Morphometric relationship of otolith, length, and weight of rainbow runner, *Elagatis bipinnulata* (Quoy and Gaimard, 1825) from Gunungkidul Coastal Waters

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Abstract. Rainbow runner, a high-value commodity caught by fishermen in southern Java, is not the main catch target. This research aimed to determine the morphological characteristics and morphometric relationship of otoliths with the length-weight of fish in rainbow runner. A total of 120 individual samples taken in October-November consisting of 68 females and 52 males from local fishermen at Sadeng Coastal Fishing Port were extracted and measured according to the Otolith shape index method with six descriptors: Form Factor (FF), Roundness (RO), Rectangularity (Rt), Circularity (C), and Aspect Ratio (AR). The otoliths were tested using the T-test, and the results showed substantial differences in the width, area, and periphery of the left and right otoliths. The results of the regression test showed that the highest relationship was between the weight of the otolith and the length of the rainbow runner, which had an R-value of 0.70, and the relationship between the weight of the otolith and the weight of the rainbow runner had an R-value of 0.51. Rainbow runner otoliths have an irregular surface, are not circular, and tend to be elongated. Otolith size varies between species, influenced by differences in calcium carbonate deposition rates, growth, age, and environmental factors.

1 Introduction

As a maritime country, Indonesia has a water area proportion of 62%, so the water area is more expansive than land. Therefore, Indonesia has an enormous and diverse fisheries resource potential, including the potential for capture fisheries production. The volume of rainbow runner catches in Indonesia in 2020 was 10,333 tons and increased in 2021 by 10,493 tons [1]. Rainbow runner fishing activities in Yogyakarta Special Region Provincial waters can occur throughout the year. Fishermen land their catches at the Sadeng Coastal Fishing Port (PPP Sadeng), with production volumes in 2020 and 2021 of 11,576 and 9,644 kg, respectively [2]. Rainbow runner (*Elagatis bipinnulata,* Quoy and Gaimard 1825) is a small pelagic fish catch fishery commodity at the PPP Sadeng. Rainbow runners, which belong to

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the Carangidae family, are fish that are widely distributed in tropical and subtropical waters [3]. They are abundant among the fishermen's catch during January-April and comprise 140 species in 32 genera [4]. Rainbow runners are fast swimmers with high migratory abilities, often found in near-surface waters, coral reefs, and offshore water, both in schooling or under solitary conditions [5].

In PPP Sadeng, Rainbow runners are often caught as bycatch from fishing vessels that catch large pelagic fish such as tuna and skipjack [6]. Usually, these ships range from 10-30 GT (sekochi boats) to more than 30 GT (inkamina fleet). Many rainbow runners are caught using purse seines [7; 8], and small quantities are caught using gill nets and trawl fishing gear [9]. This species has many consumers in the local market, so it has high commercial value [8]. In the southern coastal region of Java, rainbow runner fish also have a relatively large market, and usually, this type is called laura fish or sea milkfish. Usually rainbow runner fish are sold in traditional markets as fresh fish. This species is widespread among many people because of its delicious meat and affordable price [10].

Otoliths are organs that exist in every individual teleost fish and result from biomineralization in the fish's body [11]. Otoliths are composed of calcium carbonate ($CaCO_3$), the main component. They usually take the form of aragonite and salt deposits composed of inorganic protein material. Otoliths have three parts, namely sagittae, lapillus and asteriscus. The sagittae is the most often observed because it is the largest, and this part also has the most stable structure and the most specific characteristics. Otoliths are a permanent life history recording medium for each fish and can provide information about the environmental conditions of the fish's habitat during its lifetime [12]. Because the otolith does not undergo resorption, the calcium carbonate deposits will be permanent and continue to record information, including changes in fish habitat conditions that influence the fish's growth rate [13]. Studies on otoliths in Indonesia are still rare; thus, this research was a trailblazer for other studies. Studies on the relationship between length and weight on fish otolith morphometry have many benefits, including estimating the size of fish species that have been digested, both in length and weight. In addition, the results of otolith research provide supporting data in the study of prey-predator relationships. They can back-calculate the size of fish that have been preyed upon or digested [12]. This study also provides benefits in studies of fish stock estimation, fisheries biology, and fisheries resource management related to fish age.

2 Materials and methods

2.1 Study site

The fleet of fishing vessels measuring > 30 GT, for example, the Inkamina vessels, land their catch at PPP Sadeng, Gunungkidul, Special Region of Yogyakarta (**Fig. 1**). The Inkamina ship operates fishing gear in hand lines and purse seines, the results of which are pelagic fish, including rainbow runners. Rainbow runner samples were selected randomly from the catches of the Inkamina ship. Fish samples were collected from as many as 50 kg or more than 50 individuals monthly. Sampling time takes place in October – December 2022. Fish samples are then transported to the laboratory for data collecting, including measuring the length and weight of the fish, dissecting, taking otoliths, measuring the length and weight of otoliths, and determining sex.

