Environ Biol Fish (2011) 91:369–378
DOI 10.1007/s10641-011-9796-0

Age and growth of the small red scorpionfish, *Scorpaena notata* Rafinesque, 1810, based on whole and sectioned otolith readings

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Received: 12 March 2010 / Accepted: 16 March 2011 / Published online: 1 April 2011

Abstract Age and growth of *Scorpaena notata* from the northern Adriatic Sea were investigated by annual growth increment counts (*annuli*). Overall, age and growth were estimated from 538 specimens of *S. notata* ranging between 47 and 199 mm TL. No clear sexual dimorphism in size was observed. Annual deposition of annuli and location of the first annulus have been validated by edge analysis and daily growth increment counts, respectively. The estimated age range was between 0–16 years for female and 0–14 years for males. The estimated values of Von Bertalanffy asymptotic length L_∞ (cm) and k (years^{-1}) were 16.3 and 0.46 for males and 15.6 and 0.35 for females. Thus, males of *S. notata* appeared to attain a slightly larger size at faster rate than females. The growth performance index ranged between 1.9 and 2.1, which is in the middle of the range observed in other scorpaenids. Comparing ageing data of *S. notata* with other Mediterranean scorpaenids, a direct relationship between fish size and growth performance was observed.

Keywords Adriatic Sea · Age and growth · Scorpaenidae · *Scorpaena notata*

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Introduction

The small red scorpionfish, *Scorpaena notata* (Rafinesque 1810) (Pisces, Scorpaenidae), is a benthic sedentary species distributed in the Eastern Atlantic from the Bay of Biscay to Senegal, off Madeira, Azores and the Canary Islands, in the Mediterranean Sea and the Black Sea, where it is represented by the subspecies *Scorpaena notata afimbria* (Slasteneko 1939) (Hureau and Litvinenko 1986). Although *S. notata* is considered to be rare in the northern Adriatic Sea (Hureau and Litvinenko 1986), it was frequently found in recent surveys close to natural and artificial hard bottom (Fabi et al. 2004; Casellato and Stefanon 2008), playing likely a more important role in the benthic fish community of rocky habitat than previously thought.

The small red scorpionfish is generally less than 20 cm total length (TL), and inhabits preferably rocky bottoms inside crevices or sea grass meadows, but it is also captured by trawlers operating on sandy bottoms in the proximity of hard substrates (Hureau and Litvinenko 1986; Harmelin-Vivien et al. 1989; Morte et al. 2001; Relini et al. 2007).

Several biological aspects of *S. notata* have been studied in the Mediterranean Sea, such as diet (Harmelin-Vivien et al. 1989; Morte et al. 2001), gonad morphology (Muñoz et al. 1996, 2002a, b), fecundity and reproductive cycle (Muñoz et al. 2005) and the relationships with artificial and natural habitats (Relini et al. 2002; Ordines et al. 2009). The only data concerning age and growth are presented in a publication based on scales reading of specimens from the Gulf of Gabes (Bradai and Bouain 1990) and by a recent study of Ordines et al. (2009) about the habitat preference of *S. notata* in Balearic Islands.

The present paper aims to improve the knowledge of ageing procedure for *S. notata* by means of otolith readings, also observed after sectioning, in order to reach a better understanding of the life span and the calculation of the Von Bertalanffy growth parameters of the species, applying also indirect age validation methods to support the reliability of age estimates.

Material and methods

Samples of *S. notata* were collected in the northern Adriatic Sea between July 2004 and November 2008. The study area included both natural reefs consisting of hard substrates and artificial structures, such as off-shore gas platforms (Fig. 1). Sampling was carried out in the proximity of natural reefs and artificial structures, using a beam trawl with 40 mm cod-end stretched mesh size and trammel nets with 70 and 400 mm stretched mesh size. Hauls performed with beam trawl were randomly located over the whole sampling area. The beam trawl was generally towed at about 4.8–5.2 knots for 15–30 min on the bottom during daylight hours. Conversely, trammel nets, positioned close to artificial structures and natural reefs, were set at dusk and pulled in at dawn, with an average fishing time of 12 h.

