Age and growth of early life stages of European pilchard (*Sardina pilchardus*) from the western Adriatic Sea

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Age and growth of early life stages of sardine, Sardina pilchardus*, were investigated by microincrement counts on sagittal otoliths. Postlarval and juvenile specimens were collected in the coastal waters off Ortona (central Adriatic Sea) from December 1996 to May 1997. Otolith microstructure analysis was conducted on 286 specimens ranging from 30 to 68 mm TL. Age estimates ranged from 65 to 151 days. The precision of age estimates was tested by applying both the average percent error (APE) and the coefficient of variation (CV). The hatch date distribution, back-calculated from the date of capture, was spread over a long period lasting from September to March, with two main cohorts hatched in autumn (October) and winter (February), consistently with a long spawning season reported for this species. Applying the Gompertz growth model to the age-length data set, a growth curve was obtained for each cohort. The mean absolute daily growth rate was 0.33 mm day^{-1} and 0.22 mm day^{-1} for the autumn and winter cohorts, respectively. Otolith growth increment patterns was rather similar in the two cohorts, but they differed for the amplitude and timing of increment width deposition, being otoliths accurate proxies of larval fish growth rate. Strong seasonality of environmental features, such as water temperature and food resources, and endogenous factors linked to the larval ontogeny, largely contributed to affect the early life history stages of sardine.*

Key words: age and growth, small pelagic fish, early life, otolith reading, Adriatic Sea

INTRODUCTION

European pilchard or sardine (*Sardina pilchardus*, Walbaum, 1792) is a member of the family Clupeidae. It is a coastal pelagic and euryhaline species distributed across the northeastern Atlantic, from Iceland to Senegal, and in the Mediterranean, including the Adriatic Sea (WHITEHEAD, 1985). It represents one of the most important pelagic fish resources in the Mediterranean Sea, being one of the target species of purse seine and mid-water pair trawl fishing fleet operating across the basin. Overall, this species account for about 41% of the total catches (MORELLO & ARNERI, 2009).

From ecological perspective, the key role of small pelagic fishes has been recently reported from the northern and central Adriatic Sea, where anchovy and sardine overwhelmingly dominate the pelagic component, which has a large impact on the food chain through the transfer of energy from the lowest to the highest trophic levels. As the small pelagic species represent the intermediate trophic level, and the

negative effects of their overfishing could cause significant changes in the functioning of the entire ecosystem (COLL *et al.*, 2007).

Sardine is a serial spawner with an indeterminate annual fecundity. In females, oocytes do not mature all simultaneously, but they are released in multiple batches during a relatively long spawning season. In the Adriatic Sea, spawning occurs mainly in winter, usually between October and May. In this period, there are one or two reproductive peaks, the timing of which largely depends by environmental factors. Timing and areas of reproduction seem to be related to biotic and abiotic factors like food availability, temperature and salinity (MORELLO & ARNERI, 2009 and references therein; MANDIĆ *et al.*, 2013).

Although the presence of sardine eggs has been recorded in shallow waters of the whole Adriatic Sea, there are two main spawning grounds in the Adriatic Sea, i.e. off the island of Dugi Otok and around the Dalmatian islands extended offshore Palagruža. Occasionally, these two areas have joined together and the southern area extended westward up to the Italian coast, below Otranto. There are two physical barriers that isolate these reproductive areas, namely the Pomo pit and the South-Adriatic pit. Perhaps, the geographical separation has generated two distinct sub-populations of sardine, one in northern Adriatic Sea and another in the central Adriatic Sea, respectively (MORELLO & ARNERI, 2009 and references therein).

As far as the aggregation behaviour of sardine during ontogeny is concerned, the larvae are rather dispersed, whereas they begin to aggregate at the post larval and juvenile stages, when they move from the nursery area to be recruited for fishing. Recruits form a relatively large proportion in short-lived species like sardine, so the parental stock size exploitable each year is largely determined by the recruitment strength a year before. During ontogeny, body size at hatching, exogenous feeding and metamorphosis are considered major factors affecting survival during the larval and juvenile stages (HOUDE, 1987). The larger and faster growing individuals would be exposed for a shorter period to starvation and predation, which are probably the most important causes of mortality in fish larvae (BAILEY & HOUDE, 1989), as well as to harmful physical processes, such as advection for an unsuitable habitat or water turbulence (BERGERON, 2000). For this reasons, the knowledge of growth and mortality rates in early life stages of sardine is crucial to understanding their life strategy and to provide future recruitment success.

