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Daily otolith increments and growth rate of juvenile anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), in the south-eastern Adriatic Sea

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Daily growth rate of juvenile anchovy, Engraulis encrasicolus *(Linnaeus, 1758), was estimated in the south-eastern Adriatic Sea. Samples were taken by a commercial beach seine net and the size range of 100 sampled individuals was 5.0-6.8 cm total length. Age was estimated by counting growth increments of sagittal otoliths, which are formed with daily periodicity. Estimated ages were 41- 90 days and hatch date for each age class was back-calculated from the date of capture. The calculated instantaneous growth rate was 0.41 mm day^{-1}. Fish size at metamorphosis from late larva to juvenile was estimated to 3.2 cm total length. Parameters of the von Bertalanffy's growth function were* L_∞ *= 10.41 and* K *= 3.36*

Key words: juvenile anchovy, growth, age, otolith, Adriatic Sea

INTRODUCTION

Anchovy, *Engraulis encrasicolus* (L.), is the only species of the family Engraulidae distributed along the coasts of Europe. Its range extends from the North Sea to South Africa in the eastern Atlantic, and to the Mediterranean as well as Black and Azov Seas (WHITEHEAD, 1990). It is one of the most important commercial pelagic fish species in the Adriatic Sea, characterised by a short life span, relatively high growth rate, early sexual maturation (approximately at age one), long spawning period, high fecundity, seasonal migrations, and schooling behaviour (LA MESA *et al*., 2009). Early growth stages of this species have been studied in the Adriatic Sea for more than 100 years (REGNER, 1996). The Boka Kotorska Bay represents its natural spawning and feeding site (MERKER & VUJOŠEVIĆ, 1972; MANDIĆ *et al*., 2011). Until 2008, there were no data on juvenile anchovy biology or fisheries in the Boka Kotorska Bay (ĐUROVIĆ *et al*., 2008), although, along with the sardine, it represents the main catch of beach seine fishing with artificial light. This type of fishing is traditional in the region for centuries. Considering that the fishing

fleet for small pelagic fish is almost nonexistent in this part of the Adriatic Sea, anchovy and sardine are mainly caught with 5-6 mm mesh beach seine nets, and only juveniles are harvested (PEŠIĆ *et al*., 2010). To allow the sustainable fishing in the Boka Kotorska Bay, it is important to determine the population dynamics and to issue conservation measures to protect the species from overfishing.

Daily increment formation in otoliths was discovered by PANNELLA (1971) and it is a universal characteristic of all fishes from polar to tropical waters (CAMPANA & NEILSON, 1985). Otolith formation and growth are directly influenced by endogenous rhythms, which are synchronized with the photoperiod (*Ibid*.). One growth zone is most frequently formed during the 24-hour period (TANAKA *et al*., 1981), which allows the determination of age, growth, and mortality by analysing the otolith microstructure, particularly in larvae and juveniles. Daily increment formation in sagittal otoliths has been validated both in juvenile and adult anchovy of various body lengths (CERMEÑO *et al*., 2003). The aim of this study was to: (1) analyse daily otolith increments in juvenile anchovy from the south-eastern Adriatic Sea for the first time, and (2) assess the growth rate of juvenile anchovy in this region.

MATERIAL AND METHODS

Study area

The Boka Kotorska Bay is situated in the southern part of the eastern Adriatic (Fig. 1). It is deeply cut into the mainland, and the innermost point is 15 NM far from the open sea (MAGAŠ, 2002). The circulation of both water mass and temperature is greatly influenced by seasonal changes – during the summer the inflow of fresh water from the surrounding mountains and from small tributaries is minor, causing low circulation of the water mass, while during the winter the conditions are reverse. Maximum recorded surface temperature is 28.1°C (August) and minimum 7°C (January) (REGNER *et al*., 2000). Along with large annual temperature oscillations, a significant issue is the formation of a thin surface ice layer during the coldest winter days. The salinity is much lower than at the open sea, due to a large quantity of fresh water and numerous underwater springs distributed all around the Boka Kotorska Bay (MAGAŠ, 2002).

