

Age, growth and mortality of the toadfish, *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae), in the Bay of Cádiz (southwestern Spain)

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SUMMARY: Age, growth and mortality of the toadfish, *Halobatrachus didactylus*, were determined by examination of the whole sagittal otoliths of fish sampled in the Bay of Cádiz (southwestern Spain) from March 1999 to March 2000. A total of 844 specimens (425 males, 416 females, and 3 of indeterminate sex), ranging from 95 to 470 mm in total length were examined. Eighty-nine percent of the otoliths could be read allowing an age estimation. The opaque zone was formed between April and May coincident with the maximum reproductive peak, while the translucent zone formed mainly in summer-fall (June to December). Maximum ages for males and females were 12 and 10 years, respectively. The samples were dominated by 2- to 6-year-old specimens. Males matured at an age of approximately 2 years and females at 3 years. Fish total length and otolith radius were closely related ($r^2 > 0.80$). The von Bertalanffy growth curve was used to describe growth. The parameters were derived from back-calculated length-at-age. Significant differences in the growth parameters were found between sexes (in males $L_{\infty} = 477.06$ mm, $K = 0.15$ and $t_0 = -0.59$; in females $L_{\infty} = 363.69$ mm, $K = 0.20$ and $t_0 = -0.75$). Although the growth analysis revealed that this species is slow-growing, males reached larger sizes than females. Females appeared to experience higher natural mortality rates than males.

Keywords: age, growth, mortality, otolith, toadfish, *Halobatrachus didactylus*, Bay of Cádiz.

RESUMEN: EDAD, CRECIMIENTO Y MORTALIDAD DEL PEZ SAPO, *HALOBATRACHUS DIDACTYLUS* (SCHNEIDER, 1801) (PISCES: BATRACHOIDIDAE), EN LA BAHÍA DE CÁDIZ (SUROESTE DE ESPAÑA). – Se determinó la edad y se estimaron los parámetros de crecimiento y la tasa de mortalidad natural de *H. didactylus* con base en el análisis de los otolitos sagita de ejemplares capturados mensualmente en la Bahía de Cádiz (suroeste de España) entre marzo de 1999 y marzo de 2000. Se examinó un total de 844 ejemplares (425 machos, 416 hembras, y 3 con sexo indeterminado), con tallas que oscilaron entre 95 y 470 mm de longitud total. El 89% de los otolitos analizados resultó legible y permitió asignar un número de anillos. El crecimiento anual estuvo representado por una zona opaca y una translúcida. La zona opaca se forma entre abril y mayo, coincidiendo con el máximo pico reproductivo, mientras que la zona translúcida se forma principalmente en verano-otoño, (junio-diciembre). El recuento de zonas anuales en los otolitos permitió establecer hasta 12 grupos de edad en los machos y 10 en las hembras. En ambos casos predominaron los ejemplares con 2–6 años. Los machos alcanzan la madurez sexual, aproximadamente, a los 2 años de edad y las hembras a los 3 años. La longitud total del pez y el radio de los otolitos estuvieron fuertemente relacionados $r^2 > 0.80$. El crecimiento individual fue determinado mediante ajuste al modelo de von Bertalanffy. Los parámetros de crecimiento estimados para machos y hembras a partir de las longitudes a edad pretérita para cada edad fueron significativamente diferentes (machos: $L_{\infty} = 477.06$ mm; $k = 0.15$ y $t_0 = -0.59$; hembras: $L_{\infty} = 363.69$ mm; $k = 0.20$ y $t_0 = -0.75$). Aunque puede considerarse como una especie de crecimiento lento, los machos alcanzan mayores longitudes que las hembras. Las tasas de mortalidad natural estimadas fueron ligeramente superiores en las hembras.

Palabras clave: edad, crecimiento, mortalidad, otolito, pez sapo, *Halobatrachus didactylus*, Bahía de Cádiz.

INTRODUCTION

The toadfish, *Halobatrachus didactylus* (Schneider), is a benthic, sedentary species occurring in the eastern Atlantic Ocean from the Bay of Biscay to Ghana and in the western Mediterranean Sea (Roux, 1986). It inhabits shallow water—tidal channels and creeks, inlets, beaches—to a depth of 50 m, over soft sand, mud, seagrasses and weed beds or concealed in rock crevices (Roux, 1986; Fischer *et al.*, 1987). Owing to its euryhaline ability it is capable of colonising diverse biotopes, from brackish waters of river estuaries to hypersaline waters of salt ponds (Arias and Drake, 1990; Lopes da Cunha, 1994; Costa *et al.*, 2000; Fernández-Delgado *et al.*, 2000). It is a common fish species in the Bay of Cádiz, and although it has a relatively low commercial value, its ecological importance is quite significant, and it is considered an important component of the fish communities (Arias, 1976). In the Bay of Cádiz this species is caught by artisanal and recreational fishermen. It shows great resilience, being able to survive for hours out of water (personal observations). Though this species has been used extensively as a laboratory animal for cardiological, physiological and toxicological studies for more than three decades (see references in Palazón-Fernández *et al.*, 2001), biological information on it is scarce. In previous papers the reproductive biology and cytogenetics of the species were reported (Palazón-Fernández *et al.*, 2001; Palazón *et al.*, 2003).

Age and growth in the Batrachoididae family have been studied using various methods, including length-frequency analysis (Serafy *et al.*, 1997) and inferences from observed marks on hard parts such as vertebrae (Schwartz and Dutcher, 1963) and otoliths (Wilson *et al.*, 1982; Radtke *et al.*, 1985). Otoliths have been used extensively to age fish, and have been suggested to be the most accurate ageing structures (Six and Horton, 1977; Casselman, 1983; Campana *et al.*, 1995; Campana, 2001) because they are not susceptible to resorption, undergo no chemical alteration once formed, and are available in species which lack or have tiny scales (Mugiya and Watabe, 1977; Six and Horton, 1977; Campana, 1983).

No previous information on age, growth and mortality of *H. didactylus* exists. The objective of the present study was to estimate age, growth and mortality of toadfish by examining the sagittal otoliths.

MATERIALS AND METHODS

Samples of *H. didactylus* were collected monthly from artisanal fishermen's captures in Cádiz Bay (southwestern Spain) between March 1999 and March 2000. These fish were caught primarily with gillnets. Meristic measurements were taken on each fish, including total length (L_T) measured to the nearest mm, and whole weight (W_T) and gutted weight (W_G) measured to the nearest gram. Length and weight frequency distributions were compared by sex using the Kolmogorov-Smirnov two-sample test (Zar, 1984). Sex was established by macroscopic examination of the gonads.

