The developmental variations of the sagitta otolith in the young and mature male of a hermaphrodite polynemidae fish, *Eleutheronema tetracystylum* (Shaw, 1804)

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Abstract. The *Eleutheronema tetracystylum* is a protandrous, hermaphrodite, marine perciformes fish. The body length of this fish acts as an important diagnostic marker for male and female discrimination. The present study describes for the first time the ultrastructural characteristics on the medial surface of the sagitta otolith in different body size groups of males of *E. tetracystylum* (Polynemidae: Perciformes) using scanning electron microscopy. The sagitta is a spindle-shaped structure that includes a well-developed rostrum and a poorly developed antirostrum. The sulcus is ostio-pseudocaudal type, almost straight and devoid of the collum. The ostium is a well-developed, vase-shaped structure. The cauda includes the colliculum and a well-developed caudal bulb with several distinct growth stripes. The length of the caudal bulb is significantly correlated to the growth of the body size of the fish. The excisura major is indistinct and the excisura minor is absent. The cristae are distinct on both sides of the sulcus. The one-way ANOVA test revealed that the development of several sagitta features shows significant differences in various body size groups of *E. tetracystylum*. The growth of the sagitta length is more closely related to the fork length than the sagitta width. Therefore, the sagitta length and the caudal bulb length can be used as important predictors to evaluate the fish size. The cauda region of the sagitta in *E. tetracystylum* is unique as well as more decorative than those of another Polynemidae fish and other hermaphrodite, marine perciformes fishes. The sagitta characteristics of *E. tetracystylum* might be advantageous in the identification of the sex and the taxonomy of the hermaphrodite fish species.

Keywords. Sagitta; Protandrous fish; Scanning electron microscopy; Surface sculpture.

INTRODUCTION

The otolith is a calcareous anatomical structure in the inner ear of fish and assists in the sensation as well as the body balancing by stimulation of inner ear hair cells (Sanchez & Martinez, 2017). In the taxonomic field, the fish species identification is significantly supported by the structural characteristics of the otolith due to its longer time of degradation (Rodriguez Mendoza, 2006; Vilizzi, 2018; Mitsui et al., 2020). The otolith includes one pair of *sagittae*, *asteriscii* and *lapiil* (Popper et al., 2005). The sagittae are the largest pair in size in fish groups (except, order Siluriformes and Cypriniformes) and are included in several species-specific characteristics (Harvey et al., 2000; Kontaş & Bostanci, 2015; Yılmaz et al., 2015; Mehnanna et al., 2016). The sagitta has two surfaces which are the lateral (outer) and medial surface (inner). The medial surface of the sagitta is well-decorated with different morpwo-structural features (Smale et al., 1995; Jawad et al., 2018; Bardhan et al., 2021), which characteristically varies with the fish groups and various environmental factors in their respective habitats (Kumar et al., 2012; Omar & AMohamed, 2016; Abdulsamad, 2017; Sanchez & Martinez, 2017; Jawad et al., 2018; Pracheil et al., 2019). Several studies have dealt with the morphological descriptions of the sagitta of marine Perciformes fishes (Hunt, 1992; Smale et al., 1995; Gierl et al., 2018; Jawad et al., 2018), but no ultrastructural studies on the sagitta are available in the hermaphrodite perciformes fishes. Additionally, the perciform family Polynemidae have eight genera but the sagitta morphologies are only described by light micro-
copy in only *Polydactylus virginicus* (Santificetur et al., 2017).