Otolith microstructure analysis can be used to back-calculate the timing of first exogenous feeding and hatching, as well as to reconstruct age and day-specific histories of otolith growth as a proxy for somatic growth (BAUMANN *et al.*, 2006). Some important thresholds in early life stages development, such as notochord flexion, exogenous feeding and metamorphosis, are also marked by a significant change in otolith growth pattern (SOMARAKIS & NIKOLIOUDAKIS, 2007). In turn, they are linked respectively to changes in swimming ability, feeding regimes and schooling behaviour, which largely affect growth rate and survival.

In the present study, we estimated age and growth of *S. pilchardus* collected in the central Adriatic Sea during the transition from larval to juvenile stages by means of daily growth increments counts on sagittal otoliths, aiming to provide more insights on its early life history traits.

MATERIAL AND METHODS

Study area and field sampling

Early life stages samples of *S. pilchardus* were collected monthly from December 1996 to May 1997. The study area was located off Ortona, in the central Adriatic Sea (Fig. 1). For sampling activities, the coastal area was divided into four depth strata located at 10, 20, 30 and 40 m. Fishing grounds consisted mainly of sandy bottoms down to about 20 m depth, becoming muddy offshore. Tows were carried-out parallel to the shoreline using a local commercial fishing vessel equipped with a small-meshed semi pelagic trawl, composed of three concentric bags with a codend mesh size from 5 to 16 mm.
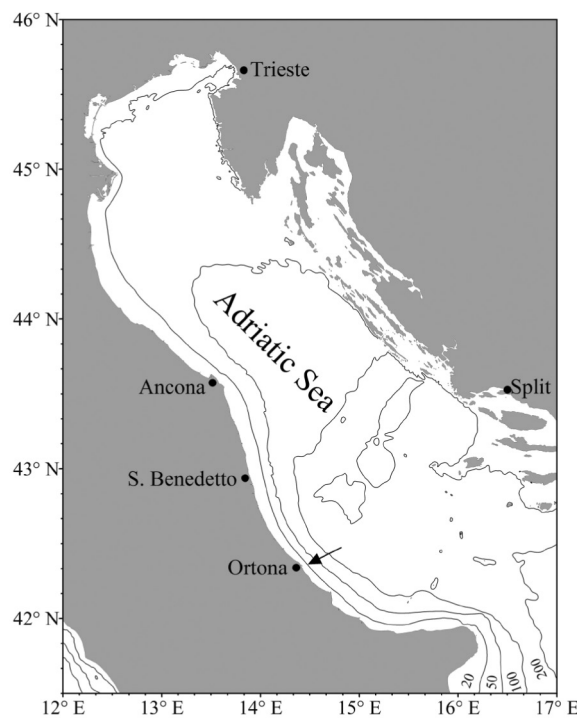
Fig. 1. Map of the Adriatic Sea, showing sampling site of early life stages of Sardina pilchardus *located off the Ortona harbour*

Each tow was carried out for 45 min at a vessel speed of 1.5-2 knots. Once caught, fish samples were immediately frozen and stored in vials.

Laboratory activities

In laboratory, postlarvae and juveniles of sardine were sorted out and measured to the nearest mm below (total length, *TL*, and standard length, *SL*). Sagittal otoliths were removed from each fish under a stereomicroscope, cleaned and mounted on glass slide (sulcus acusticus side down) with epoxy resin (Petropoxy), cured in an oven at 80°C for about 12 h. Otoliths were then ground with a metallographic abrasive paper disc lubricated with 1-3 μm alumina powder, until all increments along the counting path were readable, and finally polished with 0.05 μm alumina to obtain transverse sections without scratches.