Both sagittal otoliths were removed, rinsed and stored dry for later age determination. Otoliths were viewed with a dissection microscope under reflected light. Opaque zones and translucent zones exhibited an alternating pattern. The total number of opaque zones was recorded in order to assign an estimated age to each toadfish. Length-at-age included any growth that occurred after the last opaque zone was formed. Otoliths were read without reference to other data.

As a comparison between whole and sectioned otoliths, 179 otoliths from the full size range of the samples were sectioned. Three transverse (dorsoventral) sections from the left otolith of each fish were taken using a low-speed saw. One section was made on either side of the core, and the other encompassed the core. The sections were mounted on glass slides with thermal cement and examined through a microscope under reflected light. Immersion oil was applied to each section to enhance the legibility of the growth zones on the section.

A zone count was assigned to 83% of the sections (148 of 179). Of the 148 samples, 73% (108) agreed in zone count with the whole otoliths and 88% (130) agreed within ± 1 . Agreement was reached over the full range of ages though the majority of the samples that disagreed by 2 or more zones was primarily from fish with more than 6 zones. The Wilcoxon signed-rank test was applied to the pairs of whole and sectioned otolith ages for each fish. No significant difference was found between ages from both methods (Wilcoxon signed-rank test, $n = 148$; $P > 0.05$). Both whole and sectioned otoliths were considered reliable methods for aging toadfish for the age range of our study, so it was decided to use the whole otoliths because this method is less costly and time-consuming.

Otoliths that were completely translucent, broken, or with structural irregularities, such as unusual calcification, deterioration, and poorly defined annual growth increments, were considered unreadable and were excluded from further analysis.

Radial measurements on the distal side of the whole otoliths were taken from the core to the outer edge of each successive opaque zone and to the otolith margin when possible using an image analysis system. It was possible to measure 87% of the aged samples (658 of 754). However, samples with 11 and 12 opaque zones could not be measured because those zones were more closely formed together, making accurate measures more difficult.

The relationship of fish total length-at-capture (L_C) and otolith radius-at-capture (R_C) was described by regressing the observed total length on otolith radius. The linear equation was $L_C = a + bR_C$.

The periodicity of opaque zone formation was examined by edge-type analysis (Rocha-Olivares and Gómez-Muñoz, 1993; Newman and Dunk, 2003). The marginal edge on each otolith was examined and classified as opaque or translucent. Percentages of otoliths with opaque and translucent margins were plotted by month of capture for all months in order to locate periodical trends in zone formation. Moreover, the marginal increment ratio (MIR) was estimated for each specimen by the following formula (Araújo and Martins, 2007):

$$\text{MIR} = (R_T - R_L) / (R_L - R_{L-1})$$

where R_T is the total radius of otolith, R_L is the radius to the last annulus formed and R_{L-1} is the radius to the penultimate annulus formed.

The mean MIR and the standard deviation were computed for each month by sex for all ages combined and also for each age separately. An analysis of variance (ANOVA) was used to detect significant differences by sex, age group and month of capture. The Student-Newman-Keuls (SNK) multiple range test was used to contrast means when treatment differences were significant (Zar, 1984). Means (\pm S.D.) were plotted against month of capture, the minima indicating the month of annulus formation.

Once the periodicity and timing of zone formation were verified, the age of each fish was determined from the number of annuli, the assumed birthday, and the sampling date. Because the peak spawning period of *H. didactylus* occurs in May (Palazón-Fernández

et al., 2001), all fishes were assigned a birth date of 1 May to ensure proper year-class identification.

The back-calculated total lengths at each age were determined from the body proportional equation (Francis, 1990):

$$L_A = [(a+bR_A)/(a+bR_C)] L_C$$

where L_A = back-calculated total length to annulus A; R_A = otolith radius to annulus A; R_C = total otolith radius at time of capture; L_C = total length at time of capture; a = intercept from the linear total length–otolith radius regression; and b = slope from the linear total length–otolith radius regression.

The von Bertalanffy growth model,

$$L_t = L_\infty [1 - \exp(-K(t - t_0))],$$

was fitted to back-calculated lengths-at-ages for the most recently formed annuli (Vaughan and Burton, 1994; Potts and Manooch, 2002). Growth parameters were estimated by non-linear regression with Marquart's algorithm (Draper and Smith, 1981).

Growth models were developed separately for males and females to account for possible sex-specific growth rates. Differences in growth curves for the sexes were tested using the F test (Chen *et al.*, 1992). Individuals whose sex could not be determined because their gonads were undifferentiated, but which were determined to have one annulus, were included in the back-calculations for each sex in order to increase sample size and give more weight to the few one-year-old fish.

Longevity was calculated from Taylor's equation (Taylor 1958):

$$A_{0.95} = t_0 + \frac{2.996}{K}$$

where $A_{0.95}$ is the lifespan or age required to reach 95% of the final length (L_∞), and t_0 and K are von Bertalanffy growth parameters.

Estimates of natural mortality rates (M) were obtained using two methods. The first estimate of M was based on Taylor's formulae (Taylor, 1958)

$$M = \frac{2.996K}{2.996 + Kt_0}$$

where K and t_0 are parameters of the von Bertalanffy growth equation.

Natural mortality was also computed by the method of Pauly (1980), assuming a mean annual

water temperature (T) for the Bay of Cádiz of 18.4°C (Forja, 1990):

$$\log M = -0.0066 - 0.279 \log L_{\infty} + 0.6543 \log K + 0.4634 \log T$$

In order to compare growth performance of fish within the Batrachoididae, the parameter Φ' (Pauly and Munro, 1984) was calculated:

$$\Phi' = \log K + 2 \log L_{\infty}$$

where K is the growth constant and L_{∞} is the asymptotic length derived from the von Bertalanffy growth curve.

RESULTS

A total of 844 toadfishes (425 males, 416 females and 3 individuals of indeterminate sex) ranging in size from 95 to 470 mm total length were examined.

