Introduction

The blackbellied angler (Lophius budegassa Spinola, 1807) inhabits bottoms between 100 and 500 m depth from the British Isles to Senegal and in the Mediterranean [1]. L. budegassa in Mediterranean waters is captured in mixed trawl fisheries, with annual landings of ~5700 t (in 2009-2011).

The first growth pattern of L. budegassa using calcified structures was estimated using otoliths [2], but the growth pattern shows fewer difficulties (opacity of otoliths with age and confusing multichucks) to be distinguished in the first dorsal fin ray (illicium) [3,4]. After several age estimation workshops and calibration exchanges [5-8], the original age estimation criterion based on illicia of Dupouy et al. [9] was standardized and included in an age estimation guide for anglerfish [10]. This illicia methodology was used in most of the age estimation studies of L. budegassa, and for stock assessment purposes [11-15]. However, the absence of evidence that allows validating or corroborating the age estimation in some species has led to a misunderstanding of the real state of their stocks. Thus, the Atlantic L. budegassa stocks have not been annually assessed using age-structured models during the last years in ICES working groups owing to problems with the data quality, including those related with the illicia age estimation criterion [16]. The same criterion has been used for the Mediterranean L. budegassa. Moreover, recent studies using length-frequency analysis [14,15] have shown a faster growth pattern than that based on age estimation of illicia or otoliths in the same areas.

Corroboration of Fast Growth Rate of Blackbellied Angler (Lophius budegassa) in Spanish Mediterranean

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Abstract

The growth pattern of Lophius budegassa, an important exploited demersal fish, is analyzed in Spanish Mediterranean waters using length-frequencies: Length Frequency Distribution Analysis package (LFDA) and Modal class Progression Analysis (MPA). Age and growth are key biological aspects for the assessment of this population. The growth parameters presented may be used in the age-structured models currently used for its annual stock assessment. Individuals ranging from 3 to 128 cm were collected by means of 12 annual bottom research surveys (2004-2015). The growth curve estimated by the length-frequency methodologies was similar (Φ = 0.084 years (SLCA); L ∞ = 105.41 cm, k = 0.144, t 0 = -0.084 years (SLCA); L ∞ = 96.99 cm, k = 0.178, t 0 = -0.084 years (SLCA); L ∞ = 105.41 cm, k = 0.144, t 0 = -0.212 years (MPA). The results reveal that the growth pattern of this population is faster than those estimated by applying the traditional illicia (first dorsal fin ray) or otolith age reading. The new faster growth estimated in this study provides reasonable tracking of cohorts in the abundance-at-age matrix obtained. These cohort tracking results, beside the consistency of the growth pattern observed in the length-frequency studies, corroborate the faster growth presented. In addition, the age was estimated from a sample of illicia, and the modifications in the traditional biased age estimation criterion are shown, suggesting a new and more accurate criterion at early ages. The findings presented in this study on the growth of L. budegassa and their corroboration can help to reduce the uncertainty in the estimation of its demographic structure.

Keywords: Blackbellied angler; Lophius budegassa; Growth; Length-frequency; Mediterranean; Groundfish surveys

The growth patterns of L. budegassa previously estimated in Mediterranean have not been fully validated, although it is recommended that the growth pattern of a stock should be validated to assure its accuracy, before estimating routinely its age on CS. The age corroboration method of tracking of strong/weak year-classes used in this study compares the interval between yearly samples and the increase in the apparent modal age of a recruitment pulse as determined through annuli counts [17]. In addition, the recent results on the otolith microstructure analysis [18,19] obtained have allowed locating more precisely the real first annulus in the CS, such as otoliths and illicia. Moreover, the studies showed a faster growth pattern in the early months of this species in Mediterranean and Atlantic waters, respectively. In addition, both studies indicated that juveniles <20 cm of total length collected in early Autumn were born in the same year and, therefore, belong to age class 0. As the growth based on illicia age estimation criteria that was used in the assessment of Atlantic stocks was being underestimated, those results may be also basic to establishing a new and corroborated age estimation criterion using CS, in a similar way to that occurred in L. piscatorius [20-22].

