- REVIEW ARTICLE -

Population Structures of the Two Banded Seabream, *Diplodus vulgaris* and the White Seabream, *Diplodus sargus* Reveal New Records in Maximal Age

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Abstract

The common two-banded seabream *Diplodus vulgaris* and *Diplodus sargus* were sampled from non-commercial fishing catches around the Maltese Islands between July 2012 and December 2017. A total of 1550 (*D. vulgaris* N=1204, *D. sargus* N=346) specimens were collected. In this study, age, growth, length-weight relationship (LWR), condition factor (*K*) and relative condition factor (*Kn*) were investigated. Ages ranged from 0 to 17 years for *D. sargus* and 0 to 16 years for *D. vulgaris*. A positive allometric growth was observed for *D. sargus* while growth in *D. vulgaris* was isometric. Average Kn was found to be 0.99 and 1.01 in *D. vulgaris* and 0.97 and 1.00 in *D. sargus* for males and females respectively. The growth parameters of the von Bertalanffy equation were: L_∞=26.71 and 30.86 cm, K= 0.275 and 0.286 year^{-1} and t_o = -2.27 and -1.61 year for all individuals of *D. vulgaris* and *D. sargus* respectively. This study is the first reference on the LWR equation parameters, Kn, K and Age for these species around the Maltese Islands and establishes a new maximum age for *D. vulgaris* of 16 years.

Introduction

Diplodus vulgaris (Geoffroy, 1817) and *Diplodus sargus* (Linnaeus, 1758) are commercially important species (Beltrano et al., 2003; Gonçalves et al., 2003) which are also targeted by the recreational fishery (Agius Darmanin & Vella, 2018) in the Mediterranean Sea. They are widespread in the Mediterranean, the coast of the East Atlantic and the Black Sea (Bauchot &

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Hureau, 1986). While some authors have published in different areas for both species e.g. Dulčić et al. (2011) and Benchalel & Kara (2013), studies on the stock composition of these two species in the central Mediterranean are more limited especially *for D. sargus*.

The demographic structure of fish stocks is considered an important characteristic by the Marine Strategy Framework Directive (MFSD) for determining the health of the population with age based indicators having been shown to be more reactive to exploitation (Brunel & Piet, 2013). Fecundity of older, larger fishes is higher showing exponential increase with size (Birkeland & Dayton, 2005). Stock assessment, together with the use of genetic studies provide valuable scientific data which is essential for the management of fishery resources with studies on *D. sargus* suggesting that the population structure of this species is a complex one (González-Wangüemert et al., 2007), while a low-level structure is present in the Mediterranean region for *D. vulgaris* (Kaoueche et al., 2011).

For the rational exploitation and management of *D. vulgaris* and *D. sargus* stocks in the central Mediterranean, information on their population dynamics and biology is essential. Since age and growth within a species can vary across geographic distribution (Law & Mitcheson, 2018), this study aims to identify the length weight relationship, age structure and body condition parameters which are essential for stock assessment off the Maltese Islands while also contrasting the data with studies in other areas of the Mediterranean which may be used in the development of a management strategy.

Material and Methods

Sampling

A total of 1550 fish (*D. vulgaris* N=1204, *D. sargus* N=346) were collected from July 2012 till December 2017 during 132 sport fishing competitions in 46 coastal locations around Malta, Gozo and Comino together with 35 recreational fishing observations, 33 target sampling sessions, 2 spear fishing competitions, donations by recreational fishers and visits to fish markets (Figure 1). Sampling was continuous, several times monthly during the study period. Fish were already dead when supplied by fishermen.
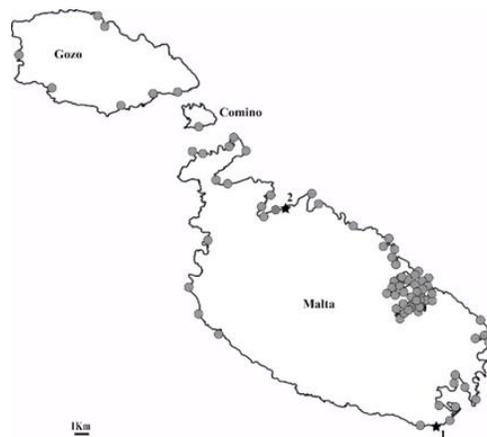
Figure 1. Map of the Maltese Islands showing sampling locations. Numbers denote where oldest specimens were caught (1=*D. vulgaris*, 2=*D. sargus*).

Fish were measured to record the total length (TL) and fork length (FL) to the 0.1cm. They were weighed to the nearest 0.01g and sexed by macroscopic and microscopic examination of the gonads. Sagittal otoliths were removed, cleaned, dried and stored for further analyses.