Male and female total length ranged from 137 to 470 mm L_T and from 95 to 366 mm L_T , respectively (Fig. 1). A higher proportion of females were smaller than males. Males dominated in the >300 mm size classes, whereas females were more abundant in the 220 – 260 mm size classes. The Kolmogorov-Smirnov test ($n_1 = 425, n_2 = 416; P < 0.01$) showed significant differences between male and female frequency distributions. The mean length of males (264.78 ± 2.96 mm) was significantly greater than that of females (238.20 ± 1.87 mm) (t test, $df = 839, P < 0.01$).

Male and female total weight ranged from 50 to 2105 g and from 30 to 1075 g, respectively. A Kolmogorov-Smirnov test ($n_1 = 425, n_2 = 416; P < 0.01$) indicated significant differences between sexes. The mean weight of males (362.44 ± 13.98 g) was significantly greater than that of females (238.68 ± 6.31 g) (t test, $df = 839; P < 0.01$).

Otoliths displayed well-defined alternating thin opaque zones and wide translucent zones under reflected light (Fig. 2). An opaque zone combined with a translucent zone was interpreted as one year's growth. Enumerating the opaque zones on the otoliths of the toadfish was relatively easy. Whole otoliths were read with little difficulty, and of the 844 otoliths sampled 754 (89%) were read successfully. As a result of the decreased growth as the fish grows older, successive annuli are progressively closer so it

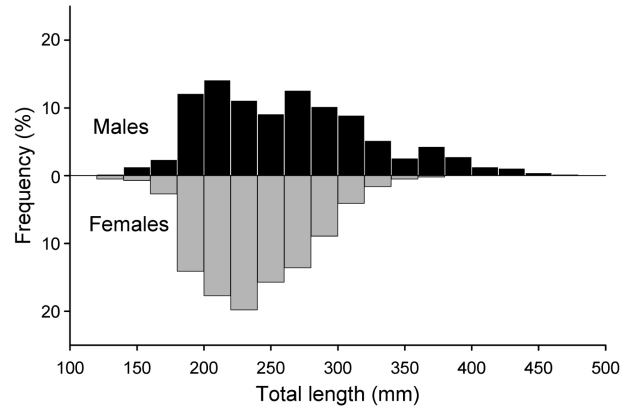


FIG. 1. – Length-frequency distributions (20-mm length classes) of male and female toadfish sampled for age determination in the Bay of Cádiz between March 1999 and March 2000.

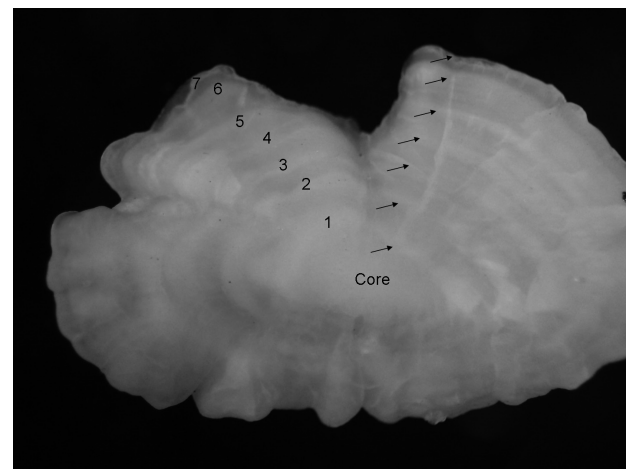


FIG. 2. – Sagittal otolith of a 7-year-old toadfish. Opaque margin, May 1999, 235 mm L_T .

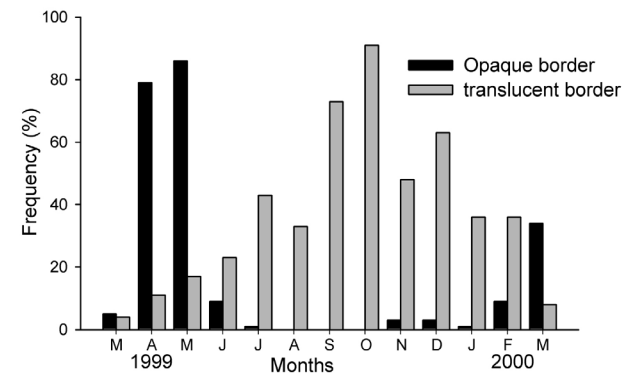


FIG. 3. – Monthly frequency of opaque and translucent margins of otoliths of *H. didactylus* sampled between March 1999 and March 2000 in the Bay of Cádiz.

was fairly difficult to observe and measure accretion of newly formed material at the margin of the otolith in older fish.

Formation of growth increments followed a seasonal pattern (Fig. 3). The opaque zones begin

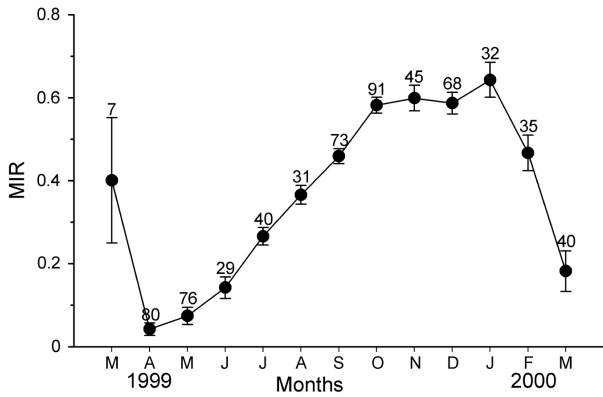


FIG. 4. – Monthly means of marginal increment ratio (MIR) of otoliths of *H. didactylus* sampled between March 1999 and March 2000 in the Bay of Cádiz. Vertical bars are ± 1 SE; numbers on the top of vertical bars are sample sizes.

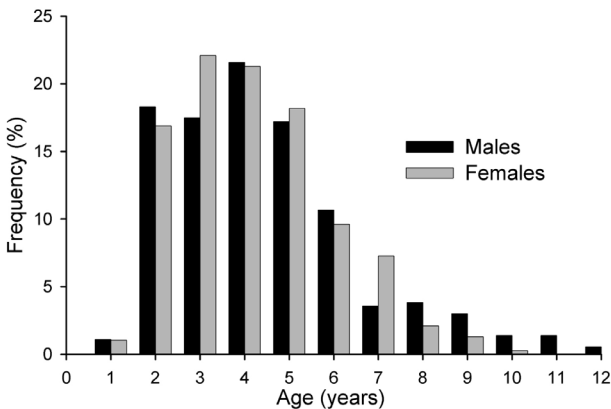


FIG. 5. – Age distribution for male and female toadfish sampled in the Bay of Cádiz between March 1999 and March 2000.

to develop in late autumn, though they are not completely formed until the next spring (April-May), coincident with peak spawning (Palazón-Fernández *et al.*, 2001). In these months, the proportion of otoliths with opaque margins was highest (>80%). A marked decline in June and July, followed by an absence of opaque margins from August to October indicates cessation of opaque annulus formation and the onset of translucent annulus formation beginning in late spring. The translucent zones were laid down mainly in summer-autumn (June-December) when growth is faster. The data suggest that only one opaque and one translucent zone are laid down per year and represent valid annual growth increments.