Increments counts and measurements were made under a light microscope set at 400x magnification, equipped with a CCD video camera connected to an image analysis system (Image Pro Plus 7.0). All growth increments were counted along the same otolith counting path, from the core to the post-rostrum. The otoliths sections were observed using green light and polarizing filters to improve the clarity of increments. In sagittal otoliths of postlarval and juvenile sardine, the light microscope reveals a pattern of alternating light (i.e. incremental or L-zone) and dark rings (i.e. discontinuous or D-zone), which together were counted as one complete growth daily increment. Small subunits laid down with a sub-daily cycle were sometimes observed within the growth increments. To minimize ageing misinterpretation due to these structures, we count only those increments with a discontinuous zone wide enough to remain visible when the micrometer is turned to slightly readjust the focus (PALOMERA *et al.*, 1988). Two increment counts were made for each sagitta and, when counts differed from each other by more than 10%, they were discarded.

From each otolith pairs, a single sagitta was randomly selected and the otolith radius (OR, μm) was measured as the maximum distance from the core to the post-rostrum. The relationship between fish size (TL, mm) and OR (μm) was estimated fitting a least-square linear regression only for the most abundant autumn cohort.

To estimate changes in the accretion rate of otoliths during ontogeny, otolith increment width along the counting path was measured every five increments from the increment closest to primordium to the otolith margin. Average otolith growth rate was then calculated by dividing the measured otolith width by the number of daily increments counted (i.e. 5).

Data analyses

Theoretically, it is possible to use the measured widths of a daily increment time series to determine both the size and the growth rate of an individual fish for each day of its life. All growth back calculation procedures are based upon the assumption of proportionality (i.e. a linear relationship) between otolith and fish sizes. In this context, we applied the Fraser-Lee

regression method (CAMPANA & JONES, 1992), which is summarized in the following equation:

$$TL_a = d + (TL_c - d) \, O_c^{-1} \, O_a$$

where TL_a and O_a are the fish size and otolith size (radius) at a certain age, TL_c and O_c are the fish size and otolith size at capture, and d is the theoretical fish size at hatching estimated from the fish size otolith radius relationship defined above.

All otolith sections were read twice by a single reader and the age estimation was obtained from the mean of the two readings. To assess ageing precision between readings, the index of average percent error (APE) (BEAMISH & FOURNIER, 1981) and the mean coefficient of variation (CV) (CHANG, 1982) were calculated. A bias plot was then computed to assess systematic differences between readings (CAMPANA *et al.*, 1995).

The relationship between fish size (*TL*, mm) and age (*t*, days) was estimated by fitting the Gompertz model, which is usually fitted for the growth of larval and juvenile stages of fishes. The Gompertz model is summarized in the following equation (CAMPANA & JONES, 1992):

$$TL = L_0 \, exp \, (k \, (1 - exp \, (- Gt)))$$

Where L_0 is the larval size at hatching, G is the instantaneous growth rate at the inflexion point of the curve, and k is a constant.

The absolute daily growth rate (mm day^{-1}) was calculated applying the following equation:

$$gt = GLt \, (lnL_\infty - lnLt)$$

where G is the instantaneous growth rate, L_∞ and Lt are the fish length at the asymptote and at age t, respectively.

The hatch date distribution was back calculated by subtracting age estimates from the date of capture. To test for differences in growth rate of juvenile sardine among different seasons, two main groups of specimens (cohorts) hatched in autumn (October) and winter (February) were selected and compared.

Finally, light microscope increment counts and width measurements were checked using the scanning electron microscopy (SEM) (GEFFEN, 1992). Sagittal otoliths were randomly selected

from a few specimens of 35-60 mm *TL*. Otolith sections were etched using EDTA, after some trials to determine the best acid concentration and time of exposure. After etching, each section was mounted on stub with thermoplastic glue and coated with colloidal silver for SEM examination.

RESULTS

Fish samples

A total of 286 late larval and juvenile sardine ranging from 30 to 68 mm were collected in the coastal area off the Ortona harbour, all within the shallower depth stratum (10 m) (Table 1). The specimens were sampled during the metamorphosis, which represents a key event in the early ontogeny of fishes. The length-frequency distribution was slightly right skewed, with the bulk of catches composed of fish ranging from 30 to 60 mm and a few larger specimens (Fig. 2).

