

## REGIONAL GROWTH DIFFERENCES IN SARDINE (*SARDINA PILCHARDUS* WALB.) LARVAE FROM ISTRIAN AND DALMATIAN COASTS

Jakov DULČIĆ

PhD., fisheries biologist, Institute of Oceanography and Fisheries - Split, 21000 Split, Šet. I. Meštrovića 63, CRO  
dr., ribiški biolog, IOR, 21000 Split, Šet. I. Meštrovića 63, CRO

### ABSTRACT

Sardine larvae were collected from October 1990 to April 1991. Growth rates were estimated from daily increments on sagitta otoliths. Otolith growth in relation to standard length and increment counts was also estimated. Growth differences were significant between Istrian and Dalmatian coasts at the same month, with growth being lower in the former. Variations among certain months in the Split area were also observed. These results are discussed in relation to temperature.

**Key words:** sardine, larvae, growth, Istria, Dalmatia  
**Ključne besede:** sardela, ličinke, rast, Istra, Dalmacija

### INTRODUCTION

The fate of various early fish stages is, according to recent theory, essential for the recruitment of adult populations. At the same time, knowledge on the growth rates of larval fish is indispensable for ecological studies of this important phase of fish life cycles, since they provide the basis for further studies of mortality, population dynamics, stock assessment, etc.

Up to the 1970s, the only way to estimate growth parameters of larval fish stages was to measure the length increase as a function of time under the controlled experimental conditions. In the early 1970s, Pannella (1971, 1974) first described the existence of daily growth structures in the otoliths of Osteichthyes, and Brothers *et al.* (1976) demonstrated that these structures were present in the otoliths of larvae and were laid down on a daily basis.

*Sardina (Sardina pilchardus)* is the traditional target of a important purse seine fishery along the eastern Adriatic coast. The Adriatic sardine has two preferred spawning areas, the northern one in a wider area between the island Dugi otok and Ancona and the southern one in the broader surroundings of Palagruža Island (Piccinetti *et al.*, 1981; Regner *et al.*, 1983; Gamulin &

Hure, 1983).

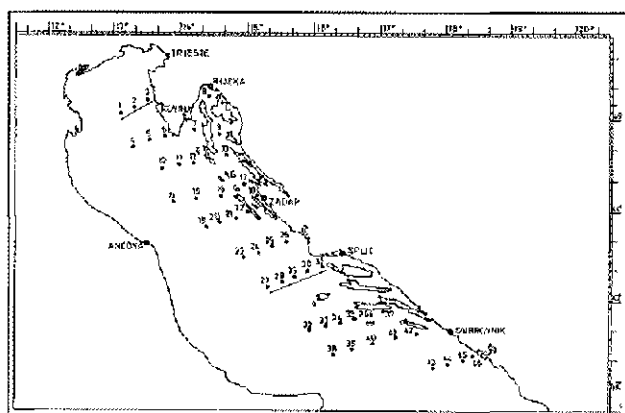
During recent years, otolith microstructure investigations have made it possible to study the age in days as well as the daily growth rate of fish larvae and juveniles. For sardine, Ré (1984) has validated the daily nature of these microincrements in sagitta otoliths, and Dulčić (1993) found that visible rings are laid down beginning at the day of hatching. Thus, in sardine larvae, the number of increments provides a direct estimation of actual age under optimal conditions. On the other hand, Ré (1983a) found that growth rates and thickness of daily units varied in relation to the time of the year in Portuguese waters.

The purpose of this study was to compare growth rates of sardine larvae between different areas of Northern and Central Adriatic and its possible seasonal variations. This would provide the basis for future studies on sardine growth parameters and on the conditions that affect the renewal of the sardine population in the Adriatic.

### MATERIAL AND METHODS

The two cross-shelf transects were sampled from October 1990 to April 1991. (Fig. 1, Table 1). A Bongo

net with a 0.2 m diameter and 250 microns mesh size was used. Double oblique tows were performed according to standard techniques (Smith & Richardson, 1977). Tow depth ranged from 10 to 150 m. Sea Surface Temperature (SST) at each station was measured. Plankton samples were fixed in 2% neutralized formal solution in seawater, with a formal pH of 7.8-8.6.



