Fish length and otolith size relationship of the *Channa striata* in Lake Rawa Pening, Central Java, Indonesia

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Abstract. The objective of this study was to examine the relationship between length, width and weight of the otolith to the total length and daily ring in otolith of the snakehead (*Channa striata* Bloch, 1793). Fish sampling was conducted monthly from October 2017 to August 2018 on Lake Rawa Pening, Central Java, Indonesia. Fish total length, then otolith length, width, and weight were measured, and sex determined. Otoliths were embedded in epoxy acrylic resins and cut from the anterior to the posterior limits, in a transversal section, using a double ceramic saw. The daily ring number was calculated under a light microscope with a magnification of 100-200. The sampling population consisted of 217 individuals, among which 85 females and 132 males. Female and male average lengths were 36.6 cm and 33.0 cm, respectively, ranging over the intervals from 24.2 to 64.8 cm and 23.4 to 64.6 cm, respectively. The average number of daily rings of the otoliths was 180 days, with a range of 108-309 days. The number of otolith daily rings between left and right and between females and males did not differ significantly.

Key Words: daily range, snakehead, length, width, weight.

Introduction. Snakehead (*Channa striata*) is a fish species that inhabits in lotic, lentic freshwater and shallow flooded areas. This fish is found in rivers or swamps in the south and Southeast Asia from India, Thailand (Jutagate et al 2013) to Indonesia. *C. striata* is the main catch target of freshwater fishermen in Sumatra, Java, and several other islands (Ndobe et al 2014). In Java, many *C. striata* inhabits the river, gutters, sand puddles, swamps, dams, reservoirs and lakes. In Lake Rawa Pening, *C. striata* inhabits areas where there are humps of water hyacinth (*Eichhornia crassipes*) or hordes of aquatic plants. Fishers use various types of fishing gear to catch *C. striata*, for example, fishing rods, traps, ring nets and blinds made of woven bamboo.

Previous research on *C. striata* has been widely carried out from various perspectives. For example, genetics of *C. striata* has been studied in Sulawesi Island (Irmawati et al 2017), and in the US (Orrell & Weigt 2005). Fish stock structure was studied by using otolith chemistry (Miyan et al 2014), and the fish growth and age were subsequently determined through otolith readings (Jutagate et al 2013). *C. striata* biological aspects were also studied on specimens inhabiting the waters of Rawa Pening lake (Puspaningdiah et al 2014), in particular their reproductive biology (Djumanto et al 2019). *C. striata* spawning was sampled from various sources in order to obtain the best derivatives (Samidjan & Rachmawati 2016). Also, the relationship of otolith size to body length or age was studied on other species of fish. For example, the relationship between fish allometric characteristics and otolith size was studied by Kumar et al (2016) on the Indian Ruff (*Psenopsis cyanea*), and by Bal et al (2018) on the bluefish (*Pomatomus saltatrix*).

Information about the relationship between otolith size and fish body length or age is crucial for addressing the fishery resources management and conservation issues, such as stock assessment. The novelty of the current study is the exploration of the triangular relationship between otolith size, body length and age of *C. striata*. The objective of this research was to obtain information about the relationship between length, width, and weight of otolith, the fish body length and the fish age. *C. striata* fishery managers can use the results of this study for management and conservation purposes. For instance, based on otolith size information, managers can identify the most vulnerable species and their vulnerability to the predator's attacks, which depend on their size and habitat.

Material and Method

Description of the study site. The fish sampling was carried out in the lake Rawa Pening, considered as the center of *C. striata* fishing in Central Java. Fishing was carried out in several locations of Bawen and Ambarawa districts (Figure 1). Sampling locations in areas with an abundant density of *E. crassipes* were relatively suitable habitats for *C. striata*. It was carried out once a month from October 2017 to August 2018 in the dry and rainy season.

