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Comparison between the opercular bones, the scales and the otoliths to investigate the growth of the brilliant pomfret (*Eumegistus illustris*) off the coast of Reunion Island (SW Indian Ocean)

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Abstract
The brilliant pomfret (*Eumegistus illustris*) occurs in the western part of the Indian Ocean and the western and central Pacific Ocean. To date, the biology of *E. illustris* has never been documented. The aim of the present study was to investigate age and growth of *E. illustris* along the coasts of Reunion Island, based on calcified structures analysis. One hundred thirty five individuals were sampled in the landings of the French local artisanal fisheries from March 2014 to March 2015. The relationships between two types of body length (Total and Standard lengths, cm) and Total weight (g) were significant ($P<0.05$). Total length-weight relationship was described by the following parameters: a=0.012 and b=3.015 without significant effect of sexual dimorphism. The oblong shape of the scales and the otoliths could explain the difficulty in using these calcified structures for ageing *E. illustris*. Consequently, the opercular bone is a good alternative calcified structure when the otoliths and scales are unsuitable especially for slowly growing fish.

Keywords: Eumegistus illustris, growth, scale, otolith, opercular bone, Indian Ocean, Reunion island

1. Introduction
The brilliant pomfret (*Eumegistus illustris,* [16]) inhabits the western part of the Indian Ocean and the western and central Pacific Ocean [9, 22], unlike the other *Eumegistus* species (*Eumegistus brevorti*) which occurs in the south-western Atlantic Ocean only [14]. These two species are distinguished one another by the vertebral count [16, 24]. *E. illustris* is present in tropical and subtropical waters. Specimens of *E. illustris* were observed from the Hawaiian Archipelago [16, 25], the Japanese archipelago [22], the Philippines archipelago [6], New Caledonia [10] and Reunion Island [20].
To date, the biology of *E. illustris* has never been documented. However, this species is targeted by artisanal fisheries at Reunion Island and it is highly desired for local consumption. Because fish age data are essential to determine recruitment, growth and mortality rates, the aim of this study was thus to investigate age and growth of *E. illustris* along the coasts of Reunion Island based on calcified structures analysis.

2. Materials and Methods
A total of 135 *E. illustris* were sampled in the landings of the French local artisanal fisheries (deep-water handline on electric reel), in the eastern part of Reunion Island (21°.10 S latitude; 55°.30 E longitude). Monthly samples were collected between March 2014 and March 2015. All fresh specimens were examined in the laboratory for Total Length (TL, cm), Standard Length (SL, cm), Total wet weight (W, g) and sex.
Preliminary to the characterization of the length-length (L-L) and length-weight (L-W) relationships, all pairs of data were plotted in order to identify and delete obvious outliers. In order to estimate the parameters of the allometric L-W relationship, its base-10 logarithm was fitted to data using a least squared linear model:

$$W = a\,L^{b} \qquad (1)$$
$$\log W = \log a + b.\log L \qquad (2)$$

Where 'a' is the intercept or initial growth coefficient and 'b' is the slope *i.e.* the growth coefficient [11, 19, 28].

Since growth of *E. illustris* has not been investigated before, calibration of the method for age and growth structure determination was needed. Different calcified structures (otolith, scale, opercular bone, vertebra…) can be used for the age determination of fishes. The choice of aging structure is critical because all structures are not suitable for age determination of a given species. For each fish, the sagittal otoliths, a few scales and opercular bones were used in order to estimate and compare the age of fish between three calcified structures. The sagittal otoliths were removed from left and right head sides and rinsed in water. Scales vary in size and growth patterns depending on the location on the fish body. Scales removed from behind the pectoral fin are least likely to be replacement scales as they are protected [7]. It is important to remove sufficient scales to provide the reader ample opportunity for accurate age assessment. Consequently, ten scales are sampled from just behind the pectoral fin per fish to ensure a minimum of readable scales. Otoliths and scales were stored dry in envelopes. Different parts of opercular (opercular and sub opercular bones) were sampled from left and right head sides. Depending of fish size, opercular were boiled during 5 to 15 minutes to clean any excess of flesh. After, the parts of the opercular were dried for at least 24 hours before storing in the same way as the scales.

