***Original Research Article***

**The use of otolith biometrics found in the stomach of purple-spotted bigeye (*Priacanthus tayenus* Ricardson, 1846) for food type identification** **of the Makassar Strait, Indonesia**

**ABSTRACT**

Identification of the type of food of purple-spotted bigeye (*Priacanthus tayenus*) is very important to analyze the dynamics of the ecosystem and trophic interaction patterns in tropical waters. The main obstacle in the study of feeding patterns is the difficulty of identifying prey species due to soft tissue degradation in the digestive tract. This study aims to analyze the use of otolith biometric parameters in identifying the type of food of purple-spotted bigeye caught in the waters of the Makassar Strait, Indonesia. The samples used were 45 purple-spotted bigeye collected from the fish landing site. Stomach content analysis was conducted with a focus on otolith type as the primary indicator of prey identification. The results were then compared with a reference collection of otolith atlases to identify the otolith type, fish name, and scientific name. Biometric calculations were then performed on intact and paired otoliths. The identification results for otolith type and shape were analyzed descriptively and narratively. The otolith shape parameters studied included form factor (FF), roundness (RO), circularity (C), rectangularity (R), ellipticity (E), aspect ratio (AR). The results showed that the combination of the six parameters was able to distinguish otoliths from various prey species. The prey families that were successfully identified included Mullidae, Ariidae, Apogonidae, Cynoglossidae, Moringuidae, Plotosidae, Synodontidae, Gobiidae, Ambassidae and Leiognathidae. The roundness and circularity variables were the most sensitive indicators in distinguishing species that had similar otolith morphology. These findings prove that biometric analysis of otoliths is an effective method for identifying the type of food of purple-spotted bigeye so that it can improve the accuracy of trophic ecology studies of demersal predators in the Makassar Strait waters.

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Keywords: Otolith biometrics, food identification, *Priacanthus tayenus*, Makassar Strait

1. **INTRODUCTION**

 The purple-spotted bigeye (*Priacanthus tayenus*), is a demersal predatory fish that plays an important role in the trophic network of marine waters. Studies on the diet of purple-spotted bigeye have been numerous, but the use of otolith biometrics as a quantitative indicator of prey composition is still relatively rarely elaborated. Kantun and Budimawan (2023) stated that otoliths as small calcified structures in fish are superior biometric markers because they contain morphological and size traces that can be used to identify prey types and estimate the length and body weight of their predators. Tuset et al. (2003) & Bani et al. (2013) discussed in detail the use of morphometric characters of otoliths including sulcus length, cauda length, and other parts for species identification with the main focus on taxonomic identification.

This research is increasingly urgent to be carried out because Kantun et al. (2024) reported the morphometric index of the asteriscus otolith of *P. tayenus* from the Makassar Strait, showing that the left and right otoliths are relatively uniform and elongated oval in shape. However, the study was limited to shape characters, and did not include prey analysis or the allometric relationship between prey otoliths and prey body size.

The study of otolith biometrics in predatory fish prey such as purple-spotted bigeye is very important because it offers a quantitative and high-precision approach in identifying trophic interactions in marine ecosystems. Otoliths are resistant to digestion processes, allowing for prey identification analysis even though their soft body structures have been destroyed. This provides advantages over conventional methods such as soft tissue-based stomach content analysis (Smale et al. 1995; Cortés 1997; Campana 2004).

More detailed biometric analysis of otoliths such as length (OL), width (OW), and shape ratio can be used to estimate prey body size, which ultimately helps model the body length structure of prey fish communities (Battaglia et al. 2010). This is ecologically relevant because prey size is closely correlated with predator energy gain, predation behavior, and ecological adaptation strategies (Scharf et al. 2000)

This research is important to support the reconstruction of marine food webs through a biometric approach that is rarely used in studies of small demersal predators such as purple-spotted bigeye. This effort is to fill the knowledge gap regarding *Priacanthus* spp. food in tropical areas such as Indonesia, which still lack otolith biometric data. In addition, it also provides basic data that is useful in the conservation and management of fisheries resources, especially in determining the role of key species in the ecosystem. Combination of otolith biometric parameters related to physical dimensions and biometric characters such as sulcus with analysis of the stomach contents of purple-spotted bigeye, this study aims to identify the type of prey based on otolith biometrics found in the stomach of purple-spotted bigeye and develop a correlation model between prey otolith size and estimated body length of purple-spotted bigeye prey in the local food chain of the Makassar Strait waters.