The *E. tetradactylum* (Polynemidae: Perciformes) is in fact a protandrous, hermaphrodite marine fish (Patnaik, 1967; Kowtal, 1972; Stanger, 1974; Motomura, 2004; Sadovy & Liu, 2008). They act as functional male in their early part of life and then act as functional female for the male and female discrimination among the individuals (Kailola et al., 1993; McPherson, 1997; Ballagh et al., 2012). The body size (fork length) of *E. tetradactylum* act as an important diagnostic marker for the male and female discrimination for the later periods of life (Patnaik, 1967; Kowtal, 1972; Stanger, 1974; McPherson, 1997; Ballagh et al., 2012). The body size (fork length) of *E. tetradactylum* act as an important diagnostic marker for the male and female discrimination among the individuals (Patnaik et al., 1967; Kowtal, 1972; Stanger, 1974; Motomura, 2004; Ballagh et al., 2012). Ballagh et al. (2012) briefly described the sagittae of *Eleutheronema tetradactylum* in a study of the relationship between the age and the growth of total body length of this fish. It has been stated that the development of the sagitta components (i.e., sulcus, ostium, rostrum, etc.) is associated with the body size (length/weight) groups of the sexually dimorphic fishes (Harvey et al., 2000; Jawad et al., 2018; Bardhan et al., 2021). However, there is no information regarding the developmental variations of the otolith morphologies among the young male and the mature male of the protandrous, hermaphroditic fishes. The present study aims to investigate detail ultrastructural characteristics of the sagitta otolith of the protandrous *Eleutheronema tetradactylum* (Shaw, 1804). A comparative developmental relationship of the various sagitta constituents between the young male and the mature male groups is conducted here. The results of the *E. tetradactylum* are also compared with the available data on the sagitta for a protandrous Polynemidae fish (*Polydactylus virginicus*) and three protandrous Sparidae fishes (*Sparidentex hastata*, *Acanthopagrus berda*, and *Acanthopagrus latus*).

**MATERIAL AND METHODS**

**Sample collection and grouping**

A total of 130 individuals of *Eleutheronema tetradactylum* (Shaw, 1804) (Polynemidae: Perciformes) were randomly collected from the fish market of Kolkata, West Bengal, India. The samples were identified by the Zoological Survey of India (ZSI), Kolkata, West Bengal, India. The body size of the individuals was examined by their fork length (i.e., the length from the anterior tip of the longest jaw to the median point of the caudal fin) (Önsoy et al., 2011; Butler et al., 2021) and was measured using a centimetre scale. The specimens were divided into four groups according to their fork length of the fishes (FL) (Jawad et al., 2011): group I (Gr-I), 11-12 cm (Mean: 11.55 ± 0.30), n = 25; group II (Gr-II), 15-16 cm (Mean: 15.54 ± 0.30), n = 45; group III (Gr-III), 19-20 cm (Mean: 19.58 ± 0.31), n = 35; group IV (Gr-IV), 23-24 cm (Mean: 23.52 ± 0.31), n = 20. The specimens in group IV were mature males and the individuals in other three groups were younger males.

**Collection of the sagitta otolith and scanning electron microscopy**

One pair of sagittae were removed from the sacculus of the inner ear of each individual of the four groups (Ruck, 1976; Jawad et al., 2018), cleaned with water and 70% ethanol, and stored dry in individual plastic tubes. For ultrastructural studies on the medial (inner) surface of the sagittae, the right sagitta was examined (Bardhan et al., 2021). The sagittae were dried and mounted on an aluminium stub using double-sided carbon tape. The sagittae were gold-coated by DWARDS, RVS coater, and analysed in an EVO18, ZEISS.

In the work, all the terminologies used for the description of the structural constituents of the medial surface of the sagitta are following Smale et al. (1995), Jawad (2007) and Bardhan et al. (2021).

**Morphometry and statistical analysis**

The measurements (mean value ± SD) of the various sagitta constituents were taken for the sagittae from the four size groups using image-processing software “ImageJ 1.51t” (Wayne Rasband, NIH, USA). The weights of sagittae were taken with a digital weight machine (Mettler Toledo ME204). A normality test using Shapiro-Wilk test was applied to check the distributions of the studied sagitta constituents among the four groups. The test met the assumption of parametric analysis and a one-way ANOVA followed by Tukey’s test was performed using XLSTAT statistical program to determine the statistical significance (P < 0.05) on the developmental differences on the growth of the studied sagitta features in the different life stages (young males to mature male) of *E. tetradactylum*.