The monthly mean MIR (sexes and all ages combined) showed a single minimum in April-May (Fig. 4). This pattern indicated the formation of one annulus per year in April-May. This same trend was exhibited by monthly means of MRI when plotted by sex, and for the different ages, respectively. ANOVA detected significant differences during the year (df

= 11,626; $P < 0.01$). The S.N.K. test showed that the MIR in April-May was significantly lower than in the rest of the year, providing further evidence that annulus formation occurred yearly in April-May. The maximum values occurred in October-December and January. The ANOVA did not show significant differences for the sexes (df = 1,646; $P > 0.05$) and/or age group (df = 8,626; $P > 0.05$).

Age distributions for the sexes are exhibited in Figure 5. The maximum age of the sampled toadfish was 12 years for males and 10 years for females. Age distribution was similar for both sexes and was skewed to the left. The population was dominated by 2- to 6- year-old fish, which represented 85% to 88% of the fish sampled. Fish younger than 2 years and older than 9 years were poorly represented. After age 7, males dominated the older age classes.

Somatic growth (L_c -mm) and otolith growth (R_c -mm) were strongly linearly related in both males ($r^2 = 0.88$) and females ($r^2 = 0.81$). ANCOVA revealed significant differences in the relationship between males and females (df = 1,721; $P < 0.001$), so separate models were fitted for each sex. Figure 6 shows the sex-specific relationship between total length and otolith radius. In both cases, an ANOVA revealed a

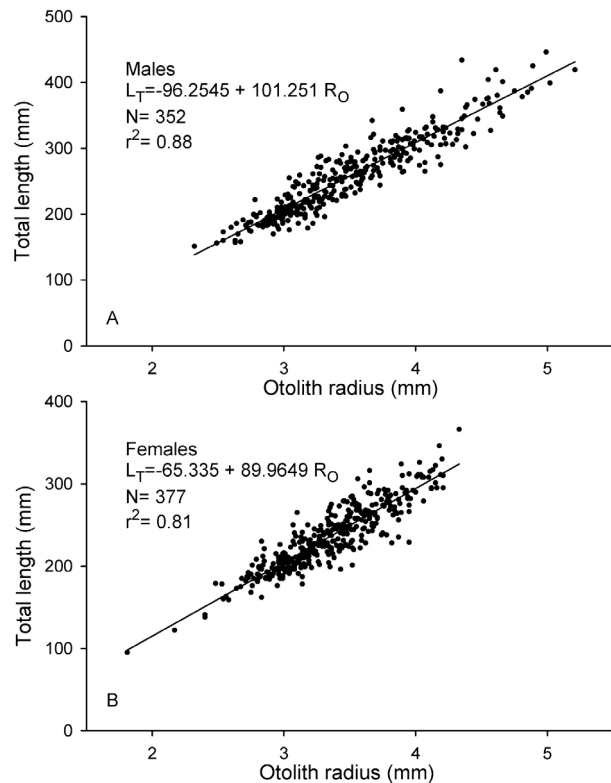
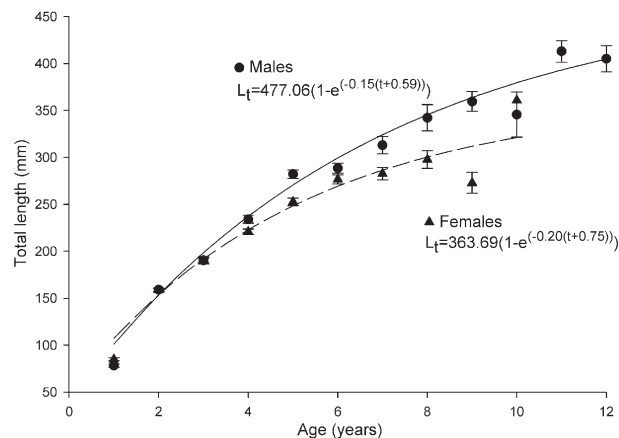


FIG. 6. – Relationship between total length at capture and otolith radius for male and female toadfish sampled in the Bay of Cádiz between March 1999 and March 2000.

TABLE 1. – Mean observed and back-calculated lengths-at-ages for male and female *H. didactylus* sampled in the Bay of Cádiz between March 1999 and March 2000.

Age class (years)	N	Observed Mean L_T	I	II	III	IV	Annulus V	VI	VII	VIII	IX
Males											
1	5	145.6	78.3								
2	66	199.7	73.8	159.1							
3	63	214.4	58.4	132.2	190.4						
4	73	248.5	61.1	130.1	187.9	234.1					
5	56	291.6	65.4	138.5	198.3	245.2	282.1				
6	29	295.5	59.7	128.7	182.8	226.2	261.2	288.5			
7	8	323.8	59.7	128.3	185.7	228.6	264.3	292.0	313.1		
8	6	343.9	67.0	142.1	202.6	241.7	274.0	302.2	325.0	342.3	
9	4	361.8	53.8	121.9	173.4	216.6	256.7	291.2	318.7	339.9	356.8
Mean			64.2	138.3	190.4	235.9	273.4	291.1	318.3	341.3	356.8
Increment				74.1	52.1	45.4	37.3	17.7	26.0	21.4	
Females											
1	3	138.9	85.1								
2	65	193.4	84.7	158.7							
3	83	216.5	71.4	137.6	190.5						
4	79	234.4	73.0	134.4	182.4	220.8					
5	62	257.2	76.5	135.1	182.9	222.0	252.5				
6	30	278.2	79.5	140.4	189.9	228.1	256.0	276.6			
7	16	283.5	70.6	131.7	174.0	206.7	234.9	261.0	282.8		
8	3	289.6	82.8	136.8	187.6	215.7	243.1	263.3	282.0	297.7	
Mean			76.1	140.4	185.3	221.0	250.6	270.5	282.7	297.7	
Increment				64.3	44.9	35.7	29.6	19.9	12.1	15.0	

FIG. 7. – von Bertalanffy growth curves derived from back-calculated age-at-lengths for male and female *H. didactylus* sampled in the Bay of Cádiz between March 1999 and March 2000.

highly significant relationship (in males $df = 1,350$ and $P < 0.001$; in females $df = 1,371$ and $P < 0.001$). Moreover, the lack-of-fit test revealed that the linear model describes the relationship satisfactorily for both males ($df = 161,189$; $P > 0.05$), and females ($df = 149,222$; $P > 0.05$), and explained 87.8% (males) and 81.1% (females) of the observed variation; therefore, a direct proportional method can be used for back-calculation of total lengths at each age.

