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The application of the otolith weight as an estimator of age in the anchovy *Engraulis encrasicolus*

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Abstract: This study deals with the potential application of otolith weight as an estimator of age in the anchovy, *Engraulis encrasicolus*, caught in the Novigrad Sea (eastern mid-Adriatic Sea) from March to October 2002. The linear relationship between otolith weight and the estimated age (determined from otolith annuli) of 312 anchovy individuals was established ($OW = 0.902\ T + 0.461$, $r^2 = 0.939$).

Résumé : *Utilisation du poids des otolithes comme estimateur de l'âge de l'anchois* Engraulis encrasicolus. Cette étude traite de l'application potentielle du poids de l'otolithe comme un estimateur de l'âge de l'anchois *Engraulis encrasicolus*, capturé dans la Mer de Novigrad (Mer Adriatique orientale) entre Mars et Octobre 2002. Une relation linéaire entre le poids de l'otolithe et l'âge (déterminé à partir des anneaux de croissance de l'otolithe), estimée sur 312 individus, a été établie ($OW = 0{,}902\ T + 0{,}461$, $r^2 = 0{,}939$).

Keywords: Otolith weight • Age • *Engraulis encrasicolus* • Adriatic Sea

Introduction

The starting point of many fish stock assessments is the estimation of the population age structure. A number of techniques have been used to determine the age structure of populations in the past. The simplest approach to ageing is by analysing the population length-frequency data (Sparre & Venema, 1998), but the most accurate method is based on the interpretation of annual rings in hard body structures such as otoliths, vertebrae, fin rays or scales, particularly for longer lifespan of species (e.g. Zorica et al., 2005; Kraljević et al., 2007). This method is time consuming and therefore expensive; readers should have a certain degree of skill and experience. Besides, in some fish species age determination is confounded due to the presence of false rings. So, an alternative, objective, economic and easier approach to fish ageing based on otolith weight is being developed (Boehlert, 1985; Pawson, 1990; Fletcher, 1991;

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Fletcher, 1995; Worthington et al., 1995a & b; Cardinale et al., 2000; Labropoulou & Papaconstantinou, 2000; Pilling et al., 2003; Pino et al., 2004, Lou et al., 2005; 2007). Otolith weight increases throughout the lifespan of an individual and can provide a quick, reliable and efficient way to estimate age, especially for young fish (Worthington et al., 1995a), unlike fish length or otolith length.

The anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), is a pelagic fish species that is abundant along the Mediterranean Sea and East Atlantic, reaching the western shores of Africa, the Skagerrak, Kattegat and British Isles. The anchovy is widely distributed in the Adriatic Sea. It occurs from the Gulf of Trieste to the Otranto Strait, but not deeper than 200 m. Its reproductive period is from April to October, with the main spawning peak occurring between June and August (Sinovčić, 1978 & 2000).

The objective of the present study was to look for alternative method of determining the age of anchovy, when the ages of a great number of individuals are required, e.g. the age composition of anchovy catches is one of pertinent information required for responsibly assessment and management of this highly important pelagic fish species in the whole Mediterranean. In this paper we present evidence for the relationship between age and otolith weight for *E.encrasicolus*, in accordance with the following objective to analyse the relationship between otolith weight and age of anchovy individuals.

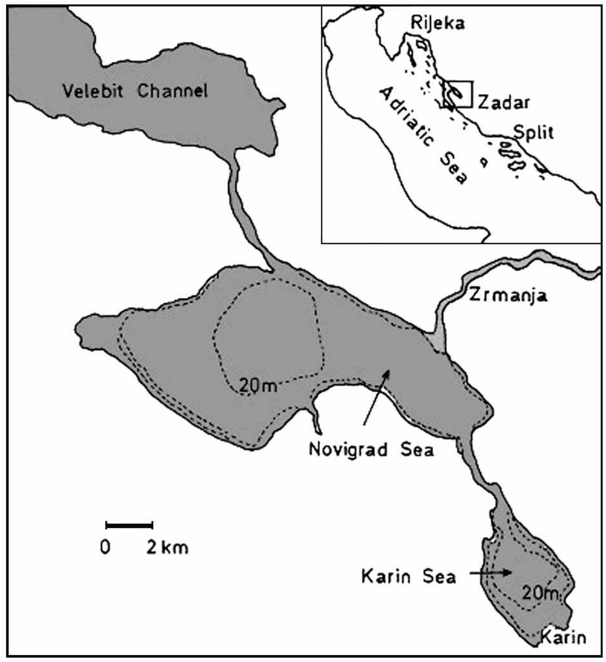

Figure 1. *Engraulis encrasicolus.* Map of the study area.

Figure 1. *Engraulis encrasicolus.* Carte de la région de l'étude.

