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Age, growth and validation of otolith morphometrics as predictors of age in the forkbeard, *Phycis phycis* (Gadidae)

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ABSTRACT

The traditional counting of annual growth zones on thick otoliths of gadiform fish requires special processing methods and is for that reason difficult, expensive and time-consuming. Therefore, this study analysed an alternative method such as the use of otolith morphometrics as predictors of age in the forkbeard, *Phycis phycis*. We examined sagittal otoliths of this fish species collected from the south-eastern Adriatic Sea (Elafiti Islands) between February 2008 and February 2009. Females and males ranged in size from 19.4 to 45.8 cm and from 14.0 to 42.0 cm of the total length (L_T), respectively. Ground otoliths displayed a concentric pattern of alternating opaque and translucent zones which were used to estimate fish ages. Edge-type analysis confirmed the formation of a single growth annulus per year.

The relationship between the total length and age was described by the von Bertalanffy growth model: $L_T = 59.08[1 - \exp(-0.24(t + 0.33))]$ for females and $L_T = 75.18[1 - \exp(-0.15(t + 0.70))]$ for males. Observed maximum age for both sexes was 5 years although most of the sampled fish were 2 years old. All measured otolith morphometric parameters (length, width and mass) were linear with fish age. The linear model explained between 61.3% and 69.0% of the variation in age. The most precise age estimations of analysed population were obtained from the otolith mass, followed by the otolith width and length. Moreover, the effectiveness of the simple otolith mass measuring technique for estimating the age of studied species was the same as of the laborious preparation and reading otoliths. It should be emphasized, however, that presented models were fitted to *P. phycis* ages 1–5 so extrapolation of data beyond this estimated range is not recommended.

1. Introduction

Fish age determination is a fundamental step in understanding of fish biology and dynamics of fish populations (Beamish and McFarlane, 1983) and is therefore essential for fisheries management. Many different techniques have been used in age determination (Lai et al., 1987; Kalish et al., 1996; Treble et al., 2008; Horn et al., 2010), but individual fish age has been estimated most frequently by a simple counting of annual rings on otoliths. Although this method provides highly precise age estimation, it has several disadvantages (Cardinale and Arrhenius, 2004). In particular, traditional age reading is subjective, laborious, time-consuming, expensive and dependent on the readers' skill and experience. Therefore, alternative methods that simplify the procedure of estimating fish age without reducing accuracy are necessary and welcomed. In that context, several studies have analysed and demonstrated a close relationship between the size of the otoliths and fish age (Worthington et al., 1995; Cardinale and Arrhenius, 2004; Pino et al., 2004; Doering-Arjes et al., 2008; Steward et al., 2009). Compared to traditional counting of annual rings on otoliths, methods that use otolith size in ageing fish are objective, fast and repeatable (Cardinale and Arrhenius, 2004) and require less skill, time and equipment (Fossen et al., 2003).

These alternative methods could be particularly important and useful for age estimation in gadiform fish because their very thick and dense otoliths require special processing methods (Deree, 1999). For example, combination of several techniques, like grinding, sawing, burning and/or slicing, is necessary before the growth increments become visible (Matarrese et al., 1998; Casas and Piñeiro, 2000; Abecasis et al., 2009). This increased complexity in otolith preparation for precise age estimation motivated the

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present study and analysis of the use of otolith measures as predictors of age in one gadiform species of the genus *Phycis*.