**Fig. 1: Sampling area and location of stations.**

**Slika 1: Zemljevid obravnavanega območja z vzorčevalnimi postajami.**

Sardine larvae ( $n=346$ ) were sorted. Standard lengths were measured to the nearest 0.1 mm. The size range was 3.0 to 22.8 mm. Size was not corrected, bearing in mind shrinkage due to capture and fixation technique. Sagitta otoliths were removed by fine steel needles and rinsed in distilled water, dried and placed in a drop of immersion oil. The counts of daily increment rings were made, depending on otolith size, at magnifications of 450x, 600x or 1000x, under a transmission light microscope. The otolith maximum radius was measured.

The number of increments were determined according to Methot (1981).

Least squares regressions between standard length vs increment counts (sl vs in), otolith radius vs standard length (or vs sl) and, otolith radius vs increment counts (or vs in) were fitted for each sample using the simple regression procedure Statgraphics. F-tests for homogeneity of variances, comparison of slopes and elevations were applied according to Snedecor & Cochran (1989) in order to determine the statistical significance of differences among samples at each region.

## RESULTS

### Standard length vs increment counts

There was linear relationship between standard length and increment counts over the size range collected at each month and in each sampled region. A simple linear growth model was fitted to each sample

individually. Monthly estimates of growth off Rovinj varied between 0.590 and 0.628 mm/day, whereas off Split they varied between 0.595 and 0.762 mm/day. Regression parameters are shown in Table 2 and graphically displayed in Fig. 2 (A-F).

Region	Sampling date	n	Size range (mm)	$\bar{T}^{\circ}\text{C}$
Rovinj	15 October	26	5.5-19.0	17.9
	25 January	59	3.0-14.6	10.2
	6 April	32	4.0-18.0	12.8
Split	27 October	55	6.7-17.3	19.1
	12 January	44	5.5-22.8	13.5
	18 April	23	4.7-13.2	14.8

**Table 1: Sampling schedule. Temperature is the average SST along transects (n and fish size range data correspond to larvae whose otoliths were analysed).**

**Tabela 1: Seznam vzorčenja. Temperatura je povprečna SST vzdolž transektov (podatki o številu in velikostnih razredih za larve z analiziranimi otoliti).**

Region	Month	Slope		Intercept		$r^2$
		mm/day	SE	mm	SE	
Split	October	0.595	0.016	5.16	0.13	0.97
	January	0.762	0.029	3.96	0.30	0.94
	April	0.726	0.086	3.51	0.66	0.77
Rovinj	October	0.590	0.027	4.91	0.28	0.95
	January	0.628	0.025	3.32	0.23	0.92
	April	0.600	0.026	3.59	0.25	0.95

**Table 2: Parameter estimates from linear regression of larval standard length (y) against increment counts (x). Estimates of slopes, standard error on slope, intercept and r-square for each region and month.**

**Tabela 2: Ocena parametrov linearne regresije standardnih dolžin larv (y) glede na število prirastkov (x). Ocena padcev, standardne napake (SE) v padcu, pre-strezne vrednosti in r-kvadrat za posamezno območje in mesec.**

### Otolith radius vs increment counts

An exponential model ( $y=e^{a+bx}$ ) provided the best fit to the data in all cases. Results are shown in Table 3 and Fig. 3 (A-F).

### Otolith radius vs standard length

The same exponential model also provided the best fit to these data as might have been expected taking into account the linear relationship between standard length and increment counts. Results are summarized in Table 4 and Fig. 4 (A-F).