Materials and methods

A total of 312 anchovy specimens were obtained monthly from commercial catch samples during the spawning season – from March to October 2002. They were caught by purse seine (mesh size of 8 mm) in the Novigrad Sea (44º15'N; 15º30'E; Fig. 1) under artificial light at night, but not during a full moon.

Total lengths (*TL*) of the specimens were measured to the nearest 0.5 cm in the laboratory. Five pairs of sagittal otoliths per 0.5 cm length group were taken from the analysed size range of each fish sample. A cross section was made in the middle between the posterior margin of the eye socket and the posterior of the operculum. Saggittal otoliths were removed by opening the otic bulla, washed in freshwater, dried and stored in plastic tubes prior to ageing. The weights of undamaged and cleaned otoliths were measured on a Mettler analytical balance (to the nearest 0.01 mg). Initially, both otoliths from 184 fish from whole sample were weighed to assess if weight of the left and right otoliths from the same fish varied.

Whole sagittal otoliths were fully immersed in an ethanol solution (96%) and observed under reflected light against a dark background. The smallest magnification (1.6x) of a binocular microscope with reflected light was used. Hyaline and opaque rings were counted in the dorso-ventral part of the concave side of the otoliths. The growth of anchovy otoliths started by the formation of the otolith nucleus, which could be light or dark, depending on whether the otolith belonged to a specimen produced by an earlier or a later spawning season parental stock. A wider opaque ring usually followed the nucleus, followed by a hyaline ring (0+). In the case of an additional marginal opaque ring besides the stated ones, we would assign the fish to the 1+ group, which means that the specimen was in the beginning of its second year of growth. An opaque nucleus with a following hyaline ring and a following opaque ring, and a marginal barely started hyaline ring was assigned to 1+. In older anchovy specimens the same rule was applied, namely, one hyaline (winter) and one opaque (summer) ring were interpreted as one year of anchovy growth.

Two readers read the otoliths on separate occasions, approximately two months apart. The readings for a given otolith were only accepted if both readers agreed upon them. In the case of a discrepancy between the two readings, a third reading was carried out. Otoliths, whose three readings differed because of false rings, were considered as unreadable and were rejected.

The relationship between otolith weight and total length of the fish was analysed using the power function:

$$OW = aTL^b \qquad (1)$$

where *OW* is the otolith weight (mg), *TL* total length of specimen (cm), *a* is a proportionality constant and *b* a regression coefficient.

The mean age for each 0.5 cm length class was determined. To obtain an otolith weight-age relationship, a linear regression was used:

$$OW = a + bT \qquad (2)$$

where *OW* is the otolith weight (mg), *T* the estimated age (years) and *a* and *b* are the y-intercept and the slope of the otolith weight-age curve, respectively.

In order to establish the validity of the otoliths weight used as an age estimator, the linear calibration between otolith weight and fish age was used. Relative age for each half-centimetre length class was calculated and was statistically compared (paired t-test, $P < 0.05$) with the real age obtained by counting the rings. Normal distribution of those values was established with Kolmogorov-Smirnov (K-S) test.

Growth parameters were estimated using the software package Statistica. The von Bertalanffy growth equation:

$$L_t = L_\infty [1 - e^{-K(t-t_0)}] \qquad (3)$$

was fitted as function of length and age data using non-linear least squares parameter estimation, where L_t = the length at age t, L_∞ = the asymptotic length, K = the growth constant, t_0 = the theoretical age at which $L_t = 0$. The growth performance index phi-prime (ϕ') was estimated to compare the growth parameters obtained in this paper with those reported by Sinovčić (1999 & 2000) who published age–length keys for anchovy from the Adriatic Sea based on growth increments determined from otoliths. This index was calculated by the equation (Munro & Pauly, 1983):

$$\phi' = \log K + 2\log L_\infty \qquad (4)$$

Results

Total length (*TL*) of 312 analysed specimens ranged from 6.0 to 14.0 cm, most frequently from 9.0 to 11.0 cm (mean 9.75 ± 1.539 cm).

Only 281 otoliths out of 312 were successfully aged. These belonged to the 0+ to 2+ years age classes; the dominant age class was 1+ (65.2%, Table 1).

The weights of otolith pairs from 184 fish were not significantly different (paired t-test comparison of two data set, $t = 0.789$, $df = 183$, $p > 0.05$); 99% of otolith pairs weighed within 0.002 g of each other. Hence, only right otolith from subsequent pairs was therefore weighted and used in further analyses.

The relationship between total length (*TL*) and otolith weight (*OW*) of the fish from age classes 0+, 1+ and 2+ was adequately described by the power function (Fig. 2):

$$OW = 0.008TL^{2.128} \quad (r^2 = 0.895) \qquad (5)$$

Table 1. *Engraulis encrasicolus.* Age-length key for individuals collected from the Novigrad Sea catch samples between March and October 2002.

