


Article

Age and Growth of *Diaphus brachycephalus* in the South China Sea Using Sagittal Otolith Microstructure

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Abstract: To obtain the growth and age characteristics of *Diaphus brachycephalus* in the South China Sea (SCS), specimens of *D. brachycephalus* were collected by a mid-water trawl in January and June 2015. The relationship between standard length (*L*) and body weight (*W*) was $W = 0.00002699L^{2.8789}$, with no significant differences between the two survey stations and the sexes. Microscopic observation of the sagittal otoliths revealed that the daily growth increments in each period were divided into the central zone, middle zone, and external zone. The age was determined by summing the daily growth increments of the three areas. The von Bertalanffy growth curve fitted to the relationship between age and *L* was shown as: $L = 65.6[1 - \exp\{-0.0132(t - 6.94)\}]$, $r^2 = 0.935$. The growth rate decreased from juvenile to adult, with a maximum rate of 0.436 mm day⁻¹. The back-calculated hatching dates of specimens were speculated to be from March to November, but predominantly occurred from April to May and from September to October.



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Keywords: *Diaphus brachycephalus*; otolith microstructure; age; growth; South China Sea

1. Introduction

The South China Sea (SCS), which covers an area of approximately 3.5 million km² and has an average depth of 1200 m, is the largest semi-enclosed marginal sea in the Pacific Ocean [1–3]. Enormous fishery resources in the SCS provide coastal countries and regions with a core fishing economy. However, the fishery supply capacity in the SCS has been declining because of excessive fishing capacity. The exploitation of mesopelagic fish resources in the SCS is still in its infancy but has great potential for future development [4–6].

Mesopelagic fishes live in the mesopelagic zone (200–1000 m). They have an estimated resource of 5–15 billion tons and are considered the most abundant vertebrates in the biosphere [7–9]. As one of the most dominant groups of mesopelagic fish, lanternfish belong to Myctophiformes; there are 348 species in 34 genera in this family, including the most common and most abundant mesopelagic fish species. They account for at least 20% of total marine fish species [10,11]. Diel vertical migration (DVM) is a well-known habit common to most lanternfish species. Lanternfish establish temporal, spatial, and biological connections between multiple water layers and act as a “biological pump” to facilitate the input of carbon into the deep ocean through DVM [8,12,13]. These fishes also act as a trophic link between zooplankton and top predators, such as seabirds, marine mammals, and other fishes. Both contribute to the energy flow and material cycling of the deep-sea food web and maintain the stability of deep-sea ecosystems [14–16].

The life span of lanternfish is short, usually 1–5 years, but they have a rapid rate of regeneration, which is an important reason that they have received attention as a fish resource [17,18]. However, the lack of age and growth information for many mesopelagic

fish species limits our knowledge and exploitation of this resource. Their age and growth can be reflected by the otolith microstructure that originates from a primordium, which is the first calcified tissue during embryonic development of osteichthyans [19,20]. Otolith microstructure reveals fish growth in the form of daily annuli formed by the differential deposits of calcium carbonate and protein. Sagittal otoliths are the largest among the three pairs of otoliths; they have more observable structures and therefore are more widely used in fish age growth research [21–23]. Moreover, sagittal otoliths are also used to study other biological and ecological features of lanternfish, such as DVM, early life history, and reproduction [22,24–26].

As the largest genus of the family, the Myctophidae genus *Diaphus* contains 77 species, which accounts for more than one-third of the species in Myctophidae [27–30]. *Diaphus brachycephalus*, also known as short-headed lanternfish, is widely distributed in tropical and subtropical waters around the world, including the SCS [11,31–34]. Several surveys conducted in different areas of the SCS had reported the presence of *D. brachycephalus* and indicated that the dominant distribution of this species is in the SCS. However, research on *D. brachycephalus* in the SCS is still in its infancy; although some studies have investigated their distribution and DVM, research on their age and growth characteristics is still lacking [34–37]. In this study, the relationship between age and growth was investigated through the standard length and sagittal otolith microstructure to estimate the growth pattern and biology of *D. brachycephalus* in the SCS. Thus, it provides a useful reference for further study of *D. brachycephalus* and scientific guidance for the exploitation of mesopelagic fish in the SCS.