1. **MATERIALS AND METHODS**

**2.1 Data collection and sample handling**

The purple-spotted bigeye used as samples amounted to 45 fish for all size representations so that the data obtained is representative. The use of this size strata is considered to obtain variations in otolith biometry. Otolith extraction was carried out by splitting the stomach of the purple-spotted bigeye, then observing and collecting the otoliths using tweezers with pointed ends. The otoliths found were separated based on the type and size of the fish being dissected. The otoliths found were then compared with the reference collection of otoliths and matched to the type of fish to find the fish's name and scientific name (Lin & Chan (2012). Handling was carried out using distilled water to clean the otoliths from the remaining membranes and mucus, then dried and put into an eppendorf bottle before the otoliths were measured and weighed.

**2.2 Otolith biometry observation**

Otolith biometric measurements were only performed on intact and undamaged otoliths and in pairs. The length and width of the otoliths were measured through observation of an Olympus Sz61 binocular microscope, as well as to identify the shape of the otoliths photographed using a binocular microscope. While each intact left and right otolith was weighed using a micro scale (OHAUS Adventurer AX223) with a sensitivity of 0.0001 mg.

Measurement of otolith length starts from the left end (posterior) based on the sulcus acusticus (midpoint) to the right (anterior) on the rostrum. Otolith width is measured from the dorsal to the ventral part with the midpoint of measurement at the sulcus acusticus (Anissa et al. 2023 and Kantun et al. 2024). The parts measured are otolith length (OL, mm), otolith width (OW, mm), otolith area (OA, mm2), and otolith perimeter (OP, mm). The radius is measured from the sulcus acusticus towards the posterior, anterior, dorsal and ventral for both the right and left otoliths.



Figure 1. Biometric measurement axes of otoliths. Otolith length (OL), otolith width (OW), otolith perimeter (OP), otolith area (OA) (Annisa et al. 2024; Kantun et al. 2024)

**2.3 Data Analysis**

The right and left otolith biometric data were tested for normality and homogeneity using the Kolmogorov-Smirnov test, to determine the significance of the differences in the right and left otolith biometric data. Determination of the otolith shape index was carried out through six descriptors consisting of form factor (FF), roundness (RO), circularity (C), rectangularity (R), ellipticity (E), aspect ratio (AR) (Ponton 2006; Aguera et al. (2011); Sadighzadeh et al. (2012: Zengin et al. (2015: Zischke et al. (2016); Aviglion et al. (2017). Wujdi 2018; Annisa et al. 2024 and Kantun et al. 2024), as shown in Table 1. Meanwhile, the observation results were analyzed using a descriptive narrative and comparative approach to the otolith reference collection (Lin & Chan 2012) to find the names and families of fish preyed on by the purple-spotted bigeye.

Table 1. Calculation of otolith shape index using biometric measurements.

|  |  |  |
| --- | --- | --- |
| Shape index | Formula | usability |
| FF | 4 π OA / Op2 | Estimates the regularity of the otolith surface, where FF=1 indicates a regular surface like a circle. FF<1 means surface is irregular |
| RO | 4 OA / π L2 | Comparing the otolith shape against the full circle shape, where RO =1 indicates a full circle shape |
| C | (OP2 / OA) | Comparing otolith shape to full circle shape  |
| RT |  OA / ( OL . OW) | Describes the variation of otolith length and width with respect to area, where Rt = 1 represents a perfectly square otolith |
| E | ( OL – OW) / (OL + OW) | Indicates a proportional change in axis |
| AR | OL / OW | Indicates the shape of otolith, where an AR value >1 indicates an otolith shape which to be elongated  |

**RESULT AND DISCUSSION**

Based on the number of samples of purple-spotted bigeye whose stomachs were dissected, 2 pairs of otoliths were found, namely a pair of sagita types and a pair of lappilus in small fish, in medium-sized fish 2 pairs of sagita type otoliths were found, 1 pair of lapillus and in large fish 6 pairs of otoliths were found, namely 3 pairs of sagita and 3 pairs of asteriscus and the rest were found to have unpaired sagita, lapillus and astericus otoliths.