Tableau 1. *Engraulis encrasicolus.* Clef de l'âge-longueur pour les individus récoltés en Mer de Novigrad entre mars et octobre 2002.

Size (cm)	Age (years)			
	0+	1+	2+	Total
6.0	2			2
6.5	1			1
7.0	8			8
7.5	21			21
8.0	17	4		21
8.5	11	8		19
9.0	11	21		32
9.5	10	22		32
10.0	6	26		32
10.5	1	32		33
11.0		31		31
11.5		25	1	26
12.0		9	4	13
12.5		3	2	5
13.0		2		2
13.5				
14.0			3	3
n	88	183	10	281
Mean total length	8.25	10.35	12.65	9.76

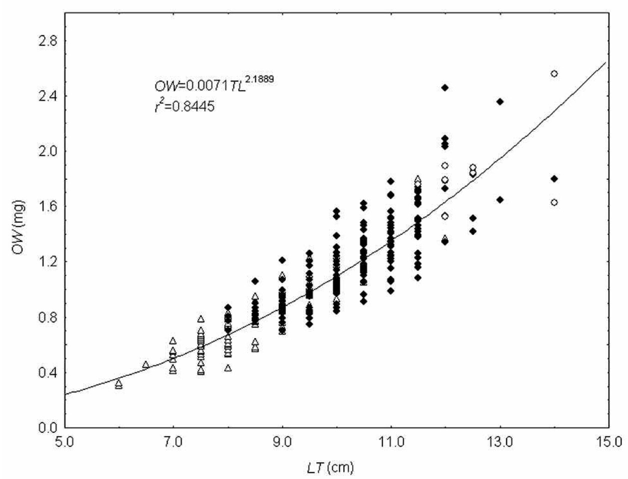

Figure 2. *Engraulis encrasicolus.* Total length (*TL*, cm) and otolith weight (*OW*, mg) relationship for age classes 0+ (Δ), 1+ (◆) and 2+ (o) estimated by annuli on the otoliths from the March - October 2002 samples realised in the Novigrad Sea

Figure 2. *Engraulis encrasicolus.* Relation entre la longueur totale (*TL*, centimètre) et le poids de l'otolithe (*OW*, mg) pour les classes d'âge 0+ (Δ), 1+ (◆) et 2+ (o) estimées par les anneaux de croissance des otolithes des individus récoltés entre mars et octobre 2002 en Mer de Novigrad.

Otolith weight increased significantly with the length of specimens.

The age-otolith weight key was given in Table 2. It is evident that there were some otolith weights overlapping within the different age classes.

The mean otolith weight and mean age for each 0.5 cm length class was presented in Figure 3. A significant linear relationship was found between otolith weight and estimated age:

$$OW = 0.902T + 0.461 \ (r^2 = 0.939, \text{n} = 280) \quad (6)$$

Otolith weight was positively correlated with total length ($r^2 = 0.894$, $P < 0.001$, $n = 281$) and age ($r^2 = 0.650$, $P < 0.001$, $n = 281$) of each specimen; larger and older fish tend to have heavier otoliths.

The von Bertalanffy growth formula was calculated for estimated age, obtained by linear recalibration between otolith weight and age, and real age for each 0.5 cm length class. Validity of the real age was corroborated with likelihood ratio test ($\chi^2 = 0.429$, df = 15, $p > 0.05$), which did not show significant difference between the growth curves obtained in this study and study presented by Sinovčić (1999). The von Bertalanffy growth curves are as follow:

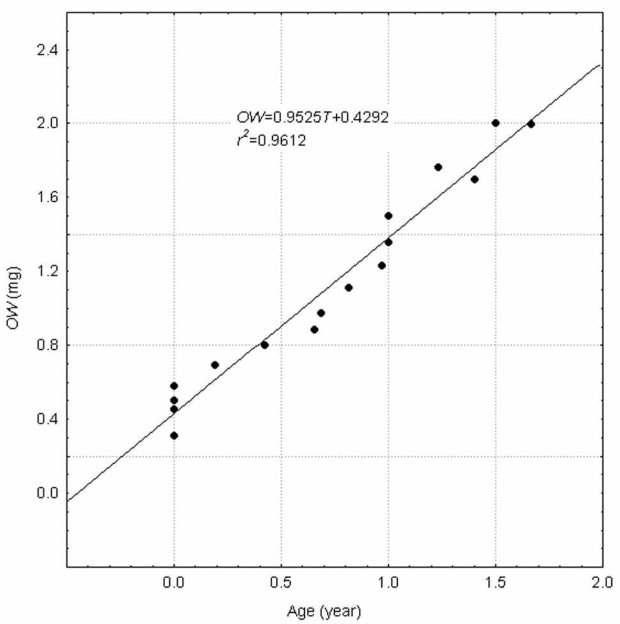

Figure 3. *Engraulis encrasicolus.* Relationship of mean otolith weight (*OW*, mg) and mean age (years) for each half centimeter length class of individuals collected from the Novigrad Sea between March and October 2002.