2. Materials and Methods

Specimens of *D. brachycephalus* were collected from two survey stations in the SCS in January and June 2015 by the R/V Nanfeng (1537 t GT, 66.7 m length, 12.4 m width, and 4.8 m draught) and a mid-water trawl (a cod-end mesh size of 10 mm, estimated mouth opening of approximately 128 m²) with a PI44 monitoring system (Kongsberg Maritime Inc., Kongsberg, Norway) to ensure that the trawl depths remained relatively constant at a 75 m water depth (Table 1). To prevent these fragile specimens from being destroyed, specimens were collected on deck and gently cleaned to remove impurities from the surface of the body. The above steps were completed as soon as possible. Then, specimens were immersed in seawater and temporarily stored at a temperature of −40 °C [38]. Biological characteristics were measured and recorded in an onshore laboratory, which included standard length (*L*, mm) from the snout tip to the last vertebra (to the nearest 0.1 mm) and body weight (*W*, g) to the nearest 0.01 g. Sex was determined by macroscopic observation of gonads [24].

Table 1. Sampling information of *Diaphus brachycephalus* in the South China Sea.

Sample Time	M/D/Y	Starting Location	Finishing Location	Trawl Depth	Mean Towing Speed
		Lat. (N)/Long. (E)	Lat. (N)/Long. (E)	(m)	(m/s)
18:17~19:51	01/27/2015	15°21.45'/115°11.07'	15°19.93'/115°04.40'	75	2.3
21:30~22:30	06/20/2015	19°48.04'/115°48.90'	19°45.44'/115°51.90'	75	1.8

Sagittal otoliths were extracted, numbered, and stored in 1.5 mL tubes filled with 75% alcohol to clean the soft tissue and organic material from the surface of the otoliths (Figure 1). The cleaned otoliths were dried for 1 h at 60 °C and then weighed to two decimal places in milligrams. After gluing and fixing with epoxy resin and hardener (EpoHeat, Buehler, Lake Bluff, IL, USA), the otoliths were longitudinally ground by a MetaServ 250 grinding machine (Buehler, Lake Bluff, IL, USA) with 280- and 800-grit sandpaper. They were further polished on top of its viewing surface using 1200- and 2500-grit sandpaper to obtain clear longitudinal sections [23,39,40].

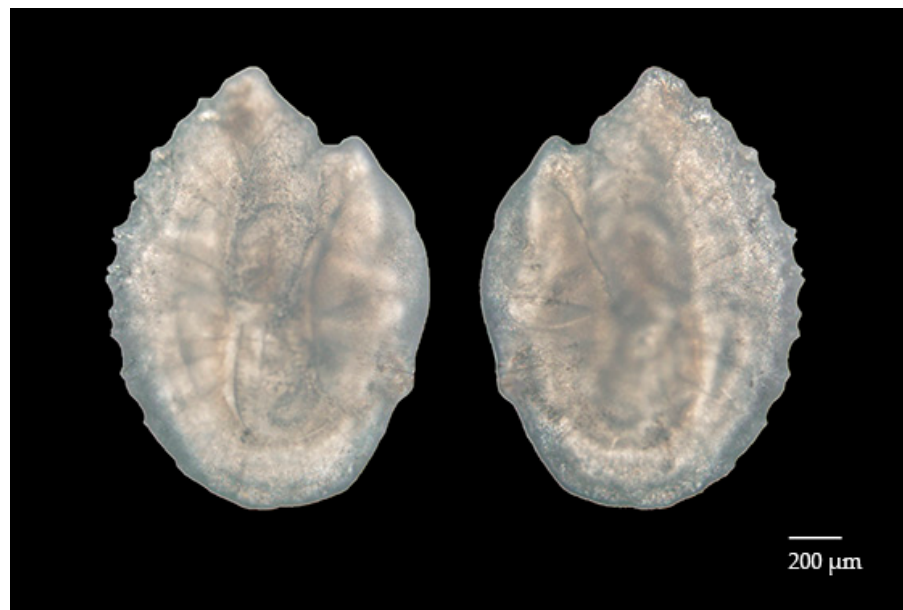


Figure 1. Sagittal otolith of *Diaphus brachycephalus* (scale = 200 μm).