**RESULTS**

**General morphology of the sagitta**

The sagitta of *E. tetradactylum* males is an oblong or spindle-shaped structure (Figs. 1, 2A-D). The medial surface of this sagitta is slightly convex and enriched with different structural features (Figs. 1, 2A-D, Tables 1-2). The ultrastructural characteristics of the various constituents on the medial surface of this sagitta is described with the following points.

**The sulcus**

The sagitta includes well-developed heterosulcoidal and ostio-pseudocaudal type sulcus (Figs. 1, 2A-D, Table 1). The sulcus is mostly straight along its length and slightly bent near posterior end (Figs. 1, 2A-D, Table 1). The anterior end of the sulcus includes a vase-shaped ostial opening (Figs. 1, 2A). The posterior end of the sulcus contains slightly swollen, bulb-shaped, closed, caudal end- termed as the caudal bulb (Figs. 1, 2A-D, Table 1).
The sulcus lacks the collum (Figs. 1, 2A-D). A distinct ‘V’-shaped ridge is developed near the junction of ostium and cauda in the sulcus groove (Figs. 1, 2A-D).

**The rostrum and antirostrum**

The rostrum part of the sagitta is well-developed while the antirostrum part is comparatively very shorter than that of the rostrum (Figs. 1, 2A-D, Tables 1-2). A distinct gap is developed between the rostrum and the antirostrum, termed as excisura major (Figs. 1, 2A-D, Tables 1-2).

**The margins and surface sculptures**

The sagitta is dorsally oval-shaped with a distinct irregular margin and is ventrally slightly curved with sinuate margin (Figs. 1, 2A-D). A distinct groove is developed near the mid-dorsal region of the dorsal margin and is termed as the mid-dorsal groove (Figs. 1, 2B-D). The mid-dorsal groove comprises a broad base and a tiny outer opening (Figs. 2B-D, Table 1). Several growth stripes are distinct in the various portions of the caudal end and the ventral wall of the ostium (Figs. 2A-D). The ridge-like cristae are well-developed on both side of the sulcus (Figs. 1, 2A-D). The caudal colliculum is present and including several small, globular concretions, whereas the ostial colliculum is absent (Figs. 1, 2A-D). A well-developed dorsal depression is observed on the medial surface of sagittae, while ventral depression is absent (Figs. 1, 2A-D).

**Variations of the sagitta morphologies in different size groups**

_Eleutheronema tetradactylum_ showed many developmental variations of different sagittae components with-
Table 1. Relative features of the sagitta characteristics of the four fork length groups of *Eleutheronema tetradactylum*.

| Sagitta Characteristics | Gr-I (11-12 cm) (n = 25) | Gr-II (15-16 cm) (n = 45) | Gr-III (19-20 cm) (n = 35) | Gr-IV (23-24 cm) (n = 20) |
|-------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Rostrum                 | Poorly developed           | Ill-developed             | Developed                 | Well-developed            |
| Antrostium              | Indistinct                 | Developed, pointed        | Developed, blunt          | Reduced                   |
| Outer margin            | Mostly smooth with very few indistinct indentations | Dorsally irregular, ventrally sinuate, much distinct indentations | Dorsally irregular, ventrally sinuate, distinct some indentations | Dorsally irregular, ventrally sinuate, distinct some indentations |
| Groove on the mid dorsal margin | Absent                      | Typical V-shaped notch    | Cylindrical with flat base | Flank-shaped notch         |
| Sulcus                  | Straight, slightly curved to caudal end. Contains indistinct growth strips. | Straight, comparatively more curved to caudal end. Contains distinct growth strips. | Straight, bent near caudal end. Contains distinct growth strips. | Straight, bent near caudal end. Contains distinct growth strips. |