The selected study species was the forkbeard, *Phycis phycis* (Linnaeus, 1766), which is widely distributed in the Mediterranean Sea and is also common in the north-eastern Atlantic from the Golf of Biscay to Morocco, south to Cape Verde, and including the Macaronesian Archipelagos (Cohen et al., 1990; Abecasis et al., 2009). It is one of the most important commercial demersal species in the Azores (Abecasis et al., 2009), which also has an ecological importance in the Mediterranean fishing industry (Farjallah et al., 2006). However, in spite of its relatively broad geographic distribution and high economic value, the life history of the forkbeard is poorly known. This study investigated age and growth of *P. phycis* from the eastern Adriatic Sea, which is particularly important since we present the first data on this subject in the entire Mediterranean. *P. phycis* is very common and abundant species around the outer islands in the middle and south-eastern Adriatic. It inhabits rocky bottoms and trawling grounds up to 370 m depth, although usually between 20 and 70 m (Pallaoro and Jardas, 2002). Moreover, the investigated gadiform is more common species in the catches than *P. blennoides* along the eastern Adriatic coast, and obtained growth parameters in this study could be important input data for stock assessment techniques and management proposals. Age estimation has been already done for the forkbeard from the Azorean archipelago, but the age counting technique and the age interpretation were laborious and time-consuming (Abecasis et al., 2009). Therefore, we also assessed the usage of three otolith parameters in age prediction of the investigated species. In order to describe which otolith measure allows the most reliable and quick prediction of *P. phycis* age, we evaluated the utility of otolith length, width and mass by comparing observed and model estimated ages. Moreover, we described how different techniques influence the precision in age determination of this commercially important fish species.

2. Materials and methods

2.1. Sample collection

Specimens of the forkbeard (*P. phycis*) were collected monthly in the south-eastern Adriatic Sea (Elafiti Islands) between February 2008 and February 2009. Fish were caught using trammel nets with 80 and 300 mm stretched mesh size (inner and outer panel, respectively). For each fish the total length (L_T) was measured to the nearest 0.1 cm, weight was measured to the nearest 0.1 g and the sex was determined by macroscopic observation of the gonads. Sagittal otoliths were removed, cleaned and stored dry in labelled envelopes for later examination and age determination. Length-frequency distributions of females and males were compared using the Kolmogorov–Smirnov two-sample test.

2.2. Otolith morphometric measurements

Prior to grinding, whole otoliths were photographed using Olympus DP-25 digital camera attached to a stereo microscope with reflected light. Otolith length and width were measured to the nearest 0.01 mm using Olympus cellÂ Imaging Software and otolith mass was weighed to the nearest 0.0001 g. Otolith length was defined as the longest axis between anterior and posterior otolith edge and otolith width as a distance from dorsal to ventral edge taken perpendicular to the length throughout the otolith focus. Differences between left and right otoliths were tested by paired *t*-test while ANCOVA was used to test for differences in otolith measures between females and males, considering fish length as a covariate.

2.3. Age and growth determination

One otolith from each pair was selected at random and ground using a polisher (Struers LaboPol-5 speed range 100–500 rpm, SiC-paper grit size 1200) along the transverse axis and through the centre until the nucleus and the growth rings were exposed. Thus prepared otoliths were photographed under reflected light against a dark background at a magnification of 1.25×, using a stereo microscope coupled with Olympus DP-25 digital camera. Growth rings were visible as alternating opaque and translucent zones and ages were assigned to fish specimens based on their counts (one opaque zone combined with one translucent zone was interpreted as one year's growth). We used images for age determination because their size and quality made it easier to interpret zoning pattern than direct observations under the stereo microscope. The annual periodicity of deposition of growth rings was examined by classifying the marginal edge of otoliths as opaque or translucent. Percentages of otoliths with opaque and translucent margins were plotted by month of capture for the whole investigated period.

For each otolith two readers independently counted the annual growth rings twice, with an interval of about one month between counts. Otoliths with poorly defined zones were considered unreadable and were discarded. Both otolith readings of an individual reader were averaged to obtain the age estimated by that reader. Age estimates were compared between each reader's first and second read and then between two readers. The precision of estimations was tested by the index of average percentage error (IAPE) (Beamish and Fournier, 1981) and the coefficient of variation (CV) (Chang, 1982). All four readings were averaged to get the mean value which presented the age of the fish and was used for the morphometric age models creation (Steward et al., 2009).

Observed length at age was described by the von Bertalanffy growth model using a non-linear least square procedure of a Gauss–Newton algorithm for female and male forkbeard: $L_T = L_\infty [1 - \exp(-K(t - t_0))]$, where L_T is the length of fish at age t, L_∞ is the estimated asymptotic length, K is a constant that determines the rate at which L_T approaches L_∞ and t_0 is the hypothetical age at zero length. The multivariate Hotelling's T^2-test was used to compare growth parameters between sexes (Bernard, 1981).