Sagittal otoliths were divided into three regions: the central zone (CZ), middle zone (MZ), and external zone (EZ), as proposed by Gigarosov and cited in subsequent studies [23,41–43]. CZ is a bright area that covers the primordium and provides a clearer record of daily growth increments during the larval stage. Among the three regions, pigmentation is most pronounced in the MZ region, such that the otoliths form a white, black, and white sandwich structure between the three regions. The reason for this phenomenon may be related to the change in the perching water layer caused by the metamorphosis of these fish. EZ records the incremental characterization of fish from the completion of metamorphosis until they are harvested, and is usually the area with the clearest, densest, and most abundant incremental characterization. Daily growth increments were counted at least three times and ended with a difference of less than 5% between the average count and each count, unless it needed to be recounted. The above counting experiment was conducted by one person using a light microscope, and the mean value was taken as the final value for analysis. Both growth increments counted in regions of CZ and MZ were extracted and used to obtain biological information at metamorphosis. The summaries of CZ, MZ, and EZ were used for age analysis [20,23,42,43].

Differences between the two regions and between the sexes were tested by one-way ANOVAs. The interaction between the two regions and sexes was also tested. Additionally, the biological characteristics of *D. brachycephalus* were discussed based on the different results of analyses. The relationship between standard length (L) and body weight (W) was described by the following equation [20,44]:

$$W = aL^b, \quad (1)$$

where L is standard length, W is body weight, and a and b are regression coefficients [45]. The relationship between daily growth increments of CZ and MZ is shown below [22,23]:

$$N_{CZ} = cN_{MZ} + d, \quad (2)$$

where N_{CZ} is the daily growth increment of CZ and N_{MZ} is the daily growth increment of MZ. c and d are regression coefficients. In addition, the daily growth increment ratio of CZ and MZ was calculated to determine the proportion of daily growth between the egg and

metamorphic stage. The growth model selection was based on the von Bertalanffy (VB) growth curve, which is expressed as follows [22,23]:

$$L_t = L_\infty[1 - \exp\{-k(t - t_0)\}], \quad (3)$$

where L_t is the standard length at age t , L_∞ is theoretical asymptotic length, k is the growth coefficient, and t_0 is the theoretical age when $L = 0$. The calculation of the above parameters was obtained by maximum likelihood estimation. The growth rate curve was transformed from the VB growth curve [23]:

$$dL/dt = L_\infty \times k \times \exp[-k(t - t_0)], \quad (4)$$

where dL/dt is the growth rate. The data were analyzed and plotted using Excel 2019, SPSS 21.0, and Origin 9.9.0.225.

3. Results

A total of 87 specimens of *D. brachycephalus* were collected, of which 46 specimens (9 males and 37 females) were collected in January 2015, and a total of 41 specimens (7 males and 34 females) were collected in June 2015.

3.1. Body Length and Weight

The standard length and body weight of the *D. brachycephalus* specimens had no significant differences ($P > 0.05$) between the two regions and between the sexes, as tested by one-way ANOVAs. The interaction between the two regions and sexes was not significant ($P > 0.05$). Therefore, the male and female specimens of the two regions were analyzed as a whole. Means \pm SD for the regions and sexes are shown in Table 2.

Table 2. Standard length (L) and body weight (W) of *Diaphus brachycephalus*.