The size distribution of the sample could be affected by the selectivity of the gears employed. Anyway, the beam trawl was towed at a speed of six knots and, hence, its selectivity was strongly reduced by the scarce opening of codend meshes. It is acknowledged that codends of such gears are virtually non-selective for the sizes of most finfish (Rotherham et al. 2008). In addition, the particular shape of *S. notata* (round body with many spines) strongly reduces the probabilities of escaping thoughout the codend meshes.

A study on gillnet selectivity carried out in Mediterranean showed that this gear is scarcely selective for *Scorpaena porcus* (Stergiou and Erzini 2002). As a matter of fact, trammel nets are widely considered less selective if compared to gillnets, and, also in this case, the presence of many spines around the body of scorpenids favour the non-selective way of capture called "entanglement". On this basis, it can be realistic to assume that the samples collected during fishing survey may be representative of the pupulation at sea.

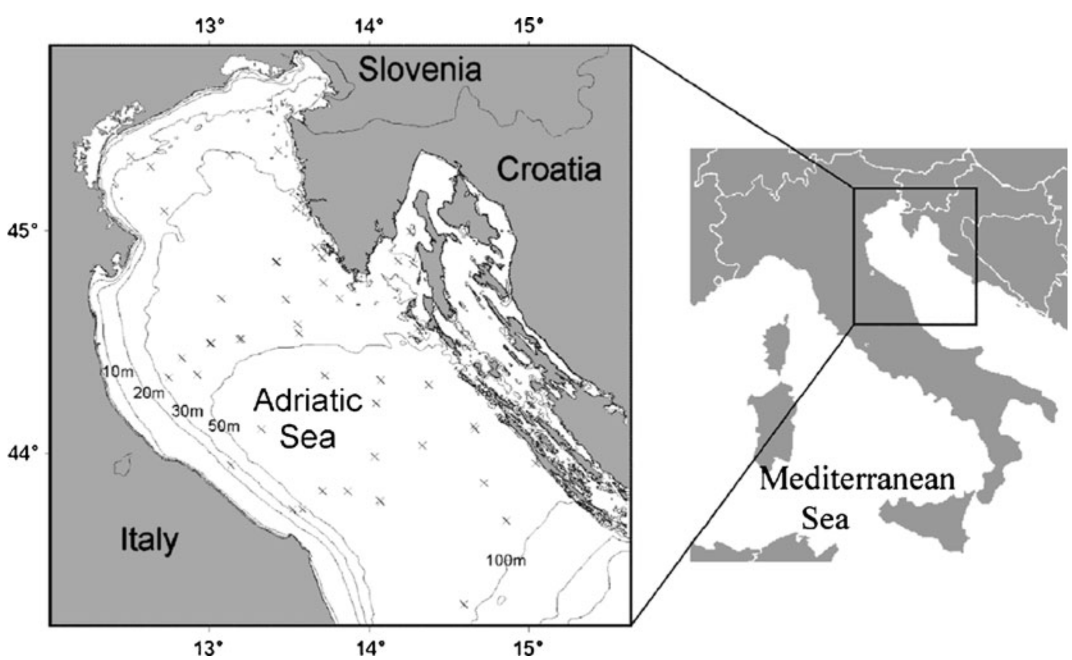

Fig. 1 Map of northern Adriatic Sea with sampling locations

In the laboratory, wet weight (W, 0.1 g), TL, measured to the lowest mm, and sex, determined by macroscopic examination of gonads were recorded for each specimen. In small specimens, gonads were observed by a light microscope to aid sex determination. The sagittal otoliths were removed from all specimens, cleaned and stored dry.

The length–weight relationship of fish was calculated both for the whole population and for each sex. The exponential equation $W=aTL^b$, where W is total weight (0.1 g), TL is total length of fish (mm) and a and b are regression parameters, was fitted to the data. The equation was linearized applying the $\log_{10}$ transformation of data to estimate the regression parameters. An *F*-test was used to test for difference between males and females in allometric indices (b) (Sokal and Rohlf 1995).