2.4. Model development and testing

Once the age was determined by counting annual growth rings, relationships between observed fish age and otolith morphometrics (length, width and mass) were constructed using the linear model. Otoliths were randomly divided into 60% and 40% subsets and the linear function was fitted to the 60% subset to produce predictive equations which allowed the estimation of fish age in the remaining 40% subset. Estimated and observed age structures were compared by the Kolmogorov–Smirnov two-sample test and precision of the estimation was tested by the IAPE and CV indices. To aid visual interpretation, normal distribution curves were fitted to the observed and estimated age structures using the maximum likelihood method.

3. Results

Of the 754 individuals sampled, 385 (51.1%) were females, 254 (33.7%) males and 115 (15.2%) individuals of indeterminate sex. Female and male total lengths ranged from 19.4 to 45.8 cm and from 14.0 to 42.0 cm, respectively. A higher proportion of males were observed in the ≤22.0 cm length classes while females were more abundant in the ≥23.0 cm length classes (Fig. 1). The Kolmogorov–Smirnov two-sample test ($n_1 = 385$, $n_2 = 254$;

Table 1
Comparison of age estimates between the two readers and between observed and estimated age models.

Comparison	Exact match (%)	Differ by 1 year (%)	Differ by >1 year (%)	IAPE (%)	CV (%)	*N*
Reader 1 a/b	85.4	14.4	0.2	3.4	4.8	427
Reader 2 a/b	68.1	30.9	1.0	6.7	9.5	427
Reader 1 (average)/Reader 2 (average)	65.7	34.3	–	4.2	6.0	427
Observed age/Age estimated from otolith length	61.4	38.6	–	10.3	14.5	171
Observed age/Age estimated from otolith width	62.6	37.4	–	9.5	13.5	171
Observed age/Age estimated from otolith mass	74.9	25.1	–	5.9	8.3	171

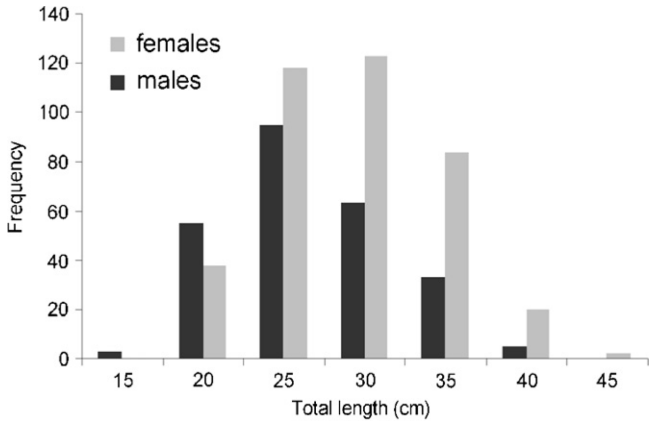

Fig. 1. Length-frequency distributions (5-cm length classes) of female and male forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands) between February 2008 and February 2009.

$P < 0.001$) showed significant differences between female and male length-frequency distributions.

3.1. Age and growth

Ground otoliths displayed a concentric pattern of alternating opaque and translucent zones around a large opaque nucleus (Fig. 2). The opaque zones were laid down mainly from March to August while the translucent zones were laid down mainly from September to February. Therefore, the formation of growth rings followed a seasonal pattern (Fig. 3). The proportion of otoliths with opaque and translucent margins was the highest (>70%) in May and November, respectively. The results indicated that one opaque and one translucent zone are laid down over a 12-month period and for that reason, represent valid annual growth rings.

Percentage agreement between the readings of the Reader 1 (85.4%) was much higher than the obtained percentage between the readings of the less experienced Reader 2 (68.1%) (Table 1). However, the exact match of the averaged readings between the two readers was relatively high (65.7%) with a quite low variability of IAPE and CV indices (4.2% and 6.0%, respectively). As a result of lack of experience, discrepancy in the mean age was more pronounced for the Reader 2 and it increased after an age of 2 years (Fig. 4).