Of the otoliths aged, 87% (658 of 754) were considered legible to record measurements from the

TABLE 2. – Growth parameters (\pm SE) derived from the von Bertalanffy growth function for *H. didactylus* sampled in the Bay of Cádiz between March 1999 and March 2000.

Parameter	Males	Females
L_∞	477.06 \pm 29.28	363.69 \pm 24.21
K	0.15 \pm 0.02	0.20 \pm 0.04
t_0	-0.59 \pm 0.21	-0.75 \pm 0.28
n	326	345
r^2	81.41	74.78

core to each successive opaque zone and the otolith margin. The mean back-calculated lengths-at-age for 1- to 9-year-old specimens are listed in Table 1. Growth increments were higher during the first year of life (mean 74.1 mm and 64.3 mm L_T for males and females, respectively). After that, the growth rates of both sexes slowed appreciably. Males and females showed similar mean lengths until age 3; afterwards, males grew faster and consequently reached larger sizes than females. The differences become more pronounced with increasing age (Table 1; Fig. 7).

The von Bertalanffy growth curves for males and females are shown in Figure 7, and the corresponding parameter estimates are listed in Table 2. Growth curves fitted very closely to the back-calculated lengths. Predicted lengths fitted well with the back-calculated length-at-age data for both males ($r^2 = 0.82$, $n = 325$) and females ($r^2 = 0.74$, n

TABLE 3. – Mean observed, back-calculated (B-C) and theoretical total lengths for male and female *H. didactylus* sampled in the Bay of Cádiz between March 1999 and March 2000.

Age	Males			Females		
	Observed	B-C	Theoretical	Observed	B-C	Theoretical
1	145.6	64.2	101.2	138.9	76.1	107.4
2	199.7	138.3	153.6	193.4	140.4	153.9
3	214.4	190.4	198.6	216.5	185.3	191.9
4	248.5	235.9	237.4	234.4	221.0	223.0
5	291.6	273.4	270.8	257.2	250.6	248.5
6	295.5	291.1	299.5	278.2	270.5	269.4
7	323.8	318.3	324.3	283.5	282.7	286.5
8	343.9	341.3	345.5	289.6	297.7	300.5
9	361.8	356.8	363.9	286.7	285.4	312.0
10	350.5	345.7	379.6	366.0	360.9	321.3
11	413.0	413.0	393.2			
12	405.0	405.0	404.9			

TABLE 4. – Estimated natural mortality rates for male and female *H. didactylus* sampled in the Bay of Cádiz between March 1999 and March 2000.

Method	Males	Females
Taylor	0.15	0.21
Pauly	0.20	0.26

= 345). Growth models for males and females were significantly different from one another (F test; $df = 3,663$; $P < 0.001$). After age 3, males were growing at a faster rate and reached larger sizes and weights than females (Fig. 7).

The estimated life span of the toadfish was 19.4 years for males and 14.2 years for females. The growth performances of this Batrachoid were 4.53 and 4.42 for males and females, respectively.

Observed lengths-at-age were consistently higher than those back-calculated and/or predicted by the growth curves (Table 3); the differences were higher at lower ages and were interpreted as a consequence of growth that occurred between annuli deposition and time of capture.

Estimates for natural mortality (M) are shown in Table 4. These estimates varied slightly depending on the method used, but both methods indicate a slightly greater mortality in females.

DISCUSSION

In the present study, sagittal otoliths were determined to be valid structures for age and growth studies in *H. didactylus*. They satisfy the criteria stressed by van Oosten (1929) and Williams and Bedford (1974): a) otoliths can readily be located

and removed, and are relatively easy to prepare, store and read; b) a recognizable pattern can be seen in the otoliths; c) growth of the otoliths was proportional to the overall growth of the fish; d) the number of growth zones increased with an increase in fish size and the marking pattern was similar for fishes of different ages; e) growth checkmarks formed once a year and at approximately the same time; and f) back-calculated lengths-at-age agree with observed lengths-at-capture.

Otoliths were also successfully used by Wilson *et al.* (1982) and Radtke *et al.* (1985) to assess age structure in the oyster toadfish *O. tau*. Radtke considered otoliths as useful structures for age and mortality estimations in this species. Similar to the results found in *O. tau*, *H. didactylus* otoliths exhibited a well-defined and consistent mark pattern consisting of one thinner opaque zone deposited during slow growth in winter and one thicker translucent zone formed during fast growth in summer.

Validation of the periodicity of ring formation is a critical step for using hard parts for age and growth studies in fish (Beamish and McFarlane, 1983; Zaboukas and Megalofonou, 2007). The marginal increment analysis used in this study indicated the time of opaque zone formation and clearly showed that annual growth increments are formed once per year. Translucent zones are formed during summer, whereas opaque zone formation begins in late autumn (November) and is completed in April-May, coincident with peak spawning. Opaque zone formation in winter-spring has been reported for sagittae of several other teleosts in the northeastern Atlantic and Mediterranean (Landa and Piñeiro, 2000; Zaboukas and Megalofonou, 2007).

