BIODIVERSITAS
Volume 26, Number 4, April 2025
Pages: 1807-1815

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260430

Morphological diversity of fish size and otolith dimension of the protandrous hermaphroditic fish *Eleutheronema tetradactylum* from Pattani Bay, Thailand

TEUKU HARIS IQBAL[1,♥], SUKREE HAJISAMAE[2], SOFIYUDIN MAAE[2], ILHAM ZULFAHMI[1]
[1]Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Universitas Syiah Kuala. Jl. Teuku Nyak Arief Darussalam, Banda Aceh 24415, Aceh, Indonesia. Tel./fax.: +62-651-7553205, ♥email: tee.hariss@usk.ac.id
[2]Department of Agricultural Science and Fisheries, Faculty of Science and Technology, Prince of Songkla University. Pattani Campus 181 Moo 6, Charoen Pradit Road, Rusamilae, Mueang 94000, Pattani, Thailand

Manuscript received: 27 February 2025. Revision accepted: 11 April 2025.

Abstract. *Iqbal TH, Hajisamae S, Maae S, Zulfahmi I. 2025. Morphological diversity of fish size and otolith dimension of the protandrous hermaphroditic fish* Eleutheronema tetradactylum *from Pattani Bay, Thailand. Biodiversitas 26: 1807-1815.* This study examines the morphological diversity of otolith dimensions across different sexes and size classes of *Eleutheronema tetradactylum* from Pattani Bay, Thailand. A total of 303 specimens (166 males, 137 females) were collected using monofilament gillnets from May to December 2021. Measurements of Standard Length (SL) and Body Weight (BW), Otolith Length (OL), Otolith Height (OH), and Otolith Weight (OW) were taken to the nearest size of 0.01 mm and weight of 0.001 g. Ordinary Least Squares (OLS) regression, Student's t-test, and One-way Analysis of Variance (ANOVA) were applied to estimate relationships and significant differences between sexes and size classes. The highest correlation was observed between SL and BW with OL in males (R^2: 0.95). Significant differences were found in otolith parameters between sexes and size classes; females exhibited significantly larger otoliths than males, and the larger size of fish was also associated with significantly larger otoliths than those of smaller fish ($P<0.01$). These findings highlight the correlation between fish size and otolith dimensions, demonstrating otolith analysis as a useful tool for growth estimation and stock differentiation of *E. tetradactylum* populations.

Keywords: Fish morphology, growth pattern, otolith relationship, otolith study, size variation, threadfin fish

INTRODUCTION

The four-fingered threadfin, *Eleutheronema tetradactylum* (Polynemidae; Perciformes), is a protandrous hermaphroditic fish. This species is extensively distributed across tropical and subtropical regions, spanning from the Persian Gulf through Bangladesh and Southeast Asia to Australian waters (Ballagh et al. 2012). Juveniles are initially considered males in the early life stage and typically inhabit estuarine environments. In contrast, adults of this species reach a certain size and are typically found in shallow marine coastal waters at depths of less than 5 meters within tropical and subtropical ecosystems (Motomura et al. 2002; Zischke et al. 2009). It is also reported that Ballagh et al. (2012) conducted a sagittal otolith analysis to explore the relationship between otolith size and fish size in *E. tetradactylum*.

Otoliths are biomineralized calcium carbonate structures located in the inner ear cavity of teleost fishes, beneath the hindbrain (Masato and Katsunori 2021; Khanali et al. 2021; Smoliński and Gutkowska 2024). They are composed primarily of more than 90% aragonite within a proteinaceous matrix (Liu et al. 2022). Otoliths are classified into three types: sagitta, lapillus, and asteriscus (Mitsui et al. 2020; Schroeder et al. 2022). The sagitta and asteriscus exhibit interspecific variation, whereas the lapillus is generally conserved across species (Samsun and Sağlam 2024).

The sagitta is the largest otolith of most teleost species, except those belonging to the orders Siluriformes and Cypriniformes. It possesses species-specific characteristics (Roy and Bardhan 2021; Pavlov 2022) and has been the primary focus of otolith morphology studies (Gao et al. 2021; Motamedi et al. 2021; Fadzli et al. 2023). The sagitta comprises two surfaces: the outer (lateral) and inner (medial) surfaces. Otolith growth is positively correlated with fish size and typically follows an allometric pattern (Badaev et al. 2023; Deville et al. 2023; Majhi et al. 2023).

Identifying the key factors influencing otolith size and shape variation is essential for advancing taxonomic, systematic, and evolutionary studies (Mitsui et al. 2020; Singh et al. 2022; Masubuchi et al. 2024). The morphology of the sagitta provides valuable taxonomic information, assisting in species identification and evolutionary research (Nelson et al. 2021; Mejri et al. 2022; Widdrington et al. 2025) as well as enhancing the understanding of fish biology and ecology (Huang et al. 2021; Ku et al. 2021; Roy et al. 2024).

Intraspecific variation in sagittal otolith dimensions (e.g. length, width, weight) has been utilized to estimate fish growth patterns (Wu et al. 2023; Widdrington et al. 2025), distinguish fish stocks (Ballagh et al. 2012; Gao et al. 2021), and classify fish species, populations, sexes, and age classes (Başusta and Khan 2021; Mejri et al. 2022; Wu et al. 2023). Additionally, otoliths provide insights into sensory adaptation (Singh et al. 2022), pigmentation patterns (Koca and Küçükköse 2023), ultrastructural characteristics (Roy and Bardhan 2021), ontogenetic changes, feeding ecology, predator-prey interactions, and trophic relationships (Mitsui et al. 2020; Majhi et al. 2023; Wu et al. 2023; Smoliński and Gutkowska 2024). The variation in otolith dimensions is also valuable for detecting hybridization (Li et al. 2021) and for studying otolith chemistry to track fish migration and life history (Liu et al. 2022; Yang et al. 2024).

Despite extensive research on otoliths, certain aspects of their morphology remain poorly understood. Notably, no study has investigated otolith variation across different size classes or life stages of protandrous hermaphroditic fish. Slower-growing individuals often exhibit disproportionately larger otoliths (Chanthran et al. 2021; Li et al. 2021; Brand et al. 2023). Conversely, Roy et al. (2024) demonstrated that in *Trichogaster fasciata*, fast-growing males possessed smaller otoliths than slower-growing females despite males being larger at the same age.