Different techniques were used to gain the most precise evaluation of the fish age: observation under transmitted light of whole scales, observation under both transmitted light and reflected light of the whole otolith and the transverse section (width: 0.4 mm) before and after polishing of the otolith, observation of opercular bones under reflected light on a black background. All viewing techniques were analysed using the TNPC software (digital processing of calcified structures, www.tnpc.fr). Oil was used as clarifying liquid.

All statistical analyses were carried out using the open-source statistical package "R" [27]. Differences were considered significant at $P<0.05$.

3. Results and Discussion

A total of 135 *E. illustris* were sampled. The length range of the fish was between 45 and 90 cm, with a mean length of 67 cm. Females ranged in size from 45.2 to 90.1 cm TL (68 ± 14 cm, $n=75$) and males ranged from 48.2 to 86.2 cm TL (66 ± 12 cm, $n=60$) (Fig 1). Significant L-L and L-W relationships were detected regardless to sex:

$$TL = 1.350 * SL - 51.874;\ r^2 = 0.98;\ P < 2.10^{-16}$$
$$W = 0.012 * TL^{3.015};\ r^2 = 0.98;\ P < 2.010^{-16}$$

Significantly, TL-SL relationship was comparable for both males and females (ANCOVA, $F(1, 131) = 0.195$, P = 0.659). The length–weight relationship showed a significant positive allometric growth, regardless of the sex. Allometric coefficient of the regression for females was not significantly different to that of males (ANCOVA, $F(1, 131) = 0.167$, P = 0.683).

Left and right sagittal whole otoliths were examined side by side to aid in discerning between growth annuli and checks. The sagittal plane of the otoliths did not provided a clear view of the annuli (Fig 2). The same results were obtained from the whole otoliths after burning. Finally, the transverse section of otolith was tested to examine the transversal plane of otolith. Otoliths were embedded in translucent polyester resin, and thin sections of 0.4 mm were obtained using a precision high-speed diamond saw. The results of transverse section of otoliths before and after polishing were no better than those from the whole otolith (Fig 2). All scales on a slide were examined to distinguish growth *circulii* from checks, but the scales were also not suitable for age determination of *E. illustris* (Fig 2). Subsequently, the parts of the opercular bone (opercular and sub opercular bones) were tested. The sub opercular bone was suitable to be used to estimate the age of *E. illustris* (n=11; Fig 2). The Age/Length key showed similar growth patterns between females and males (Fig 3).

To date, the L-L and the L-W relationships of *E. illustris* have never been documented. Only maximum observed total length data were reported in the Japanese Archipelago (SL=47 cm; [22]) and in southern Mindanao at the Philippines (TL=60 cm, [6]). During this study, the specimens were sampled in the landings of the French fisheries in Reunion island from deep-water handlines on electric reels and the largest specimen was measured at 90.1 cm (TL, Female) and the heaviest fish weighed 9.87 kg (TL=85.4 cm, Female). Morphometric feature relationships of fish are a necessary index to enable a conversion of one variable to the other, which are essential in fisheries management [19]. All body measurements (TL, SL and W) of *E. illustris* are significantly correlated and so for this species it is possible to estimate 1 parameter from the other. Seasonal or annual differences in L-W relationships can be generally related to reproduction (gonad development and spawning period) or feeding activities (food availability and feeding rate) [4, 30, 31] but attributed also to differences in sampling, especially length ranges. In this study, the fish were sampled monthly during one year to limit the seasonality effect. According to Pauly and Gayanilo [26] and Froese [11], 'b' values may range from 2.5 to 3.5 for fish, which is the case for the values estimated in this study. Sexual dimorphism did not influence significantly the L-L and the L-W relationships of *E. illustris.* Consequently, it is possible to a convert one variable to another regardless of sex.