The weight of both left and right sagitta was recorded with an accuracy of 0.1 mg and compared using a *t*-test for paired comparisons (Sokal and Rohlf 1995). As no difference was found (Student's *t*-test, d.f.=806, $t=1.647$, $p>0.05$), the maximum (OD_{max}; 0.01 mm) and the minimum diameter (OD_{min}; 0.01 mm) were measured from one randomly selected sagitta (left or right) on the whole sample, under a stereomicroscope coupled to a video camera using an image analysis software (OPTIMAS 6.5). The relationship between TL and OD_{max} and OD_{min} was investigated by linear regression analysis.

Otoliths were immersed in ethanol with distal face up and the annuli were counted using a binocular microscope under reflected light against a dark background (magnification: ×25 and ×40). The nucleus and the opaque zones of otolith appeared as light rings and the translucent or hyaline zones as dark rings. The combination of each opaque and subsequent translucent zone was considered to be an annulus, as observed in other scorpaenids (Massutí et al. 2000; López Abellán et al. 2001).

In larger fish, the annulation pattern was difficult to discriminate due to considerable otolith thickness. As a consequence, these otoliths were embedded in epoxy resin and transversally sectioned. Otolith sections were then polished with 0.05 μm alumina paste and read under reflected light following the same aforementioned procedure. To compare the two readings procedures also in smaller otoliths, a representative sample of them was read directly as a whole and then sectioned. As the age estimates were the same, the sectioning practice was carried out only on the thick otoliths of larger (older) fish.

The count path of annuli in whole otoliths was generally from the nucleus towards the tip of the rostrum, where the deposition of seasonal rings appeared to start. Otoliths were firstly read by one reader, without any ancillary data on fish size. A second reading was carried out a week later by the same reader.

When readings differed by one or more annuli, a third reading was made; if the third reading differed from the previuos two, the otolith was discarded. The index of average percent error (APE) (Beamish and Fournier 1981), as well as the mean coefficient of variation (CV) (Chang 1982), were calculated to estimate the relative precision between readings.

To assess the annual nature of ring deposition, i.e. the accuracy of age estimates, two different indirect or semi-direct methods were applied. To validate specimens aged 0, i.e. fish with sagittae composed of only an opaque nucleus, some otoliths were randomly selected for microincrements (daily rings) counting. Following other studies on scorpaenids (Laidig and Ralston 1995; Massutí et al. 2000), we assumed that microincrements were laid down daily, providing the true age (in days) of aged specimens. Otoliths were set in moulds, embedded in epoxy resin and ground to obtain sagittal sections. They were then polished with 0.05 μm alumina paste and the microincrements counted under a light microscope at magnification ×400–630.

To validate the seasonality of deposition of opaque and translucent zones, the relative frequency of an opaque zone on the otolith margin was plotted by month (Beckman and Wilson 1995; Panfili and Morales-Nin 2002). The cycle in formation of the opaque and translucent zones should equal 1 year in true annuli (Campana 2001).

Once the age estimates were validated, the Von Bertalanffy growth function was fitted to the age-length data using the routine FISHPARM from the statistical package FSAS (Saila et al. 1988), which implements the Marquardt algorithm for non-linear least squares parameter estimation. The Von Bertalanffy growth parameters (L_∞, k and t_0) were calculated for each sex and for the whole population (including unsexed fish). Finally, the growth performance index ($\Phi'=2 \log L_\infty + \log k$) (Munro and Pauly 1983), was calculated to compare the growth of *S. notata* with other scorpaenids.

Results

Overall, 570 specimens of *S. notata* were collected, 239 females, 302 males and 29 unsexed individuals. The sex ratio differed significantly from 1:1, males being more abundant than females (χ^2=7.3, d.f.=1, p<0.01). Males and females had similar size ranges, 65 to 198 mm and 47 to 199 mm, respectively, and size range of unsexed fish was between 75 and 155 mm (Fig. 2). Individual fish weights varied between 1.7 and 169.0 g for females and between 5.4 and 143.7 g for males.