The aforementioned findings from otolith microstructure analysis of L. budegassa, along with the available time series from annual surveys of this species in Western Mediterranean, could help us make progress in the knowledge of its growth pattern.

The objective of the present study is (i) to present the growth pattern of L. budegassa in Spanish Mediterranean waters based on length-frequency analysis of a 12-year time series of surveys, (ii) to corroborate this growth pattern by cohorts tracking, and (iii) to apply the findings obtained in previous studies and in this study to establish a new age estimation criterion in illicia.
Materials and Methods

Sampling

A total of 6,466 specimens of *L. budegassa* were collected in 12 annual bottom trawl research surveys (MEDITS) conducted on board the R/V “Cornide de Saavedra” in Western Mediterranean (Spanish) waters (FAO Subarea 37.1.1) from the Strait of Gibraltar to Cape Creus (GSA01, GSA02, and GSA06), and Balearic Islands (GSA05). The mean number of hauls per year in GSA01 and GSA02, GSA05 and GSA06 was 45, 52, and 85 hauls respectively; according to that established in the MEDITS project [23]. These surveys were performed between April and June during 12 years, from 2004 to 2015, covering a depth range of 30-800 m (Figure 1).

At each haul, all *L. budegassa* caught were measured (total length) in centimeter, and illicium was removed for age estimation. Their sizes ranged from 3 to 106 cm in total fish length, representing most of the length range of *L. budegassa* caught by the fleet (Table 1).

![Figure 1: Sampling stations from 2004 to 2015 in Spanish Mediterranean waters.](Image)

Length-frequency analysis

Several methods of length-frequency analysis were used to infer growth parameters from the apparent shift of the modes in the time series of unsexed length-frequency samples:

(i) The software package Length Frequency Distribution Analysis (LFDA) [24] was used by applying three methods [Shepherd’s Length Composition Analysis (SLCA), Electronic Length Frequency Analysis (ELEFAN), and the Projection Matrix Method (PROJMAT)] to the length-frequency distributions of each survey to obtain the most objective von Bertalanffy growth parameters. The SLCA method compares each observed length-frequency distribution with a length-frequency distribution that would be expected for provided values of the growth parameters *L*∞ and *k*. A goodness-of-fit score is then calculated using a certain test function, and the best estimates of *L*∞ and *k* are those that correspond with a maximum value of the score function. The ELEFAN method [25] works by restructuring the length-frequency data in a way that emphasizes the peaks and troughs in the data and then calculating a function of the proportion of available peaks and troughs that can be explained by a von Bertalanffy growth curve with specific parameters. PROJMAT projects one observed length-frequency distribution forward in time, based on an assumed set of growth parameters, to obtain a prediction of what that length-frequency distribution should have looked like at the time the second observed length-frequency was collected. The best estimates of the growth parameters are those that lead to the best fit between the observed and predicted distributions.

(ii) Modal class Progression Analysis (MPA) using Bhattacharya’s method [26], and included in the FISAT II program [27], allowed to split the observed length-frequency distribution in distributions of distinct cohorts and thus to estimate their mean lengths at (relative) age. The length-frequency distribution of each survey was analyzed. A grouped length-frequency distribution resulting from grouping the distributions of all surveys from the time series was analyzed as well.

Abundance indices at age and tracking cohorts

A matrix of abundance indices by age group and year was first estimated by each methodology (LFDA and MPA) to check cohorts tracking along the years. A matrix was calculated using the slicing method [28] that was applied to the stratified length compositions of the surveys using the growth parameters calculated by LFDA. In the case of MPA, the matrix of abundance indices by age and year was directly obtained from the results of FISAT II. For checking the ability of cohort tracking along years and ages, the standardized abundance proportions at age of both matrices were estimated. Moreover, the correlation coefficients estimated over years between two consecutive ages from the same cohorts ([Uα,y vs. Uα+1,y+1]), where U is the abundance index, α is the age, and y is the year) [29] were then analyzed.