- **V-shaped impression at the junction of ostium and cauda**
  - Indistinct
  - Developed
  - Well-developed

- **Ostium**
  - Well developed, vane-shaped, almost smooth surface
  - Well developed, wider anteriorly, vane-shaped, rough surface with indistinct growth strips
  - Well developed, wider, vane-shaped, rough surface with some distinct growth strips
  - Well developed, wider, vane-shaped, mostly smooth surface with very few growth stripes

- **Cauda**
  - Almost cylindrical posteriorly
  - Posteriorly bulb-shaped with prominent growth stripes
  - Posteriorly bulb-shaped with prominent growth stripes
  - Posteriorly hockey stick like, indistinct growth stripes

- **Caudal colliculum**
  - Elongated, decorated with scattered button like structures
  - Elongated, decorated with scattered and distinct small globular concretion
  - Present, the concretion restricted near posterior end
  - Present, including fine granular sculptures

- **Dorsal depression**
  - Shallow
  - Deep, wider
  - Shallow
  - Reduced

- **Posterior end**
  - Smooth, pointed, single lobed
  - Smooth, blunt, crown shaped with three lobes
  - Smooth, blunt, single lobed
  - Smooth, pointed, crown shaped with three lobes

Table 2. The morphometry (mean value ± SD) of sagitta constituents of the four fork length groups of *Eleutheronema tetradactylum*.

| Sagitta features | Gr-I (n = 25) | Gr-II (n = 45) | Gr-III (n = 35) | Gr-IV (n = 20) |
|------------------|--------------|---------------|----------------|--------------|
| Sagitta length (mm) | 4.31 ± 0.58a | 5.14 ± 0.63b | 5.72 ± 0.71c | 6.48 ± 0.58c |
| Sagitta width (mm) | 2.01 ± 0.26a | 2.50 ± 0.32b | 2.51 ± 0.42a | 2.64 ± 0.43a |
| Sagitta weight (mg) | 3 ± 0.19a | 6 ± 0.43b | 8 ± 0.43a | 8 ± 0.66a |
| Rostrum length (µm) | 0.20 ± 0.05a | 0.34 ± 0.04b | 0.19 ± 0.05c | 0.27 ± 0.03d |
| Antrostum length (µm) | 0.11 ± 0.03a | 0.20 ± 0.04b | 0.22 ± 0.02c | 0.11 ± 0.05d |
| Sulcus length (µm) | 2.89 ± 0.02a | 3.1 ± 0.01b | 2.91 ± 0.02c | 2.72 ± 0.03c |
| Sulcus width (µm) | 0.35 ± 0.02a | 0.32 ± 0.02b | 0.31 ± 0.02c | 0.30 ± 0.01d |
| Sulcus depth (µm) | 0.07 ± 0.01a | 0.10 ± 0.01b | 0.10 ± 0.02c | 0.10 ± 0.02c |
| Width of excisura major (µm) | 0.75 ± 0.01a | 0.10 ± 0.01b | 0.97 ± 0.01c | 0.81 ± 0.01c |
| Ostium length (µm) | 0.67 ± 0.04a | 0.9 ± 0.02b | 0.88 ± 0.02c | 0.66 ± 0.01c |
| Ostium width (µm) | 0.38 ± 0.04a | 0.6 ± 0.01b | 0.38 ± 0.01c | 0.53 ± 0.01c |
| Cauda length (µm) | 2.19 ± 0.02a | 2.2 ± 0.02b | 2.03 ± 0.02c | 2.00 ± 0.02c |
| Cauda width (µm) | 0.35 ± 0.01a | 0.30 ± 0.01b | 0.33 ± 0.01c | 0.31 ± 0.01c |
| Length of caudal bulb (µm) | 0.45 ± 0.02a | 0.60 ± 0.02b | 0.70 ± 0.02c | 1.29 ± 0.02d |
| Width of the groove of mid dorsal margin (µm) | 21.89 ± 1.2a | 18.03 ± 1.52a | 18.03 ± 1.52a | 18.03 ± 1.52a |
| Depth of the groove of mid dorsal margin (µm) | 18.47 ± 0.61a | 10.58 ± 1.2c | 14.09 ± 1.28a | 14.09 ± 1.28a |
| Width between posterior caudal end and the postero-ventral margin (µm) | 0.13 ± 0.01a | 0.10 ± 0.01c | 0.14 ± 0.01c | 0.11 ± 0.01d |
| Width of crista superior-dorsal margin (µm) | 0.52 ± 0.05a | 0.60 ± 0.01b | 0.56 ± 0.01c | 0.54 ± 0.01d |
| Width of crista inferior-ventral margin (µm) | 0.59 ± 0.02a | 0.70 ± 0.02b | 0.61 ± 0.02c | 0.52 ± 0.03d |