The relationships between TL (mm) and W (0.1 g) was calculated for each sex and for the whole population including unsexed fish, as summarized in the following equations:

$$W = 0.000017TL^{3.07},\ n = 239,\ r^2 = 0.97 \qquad \text{females}$$
$$W = 0.000016TL^{3.09},\ n = 302,\ r^2 = 0.98 \qquad \text{males}$$
$$W = 0.000017\ TL^{3.08},\ n = 570,\ r^2 = 0.96 \qquad \text{total}$$

The coefficient b was not significantly different between sexes (F-test, F_{1-536}=0.236, p>0.1), and nearly isometric.

Maximum and minimum otolith diameters (OD_{max}, OD_{min}) varied linearly with fish length, according to the following equations:

$$OD_{max} = 0.47\ TL + 0.55, \qquad n = 570, \qquad r^2 = 0.89$$
$$OD_{min} = 0.17\ TL + 0.65, \qquad n = 570, \qquad r^2 = 0.76$$

The annulation pattern of otoliths was composed of alternating opaque and translucent zones. Outside the opaque nucleus, the first 2–3 rings were wide and easily recognizable, followed by rings of decreasing thickness towards the otolith margin (Fig. 3). In larger fish (>150 mm), the outer rings close to the margin were difficult to discriminate, so the transverse sections were helpful to obtain a more reliable count of annuli (Fig. 4).

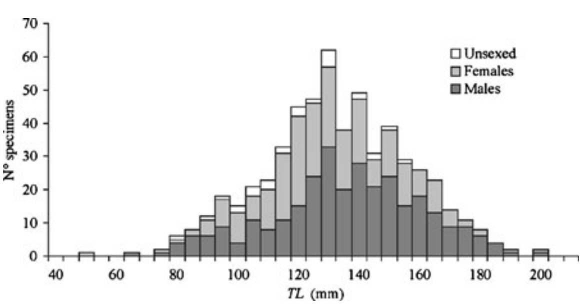

Fig. 2 Length frequency distribution of *Scorpaena notata* sampled in the northern Adriatic Sea

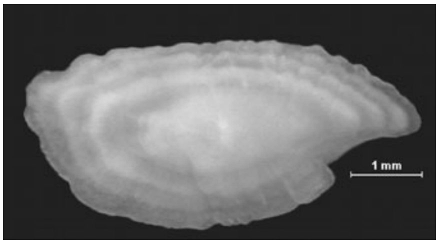

Fig. 3 Photograph showing the annulations pattern of *Scorpaena notata* sagittal otolith, 3-years-old male, 112 mm total length (TL)

Microincrement counts to validate fish aged 0 (i.e. young of the year) were carried out on ten specimens ranging between 47 and 96 mm TL. The sagittae of *S. notata* showed the typical pattern of light and dark alternated microincrements, representing daily growth rings (Fig. 5). A continuous series of concentric rings of increasing size, ranging from 1.0 to 3.6 μm, were observed from the core to the otolith margin. Accessory primordia were also observed in some specimens. The age estimates ranged from 140 to 300 days, validating all otoliths aged 0 characterized by an opaque nucleus surrounded by a more or less developed translucent zone.

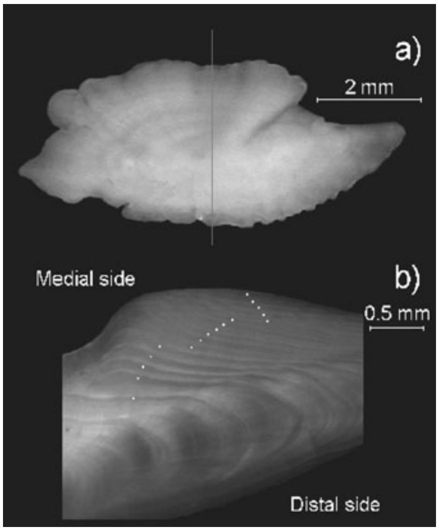

Fig. 4 Photographs of (**a**) surface and (**b**) cross-section otolith of *Scorpaena notata*, 16-years-old female, 165 mm total length (TL). Vertical line indicate plane observed by cross-section. Dots denote annulus from 1st to 16th