Based on the annual growth ring counts of 427 (56.6%) readable otoliths, forkbeard ages ranged from 1 to 5 years. Most of the fish were 2 years old, accounting for 62.5% of the total sample. Fish younger than 2 years were poorly represented in the sample. Moreover, twenty-nine forkbeards were older than age 3, with age 5 represented with only five individuals. The von Bertalanffy growth curves and equations for observed lengths at age of females and males are shown in Fig. 5. Hotelling's T^2-test showed significant differences in the growth parameters between females and males ($T^2 = 106.930 > T_0^2 = 7.915$).

3.2. Model development and testing

No significant differences in morphometric measures (length, width and mass) were found between left and right otoliths (paired *t*-test, $P > 0.05$ for all measures) and between females and males (ANCOVA; $P > 0.05$ for all measures) and for that reason, data were pooled for both sexes and mean values for each otolith pair were used in further analysis.

Relationships between otolith morphometrics and observed age for the subsample of 256 forkbeards are presented in Fig. 6. The linear model explained between 61.3% and 69.0% of the variation in age. Based on the equations provided in Fig. 6, age of the remaining 171 fish specimens was estimated from the otolith morphometrics and the age structures were produced. No significant differences were found when the otolith mass was used for the age estimation (Kolmogorov–Smirnov two-sample test; $P > 0.05$), but this was not the case for the otolith length and width (Kolmogorov–Smirnov two-sample test; $P < 0.05$ for both measures). Comparison of the

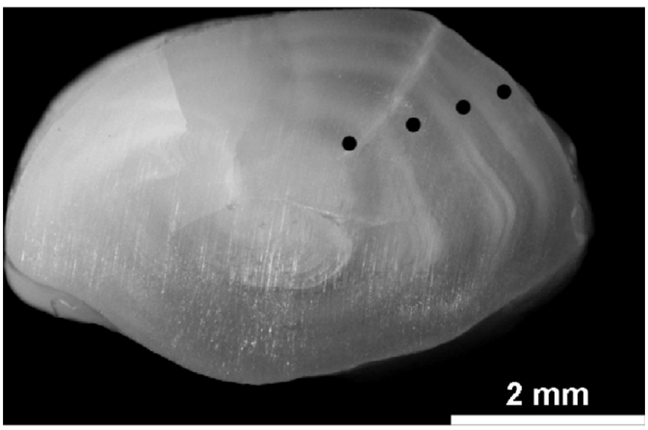

Fig. 2. Sagittal otolith of a 4-year-old forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands), with 34.5 cm L_T.

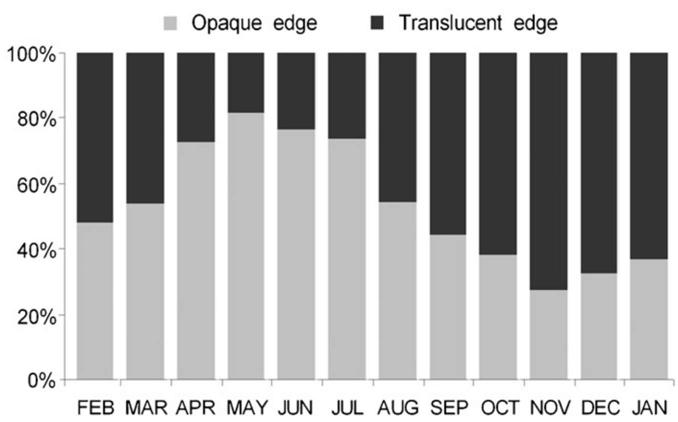

Fig. 3. Annual variation pattern of the percentage of opaque and translucent edges of otoliths of the forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands) between February 2008 and February 2009.