M/D/Y	Sexes	L (mean \pm SD, mm)	W (mean \pm SD, g)	n
01/27/2015	male	49.6 \pm 6.2	1.98 \pm 0.62	9
01/27/2015	female	50.4 \pm 5.1	2.27 \pm 0.57	37
06/20/2015	male	49.6 \pm 3.5	2.15 \pm 0.42	7
06/20/2015	female	49.7 \pm 2.7	2.15 \pm 0.42	34

All specimens (87 in total) were used to describe the relationship between L and W (males, 16; females, 71). L of *D. brachycephalus* in the SCS ($n = 87$) ranged 32.0–58.0 mm, mean 50.0 ± 4.3 mm, and W of specimens ranged 0.71–3.21 g, mean 2.14 ± 0.51 g. The L – W relationship (Figure 2) of *D. brachycephalus* was as follows:

$$W = 0.00002699L^{2.8789}, r^2 = 0.905. \quad (5)$$

3.2. Sagittal Otolith Microstructure

In total, 73 pairs of clear longitudinal sections of the sagittal otoliths were collected because of losses during extraction and grinding. Females accounted for 59 sagittal otoliths, of which 30 were from January 2015 and 29 were from June 2015. Additionally, 7 sagittal otoliths from males were obtained from each region. The one on the right was selected for microscopic observation. Sagittal otolith microstructure of *D. brachycephalus* is composed of CZ, MZ, and EZ (Figure 3). These three zones were wrapped around from the inside out, which is visible through light micrographs. Moreover, a number of daily growth increments were found in MZ with abnormal dark colors and shapes. Daily growth increments of CZ, MZ, and EZ were counted for all 73 specimens. Additionally, the daily growth increments of CZ and MZ fluctuated within a relatively small range 16–26 (mean = 20.3 ± 2.6) and

12–20 (mean = 16.0 ± 1.9), respectively. The ratio of CZ/MZ was 1.27, and the relationship between daily growth increments of CZ and MZ was fitted as:

$$N_{CZ} = 0.05029N_{MZ} + 13.9574, r^2 = 0.0714, P > 0.05. \quad (6)$$

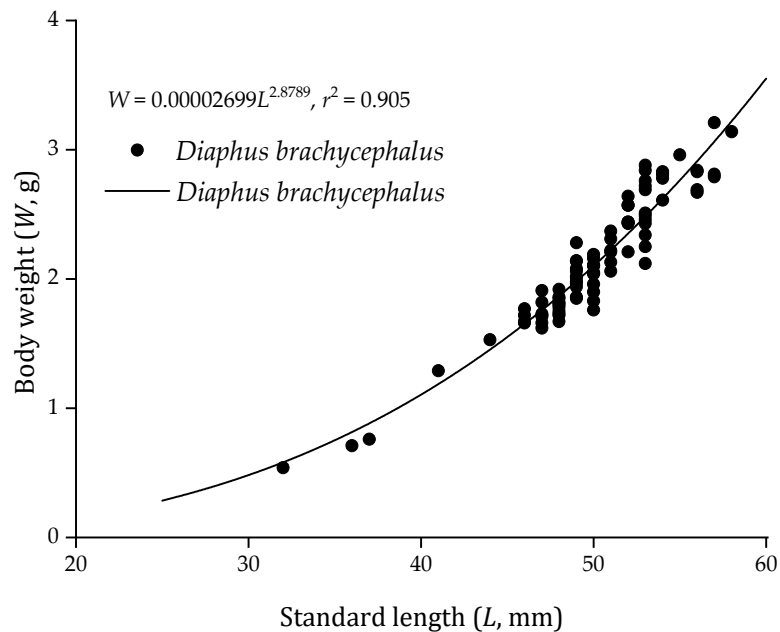


Figure 2. Relationship between standard length and body weight of *Diaphus brachycephalus* ($n = 87$) in two regions of the South China Sea.