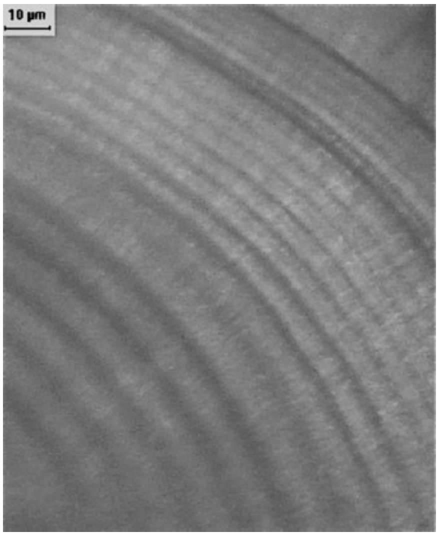

Fig. 5 Photomicrograph of otolith microstructure in the core region, showing the typical pattern of alternating light and dark increments assumed to be deposited daily. *Scale bar* = 10 µm

The edge analysis, performed on the whole fish sample, validated the annual deposition of each annulus formed by an opaque and translucent zone. In particular, the opaque zones were laid down from April to August, whereas the translucent zones were laid down from September to March (Fig. 6).

Out of 570 otoliths examined, only 32 (approximately 5%) were discarded, because they were either unreadable or provided different age estimates between readings. Counting variability indices, CV and APE, were both quite low (11.6% and 8.2%, respectively), indicating that the ageing procedure adopted gave a reasonable level of consistency (or reproducibility) between readings.

Age estimates ranged between 0 and 14 years for males and between 0 and 16 years for females (Table 1). However, the fish sample was mainly composed of 1–4-year-old fish, representing 80% and 72% of males and females, respectively. The Von Bertalanffy growth function was fitted to the age-length data pairs for each sex, and for the whole population (Table 2, Fig. 7). The parameters estimated were significantly different between sexes only if considered together, with male showing L_∞, k and t_0 slightly higher than females (Table 3). The growth performance index (Φ'), calculated for each sex, is reported in Table 2. Lengths-at-age, calculated from the Von Bertalanffy growth function, provides estimates of growth increments by age. The annual growth rate ranged between 19.2 and 0.2 mm for females and 28.8 and 0.1 mm for males in the estimated age range (Table 4).

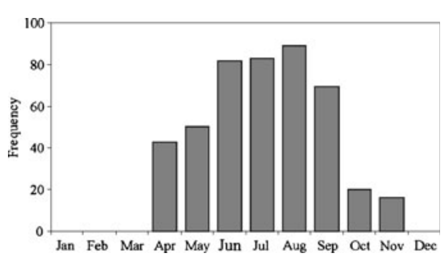

Fig. 6 Monthly change in relative frequency of the opaque zone and translucent zone on the otolith margin of *Scorpaena notata*

Discussion

Comparing the results of the age reading reported in the present paper with those obtained in other published works on small red scorpionfish, it is possible to discover evident discrepancies (Table 2). The ageing methodology (scales reading) adopted by Bradai and Bouain (1990), as well as the relatively narrow fish size range composed of small individuals, could have led to an underestimate of the life span of *S. notata* from the Gulf of Gabes. Indeed, differently from scales, otoliths are one of the few calcified structures that is nonskeletal and their growth is maintained even through periods when somatic growth is nonexistent (Maillet and Checkley 1990; Campana and Thorrold 2001). The advantage of a continuous growth pattern is most evident in studies of old fish, in which annulus counts from scales grossly underestimate those visible in the otolith (Beamish and McFarlane 1995). Instead, the study from Balearic Islands (Ordines et al. 2009) was based on otolith readings carried out on a large fish sample (947 individuals), characterized by a wide size range comparable to the Adriatic sample. However, age estimates were performed on whole otoliths, which again could have led to an underestimate of age in older (larger) specimens of *S. notata*, considering that generally otoliths of these individuals are extremely opaque and too thick to allow a reliable estimate of rings close to the otolith margin.