* Lack of the structures in the group. The same letters after the mean values mean the absence of significant differences between the groups.

**Note:**

- Table 1 and Table 2 demonstrate the comparison of sagitta characteristics among four fork length groups of *Eleutheronema tetradactylum*.
- The data show a relative developmental variation in the four different body size groups (Table 2). Normality test shows that all the studied sagitta features are normally distributed in the four body size groups (Table 3).
- The development of the rostrum and antrostium are relatively different among the four body size groups (Figs. 2A-B, 6A-B; Table 2). The growth stripes in different parts of the sulcus are prominent in the groups with larger specimens (Table 1). It is observed that the margins and marginal sculpture of the sagittae are var-
ied with the increment of the total body length of fish-
es (Figs. 2A-D; Table 1). The outer margins (i.e., dorsal
and ventral margins) and their marginal sculptures (i.e.,
smooth margin, irregular margin, etc.) of the sagittae are
varied with the increment of the total body length of the
fishes (Figs. A-D; Table 1). The dorsal margin of the sagitta
in the individuals in group I is mostly smooth while it is
developed as characteristically irregular in the groups of
the larger specimen (Figs. 2A-D; Table 1). The ventral mar-
gin of the sagitta in the groups (i.e., Gr-II, Gr-III, and Gr-IV)
with larger specimens are the sinuate type with very dis-
tinct several marginal indentations which are very indis-
tinct and few in the sagitta of the individuals in group I
(Figs. 2A-D; Table 1). The groove on the mid-dorsal mar-
gin of sagittae is absent in group (Gr-I) with smaller spec-
imens while it is characteristically very distinct with var-ious shapes and sizes in groups (Gr-II, Gr-III, and Gr-IV)
with larger specimens (Tables 1-2). A ‘V’-shaped ridge is
developed near the junction of ostium and cauda in the
sulcus and is well-developed in groups with larger spec-
imens (Asterisk, Figs. 2A-D; Table 1). Development of the
caudal bulb (Cbl) at the caudal end is significantly and
positively correlated to the increment of the total body
length (Figs. 2A-D, 7B; Tables 1-2). The caudal bulb is
measured of 1.29 ± 0.02 µm in length in the individuals in
group IV (Gr-IV; 23-24 cm FL) (Fig. 2D) and 0.45 ± 0.02 µm
in length in the specimens in group I (Gr-I; 11-12 cm FL)
(Figs. 2A, D; Table 2). The development of the sagitta

Table 3. The tabular representation of the p-value of all corresponding sagitta constituents of the normality test (Shapiro-Wilk test). Individual p-value is greater
the alpha value (0.05). All the variables are normally distributed in all the groups of Eleutheronema tetradactylum.