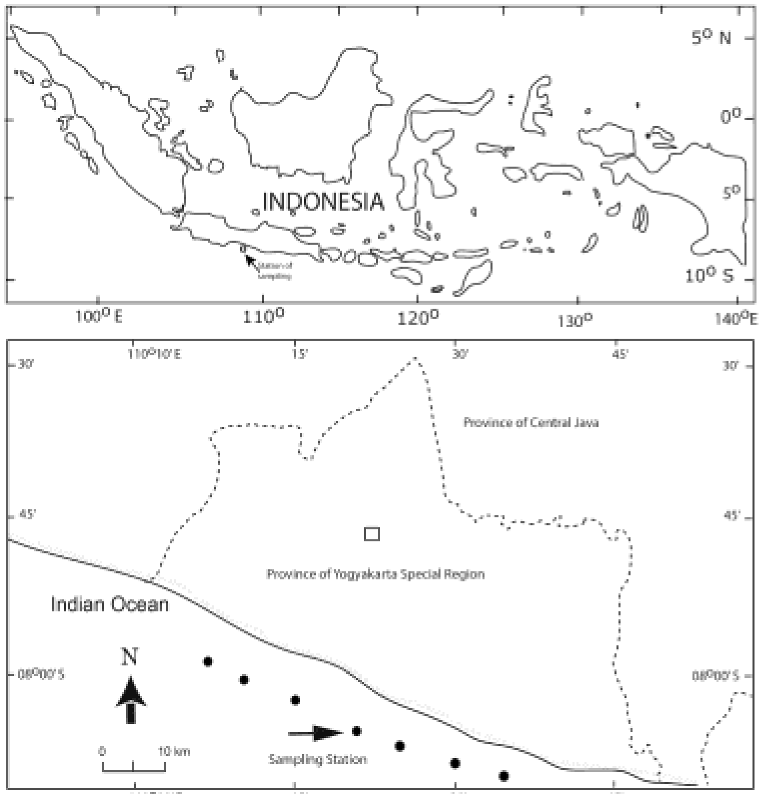


Fig 1. Map of locations for catching rainbow runners using hand lines or purse seines in the waters south of Java. The fishing ground location is > 12 miles from the coastline.

2.2 Otolith morphometric data collection

Otolith collection begins by weighing the rainbow runner using an electric scale with an accuracy of 0.1 g, then measuring its total length using a stainless-steel ruler with an accuracy of 0.1 cm. Next, the fish was positioned on its back, the operculum opened as wide as possible, and the gill sieves were cut and removed. The upper and posterior oral cavities were cleaned, and the first vertebra was gently broken so that the otoliths were exposed and pushed out of the sac. The otolith is then taken using tweezers and cleaned of attached membranes and mucus. Next, the otoliths were cleaned, dried, and stored in jars equipped with identification marks. Observations were continued by determining the sex of the fish through primary characteristics, namely observing the gonads, which consist of testes or ovaries [14]. Each otolith was weighed using an analytical balance with an accuracy of 0.001 g to obtain information on the otolith weight (OW). Then, the otoliths were documented using a 25-megapixel resolution camera. Using ImageJ software, otolith morphometric data was collected (Fig. 2), namely otolith length (OL), otolith width (OWi), otolith area (OA), and otolith periphery (OP).

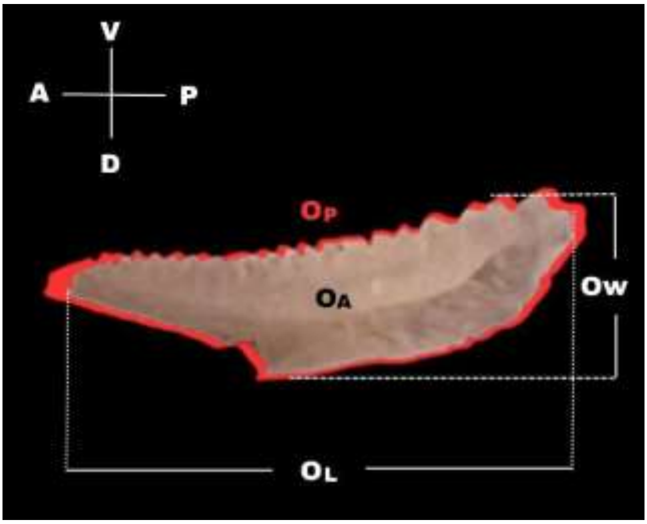


Fig. 2. Measurement and collection of otolith morphometric data on the rainbow runner.