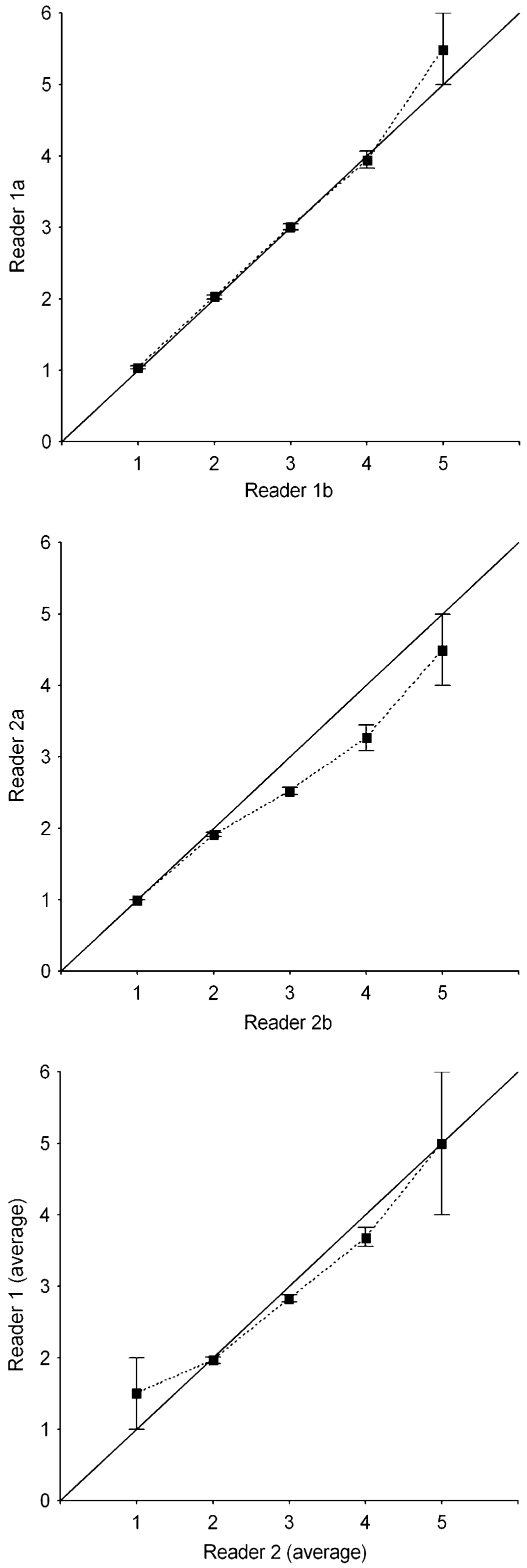

Fig. 4. For each age-group determined at one reading (x-axis), the figure shows mean age with ± 1 SE determined at another age reading of the same otoliths (y-axis). The solid line indicates identical ages.

observed and estimated age structures from the otolith mass is given in Fig. 7.

In general, the most precise age estimations were obtained from the otolith mass (IAPE = 5.9%; CV = 8.3%), followed by the otolith width (9.5%; 13.5%) and length (10.3%; 14.5%) (Table 1). Models predicted the same age as the readers for 61.4–74.9% of the fish. The best model (estimated from otolith mass) produced the higher percentage of agreement (74.9%) than that obtained by the averaged readings between two readers (65.7%) (Table 1). Moreover, if we omit models estimated from otolith length and width, differences of one year in age estimation were higher between the two readers (34.3%) than between the observed and the model estimated age from otolith mass (25.1%).

4. Discussion

The seasonal variation of otolith opaque and translucent edges shown in this study, demonstrated annual opaque zones formation in the otoliths of *P. phycis*. Therefore, we have found the interpreting annual growth rings in ground otoliths to be a valid method in ageing of this gadiform fish. The same results, including data on timing of opaque zone formation, have been obtained by Abecasis et al. (2009) who found a high percentage of *P. phycis* otoliths with opaque edges during spring and summer months (Azorean archipelago).

The age estimation in forkbeard was somewhat difficult due to the otolith thickness, making it necessary to carry out special processing method, such as grinding. Abecasis et al. (2009) suggested that combination of sectioning and burning of otoliths is the best method for this species ageing. Mentioned authors supported this conclusion by presenting low values of IAPE (2.6%) and CV (2.6%) indices. The both indices in the present study took a slightly higher value than the mentioned results of Azorean forkbeard and this may be caused by the counting skills of readers (Gunn et al., 2008). However, IAPE (4.2%) and CV (6.0%) for averaged readings between our two readers are still lower than the average values obtained in many ageing studies (Campana, 2001). In addition, we used digitized pictures instead of stereo microscope readings and according to Cailliet et al. (1996), this could also result in a higher precision of age estimations. Repeated observations under the stereo microscope can be affected by different light conditions and placements of the otoliths, none of which affect images (Fossen et al., 2003). Therefore, the otolith processing method in the present study is considered to be both reliable and precise while the ageing is regarded as consistent.