Growth parameters

The von Bertalanffy growth function (VBGF) [30] was estimated according to

\[ L_t = L_\infty \left(1-e^{-k(t-t_0)}\right) \]

where \(L_t\) is the mean fish length at age \(t\), \(L_\infty\) is the mean asymptotic fish length, \(k\) is the instantaneous growth coefficient, \(t\) is the age, and \(t_0\) is the age at which the mean fish length is 0.

The growth parameters were provided directly by LFDA. Moreover, in the MPA method, the parameters were calculated from the mean lengths at the age obtained.
Table 2: *L. budegassa* growth parameters from VBGF and mean lengths at age obtained in different studies based on length-frequency analysis and age estimation in Mediterranean waters. A reference study based on illicia in Atlantic is also included. Some mean lengths (~20, ~50 and ~70 cm) estimated in each study are joined by lines, allowing a simple visual comparison of the growth pattern among studies.
A fractional age was estimated as the difference between the birthdate and the capture date, adding this fraction to the estimated age (i.e., during the season of the surveys, mainly May-June, the specimens should be approximately +0.38 years older at each age group). The model was fitted to those data (mean lengths and age) using the Levenberg-Marquardt algorithm for least-squares minimization.

The growth performance index (Φ') [31] was calculated to compare the growth parameter values with previous references of *L. budegassa*, and thus to check if our growth was also consistent with those reported.

**Age estimation in illicia**

The growth pattern estimated in this study from length-frequencies was checked to determine whether it could be similar to that based on the age estimation in illicia. A subsample of 43 illicia from specimens caught in the surveys were selected, and their ages were estimated. We selected the specimens whose lengths were next to the first modal lengths obtained in the length-frequency analysis (~9, ~20, and ~30 cm), to check whether they match with each of the different first age groups when they are aged.

The preparation and age estimation of the illicia were performed following the standardized age estimation methodology of Duarte et al. [9]. The recent recommendations of not counting the first increment observed in the illicia, based on the micro-increments results [18, 19], were considered in the age estimation. The widths of the growth increments observed on the illicia were also measured to check their consistency among specimens.

**Results**

*L. budegassa* was relatively abundant in the catch in the year 2004 and particularly in 2015, with the lowest values in 2007-2009, and was maintaining values between 300 and 500 specimens by survey in most of the time series (Table 1).

**Length-frequency analysis**

The growth patterns obtained by the length-frequency methods (i and ii) were similar (Table 2):

(i) LFDA package. The best estimates of the growth parameters calculated by LFDA using the three options (SLCA, ELEFAN, and PROJMAT) were selected (Table 2) using a maximization algorithm to find the best fits to the length distribution. SLCA and ELEFAN showed consistent results, while PROJMAT offered unrealistic parameters ($k = 0.05, L_\infty = 101$). Therefore, the mean lengths at age for SLCA and ELEFAN were estimated (Table 2) from the respective growth parameters previously obtained.
An interannual variability in the modal lengths at first age classes can be observed in Figure 2. Consequently, the first modal length in the years 2009 and 2013 (~6-7 cm) and the second mode in 2010, 2011, and 2014 (~20 cm) are smaller modal lengths than those observed in most years of the time series (~9 and ~22 cm).

Tracking cohorts

The abundance indices by age group and year obtained from the SLCA, ELEFAN, and MPA were used to build the abundance-at-age matrix. The results obtained by SLCA and ELEFAN were almost identical, and therefore the following analyses were performed using only one of them (SLCA). In addition, the MPA results were similar to these. However, they showed a better tracking of most of the cohorts (Figure 3). Abundant year classes, as those of 2003, 2004, 2010 (MPA), and 2013 were able to be tracked in many of the available years of the time series, and the scarce year classes of 2007, 2008, 2009, and 2011 (MPA) were also detected (Figure 3). However, some mismatch in the cohort tracking over time was also found, and the abundances at age in some years presented some inconsistencies compared with the rest of the abundance index of the cohort, as age 0 in the scarcely abundant 2008 and 2009 year classes (Figure 3).