| Sagitta features                  | Gr-I          | Gr-II         | Gr-III        | Gr-IV          |
|-----------------------------------|---------------|---------------|---------------|---------------|
| Sagitta length (OL)               | 0.1109        | 0.9789        | 0.8203        | 0.1568        |
| Sagitta width (OW)                | 0.9999        | 0.5031        | 0.4604        | 0.0834        |
| Sagitta weight (Owt)              | 0.5298        | 0.9370        | 0.9370        | 0.5043        |
| Sulcus length (SL)                | 0.7560        | 0.8294        | 0.8151        | 0.9585        |
| Sulcus width (SW)                 | 0.7495        | 0.7475        | 0.3928        | 0.2182        |
| Sulcus depth (SD)                 | 0.3271        | 0.7431        | 0.5836        | 0.5157        |
| Rostrum length (RL)               | 0.5974        | 0.7979        | 0.2717        | 0.9620        |
| Antrostrosum length (Art)         | 0.2908        | 0.2562        | 0.8433        | 0.1350        |
| Width of excisura major (ExW)     | 0.4184        | 0.5459        | 0.3080        | 0.8703        |
| Ostium length (Osl)               | 0.6848        | 0.6350        | 0.3193        | 0.4152        |
| Ostium width (Osw)                | 0.7833        | 0.7413        | 0.6966        | 0.2200        |
| Cauda length (CI)                 | 0.6471        | 0.6286        | 0.6436        | 0.7450        |
| Cauda width (CW)                  | 0.9874        | 0.8120        | 0.4275        | 0.6759        |
| Length of caudal bulb (Cbl)       | 0.9882        | 0.2584        | 0.1071        | 0.3785        |
| Width between posterior caudal end and the postero-ventral margin (CPVM) | 0.8648 | 0.0707 | 0.4367 | 0.2636 |
| Width of crista superior-dorsal margin (Csd) | 0.1735 | 0.8698 | 0.1961 | 0.5733 |
| Width of crista inferior-ventral margin (Civ) | 0.9963 | 0.9223 | 0.3159 | 0.1187 |
Figure 5. Relationships of the body size groups with different sagitta features: (A) sagitta length (OL); (B) sagitta width (OW); (C) sagitta weight (Owt); (D) sulcus length (SL); (E) sulcus width (SW); (F) sulcus depth (SD).
Figure 6. Relationships of the body size groups with different sagitta constituents: (A) rostrum length (RL); (B) antirostrum length (ArL); (C) width of excisura major (ExW); (D) ostium length (Ol); (E) ostium width (Ow); (F) cauda length (Cl).
length is significantly increased with the growth of total body length of fishes (Figs. 3, 5A; Table 2). The sagitta width is slightly increased with the growth of total body length and their developmental relationship is not significant (Fig. 5B; Table 2). The sagitta weight is initially increased with the increment of the total body length and after a certain body size (19-20 cm FL), its development remains the same or insignificantly developed as body length increases (Figs. 2A-D, 3, 5C; Tables 1-2). The development of the caudal length (Cl) and sulcus width (SW) is negatively correlated to the total body length (Figs. 4, 5E, 6F; Table 2). The sulcus depth (SD) and the width of the crista inferior to the ventral margin (CrvI) are larger in groups with smaller individuals than those in groups with larger individuals (Figs. 2A-D, 4, 5E, 8A; Table 2).

In the present study, it is observed that the sagitta morphologies of male *E. tetradactylum* have several relative relationships in respect of hermaphroditism with another protandrous Polynemidae fish (*Polydactylus virginicus*) and three protandrous Sparidae fishes (*Sparidentex hasta*, *Acanthopagus berda*, and *Acanthopagus latus*) irrespective of their male/female discriminations (Table 4). This comparative study showed that the sagitta morphologies are closely similar, but with some significant species-specific differences within the Polynemid fishes while they are characteristically different among the species of other protandrous Perciformes fishes (Table 4). The morphostructural and morphometric analysis advocates that the growth of the sagitta length and caudal bulb length are significantly increased with the increment of the total body length than that of the sagitta width and weight (Figs. 3, 5A-C, 7B; Table 2). The sagitta length, weight, and caudal bulb length may be used as important predictors to evaluate the body size of *E. tetradactylum*.