This present study aims to elucidate the morphological diversity between otolith parameters of Otolith Length (OL), Otolith Height (OH), and Otolith Weight (OW) with Standard Length (SL) and total Body Weight (BW) of *E. tetradactylum* across different sexes and size classes from Pattani Bay, Thailand, to address these knowledge gaps. The findings of this study will contribute to baseline biological knowledge and serve as a reference for future research on the management and conservation of this ecologically and economically significant species.

MATERIALS AND METHODS

Study area

The study was conducted in the nearshore waters of Pattani Bay, Pattani Province, located in the southern region of Thailand (Figure 1). Pattani Bay is a 74 km^2 semi-enclosed estuarine system located in the lower part of Gulf of Thailand, characterized by a tropical monsoonal climate. The average water depth ranges from 0.2 to 1.5 m, with a maximum depth of 5 m at the bay's mouth (Hajisamae et al. 2006).

Procedures

Fish sampling

Adult specimens of *E. tetradactylum* were collected using a monofilament gillnet with a mesh size of 4.5 cm stretched, a depth of 5 m, and a total length of 540 m over a continuous eight-month period (May-December 2021). Juvenile and smaller-sized *E. tetradactylum* specimens were sampled using a shrimp net in nearshore areas immediately following the primary sampling. A total of 303 specimens (166 males and 137 females) were collected, stored in insulated cool boxes, and transported to the laboratory for further analysis.

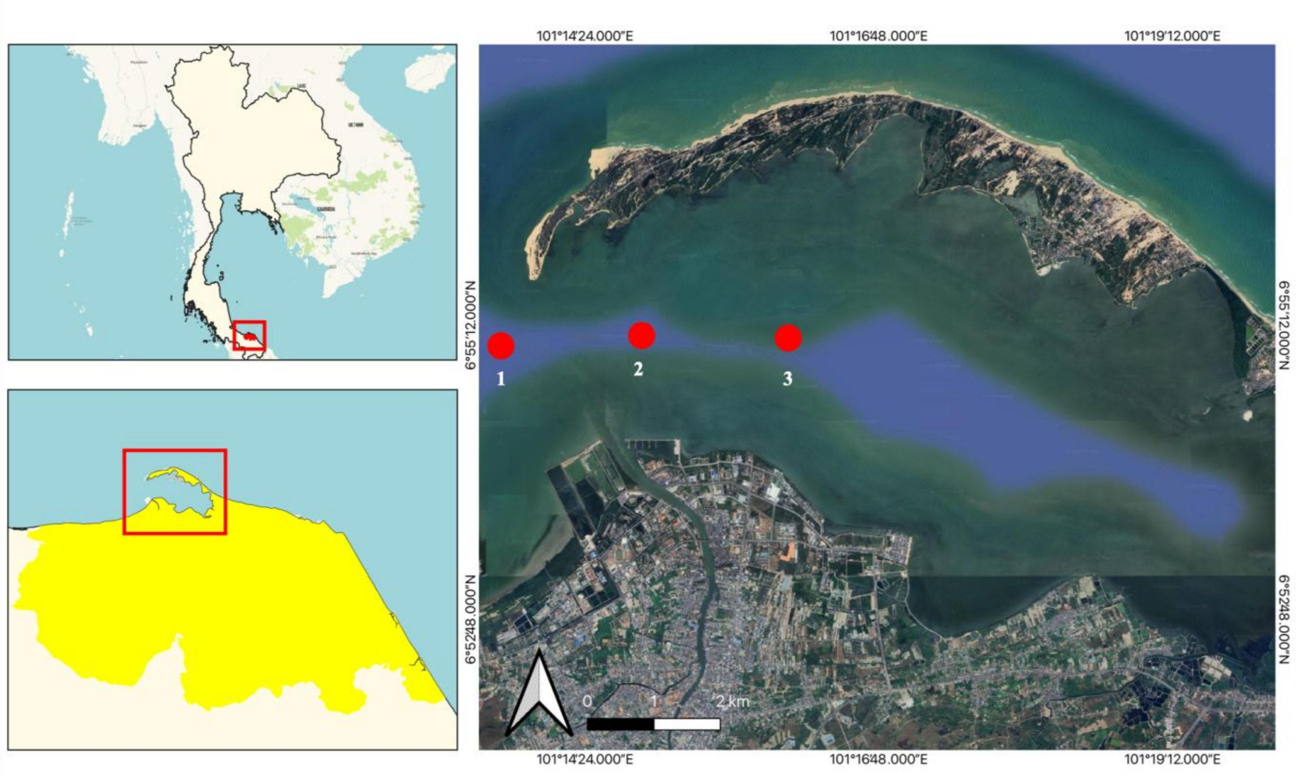

Figure 1. Maps of the sampling area in Pattani Bay, Pattani Province, Thailand. Site: 1: Outer bay; 2: Middle bay; 3: Inner bay

Laboratory works

In the laboratory, Standard Length (SL) was measured to the nearest 0.01 cm using a digital vernier caliper, and total Body Weight (BW) was determined with an electronic balance to the nearest 0.01 gram (g). The specimens were categorized into three distinct size classes statistically based on their Standard Length (SL) in centimeters: small (4.77-18.35 cm), medium (19.11-32.31 cm), and large (32.34-46.11 cm), respectively. These categories were also similar to those in a previous study (Iqbal et al. 2023). Sagittal otoliths were extracted from the hindbrain using medical tweezer forceps, rinsed in distilled water, dried with tissue, and stored in vial tubes. Otoliths were photographed using a Nikon SMZ 745T microscope with the iWorks 2.0 software (NAHWOO, Version 20.16.306). Otolith Weight (OW) was measured to the nearest 0.0001 g using a digital balance (OHAUS PR Series 224/E). Subsequently, Otolith Length (OL) and Otolith Height (OH) were measured to the nearest 0.001 mm using the Digimizer image analysis software (Version 4.3.4) on the meter scale (Figure 2). The same person conducted all measurements to minimize the potential for measurement bias.

Data analysis

The relationships between SL, BW, and Otolith Parameters (OL, OH, and OW) were estimated for each sex and size class using Ordinary Least Squares (OLS) regression, following the methodology recommended by Kilmer and Rodríguez (2017) for allometric studies and functional trait relationships. Differences in otolith shape were analyzed using multivariate statistical methods suitable for flat structures, which can be accurately represented in two-dimensional space and imagery.

$$y = a + bx$$

$$a = y - bx$$

$$b = r\,\frac{s_y}{s_x}$$

Where, y represents the dependent variable, x denotes the independent variable, a corresponds to the intercept, and b represents the slope of the linear regression model. The r denotes the correlation coefficient, and the standard error is represented by s.