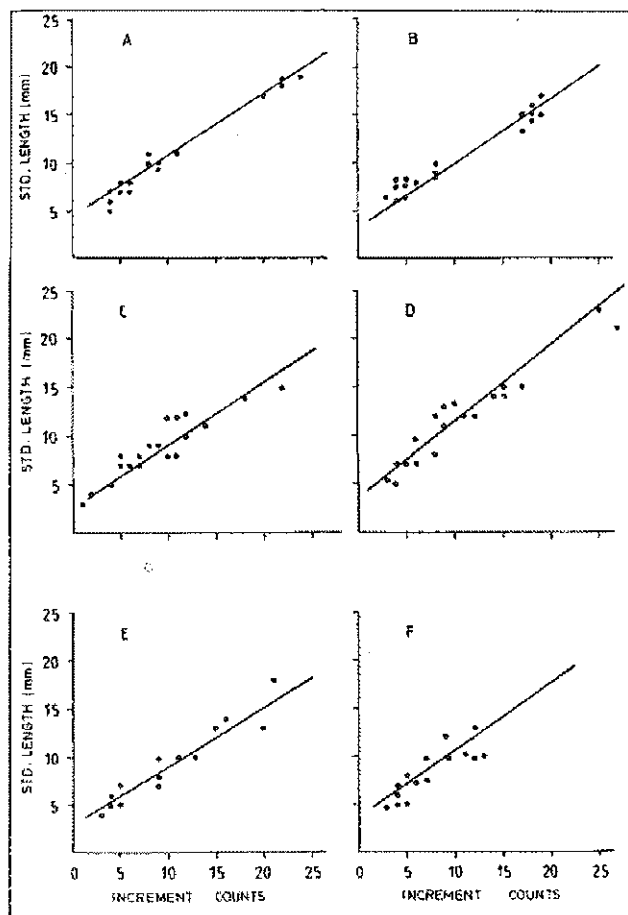


Fig. 2: (A-F) Relationships between standard length and increments counts (Rovinj, A-October, C-January, D-April; Split, B-October, D-January, F-April).

Slika 2: (A-F) Odnos med standardno dolžino in številom prirastkov (Rovinj, A- oktober, C-januar, D-april; split, B-oktober, D-januar, F-april)

Region	Month	Intercept					
		b	SE	a	SE	( $\mu\text{m}$ )	$r^2$
Rovinj	October	0.078	0.002	1.92	0.03	6.83	0.98
	January	0.084	0.003	1.87	0.03	6.53	0.93
	April	0.073	0.003	1.94	0.03	6.95	0.96
Split	October	0.084	0.002	1.97	0.02	7.29	0.96
	January	0.084	0.002	1.96	0.03	7.09	0.97
	April	0.066	0.006	2.03	0.05	7.61	0.85

Table 3: Parameter estimates from exponential regression ( $y=e^{a+bx}$ ) of otolith radius ( $y$ ) against increment counts ( $x$ ). Intercept values correspond to the otolith radius at hatching.

Tabela 3: Ocena parametров eksponentne regresije ( $y = e$ ) premera otolitov ( $y$ ) glede na število prirastkov ( $x$ ). Prestrezne vrednosti so v skladu s premerom otolitov pri izvalitvi.

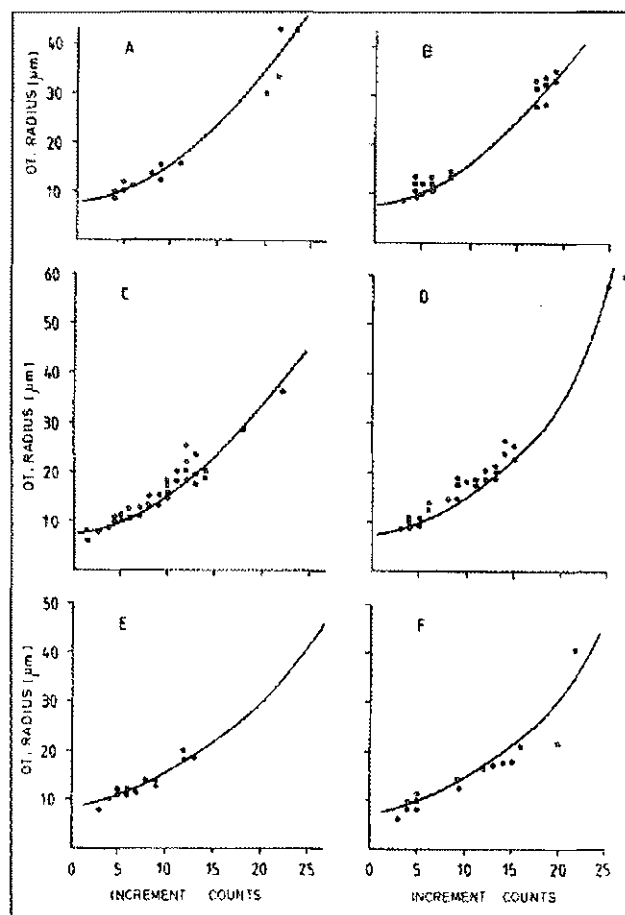


Fig. 3: (A-F) Relationships between otolith radius and increment counts (Rovinj, A-October, C-January, D-April; Split, B-October, D-January, F-April).