The results of the analyses performed with SLCA and MPA agree well, showing that the bulk (~87-91%) of the catches in number from most of the surveys was composed by specimens between 0 and 2 years old (Table 3); age 3 contributes ~6%, and ages 4 and 5 represent ~2% each. The abundance indices of the older ages are typically integrated by a few individuals. Consequently, small alterations owing to the capture of a few more individuals, or some less, in those ages in some survey, can make tracking the cohorts difficult in older ages.

The results of the correlations showed values greater than 0.5 between the pairs of the correlative age groups compared, being only significant to those greater than 0.6 (ages 0-1 and 2-3 from SLCA analysis and 2-3 and 4-5 from MPA) (Table 3).

The overall results of cohort tracking of the abundance indices at first age classes corroborate that the growth pattern obtained in this study from length-frequency analysis could be considered valid up to age 2 (Figure 4), showing a high grade of consistency.

Table 3: Results of the correlations performed between the abundances of consecutive age groups of L. budegassa belonging to the same year class. Abundances estimated from the length-frequency analysis using (a) MPA and (b) SLCA. P-values and levels of significance: (***) p≤0.01; (*) p≤0.05.
Age estimation in illicia

Two first (closer to the illicium core) well-marked growth increments were observed in the most of the illicia sections from the groups of specimens of ~9, ~20, and ~30 cm in fish length. These increments showed mean widths of 66 (±10) and 110 μm (±13), respectively (Figure 6). The next (outward) well-marked growth increment in the illicia showed a mean width of 179 μm (±18) and was observed only in the group of illicia from L. budegassa of ~20 and ~30 cm in length. Another well-marked growth increment was observed further outward from the illicia, showing a mean width of 281 μm (±17), and it was present only in the group of specimens of ~30 cm in fish length (Figure 6). The similarity of the measurements obtained at each growth increment, showing low SD values, demonstrates the consistency of them.

Discussion

The growth patterns of L. budegassa estimated in this study using several length-frequency analyses are similar, and they are also similar to those estimated previously in the same area using that methodology, providing consistency to the growth obtained by length-frequencies.

The size range analyzed represents most of the sizes of this population caught by the fleet. The length-frequency methods are primarily suited for young, fast-growing fish where the length modes for each age are easily distinguished [17] and, thus their use in this study has allowed us to move forward in the knowledge of the growth pattern of the early years of life of L. budegassa. This pattern estimated from length-frequency analysis has been corroborated at first ages by the cohort tracking performed in the matrix of abundance indices.

The growth patterns based on length-frequencies estimated in Mediterranean studies [14,15] are clearly faster than those based on the traditional age estimation criterion using illicia or using otoliths. Our corroborated faster growth pattern based on length-frequency analysis contributes significantly for establishing a new and more accurate age estimation criterion in the early years of L. budegassa by using illicia.

Figure 4: Catch curve by cohort of L. budegassa. Abundances (individuals × 30 min haul⁻¹) estimated from the length-frequency analysis using (a) MPA and (b) SLCA. For a better viewing, only the cohorts from 2001 to 2013 are shown.

Figure 5: VBGF of L. budegassa estimated in the present and previous studies in the Mediterranean Sea. A reference study based on illicia in Atlantic (Dupouy et al., 1986) is also included.

Catch curves by cohort of L. budegassa were estimated, and the abundance indices showed a drop from age 1 (Figure 4). Although the catch curves by cohort calculated by MPA and SLCA are similar (Figure 4), a higher dispersion in the abundance indices is observed by MPA.