Table 1. *Monthly samples of early life stages of* Sardina pilchardus *collected off the Ortona Harbour between December 1996 and May 1997. N = number of specimens; TL = total length*

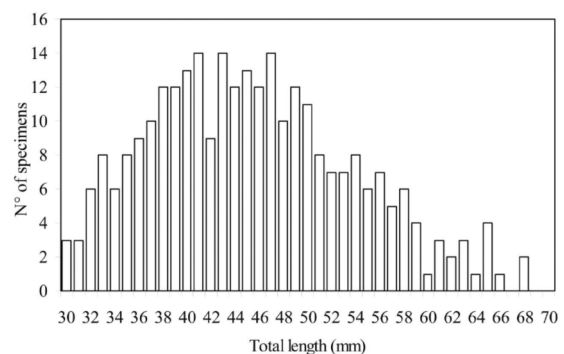| Month	N	TL (mm)	Mean TL (mm)
December	53	32-56	42.6
January	141	32-68	48.3
February	44	33-58	41.9
March	34	40-68	48.3
April	-	-	-
May	14	30-34	31.8

Fig. 2. *Length frequency distribution of early life stages of* Sardina pilchardus *sampled from December 1996 to May 1997*

Otolith microstructure

Sagittal otoliths in early life stages of sardine had an elliptical shape, with a smooth posterior margin and an early short rostrum on the opposite side. The primordium was generally encircled by two or three faint increments, which were deposited after the beginning of exogenous feeding according to previous authors (e.g. RE', 1987; PALOMERA *et al.*, 1988). Subsequently, increments became more regular and clear, and they showed the typical bipartite structure. Growth increments close to the primordium appeared thin and evenly spaced, becoming progressively wider to decrease again towards the margin. On average, increments width ranged from 1.8 to 5.9 µm. As increments width increased, small subunits became visible, probably laid down with a sub daily periodicity. The SEM analysis confirmed the bipartite structure of increments and their width pattern, as revealed by the standard light microscopy (Fig. 3).

The linear model fitted to the fish length (*TL*) and otolith radius (*OR*) gave the following relationship (Fig. 4):

$$OR = -27.7 + 8.43\ TL \quad (n = 266 \quad r^2 = 0.87)$$

The linear fitting explained most of the total variance between the two parameters (87%), supporting us to back calculate fish growth applying the Fraser-Lee procedure.
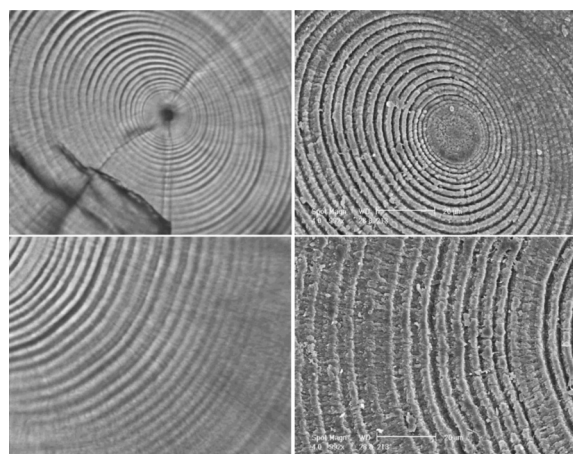

Fig. 3. Micrographs showing growth increment patterns of sagittal otoliths of Sardina pilchardus *using light microscopy (right) and the scanning electron microscopy (SEM) (left).*
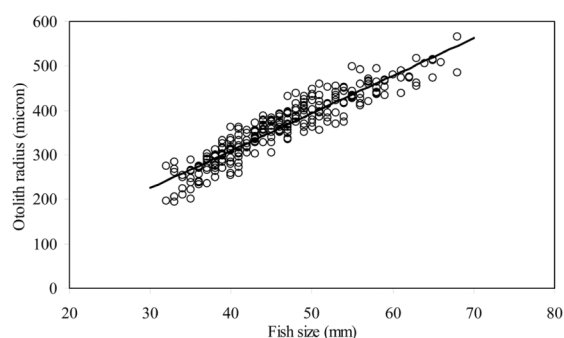

Fig. 4. Fish size-otolith radius relationship of Sardina pilchardus *fitted by the linear regression analysis from the autumn cohort*

Age and growth rate

Overall 280 specimens of sardine were successfully aged (98%). Based on the assumption that growth increments were laid down with daily periodicity and starting from hatching, age estimates of the whole sample ranged from 65 to 151 days. Age-length key is summarized in Table 2. Indices of ageing precision APE and CV were relatively low (0.96% and 1.36%, respectively), indicating the goodness of the ageing procedure adopted and a good consistency or reproducibility among readings. No bias between readings was present across the whole age range estimated.