Slika 3: (A-F) Odnos med premerom otolitov in številom prirastkov (Rovinj, A-oktober, C-januar, D-april; Split, B-oktober, D-januar, F-april).

Region	Month	b	SE	a	SE	$r^2$
Rovinj	October	0.128	0.005	1.31	0.05	0.97
	January	0.128	0.005	1.49	0.05	0.92
	April	0.118	0.004	1.53	0.05	0.96
Split	October	0.137	0.005	1.30	0.05	0.95
	January	0.106	0.003	1.56	0.03	0.96
	April	0.080	0.008	1.81	0.07	0.84

Table 4: Parameter estimates from exponential regressions ( $y=e^{a+bx}$ ) of otolith radius ( $y$ ) against standard length ( $x$ ).

Tabela 4: Ocena parametров eksponentnih regresij ( $y = e$ ) premera otolitov ( $y$ ) glede na standardne dolžne ( $x$ ).

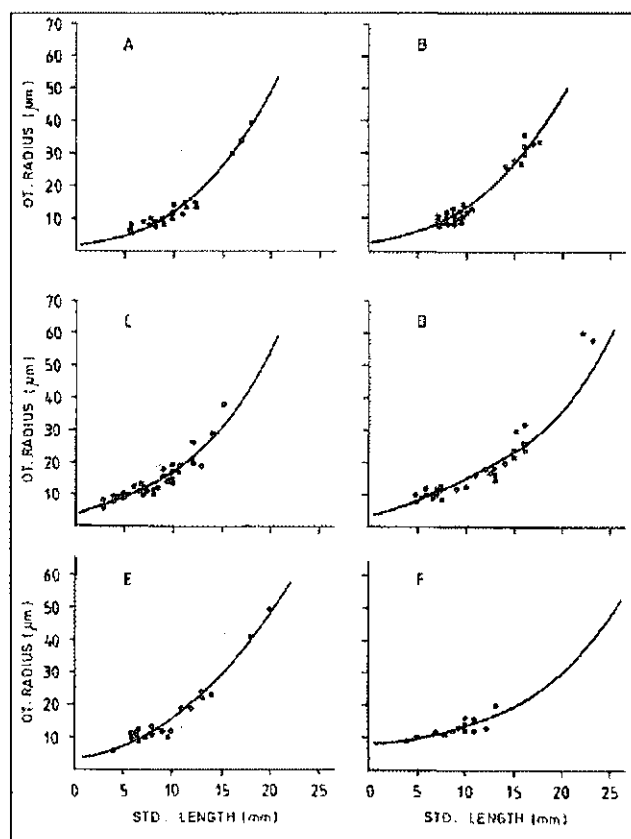


Fig. 4: (A-F) Relationships between otolith radius and standard length (Rovinj, A-October, C-January, D-April; Split, B-October, D-January, F-April).

Slika 4: (A-F) Odnos med premerom otolitov in standardno dolžino (Rovinj, A-oktober, C-januar, D-april; Split, B-oktober, D-januar, F-april).

#### F-test

To apply the F-test, log transformations of exponential regressions have been made (Tables 5, 6, 7).

Rovinj samples (sl vs in) showed no significant differences in slopes. Only the intercept with the y-axis in the April sample was different. However, this result could be an effect of an underestimation of actual age in that month. Variances were homogeneous in all cases. There was no difference between January and April samples off Split, whereas the October sample showed differences compared with the other months. However, these differences are doubtful because the variances were heterogeneous. No differences were found among the three regressions fitted for the Rovinj samples (ln or vs in). The slopes also did not differ between the three months off Split. These elevations were similar in January and April, but October elevations differed significantly from these. The slopes did not differ among Rovinj samples (ln or vs sl), whereas elevations showed significant differences. In Split, the results were similar

to those obtained for standard length vs increment counts regressions. There was no difference between January and April, whereas the October sample differed from these in both elevations and slopes.

Larval growth rates were higher in Split than in Rovinj. Both larval standard length and otolith radius at hatching were higher in Split as well.

F-tests were applied in the same way as with individual samples to compare slopes and elevations between regions (Table 8). Slopes of standard length vs increment counts were significantly different, in spite of heterogeneity of variances. Slopes of ln otolith radius vs standard length also differed significantly, whereas slopes of ln otolith radius vs increment counts did not differ. In this case, however, intercepts were significantly different.