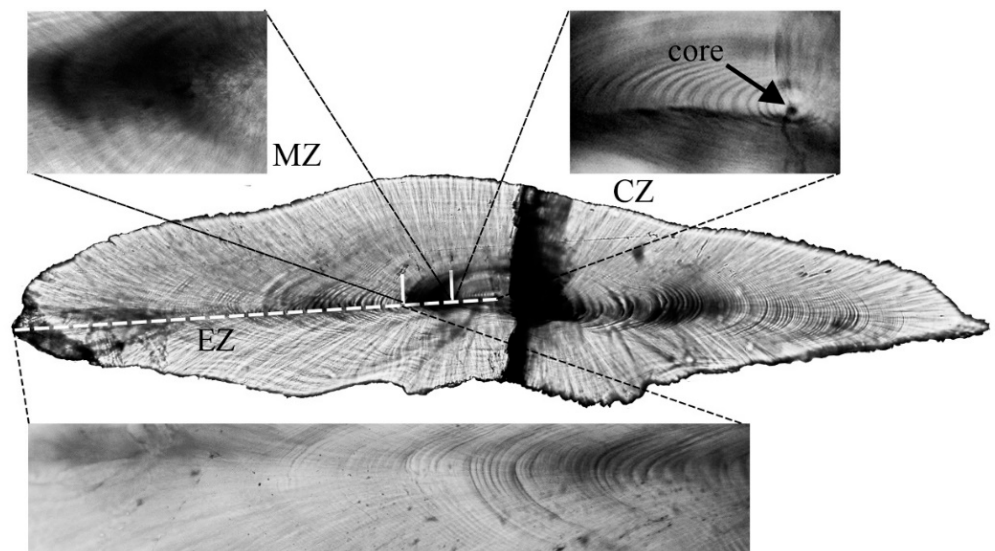


Figure 3. Light micrographs of a sagittal otolith from *Diaphus brachycephalus*: central zone (CZ), middle zone (MZ), and external zone (EZ). The shaded area across the otolith on the right was a fracture caused by dehydration due to the action of alcohol when the otolith was preserved and did not affect the observation of daily growth increments.

Daily growth increments in EZ ranged 18–137. The results showed that daily growth increments of *D. brachycephalus* did not significantly differ between the regions or between the sexes (One-way ANOVA, $P > 0.05$).

3.3. Age and Growth

The ages of *D. brachycephalus*, ranging 59–168, were obtained by summing all of the daily growth increments in CZ, MZ, and EZ. There were non-significant differences between the two regions and sexes (one-way ANOVA, $P > 0.05$). Additionally, their interaction between the two regions and sexes was not significantly different ($P > 0.05$). Mean ages of males and females in January 2015 were 115 ± 27 days and 119 ± 25 days, respectively. The mean ages of males and females in June 2015 were 119 ± 15 days and 115 ± 13 days, respectively. Moreover, the interaction between the two regions and sexes was not significant ($P > 0.05$). The VB growth curve for *D. brachycephalus* was determined by fitting standard length and age (Figure 4):

$$L = 65.6[1 - \exp\{-0.0132(t - 6.94)\}], r^2 = 0.935, \quad (7)$$

where L is standard length, t is the age in days (summary of daily growth increments in CZ, MZ, and EZ). Fitting the relationship between age and L , the growth rate curve was shown in (Figure 5):

