Research Article

Age composition and growth parameters of areolate grouper, *Epinephelus areolatus* and six-bar grouper, *E. sexfasciatus* (Teleostei: Serranidae) in the Terengganu waters, Malaysia

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**Abstract:** The age and growth of areolate grouper, *Epinephelus areolatus* and six-bar grouper, *E. sexfasciatus* in the Terengganu waters, Malaysia, were estimated by analysis of sectioned sagittal otoliths. A total of 795 grouper samples were collected monthly for 12 months from February 2014 to January 2015 in two main fishing landing ports of the Terengganu. The recorded results showed that the ages ranged were from 1–9 and 2–9 years, for *E. areolatus* and *E. sexfasciatus*, respectively, for. The length-at-age data fitted to the von Bertalanffy growth model revealed that the growth function was TLt = 86.66 [1 - e−0.06(t+2.32)] for *E. areolatus* and TLt = 33.86 [1 - e−0.44(t+0.35)] for *E. sexfasciatus*. The high proportion of immature fish in the catch and the lack of adults near maximum size indicate that the population of *E. areolatus* in particular is in danger of being overfished and management measures should be urgently implemented to reduce the catch of immature fish and to preserve an adequate breeding stock of larger adults of these and other groupers of commercial importance.

**Keywords:** Malaysian fisheries, Groupers, Otolith, Reef fishes, von Bertalanffy growth function.


**Introduction**

Groupers (Serranidae: Epinephelinae) are considered a high-value species in the coastal fisheries of tropical and subtropical areas (Sadovy de Mitcheson et al. 2013). Worldwide, the population of reef fishes is decreasing largely due to chronic overfishing and the use of fishing gears that damage their spawning habitat (Sadovy de Mitcheson et al. 2013). Groupers are vulnerable to overfishing because of their high site fidelity and their habit of forming of spawning aggregations (Erisman et al. 2007). They are also generally slow to mature and long-lived (Sadovy de Mitcheson et al. 2013).

The areolate grouper, *Epinephelus areolatus* (Forsskål, 1775) and six-bar grouper, *E. sexfasciatus* (Valenciennes, 1828) are the most frequently-caught grouper species in the waters of Terengganu State, eastern Peninsular Malaysia (Abdul Kadir et al. 2016), but biological information for these species is limited. Published studies are available for *E. areolatus* in Malaysian waters for reproduction (Mahmoud 2009; Zubair et al. 2011; Abdul Kadir et al. 2016), and otolith measurements (Mat Piah et al. 2017). Growth parameters have been determined for the species in the Gulf of Suez (Abd-Allah et al. 2015). No biological data for *E. sexfasciatus* have been reported in the areas. As a result, *E. sexfasciatus* is listed as ‘Data Deficient’ in the IUCN Red List (Russel et al. 2008).

In Malaysia, the growth parameters of fish are mostly estimated using length frequency data that are fitted into FAO-ICLARM Stock Assessment Tools.
(FiSAT II) (e.g. Simon et al. 2010; Jamon et al. 2016). This is in part due to the difficulty in estimating the age of fish from the otoliths in the tropical fishes, (Jiménez-Badillo 2006; Holloway et al. 2015). However, growth estimates of fish by otolith structure is more reliable than the use of length-frequency especially in long-lived, slow-growing species (Bilgin et al. 2012). This study is the first attempt to determine the age composition of *E. areolatus* and *E. sexfasciatus* in Malaysia from sectioned otoliths, and to estimate their growth parameters from the length-at-age data obtained in order to understand their population status in the area.

**Materials and Methods**

**Study area and sampling:** Grouper samples were collected at monthly intervals from commercial catches landed at the two main fishing ports in Terengganu, Malaysia from February 2014 to January 2015. Total length (TL) of each fish was measured to the nearest centimetre (cm) and the weight was measured to the nearest 0.01 gram (g).

**Otolith extraction and preparation:** Left and right sagittal otoliths were extracted using the “up through the gills method” as described by Secor et al. (1992). Otoliths were cleaned gently with water to remove attached tissues and fluid, air dried, and stored in vials.