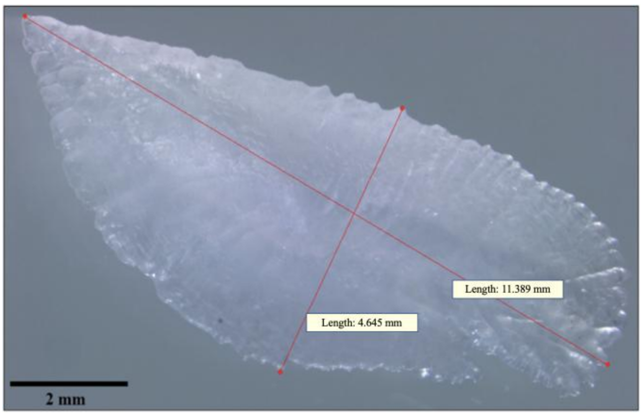

Figure 2. The measurements of otolith length (longest line) and otolith height (shortest line) of *Eleutheronema tetradactylum*

The degree of the association between variables was assessed using the coefficient of determination (R^2). Additionally, a T-test was performed to evaluate whether significant differences exist in the b values across sexes and size classes, as derived from the linear regression analysis (Sokal and Rohlf 1987).

$$T_s = (b - 3)/S_b$$

Where, T_s is the t-test value, b represents the slope parameter, and S_b denotes the standard error of b value (the slope). The b values indicate three distinct growth patterns: positive allometric growth (b>3.0), negative allometric growth (b<3.0), and isometric growth (b: 3.0) (Ricker 1973).

Student's t-test was employed to evaluate the statistical significance of differences in otolith parameters (OL, OH, and OW) between males and females of *E. tetradactylum*. Additionally, One-way Analysis of Variance (ANOVA) was conducted to examine variations in otolith parameters among different size classes of fish (small, medium, and large) of *E. tetradactylum*. When significant differences were detected among size classes, a post hoc Tukey's Honestly Significant Difference (HSD) test was applied to determine pairwise differences.

Prior to statistical analysis, otolith measurements (OL, OH, and OW) were standardized to the standard body length of *E. tetradactylum* using the allometric equation proposed by Lombarte and Lleonart (1993), as follows:

$$M_{adj} = M\left(\frac{L_s}{L_0}\right)^b$$

Where, M_{adj} represents the size-adjusted measurement, M denotes the original otolith measurement; Ls corresponds to the overall mean Standard Length (SL) of the fish, L_0 represents the individual SL of the fish, and parameter b is the slope derived from the regression of log-transformed M against log L_0. Prior to analysis, all morphometric measurements were logarithmically transformed to improve normality and minimize residual heteroscedasticity. Statistical analyses were conducted using Paleontological Statistics (PAST) software version 4.03 (Hammer et al. 2001).

RESULTS AND DISCUSSION

General characteristics

The Standard Length (SL) and Body Weight (BW) of *E. tetradactylum* specimens ranged from 4.77 cm to 46.11 cm and between 1.98 g and 1,300 g, respectively. Otolith measurements for Otolith Length (OL) ranged from 1.64 mm to 12.20 mm, Otolith Height (OH) from 0.86 mm to 5.82 mm, and otolith weight (OW) from 0.0003 g to 0.0580 g. A comprehensive summary of SL, BW, and otolith parameters (OL, OH, and OW) of *E. tetradactylum* across the entire sampling period is presented in Table 1.

Table 1. Summary descriptives of fish body sizes and otolith parameters of male, female, small, medium, and large size classes of *Eleutheronema tetradactylum*

Sex/Size	Fish size/otolith parameters	Min-Max	Mean±SD	95% CI
Male (n: 166)	Standard length (cm)	4.77-24.14	10.52±6.31	9.55-11.49
	Body weight (g)	1.98-273.49	47.34±73.30	36.11-58.58
	Otolith length (mm)	1.64-8.72	4.05±2.45	3.68-4.43
	Otolith height (mm)	0.86-4.02	1.85±1.17	1.68-2.04
	Otolith weight (g)	0.0003-0.0189	0.0044±0.005	0.0036-0.0053
Female (n: 137)	Standard length (cm)	22.1-46.11	30.22±5.46	29.3-31.15
	Body weight (g)	200.3-1300	523.14±263.39	478.64-567.65
	Otolith length (mm)	6.25-12.20	8.81±0.92	8.66-8.97
	Otolith height (mm)	2.85-5.82	3.96±0.45	3.89-4.05
	Otolith weight (g)	0.0144-0.0575	0.0279±0.01	0.0262 0.0297
Small (n: 129)	Standard length (cm)	4.77-18.35	7.43±2.78	6.95-7.92
	Body weight (g)	1.98-132.12	11.36±23.99	7.18-15.54
	Otolith length (mm)	1.649-8.107	2.932±1.394	2.69-3.17
	Otolith height (mm)	0.862-3.869	1.328±0.688	1.21-1.45
	Otolith weight (g)	0.0003-0.0104	0.0017±0.0019	0.001-0.002
Medium (n: 135)	Standard length (cm)	19.11-32.31	25.73±3.54	25.13-26.33
	Body weight (g)	98.15-900	336.44±142.71	312.14-360.73
	Otolith length (mm)	6.255-10.115	8.334±0.566	8.24-8.43
	Otolith height (mm)	2.856-4.546	3.79±0.327	3.73-3.85
	Otolith weight (g)	0.0102-0.0563	0.0206±0.0066	0.019-0.02
Large (n: 39)	Standard length (cm)	32.34-46.11	37.31±4.37	35.89-38.73
	Body weight (g)	376.81-1300	837.06±269.59	749.67-924.45
	Otolith length (mm)	7.97-12.201	9.692±1.091	9.34-10.05
	Otolith height (mm)	3.586-5.83	4.341±0.54	4.17-4.52
	Otolith weight (g)	0.027-0.058	0.04±0.009	0.037-0.043
Overall (n: 303)	Standard length (cm)	4.77-46.11	19.42±11.47	18.13-20.73
	Body weight (g)	1.98-1300	262.47±300.73	228.48-296.47
	Otolith length (mm)	1.64-12.20	6.20±3.04	5.86-6.55
	Otolith height (mm)	0.86-5.82	2.81±1.39	2.65-2.97
	Otolith weight (g)	0.0003-0.0575	0.015±0.01	0.0135-0.0167