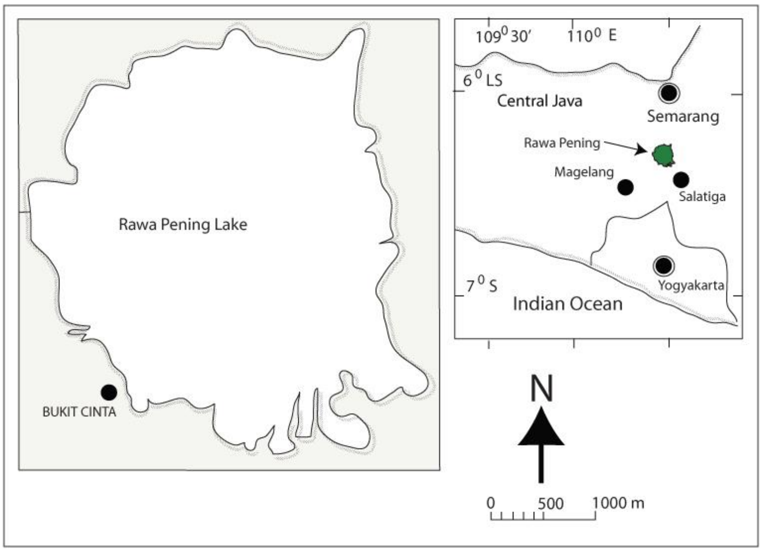

Figure 1. Map of Lake Rawa Pening, Central Java, where *Channa striata* sampling was made with bamboo blinds. The fishing gear was operated in all areas of lake waters with aquatic weeds in abundance.

Materials and tools. *C. striata* samples used in this study were obtained by catching fish using bamboo slats that were knitted into blinds. About 5-7 local fishermen operated fishing gear from morning to evening. The hyacinth groups were caged up using bamboo blinds measuring 25-50 m long and 1.5 m high as a fence. Next, the water hyacinth was removed from the cage, and the size of the fence circle was gradually reduced. The *C. striata* trapped in this manner was collected and stored in a cool box filled with ice cubes, and then brought to the laboratory.

Fish samples observation. The total length (TL) of *C. striata* individuals were then measured using a ruler with an accuracy of 0.1 cm and their weight was measured using a digital scale with an accuracy of 0.1 g. Because *C. striata*'s secondary characteristics cannot be distinguished visually, their sex was differentiated based on gonads (ovarian or testicular) presence determination and observation through abdominal surgery. The results of otolith length and weight measurements between males and females showed no significant difference (t-test, $P<0.05$) so that subsequent analyzes did not distinguish between males and females.

Next, the branchiostegal bone was cut so that the oral cavity was opened. The head cavity was cut and opened so that the otolith was exposed. Sagitta otoliths were taken using tweezers, cleaned, and stored dry in a numbered plastic bottle. The length of the otolith (OL) and width (OW) was measured with a caliper with an accuracy of 0.01

mm (Figure 2). Otolith length was measured from the anterior end at point A through the primordium to the posterior edge at point P. The width of the otolith was the furthest distance perpendicular to the length that passes through the primordium. The weight of the otolith (OM) was measured using a standard analytical balance with an accuracy of 0.1 mg.

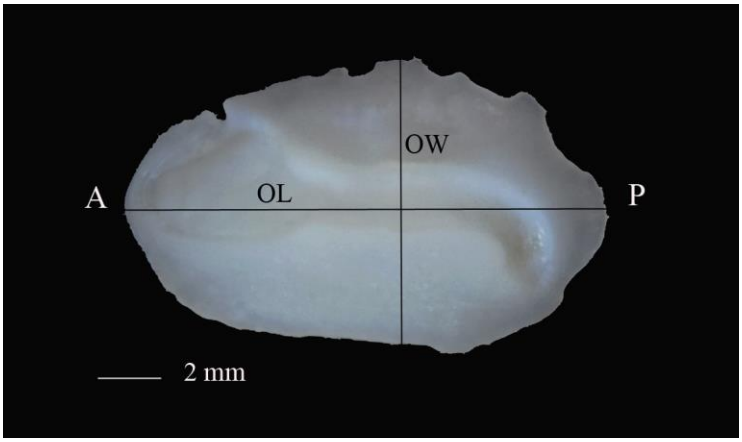

Figure 2. Ventral and inner view of the *Channa striata* right sagittal otolith, and procedures for measuring the length and width of the otolith.