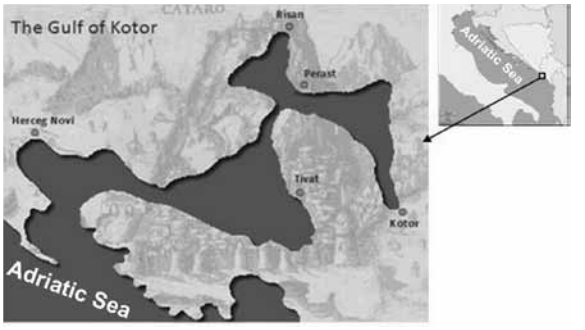

Fig 1. Map of the Boka Kotorska Bay (southeastern Adriatic Sea)

A sample of 100 juvenile anchovy was collected on October 19, 2005 at the location Orahovac (42°29'13.3"N, 018°45'20.4"E) within the Boka Kotorska Bay during the night. A commercial beach seine net (5 mm stretched mesh size) with artificial light was used. Total length (*TL*) to the nearest 0.1 cm, and weight (*W*) to the nearest 0.01 g, were measured for each individual fish in the laboratory. A pair of sagittal otoliths was removed under the Wild Heerbrugg binocular microscope and cleaned in distilled water, dried on filter paper, mounted on slides, and fixed with colourless nail varnish for 24 hours. Next, they were grinded and polished under the microscope with nail files of different granulation, from coarse to fine.

Each daily increment has two zones, a discontinuous or matrix-rich zone (*D*-zone) and incremental zone (*L*-zone); under the transmitted light, the *D*-zone is opaque (dark), and the *L*-zone is translucent (light) (STEVENSON & CAMPANA, 1992). Reading of otoliths is generally very difficult due to the presence of small sub-daily increments. For this reason, only the otoliths with optimum increment definition and clarity should be taken into account (MORALES-NIN, 1992).

The rings were counted from the core along the post-rostrum axis and the maximum length from post-rostrum to rostrum (*OL*) was measured under a Carl Zeiss Axio Imager with a

digital camera connected to the monitor, at 400x magnification. Each otolith was read twice by two independent observers. When there was any discrepancy between the two readings, a third reading was carried out. Out of a total number of 100 otolith pairs, 88 were readable. Individuals were sorted into length classes of 0.1 cm; mean age in days was estimated.

The date of hatching for each length class was back-calculated by subtracting the estimated mean age in days from the date of capture.

Growth curves of the von Bertalanffy's growth function (VBGF) were generated with the Simply Growth programme (PISCES Conservation Ltd, 2002), which estimates parameters by non-linear regression using the Levenberg–Marquardt method. To test the overall growth performance and to overcome the problem of correlation between growth parameters K and L_∞, the phi-prime index (φ') was calculated (MUNRO & PAULY,1983; PAULY & MUNRO, 1984):

$$\varphi' = \log_{10} K + 2\log_{10} L_\infty$$

where K is Brody's growth coefficient and L_∞ is asymptotic length.

The relationship between fish size (*TL*) and age (days) was assessed by fitting a linear equation, in the following form: $TL = a + b$ age (days) where *TL* is the total fish length (mm), *a* is the fish size at metamorphosis from late larva to juvenile and *b* is the daily growth rate of juvenile anchovy.

The growth rate of otoliths was estimated using the linear regression model*:* $OL = a\ TL + b$, where *TL* is the total length of the fish (cm), *OL* is the maximum otolith length (mm), *a* is the angular coefficient characterizing the growth rate of the otolith and *b* is a constant for the observed species. Data of otolith length at age were fitted to the Gompertz growth model (RICKER, 1979).

Length-weight relationship was calculated as $W = aTL^b$, where *TL* is total length in cm, *W* is weight in g, *a* intercept, and *b* slope. Determination coefficient r^2 and correlation coefficient *r* were also calculated.