The results of the measurement of otolith biometry samples found in the stomach of the purple-spotted bigeye were 11 pairs of intact otoliths, with varying sizes for the left and right otoliths. After being compared with the otolith reference (Lin & Chan 2012), 8 types of fish were found from 11 pairs of otoliths, *Awaous melanocephalus,* *Plotosus lineatus, Upeneus moluccensis, Arius maculatus, Apogon angustatus, Leiognathus equlus. Arnoglossus polyspilus, Moringua abbreviate.* Otoliths of the *Awaous melanocephalus* were found in 3 pairs and otoliths of *Plotosus lineatus* were found in 2 pairs.

The size of the otoliths found in the stomach of the swanggi fish based on the size of the fish samples as presented in Tables 2 to 4

Table 2. Size of otoliths found in the stomachs of small fish

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Fish name | Length (mm) | Weight (g) | Width size (mm) |
|  | *Awaous melanocephalus* | 1,188 ± 0,0035  | 0,00115 ± 0,0004  | 1,204 ± 0,0247  |
|  | *Plotosus lineatus* | 1,190 ± 0,0057  | 0,00105 ±0,0005  | 1,116 ±0,0615  |

Table 3. Otolith sizes found in the stomachs of medium-sized fish

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Fish name | Length (mm) | Weight (g) | Width size (mm) |
|  | *Upeneus moluccensis* | 0,980 ±0,0042 | 0,00180 ±0,00180 | 0,979 ±0,0304 |
|  | *Arius maculatus* | 0,821 ±0,0721 | 0,0005 ±0,0003 | 0,696 ± 0,0516 |
|  | *Apogon angustatus* | 1,270 ±0,0247 | 0,00275 ±0,0002 | 1,322 ± 0,1146 |

Table 4. Sizes of otoliths found in the stomachs of large fish.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| No | Fish name | Length (mm) | Weight (g) | Width size (mm) |
|  | *Leiognathus equlus* | 1,201 ± 0,0205 | 0,00170 ± 0,0003 | 1,213 ± 0,076 |
|  | *Awaous melanocephalus* | 0,952 ± 0,0601 | 0,00125 ±0,0011 | 1,041 ± 0,170 |
|  | *Arnoglossus polyspilus* | 1,170 ± 0,2482 | 0,00135 ±0,0015 | 1,412 ± 0,106 |
|  | *Moringua abbreviata* | 1,209 ±0,3033 | 0,0021 ± 0,0025 | 1,460 ± 0,054 |
|  | *Plotosus lineatus* | 0,943 ± 0,0721 | 0,0095 ± 0,0008 | 0,904 ± 0,107 |
|  | *Awaous melanocephalus* | 0,999 ± 0,0071 | 0,0015 ± 0,0008 | 0,949 ± 0,037 |

The diversity of otolith types and sizes found in the stomachs of predators, such as the swanggi fish (epipelagic predators), reflects the variation in the types and sizes of prey they consume. This diversity of otolith types reflects that predators feed on a variety of fish species, both those living at the bottom (demersal), the water column (pelagic), and coastal environments. The variation in otolith size indicates that predators consume prey at various ontogenetic stages (juvenile to adult). This pattern illustrates an opportunistic feeding strategy, in which predators capture prey based on its availability in the habitat. According to Battaglia et al. (2010), different otolith sizes in the stomach indicate variations in prey body size, and this helps to indirectly estimate prey biomass. Furthermore, Bernal-Duran & Landaeta (2017) emphasized that the composition of otoliths found can reflect predator feeding preferences, seasonal changes in prey availability, and different habitat uses by predators.

The discovery of abundant fish otoliths in the stomach of the purple-spotted bigeye (*Priacanthus tayenus*) provides important indications regarding the feeding pattern, prey preferences, and ecological position of this species in tropical aquatic ecosystems. The presence of large numbers of otoliths in the stomach of the purple-spotted bigeye is related to the feeding preference for small demersal fish and the level of otolith resistance to degradation in the digestive tract of predators (Frost & Lowry, 1980; Tollit et al., 2003). These small fishes, especially from families such as Mullidae, Apogonidae, and Leiognathidae, generally have a body size suitable for being swallowed whole, so their relatively intact otolith remains are found in the stomachs of predators (Byrd et al. 2020).