Note: Min: Minimum; Max: Maximum; SD: Standard Deviation; CI: Confident Interval

Relationship between fish size and otolith size

The relationship between fish size parameters (SL and BW) and otolith parameters (OL, OH, and OW) of *E. tetradactylum* was analyzed across different groups, including the overall population, males, females, and size classes (small, medium, and large), as summarized in Tables 2 and 3. The highest correlations were observed between SL and BW with OL in male fish, with both regression coefficients (R^2) reaching 0.958. Conversely, weaker relationships were identified based on adjusted R^2 values (R^2<0.500) in the following cases: SL and BW with OL in medium-sized fish, SL and BW with OH in females, medium and large-size classes, and SL and BW with OW in medium-sized fish. Additionally, the relationship between fish size and otolith dimensions exhibited negative allometric growth, with *b* values consistently below 3.0 (*b*<3.0).

Results from t-tests indicated statistically significant differences (*P*<0.01) in the estimated of *b* values between males and females, as well as among the small, medium, and large size classes for all examined relationships, except for the relationships between SL and BW with OH in medium-sized fish, where no significant differences were detected (*P*: 0.116 and *P*: 0.188, respectively).

Relationship between different otolith dimensions

The estimation of the relationships between OL vs OH, OL vs OW, and OH vs OW indicated a negative allometric growth pattern (*b*<3.0). The strongest correlations were observed between OH vs OL, OL vs OW, and OH vs OW in males and the small-size class (R^2>0.90). In contrast, the weakest correlations were found for OH vs OL, OL vs OW, and OH vs OW in females and the medium-size class (R^2<0.500). Results from the t-test revealed that all *b* values exhibited significant differences across all otolith size parameters (*P*<0.01), except for OH vs OW in the medium size class (*P*: 0.137). Details of the relationships between otolith parameters are presented in Table 4.

Result of statistical analysis of otolith parameter

Table 5 presents the statistical analysis of otolith size in male and female specimens. The mean size±Standard Deviation (SD) for OL was measured as 0.661±0.184 mm and 0.99±0.039 mm, whereas OH was 0.425±0.157 mm and 0.695±0.039 mm, and OW was 0.002±0.002 g and 0.012±0.004 g in males and females, respectively. Results of the t-test revealed statistically significant differences in all otolith parameters between male and female specimens (*P*<0.01), with females exhibiting significantly larger otoliths than males. All otolith parameters (OH, OL, and OW) clearly undergo significant changes when the fish transition from male to female, with larger otoliths occurring during the sex change.

Table 6 summarizes the mean size±SD of small, medium, and large otoliths. The mean OL was 0.578±0.109 mm, 0.969±0.027 mm, and 1.027±0.044 mm. Corresponding OH values were 0.354±0.095 mm, 0.679±0.031 mm, and 0.726±0.043 mm, while OW values were 0.001±0.001 g, 0.009±0.003 g, and 0.017±0.004 g for small, medium, and large otoliths, respectively.

Analysis of Variance (ANOVA) demonstrated significant differences in otolith parameters among the three size categories (*P*<0.01). These findings indicate that larger fish possess proportionally larger otoliths than smaller individuals. Furthermore, post hoc analysis using Tukey's Honestly Significant Difference (HSD) test confirmed highly significant differences (*P*<0.01) among all pairwise comparisons of fish size and otolith parameters.

Table 2. Relationship between the fish standard length (cm) and the otolith parameter of *Eleutheronema tetradactylum*

Standard length (cm)		**Ordinary least squares regression**			**Student's t-test for *b***	
Otolith	**Sex/Size**	**Slope (*b*) ±SE**	**Intercept (*a*)±SE**	**Adjusted R^2**	***t***	**P value**
OL (mm)	Male	0.876±0.014	-0.221±0.014	0.958	61.433	<0.001
	Female	0.393±0.033	0.404±0.049	0.509	11.851	<0.001
	Small	0.962±0.030	-0.298±0.027	0.890	32.052	<0.001
	Medium	0.180±0.036	0.712±0.051	0.159	5.021	<0.001
	Large	0.666±0.100	-0.026±0.159	0.540	6.602	<0.001
	Overall	0.729±0.008	-0.083±0.011	0.956	81.629	<0.001
OH (mm)	Male	0.742±0.014	0.322±0.015	0.940	50.869	<0.001
	Female	0.315±0.037	0.224±0.056	0.338	8.315	<0.001
	Small	0.824±0.029	-0.396±0.027	0.857	27.662	<0.001
	Medium	0.070±0.044	0.578±0.063	0.018	1.580	0.11649
	Large	0.584±0.108	-0.198±0.170	0.441	5.411	<0.001
	Overall	0.604±0.008	-0.193±0.011	0.938	67.546	<0.001
OW (g)	Male	0.011±0.001	-0.009±0.001	0.926	45.39	<0.001
	Female	0.052±0.002	-0.066±0.004	0.734	19.313	<0.001
	Small	0.007±0.001	-0.005±0.001	0.852	27.072	<0.001
	Medium	0.033±0.002	-0.039±0.004	0.501	11.558	<0.001
	Large	0.062±0.006	-0.082±0.010	0.717	9.698	<0.001
	Overall	0.019±0.001	-0.016±0.001	0.816	36.646	<0.001

Table 3. Relationship between fish body weight (g) and otolith parameter of *Eleutheronema tetradactylum*