The periodicity of opaque zone formation, and its relation with reproductive activity observed in *H. didactylus*, have also been reported in other fish species such as the red snapper *Lutjanus purpureus* (Poey) (Lima, 1965), the swordfish, *Xiphias gladius* Linnaeus (Tserpes and Tsimenides, 1995), the big-eye tuna, *Thunnus obesus* (Lowe) (Sun *et al.*, 2001), and the Indo-Pacific sailfish, *Istiophorus platypterus* (Shaw) (Chiang *et al.*, 2004). A possible explanation for this would be that, during the spawning season, energy may be allocated primarily for gamete production instead of somatic growth, causing a slowdown in growth rates. Furthermore, during the spawning season male toadfishes may decrease their feeding activity while they guard nests and take care of their eggs and fry. This hypothesis, although

plausible for adult fish, cannot explain the formation of annuli in young immature fish so annulus formation is not necessarily a direct consequence of the reproductive activity, and must also be related to seasonal patterns of growth rather than reproduction. Many factors other than spawning can affect growth rates of fish, including seasonal changes in environmental factors, mainly water temperatures, feeding regime and related effects on metabolism, as observed in other fish species (Casselman, 1983; Morales-Nin and Ralston, 1990; Newman and Dunk, 2003; Kingsford and Hughes, 2005), although the determining factor is unknown.

Overall, the numbers of sampled males and females were nearly equal, but males were predominant in samples greater than 7 years old. The data also suggest a difference in maximum length and age attained by the sexes. The oldest male collected was 12 years old, whereas the oldest female was 10 years old. A similar pattern was observed in the oyster toadfish, *O. tau*, by Radtke *et al.* (1985), who concluded that the differences in sex-specific numbers by age for *O. tau* resulted from females experiencing a higher mortality rate as a result of the increased energy investment for gametes in females. Schwartz and Dutcher (1963) and Radtke *et al.* (1985) reported similar maximum ages for *O. tau* (males, 12 years; females, 9 years) to that observed in *H. didactylus*.

Female toadfish matured at a larger size and at a greater age (191 mm, age 3) than males (160 mm, age 2). Similar to the observations of Armstrong *et al.* (1992) for the goosefish, *Lophius americanus* Valenciennes, female toadfish may require a larger body size to accommodate the large eggs. GSI in toadfish females can be as high as 40% gutted weight (Palazón-Fernández *et al.*, 2001). The slowing of growth coincides with age at first maturity and support the argument of Day and Taylor (1997) and Kritzer (2004) that maturation represents a pivotal physiological transformation. Consequently, a fundamental shift in the growth trajectory occurs and reproductive development is at the expense of somatic growth.

Toadfish are medium-lived fish, and the maximum ages for each sex must be near the maximum age for the species owing to the low fishing pressure on the population. Toadfish reach sexual maturity at 160 mm L_T for males and 191 mm L_T for females (Palazón-Fernández *et al.*, 2001), which correspond to 2.1 and 3.0 years of age for males and females, respectively. As we can see in Figure 5, 99% of the

males and 82% of the females had reached sexual maturity at time of capture, so the fishery is mainly based on adults.

In this study the male lifespan was greater than that of females, and the males attained a larger size than females. During the reproductive station, males stay in the nesting sites guarding the eggs and fry. This behaviour could render the males less vulnerable to predation or fishing. The presence of larger males in the population may have a selective advantage during the reproductive season if they must defend a spawning pit or brood against potential predators. Because sperm production requires less energy than egg production (Bateman, 1948; Jalaibert and Zohar, 1982), the slowed growth observed in females compared with males is likely due to differences in energy budgets during the reproductive season. Moyle and Cech (1988) indicated that the differences in length between the sexes are the most common form of sexual dimorphism among fishes. Schwartz and Dutcher (1963) and Radtke *et al.* (1985) found similar differences between the sexes for *O. tau* caught in Maryland and York River, respectively, with females growing at a lower rate and attaining younger ages than males. Wilson *et al.* (1982) noted no sex related differences in growth for a population of *O. tau* in South Carolina, although they found differences in maximum age attained by the sexes, which were lower for females.

The strong association between the size of the fish and otolith radius found in *H. didactylus* indicates that otoliths grow proportionally to toadfish length. This is an important assumption in growth studies using hard parts and allows for back-calculation. This relationship also indicates that otoliths are useful structures for age estimation and as indicators of the past growth history of the species. Similar results were reported for *O. tau* by Radtke *et al.* (1985), who found a strong association between otolith dimension and body growth and stated that otolith length could be chosen as the best parameter for estimation of fish total length. The strong relationship between otolith and fish growth found in most fish is an essential step for back-calculation and one of the criteria why otoliths have been suggested to be one of the most accurate ageing structures (Manooch, 1987; Wootton, 1990).

Upon reviewing the back-calculated data (Table 1), we found some evidence of Lee's phenomenon (Lee, 1912) in the collection of fish. The linear regression of the radius to the first opaque zone on the

TABLE 5. – Comparisons of estimates of the von Bertalanffy growth parameters, ϕ' value and natural mortality rates reported by various authors for some batrachoid species.

Species	L_{∞}	K	t_0	ϕ'^1	M^2	Investigators
<i>Opsanus beta</i> ³	300	0.168	-0.09	4.18	0.17	Seraffy <i>et al.</i> , 1997
<i>Opsanus beta</i> ³	300	0.218	-0.27	4.29	0.22	Seraffy <i>et al.</i> , 1997
<i>Opsanus tau</i> ♂	407.5	0.15	-0.33	4.40	0.15	Radtke <i>et al.</i> , 1985
<i>Opsanus tau</i> ♀	271.5	0.39	-0.44	4.46	0.41	Radtke <i>et al.</i> , 1985
<i>H. didactylus</i> ♂	477.1	0.15	-0.59	4.53	0.15	This study
<i>H. didactylus</i> ♀	363.7	0.20	-0.75	4.42	0.21	This study

¹ values calculated from author's data; ² values calculated from author's data using the equation of Taylor (1958); ³ both sexes.

number of opaque zones on the otolith did produce a slope significantly different from 0 ($P < 0.01$) but the model explained very little of the variation (2.3%). There was such a spread in the data at each count, and small sample sizes on the two tails that the difference is very slight and probably of limited biological significance.

Growth parameters obtained in the present work represent the first estimations of toadfish growth. The von Bertalanffy growth equations fit the back-calculated lengths very well. Table 5 shows the population parameters from the von Bertalanffy growth curves compared with those of other batrachoididae. The growth rates of toadfish in our study were within the range of those estimated for other members of the family in temperate waters. Even though differences can be observed between the population parameters, the similarity of the ϕ' values indicates the existence of a similar growth pattern. This result supports the statement of Sparre *et al.* (1989) that the ϕ' values must be similar in members of the same family.

