defined as Ln(M)=-0.0152- $0.297 \text{ Ln}(L\infty) + 0.654 \text{ Ln}(k) + 0.642 \text{ Ln}(T)$ . Here, M represents the annual natural mortality rate, L\infty is the fish's infinite length (in cm), K is the growth curve parameter from the von Bertalanffy growth equation, and T denotes the average environmental temperature (Sparre and Venema, 1998). The average annual surface water temperature in this area was determined to be 26°C (Kaymaram et al., 2009, 2014). Total mortality (Z) was derived from length-converted catch Fishing mortality curves. (F) calculated using the formula F=Z-M, where Z represents total mortality, F

denotes fishing mortality, and M stands for natural mortality. The exploitation rate (E), which indicates the proportion of fishing mortality to total mortality, was computed using the equation E=F/Z (Sparre and Venema, 1998).

Yield per recruit and biomass per recruit The relative yield per recruitment was assessed in relation to the fishing mortality coefficient or exploitation rate. In this equation, E represents the exploitation coefficient, U denotes the exploitation rate, M stands for the natural mortality coefficient, F indicates the fishing mortality coefficient, and Lc is equivalent to Lc50 (Gayanilo and Pauly, 2003). In the relative biomass addition, recruitment (B/Rp) was calculated using the following equation:

Y/R = EU M/K (-3 U/(1+m) + 3U2 / (1+2m) + U3/(1+3m)  $U = 1 - (LC/L\infty) ; M = (1-E)/(M/K) = (K/Z); E = F/Z$ B/R = Y/R/F

#### *Length-based reference point*

The length-based reference points are outlined as follows: the percentage of mature fish in the catch (Pmat), the percentage of fish harvested at the optimal length (Popt), and the percentage of megaspawners (those measuring between 1.1 Lopt and Lmax in the catch length composition) (Pmega). Together, these are known as the Pobj value (Cope and Punt, 2009). The values for optimal fishing length (Lopt) are calculated as described by Froese and Binohlan (2000):

Lopt=Linf (3/(3+M/K))

Pmega=Lopt+10%

The size at first maturity (denoted as 'Lm50') was calculated by the equation: Fork length of all individuals was used to estimate the size at first maturity (Froese and Binohlan, 2000):

 $\log(LM) = 0.8979 \times \log L \infty - 0.0782$ 

#### Fish stock condition

The condition of fish stocks was assessed using the equation {(Heaviness×value/full value)}×100%, with the full value set at 55. Each indicator was assigned varying levels

of heaviness based on its importance, and these indicators were further broken down into sub-indicators with different values. The product of the indicator's heaviness and its value were then calculated. The stock condition was categorized using the following references: a stock condition of ≥85–100% is considered very good; a stock condition of <85–65% is deemed good; and a stock condition of <65% is classified as low (Mallawa *et al.*, 2015; Haruna *et al.*, 2018) (Table 1).

Table 1: Survey of stock condition based on its population indices.

Indicator	Range	Heaviness	Value	Heaviness × value		
Size Structure	Small fish Small - medium fish Medium - Large fish	2	1 3 5	10		
Number of age groups	Less than three Three to five More than five	2	1 3 5	10		
The rate of fishing mortality	More than two Between one and two Less than one	2	1 3 5	10		
The rate of exploitation	More than 1 Between 1 and 0.5 Less than 0.5	1	1 3 5	5		
The rate of population growth	Less than 0.5 per year Between 0.5 - 0.75 per year More than 0.75 per year	1	1 3 5	5		
The percentage of eligible catch size	Less than 30 % Between 30 % and 50 % More than 50 %	2	1 3 5	10		
Yield per Recruitment	Y/R now> Y/R optimal Y/R now = Y/R optimal Y/R now< Y/R optimal	1	1 3 5	5		
Full value (Total)	-	-	-	55		

#### Spawning potential ratio (SPR)

The Spawning Potential Ratio (SPR) serves as a biological benchmark for assessing the population status of a species. The established SPR reference points are: SPR 20% as the threshold limit and SPR

40% as the desired target (Hordyk *et al.*, 2015a). To calculate the SPR, one must consider various life history traits. The definition of SPR is provided in the work of Hordyk *et al.* (2015b):

SPR =  $(\sum (1-LX)^{((M/K)[(F/M)+1])}L_x^b)/(\sum [(1-L_X)])(L_x^b^{(M/K)})$ , Where, Lx is fork length; M is natural mortality; k is growth rate; F is fishing mortality, and b is an exponent usually close to 3.