2.3 Data analysis

The otolith shape index is described using six descriptors: Form Factor (FF), Roundness (RO), Circularity or Compactness (C), Rectangularity (Rt), Ellipticity (E), and Aspect Ratio (AR). Table 1 presents the calculation formulas and information for each descriptor.

Table 1. Otolith shape index formula and description.

Shape Index	Formula	Description
FF	$4\pi OA/OP\ 2$	Estimating regularity on the otolith surface, where FF = 1 indicates a regular surface such as a circle, while the FF value < 1 indicates an irregular surface.
RO	$4OA/\pi OL2$	Compare the shape of the otolith to the circle shape full, where RO = 1 indicates a full circle shape.
C	$OL\ 2/OA$	Compare the shape of the otolith to the shape of a full circle
Rt	$OA/(OL.\ OW\text{i})$	Describes variations in the length and width of the otolith relative to the area, where Rt = 1 describes the otolith as a perfect square.
E	$\frac{OL - OW\text{i}}{(OL + OW\text{i})}$	Indicates a proportional change in the axis.
AR	$OL/OW\text{i}$	It shows the shape of the otolith, where an AR value > 1 indicates the form of the otolith tends to be elongated.

Then, differences in male-female and left-right otolith morphometry were analyzed using the T-test with a 95% confidence level. The initial hypothesis (H0) is that there is no significant difference between male-female and left-right otoliths. If the P value exceeds the alpha value (0.05), then the initial hypothesis (H0) is accepted, whereas if the P value is smaller than the alpha value (0.05), then the initial hypothesis (H0) is rejected. Next, a linear regression test analyzed the relationship between otolith morphometrics and fish length-weight. The linear equation y = ax + b was used to analyze the correlation coefficient (R) of the otolith's length, width, area, and periphery with the total length and weight of the fish with a 95% confidence level to each correlation.

3 Result

One hundred twenty samples were collected from the fishermen, consisting of 68 females and 52 males. The males' length varies from 30.0 to 56.3 cm, averaging 45.92 cm. Their weight ranges from 208.7 to 1427.5 g, with an average of 847.4 g. In females, the length varies from 34.5-55.6 cm with an average of 48.2 cm and a weight range of around 310.1-1450 g with an average of 888.4 g, which is significantly more significant compared to their counterparts on average.

Out of 120 individual samples, only 89 pairs of otoliths were collected from 52 females and 37 males. Sixteen pairs of female otoliths and 15 pairs of male otoliths were damaged during the collection process, so they were excluded from this analysis to maintain clarity.

3.1 Otolith shape index

The otolith shape index describes the shape of fish otoliths by calculating data on the length, width, area, and periphery of the otolith using six descriptors, including Form Factor (FF), Roundness (R0), Circularity (C), Rectangularity (R), Ellipticity (E), and the average value of the shape index, which is presented in Table 2.

Table 2. Determine the rainbow runner otolith shape using the shape index.

Shape Index	Min	Max	Average	description
Form Factor (FF)	0.311	0.582	0.400	< 1, Irregular otolith surface
Roundness (R0)	0.20	0.36	0.256	≠ 1, otoliths do not form a complete circle
Circularity (C)	21.567	40.413	31.792	-
Rectangularity (Rt)	0.777	0.767	0.753	≠ 1, the otoliths do not form a perfect square
Ellipticity (E)	0.444	0.65	0.579	-
Aspect Ratio (AR)	2.60	4.72	3.773	> 1, the shape of the otolith tends to be elongated