One of the main problems facing age and growth estimates is the selection of the most suitable structure and technique to age the fish. Although comparisons between scales and otoliths have been relatively common [1], more comprehensive studies concerning teleost fish that involve multiple structures are rarer [15, 17, 23]. In this study, the observed results identified scales and otoliths as unsuitable calcified structure for age determination of *E. illustris* regardless of the preparation technique. Opercular bones were reported to be most suitable structure for ageing the important freshwater fish as Common carp (*Cyprinus carpio* [23]) common pike (*Esox lucius* [12]), African carp (*Labeo senegalensis* [5]), catla (*Catla catla* [17]), European perch (*Perca fluviatilis* [29]) and silver carp (*Hypophthalmichthys molitrix* [18]). However, Elzey *et al.* [8] showed that the opercular underestimated the age of the American shad (*Alosa sapidissima*) in relation to the otoliths and scales. In the same way, the opercular of thinlip grey mullet (*Liza ramada)* were reported to be an unsuitable structure for ageing [13]. Conversely, the ageing of other marine species such as monkeyface prickleback (*Cebidichthys violaceus* [21]) and totog (*Tautoga onitis* [3]), could be realized by opercular bones as well as otoliths.

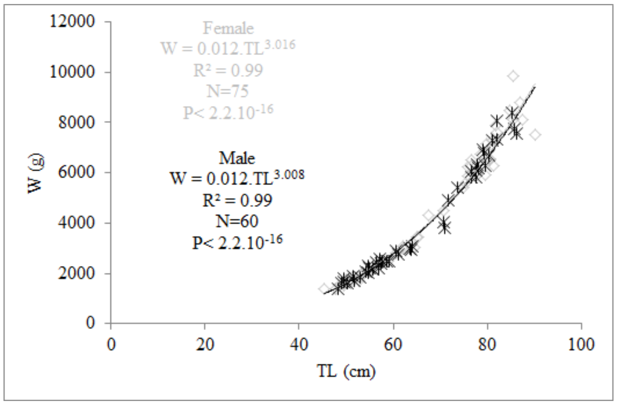

Fig 1: The Length-weight relationships of *E. illustris* from Reunion Island: females (grey dashed line) and males (solid dark line) fitted to the data (n=94).

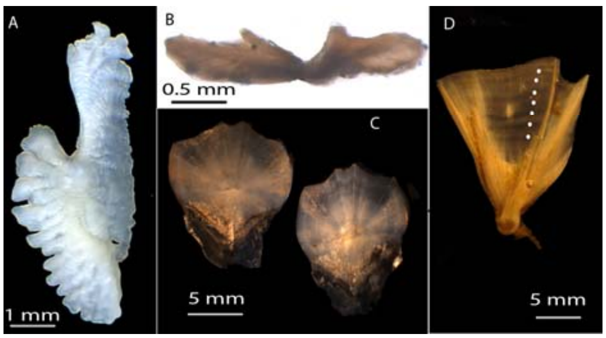

Fig 2: Different calcified structures (a: whole otolith, b: transverse section of otolith, c: scales and d: subopercular bone with 7 annuli for ageing one *E. illustris* individual (Female).

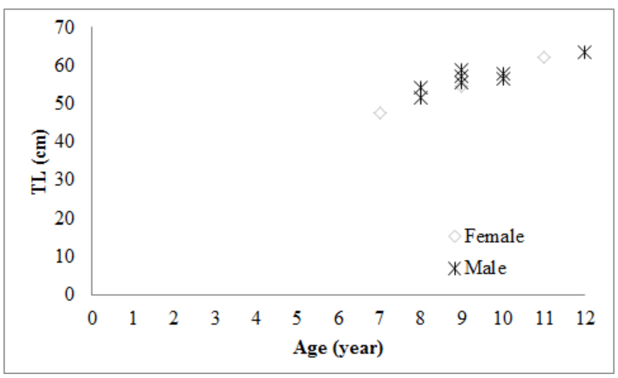

Fig 3: Growth data of *E. illustris* from Reunion Island.

4. Conclusions

In this study, the oblong shape of the scales and the otoliths could explain the difficulty in using these calcified structures to age the fish. Consequently, the opercular bone is a good alternative calcified structure when the otoliths and scales are unsuitable especially for slowly growing fish [2] as shown in the first growth data of *E. illustris.*

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