RESULTS

The age of sampled individuals ranged from 41 to 90 days, the length from 5.0 to 6.8 cm, the weight from 0.59 to 1.83 g, and the length of otoliths from 1.42 to 1.95 mm (Table 1).

	TL (cm)	W (g)	AGE (days)	OL (mm)
Min	5.00	0.59	41.00	1.42
1st Qu.	5.50	0.83	56.00	1.55
Median	5.80	0.98	63.00	1.67
Mean	5.79	1.00	64.56	1.66
3rd Qu.	6.10	1.16	71.50	1.77
Max.	6.80	1.83	90.00	1.95

Table 1. Summary of statistical data on total length (TL), weight (W), age (AGE), and otolith length (OL) of sampled juvenile anchovy individuals in the Boka Kotorska Bay

The estimated dates of hatching for sampled juvenile anchovy, back-calculated from the date of capture, start from 8-Sept-2005 and end on 21-July-2005 (Table 2).

The length-at-age relationship curve is shown in Fig. 2. The mean age in years for each length class was used as entry data for the estimation of the VBGF parameters. The calculated growth performance index (φ') was 2.560.

The daily growth rate of juvenile anchovy was estimated by fitting a linear regression to the entire length-age data set. The relationship between fish size (*TL*) and age (days) was

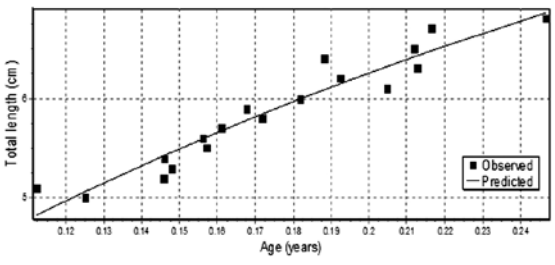

Fig. 2. Length-at-age of sampled juvenile anchovy individuals in the Boka Kotorska Bay (TL = 10.41 x (1 – e –3.36 (t +0.074)); $r^2 = 0.84$, $P < 0.001$, $n = 88$)

Table 2. Estimated mean age and date of hatching for each length class of sampled juvenile anchovy individuals in the Boka Kotorska Bay

Length class TL (cm)	Date of capture	Mean age (days)	Mean age (years)	Date of hatching	n
5.0	19-Oct-05	45.67	0.125	3-Sep-05	3
5.1	19-Oct-05	41.00	0.112	8-Sep-05	1
5.2	19-Oct-05	53.25	0.146	26-Aug-05	4
5.3	19-Oct-05	54.00	0.148	26-Aug-05	6
5.4	19-Oct-05	53.29	0.146	26-Aug-05	7
5.5	19-Oct-05	57.33	0.157	22-Aug-05	6
5.6	19-Oct-05	57.00	0.156	23-Aug-05	6
5.7	19-Oct-05	58.80	0.161	21-Aug-05	9
5.8	19-Oct-05	62.75	0.172	17-Aug-05	8
5.9	19-Oct-05	61.20	0.168	18-Aug-05	5
6.0	19-Oct-05	66.40	0.182	13-Aug-05	5
6.1	19-Oct-05	74.75	0.205	5-Aug-05	8
6.2	19-Oct-05	70.25	0.192	9-Aug-05	8
6.3	19-Oct-05	77.67	0.213	2-Aug-05	3
6.4	19-Oct-05	68.67	0.188	11-Aug-05	4
6.5	19-Oct-05	77.33	0.212	2-Aug-05	3
6.6	–	–	–	–	0
6.7	19-Oct-05	79.00	0.216	1-Aug-05	1
6.8	19-Oct-05	90.00	0.247	21-Jul-05	1

described as: $TL = 32.14 + 0.41$ age (days), $r^2 = 0.92$, $n = 88$. The daily growth rate within the analysed size range, calculated by pooling fish of all ages, was 0.41 mm day^{-1}, and the estimated fish size at metamorphosis from late larva to juvenile was 32.14 mm.

Otolith length was linearly related to total fish length and the regression parameters were highly significant: $OL = 0.027\ TL + 0.12$, $r^2 = 0.77$, $P<0.001$, $n = 88$ (Fig. 3).