Growth parameters

The unsexed VBGF parameters and mean lengths at age obtained by length-frequency analyses are shown in Table 2 and Figure 5. The growth rates and the mean lengths at age obtained in this study from LFDA (SLCA and ELEFAN) and MPA were very similar, showing values of the growth performance index (Φ’) = 3.2 (Table 2). The growth of L. budegassa estimated in previous studies in Mediterranean waters is also shown in Table 2 and Figure 5.
The availability of a time series of fishery-independent data of *L. budegassa* from trawl research surveys has been relevant in this study. The research surveys, despite the cost and scarcity of samples in some population fraction of this species, are standardized and do not have the shortcomings of fishery information [32].

### Lengths at age

Validation of age and growth pattern should be an obligatory step in all sclerochronological studies [33]. Length-frequency analyses are considered an indirect validation method that allows one to identify various age groups present in the catch, although it does not validate the periodicity of the hard part incremental growth pattern [33]. The length-frequency analyses do not allow one to know accurately the age that corresponds to each modal class estimated. However, other types of studies, as the micro-increment studies in CS, help to establish the age of the first modal length. The growth rate during the first half year of *L. budegassa* in Mediterranean waters was estimated at ~0.9 mm/day by otolith micro increments [18]. Thus, the modal class of ~9 cm in May estimated in this study may correspond to the individuals born 81-91 days before their capture, resulting in a possible hatching distribution around February. This agrees well with the estimated spawning season in the Mediterranean, focused in winter (November-March), with a peak in January [34,35].

Therefore, the modal class of ~9 cm in May estimated in this study should correspond to the age group 0. Considering the significant correlation between the abundance indices of successive early age classes demonstrated in this study, the estimated modal lengths of the following age groups, age 1 (~22 cm) and age 2 (~32 cm), can be considered as corroborated. This new growth rate in the early ages is faster than those previously published using CS.

The interannual variability in the modal lengths at age class 0 (from 6 to 11 cm) observed in the time series of length-frequencies can mainly be explained by the interannual variation in the spawning season and hatching time. Interannual variability in the modal length at some age groups, such as the age 0, has also been observed in the congener *L. piscatorius* [36]. The age classes 0 with clearly smaller modal lengths than the mean value in our time series (those of years 2009 and 2013) can also be tracked, as smaller lengths than the mean, in the corresponding age classes 1 in the following years (2010 and 2014). This tracking of unusually small individuals of the age group 0 is another piece that also supports the consistency of the overall cohort tracking analyzed in this study.

### Tracking cohorts

The results in the cohorts tracking shown in the matrix of abundance indices and the significant correlations at first ages support the growth pattern based on the length distribution analyses presented in this study.

Scarce abundance of specimens older than 3 years was observed in these surveys, and an overall decline of the abundance indices in the time series along most of age classes was also indicated (Figure 4). The increase from age 0 to age 1 may be related so that age 0 is not fully recruited into the survey. The decrease with the age in the abundance indices of surveys along most age groups and an increase in the first age have also been observed in other benthic fish as the megrim and four-spot megrim [37]. In those species, age 0 was not fully recruited into these types of demersal surveys, explaining the increase from age 0 to age 1.

The poor correlation between some age classes analyzed may be due, in some degree, to the different catchability of them. Part of individuals of early age groups of *L. budegassa* could occupy other areas or depths not so accessible for these surveys. Different geographical and bathymetric distribution among age classes has also been observed in *L. piscatorius* and may influence the correlation between the abundances and some successive ages [36].

The interannual variability owing to the different conditions occurred in each survey may have affected differently. The variability may have affected the capture of particular age classes. In addition, it may have contributed to certain irregularities in the cohort tracking and some low values of correlation observed.

Comparing the length-frequency methods used in this study, MPA is more subjective than that performed with SLCA; however, when using MPA, a specific growth pattern for each year can be estimated. Thus, the resulting abundance indices by age from MPA offer a more specific view...
of the demographic structure of each year and each cohort than that estimated by LFDA, which shows a global growth pattern for the entire time series, and their abundance indices for each year are estimated by slicing. The better overall tracking of most of the cohorts shown by MPA than by SLCA in Figure 3 can be related with that fact. The higher dispersion and less uniformity of values observed in the cohort pattern estimated by MPA compared to SLCA (Figure 4) also may be influenced by the aforementioned, showing a more specific pattern for each year than those obtained by SLCA.