$$dL/dt = 65.6 \times 0.0132 \times \exp[-0.0132(t - 6.94)]. \quad (8)$$

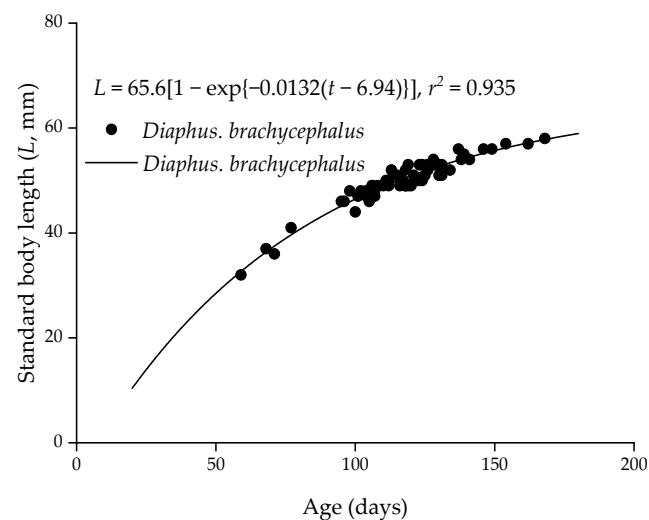


Figure 4. The von Bertalanffy growth curve fitted to the relationship between age and standard length in *Diaphus brachycephalus* ($n = 73$) in the South China Sea.

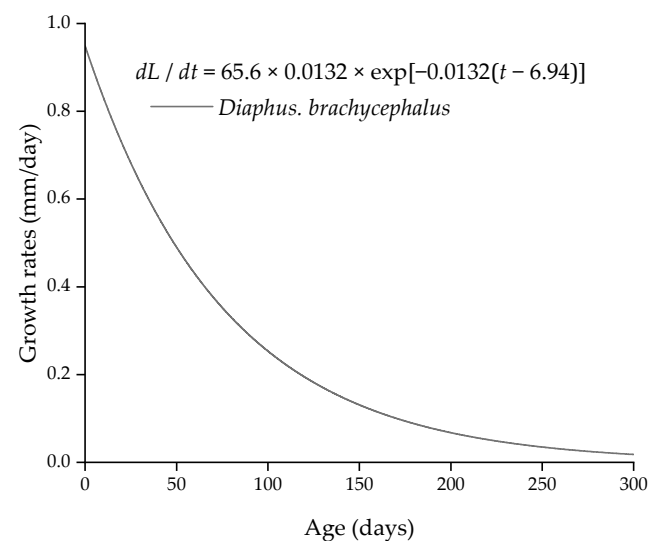


Figure 5. Growth rate for standard length as dL/dt for *Diaphus brachycephalus* in the South China Sea.

The maximum growth rate was $0.436 \text{ mm day}^{-1}$ at the age of 59 days, when the standard length of *D. brachycephalus* was 32.0 mm and the body weight was 0.54 g. Based on age and date of capture, the back-calculated hatching dates of *D. brachycephalus* were distributed from March to May and from August to November, with two peak spawning periods concentrated from April to May and from September to October (Figure 6).

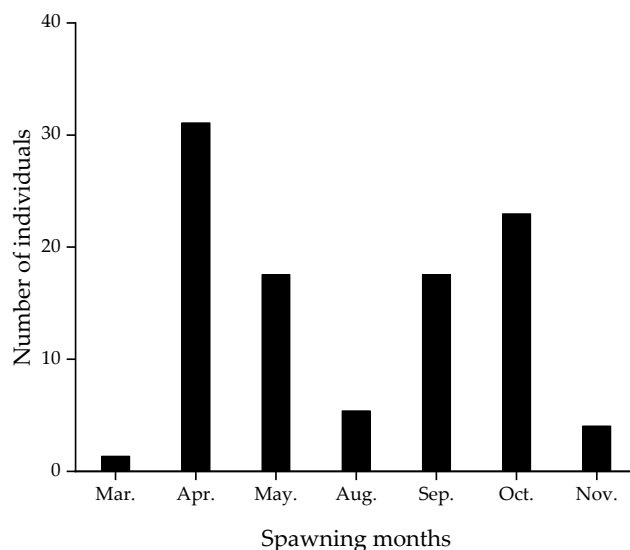


Figure 6. Speculated spawning times of *Diaphus brachycephalus* specimens in the South China Sea.