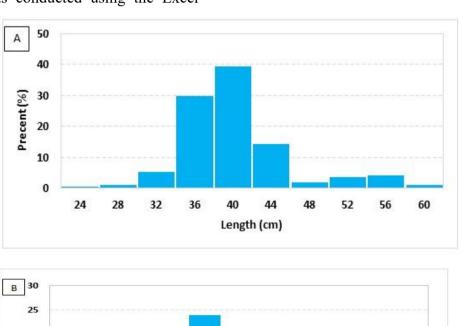
To estimate the Spawning Potential Ratio (SPR) using certain models, its assumed

that fish selectivity follows an asymptotic or logistic pattern without any age-related length variation. The fishing mortality to natural mortality ratio (F/M) can be inferred from the size distribution of the catch (Hordyk et al., 2015b). connection between F/M and SPR is shaped by the selectivity parameters and follows an asymptotic curve. The Length-Based Spawning Potential Ratio (LB-SPR) model employs maximum likelihood estimation to determine the selectivity curve, presumed to be logistic and defined by the parameters SL50 and SL95, as well as the relative fishing mortality (F/M). These estimates are then utilized to compute the SPR. The LB-SPR analysis was conducted using the LB-SPR package in R (Carruthers and Hordyk, 2018). Data analysis was conducted using the Excel

program, R Studio software (version 1.1.46), and the TropFishR package.

#### Results

A length frequency analysis of Frigate tuna (FRT) and Bullet tuna (BLT) was conducted on 2,409 fish. The shortest fish of *A. thazard* and *A. rochei* measured 38 and 32 cm, while the longest reached 60 and 44 cm, respectively. The average lengths (±SD) for these species were 35±15 cm and 32±10 cm, respectively. The fish length data were organized into groups of 4 and 2 cm, with the most common lengths being in the 36-40 cm and 31-33 cm ranges, as shown in Figure 2.



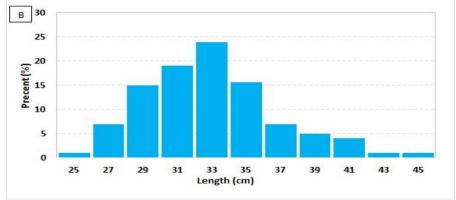


Figure 2: The percentage frequency in different length categories of A. thazard (A) and A. rochei (B) in the Oman Sea.

Growth and mortality indices were including calculated, infinite length (L∞=61 and 45 cm), growth coefficient and 0.46  $(vr^{-1})$ , growth performance index ( $\Phi$ =3.18 and 2.93), natural mortality (M=0.77 and 0.68 (yr<sup>-1</sup>)), fishing mortality (F=0.25 and 0.1 (yr<sup>-1</sup>)),

total mortality ( $Z=1.02\pm0.09$  and  $0.78\pm0.09$  ( $yr^{-1}$ )), and exploitation coefficient (E=0.25 and 0.13 ( $yr^{-1}$ )), as well as length at time zero (-0.33 and 0.32  $yr^{-1}$ ) (Fig. 3).

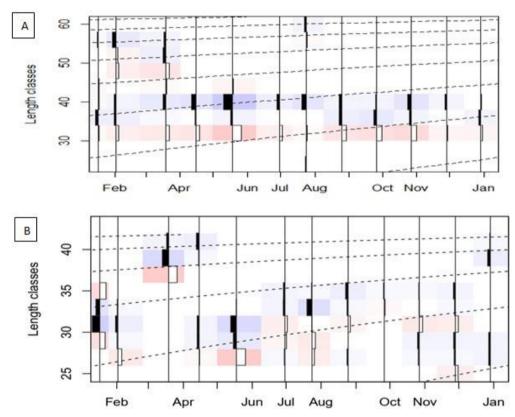


Figure 3: Growth curve derived from frequency data of A. thazard (A) and A. rochei (B) in the Oman Sea.

LC or LC50 refers to the length at which there is a 50% chance of catching fish of that size (35 cm for A. thazard and 18 cm for A. rochei in this study). The relative production per recruitment was Y/Rp=0.03 for A. thazard and 0.04 for A. rochei, while the relative biomass per recruitment was B/Rp = 0.75and 0.85 (per year), respectively. In general, the estimates showed that the exploitation rates were U=0.95 and 0.70 (per year), while the fishing mortality at maximum sustainable yield was estimated to be FMSY=0.7 and 0.55 (per year) for these fish stocks (Fig. 4 and Table 2), respectively.

Length-based reference points for *A. thazard* and *A. rochei* species in the Oman Sea are as follows: percentage of mature fish in the catch (Lmat=30 cm, Lmat=26 cm), percentage of fish caught at the optimum length for harvest (Lopt=37 cm, Lopt=28 cm), and percentage of megaspawners in the catch (Lmega=41 cm, Lmega=31 cm), respectively (Fig. 5 and Table 3).