The length of the otolith (OL) is the longest distance between the most anterior (A) and posterior (P) points. Otolith width (OW) is the longest distance between the ventral and dorsal edges, perpendicular to the long line.

Otolith was embedded in epoxy acrylic resin and allowed to harden. Then the otolith was positioned dorsally facing upwards and cut anteriorly-posteriorly using a double-mounted ceramic saw with a speed of around 3,000 rpm. The surface of the otolith sheet was smoothed using abrasive sandpaper no. 1,200, and the dust was cleaned using wet tissue. Furthermore, the otolith was placed on top of the object-glass and coated with nail polish to increase the brightness. The number of daily rings contained in the otolith was counted under a transmitted light microscope with magnification 100-200 times. Experienced readers conducted daily ring counting, performed on each sheet of otolith three times and then averaged. The number of daily rings shows the age (day) of the fish. The results of readings between right and left otoliths showed no significant difference (t-test, $P<0.05$), so the right otoliths were used for analysis.

Statistical analysis. Some otoliths were broken during the process of collecting from the head cavity, or during the process of making sheets otolith piece. Besides, the daily ring of some otoliths cannot be observed due to the irregular forming of the daily ring. Only otoliths that were good, undamaged and that can show the daily ring loop were subjected to statistical analysis. The number of otoliths that were analyzed every month was about 30 pairs. On the OL, OW, OM values between left and right samples, as well as on data provided by male and female specimens' observation, Student's t-tests were performed at a 5% statistical significance level.

Statistical analysis was performed on linear and non-linear regression correlations using the program SPSS version 22. The relationship of total length (TL) and otolith length (OL), then total length (TL) to otolith width (OW), between fish age (DR) and the total length (TL), and between age (DR) and otolith length (OL) were analyzed by the linear equation $y = ax + b$. Next, the relationship between fish age (DR) and otolith weight (OM) was analyzed using the equation $y = ax^b$. The level of significance of all statistical analyzes was 5%. The value of b for each correlation equation was tested by Student's t-test (95% confidence level).

Results. A total number of 217 individuals, consisting of 85 females and 132 males, were analyzed during this study. The length of the male fish was distributed over the range of 23.4-64.6 cm, with an average of 33.0 cm and the length of the female fish was distributed over the range of 24.2-64.8 cm, with an average of 36.6 cm. The average daily ring on otolith was 180 days, with the range 108-309 days. Overall, the fish caught are less than one year old.

For the otolith of the right-side, the average values (mean±sd) were: length of 11.6±1.9 mm, width of 6.9±1.0 mm, and weight of 169.1±92.1 mg, almost the same as for the left-hand otoliths: length of 11.6±2.0 mm, width of 6.6±1.9 mm and weight of 179.1±32.9 mg. No significant difference was found between the left and right sides (t-test, $0.05<P$). Therefore, only the right otoliths were used in all statistical analyzes.

The female otolith the average values (mean±sd) were: length of 11.7±2.2 mm, width of 7.5±1.2 mm, and weight of 165.1±136.9 mg, while for the male otolith they were: length of 11.2±1.8 mm, width of 6.7±0.9 mm, and weight of 142.7±116.9 mg. No significant difference was found between the female and male otolith values (t-test, $0.05<P$). Therefore, further statistical analysis was performed on the combination of both female and male otoliths.

Regression analysis of the relationships between total length and otolith length, between total length and otolith width, between total length and daily ring, and between the daily ring and otolith length showed positive and linear patterns. The linear regression relationship between total length (TL) and otolith length (OL) was estimated to form the equation, as shown in Figure 3A. There was a positive and strong relationship between the increase in total length and otolith length (r=0.85). An increase of otolith 0.269 mm will follow each growth of one cm in length. Furthermore, the average total length growth will affect the growth of otolith length by 85%.