4. Discussion

In this study, there were more female than male *D. brachycephalus* specimens collected in the SCS, with a ratio of females to males of approximately 4.44. This is a trait thought to be common in many species of lanternfish; a large number of females would produce abundant eggs to guarantee a high population recruitment rate, such as in *D. garmani*, *D. chrysohynchus*, and *Myctophum asperum* [17,22,46–48]. In our study, the range of standard length and body weight of *D. brachycephalus* (L , 32–58 mm and W , 0.71–3.21 g) were larger than the finding of Lopez-Perez in the Atlantic (L , 12–47 mm and W , 0.02–0.75 g) [49]. Additionally, there were neither sex nor regional differences ($P > 0.05$) in *D. brachycephalus* from the SCS. Gibbs also concluded that *D. brachycephalus* had no sexual dimorphism in body size [50]. Thus, based on the premise that there was no significant difference in body weight and sex, our collection could be used to analyze the relationship between L – W despite only including a few males. These specimens showed that *D. brachycephalus* had negative allometric growth, whereas most species of mesopelagic fish have isometric growth, such as *Benthoosema pterotum*, *Ceratospiculus warmingii*, *Hygophum benoiti*, and *Hygophum hygomii* [23,25,51].

Interestingly, our findings were opposite to those of Froese’s survey in the Atlantic [43]. The L – W relationship in the SCS and Atlantic was $W = 0.000026999 \times L^{2.8789}$ and $W = 0.00000131 \times L^{3.39}$, respectively, suggesting that *D. brachycephalus* had negative allometric growth in the SCS and positive allometric growth in the Atlantic. This phenomenon also occurred in *M. punctatum* from different regions, with the following relationships between L – W : $W = 0.00000057 \times L^{3.36}$ in the Atlantic and $W = 0.000014 \times L^{2.97}$ in the Mediterranean Sea [25,51]. The differences in the environment and temperature of the ocean may be the main reasons for the differences in the L – W relationships among lanternfish. By sorting out the biological characteristics of *D. brachycephalus*, we found that the length of juveniles of *D. brachycephalus* ranged 10–26 mm, subadults 25–40 mm, and adults were generally less than 61 mm [51,52]. Such evidence might explain the discrepancy between our results and those of Lopez-Perez; the Lopez-Perez study might have focused more on the subadult stage of *D. brachycephalus* and the influence of geographical differences [49].

CZ and MZ document lanternfish developmental processes during their juvenile years. CZ covers the embryonic development of a fertilized egg and ends with hatching. The formation of MZ is still uncertain, but it is generally believed that larval fishes migrate to deep waters where they complete their metamorphosis and thus become subadults [22,23,53]. In the absence of accurate descriptions of egg information for *D. brachycephalus*, we refer to spawning information for other species of mesopelagic fish in the genus *Diaphus*. The presence of hydrated oocytes signals that the fish are about to spawn; thus, in the absence of direct collection of eggs from water, the layer of water in which females with hydrated oocytes are collected can be considered the layer in which spawning takes place [46,54,55]. When the gonads of the specimens were examined, 32.4% of the females were found to have hydrated oocytes and the rest were in a state of ovarian development close to imminent spawning. Therefore, *D. brachycephalus* is likely to spawn in surface waters. Gibbs found *D. brachycephalus* larvae at depths from 150 m to 250 m, suggesting that MZ formation was influenced by metamorphosis and DVM [50].