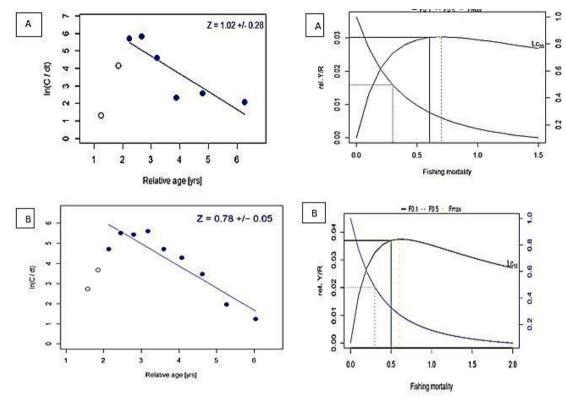
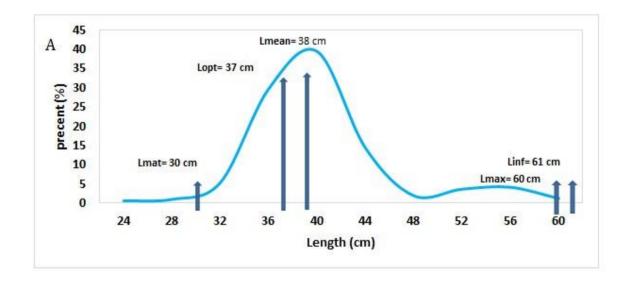


Figure 4: Total mortality curve and fishing mortality at maximum sustainable yield of Fmsy of A. thazard (A) and A. rochei (B) in the Oman Sea.

Table 2: Comparison of population characteristics of A. thazard and A. rochei.

Species	L∞ (cm)	Lmax (cm)	K (yr -1)	t <sub>o</sub> (yr <sup>-1</sup> )	Φ'	M (yr <sup>-1</sup> )	F (yr <sup>-1</sup> )	Z (yr <sup>-1</sup> )	E
A. thazard	61	60	0.40	-0.33	3.18	0.77	0.25	1.02	0.25
A. rochei	45	43	0.58	-0.32	2.93	0.68	0.1	0.78	0.13



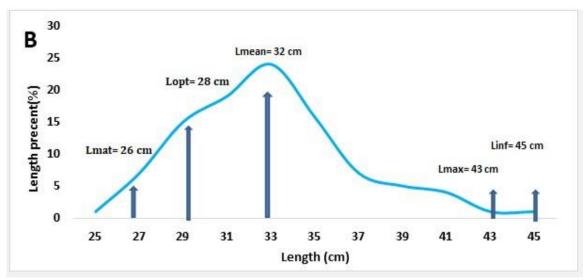
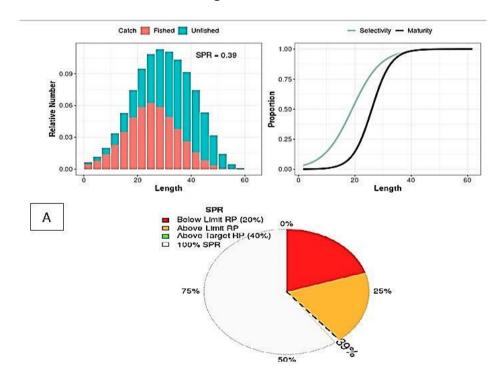


Figure 5: Length-based reference point of A. thazard (A) and A. rochei (B) in the Oman Sea.

### The stock status

According to Table 2, these species are mostly in good condition based on stock condition indicators from different population parameters. While some factors, like size structure, number of age

groups, and mortality rates, fall below the optimal average, others exceed it. The fish stock condition index values calculated were 71% ( $39/55\times100$ ) and 72% ( $40/55\times100$ ) (Fig. 6; Table 3).



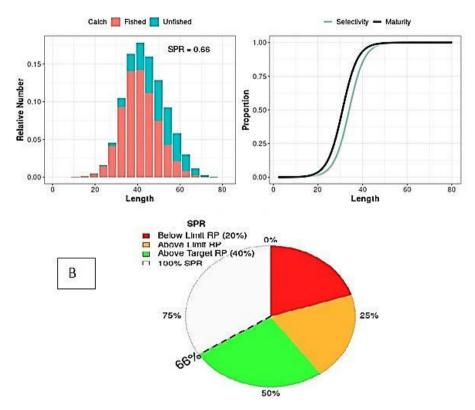


Figure 6: The spawning potential based on length (LBSPR) of *A. thazard* (A) and *A. rochei* (B) in the Oman Sea.