Our estimate of M is similar to the figures estimated for other batrachoid species (Table 5) except for *O. tau* females, which have a higher mortality ($M = 0.41$) than males of the same species, and to the estimations made for the rest of the batrachoids. Similar to our observations in *H. didactylus*, *O. tau* females show a heavier mortality than males (Radtke *et al.*, 1985). This may be caused by increased predation due to their smaller size, to behavioural or distributional differences that make them more susceptible to predation or fishing effort, or to the existence of higher environmental pressures acting on the females. Another possibility is that they simply reach senescence before males.

Many factors can affect growth rates of fish, including differences in the seasonality of spawning, environmental factors, amount and size of food, and genetics (Weatherly and Gill, 1987; Moyle and Cech, 1988). Differences in reproductive traits (Palazón-

Fernández *et al.*, 2001; Modesto and Canario, 2003), as well as genetic and morphological differentiation (Marques *et al.*, 2006) between geographical regions in the range of distribution of the toadfish, may indicate that growth rates of the species also differ. Future research is needed to compare growth and mortality of toadfishes from different areas in their range of distribution.

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REFERENCES

- Araújo, J.N. and A.S. Martins. – 2007. Age, growth and mortality of white grunt (*Haemulon plumieri*) from the central coast of Brazil. *Sci. Mar.*, 71(4): 793-800.
- Arias, A. – 1976. Contribución al conocimiento de la fauna bentónica de la Bahía de Cádiz. *Inv. Pesq.*, 40 (2): 355-386.
- Arias, A. and P. Drake. – 1990. *Estados juveniles de la ictiofauna en los caños de las salinas de la Bahía de Cádiz*. Instituto de Ciencias Marinas de Andalucía, Junta de Andalucía, Cádiz.
- Armstrong, M.P., J.A. Musick and J.A. Colvocoresses. – 1992. Age, growth and reproduction of the goosefish, *Lophius americanus* (Pisces: Lophiiformes). *U.S. Fish. Bull.*, 90: 217-230.
- Bateman, A.J. – 1948. Intra-sexual selection in *Drosophila*. *Heredity*, 2: 349-368.
- Beamish, R.J. and G.A. McFarlane. – 1983. Validation of age determination estimates: the forgotten requirement. In: E. Prince and L. Pulos (eds.), *Proceedings of the international workshop on age determination of oceanic pelagic fishes: Tunas, Billfishes, and Sharks*. NOAA Tech. Rep. NMFS, 8: 29-33.
- Campana, S.E. – 1983. Calcium deposition and otolith check formation during periods of stress in coho salmon, *Oncorhynchus kisutch*. *Comp. Biochem. Physiol.*, 75a: 215-220.
- Campana, S.E. – 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.*, 59: 197-242.
- Campana, S.E., M.C. Annand and J.I. McMillan. – 1995. Graphical and statistical methods for determining the consistency of age