Region	Months	Hom. Variances	Slopes	Elevations
Rovinj	Oct/Jan	F=1.00(24.57) P>0.5	F=1.07(1.81) P>0.25	F=42.21(1.81) P<0.005
Rovinj	Oct/Apr	F=1.22(24.30) P>0.5	F=0.07(1.54) P>0.25	F=34.59(1.55) P<0.005
Rovinj	Jan/Apr	F=1.18(57.30) P>0.5	F=0.58(1.87) P>0.25	F=0.08(1.88) P>0.25
Split	Oct/Jan	F=3.75(42.53) P<0.01	F=26.6(1.95) P<0.005	-
Split	Oct/Apr	F=3.92(21.53) P<0.01	F=4.40(1.74) 0.005<P<0.025	F=12.9(1.75) P<0.005
Split	Jan/Apr	F=1.03(21.42) P>0.5	F=0.15(1.63) P>0.25	F=6.06(1.64) 0.005<P<0.025

Table 5: Results of F-tests from comparisons of standard length vs increment counts regressions within each region.

Tabela 5: Rezultat F testov iz primerjav standardne dolžine in regresij števila prirastkov znotraj posameznega območja.

#### DISCUSSION

Reading the increments in sagitta otoliths of sardine larvae provides a good estimation of actual age because increments are laid down daily from the day of hatching (Dulčić, 1993). Ré (1983a) suggested that daily rings occurred in sardine sagittae not earlier than after the yolk-sac absorption (3-5 days after hatching). The deposition of the first increment in fish otoliths seems to be related to the duration of embryonic development, and otolith increments formed at different developmental stages were characteristics of the species being studied. Some species hatch with increments already formed, while others do not form increments until yolk-sac absorption.

Our results from estimating body growth rates indi

Region	Months	Hom. Variances	Slopes	Elevations
Rovinj	Oct/Jan	F=1.72(57.24)	F=2.54(1.81)	F=0.10(1.82)
		0.05<P<0.2	0.1<P<0.25	0.1<P<0.25
Rovinj	Oct/Apr	F=1.14(30.24)	F=1.57(1.54)	F=1.22(1.55)
		P>0.5	0.1<P<0.25	P>0.25
Rovinj	Jan/Apr	F=1.51(57.30)	F=6.72(1.87)	F=1.77(1.88)
		0.2<P<0.5	P>0.25	P<0.005
Split	Oct/Jan	F=1.27(42.53)	F=0.00(1.95)	F=71.32(1.96)
		P>0.5	0.005<P<0.025	P<0.005
Split	Oct/Apr	F=1.05(53.21)	F=6.51(1.74)	F=60.36(1.75)
		P>0.5	0.005<P<0.025	P<0.005
Split	Jan/Apr	F=1.32(42.21)	F=5.61(1.63)	F=5.71(1.64)
		P>0.5	0.005<P<0.0025	0.005<P<0.025

**Table 6: Results of F-tests from comparisons of  $\ln$  otolith radius vs increment counts regressions within each region.**

**Tabela 6: Rezultati F testov iz primerjav  $\ln$  radija otolita in regresij števila prirastkov znotraj posameznega območja.**

cated regional growth differences of Adriatic sardine larvae. The sampling sites were divided into two regions - the Istrian and Dalmatian coasts - based on the distribution of sardine eggs and larvae (Regner *et al.*, 1987). The growth rate was the same (0.59mm/day) in both regions in October and January. Growth was significantly higher in Split (0.76mm/day) than in Rovinj (0.61 mm/day) in January and April. The dominant factors influencing growth of fish larvae are temperature and food availability. The possible factor which produced the different growth rates observed here is the temperature difference recorded between the two regions. Houde (1989) reported that a 1°C increase in temperature will cause on average a 1% increase in specific growth rate. A comparison of the positions of sardine spawning centers and productive zones showed spawning centers to be located for the most part in the zone where production is lowest (oligotrophic area) (that zone includes stations in the Central Adriatic) (Regner *et al.*, 1987). That temperature distribution during winter is apparently the main reason. Based on long - term investigations, sardine spawns at temperature range from 11 to 22°C, with a maximum between 11 and 12.9°C (Karlovac, 1967). Since the temperature drops below 10°C in the northern Adriatic in winter (Buljan & Zore-Armanda, 1976), sardine cannot spawn in this productive zone for the major part of their spawning season. Dulčić (1993) found the greatest body length increment on the ninth day (0.92 mm) in the central Adriatic, but in this case all values for growth rates were obtained by Gompertz function. In January 1982, Ré (1983b) found a growth rate of sardine larvae in Portuguese waters lower (0.41