The linear regression relationship of the total length and otolith width formed the equation, as shown in Figure 3B. The linear regression relationships of total length to otolith length and total length to otolith width had the same regression coefficient and determination coefficient. It showed that the growth of total length had an equally strong correlation to the growth of otolith length and otolith width.

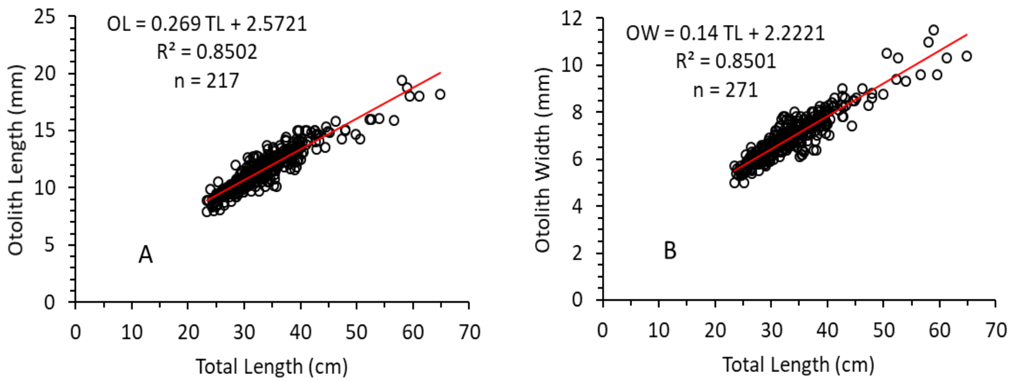

Figure 3. The linear regression relationship between the total length (TL, cm) and the otolith length (OL, mm).

The linear regression of daily range to the total length formed the equation, as shown in Figure 4A. It showed a positive and strong relationship between the increase in the daily ring to the total length (R=0.58). The growth of 0.154 cm in total length will follow an increase of age in one day. Furthermore, the daily range and the otolith length formed the regression equation, as seen in Figure 4B. The linear regression relationship between daily range and otolith length was stronger (R=0.66) than between daily range and total length (R=0.58).

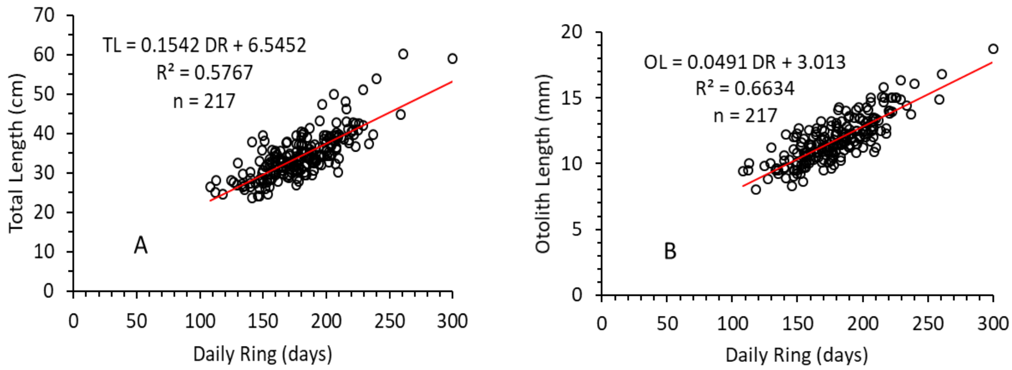

Figure 4. The linear regression relationship between the total length (TL, cm) and the daily ring (age, day).

The relationship between total length and weight of otolith is shown in Figure 5. Based on the regression analysis obtained between the total length and weight of the otolith, it forms the power equation, as shown in Figure 5. The correlation coefficient between total length and otolith weight (R=0.83) shows a strong relationship of growth in total length and an increase in otolith weight.