Interestingly, S. *notata* did not show a clear sexual size dimorphism in growth, although males may have a slightly greater size at age than females. This is in

Table 1 Age length key of *Sorpaena notata* sampled in the northern Adriatic Sea, and the number of fish (*n*)

Lenght class (mm)	Age class (year)																
Females	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
40	1																
50																	
60																	
70																	
80	2	3															
90	3	11	1														
100		4	5	6													
110		8	13	13	3			1									
120		1	15	18	6	1	1	2	2								
130			6	10	11	2	1	1	2	1		1					
140			3	5	6	1	1	1	2		2	2					
150			1	3	5	2		3	3		2	1	2	1		1	
160				1	3	2	1		1	2	1	2	1	1		1	1
170						1					1			1			
180					1				1								
190																	1
n	6	27	44	56	35	9	4	8	11	3	6	6	3	3	–	2	2
Males																	
60	1																
70	1	2															
80	6	3															
90	3	11		1													
100		9	6	2													
110		5	3	6	2												
120		5	20	15	4												
130		1	18	24	7	2	1	2	1								
140			6	21	12	4	1	2	1			1					
150			2	15	8	1		1	2	1							
160			1	2	10	3	1	1	3		3			1	1		
170				1	3	1		1	2	2	2				1		
180					2	1				1		1	1	1			
n	11	36	56	87	48	12	3	7	9	4	5	2	1	2	2	–	–

Table 2 The von Bertalanffy growth parameters, number of specimens, growth performance indices (Φ′), size and age ranges observed for *Scorpaena notata*. The asymptotic standard errors of the estimates are shown between brackets

Authors	Area	Method	Sex	L_∞ mm	k year^{-1}	t_0 year	*n*	Φ′	Age ranges years	Size ranges mm
Present study	Northern Adriatic Sea	Whole otoliths and sectioned otoliths	M	163.2 (3.0)	0.46 (0.5)	−1.17 (0.21)	285	2.08	0–14	65–198
			F	155.7 (3.3)	0.35 (0.5)	−2.06 (0.43)	225	1.92	0–16	47–199
			Total	157.2 (2.1)	0.44 (0.4)	−1.37 (0.19)	538	2.03	0–16	47–199
Bradai and Bouain (1990)	Gulf of Gabes	Scales	Total	139.48	0.116	−1.378	85	1.35	1–6	50–112[a]
Ordines et al. (2009)	Balearic Islands	Whole otoliths	M	179	0.236	−1.742	476	1.88	0–8	40–179
			F	179	0.195	−2.269	471	1.80	0–6	40–179
			Total	179	0.216	−1.946	947	1.84	0–8	40–179