Growth pattern and parameters

It was considered more suitable to perform a comparison among the studies performed on *L. budegassa* based on the mean lengths at age [13,38] and the growth performance indices (Φ'), than only a comparison of the growth parameters, owing to the parameters L∞ and k of VBGF being inversely correlated and dependent on the age range of the studied population. Thus, two different groups of growth patterns are reflected in those studies: a faster growth, with mean lengths of approximately 30, 48-58, and 63-74 cm at ages 2, 5, and 8, respectively, come from length-frequency analyses [13,14; present study]; and a slower growth, with mean lengths of 16-22, 29-34, and 40-49 cm for those aforementioned age classes was estimated in CS (illicia and otoliths) age estimation studies [2,14,15] (Table 2, Figure 5). Two groups of values of Φ' can also be distinguished: from length-frequency studies (3.1-3.2) and from studies based on age estimation using CS (~2.8) (Table 2).

Our length-frequency results, corroborated at early age groups by cohort tracking, are consistent with those estimated in previous studies using length-frequencies and provide more certainty in the parameters obtained, showing a faster growth.

Regarding the differences between both groups of growth patterns, the mean lengths at age 5 from CS aging studies were similar to those estimated at age 2 using length-frequency analyses (30-35 cm) (Table 2). Although oceanographic factors and feeding in each studied area may influence growth differences among areas, the different methodology (CS vs. length-frequency analysis) may have a higher influence on it than geographical or annual variation in growth. The faster growth at first ages estimated in the length-frequency studies may indicate that at least two growth increments that were being counted as annuli in CS could not be really annual.

Scarce information on direct validation of the growth of *L. budegassa* is available. Only data of two tagged and recaptured specimens (40 and 71 cm in length) in Atlantic waters are known, showing annual growth rates of 5 and 1 cm, respectively [8]. Those annual growth rates estimated in other waters are lower than that estimated from length-frequency in Mediterranean studies (~9 and ~5 cm, respectively, for the aforementioned lengths), and they are closer to the 5 and 3 cm from CS studies. The variability of the growth among individuals and the difference of areas studied should be considered in the comparison. Furthermore, an overview on the overall growth rate of this species cannot be achieved with information from only two recaptured fish, and more recaptures are essential to draw a conclusion about it.

Age estimation in illicia

Azevedo et al. [16] found inconsistencies in the cohort tracking of catch at age time series, when the traditional standardized aging criterion of illicia [10] was applied in the Iberian Atlantic stock. This criterion considers a diameter of ~60-100 μm for the first annulus, for a section obtained at 5 mm from the base of the illicia. However, the results of the micro-increment study [18] and the faster growth pattern estimated in this study from length-frequencies indicate that the first two well-marked growth increments observed in illicia (widths of ~55-76 and ~97-123 μm, respectively) should not be considered as annuli. The new first annulus that is shown in this study is likely of width ~161-197 μm, and the second one is of width ~264-298 μm. Further studies on the growth pattern in illicia are essential. However, these results about the growth of the first ages may begin to allow improvement in the age estimation criterion.

Considering the importance of the age and growth to assessment practices of this species, the present study can be considered as a new step in it. These results, together with others from alternative methods to the age estimation (tag-recapture, microstructure analyses of CS, more length-frequency analyses in other areas, etc.) will be useful to enhance that knowledge and to establish a wider basis for a more accurate and validated age estimation criterion using CS.

The use of validated information of age and growth allows obtaining more real results from an age-structured stock assessment, and may allow a better management of the resource. The use of not validated age-growth data has led to serious misunderstanding of population dynamics and management strategies in several species [39].

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