The hatch date distribution, back-calculated from the age estimates and dates of capture, was spread over an extended period lasting from September to March. Nevertheless, two main peaks characterized the overall distribution, representing two main cohorts hatched in autumn (October 1996) and winter (February 1997), respectively (Fig. 5).

The Gompertz growth function was fitted to the age-length data for each of the two main cohorts (Fig. 6). The relationships between fish size *TL* (mm) and age (days) estimated for the two cohorts were summarized below:

$$TL = 7.22\ exp\ (2.05\ (1 - exp\ (- 0.02\ age)))$$
$$(n = 266)$$
autumn cohort
$$TL = 8.26\ exp\ (2.03\ (1 - exp\ (- 0.01\ age)))$$
$$(n = 14)$$
winter cohort

Table 2. Age-length key of early life stages of Sardina pilchardus *collected off the Ortona Harbour. TL = total length*

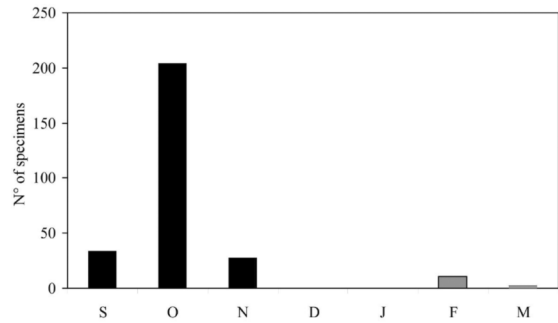| TL (mm)	Age (months)		
	2-3	3-4	4-5
30	3		
31	3		
32	6		
33	7	1	
34	5	1	
35	5	3	
36	4	4	
37	4	5	
38	7	4	
39	2	9	
40	5	7	1
41	6	7	1
42	1	6	2
43	4	6	3
44	1	8	3
45	3	8	2
46	2	6	4
47	3	7	4
48	1	8	1
49	1	9	1
50	2	6	3
51	1	6	1
52	3	4	
53	1	6	
54	2	6	
55		6	
56	2	5	
57		5	
58		5	1
59		3	1
60		1	
61		3	
62		2	
63		3	
64		1	
65		2	2
66		1	
67			
68		1	1

Fig. 5. Monthly hatch date distribution of Sardina pilchardus *back-calculated from age estimates and date of capture, showing the autumn (black bars) and winter (grey bars) cohorts*
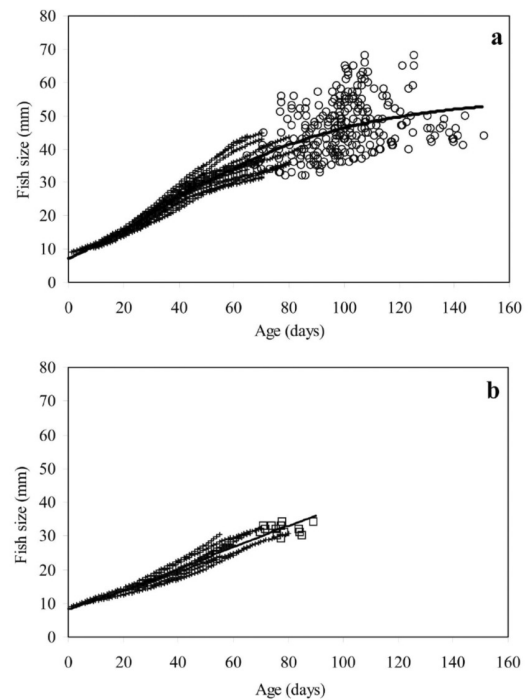

Fig. 6. Gompertz growth curves (solid lines) fitted for early life stages of Sardina pilchardus *calculated for autumn (a) and winter (b) cohorts. Circles and squares represent age-length data pairs estimated by growth increment counts, small crosses represent age-length data pairs back-calculated applying the Fraser-Lee regression method*

For the autumn cohort, the absolute daily growth rate (gt) ranged between 0 mm (i.e. at the asymptote) and 0.48 mm day^{-1} (i.e. at the inflection point of the logistic curve), with a mean value of 0.33 mm day^{-1}. For the winter cohort, the absolute daily growth rate (gt) ranged between 0 mm and 0.33 mm day^{-1}, with a mean value of 0.22 mm day^{-1}.