Body weight (g)		**Ordinary least squares regression**			**Student's t-test for *b***	
Otolith	**Sex/Size**	**Slope (*b*)±SE**	**Intercept (*a*) ±SE**	**Adjusted R^2**	***t***	**P value**
OL (mm)	Male	0.270±0.004	0.352±0.006	0.954	58.749	<0.001
	Female	0.147±0.012	0.595±0.034	0.495	11.517	<0.001
	Small	0.287±0.009	0.339±0.008	0.871	29.394	<0.001
	Medium	0.056±0.010	0.828±0.026	0.170	5.2281	<0.001
	Large	0.221±0.036	0.384±0.105	0.502	6.1181	<0.001
	Overall	0.229±0.002	0.389±0.005	0.959	84.878	<0.001
OH (mm)	Male	0.228±0.004	0.164±0.006	0.933	47.853	<0.001
	Female	0.119±0.014	0.375±0.038	0.333	8.2163	<0.001
	Small	0.245±0.009	0.151±0.008	0.834	25.342	<0.001
	Medium	0.017±0.013	0.634±0.033	0.012	1.3231	0.18807
	Large	0.213±0.035	0.105±0.102	0.496	6.0361	<0.001
	Overall	0.189±0.002	0.198±0.005	0.940	68.888	<0.001
OW (g)	Male	0.003±0.001	-0.002±0.001	0.921	43.998	<0.001
	Female	0.019±0.001	-0.040±0.002	0.693	17.491	<0.001
	Small	0.002±0.001	-0.001±0.001	0.842	26.058	<0.001
	Medium	0.009±0.001	-0.015±0.002	0.468	10.830	<0.001
	Large	0.022±0.001	-0.048±0.005	0.778	11.402	<0.001
	Overall	0.005±0.001	0.004±0.001	0.796	34.326	<0.001

Note: OL: Otolith Length; OH: Otolith Height; OW: Otolith Weight; SE: Standard Error; *T*: Student's t-test value

Table 4. Relationship between different otolith parameters (length, height, and weight) of *Eleutheronema tetradactylum*

Otolith	Sex/Size	Ordinary least squares regression			Student's t-test for *b*	
		Slope (*b*)±SE	Intercept (*a*)±SE	Adjusted R^2	*t*	P value
OL vs OH	Male	0.844±0.010	-0.133±0.007	0.974	79.602	<0.001
	Female	0.789±0.050	-0.087±0.050	0.642	15.57	<0.001
	Small	0.837±0.021	-0.129±0.012	0.92	38.91	<0.001
	Medium	0.751±0.075	-0.049±0.072	0.4291	9.99	<0.001
	Large	0.844±0.078	-0.142±0.080	0.7576	10.75	<0.001
	Overall	0.828±0.006	-0.124±0.005	0.981	126.93	<0.001
OL vs OW	Male	0.012±0.0003	-0.006±0.0002	0.894	37.361	<0.001
	Female	0.077±0.006	-0.064±0.006	0.483	11.239	<0.001
	Small	0.007±0.0002	-0.003±0.0001	0.898	33.56	<0.001
	Medium	0.037±0.0085	-0.027±0.008	0.123	4.33	<0.001
	Large	0.061±0.0089	-0.045±0.009	0.558	6.84	<0.001
	Overall	0.023±0.0008	-0.012±0.0007	0.715	27.493	<0.001
OH vs OW	Male	0.014±0.0004	-0.004±0.0001	0.88	34.706	<0.001
	Female	0.066±0.007	-0.034±0.005	0.342	8.3934	<0.001
	Small	0.008±0.0002	-0.002±0.00009	0.893	32.62	<0.001
	Medium	0.011±0.007	0.0008±0.005	0.016	1.49	0.13735
	Large	0.060±0.009	-0.026±0.007	0.517	6.29	<0.001
	Overall	0.028±0.001	-0.008±0.0006	0.696	26.308	<0.001

Note: OL: Otolith Length; OH: Otolith Height; OW: Otolith Weight; SE: Standard Error; *T*: Student's t-test value

Table 5. Student's t-test analysis on mean value ±SD of fish size and otolith size between male and female of *Eleutheronema tetradactylum*

Otolith parameters	Mean±SD (Male)	Mean±SD (Female)	*t*	P value
OL (mm)	0.661±0.184	0.99±0.039	20.514	<0.001
OH (mm)	0.425±0.157	0.695±0.039	19.548	<0.001
OW (g)	0.002±0.002	0.012±0.004	25.277	<0.001

Note: OL: Otolith Length; OH: Otolith Height; OW: Otolith Weight; SD: Standard Deviation; *T*: Student's t-test value

Table 6. One-way ANOVA test on mean value ±SD of fish size and otolith size between small, medium, and large size classes of *Eleutheronema tetradactylum*

Otolith Size	Mean ±SD (S)	Mean ±SD (M)	Mean ±SD (L)	SS	MS	F	P value
OL (mm)	0.578±0.109	0.969±0.027	1.027±0.044	12.228	6.114	1086	<0.001
OH (mm)	0.354±0.095	0.679±0.031	0.726±0.043	8.391	4.196	931.3	<0.001
OW (g)	0.001±0.001	0.009±0.003	0.017±0.004	0.009	0.005	864.5	<0.001

Note: S: Small; M: Medium; L: Large; OL: Otolith Length; OH: Otolith Height; OW: Otolith Weight; SD: Standard Deviation; SS: Sum of Square; MS: Mean Square; F: F-test value

Discussion

In the present study, the relationship between fish length and weight and otolith parameters was analyzed using the Ordinary Least Squares (OLS) regression model. The results demonstrated that otolith parameters exhibited a linear correlation with fish length and weight, consistent with findings reported for *Umbrina cirrosa* in the eastern Mediterranean Sea (Başusta and Khan 2021), *Forsterygion nigripenne* in Otago, New Zealand (Taddese et al. 2021), *Aphanius furcatus* from the Hormuzgan Basin Southern Iran (Motamedi et al. 2021) and *Chaunax abei* in Suruga Bay, Japan (Şenbahar et al. 2024). Furthermore, sexual dimorphism in otolith parameters has been investigated in several marine species, including the shi drum (*U. cirrosa*), killifish (*A. furcatus*), and toadfish (*Porichthys notatus*) (Mitsui et al. 2020; Başusta and Khan 2021; Motamedi et al. 2021).

These study findings indicate that female *E. tetradactylum* exhibits larger otoliths compared to their male counterparts. The increased otolith size in females may be more closely associated with enhanced auditory acuity rather than heightened sound sensitivity (Lackmann et al. 2022), which is characterized by a lower threshold and a broader range of frequency responses (Bose et al. 2018; Chanthran et al. 2021; Koca and Küçükköse 2023). Motamedi et al. (2021) reported a similar pattern in *A. furcatus*, where females possessed larger otoliths than males, likely due to differential growth rates during early developmental stages (Brand et al. 2023; Itoh 2024). The larger otolith shape in *E. tetradactylum* is initially developed in male individuals and remains unchanged in females after sex change (Itoh 2024; La Mesa and Eastman 2024). Furthermore, under the influence of sensory drive, female fish are more likely to select males whose mating signals can be easily detected through their sensory mechanisms (Li et al. 2021; Daban and Şen 2024).