Despite obtained high agreement percentage between the averaged readings of Reader 1 and Reader 2, the effect of readers' experience, as important drawback of traditional otolith reading (Pilling et al., 2003; Pino et al., 2004; Steward et al., 2009), was also noted in this study. None of the readers had previously aged gadiform species, but Reader 1 was more experienced in fish ageing using otoliths. Therefore, this reader was more confident and consistent in *P. phycis* ageing, what was also reflected in a higher agreement between readings of Reader 1 than of less experienced Reader 2. Most of the differences between readers were caused usually by the location of the first annulus and identification of false annuli. In addition, the differences between readers usually increase in older fish (Peltonen et al., 2002; Steward et al., 2009); however, we did not observe such a clear trend, probably due to the low number of estimated age classes.

The oldest forkbeard we aged (5 years) was much younger than the maximum reported age of 18 years for this relatively slow growing and long lived species in Azorean archipelago (Abecasis et al., 2009). This may have been due to the limited sampling of both small and large individuals in the present study. In particular, Adriatic sampled population size structure is probably a reflection of the sampling fishing techniques using trammel nets. According to Pallaoro and Jardas (2002), smaller specimens of the forkbeard are usually found in shallow waters while the larger individuals prefer sandy and muddy bottoms in deeper areas and therefore, are

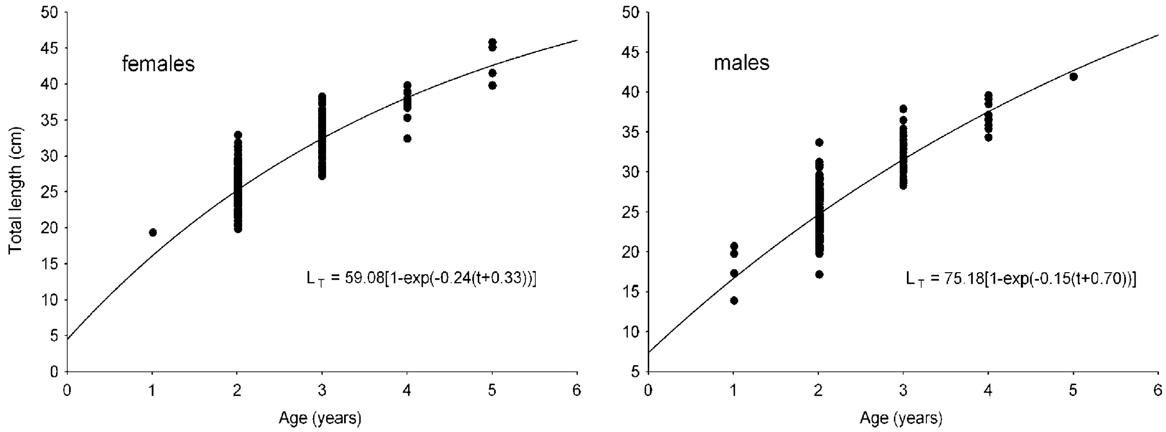

Fig. 5. Observed length at age data fitted with von Bertalanffy growth curves for females and males of the forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands).

not common at the fishing depths covered in this study. Despite noted, the investigated gadiform is in general highly represented in the trammel net catches (Goñi et al., 2008) due to the fact that *P. phycis* is the night active predator (Morato et al., 1999) and thus vulnerable to capture in mentioned nets. On the other hand, Abecasis et al. (2009) collected *P. phycis* in the Azores from bottom longline fisheries which operate in the greater depths but also reported that individuals larger than 60 cm L_T were scarce in the sample. This lack of larger individuals in both studies could be the result of their natural scarceness in the populations but also a direct consequence of a high fishing pressure.