Figure 3. *Engraulis encrasicolus.* Relation entre le poids moyen de l'otolithe (*OW*, mg) et l'âge moyen (années) pour chaque classe de longueur (demi-centimètre) des individus récoltés en Mer de Novigrad entre mars et octobre 2002.

Table 2. *Engraulis encrasicolus.* Age-otolith weight key for individuals collected from the Novigrad Sea catch samples between March and October 2002.

Tableau 2. *Engraulis encrasicolus.* Clef de l'âge-poids de l'otolithe pour les individus récoltés en Mer de Novigrad entre mars et octobre 2002.

Otolith weight (mg)	Age (years)			
	0+	1+	2+	Total
0.30 – 0.59	17			17
0.60 – 0.89	44	22		66
0.90 – 1.19	24	63		87
1.20 – 1.49	3	59		62
1.50 – 1.79		30	3	33
1.80 – 2.09		5	6	11
2.10 – 2.39		2		2
2.40 – 2.69		2	1	3
n	88	183	10	281
Mean otolith weight	0.73	1.22	1.85	1.09

Estimated age:

$$TL = 18.73\,[1 - e^{-0.48(t+0.903)}] \quad (r^2 = 0.999) \quad (7)$$

Real age:

$$TL = 19\,[1 - e^{-0.45(t+0.979)}] \quad (r^2 = 0.979) \quad (8)$$

The results demonstrated that the growth model provided a good fit to the data, as their coefficients of determination were highly significant ($r^2 > 0.978$). The estimated growth performance index or phi-prime test (ϕ') for both growth curves was 2.2. Kolmogorov- Smirnov test ($d = 0.11$, $P > 0.05$) obtained on two data sets (estimated age and real age) confirmed normal distribution for both data sets, while paired t-test showed that there was no significant difference between those two sets of age data ($t = 0.789$, df = 15, $P > 0.05$).

Discussion and conclusion

The weight of otoliths as reliable and quick predictor of age is not new, having been suggested nearly 40 years ago by Templeman and Squires (1956). This study was initiated in order to provide alternative method of age determination in anchovy populations inhabiting the eastern part of the Adriatic Sea (Novigrad Sea) in the case when quicker age data are needed.

The hypothesis that the otolith weight of anchovies provides an estimation of their age is supported by this study. The relationship between fish total length (*TL*) and otolith weight (*OW*) was described by a power function indicating that the otolith weight of the anchovy increased with increasing total length. After a certain length, otolith weights increased with a reduced rate of increase in total length. Other studies (Boehlert, 1985; Gauldie, 1988;

Fletcher, 1991 & 1995; Worthington et al., 1995b; Pino et al., 2004, Lou et al., 2007) have indicated that the average weight of otoliths in older fish continued to increase, in contrast to variables such as fish length and weight. They tried to reach its asymptotic value during the fish growth. Hence, otolith growth can be closely related to time and age, respectively otolith weight – age relationship and could potentially provide reliable age estimates (Reznick et al., 1989).

The parameters of the von Bertalanffy growth function reported by our study for real age of *E. encrasicolus* were similar to those presented by Sinovčić (1999); the estimated growth performance indexes from the results of both studies are equal ($\phi' = 2.2$); the growth curves did not show significant difference between both studies.

The average weight of anchovy otoliths increased linearly with age, at least over the range of 0+ to 2+ ages. This implies that otolith weight may provide a method of ageing, as suggested in many previous studies (Boehlert, 1985; Reznick et al., 1989; Pawson, 1990; Worthington et al., 1995a & b; Cardinale et al., 2000; Labropoulou & Papaconstantinou, 2000; Pilling et al., 2003; Pino et al., 2004, Lou et al., 2005; 2007). The overlap in otolith weight between age classes was noticed and could be caused by a real overlap in otolith weight among different ages of fish. The estimated age of anchovy population, using the otolith weight as an age estimator, did not show statistically significant differences between the real age defined by otolith annuli reading of this species.

The application of otolith weight in ageing provided some advantages. Namely, the collection of otolith weight does not require any processing technique and can be easily performed, thus a much larger otolith sample could be analysed. Moreover, this alternative ageing method is not time consuming. The precision of this ageing approach depends on the accuracy of the real age determination before such considerations as only annuli otolith reading can provide reliable age of fish. Otolith weight can be used only in the case if initial calibration was done and coordinated with studied data. The accuracy of predicted age structure would be higher if a higher sample size were used. This method may be sufficiently accurate for generating age structure stock assessment methods in purpose of monitoring of population state and its management.

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