Table 3: Comparison of length-based reference point and fish Stock condition of A. thazard and A. rochei.

Species	Lmat (cm)	Lmean (cm)	Lopt (cm)	Lmega (cm)	LBSPR	Stock condition		
A. thazard	30	38	37	41	0.39 (0.35-0.44)	Medium (yellow color)		
A. rochei	26	32	28	31	0.66 (0.59-0.77)	Good (green color)		

The ratio of spawning potential based on length (LBSPR) of *A. thazard* and *A. rochei* was 0.39 (0.35-0.44) and 0.66 (0.59-0.77), respectively. Based on the ratio of the LBSPR index, total mortality and exploitation coefficient can be a reason for underfishing (under exploitation). The present study showed that the Frigate tuna and Bullet tuna stock has not reached 'overfished' status.

#### Discussion

Table 3 compares the biological indices of Frigate tuna and Bullet tuna stocks from this study with another global research.

The infinite length of Frigate tuna in the Oman Sea is greater than that on the east coast of India, Indonesia, and Taiwan. The growth rate in the Oman Sea is higher than in other parts of the Indian Ocean and Persian Gulf. The L∞ values here exceed those by Darvishi et al. (2020), while the K lower, possibly values are due overfishing sampling or methods. Variations in infinite length and growth rate are shaped by each area's unique ecological traits (King, 2007). These differences are often due to the amount and quality of food available and the climate (Bartulovic et al., 2004). Fish growth is

influenced by age, season, year, feeding type, physiological state, food availability, and reproductive cycles (Lalèyè, 2006).

The Growth Performance Index ( $\Phi'$ ) was recorded at 3.18, lower than the figures reported by Darvishi *et al.* (2020) for this region (Table 2). The differences in  $\Phi'$  values stem from the relationship between infinite length and growth rate, showing consistent changes over time and various sizes. Environmental differences and latitude shifts can influence infinite length and growth rate. As a result,  $\Phi'$  values may vary not only between regions but also within the same region over different periods due to changing environmental factors (King, 2007).

The natural mortality rate for A. thazard species was higher than the fishing mortality rate. The F/FMSY ratio was below one, indicating no overfishing (Arrizabalaga et al., 2012). Both the exploitation coefficient and rate were below 0.5, suggesting capture fisheries were below optimal levels. These indices should remain under 0.5, and fishing mortality should be less than natural mortality to avoid overfishing (Sparre and Venema, 1998; King, 2007). The IOTC (2019b) indicates that BLT fisheries management in the Indian Ocean lacks sufficient data to estimate BLT resources. The IOTC (2023) documented that BLT fisheries management in the Indian Ocean lacks sufficient data (unknown stocks). Reports suggest that BLT resources in Indian waters are overexploited, with an exploitation rate of 80.0% (Jasmine, 2013). There Research on A. rochei in the Oman Sea, particularly in the Indian Ocean, is scarce and was conducted by Asrial et al.

(2021). Therefore, further studies on BLT resources and population in the northern Oman Sea are necessary.

Research conducted in the Indian Ocean has reported the ranges for natural mortality, fishing mortality, total mortality, and exploitation coefficient as follows: 0.90 to 1.65 per year, 0.20 to 4.89 per year, 1.13 to 5.98 per year, and 0.1 to 0.7 per year, respectively (Pillai and Ganga, 1985; Lu et al., 1991; Abdussamad et al., 2005; Ghosh et al., 2010; Ghosh et al., 2012; Tao et al., 2012; Calicdan-Villarao et al., 2017; Mudumala et al., 2018; Lelono and Bintoro, 2019; Darvishi et al., 2020; Zapadaeva, 2021; Vieira et al., 2022). The key factors influencing the pressure on fish stocks include 1) the volume of fish caught and harvested, and 2) environmental conditions that impact survival and access to fishery resources (Hashemi et al., 2020, 2021, 2023; Hashemi and Doustdar, 2024). The findings indicate that the maximum lifespan of A. thazard and A. rochei is approximately seven and five years, calculated using the formula tmax=t0+3/K (Froese and Pauly, 2024). According to the criteria established by the American Fisheries Society (Cheung et al., 2004), and by comparing the results of this study criteria, with those the extinction vulnerability of these species was assessed to be medium (Table 4).

Table 4: Comparison of biological characteristics of *A. thazard* and *A. rochei* with other studies around the Indian Ocean.