**DISCUSSION**

The detailed morphostructural characteristics of the medial surface of the sagittae of the male *E. tetradacty-
The development of the sagitta constituents varies in different stages of the sexual maturity of the male *E. tetradactylum*. This kind of developmental differences in different body size groups are also reported in other Perciformes fishes; i.e., *Chlorurus sordidus* (Jawad et al., 2018), *Anabas testudineus* (Bardhan et al., 2021), *Umbrina cirrosa* (Başusta & Khan, 2021). The characteristics of various sagitta constituents of *E. tetradactylum* slightly differ from those of the other Polynemidae fish (e.g., *Polydactylus virginicus*, Santificetur et al., 2017) and considerably differ from other protandrous marine Perciformes fishes (e.g., *Sparidentex hasto*, *Acanthopagrus berda*, and *Acanthopagrus latus*; Abdulsamad, 2017) (Table 4). The sagitta of *E. tetradactylum* is a spindle-shaped structure, however this varies with the protandrous *Polydactylus virginicus* and the three protandrous Sparidae fishes (Table 4). The medial surface of the sagitta comprises a well-developed sulcus groove as also reported in other fishes (Dehghani et al., 2016; Omar & Moselhy, 2016; Abdulsamad, 2017; Jawad et al., 2018; Khedher & Fatnassi, 2018), and this groove may assist for connecting the medial surface with the sensory cells of the internal ears (Popper & Hoxter, 1981; Popper & Lu, 2000). A number of sulcus morphologies of *E. tetradactylum* are characteristically identical with another marine as well as freshwater Perciformes fishes (Smale et al., 1995; Bremm & Schulz, 2014; Omar & AMohamed, 2016; Omar & Moselhy, 2016; Abdulsamad, 2017; Jawad et al., 2018; Bardhan et al., 2021). Hunt (1992) stated that the otolith morphologies between male and female fishes are almost the same, whereas several authors reported that there are some structural differences between these genders in many fish species (Vallisneri et al., 2008; Bostanci et al., 2012; Kontaş & Bostanci, 2015). In the present study it is observed that the development of sagittal constituents varies also with different body size groups in a particular gender (e.g., male fish).

The development of the medial surface sagitta structures on of *E. tetradactylum* is characteristically variable among different body-size groups, as described in other fishes (Jawad et al., 2018; Bardhan et al., 2021). However, several sagitta features of *E. tetradactylum* and *Polydactylus virginicus* (Santificetur et al., 2017) are mostly similar (Table 4) and possibly the common identifying features of the sagitta in the Polynemidae species. In the present study, we show that the sagittae of the smaller fish group possess entire smooth surface and marginal sculpture, whereas those sagitta features considerably differ with the increase of the total body length, probably due to various pattern of the calcium carbonate crystals deposition (Campana & Thorrold, 2001; Schwarzhans & Grenfell, 2002; Volpedo & Echevarría, 2003; Vilizzi, 2018; Pracheil et al., 2019).

It has been suggested that the increase of the otolith weight is significantly proportional to the total body length of the individuals in some fish groups but the length and width of the otolith are not (Gümüş & Kurt, 2009; Bardhan et al., 2021). In the present study, the