- determinations. *Trans. Am. Fish. Soc.*, 124: 131-138.
- Casselman, J.M. – 1983. Age and growth assessment of fish from their calcified structures- techniques and tools. *NOAA Tech. Rep. NMFS*, 8: 1-17.
- Chen, Y., D.A. Jackson and H.H. Harvey. – 1992. A comparison of von Bertalanffy and polynomial functions in modeling fish growth data. *Can. J. Fish. Aquat. Sci.*, 49: 1228-1235.
- Chiang, W., C. Sun and S. Yeh. – 2004. Age and growth of sailfin (*Istiophorus platypterus*) in waters off eastern Taiwan. *Fish. Bull.*, 102(2): 251-263.
- Costa, J.L., G. Silva, P. Almeida and M.J. Costa. – 2000. Activity and diet of *Halobatrachus didactylus* (Bloch & Schneider, 1801) adults in the Tagus estuary. *Thalassas*, 160: 21-25.
- Day, T. and P.D. Taylor. – 1997. Von Bertalanffy's growth equation should not be used to model age and size at maturity. *Am. Nat.*, 149: 381-393.
- Draper, N.R. and H. Smith. – 1981. *Applied Regression Analysis*, 2nd ed. John Wiley and Sons, Inc, New York.
- Fernández-Delgado, C., P. Drake, A.M. Arias and D. García-González. – 2000. *Peces del Parque Nacional de Doñana y su entorno*. Ser. Téc. Minist. Medio Ambiente, Madrid.
- Fischer, W., M. Schneider and M. Bauchot. – 1987. *Fiche FAO d'identification des especes pour les besoins de la peche. Mediterranée et Mer Noire*, vol II. Vertebres. FAO, Rome.
- Forja, J. – 1990. *Regeneración bentónica de nutrientes en la Bahía de Cádiz*. Ph. D. thesis, Univ. Cádiz, Spain.
- Francis, R.I.C.C. – 1990. Back-calculation of fish length: a critical review. *J. Fish Biol.*, 36(6): 883-902.
- Jalabert, B. and Y. Zohar. – 1982. Reproductive physiology in cichlid fishes, with particular reference to *Tilapia* and *Sarotherodon*. In: R.S.V Pullin and R.H. Lowe-McConnell (eds.), *The biology and culture of tilapias*. ICLARM Conf. Proc. 7: 129-140. International Center for Living Aquatic Resources Management, Manila, Philippines.
- Kingsford, M.J. and J.M. Hughes. – 2005. Patterns of growth, mortality and size of the tropical damselfish *Acanthochromis polyacanthus* across the continental shelf of the Great Barrier Reef. *Fish. Bull.*, 103: 561-573.
- Kritzer, J.P. – 2004. Sex-specific growth and mortality, spawning season, and female maturation of the stripey bass (*Lutjanus carponotatus*) on the Great Barrier Reef. *Fish. Bull.*, 102: 94-107.
- Landa, J. and C. Piñeiro. – 2000. Megrim (*Lepidorhombus whiffiagonis*) growth in the North-eastern Atlantic based on back-calculation of otolith rings. *ICES J. Mar. Sci.*, 57: 1077-1090.
- Lee, R.M. – 1912. *An investigation into the methods of growth determination in fishes*. *Cons. Int. Explor. Mer Publ. Circumstance*, 63: 1-34.
- Lima, F.R. – 1965. Crescimento do pargo (*Lutjanus aya* Block, 1795). Aspectos quantitativos. *Bol. Est. Pesca*, 5: 33-42.
- Lopes da Cunha, P.G. – 1994. *Estrutura e dinâmica da ictiofauna do estuário do Sado*. Ph. D. thesis, Lisbon Univ., Portugal.
- Manooch, C.S. III. – 1987. Age and growth of snappers and groupers. In: J.J. Polovina and S. Ralston (eds.), *Tropical snappers and groupers: biology and fisheries management*, pp. 329-374. Westview Press Incorporated, Boulder, Colorado.
- Marques, J.F., A.L. Rego, J.L. Costa, M.J. Costa and H.N. Cabral. – 2006. Genetic and morphologic differentiation of the Lusitanian toadfish (*Halobatrachus didactylus*) between estuarine and coastal areas in Portugal. *Sci. Mar.*, 70(4): 749-758.
- Modesto, T. and A. Canario. – 2003. Morphometric changes and sex steroid levels during the annual reproductive cycle of the Lusitanian toadfish, *Halobatrachus didactylus*. *Gen. Comp. Endocrinol.*, 131: 220-231.
- Morales-Nin, B. and S. Ralston. – 1990. Age and growth of *Lutjanus kasmira* (Forsk.) in Hawaiian Waters. *J. Fish Biol.*, 36(2): 191-203.
- Moyle, P.B. and J.J. Cech, Jr. – 1988. *Fishes: an introduction to ichthyology*, 2nd ed. Prentice-Hall, Inc, Englewood Cliffs, N.J.
- Mugiya, Y. and N. Watabe. – 1977. Studies on fish scale formation and resorption. II. Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, *Carassius auratus*, and the killifish *Fundulus heteroclitus*. *Comp. Biochem. Physiol.*, 57a: 197-202.
- Newman, S.J. and I.J. Dunk. – 2003. Age validation, growth, mortality, and additional population parameters of the goldband snapper (*Pristipomoides multidens*) off the Kimberley coast of northwestern Australia. *Fish. Bull.*, 101(1): 116-128.
- Palazón-Fernández, J.L., A.M. Arias and M.C. Sarasquete. – 2001. Aspects of the reproductive biology of the toadfish *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae). *Sci. Mar.*, 65(2): 131-138.
- Palazón, J.L., M. Nirchio and C. Sarasquete. – 2003. Conventional karyotype and nucleolar organiser regions of the toadfish *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae). *Sci. Mar.*, 67(4): 445-449.
- Pauly, D. – 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. *J. Cons. Int. Explor. Mer*, 39: 175-192.
- Pauly, D. and L. Munro. – 1984. Once more on growth comparison in fish and vertebrates. *Fishbyte*, 2(1): 21.
- Potts, J. and C.S. Manooch III. – 2002. Estimated ages of red porgy (*Pagrus pagrus*) from fishery-dependent and fishery-independent data and a comparison of growth parameters. *Fish. Bull.*, 100: 81-89.
- Radtke, R., M. Fine and J. Bell. – 1985. Somatic and otolith growth in the oyster toadfish (*Opsanus tau* L.). *J. Exp. Mar. Biol. Ecol.*, 90: 259-275.
- Rocha-Olivares, A. and V.M. Gómez-Muñoz. – 1993. Validation of otolith age determination of the pacific red snapper *Lutjanus peru* (Perciformes: Lutjanidae) in La Paz Bay and adjacent waters, B.C.S., Mexico. *Cienc. Mar.*, 19(3): 321-331.
- Roux, C. – 1986. Batrachoididae. In: P.J.P. Whitehead, M.L. Bauchot, J.C. Hureau, J. Nielsen and E. Tortonese (eds.), *Fishes of the North-eastern Atlantic and the Mediterranean*. pp. 1360-1361. UNESCO, Paris.
- Schwartz, F.J. and B.W. Dutcher. – 1963. Age, growth, and food of the oyster toadfish near Solomons, Maryland. *Trans. Am. Fish. Soc.*, 92(2): 170-173.
- Serafy, J.E., T.E. Hopkins and P.J. Walsh. – 1997. Field studies on the ureogenic gulf toadfish in a subtropical bay. Patterns of abundance, size composition and growth. *J. Fish Biol.*, 50(6): 1258-1270.
- Six, L.D. and H.F. Horton. – 1977. Analysis of age determination methods for yellowtail rockfish, canary rockfish, and black rockfish off Oregon. *Fish. Bull.*, 75: 405-414.
- Sparre, P., E. Ursin and S. Venema. – 1989. Introduction to tropical fish stock assessment. Part 1 Manual. *FAO Fish. Tech. Pap.*, 306 (1): 1-337.
- Sun, C.L., C.L. Huang and S.Z. Yeh. – 2001. Age and growth of the bigeye tuna, *Thunnus obesus*, in the western Pacific Ocean. *Fish. Bull.*, 99: 502-509.
- Taylor, C.C. – 1958. Cod growth and temperature. *J. Cons. Int. Explor. Mer*, 23: 366-370.
- Tserpes, G. and N. Tsimenides. – 1995. Determination of age and growth of swordfish, *Xiphias gladius* L. 1758, in the eastern Mediterranean using anal fin spines. *Fish. Bull.*, 93: 594-602.
- Van Oosten, J. – 1929. Life History of the lake herring (*Leucichthys arcti* LeSueur) of Lake Huron as revealed by the scales, with a critique of the scale method. *Bull. U.S. Bur. Fish.*, 44: 265-448.
- Vaughan, D.S. and M.L. Burton. – 1994. Estimation of von Bertalanffy growth parameters in the presence of size-selective mortality: a simulation example with red grouper. *Trans. Am. Fish. Soc.*, 123: 1-8.
- Weatherly, A.H. and H.S. Gill. – 1987. *The biology of fish growth*. Academic Press, Orlando, FL.
- Williams, T. and B.C. Bedford. – 1974. The use of otoliths for age determination. In: T. Bagenal (ed.), *The proceedings of an international symposium on the ageing of fish*, pp. 114-123. Unwin Brothers Limited, Surrey, England.
- Wilson, C.A., J.M. Dean and R. Radtke. – 1982. Age, growth, and feeding habits of the oyster toadfish, *Opsanus tau* (Linnaeus) in South Carolina. *J. Exp. Mar. Biol. Ecol.*, 62: 251-259.
- Wootton, R. – 1990. *Ecology of fishes*. Chapman and Hall, New York.
- Zaboukas, N. and P. Megalofonou. – 2007. Age estimation of the Atlantic bonito in the eastern Mediterranean Sea using dorsal spines and validation of the method. *Sci. Mar.*, 71(4): 691-698.
- Zar, J.H. – 1984. *Biostatistical Analysis*. 2nd ed. Prentice Hall, Englewood Cliffs.

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