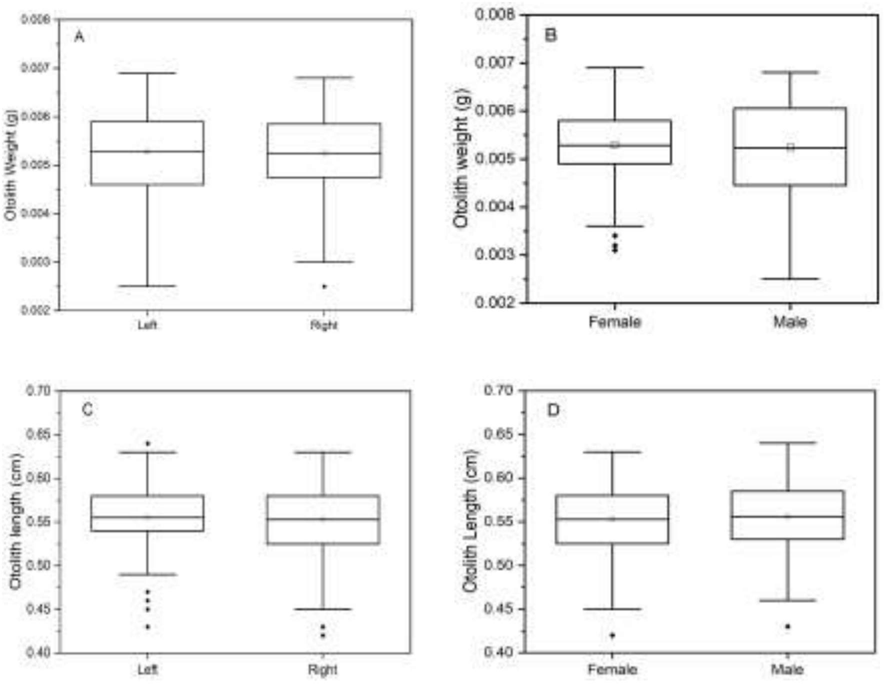
Based on the results of the shape index calculations presented in Table 2, otolith shape index values were obtained with six types of descriptors. The first descriptor is the form factor to determine the regularity of the otolith surface, and the average result obtained is 0.4. This value is less than one, so the rainbow runner otolith surface is irregular. The second descriptor is Roundness, a circle-shaped indicator with an average value of 0.256. The otolith is said to resemble a circle if the roundness value is 1, so a roundness value of 0.256 indicates that the rainbow runner otolith does not form a complete circle.

The following descriptor is Circularity, which shows the ratio between the rainbow runner otolith and a full circle, namely 31.792. Then, the next descriptor is Rectangularity, which describes the shape of a square. The otolith is declared completely square if the rectangularity value is 1. The average rectangularity value is 0.753, where this value is not equal to 1, so the shape of the rainbow runner otolith does not form a perfect square. The fifth descriptor is Ellipticity, which indicates an elliptical shape between a circle and a square. The ellipticity value of the rainbow runner otolith is 0.579, so it is not oval. Next, the final descriptor is the Aspect ratio, which has an average value of 3.733. This value is more than one so that the shape of the rainbow runner otolith tends to be elongated, not rounded, not square, or not elliptical.

3.2 Differences in the shape of female and male otoliths, as well as left and right.

Each pair of rainbow runner otoliths obtained varied in length, width, area, periphery, and weight. The left rainbow runner otolith has an average length (OL) of 0.555 ± 0.042 cm, while the right is 0.553 ± 0.044 cm. The left otolith width (OWi) is 0.143 ± 0.012 cm, while the right is 0.151 ± 0.012 cm. The left otolith area (OA) size is 0.59 ± 0.06 cm^2, while the right is 0.64 ± 0.07 cm^2. The periphery of the otolith perimeter (OP) is 1.407 ± 0.128 cm, while the right one is 1.375 ± 0.109 cm. The left otolith weight (OW) was 0.0053 ± 0.0009 g, while the right was 0.00052 ± 0.0009 g.

Furthermore, the morphometry of otoliths between sexes, namely between females and males, also has variations in size. Female otoliths have an average length (OL) of 0.553 ± 0.04 cm, while males are 0.556 ± 0.046 cm. The size of the otolith width (OWi) in females ranges from 0.1485 ± 0.0108 cm, while in males, it is 0.144 ± 0.047 mm. The area size (OA) of females is 0.611 ± 0.062 cm^2, while that of males is 0.613 ± 0.088 cm^2. Furthermore, the size of the otolith perimeter (OP) in females is 1.3956 ± 0.113 cm, while in males, it is 1.3841 ± 0.1281 cm. The otolith weight (OW) of females was 0.0053 ± 0.0008 g, while that of males was 0.0051 ± 0.0011 g. The morphometry of the right and left otoliths and female and male rainbow runners that landed at PPP Sadeng are presented in Fig. 3.