One of the otoliths was selected and embedded in BUEHLER epoxy resin (20-8128-032) and BUEHLER epoxy hardener (20-8128-088) with a mixture ratio of 5:1. Since there is no difference in characteristics between the left and right otolith (Mat Piah et al. 2017), only the left otolith was analysed. The right otolith was used only if the left was not extracted undamaged from the samples. The embedded samples were set aside to harden for one to two weeks before the sectioning process started.

For the sectioning process, a BUEHLER IsoMet 1000 precision saw equipped with a diamond wafering blade of 0.5mm thick was used to section the otolith along the dorsal-ventral line directly through the nucleus to obtain two thin wafers. The sections were then mounted on glass slides using similar resin used for embedding, and covered by a cover slip. The mounted glass slides were set aside for one to two days to allow the resin to harden before reading.

**Age determination:** The sectioned otoliths showed alternating opaque and translucent zones when viewed under reflected light with black background as shown in Figure 1. The interpretation of otolith growth, reading, identification of the first annual increment, and subsequent increments was conducted by following the guidelines provided by Francis et al. (1992).

Opaque zones were counted in along the dorsal plane between the primordium and the otolith edge.
(as shown in Figure 1), and the first deflection point in the sagitta-subcupular meshwork fibre zone (SMF) assisted in identifying the starting of the opaque zone of the first increment (Francis et al. 1992). Opaque zones of the otolith sections were counted by two readers to limit observer bias. The otolith samples were read in random order without knowledge of the meristic information. Pothin et al. (2004) reported some species of the genus *Epinephelus* are protogynous hermaphrodites, thus determination of sex by macroscopic observation of the gonads may be uncertain or inaccurate. For this reason, we estimated growth parameters for all samples combined rather than by sex.

**Timing of opaque zone formation in the otolith:** Marginal increment is defined as the fraction of the distance between the last and the next-to-last increment (Tariche et al. 2015). Otoliths were measured along the proximal (internal) medial surface, ventral to the sulcus, where the annuli were clearest. We were unable to include all samples in the marginal increment analysis due to fracturing of some otolith section edges during processing. A total of 115 specimens of *E. areolatus* and 157 specimens of *E. sexfasciatus* were analysed successfully.

**Data analysis:** Age and length data was fitted to the von Bertalanffy (1938) growth model for length:

\[ L_t = L_\infty \left(1 - \exp \left[-K (t-t_0)\right]\right) \]

Where \(L_t\) is length at age \(t\), \(L_\infty\) is the asymptotic length that the species would reach if it lived indefinitely, \(K\) is the growth coefficient and defines the growth rate towards \(L_\infty\), \(t\) is age of the fish, and \(t_0\) is theoretical age at zero length. The von Bertalanffy growth curves were fitted to the age and length data using two iterative least square procedures (SPSS non-linear regression procedure (Norusis 1995). Growth performance indexes (Munro & Pauly, 1983) were calculated as:

\[ \Theta = \log (K) + \left(2 \times \log \infty \right) \]

**Results**

A total of 666 otoliths were analysed with 499 specimens for *E. areolatus* and 167 specimens for *E. sexfasciatus*. The total length of *E. areolatus* ranged between 17.0-44.8 cm with a mean of 28.15±6.47 cm. The corresponding body weight ranged between 41.51-1289.9 g with a mean of 326.62±204.44 g. The total length range of *E. sexfasciatus* was 15.6 cm to 33.9 cm, with a mean of 27.34±3.75 cm. The body weight ranged from 20.4 g to 562.3 g with a mean of 336.69±111.507 g. Figure 2 showed the mean total length of *E. areolatus* and *E. sexfasciatus* sampled in the Terengganu waters.

The maximum observed age for *E. areolatus* in this study was nine years old with a length of 44.0 cm while the minimum age was one year old with a length of 17.0 cm (Table 1). The maximum observed age of *E. sexfasciatus* was also nine years old with a length of 32.0 cm; however, the minimum age was two years old with a length of 17.0 cm (Table 2). Modal ages of both *E. areolatus* and *E. sexfasciatus* landed from Terengganu waters were four years old, comprising 27.45 and 33.53% of the catch of each species (Tables 1, 2).