The forkbeard showed sexual dimorphism in growth and while this is the common feature among related gadiform species such as *P. blennoides* (Casas and Piñeiro, 2000), this was not the case for the Azorean specimens (Abecasis et al., 2009). In addition, when comparing the parameters obtained by this study with those obtained by Abecasis et al. (2009), we become aware of large differences in L_∞, K and t_0. While we reported that values of L_∞ for females and males are around 60 and 75 cm in L_T, respectively, these values increase to more than 80 cm in L_T for both sexes in the Azores. Observed differences can be due to the different population size structures or even different fishing pressures in two areas. Moreover, we acknowledge that estimated values of L_∞ for females and males in this study are inconsistent with maximum observed total lengths for both sexes. It is possible that our sample size of males was relatively small for modelling growth and that more data, particularly from older fish, would improve our estimates. On the other hand, we also have to note that estimated asymptotic length for *P. phycis* males is close to the historical record of 74 cm L_T for this species (Pinho, 2003). As a consequence of all mentioned, growth rates (K) estimated for the Adriatic population were higher while the values of t_0 were much lower than the estimates presented by Abecasis et al. (2009). The additional explanation is that lack of smaller individuals may influence the values estimated for t_0 and exactly this was the case for the Azorean population in which no one-year-old individuals were determined.

Worthington et al. (1995) concluded that less precise, but economic methods for estimating the age of individual fish can provide better estimates of age structures than precise, but expensive methods. In accordance with this, results of numerous studies have shown that otolith size can be efficiently used instead of otolith ring counts in fish ageing, but also emphasized that some otolith dimensions may be more useful than the others (Steward et al., 2009 and references therein). Therefore, having in mind that age estimation in gadiform fish can present some difficulties due to the otolith thickness; we assessed the utility of few otolith morphometrics as a quick and reliable predictor of *P. phycis* age.

The average otolith length, width and mass increased with the age of forkbeard, implying that these dimensions may provide a satisfactory method of ageing. All measured otolith morphometrics increased linearly with age throughout the life of the investigated fish and we have found that the best age predictor was the otolith mass. Many authors have emphasized the importance of otolith mass as age predictor and also pointed out that this otolith measure could constitute a valid and simple method to estimate age structures of fish populations (Cardinale and Arrhenius, 2004 and references therein). According to Secor and Dean (1989), one of the advantages of using otolith mass in fish age predictions is that average value of this measure usually continues to increase in older fish, in contrast to fish length, weight or otolith length. However, in order to evaluate the utility of any otolith morphometric for age estimations, both Pilling et al. (2003) and Steward et al. (2009) advised that it is necessary to compare estimated and observed fish population structures. We have followed this advice in our study and no significant difference was found only between the age structure estimated from the otolith mass and those by counting otolith annuli. Therefore, this result confirmed that method using otolith mass is a feasible approach to estimate *P. phycis* age structure. Weighting of otoliths requires only precise laboratory scale and the quite rapid procedure of measuring otolith mass could be the best choice if we have in mind its effectiveness and the same level of accuracy as the time-consuming grinding and annuli counting. Moreover, this method has an additional advantage of relatively constant otolith mass growth rate with fish age, which could be useful if sample size range is limited.

Although morphometric models may contain some ageing errors, they could still better predict age of fish species than otolith annuli counting (Worthington et al., 1995) and this is based on the possibility of processing larger sample in a relatively short time. Further on, Cardinale et al. (2000) suggested that such morphometric models are probably more objective method for age estimation because of its constant error which reduces the bias caused by annuli interpretation of different readers characterised with a variable error. These relatively fast and inexpensive morphometric models reduce the cost, effort and subjectivity but could also improve management of fish stocks and preserve fisheries that might be lost because of limited funds for basic researches (Steward et al., 2009). In conclusion, all data presented in this paper can be applicable to further ageing investigations of the forkbeard. Moreover, the obtained growth parameters may significantly