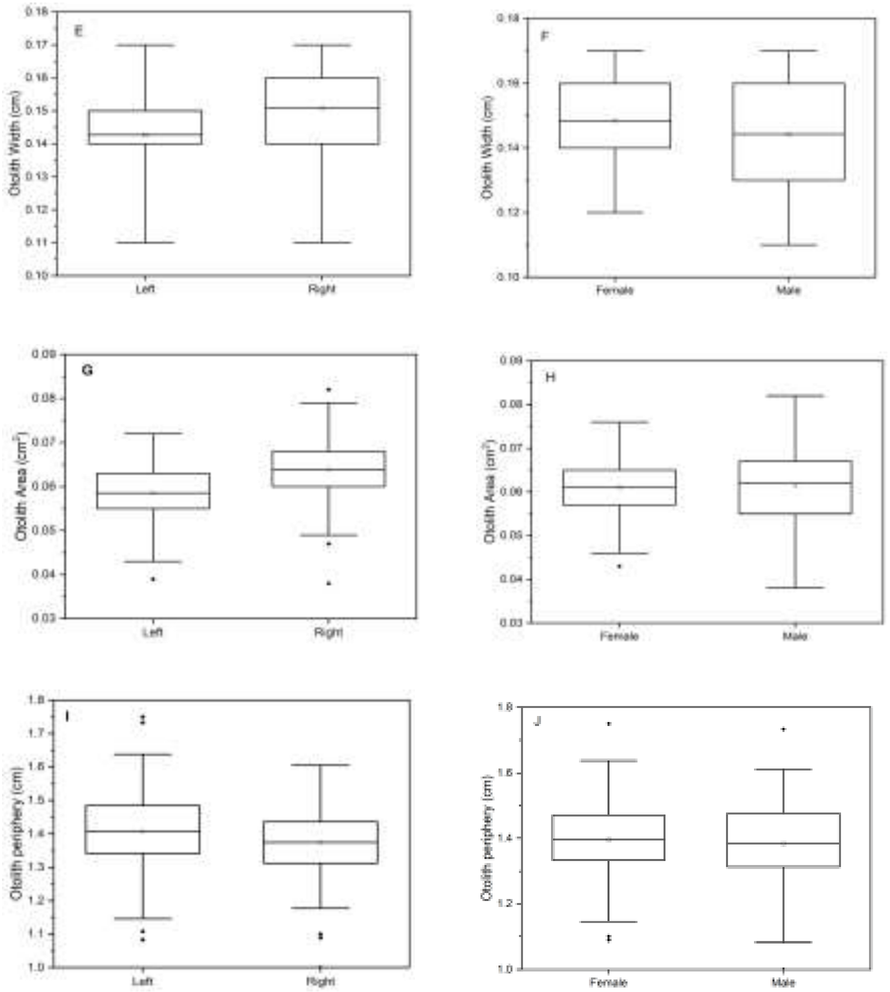


Fig. 3. Distribution of morphometric parameters of Rainbow runner otoliths between the left and right sides (left panel), as well as between females and males (right panel), namely otolith weight (panels A, B), otolith length (panels C, D), otolith width (panels E, F), otolith area (Panels G, H) and otolith periphery (panels I, J), respectively.

Based on a statistical analysis of otolith morphometrics between left and right, as well as between males and females, which includes length (OL), width (OWi), area (OA), periphery (OP), and weight (OW), it shows that the calculated t value is < t table, except between otolith width, otolith area, and otolith periphery between left and right shows the estimated t value > t table. The initial hypothesis (H0) was accepted, which means that the morphometrics of rainbow runner fish otoliths between sexes and between sides do not have significant differences, except for the size of width, area, and periphery between females and males. There are substantial differences. Morphometric comparisons of left and right otoliths and females and males in rainbow runner fish are presented in Table 3.

Table 3. Results of the otolith morphometry t-test between left and right and between males and females.

Parameter	Testing	Average	Standard deviation	T-statistic	Prob> t	Note
Otolith Weight (g)	Left X Right	0.00526	0.000091	1.6517	0.01219	Not significance
Otolith length (cm)	Left X Right	0.5504	0.0424	1.0761	0.284	Not significance
Otolith Width (cm)	Left X Right	0.14676	0.01266	-8.708	1.7751	Significance
Otolith Area (cm^2)	Left X Right	0.0611	0.00734	-12.560	3.0385	Significance
Otolith periphery (cm)	Left X Right	1.39088	0.11957	3.93048	1.969	Significance
Otolith Weight (g)	Female X Male	0.00524	0.000933	0.14749	0.88316	Not significance
Otolith length (cm)	Female X Male	0.55514	0.4136	-0.27939	0.76978	Not significance
Otolith Width (cm)	Female X Male	0.14563	0.01278	1.24958	0.21556	Not significance
Otolith Area (cm^2)	Female X Male	0.06073	0.00739	-1.8423	0.28193	Not significance
Otolith periphery (cm)	Female X Male	1.39785	0.12083	1.44568	0.15267	Not significance

3.3 Morphometric relationship of otoliths with fish body length and weight

The relationship between otolith morphometrics and the total length of rainbow runner fish was analyzed using linear regression analysis. The results of linear regression analysis showed a positive and linear relationship pattern between total length (TL) and otolith morphometry, which included length (OL), width (OWi), area (OA), otolith periphery (OP), and otolith weight (OW). The form of the linear regression relationship is presented in Fig. 4.