In addition, the chemical and morphological properties of otoliths, which are mostly composed of calcium carbonate in the form of aragonite or vaterite, make their structure resistant to digestive enzymes and the acidic environment in the stomach of predators (Campana, 1999). This makes otoliths a very good indicator for identifying prey types taxonomically even to the species level. Thus, the finding of otolith dominance in the stomach of the purple-spotted bigeye confirms the importance of otolith analysis methods in the study of the food ecology of predators living in the sea, especially in tropical areas that have a high and complex diversity of prey species.

The otolith shape index was calculated by referring to six descriptions proposed by Aviglion et al. (2017). The six descriptions of otolith shape obtained in this study are as shown in Table 2. The data in Tables 2 to 4 after being statistically tested using the t-test showed that there was no difference between the otolith shape index on the left and right sides of the fish (P> 0.05). The results shown here are only the representation of the sagitta, lapillus and asteriscus types of otoliths based on the size of the purple-spotted bigeye (small, medium and large) used as samples as shown in Tables 5 to 7.

Table 5. Index of otolith shape of sagitta type of small fish for the left and right otoliths of purple-spotted bigeye small found during the study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Otolith Shape Index | Indication of calculation results | Otolith image | Types of fish *Awaous melanocephalus* |
| Right | Left |
| FF  | 3,837 | 3,732 | FF value > 1 indicates that the otolith has a shape that is not perfectly round and an irregular surface. |  |  |
| RO | 1,026 | 1,001 | An RO value ≠ 1 indicates that the circle is not full |
| C | 3,275 | 3,367 | The otolith shape is irregular with incomplete circle conditions |
| Rt | 0,785 | 0,785 | Rt value < 1 describes otoliths that are square but not perfect, but the right and left are very symmetrical. |
| E | 0,013 | 0,011 | Indicates a proportional change in the axis |
| AR | 1,114 | 1,023 | AR value > 1 indicates that the otolith shape tends to be elongated.. |

Information: FF = *Form factor*, RO = *Roundness*, C = *Circularity* atau *compactness*, Rt = *Rectangularity*, E = *Ellipticity*, dan AR = *Aspect ratio*

Table 6. Index of otolith shape of lapillus type of medium-sized fish for the right and left otoliths of purple-spotted bigeye found during the research.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Otolith Shape Index | Indication of calculation results | Otolith image | Types of fish *Arius maculatus* |
| Right | Left |
| FF  | 2,076 | 2,301 | FF value > 1 indicates that the otolith has a shape that is not perfectly round and an irregular surface. |  |  |
| RO | 0,857 | 0,839 | An RO value ≠ 1 indicates that the circle is not full |
| C | 6,052 | 5,461 | The otolith shape is irregular with incomplete circle conditions. |
| Rt | 0,786 | 0,785 | Rt value < 1 describes otoliths that are square but not perfect. |
| E | 0,078 | 0,087 | Indicates a proportional change in the axis. |
| AR | 1,168 | 1,191 | AR value > 1 indicates the shape of the otolith tends to be elongated. |

Information: FF = Form factor, RO = Roundness, C = Circularity atau compactness, Rt = Rectangularity, E = Ellipticity, dan AR = Aspect ratio

Table 7. Index of the otolith shape of the asteriscus type of large fish for the right and left otoliths of the purple-spotted bigeye found during the study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Otolith Shape Index | Indication of calculation results | Otolith image | Types of fish *Plotosus lineatus* |
| Right | Left |
| FF  | 2,604 | 3,08 | FF value > 1 indicates that the otolith has a shape that is not perfectly round and an irregular surface. |  |  |
| RO | 0,928 | 0,961 | An RO value ≠ 1 indicates that the circle is not full |
| C | 4,826 | 4,082 | The otolith shape is irregular with incomplete circle conditions. |
| Rt | 0,785 | 0,785 | Rt value < 1 describes otoliths that are square but not perfect. |
| E | 0,037 | 0,008 | Indicates a proportional change in the axis. |
| AR | 1,077 | 1,015 | AR value > 1 indicates the shape of the otolith tends to be elongated. |  |  |

Information: FF = Form factor, RO = Roundness, C = Circularity atau compactness, Rt = Rectangularity, E = Ellipticity, dan AR = Aspect ratio

Referring to the otolith shape index as seen in Table 5, it was obtained that the surface of the sagittal otolith of the *Awaous melanocephalus,* both on the right and left, showed results with an irregular surface appearance, an incomplete circle shape, an irregular otolith shape with an incomplete circle condition, a square but imperfect otolith shape, there was a proportional change in the axis and the otolith shape tended to be elongated.