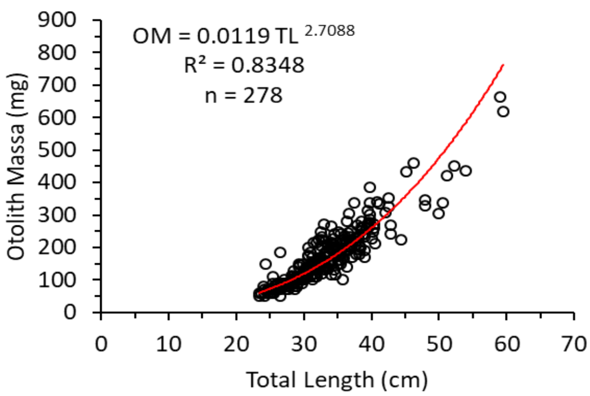

Figure 5. The relationship between total length (TL, cm) and otolith weight (OM, mg).

Discussion. The average length and the width and weight of otoliths between the right and left side, respectively, were not significantly different. These results were also consistent with measurements of the right and left otolith in the family of Sciaenidae (Waessle et al 2003), in mullets (Dehghani et al 2015), even in majorra species (Cruz-Agüero et al 2016). Right and left otoliths grow at equal speeds showing that the deposition of carbonate material on the right and left otoliths are equivalent. However, different results were shown by otolith morphometry between the right and the left in the *Diplomus puntazzo* (Bostanci et al 2016). On the left otolith, the length and perimeter were longer, but the width, thickness, and weight were smaller than the right. The morphological characteristics of otolith vary significantly in fish species and populations (Santana 2018), and the information about the morphology and shape of sagittal otoliths is limited (Bostanci et al 2016). One reason for the difference in morphometry of right and left otoliths was thought to be the difference in the density of otolith vaterite (See et al 2016). The difference in the size of the right and left otoliths may be caused by differences in species, habitat, food availability, and possibly physiochemical factors of environmental waters (Dehghani et al 2015). Besides, several factors can produce differences in the morphometry of right and left otoliths, including stadia in fish life and intrinsic characters.

The sizes of the otoliths were not significantly different between *C. striata* male and female, indicating that the sex disparities do not affect the morphometry of the otolith. The rate of otolith and somatic growth in males and females had similar proportions. These results were different from those reported in the catfish *Genidens*

genidens (Maciel et al 2019), where the male otoliths were proportionally longer, wider, and thicker than the female otoliths. In the same cohort, male otoliths were larger, reflecting higher growth rates.

Research on regression of the relationship between total length to otolith length and age was mostly conducted in marine fish species (Dehghani et al 2015; Kumar et al 2016; Cruz-Agüero et al 2016). Even research on the regression of the relationship of the body size with otolith size and age of freshwater fish living in tropical waters is very rare (See et al 2016). Some studies were conducted on the regression of the relationship of body length with otolith length for certain freshwater fish. However, research on body length or otolith size on age is still limited or even non-existent (Miyan et al 2014).

Research on *C. striata* otoliths in the tropics is still very limited in number due to various factors. Research on the chemical structure of the *C. striata* otolith has been carried out by (Miyan et al 2014), while (Odenkirk et al 2013) studied the annual ring in order to determine the growth parameters of *C. striata*. Observation of the number of daily rings in *C. striata* otolith in order to determine age is still infrequent due to a lack of equipment and to technical difficulties in preparing otolith pieces.

Fish age can be traced through the reading of the signs present on the hard structure of fish such as scales and otoliths. Fish species living at high latitudes have an annual ring that can appear on scales or otoliths. However, *C. Striata* that lives in the tropics does not have a bright yearly ring because of a stable ambient temperature. Besides, fish in the tropics have a high metabolic rate, most fish being relatively short-lived. *C. striata* that are less than one year old do not have bright annual rings, therefore it is challenging to determine their age through a daily ring.