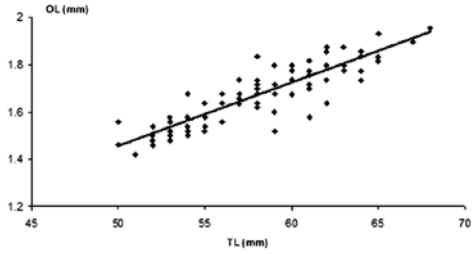

Fig. 3. Relationship between the total length and the otolith length (TL – OL) of sampled juvenile anchovy individuals in the Boka Kotorska Bay

Calculated anchovy *OL* at the end of the first growth season using the Gompertz model was 1918 µm and a maximum growth was found at 62 days after hatching (Fig. 4). A high variability was found within the sample, as can be seen by the low coefficient of determination ($r^2 = 0.59$).

The length-weight relationship of the sampled individuals was: $W = 0.0028\ L^{3.3445}$; $r^2 = 0.93$, $P < 0.001$, $n = 88$ (Fig. 5).

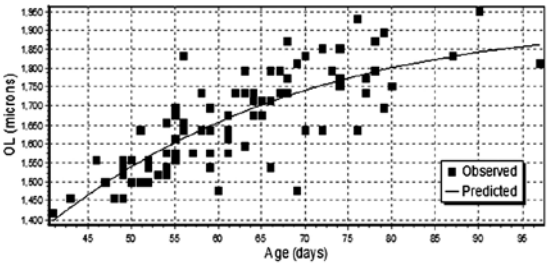

Fig. 4. Gompertz curve fitted to otolith lenght (OL) at age data; $L_\infty = 1918$ µm, (asymptotic otolith length), $k = 0.036$ (growth coefficient), and the length at the inflection point was $I = 61.86$ µm

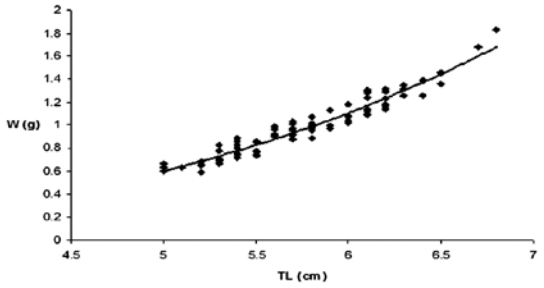

Fig. 5. Length-weight relationship of sampled juvenile anchovy individuals in the Boka Kotorska Bay

DISCUSSION

Validation of age structure and biomass of exploited fish populations are key parameters for maintenance of sustainable fisheries (CERMEÑO *et al*., 2003). Anchovy is one of the most studied species both in the Adriatic Sea and in the entire Mediterranean. There are a number of studies on anchovy adults and larvae, while on the other hand, very little is known about its juvenile phase.

The length of individual fish in the present study ranged from 50 to 68 mm. The metamorphosis from postlarval to juvenile phase occurred at length of 32.41 mm. It is somewhat smaller than the length estimated for the northern Adriatic Sea, which is 36.87 mm (DULČIĆ, 1997) or for the central Adriatic Sea, which is 35-40 mm (LA MESA *et al*., 2009). Both authors obtained samples from the open sea, a fact that could help explain the lower values obtained from the Boka Kotorska Bay. Bay areas are known to be richer in nutrients than the open sea waters, and enable faster growth and development of young individuals.

Since PANNELLA (1971) discovered daily otolith increments, RÉ (1987), PALOMERA *et al*. (1988), REGNER & DULČIĆ (1990), and DULČIĆ (1997) confirmed the formation of daily otolith increments in anchovy larvae. CERMEÑO *et al.* (2003) were the first to prove their formation in juvenile and adult anchovy.