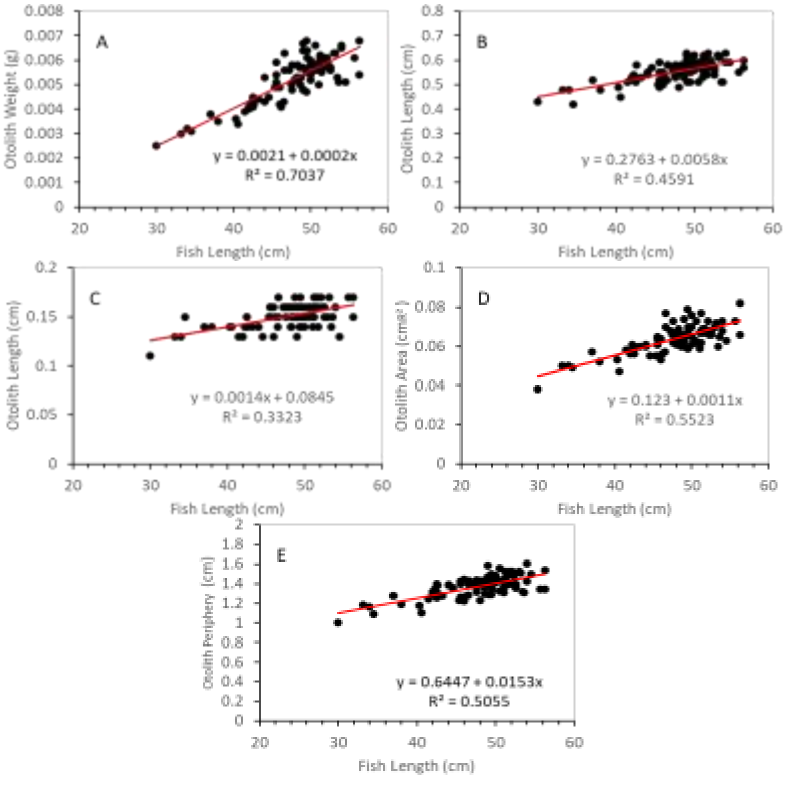


Fig. 4. Linear regression relationship between total length (TL) and otolith morphometrics, namely (A) otolith weight, (B) otolith length, (C) otolith width, (D) otolith area, and (E) otolith periphery.

Fig. 4 shows the relationship between rainbow runner length growth and otolith morphometrics, including otolith weight (OW), otolith length (OL), otolith width (OWi), otolith area (OA), and otolith periphery (OP). Based on the regression equation, the R^2 value for each relationship is obtained, varying between 0.33 and 0.70. The highest R^2 value is shown in the relationship between otolith weight (OW) and total length, with a value of R^2=0.7037. Then, the close relationship between total length and otolith area was shown to be quite strong with a value of R^2=0.5523, while between total length and otolith, the periphery was also quite strong with a value of R^2=0.505. Furthermore, the total length and otolith length show a less strong relationship, with a value of R^2=0.4591. The relationship between total length and otolith width is relatively weak, with a value of R^2=0.3323.

The relationship between individual rainbow runner weight growth and otolith morphometrics, including otolith weight (OW), otolith length (OL), otolith width (OWi), otolith area (OA), and otolith periphery (OP), is presented in Fig. 5.