[a] Standard lengths

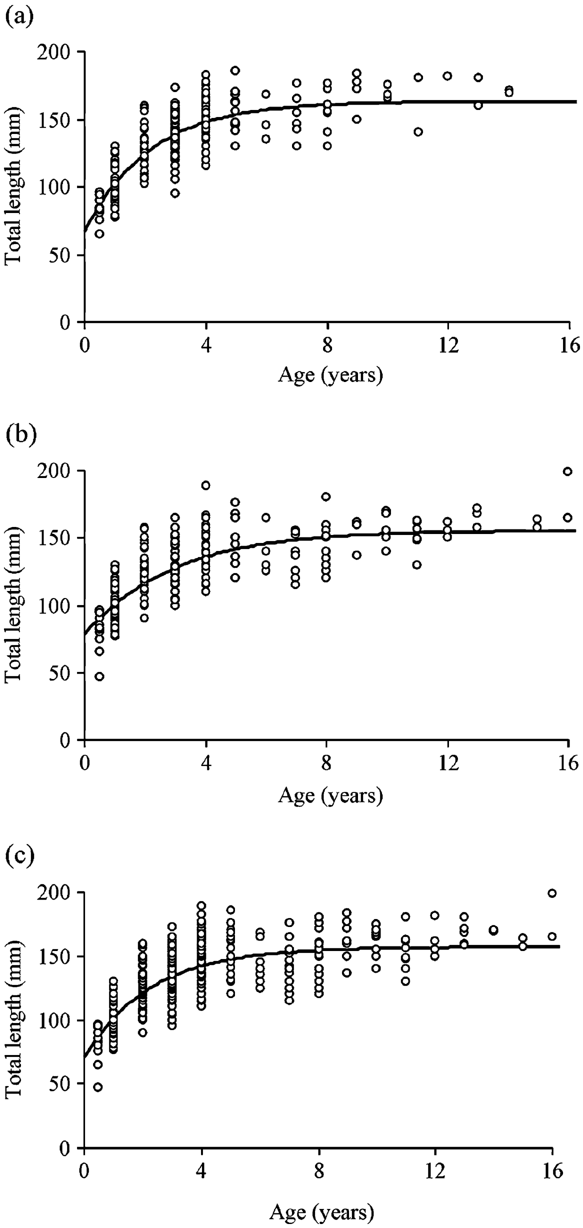

Fig. 7 von Bertalanffy growth curves fitted to the length-age data of *Scorpaena notata* **a** males, **b** females and **c** whole sample

contrast to most other Scorpaeniformes, females being often larger than males (Wyllie Echeverria 1986; Lenarz and Wyllie Echeverria 1991).Generally, it is rather common in fish that a larger size in females increase their fecundity, while the same may not be true in males (Berglund et al. 1986). In the case of *S. notata*, the documented low fecundity (Muñoz et al. 2005), compared to other Scorpaeniformes, such as *Trigla lyra* (Muñoz 2001) or *H. dactylopterus* (Muñoz and Casadevall 2002), could be the reason of the absence of sexual size dimorphism.

As reported for *H. dactylopterus* (Massutí et al. 2000), *S. notata* exhibited a double mechanism of formation of seasonal growth rings, corresponding to different stages of life, namely immature/juvenile and adult fish. The change in deposition pattern was

Table 3 Likelihood ratio test comparison of Von Bertalanffy parameters estimated for males and females of *Scorpaena notata* sampled in the northern Adriatic Sea. *d.f.* = degree of freedom; * = significant at α=0.01

Null hypothesis	*d.f.*	Log residual sum of squares	χ^2	*p*-values
$L_{\infty♂}=L_{\infty♀}$	1	−0.004	2.275	0.131
$k_{♂}=k_{♀}$	1	−0.004	2.223	0.136
$t_{0♂}=t_{0♀}$	1	−0.007	3.550	0.060
$L_{\infty♂}$, $k_{♂}=L_{\infty♀}$, $k_{♀}$	2	−0.063	32.172	0.000*
$L_{\infty♂}$, $t_{0♂}=L_{\infty♀}$, $t_{0♀}$	2	−0.036	18.554	0.000*
$k_{♂}$, $t_{0♂}=k_{♀}$, $t_{0♀}$	2	−0.009	4.449	0.108
$L_{\infty♂}$, $k_{♂}$, $t_{0♂}=L_{\infty♀}$, $k_{♀}$, $t_{0♀}$	3	−0.093	47.340	0.000*

Table 4 Estimated values of fish length-at-age for each sex of *Scorpaena notata* derived from the Von Bertalanffy equations