Variation of otolith growth increments width

Along the otolith counting path, growth increments width ranged from 2.2 μm to 5.9 μm and from 1.8 μm to 3.5 μm for the two cohorts hatched in autumn and in winter, respectively (Fig. 7). The curves showed the same sinusoi-
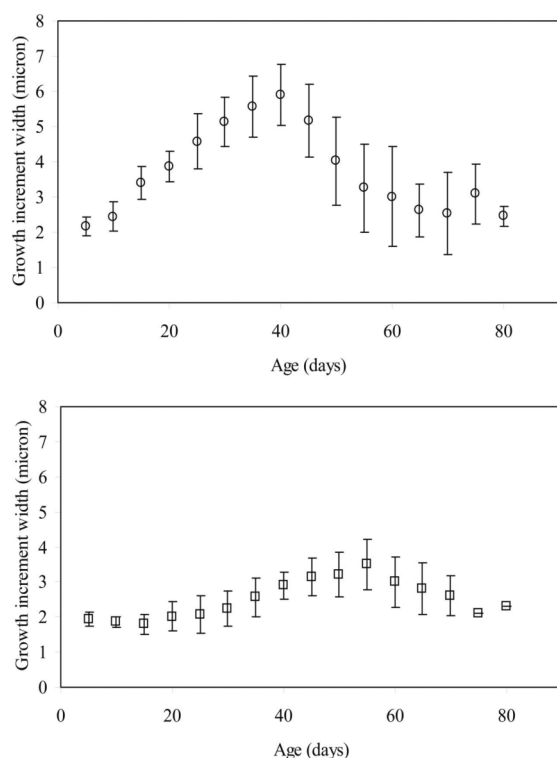
Fig. 7. Otolith increment width pattern (mean ± SD) during the early growth of Sardina pilchardus *calculated for autumn (above, circles) and winter (below, squares) cohorts*

dal shape and similar trend for the two cohorts considered, evidencing a rather slow increase until reaching a maximum value, followed by a rapid decrease. However, amplitude of growth increment width and timing of maximum peak were rather different between the two cohorts, being bigger and occurring earlier in the autumn cohort than in the winter cohort.

DISCUSSION

Comparing present data with the relatively few previous studies on age and growth of early life stages of sardine based on otolith microstructure analysis, there is a good consistency among different areas of the Adriatic Sea in terms of growth rates and spawning/hatching periods. Based on the Gompertz growth function, the daily growth rates calculated from the two cohorts sampled in the central Adriatic Sea in the present study were similar to those

reported by PANFILI *et al.* (2010), who estimated 0.21 and 0.27 mm day^{-1} in sardine late larval populations sampled in the northern (Po area) and southern (Gulf of Manfredonia) Adriatic Sea. Despite of different environmental conditions characterizing different areas of Adriatic Sea, hatching period of sardine is rather similar across their distribution and occurs in the winter season, with peaks in October in the central Adriatic Sea (present data), in November in the northern Adriatic Sea and in December in the southern Adriatic Sea (PANFILI, 2012). This confirms that sardine is a typical winter spawner, with a low variability locally driven by different environmental conditions.

Daily growth rates of sardine at an earlier stage of development than our samples are consistently higher and rather variable across their range of Mediterranean distribution. In particular, growth rates of yolk-sac and early postlarval sardine approximately ranged from 0.28 to 0.92 mm day^{-1} in the eastern Adriatic Sea (DULČIĆ 1997), in the Alboran Sea (ALEMANY *et al.*, 2006), in the Vigo region (ALVAREZ & BUTLER, 1992), in the Balearic Islands (ALVAREZ & MORALES-NIN, 1992), in the Gulf of Gabes (KHEMIRI & GAAMOUR, 2005) and in the Thyrrenian Sea (ROMANELLI *et al.*, 2002).