The length and width of the otolith had a strong correlation with the total length of the fish. These results were consistent with the results of research on mullet (See et al 2016), in Sciaenidae (Waessle et al 2003) and *Sardinella sindensis* (Dehghani et al 2015). The *C. striata* age estimation method was mostly done by analyzing length frequencies or using annual rings in scales or otolith. The current study used the daily ring number in otolith to trace the age of the *C. striata*, based on the observation that the deposition of aragonite crystalline material on otolith takes place every day, a practical method applicable to the fishery stock assessment.

The form of the regression equation of otolith length to total length, and otolith length to daily ring, and between otolith weight to daily ring, had a powerful and positive relationship. The regression relationship between total length and otolith length or otolith width had the strongest coefficient, followed by a regression relationship between total length and otolith weight. The lowest relationship was recorded between the daily ring to the otolith length or total length. According to the regression equations, the otolith length, width and weight can be used to estimate the fish length and daily ring or age.

Total length had a strong correlation with otolith length and width, while age had a strong relationship to total length and otolith length. Also, total length correlates with otolith weight. This result is supported by a research on 14 species of the Geridae family, showing a linear and very strong relationship between fish length and otolith length, as indicated by the coefficient of determination $R^2>0.85$ (Cruz-Agüero et al 2016). The existence of a strong relationship of otolith size with body length and age is supported by the results of research studies on *Psenopsis cyanea* (Kumar et al 2016), on *Prochilodus lineatus* (Santana et al 2018) and on other species (See et al 2018; Dehghani et al 2015).

The results of the current study can be effectively used in several fields, such as fish stock estimation, fisheries biology or fisheries management related to fish age. Analysis of the age in *C. striata* was mostly done by length-frequency analysis methods that require relatively low cost, simple equipment, and less labor, but the results were less accurate. *C. striata* age analysis using the annual ring was more accurate but can only be conducted for fish that are more than a year old. In most less than a year old tropical fish, including *C. striata*, the annual ring is not visible. Age analysis using daily rings on otoliths has several disadvantages, in *C. striata*, as well as in some other fish species. For example, the daily ring is revealed by cutting the otolith in parallel to the anterior-posterior canal, requiring complex processes and equipment. Also, there are some constraints of the daily ring reading: it is can be read only when cutting the otolith along the anterior-posterior canal, while cutting it transversally produces a blurred daily ring. Likewise, if the cutting of the otolith shifts slightly from the canal, the daily ring is seen again blurry, making the reading difficult. Furthermore, the contrast between hyaline and opaque layers of the daily ring otolith was not strong. Presumably, the metabolic rate between day and night is relatively equal. *C. striata* is actively foraging at night, when the temperature is lower, thereby reducing its metabolism. As a result, food input is high at night and metabolic activity during the day is low. Thus, deposits of carbonate material in the otolith that balance between day and night cause the daily ring layer to be less bright.

Conclusions. There were similarities in size between the right and left otoliths, and between male and female otoliths. The regression relationship between total length and otolith length, total length and otolith width, total length and daily ring, otolith length and daily ring form a linear regression equation, while the regression relationship between total length and otolith weight follows a power law. The regressions between otolith length or width and the total length and daily ring and between the total length and the otolith weight have a positive and powerful relationship. The strong regression relationship between otoliths to body length and age is a useful tool for assessing fish population growth patterns and fish resources management, and conservation.

Acknowledgements. The author is grateful to the Dean Faculty of Agriculture, Gadjah Mada University, for providing the funds needed for the conduction of this study. Next, the author also would like to thank Ms. Atik Murjiyanti and Anissa Dwi Rahmadhanti for helping to collect *C. striata* in the waters of Lake Rawa Pening and for analyzing the samples in the laboratory.

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Received: 20 April 2020. Accepted: 07 July 2020. Published online: 16 July 2020.
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How to cite this article:
Djumanto, 2020 Fish length and otolith size relationship of the *Channa striata* in Lake Rawa Pening, Central Java, Indonesia. AACL Bioflux 13(4):1917-1924.