In our study, CZ ranged 16–26 days (mean = 20.3 days). CZ varies between different species; for example, 33–43 days in *Myctophum nitidulum*, 80–139 days in *Tarletonbeania crenularis*, 22–32 days in *B. pterotum*, and 14–40 days in *C. warmingii* [23,41–43]. Additionally, MZ ranged 12–20 days (mean = 16 days), which was similar to *Lampanyctodes hectoris* (5–9 days), *Notoscopelus resplendens* (mean = 23 days), and *M. asperum* (mean = 10 days) [22,47,56]. MZ also varies considerably between lanternfish; for example, 51–102 days in *Tarletonbeania crenularis* and 24–54 days in *C. warmingii* [42,57]. In addition, there was no significant correlation between the daily increments of CZ and MZ in our study, and the ratio of the mean increments in the CZ/MZ of *D. brachycephalus* sagittal otoliths was 1.27. The same weak correlation between EZ and MZ was found in other studies, with the ratio between the two areas fluctuating around 1.00 [23,42,43]. For *D. brachycephalus*, the lower and more concentrated daily growth of CZ and MZ may be due to a combination of the marine environment in the SCS and the reproductive strategy of the population. That is, to maintain a relative balance in population dynamics, small individual fish of *D. brachycephalus* need to rapidly develop in preparation for entering the reproductive phase as soon as possible.

The VB growth curve objectively reflects the growth of *D. brachycephalus*. Age and standard length were used as parameters to fit the growth equation and study the growth rate of *D. brachycephalus*. The L_{∞} (65.6, mm) of the growth curve was larger than the maximum standard length (58 mm), whereas L_{∞} was greater than the currently known maximum standard length (61 mm) [52]. The VB growth curve objectively reflected the growth of *D. brachycephalus*, with $r^2 = 0.935$. Based on the available *D. brachycephalus*, the growth rate of juveniles was faster than that of adults, with a maximum growth rate of $0.436 \text{ mm day}^{-1}$, which was faster than other lanternfish, such as *S. californiensis* (0.31 mm day^{-1}), *M. asperum* (0.3 mm day^{-1}), and *C. warmingii* (0.35 mm day^{-1}) [22,23,57,58]. However, benefiting from the distribution of specimens at various age stages from larval to adult, especially including the larval stage, Sarmiento-Lezcano argued that the otolith weight–age relationship of *N. resplendens* was more in line with the Gompertz curve, whereas the relationship between standard length and age fitted well in both the Gompertz and the VB growth curve [56]. In the future, we need to know more about the distribution characteristics of *D. brachycephalus* to obtain larval specimens. The spawning of *D. brachycephalus* was mainly concentrated in April to May and September to October, suggesting that *D. brachycephalus* had at least two peak spawning periods in one year; however, this is based on the assumption of two surveys. We cannot rule out the possibility of spawning in other seasons, which will require investigation over a longer time span.

There are many gaps in the research on the microstructure of lanternfish otoliths that need to be addressed. In future studies, otolith preservation techniques will need to be fully considered, in addition to the integrity of the specimens at all age stages; a long-time span is required for such investigations. Alcohol is the most commonly used preservation

solvent in otolith studies. Because of the narrow shape of the otoliths in *D. brachycephalus*, they would have fractured under the dehydrating effect of alcohol. Furthermore, Moku found that the preservation techniques of alcohol or formalin, which are popular at this stage, cause the otoliths to shrink and affect the assessment of age [59]. Therefore, the preservation method of otoliths needs to be further improved.

5. Conclusions

This study confirmed the age and growth of *D. brachycephalus* in the SCS for the first time by analysis of L , W , and sagittal otolith microstructure. The relationship between standard length (L) and body weight (W) was $W = 0.00002699L^{2.8789}$, with non-significant sex differences. Moreover, *D. brachycephalus* was determined to have a short hatching period and metamorphosis period on the basis of the different areas of the sagittal otolith (CZ, MZ, EZ). The von Bertalanffy (VB) growth curves fitted to the relationship between age and L were shown as: $L = 65.6[1 - \exp\{-0.0132(t - 6.94)\}]$, $r^2 = 0.935$. We successfully obtained a faster growth rate during the juvenile period of *D. brachycephalus* as a result of the VB growth curve fitted with L and age, and the growth rate curve for L was dL/dt . The results indicated that *D. brachycephalus* is a short-lived small fish, and the microstructure of otoliths could well reflect the age and growth of *D. brachycephalus*.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the South China Sea Fisheries Research Institute Animal Welfare Committee (approval code: 2019/002 and approval date: 1 March 2019).

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