Indian Ocean.										
References	Species	Region	L∞ (cm)	K (yr <sup>-1</sup> )	t <sub>o</sub> (yr <sup>-1</sup> )	Φ'	M (yr <sup>-1</sup> )	F (yr <sup>-1</sup> )	Z (yr <sup>-1</sup> )	E
Pillai and Ganga, 1985	A. thazard	India	53	0.98	-0.01	3.42	1.52	3.91	5.43	0.7
Lu et al.,1991	A. thazard	Taiwan	48	0.51	-0.35	-	0.9	0.23	1.13	0.1
Abdussamad et al., 2005	$A$ . $\it thazard$	India	53	0.83	-	-	-	-	-	-
Ghosh <i>et al.</i> , 2010	A. thazard	India	46	0.93	-0.01	-	1.48	4.49	5.97	0.75
Ghosh <i>et al.</i> , 2012	A. thazard	India	57	1.2	0.007	-	1.65	4.89	3.24	0.66
Tao <i>et al.</i> , 2012	$A$ . $\it thazard$	Taiwan	48	0.52	-0.33	-	0.91	0.4	1.31	0.2
Calicdan- Villarao <i>et al.</i> , 2017	A. thazard	Philippines	46	0.7		5.2	-	-	-	-
Mudumala <i>et al.</i> , 2018	A. thazard	India	47	1.3	-0.23	3.33	1	1.43	4.43	0.77
Lelono and Bintoro, 2019	A. thazard	Indonesia	45	0.58	-0.26	4.8	-	-	-	-
Darvishi <i>et al.</i> , 2020	A. thazard	Persian Gulf and Oman sea (Iran)	53	0.8	-1.17	5.4	-	-	-	-
Zapadaeva, 2021	A. thazard	Atlantic Ocean	48	0.48	-	5.1	-	-	-	-
Vieira <i>et al.</i> , 2022	A. thazard	Brazil	49- Male 47- female	0.35 0.47	-2 -1.47	5	-	-	-	-
Present study	A. thazard	Oman Sea (Iran)	61	0.40	-0.33	3.18	0.77	0.25	1.02	0.25
Present study	A. rochei	Oman Sea (Iran)	45	0.58	-0.32	2.93	0.68	0.1	0.78	0.13

The conditions of *A. thazard* and *A. rochei* were 71% and 72%, respectively, indicating favorable fish stock conditions, as values below 65% suggest poor conditions (Mallawa *et al.*, 2015; Haruna *et al.*, 2018). Additionally, the length frequency of these species showed that less than 50% had reached maturity (Lmat), which is not considered a negative value. The LBSPR rates for this species are 0.39 (39%) and 0.66 (66%). Based on the ratio of LBSPR index and the value of fishing

and total mortality, it can be a reason for no overfishing. The LBSPR model calculates the spawning potential ratio (SPR), suggesting that values under  $0.2 \approx 0.5 \text{ B/B}$ msy) signify depleted stocks, while values over 0.4 (≈1.0 B/BMSY) indicate wellmaintained stocks. It's a useful method for evaluating fish stocks with limited data (Hordyk et al., 2015a; Hashemi and Doustdar, 2024). In simple terms, scientists have developed methods to easily and affordably measure the size and characteristics of exploited stocks, such as the length-based spawning potential ratio method (Hordyk et al., 2015b). Hommik et al. (2020) stress that Spawning Potential Ratio (SPR) estimates should be assessed against established benchmarks for healthy fisheries and the specific context of each fishery. Goodyear (1993) identifies an SPR threshold of 0.20 as the critical limit for maintaining adequate stock productivity. Additionally, Mace (1994) notes that an SPR value of 0.40 is generally used as a proxy for Maximum Sustainable Yield (MSY) in national fisheries legislation. For A. rochei in the Oman Sea (Iranian waters), the annual SPR is estimated to be around 0.66, suggesting that A. rochei is exploited less than its optimal conditions. The findings also indicate that a low number of juvenile A. rochei are being harvested before reaching maturity, possibly due to the use of multi-gear fishing methods in these ecosystems.

#### Conclusion

These parameters help managers fisheries in the Director position to protect the population of Frigate tuna (FRT) and Bullet tuna (BLT) in the northern Oman Sea. A scenario of underfishing may arise with the increase in average size and LBSPR index, along with a decrease in fishing and overall mortality rates. The research indicates that the population of BLT and FRT species is currently underfished and not experiencing overfishing. It is recommended to establish clear guidelines for the utilization and stewardship of A. thazard and A. rochei in this area. The insights gained from this research are poised to assist in the

management and sustainable exploitation of the stock of these species. The findings of this study can help in the management and sustainable harvest of these species stocks.

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#### **Conflicts of interest**

The authors have declared no conflict of interest.

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