In *E. tetradactylum*, which undergoes sex change, otoliths may play a crucial role in communication and social structuring within the population (Gao et al. 2021; Motamedi et al. 2021; Pavlov 2022). It is also suggested that males of *E. tetradactylum* that transition to females may exhibit faster growth rates prior to reaching the size or age threshold for sex change, potentially explaining their relatively smaller otolith size before transformation (Li et al. 2021; Singh et al. 2022; Xuan et al. 2023; Khanali et al. 2021; Masubuchi et al. 2024). Furthermore, the observation that otoliths in sex-changing males were already smaller suggests that early growth differences during the first year may influence their eventual development into terminal females (Nelson et al. 2021; Wu et al. 2023; Wilson and Altenritter 2024)

Phenotypic plasticity in fish is influenced by various ecological factors, including diet quality and quantity, food availability, and phylogenetic biomineralization (Roy and Bardhan 2021; Deville et al. 2023; Majhi et al. 2023; Masubuchi et al. 2024). Additionally, population-level and genetic variations, differences in growth rates between sexes, and ontogenetic changes contribute to the physiological mechanisms underlying sexual dimorphism in fish (Başusta and Khan 2021; Motamedi et al. 2021; Badaev et al. 2023).

Environmental factors such as water salinity, temperature, and depth have been shown to influence variations in otolith shape (Lombarte and Lleonart 1993; Masato and Katsunori 2021; Smoliński and Gutkowska 2024). Additionally, these factors play a significant role in the morphological modification of the sulcus acusticus region of the sagittal otolith, which is associated with enhanced auditory perception (Roy and Bardhan 2021; Nelson et al. 2021). In the present study, field observations indicated that larger individuals of *E. tetradactylum* were more frequently found in deeper waters with lower temperatures compared to those inhabiting shallower regions. Furthermore, otoliths from individuals residing in warmer waters tended to be relatively larger and heavier than those from colder environments (Mejri et al. 2022; Fadzli et al. 2023; Samsun and Sağlam 2024).

These study findings indicate a significant variation in otolith parameters across different size classes of *E. tetradactylum*, suggesting a general ontogenetic shift in otolith morphology. The development of the sagittal otolith exhibits stage-specific variations associated with sexual maturity in *E. tetradactylum* (Roy and Bardhan 2021). Ontogenetic changes in otolith shape are attributed to shifts in the growth acceleration patterns of the fish (Mitsui et al. 2020; Singh et al. 2022; Masubuchi et al. 2024). A positive correlation was observed between fish size and otolith weight, indicating proportional growth. The sagittal otoliths of smaller individuals of *E. tetradactylum* are characterized by a smooth surface and marginal sculpture, which represent plesiomorphic traits. In contrast, larger individuals exhibit substantial morphological modifications, likely due to the progressive deposition of calcium carbonate crystals (Huang et al. 2021; Ku et al. 2021; Roy and Bardhan 2021; Pavlov 2022; Fadzli et al. 2023).

The relationship between otolith size and fish body parameters has been well-documented, with studies demonstrating a significant correlation between otolith length and weight with body length and weight in various fish taxa (Mitsui et al. 2020; Nelson et al. 2021; Singh et al. 2022; Badaev et al. 2023; Brand et al. 2023; Majhi et al. 2023). In the present study, a positive correlation was observed between otolith length and body length, as well as between otolith length and body weight, in *E. tetradactylum*, indicating that otolith size increases proportionally with somatic growth. Notably, the otolith length exhibited a stronger correlation with body length than otolith height, particularly in medium-sized individuals. These findings align with previous studies on *E. tetradactylum* (Roy and Bardhan 2021) and other fish genera, including *Coelorinchus* and *Merluccius* (Lombarte and Lleonart 1993), Aphaniidae (Motamedi et al. 2021), as well as Sillaginidae (Yang et al. 2024).

It is hypothesized that otolith formation may reach a developmental threshold at a specific body length due to stabilization in otolith weight. Furthermore, variations in the relationship between otolith and body size may be species-specific and influenced by population dynamics (Roy and Bardhan 2021; Xuan et al. 2023; Yang et al. 2024; Widdrington et al. 2025). In conclusion, this present study represents the first investigation into the relationship between otolith parameters and the size (length and weight) of *E. tetradactylum* in the Gulf of Thailand, with a specific focus on Pattani Bay. Among the measured otolith parameters, otolith length was identified as the most reliable predictor of fish length and weight, considering variations in sex and size classes. The findings clearly indicate a positive correlation between fish size and otolith dimensions, with larger individuals possessing proportionally larger otoliths. Additionally, female *E. tetradactylum* exhibited significantly larger otoliths compared to males. Given the direct relationship between otolith size and fish growth, otolith parameter analysis provides essential insights into the growth dynamics of *E. tetradactylum.* It serves as a valuable tool for estimating fish length and weight. Furthermore, these parameters can be used for differentiating fish stocks within *E. tetradactylum* populations.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Faculty of Science and Technology, Prince of Songkla University, Thailand, and the TUYF Charitable Trust, Hong Kong, for their financial support.

REFERENCES

Badaev OZ, Chernienko IS, Ovsyannikova SL. 2023. A comparative analysis of age estimates for Greenland halibut, *Reinhardtius hippoglossoides matsuurae*, from the northwestern Pacific Ocean based on different methodological approaches. Russ J Mar Biol 49 (7): 616-625. DOI: 10.1134/S1063074023070027.

Ballagh AC, Welch DJ, Newman SJ, Allsop Q, Stapley JM. 2012. Stock structure of the blue threadfin (*Eleutheronema tetradactylum*) across northern Australia derived from life-history characteristics. Fish Res 121: 63-72. DOI: 10.1016/j.fishres.2012.01.011.

Başusta N, Khan U. 2021. Sexual dimorphism in the otolith shape of shi drum, *Umbrina cirrosa* (L.), in the eastern Mediterranean Sea: Fish size-otolith size relationships. J Fish Biol 99 (1): 164-174. DOI: 10.1111/jfb.14708.

Bose AP, McCallum ES, Raymond K, Marentette JR, Balshine S. 2018. Growth and otolith morphology vary with alternative reproductive tactics and contaminant exposure in the round goby *Neogobius melanostomus*. J Fish Biol 93 (4): 674-684. DOI: 10.1111/jfb.13756.