Region	Months	Hom. Variances	Slopes	Elevations
Rovinj	Oct/Jan	F=1.11(57.24)	F=0.00(1.81)	F=66.03(1.82)
		P>0.5	P>0.25	P<0.005
Rovinj	Oct/Apr	F=1.45(24.30)	F=2.00(1.54)	F=30.39(1.55)
		0.2<P<0.5	0.1<P<0.25	P<0.005
Rovinj	Jan/Apr	F=1.61(57.30)	F=1.94(1.87)	F=16.97(1.88)
		0.05<P<0.2	0.1<P<0.25	P=0.005
Split	Oct/Jan	F=1.04(53.42)	F=29.44(1.95)	-
		P>0.5	P<0.005	-
Split	Oct/Apr	F=1.47(53.21)	F=32.55(1.74)	-
		0.2<P<0.5	P<0.005	-
Split	Jan/Apr	F=1.41(42.21)	F=8.02(1.63)	F=0.16(1.64)
		0.2<P<0.5	0.005<P<0.025	P>0.25

**Table 7: Results of F-tests from comparisons of  $\ln$  otolith radius vs standard length regressions within each region.**

**Tabela 7: Rezultati F testov iz primerjav  $\ln$  radija otolita z regresijami standardne dolžine iz primerjav posameznega območja.**

mm/day) than the value registered in December 1982 in the same area (0.49mm/day) (Ré 1984).

An exponential model best fit the relationship between otolith radius and increment counts and between otolith radius and standard length. Uncoupling between growth larval length and otolith radius was found, but no uncoupling was observed in the relations between otolith radius and increment counts. This is due to the variation of otolith growth rate in relation to larval growth rate. Despite the direct relationship between otolith growth rate and larval growth, otoliths from larvae with high growth rates grow relatively slower than those from larvae with lower growth rates. In recent years there have been several reports indicating that somatic and otolith growth are not coupled, so that slow-growing fish produce larger otoliths than fast-growing fish (Secor & Dean, 1989; Reznick *et al.*, 1989; Wright *et al.*, 1990).

Variables	Hom. Variances	Slopes	Intercepts
S.L./ln.C.	F=2.03(65.89)	F=21.8(1.154)	-
	P<0.01	P<0.005	-
Ln Ot.R./ln.C.	F=1.08(89.65)	F=0.97(1.154)	F=110.5(1.155)
	P>0.5	P>0.25	P<0.005
Ln. Ot.R./S.L.	F=1.05(89.65)	F=20.0(1.154)	-
	P>0.5	P<0.005	-

**Table 8: Results of F-tests from comparisons between Split and Rovinj regressions (January and April).**

**Tabela 8: Rezultati F testov iz primerjav med splitskimi in rovinjskimi regresijami.**

The intercepts of standard length vs increment counts linear regressions with the y-axis represent the theoretical standard lengths and otolith radius of the larvae at hatching, respectively. Most of these values are very similar to those obtained from newly hatched larvae, about 3.5 mm for standard length and 6-7  $\mu$ m for otolith radius (Miranda *et al.*, 1990). Intercept estimates from linear regressions in October (Split) and January

(Rovinj) - the months with the lower growth rates - were clearly higher than actual values. This fact could be due to a slight underestimation of real age, and this underestimation could be explained by the deposition of very faint increments in the first days after yolk-sac depletion; such faint increments would not be visible with this technique (Campana *et al.*, 1987).

## POVZETEK

Zbiranje larv mediteranske sardele je potekalo od oktobra 1990 do aprila 1991. Stopnje rasti so bile ocenjene na osnovi dnevnih prirastkov na sagitnih otolithih. Izračunana je bila tudi rast otolitov glede na standardne dolžine in stevilo prirastkov. Pomembne razlike v rasti larv med istrskimi in dalmatinskimi obrežnimi vodami so bile zabeležene v istem mesecu. Tedaj je bila rast larv manjša v istrskih vodah. Razlike med določenimi meseci so bile ugotovljene tudi v splitskem območju. Rezultati so obravnavani glede na temperaturo.