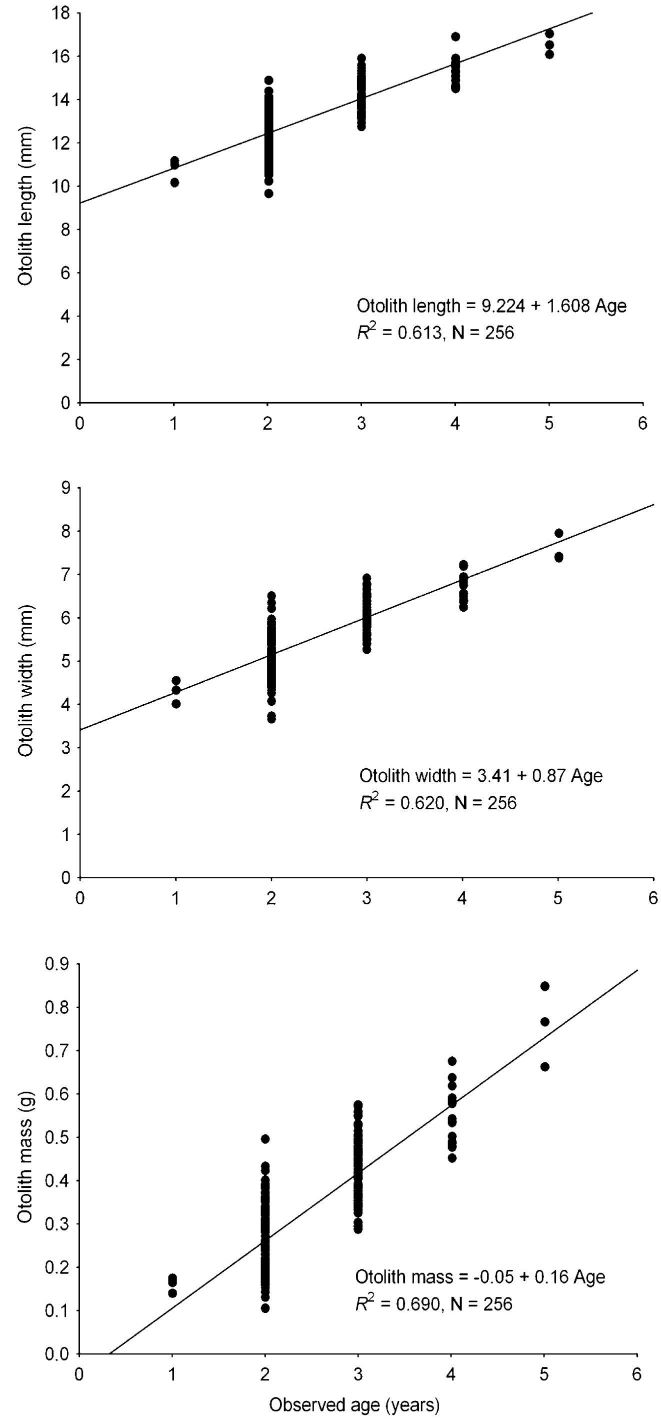

Fig. 6. Relationships between otolith morphometrics and observed age of the forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands). Equations, coefficient of determination (R^2) and the size of analysed subsample (N) are provided for the each relationship.

support future stock assessment and management proposals while calibrated morphometric model allows for quick, inexpensive and precise estimation of *P. phycis* age structure. However, the model obtained in this study is only useful for the ages modelled (1–5-years-old fish) and this should be kept in mind by users who may wish to apply this morphometric model to predict the age of the forkbeard.

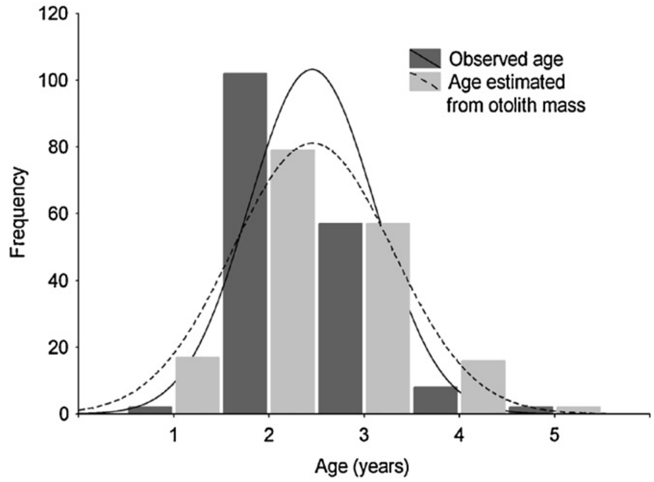

Fig. 7. Comparison of the observed and estimated age structures from otolith mass of the forkbeard, *Phycis phycis* sampled in the south-eastern Adriatic Sea (Elafiti Islands).

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