Brand M, Spotowitz L, Mark FC, Berge J, Langhelle EL, Węsławski JM, et al. 2023. Age class composition and growth of Atlantic cod (*Gadus morhua*) in the shallow water zone of Kongsfjorden, Svalbard. Polar Biol 46 (1): 53-65. DOI: 10.1007/s00300-022-03098-1.

Chanthran SS, Lim PE, Poong SW, Du J, Loh KH. 2021. Relationships between sagittal otolith size and body size of *Terapon jarbua* (Teleostei, Terapontidae) in Malaysian waters. J Oceanol Limnol 39: 372-381. DOI: 10.1007/s00343-019-9193-7.

Daban İB, Şen Y. 2024. Some notes on morphometry and population biology of shi drum *Umbrina cirrosa* juveniles in the Marmara Sea, Türkiye. Thalassas 40 (1): 581-591. DOI: 10.1007/s41208-024-00670-w.

Deville D, Kawai K, Fujita H, Umino T. 2023. Ecomorphology of three closely related Sebastes rockfishes with sympatric occurrence in Seto Inland Sea, Japan. Hydrobiologia 850 (18): 4049-4066. DOI: 10.1007/s10750-023-05286-4.

Fadzli MH, Mat-Jaafar TNA, Sharikin ASAA, Pau TM, Latiff NAA, Piah SAM, Mat-Piah R. 2023. Length frequency distribution, length-weight relationships and growth parameters of coastal trevally *Turrum coeruleopinnatum* (Rüppell, 1830) caught from Terengganu waters, Malaysia. Thalassas 39 (2): 903-913. DOI: 10.1007/s41208-023-00554-5.

Gao MH, Wu ZQ, Huang LL, Tan XC, Liu H, Rad S. 2021. Otolith shape analysis and growth characteristics in larval and juvenile *Squalidus argentatus*. Environ Biol Fish 104: 937-945. DOI: 10.1007/s10641-021-01125-4.

Hajisamae S, Yeesin P, Chaimongkol S. 2006. Habitat utilization by fishes in a shallow, semi-enclosed estuarine bay in southern Gulf of Thailand. Estuar Coast Shelf Sci 68: 647-655. DOI: 10.1016/j.ecss.2006.03.020.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4 (1): 1-9. DOI: 10.1086/519781.

Huang YF, Song BL, Deng TH, Wang Q, Shen Q, Liu LG. 2021. Ontogenetic development, allometric growth patterns, and daily increment validation of larvae and juvenile *Culter alburnus*. Environ Biol Fishes 104: 1593-1610. DOI: 10.1007/s10641-021-01181-w.

Iqbal TH, Hajisamae S, Lim A, Jantarat S, Wang W-X, Tsim KWK. 2023. Feeding habits of four-finger threadfinfish, *Eleutheronema tetradactylum*, and its diet interaction with co-existing fish species in the coastal waters of Thailand. PeerJ 11: e14688. DOI: 10.7717/peerj.14688.

Itoh T. 2024. Can sex differences in spatiotemporal distribution and age composition explain the female-biased sex ratio observed in the catch of butterfly kingfish *Gasterochisma melampus*? Fish Sci 90 (5): 723-732. DOI: 10.1007/s12562-024-01795-w.

Khanali F, Alavi-Yeganeh MS, Nasri M. 2021. Relationship between otolith measurements against length and weight body of four ponyfish species. Thalassas 37 (2): 701-704. DOI: 10.1007/s41208-021-00342-z.

Kilmer JT, Rodríguez RL. 2017. Ordinary least squares regression is indicated for studies of allometry. J Evol Biol 30 (1): 4-12. DOI: 10.1111/jeb.12986.

Koca HU, Küçükköse AG. 2023. Otolith biometry of pikeperch *Sander lucioperca* from the Lakes Region of Turkey. Inland Water Biol 16 (2): 193-197. DOI: 10.1134/S1995082923020116.

Ku JE, Lee SI, Kim DN. 2021. Age and growth of Southern Bluefin Tuna, *Thunnus maccoyii*, based on otolith microstructure. Ocean Sci J 56: 413-423. DOI: 10.1007/s12601-021-00041-z.

La Mesa M, Eastman JT. 2024. Assessing current knowledge and future challenges of age determination, life span and growth performance in notothenioid fishes: A review. Rev Fish Biol Fish 34 (2): 575-596. DOI: 10.1007/s11160-023-09829-9.

Lackmann AR, Bielak-Lackmann ES, Jacobson RI, Butler MG, Clark ME. 2022. Otolith allometry informs age and growth of long-lived Quillback *Carpiodes cyprinus*. Environ Biol Fishes 105 (8): 1051-1064. DOI: 10.1007/s10641-022-01315-8.

Li W, Zhang C, Tian Y, Liu Y, Liu S, Tian H, Cao C. 2021. Otolith shape analysis as a tool to identify two pacific Saury (*Cololabis saira*) groups from a mixed stock in the high-seas fishing ground. J Ocean Univ China 20: 402-408. DOI: 10.1007/s11802-021-4541-6.

Liu C, Zhang C, Tian Y, Wang L, Lin L, Li Y, Watanabe Y. 2022. Otolith microstructure analysis reveals different growth histories of Japanese Sardine (*Sardinops melanostictus*) in the Oyashio Waters. J Ocean Univ China 21 (1): 236-242. DOI: 10.1007/s11802-022-4840-6.

Lombarte A, Lleonart J. 1993. Otolith size changes related with body growth, habitat depth and temperature. Environ Biol Fishes 37: 297-306. DOI: 10.1007/BF00004637.

Majhi BM, Lianthumluaia, Sarkar UK, Nath AK. 2023. Understanding relationship between length and weight with otolith dimension of *Tenualosa ilisha* from Hooghly River (India). Inland Water Biol 16 (6): 1134-1140. DOI: 10.31857/S0320965223060220.

Masato U, Katsunori T. 2021. Life-history traits of a widely distributed coral reef fish, *Mulloidichthys flavolineatus* (Mullidae), Okinawa Island, southern Japan. Environ Biol Fishes 104 (12): 1559-1574. DOI: 10.1007/s10641-021-01168-7.

Masubuchi T, Kawano M, Shimose T, Yagi Y, Kanaiwa M. 2024. Age, growth, and estimation of the age-length key for Japanese flounder *Paralichthys olivaceus* in the southwestern Sea of Japan. Fish Sci 90 (3): 379-395. DOI: 10.1007/s12562-024-01765-2.