Age (years)	Females		Males	
	TL (mm)	Annual growth (mm)	*TL* (mm)	Annual growth (mm)
0	89.1		84.4	
1	108.3	19.2	113.2	28.8
2	121.9	13.7	131.5	18.3
3	131.7	9.7	143.1	11.6
4	138.6	6.9	150.5	7.4
5	143.5	4.9	155.1	4.7
6	147.0	3.5	158.2	3.1
7	149.5	2.5	160.1	1.9
8	151.3	1.8	161.2	1.1
9	152.6	1.3	161.9	0.7
10	153.5	0.9	162.4	0.5
11	154.1	0.6	162.7	0.3
12	154.6	0.5	162.9	0.2
13	154.8	0.3	162.9	0.1
14	154.9	0.3	163.0	0.1
15	155.1	0.2		
16	155.3	0.2		

observed at about 2–3 years, in concomitance with the attainment of first sexual maturity, which in the northern Adriatic Sea population took place between 10 and 14 cm TL (G. Scarcella, pers. comm.). Furthermore, we frequently found false rings within the first 3–4 true annuli, as commonly observed in some species of *Helicolenus* (Massutí et al. 2000; Sequeira et al. 2009). Similarly, the need of otolith sectioning for ageing purposes has been already reported for other scorpaenids, such as *Scorpaena elongata* (Gancitano and Ragonese 2008), *Helicolenus dactylopterus* (White et al. 1998; Allain and Lorance 2000), *Sebastes marinus* and *Sebastes mentella* (Stransky et al. 2005). The relationships between otolith diameters and fish size was linear, as reported for other species of *Scorpaena* (i.e. *S. elongata* and *S. maderensis*) (La Mesa et al. 2005; Rizzo et al. 2003) and for some Sebastidae (Wyllie Echeverria 1987), indicating a proportionality between the two mentioned dimensions.

The seasonal deposition of opaque and translucent zones in the whole sample, as well as the microincrement counts of young-of-the-year specimens, supported the validation of ageing fish by counting annuli. The fall-winter deposition of the translucent zone, supposed to occur during a slow growth period, took place when local sea temperatures reach minimum values (Artegiani et al. 1997). The precision of age estimates was comparable to that reported for species of similar longevity, such as *Pontinus kuhlii* (López Abellán et al. 2001), and within the range suggested by Campana (2001).

Considering the Von Bertalanffy growth parameters estimated for the northern Adriatic population of *S. notata*, the negative t_0 and the low value of L_∞ compared to the maximum size of fish caught were probably due to the relatively low abundance of large and small fish in the sample. Anyway, the longevity of about 15 years can be considered a reliable estimate of the maximum age attainable by the species, as the maximum size of fish aged is close to that reported in several localities of the Mediterranean Sea (Dulcic and Kraljevic 1996; Merella et al. 1997; Morey et al. 2003; Karakulak et al. 2006). This estimate falls within the wide range of longevity found in other Mediterranean scorpaenids, such as *S. porcus* and *S. maderensis*, which attain respectively 11 and 5 years of age (Jardas and Pallaoro 1992; La Mesa et al. 2005), and *S. elongata* and *H. dactylopterus*, which attain more than 30 years (Massutí et al. 2001; Gancitano and Ragonese 2008).

The index of growth performance (Φ') is a useful tool for comparing the growth curves of different populations of the same species and/or of different species belonging to the same order (Sparre et al. 1987). In Mediterranean scorpaenids, the growth performance ranged from 1.68 in *S. maderensis* (La Mesa et al. 2005), to 2.03 in *S. notata* (present study), 2.07 in *H. dactylopterus* (Massutí et al. 2000), 2.16 in *S. porcus* (Jardas and Pallaoro 1992), 2.41 in *P. kuhlii* (López Abellán et al. 2001) and 2.45 in *S. elongata* (Gancitano and Ragonese 2008), indicating a direct relationship between fish size and growth performance, as observed elsewhere in the genus *Sebastes* (Love et al. 2002).

Acknowledgements We thank the staffs of Marine Environment Management Unit and Population Dynamic Unit of ISMAR-CNR Ancona who respectively contributed to the sample collection and otoliths processing. We thank also Lesley Farley for the English revision of the text and two anonymous referees for their suggestions and criticism that clearly improved an earlier version of this manuscript.

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