Table 6 also shows that the surface of the otolith lapillus of the *Arius maculatus*, both on the right and left, produced results with an irregular surface appearance, an incomplete circle shape, an irregular otolith shape with an incomplete circle condition, a square but imperfect otolith shape, there was a proportional change in the axis and the otolith shape tended to be elongated.

Table 7 shows that the surface of the otolith lapillus of the *Plotosus lineatus*, both on the right and left, produced results with an irregular surface appearance, an incomplete circle shape, an irregular otolith shape with an incomplete circle condition, a square but imperfect otolith shape, there was a proportional change in the axis and the otolith shape tended to be elongated.

The condition of the otolith shape index of the sagitta, lapillus and asteriscus types found in this study is thought to be caused by the dimensions of the otolith, namely the length, width, area and radius which are the basic data in calculating the otolith shape index. The otolith shape index can help in identifying and determining the type of food. Identification of fish species, especially fish that are prey and have been digested, so that the relationship between prey and predator in the network and food chain in marine ecosystems can be detected properly. Similar research was conducted by Wujdi et al. (2018); Kantun et al. (2024) also used the otolith shape index in identifying food types in the food chain.

The shape of the otolith can also be important information related to ecological aspects between predators and prey (Campana & Casselman 1993). The shape of the otolith is also related to the history of the type and eating habits of fish (Gagliano & McCormick 2004) so ​​that it contributes to the development of the shape and microstructure of the otolith. The shape of the otolith is influenced by abiotic factors (temperature), environmental parameters (food availability) and biotic factors, namely the individual genotype (Vignon & Fabien 2010).

The varying otolith shapes are closely related to the genetics and ecology of fish as well as the biological behavior of fish (Battaglia et al. 2010), ontogenetics, and environmental factors such as temperature, habitat, seasonal variations and food (Campana 2004). Otolith growth is highly dependent on fish growth and otolith shapes vary along with fish feeding patterns in nature (Cortes 1997). In this regard, observation and identification of otolith shape indices can also be carried out on other species using the same method provided that the otoliths are still intact.

Each fish species has a certain morphometric size (length and weight) and characteristics (shape), where otolith morphometrics have been studied to identify growth and environment and life history (Darmanto 2019). When fish experience changes in length, the circles on the otolith also increase until they reach asymptote (saturated growth), the shape of the dark and light growth circles on the otolith are some that are far from the core and some that are close to the otolith core (Mehanna & Farouk 2021). This is related to the recording of fish events during their lifetime (Aisyah 2019). However, Bani et al. (2013), revealed that the thickness and level of roundness formed in the otoliths of pelagic fish with the habit of being active swimmers so that they have a thin and elongated sagittae otolith shape, while demersal fish have a thick otolith shape due to limitations in swimming.

The otolith shape index of fish can be influenced by abiotic factors (temperature), environmental parameters (food availability) and biotic factors, namely individual genotype (Vignon & Fabien 2010). Otolith growth is highly dependent on fish growth and otolith shape which varies along with fish feeding patterns in nature (Mille et al. 2016). Otolith shape can differ systematically between the right and left otoliths and can vary geographically due to environmental conditions (Mahe et al. 2018).

Changes in the otolith shape index of fish can be caused by global warming (Kantun & Budimawan 2023). Kelig et al. (2019), explained that global warming contributes to a decrease in the production of polyunsaturated fatty acids EPA and DHA so that it can affect the physiological aspects of fish, especially otolith morphogenesis. Changes in morphogenesis can be a characteristic of fish stocks characterized by differences in environmental conditions and genetic composition. These differences cause variations in otolith growth, resulting in variations in shape and allowing stock discrimination (Cadrin et al. 2014).

**CONCLUSIONS**

The biometric characteristics of sagitta, lapillus and asreriscus otoliths found in the stomach of purple-spotted bigeye for the left and right sides based on the otolith shape index obtained an irregular appearance, incomplete circle, irregular square shape and the axis tends to be elongated. The results of the comparison with the reference collection of otoliths found prey species from the families Mullidae, Ariidae, Apogonidae, Cynoglossidae, Moringuidae, Plotosidae, Synodontidae, Gobiidae, Ambassidae and Leiognathidae.

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