In most species, the otolith formation is related to hatching, onset of activity, or first feeding (JOH *et al*., 2005). The age of the first increment is species-specific (BROTHERS *et al*., 1976, AOKI & MIYASHITA, 2000). Due to the lack of data for anchovy, in their previous studies RÉ (1987), PALOMERA *et al*. (1988), and DULČIĆ (1997) assumed that the formation of the first two rings, layed down at the end of the yolk-sac absorption occurred after second day of hatching and that it is related to the onset of the first feeding, since REGNER (1985) found that anchovy larvae completed the yolk-sac absorption at two days of age, while BROTHERS *et al.* (1976) reported that the deposition of the first increments in the sagittal otholits of anchovy larvae is related to

Table 3. Length of one-year old juvenile anchovy in different regions of the Mediterranean, from the period 1977-2011

Area	Length at age 1	Method	Source
Southeast Adriatic (Montenegro)	10.12	Otolith	This study
Strait of Sicily (Italy)	11.60	Otolith	BASILONE *et al.* (2004)
Catalonian littoral (NE Spain)	11.00	Otolith	MORALES-NIN & PERTIERRA (1990)
Catalonian littoral (NE Spain)	11.88	Otolith	PERTIERRA (1987)
Gulf of Cádiz (SE Spain)	11.14-11.31	Freq. analysis	BELLIDO *et al*. (2000)
Central-north Adriatic (Croatia)	11.85	Otolith	SINOVČIĆ (1998, 2004)
Gulf of Cádiz (SE Spain)	10.57	Scale	RODRIGUEZ-RODA (1977)
Bay of Biscay (NW Spain)	16.83	Otolith	ALDANONDO *et al.* (2011)
Bay of Benisaf (SW Algeria)	12.87	Otolith	BACHA *et al.* (2010)

the start of the active feeding. A recent study of ALDANONDO *et al*. (2008) on anchovy larvae showed that the first regular increments were formed at hatching; hence, we decided to follow this study in back-calculation of the hatching date.

The sexual maturity signifies the transition from the juvenile to the adult life phase. According to many authors, the majority of anchovy populations (95%) become sexually mature at the end of the first year of life (MOTOS, 1996; BACHA *et al*., 2010; SINOVČIĆ, 2000, 2001). For the Adriatic Sea, 95% of the population of the anchovy is mature at 10.8 cm (SINOVČIĆ, 1998), which is very similar to the value we obtained (L_∞ = 10.41). The published data on juvenile anchovy length at the end of the first year of life (≅ 1) from different regions of the Mediterranean are presented in Table 3.

The length of anchovy at the end of the first year of life that we estimated for the juvenile anchovy from the south-eastern Adriatic Sea is similar to the values estimated for other parts of the Mediterranean, except for the value in the Bay of Biscay by ALDANONDO (2011), where the growth of anchovy was exceptionally high. In all compared studies, the von Bertalanffy's growth model was used.

The estimated value of K = 3.36 in the present study is quite high. The probable reason is because the analysed length range of anchovy is small, and it is well known that K is inversely proportional to the analysed range of lengths. For the juvenile anchovy in the central Adriatic K = 0.82 (SINOVČIĆ, 2000), but the length range of analysed specimens was much wider. However, the high value for K = 2.44 was estimated by PADOAN (1963) for adult anchovy from the north Adriatic.

The growth performance index can be used to distinguish ecosystems since it is linked to metabolism, food consumption, and energetics of the habitat (MUNRO & PAULY, 1983). Value obtained in this study (2.560) is somewhat higher than the value obtained for the central Adriatic (2.331) (SINOVČIĆ, 2000), and lower than the value for the northern Adriatic (2.757) (PADOAN, 1963). In the Mediteranean, the value of the phi prime index (φ') similar to the value from our study was found in the Gulf of Cadiz (2.503), which also represents a nursery area for the anchovy (BELLIDO *et al*., 2000). In other parts of the Mediterranean, the value of this index is smaller: for the Strait of Sicily it is 2.016 (BASILONE *et al*., 2004), for the Gulf of Lion it is 2.106 (CAMPILLO, 1992), for the central Ionian Sea it is 2.194 (MACHIAS *et al*., 2000), and for the west Algerian coast it is 2.260 (BACHA *et al.,* 2010).