![Figure 8](image-url)
weight of the sagitta in the *E. tetradactylum* is increased with the growth of total body length of the individuals in the young male groups with smaller body size (*i.e.*, the sagitta weight in group I fishes is lesser than that in the group II), while this increment of the otolith weight is restricted in the mature male group with larger body size (*i.e.*, the sagitta weight in the group III fishes is almost similar to that in the group IV). Furthermore, the morphometric measurements of the sagitta in *E. tetradactylum* reveal that the length of the otolith is significantly related to the total body length of the fishes instead of the otolith width and also reported in other fish families such as *Nototheniidae* (Lombarte et al., 1991), *Merluccidae* (Lombarte & Lleonart, 1993), *Labridae* (Skeljo & Ferri, 2012), *Sciaenidae* (Carvalho et al., 2012); *Cyprinidae* (Kontaş & Bostanci, 2015), *Sparidae* (Lombarte & Lleonart, 1993); *Labridae* (Skeljo & Ferri, 1991), *Merluccidae* (Santificetur et al., 2017) reveal that the length of the otolith is significantly related to the total body length of the individuals in the young male groups with smaller body size (*i.e.*, the sagitta weight in group I fishes is lesser than that in the group II), while this increment of the otolith weight is restricted in the mature male group with larger body size (*i.e.*, the sagitta weight in the group III fishes is almost similar to that in the group IV). Furthermore, the morphometric measurements of the sagitta in *E. tetradactylum* reveal that the length of the otolith is significantly related to the total body length of the fishes instead of the otolith width and also reported in other fish families such as *Nototheniidae* (Lombarte et al., 1991), *Merluccidae* (Lombarte & Lleonart, 1993), *Labridae* (Skeljo & Ferri, 2012); *Cyprinidae* (Kontaş & Bostanci, 2015), *Sparidae* (Khedher & Fatnassi, 2018), *Sciaenidae* (Carvalho et al., 2012). It is assumed that this formation in the sagitta may be completed at a certain body length due to constant weight. Additionally, the development of the caudal bulb is directly proportional to the growth of total body length in the *E. tetradactylum*. It is presumed that the relationship between the total body length and various characteristics of the otolith may be varied with the fish species and their relative habitats (Jawad, 2007; Jawad et al., 2018; Khedher & Fatnassi, 2018; Bardhan et al., 2021).

The sagitta features of the male *E. tetradactylum* shows some characteristic similarities with other marine perciformes fishes irrespective of their male/female gender specificity (Ballagh et al., 2012; Kontaş & Bostanci, 2015; Avigliano et al., 2016; Omar & AMohamed, 2016; Omar & Moselhy, 2016; Santificetur et al., 2017; Abdulsamad, 2017; Khedher & Fatnassi, 2018; Jawad et al., 2018). In the present study, sagitta characteristics of the male *E. tetradactylum* are described and may have some variations with those of the female individual but this requires further investigations. The results of the current study advocate that the sagitta features in different maturation phase of maleness of *E. tetradactylum* may be convenient for future studies of the otolith of other protandrous, hermaphroditic fishes and ultimately find out the relatedness among the species as well as male and female discriminations of the Polynemidae family in respect of systematics and gender choice respectively.

### ACKNOWLEDGMENTS

The authors thank principal Prof. Mohua Das, subject coordinator Prof. Debarati Mukherjee, and all other faculty members of the Department of Zoology, Women's College Calcutta, Kolkata, West Bengal, India, for their help in this study. The authors are grateful to all members of SEM Unit, Zoological Survey of India (ZSI), Kolkata, for their help with the scanning electron microscopy studies. The authors are thankful to Dr. Amit Mukhopadhyay, Mollusca Section, Zoological Survey of India (ZSI), Kolkata, for his constructive suggestions in this work. The authors are also thankful to the Fish Division, Zoological Survey of India (ZSI), Kolkata, for the identification of the studied fish in this work. The authors are also grateful to the reviewers and editorial team of the manuscript for their constructive suggestions.

### AUTHORS’ CONTRIBUTIONS

**S.R.:** Design the work, dissection of first part of the otolith, guidance of dissection, SEM preparation and photography, arrangement and analysis of figures, illus-
tration steps and microscopy, statistical analysis, graphical representations, and part of the description, supervision on the whole work, final revision of the whole manuscript.  

**I.B.:** Collection of the samples, dissection of the sagitta otolith from fish, schematic drawing of the otolith, SEM preparation, morphological and morphometry data collection, arrangement of text, table and references.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

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