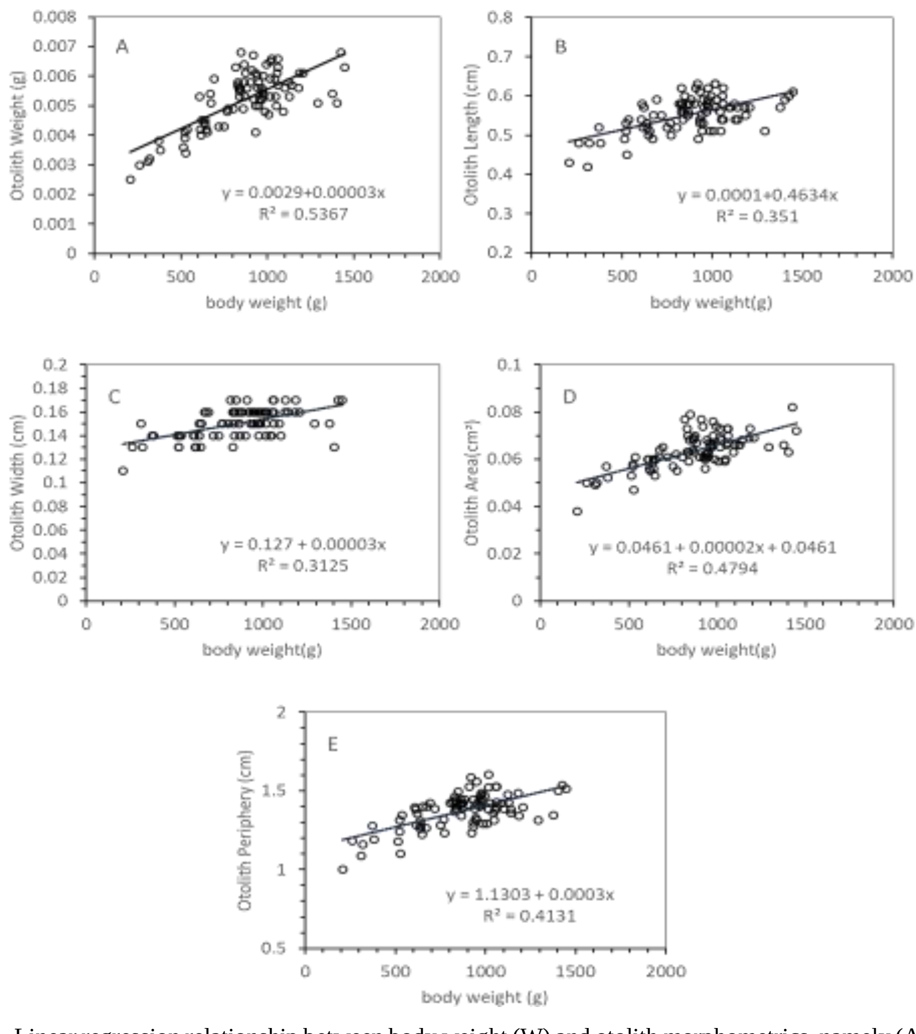


Fig. 5. Linear regression relationship between body weight (W) and otolith morphometrics, namely (A) otolith weight, (B) otolith length, (C) otolith width, (D) otolith area, and (E) otolith periphery.

Figure 5 shows the relationship between the growth of body weight and otolith morphometrics consisting of otolith weight (OW), otolith length (OL), otolith width (OWi), otolith area (OA), and otolith periphery (OP), showing a positive relationship with the relationship coefficient varies. Based on the regression equation, each relationship's coefficient of determination (R^2) ranges from 0.31 to 0.54. The highest R^2 value was shown in the relationship between otolith weight (OW) and body weight (W), with an R^2 value = 0.54. Then, the weakest relationship is between otolith width (OWi) and body weight (W), with a value of $R^2 = 0.31$.

4 Discussion

The shape index analysis (Table 2) shows that the rainbow runner otolith has an irregular surface (FF = 0.4 < 1), has a shape that is not entirely round (R0 = 0.256 ≠ 1), the ratio of the shape of the otolith to a full circle has a value 31.792. The shape of the rainbow runner otolith also does not form a perfect rectangle (Rt = 0.753 ≠ 1). The otolith shape relative to the elliptical shape has a value of 0.579, so it is not oval, and the rainbow runner otolith shape tends to be elongated (AR = 3.773 > 1). The form of the rainbow runner otolith tends to be elongated, then grows maximally in the anterior part until it maximally fills the anterior cavity, and then increases in the dorsal position. The ventral part tends to be more jagged than the dorsal part. The shape of otoliths in Carangidae can be classified into three forms, namely fusiform, sagittiform, and lanceolate [15]. The diversity of fish otolith shapes is influenced by how fish live in different environments [16] and diverse body structures.

Based on the results of the T-test between male and female rainbow runner otoliths, there were no significant differences. Research on otoliths in fish in the Carangidae family, such as *Selar crumenophthalmus* [17, 18], found no real differences between male and female otoliths. Likewise, in other carangidae family (*Trachinotus ovatus*) [19]. In testing the left and right rainbow runner otoliths with data on otolith weight (OW) and otolith length (OL), there were also no significant differences, whereas in data on otolith width (OWi), otolith area (OA) and otolith periphery (OP) found substantial differences. It was found that the left rainbow runner otolith was wider than the right otolith. This difference was also found in *Carangoides ferdau*, *Carangoides malabaricus*, and *Gnathanodon speciosus* [20]. *Barbus tauricus* also has a significant difference between the left and right otoliths. The right otolith was found to be larger than the left otolith. Likewise, in 4 different species of fish from the Carangidae family, the species *Carangoides coeruleopinnatus* has a significant difference between left and right otoliths, while in *Carangoides malabaricus*, significant differences were found in otolith width [21].