It is known that anchovy grows fastest during the first months of life. The growth slows down when yearlings approach their first winter, due to influence of the lower temperatures combined with other unfavourable environmental factors (WILHELM *et al*., 2005; FEY, 2006). Therefore, the question whether there is a specific growth phase during the juvenile stage or it is only the matter of the winter slow down, still remains unanswered. The instantaneous growth rate for juvenile anchovy in the present study was estimated to be 0.41 mm day^{-1}. For anchovy from the central Adriatic it was 0.55 mm day^{-1} (November) (LA MESA *et al.*, 2009), and for anchovy from the Bay of Biscay it was 0.4-1.0 mm day $^{-1}$ (September-October) (ALDANONDO *et al.*, 2011).

Otolith length was linearly related to the total length of juvenile anchovy. This parameter can also serve as an indicator of growth in anchovy. The coefficient of determination between the total fish length and otolith length is relatively high (r^2 = 0.769) and regression parameters were highly significant (P < 0.001). Similar values were obtained by ZORICA *et al.* (2010).

Otolith growth was similar to that estimated by ALDANONDO *et al.* (2011). However they measured the otolith radius instead of the otolith length as we did, and found that at the end of the first growth season it was 1759 µm. A maximum growth was found at 56 days after hatching, compared to 62 days in the present study.

The allometric coefficient (b), which represents the body form, is directly related to weight affected by environmental and intrinsic factors such as temperature, food supply, spawning conditions, sex, age, etc. (LE CREN, 1951; RICKER, 1975). The estimated allometric coefficient for

the juvenile anchovy in this study was 3.345, which indicates a positive allometric growth. The similar value of $b = 3.190$ was estimated for the juvenile anchovy in the central-eastern Adriatic Sea by SINOVČIĆ (1998), and of $b = 3.211$ by SINOVČIĆ & ZORICA (2006), while the value of $b = 3.341$ was estimated in the north and central-eastern Adriatic Sea by TIČINA *et al*. (2006). The value of $b = 3.529$ was estimated in the southern Adriatic Sea by CASAVOLA *et al*. (1981). This similarity is possibly the consequence of the similar environmental factors influencing the growth of the juvenile anchovy in the Adriatic Sea.

This study represents the first assessment of the von Bertalanffy's growth parameters based on daily otolith increment readings in the juvenile anchovy from the eastern Adriatic. Observed age of the studied population was used for estimation of parameters L_∞, K, and t_0, in order to provide a more accurate data on population growth. Identifying the parameters of anchovy growth will lead to the estimates of relevant parameters of population dynamics and to better understanding of the long-term changes of the juvenile anchovy stock size in the Boka Kotorska Bay.

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Dnevni naraštajni prstenovi u otolitima i stopa rasta juvenilnog inćuna, *Engraulis encrasicolus* (Linnaeus, 1758), u jugoistočnom Jadranu

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SAŽETAK

Dnevna stopa rasta juvenilnog inćuna, *Engraulis encrasicolus* (Linnaeus, 1758), procijenjena je u jugoistočnom Jadranu. Uzorci su sakupljani komercijalnom obalnom mrežom potegačom, a raspon veličine kod 100 uzorkovanih jedinki iznosio je 5.0-6.8 cm ukupne dužine. Dob je procijenjena brojenjem dnevnih naraštajnih prstenova na sagitalnim otolitima. Procijenjena starost kolebala je između 41- 90 dana, a nadnevak izvaljivanja ličinke za svaki starosni razred bio je naknadno izračunat prema datumu ulova. Izračunata trenutna stopa rasta iznosila je 0.41 mm dan^{-1}. Veličina ribe za vrijeme metarmofoze iz kasnog stadija ličinke u juvenilni stadij procijenjena je na 3.2 cm ukupne dužine. Parametri u von Bertalanffy-jevoj jednadžbi rasta bili su L_∞ = 10.41 i K = 3.36.

Ključne riječi: juvenilni inćun, rast, starost, otolit, Jadran