The analysis shows that the asymptotic length (\(L_\infty\)) of *E. areolatus* was estimated at 86.66 cm, growth coefficient (\(K\)) was 0.06 year\(^{-1}\), and \(t_0\) was 2.32 years. For *E. sexfasciatus*, \(L_\infty\) was 33.86 cm, \(K\) was 0.44 year\(^{-1}\), and \(t_0\) was 0.35 years. Von Bertalanffy growth curves for *E. areolatus* (n=499) and *E. sexfasciatus* (n=167) from the Terengganu waters, South China Sea, Malaysia.
and *E. sexfasciatus* (n=167) were fitted as shown in Figures 3 and 4. Growth performance indices of *E. areolatus* was 2.65 and 2.70 for *E. sexfasciatus*.

Marginal increment analysis of *E. areolatus* (n=115) showed that the highest ratio of outer band width to previous band width occurred in February (Fig. 5). For *E. sexfasciatus*, (n=157), the highest ratio of outer band width to previous band width was observed in January (Fig. 5). No value could be obtained for the month of August due to sampling error which questioned the sampled integrity, and also in December due to unfavourable weather condition because of monsoon season in the East Coast of peninsular Malaysia which lead to no
Table 2. Age length key for sixbar grouper, *Epinephelus sexfasciatus* from the Terengganu waters, South China Sea, Malaysia (n=167).

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<th>Length class (cm)</th>
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Fig.3. The von Bertalanffy growth curves of areolate grouper, *Epinephelus areolatus* from the Terengganu waters, Malaysia (n=499).

Fig.4. The von Bertalanffy growth curves of six-bar grouper, *Epinephelus sexfasciatus* from the Terengganu waters, Malaysia (n=167).
sample available for that respective month.

### Discussion

Estimation of age and growth parameters are important for fish population assessment. Previous studies on sub-family Epinephinae stocks in many region have mainly focused on population parameters and biological aspects on some other species of this family such as yellowedge grouper, *E. flavolimbatus* (Manickchand-Heileman and Philip 2000), brownsotted grouper, *E. chlorostigma* (Alkahem et al. 2004), tropical grouper, *E. merra* (Pothin et al. 2004), snowy grouper, *E. niveatus* (Costa et al. 2012), dusky grouper, *E. marginatus* (Condini et al. 2014), blue spotted seabass, *Cephalopholis taeniops* (Tariche et al. 2015), bar-cheeked coral trout, *Plectropomus maculatus* (Ferreira and Russ 1992) and common coral trout *P. leopardus* (Loubens 1980; McPherson et al. 1988). Information on age and growth of groupers in Malaysia is scarce and this is the first study on age and growth of *E. areolatus* and *E. sexfasciatus* in Malaysia.

This study found that *E. areolatus* is a slow-growing species. The maximum size of an individual in this study was 44cm. Moran et al. (1988 cited by Froese & Pauly 2018) reported that the maximum length of *E. areolatus* caught in North-western Australia is 47cm and Abd-Allah et al. (2015) reported that the species in the Gulf of Suez had a growth coefficient of 0.154year⁻¹ and an asymptotic length of 66.55cm. Hassan (1988) cited in Abd-Allah et al. (2015) recorded a growth coefficient of the same species at 0.13year⁻¹ and $L_\infty$ of 78.92cm (TL). This may due to the different regions of fish sampled where fish has potential for differences in growth characteristics. The variability in growth of *E. areolatus* from various locations also might be influenced by several factors, including differences in mortality rates, environmental conditions or genetic variation (Dutka-Gianelli & Murie 2001). In contrast the growth of *E. sexfasciatus*, is much faster. This is similar to the parameters calculated for *E. sexfasciatus* from the Philippines (Pauly and Ingles 1981) where $L_\infty$ was 30.9cm and $K=0.510$year⁻¹. As a number of individuals in this study were close to $L_\infty$ in size, there is greater precision in this estimate although the single nine-year-old individual exerts a strong influence on the final value of $L_\infty$.