Mejri M, Bakkari W, Tazarki M, Mili S, Chalh A, Shahin AAB, Ben Faleh AR. 2022. Discriminant geographic variation of saccular otolith shape and size in the common Pandora, *Pagellus erythrinus* (Sparidae) across the Gulf of Gabes, Tunisia. J Ichthyol 62 (6): 1053-1066. DOI: 10.1134/S0032945222060169.

Mitsui S, Strüssmann CA, Yokota M, Yamamoto Y. 2020. Comparative otolith morphology and species identification of clupeids from Japan. Ichthyol Res 67: 502-513. DOI: 10.1007/s10228-020-00746-6.

Motamedi M, Teimori A, Iranmanesh A. 2021. Ontogenetic pattern, morphological sexual and side dimorphism in the saccular otolith of a scaleless killifish *Aphanius furcatus* (Teleostei: Aphaniidae). Acta Zool 102 (1): 38-50. DOI: 10.1111/azo.12313.

Motomura H, Iwatsuki Y, Kimura S, Yoshino T. 2002. Revision of the Indo-West Pacific polynemid fish genus *Eleutheronema* (Teleostei: Perciformes). Ichthyol Res 49 (1): 47-61. DOI: 10.1007/s102280200005.

Nelson TR, Hightower CL, Coogan J, Walther BD, Powers SP. 2021. Patterns and consequences of life history diversity in salinity exposure of an estuarine dependent fish. Environ Biol Fishes 104: 419-436. DOI: 10.1007/s10641-021-01080-0.

Pavlov DA. 2022. Otolith morphology in gibel carp *Carassius gibelio* and crucian carp *C. carassius* (Cyprinidae). J Ichthyol 62 (6): 1067-1080. DOI: 10.1134/S0032945222060200.

Ricker WE. 1973. Linear regressions in fishery research. J Fish Res Board Can 30 (3): 409-434. DOI: 10.1139/f73-072.

Roy S, Bardhan I. 2021. The developmental variations of the sagitta otolith in the young and mature male of a hermaphrodite polynemidae fish, *Eleutheronema tetradactylum* (Shaw, 1804). Pap Avulsos Zool 61: 1-12. DOI: 10.11606/1807-0205/2021.61.78.

Roy S, Roy UG, Ghorai N, Saha SK. 2024. Developmental variations of sagitta otolith in different body size groups of *Trichogaster fasciata* (Bloch and Schneider, 1801). Zoomorphology 143 (2): 415-431. DOI: 10.1007/s00435-024-00646-7.

Samsun S, Sağlam NE. 2024. Determination of growth and reproduction characteristics of garfish (*Belone euxini* Günther, 1866) in the South Black Sea. Thalassas 40 (2): 1041-1051. DOI: 10.1007/s41208-024-00699-x.

Schroeder R, Schwingel PR, Correia AT. 2022. Population structure of the Brazilian sardine (*Sardinella brasiliensis*) in the Southwest Atlantic inferred from body morphology and otolith shape signatures. Hydrobiologia 849 (6): 1367-1381. DOI: 10.1007/s10750-021-04730-7.

Şenbahar AM, Eto A, Yokota M. 2024. Body and otolith morphometrics of sea toad *Chaunax abei* (Le Danois, 1978) in Suruga Bay, Japan. Thalassas 40 (2): 755-766. DOI: 10.1007/s41208-024-00715-0.
Singh M, Kashyap A, Ansari JA, Serajuddin M. 2022. Spatial variations in the shape and chemistry of sagittal otoliths in *Channa punctatus* (Channidae) populations of Ganga Basin, India. Inland Water Biol 15 (3): 249-261. DOI: 10.1134/S1995082922030142.
Smoliński S, Gutkowska J. 2024. Otolith biochronology for the long-term reconstruction of growth and stock dynamics of fish. Rev Fish Biol Fish 34 (1): 405-419. DOI: 10.1007/s11160-023-09820-4.
Sokal RR, Rohlf FJ. 1987. Introduction to Biostatistics, 2nd Ed. Freeman Publication, New York.
Taddese F, Reid M, Heim-Ballew H, Jarvis MG, Closs GP. 2021. Otolith chemistry of triplefin *Forsterygion nigripenne* indicates estuarine residency. Fish Sci 87: 271-281. DOI: 10.1007/s12562-021-01501-0.
Widdrington JB, Reis-Santos P, Morrongiello JR, Macdonald JI, Wakefield CB, Newman SJ, Gillanders BM. 2025. Otolith growth chronologies reveal distinct environmental sensitivities between and within shallow- and deep-water snappers. Rev Fish Biol Fish 35 (1): 217-240. DOI: 10.1007/s11160-024-09898-4.
Wilson KB, Altenritter ME. 2024. A multi-metric approach to characterize variability in yellow perch (*Perca flavescens*) use of Lake Ontario and two barrier-protected coastal wetlands. Environ Biol Fish 107 (5): 567-582. DOI: 10.1007/s10641-024-01552-z.
Wu R, Zhu Q, Katayama S, Tian Y, Li J, Fujiwara K, Narimatsu Y. 2023. Early life history affects fish size mainly by indirectly regulating the growth during each stage: A case study in a demersal fish. Mar Life Sci Technol 5 (1): 75-84. DOI: 10.1007/s42995-022-00145-y.
Xuan Z, Jiang T, Liu H, Chen X, Yang J. 2023. Otolith microchemical evidence revealing multiple spawning site origination of the anadromous tapertail anchovy (*Coilia nasus*) in the Changjiang (Yangtze) River Estuary. Acta Oceanol Sin 42 (1): 120-130. DOI: 10.1007/s13131-022-2135-9.
Yang T, Xiao P, Jiang X, Zhang Q, Zhao Y. 2024. Otolith morphometrics and variations between two populations of *Sillago sinica* (Perciformes, Sillaginidae) in the East China Sea and the Yellow Sea. Thalassas 40 (2): 1007-1017. DOI: 10.1007/s41208-024-00711-4.
Zischke MT, Cribb TH, Welch D, Sawynok W, Lester RJG. 2009. Stock structure of blue threadfin *Eleutheronema tetradactylum* on the Queensland east coast, as determined by parasites and conventional tagging. J Fish Biol 75 (1): 156-171. DOI: 10.1111/j.1095-8649.2009.02277.x.