## REFERENCES

- Brothers E.B., Mathews C.P., & Lasker R. 1976. Daily growth increments in otoliths from larval and adult fishes. *Fish. Bull. U.S.*, 74: 18.
- Buljan M., & Zore-Armanda M. 1976. Oceanographical properties of the Adriatic sea. *Oceanogr. Mar. Biol. Ann. Rev.*, 14: 11-98.
- Campana S., Gagne J., & Munro J. 1987. Otolith microstructure of larval herring (*Clupea harengus*): image or reality? *Can. J. Fish. Aquat. Sci.*, 44: 1992-1929.
- Dulčić J. 1993. Larval growth of sardine, *Sardina pilchardus* Walbaum, 1792, larvae in the eastern Adriatic. *Oebalia*, vol. XIX: 115-125.
- Gamulin T., & Hure J. 1983. Mriješćenje i mrestilišta pelagičnih riba u Jadranskom moru. *Acta Adriat.*, 24: 97-131.
- Houde E.D. 1989. Comparative growth, mortality and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fisheries Bulletin U.S.*, 87: 471-495.
- Karlovac J. 1967. Etude de l'ecologie de la sardine (*Sardina pilchardus* Walb.) dans la phase planctonique de sa vie en Adriatique moyenne. *Acta Adriat.*, 13 (2): 109 pp.
- Methot R. 1981. Growth rates and age distribution of larvae and juvenile northern anchovy, *Engraulis mordax*, larvae in the sea. Ph.D. Thesis UC San Diego.
- Miranda A., Cal R.M., & Iglesias J. 1990. Effect of temperature on the development of eggs and larvae of sardine *Sardina pilchardus* Walbaum in captivity. *J. Exp. Mar. Biol. Ecol.*, 140: 69-79.
- Pannella G. 1971. Fish otoliths daily growth layers and periodical patterns. *Science (Wash. D.C.)*, 173: 1124-1127.
- Pannella G. 1974. Otolith growth patterns: An aid in age determination in temperate and tropical fishes. In: T.B. Bagenal (Ed.). *The ageing of fish*, D: 28-30.
- Piccinetti C., Regner S., & Specchi M. 1981. Distribution des oeufs de sardine en Adriatique. *Rapp. Comm. int. Mer Médit.*, 27 (5): 167-170.
- Regner S., Piccinetti C., & Specchi M. 1983. Estimate of spawning biomass of sardine in the northern and central Adriatic from 1979 to 1982 by means of egg surveys. *FAO Fish. Rep.*, 290: 223-232.
- Regner S., Regner D., Marasović I., & Kršinić F. 1987. Spawning of sardine, *Sardina pilchardus* (Walbaum, 1792), in the Adriatic under upwelling conditions. *Acta Adriat.*, 28 (1-2): 161-198.
- Reznick D., Lindbeck E., & Bruga H. 1989. Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). *Can. J. Fish. Aquat. Sci.*, 46: 108-112.
- Ré P. 1983a. Daily growth increments in the sagitta of pilchard larvae, *Sardina pilchardus* (Walbaum, 1792) (*Pisces: Clupeidae*). *Cybiurn*, 7 (3): 9-15.
- Ré P. 1983b. Growth of pilchard larvae *Sardina pilchardus* (Walbaum, 1792) in relation to some environmental factors. *Inv. Pesq.*, 47 (2): 277-283.
- Ré P. 1984. Evidence of daily and hourly growth in pilchard larvae based on otolith increments, *Sardina pilchardus* (Walbaum, 1792). *Cybiurn*, 8 (1): 33-38.
- Secor D.H., & Dean J.M. 1989. Somatic growth effect on the otolith-fish size relationship in young pond-reared striped bass, *Morone saxatilis*. *Can. J. Fish. Aquat. Sci.*, 46: 113-121.
- Smith P., Richardson P. 1977. standard techniques for pelagic eggs and larvae surveys. *FAO Fish. Tec. Papers*, 175: 100 pp.
- Snedecor G., Cochran W. 1989. Statistical methods. Iowa State University Press, Ames, Iowa.
- Wright P.J., Metacalfe N.B., & Thorpe J.E. 1990. Otolith and somatic growth rates in Atlantic salmon parr, *Salmo salar* L.: evidence against coupling. *Journal of Fish Biology*, 36: 241-249.