Some groupers may reach a maximum age of more than 60 years (Reñones et al. 2007). The growth parameters obtained by otolith analysis in this study revealed that *E. areolatus* is a slow-growing species with $K=0.06$year⁻¹, and using a simple approximation of $t_{\text{max}} = 3/K$ (King 1995), the lifespan of this species may be around 50 years. When comparing to this value of maximum age in the sample it is clear that the fishery catch comprises mostly young age groups. Growth performance indices (Munro & Pauly 1983) of *E. areolatus* and *E. sexfasciatus* were compared with the other stock from the same species available on Fishbase (http://www.fishbase.org). The growth performance Index of *E. areolatus* ($\Theta=2.65$) is in the middle of other growth performance index recorded for the same species from other region such as New Caledonia ($\Theta=2.41$), Kuwait ($\Theta=2.64$), Gulf of Suez, Egypt ($\Theta=2.83$) and Red Sea, Egypt ($\Theta=2.91$). For *E. sexfasciatus*, the value of Munro Growth

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**Fig.5.** The ratio of the outer width to the previous band width for areolate grouper, *Epinephelus areolatus* (n=115) and *E. sexfasciatus* (n=157) from Pulau Kambing and Kuala Dungun fish landing ports. Notes: No sample was able to be analysed in August and December.
Performance Index was 2.70, slightly higher the value recorded for fish from Visayan Sea, Phillipines at 2.69.

The formation of opaque and translucent zones in the otoliths may be influenced by many factors such as seasonal variations in water temperature, photoperiod, feeding, and reproduction (Manickchand-Heileman & Phillip 2000; Alkahem et al. 2004). Marginal increment analysis revealed that new opaque zones start forming around the end of the Northeast Monsoon season in Malaysia, which occurs between the months of November and February (Yaakob & Chao 2005). During the monsoon sea temperature tend to be lower (Yaakob & Chau 2005) but runoff may also increase food availability to coastal fish through elevated marine productivity from elevated nutrients and organic inputs. However, there was high variation in increment ratios and mean values never dropped below 0.3 in any month suggesting that opaque zone formation may occur over an extended time that is variable between individuals. More definitive validation studies are therefore recommended for these species. Nevertheless, the results highlight the risk of intense overfishing of the E. areolatus population and immediate management intervention is required. The E. sexfasciatus population is at lower risk, but early intervention is needed to prevent exploitation from exceeding sustainable levels especially if other species become scarcer.

This study describes the growth parameters of the two most dominant groupers in Terengganu waters, South China Sea by examining sectioned otoliths. The information derived will add more knowledge on the species and will be beneficial for the management of the species in the future for sustainability of grouper fisheries.

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مقاله پژوهشی

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چکیده:
سن و رشد دو گونه از هامورماهیان Epinephelus areolatus و E. sexfasciatus از آب‌های ترنگانو در مالزی با استفاده از برش گیری عرضی از اتولیت تخمین گردید. تعداد 795 نمونه به گونه‌بندی و به مدت 12 ماه از فوریه 2014 تا ژانویه 2015 از دو اسکله ماهی‌گیری ترنگانو جمع‌آوری گردید. نتایج نشان داد که سن ماهیان مورد مطالعه برای گونه E. areolatus و E. sexfasciatus ترتیبی به ترتیب با تگذشته 86.66 و 33.56 حداقل در سن نوزادان به ترتیب 86.66*0.06(t+2.32) and 33.86*0.44(t+0.35)TLa می‌باشد (TLa در یک متر مربع) که این نتایج مقداری رشد با توجه به کاهش میزان رشد سنی ماهیان از آب‌های ترنگانو نشان می‌دهد که پایگاه گونه‌های این گروه در معرض نواحی بسیار کم جمعیت ماهی‌های نابالغ در صید و کمبود ماهیان بالغ نزدیک به حداکثر اندازه نشان می‌دهد که جمعیت گونه‌های این گروه در معرض نواحی بسیار کم جمعیت ماهی‌های نابالغ و کمیابی ماهی‌های بالغ و سایر هامورماهیان که ارزش تجاری دارند، مورد نیاز است.

کلمات کلیدی: شیلات مالزی، هامورماهیان، اتولیت، ماهیان صخره‌ای، عملکرد صید، فنون پترنالوگی.