Based on the validation of daily growth increment formation in sardine larvae (e.g. RE', 1983, 1984; PANFILI, 2012) and strong proportionality between otolith and fish size (present data), otolith growth patterns can be used as a proxy of somatic growth throughout key events in early ontogeny, such as fin formation and metamorphosis (e.g. LA MESA *et al.*, 2009). Looking at the width increment patterns and growth curves estimated in the present study for the two cohorts hatched in autumn and winter, fish body growth and increment width increased until the age of about 40-50 days, which corresponded to a size of 20-25 mm, then rapidly decreased afterwards. According to RUSSELL (1976), the onset of fin rays formation in larval sardine, which allows the transition from passive transport to active swimming in the water column, takes place exactly within this size range. Perhaps major energy reserves are employed for morpho-func-

tional processes until the end of metamorphosis, resulting in a decrease of somatic growth rate and, in turn, of otolith increment width.

Temperature and food availability have been generally considered as the main environmental factors affecting larval growth (BUCKLEY 1984; HEATH, 1992), and their importance has been demonstrated for sardine larvae development in some Mediterranean populations (e.g. RAMI-REZ et al., 2004; ALEMANY et al., 2006; CATALAN et al., 2006). Larval growth rate and otolith increment width patterns estimated for the two main cohorts described here largely supports findings of previous studies. Consistently, the cohort hatched in autumn (October) showed higher growth rate and wider increment width than the cohort hatched in winter (February), when water temperature dropped down (ARTEGIANI et al., 1997). The temporal trend of the monthly averaged chlorophyll a concentration estimated from remote sensing data for central Adriatic basin gave further support to present data, as it started to increase in October and reached a peak in January, slightly decreasing afterwards (POLIMENE et al., 2006).

Other than by daily growth rate, the two cohorts largely differed in terms of relative abundance of larvae survived until catch dates, assuming the same gear selectivity throughout the whole sampling period. Sardine belonging to the autumn cohort overwhelmingly dominate, accounting for more than 95% of total catches. Hence, early larval growth rate in sardine would largely contribute to determine juvenile survival and, ultimately, the strength of adult population recruitment. In the Alboran Sea, the sardine postlarvae that survived until the juvenile stage were those with faster growth during larval stages and thus bigger at a specific age (ALEMANY et al., 2006), supporting "the bigger/faster is the better" hypothesis (MILLER et al., 1988; FOLKWORD & HUNTER, 1986).

In conclusion, some key ontogenetic steps of early life history of sardine, such as hatching, notochord flexion and the transition from larval to postlarval and juveniles stages are not only marked by changes in body morphology and behaviour, but also by changes in otolith growth pattern. A combined effect of seasonality of key environmental characteristics, such as water temperature and productivity, and endogenous factors, like the metamorphosis processes and inherent behavioural changes, largely affect the larval body growth rate and otolith increments deposition.

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Starost i rast ranih razvojnih stadija srdele
(*Sardina pilchardus*) u zapadnom Jadranu

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SAŽETAK

Istraživani su starost i rast ranih razvojnih stadija srdele *Sardina pilchardus*, te mikro prirast na sagitalnim otolitima. Ličinke i juvenilni uzorci prikupljeni su u obalnim vodama Ortone (srednji Jadran) od prosinca 1996. do svibnja 1997. godine. Analiza mikrostrukture otolita provedena je na 286 jedinki u rasponu od 30 do 68 mm ukupne dužine (TL). Procijenjena starost kolebala je od 65 do 151-og dana. Preciznost procjene starosti testirana je primjenom prosječnog postotka pogreške (APE) i koeficijenta varijacije (CV). Datum izvaljivanja ličinki bio je naknadno izračunat prema datumu ulova koji traje od rujna do ožujka, s dvije glavne skupine koje su se izvalile u jesen (listopad) i tijekom zime (veljača), konzistentno s ustanovljenom dugom sezonom mrijesta za ove vrste.

Primjenom Gompertz modela rasta na skup podataka o starosno-duljinskom odnosu dobivena je krivulja rasta za svaku skupinu. Apsolutna srednja vrijednost dnevnog rasta iznosila je 0.33 mm dan[-1] i 0.22 mm dan[-1] za jesenske i zimske skupine, respektivno. Prirast otolita bio je prilično sličan u dvije skupine, mada se razlikovao s obzirom na vrijeme formiranja i učestalost istih. Jak utjecaj sezonskih ekoloških značajki, kao što su temperatura vode i izvor hrane, te endogeni čimbenici povezani s ontogenezom ličinki, u velikoj mjeri utječu na rane razvone stadije srdele.

Ključne riječi: starost i rast, mala plava riba, ličinke i mlađ, očitavanje otolita, Jadransko more