In testing the relationship between rainbow runner length and otolith morphometric data, the coefficient of determination with the highest R^2 value was obtained, namely the relationship between fish length and otolith weight. The relationship between fish length and otolith width showed the lowest R^2 value. Likewise, in the relationship with the weight of the rainbow runner, it is shown that the highest R^2 value is the relationship with the weight of the otolith, and the lowest value is the relationship between the otolith's weight and the otolith's width. Most studies on the relationship between fish length and weight and otolith morphometrics show that the highest relationship is between fish length and weight and otolith length. However, otoliths are parts of the internal organs of fish that have very species-specific morphometrics, so this relationship cannot be applied to every fish species [22; 23]. Fish length and weight growth can be directly or indirectly related to the growth of certain otoliths of fishes [24]. In *Rastrellinger kanagurta*, the highest relationship between length or weight and otolith morphometrics is otolith weight (OW) with an R^2 value of 0.69 [25]. The relationship between total length and otolith morphometry in *Pomatomus saltatrix* obtained the highest R^2 value between fish length and otolith weight, with an R^2 value = 0.87 [26]. In

Sperata aor and *Labeo bata*, the highest relationship between fish length and weight and otolith morphometrics is shown by the relationship between fish length and otolith weight with R^2 values of 0.96 and 0.87, respectively [27]. The results of the statistical analysis on several fish species show different results. It is because of the growth rate of each fish species and the influence of fish habitat as an external factor [28].

The shape of the otolith for each fish species is, and its morphometric growth is influenced by genetics and the environment in which the fish lives [29]. Otolith development is regulated by two factors, namely genetic conditions that control the shape of the otolith and environmental conditions that regulate the materials deposited during otolith formation [30]. Individuals of the same species living in different areas are likely to have different otolith shapes and sizes because of their different growth rates [31]. If the growth rate is fast, the otolith tends to grow elongated, whereas if the growth rate is slow, the otolith tends to have a rounded shape [32]. However, otolith growth and somatic growth are not always directly proportional. Otolith growth continues even though somatic growth slows down. Individuals with slow somatic growth often have larger otolith sizes [33, 34].

The speed of growth and otolith formation is influenced by external factors such as changes in temperature [34], water depth [23-25, 35-36], food and mineral availability [25,29, 35-37], habitat type [35], salinity and heat waves in water bodies [37], as well as upwelling processes [23]. Migration activity is also a factor that influences otolith growth [29]. The type of fish's swimming activity can be seen from its otoliths [37, 38]. Usually, fish with high or fast swimming activity have smaller otolith shapes than fish with low swimming activity [32]. As mentioned, otolith length is the most common comparison to show the length- fish weight by otolith morphometrics because growth is directly proportional. However, the increase in otolith length will be directly proportional to the length of the otolith up to its maximum size; then, the otolith will tend to grow thicker [32, 37].

The correlation between individual length and body weight on otolith morphometrics shows a positive relationship; namely, increasing fish body length and weight will cause an increase in otolith morphometric size. The energy from the prey obtained by each fish would be used to synthesize and deposit calcium carbonate and protein proportionally in the otoliths [38]. Small fish have a small otolith structure; larger fish will have larger otoliths. However, there are variations in size; namely, otoliths with greater weight can be found in fish with the same body length and body weight, and vice versa. Otolith weight has the largest coefficient of determination for the length and body weight of fish, so otolith weight can be used to estimate the length and body weight of fish. In general, the morphometric parameters of otoliths in rainbow runners have a low coefficient of determination caused by the very low deposition rate of calcium carbonate material in the otolith and the irregular shape of the otolith [39].

5 Conclusion and suggestions

The shape of the rainbow runner otolith has an irregular surface, does not form a full circle, does not form a perfect square, and its shape tends to be elongated. No significant differences were found between male and female otoliths. Significant differences were found in the otolith's width, area, and periphery between the right and left. The highest relationship between fish length and weight and otolith morphometrics is shown in the relationship between fish length and otolith weight and fish weight with otolith weight.

The specific shape of the rainbow runner otolith can be used for species identification. However, it is necessary to study otoliths of similar fish and other species in other habitats and conduct further research regarding stock and population structure estimates using otolith morphology and morphometric data.

This study is part of umbrella research involving several students regarding the population dynamics of several fish species in Yogyakarta waters. Therefore, the author would like to thank Inayah Sari Nastiti [40], who has helped collect fish and otolith sample data, some of which have been used for writing this thesis. We would also like to thank the Dean of the Faculty of Agriculture UGM, who has approved and funded collaborative research between lecturers and students to accelerate student graduation.

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