Age determination

Very small otoliths were immersed in glycerine (Butler, 1992) and read whole. Age estimates were determined by counting the number of opaque bands from the nucleus to the margin under a compound microscope at x10 magnification using reflected light against a dark background (Mendoza, 2006). Larger otoliths were embedded in resin and sectioned at a thickness of 1mm using an Accutom precision cutter, then sanded and polished manually. Sections were mounted on a slide using DPX medium. The annual increments were counted for larger otoliths under a stereo microscope by passing light through the section. In both methods, when both readings of the same otolith were different, another reading was performed. The otolith was discarded and considered unreadable if this third reading was not conclusive (Lorenzo et al., 2002). A subsample for each species was used to determine the percentage of otoliths with opaque margin for each month using otoliths sampled in 2016 (largest sample year) for *D. vulgaris*, while an extra year (2017) was added for *D. sargus.* The annual ring formation was determined through this methodology (Pajuelo & Lorenzo, 2001).

Length-weight relationship

Length-to-weight relationship was calculated using the equation:

$$W = aL^b \text{ (Le Cren, 1951)} \tag{1}$$

Where L is the total fish length in cm, W is the weight in grams, a is the intercept and b is an exponent indicating isometric growth when equal to 3. Linear regression was used to estimate a and b using the transformed equation:

$$\text{Log10 } (W) = \log10 (a) + b \log10 (L) \tag{2}$$

The null hypothesis of the isometric growth (H0: b =3) was tested using t-test at the 0.05 significance using the equation:

$$t = b - 3/S.E(b) \tag{3}$$

where t is t statistic, S.E (b) is the standard error of b obtained from linear regression for testing significant differences among slopes (b) between two regressions for the same species (Morey et al., 2003). The determination coefficient (r^2) was used to determine the strength of each relationship (Zar, 1984). All data was tested, and the outliers removed prior to each linear regression analysis for best model fit. The von Bertalanffy (VB) growth equation:

$$L_t = L_\infty (1 - e^{(-k(t-t_0))}) \tag{4}$$

Where L_t = total length at age t, K is a rate constant with units of reciprocal time, L_∞ represents the asymptotic length (mean maximum length), t_0 represents the theoretical age at length

0, L_{zero} = length at birth was used to describe the growth of both *Diplodus* species. The asymptotic weight W_∞ was calculated using the equation:

$$W_\infty = q * L_\infty^3 \tag{5}$$

where the parameter q, is called the condition factor calculated from the LWR. Fulton's condition factor was determined by using the equation:

$$K = 100W/L^b \tag{6}$$

Where, W is the weight in grams, L is length in cm and b is an exponent of the length-weight relationship (Nash et al., 2006). The relative condition factor (Kn) was calculated using the equation:

$$Kn = W_t/W_e \tag{7}$$

suggested by Le Cren (1951). Where W_t is the observed body weight and W_e is the theoretically estimated weight calculated from the length-weight relationship. The difference between Kn and K is that the former is measuring the deviation of an individual from the average weight for length, while K assumes that the exponent b is a constant where the weight of the fish is simply proportional to the cube of the length (Le Cren, 1951). The growth performance was determined using the growth index (Φ') using the equation by Sparre and Venema (1992);

$$\Phi' = \ln K + 2\ln L_\infty \tag{8}$$

to compare the growth parameters $L\infty$ and K estimated in this study with data published from other regions. All statistical analyses were performed with SPSS software package version 24 and a significance level of 0.05 was accepted.

Results

Length-weight relationship

The 1550 fish examined, (*D. vulgaris* N=1204, *D. sargus* N=346) consisted of 114 (*D. vulgaris* =76, *D. sargus* =38) males, 277 females (*D. vulgaris* =188, *D. sargus* =89), 85 hermaphrodites (*D. vulgaris* =22, *D. sargus* =63), 961 fish unidentified (*D. vulgaris* =805, *D. sargus* =156) and 113 *D. vulgaris* had decomposed gonads and could not be sexed. Total length of *D. vulgaris* ranged from 6.7 to 30.7 cm (mean 14.8 ±4.1 cm) while that for *D. sargus* ranged from 6.0 to 36.4cm (mean 18.93 ±6.48 cm).

Linear regressions fitted to estimate the WLR were significant for both species (p<0.001). Student t-test showed that the 'b' values obtained in *D. vulgaris* for males (M) and females (F) were significantly different from 3 (M, t=-5.667, $t_{(2)0.05, 75}$ =1.96; F, t=-4.303, $t_{(2)0.05, 189}$ = 1.96) indicating a negative allometric growth (A-) while for all individuals (juveniles, males, females and hermaphrodites), an isometric growth was observed (t=1.250, $t_{(2)0.05, 1193}$ = 1.96). In *D. sargus,* values for males and females were not significantly different (M, t=-0.854, $t_{(2)0.05, 39}$ =1.96; F, t=1.50, $t_{(2)0.05, 89}$ = 1.96); while for all the individuals a positive allometric growth (A+) was

observed (t=4.000, ($t_{(2)0.05, \, 346}$= 1.96) (Table 1). The was no significant difference between the slope (b-value) of the total length-total weight regression of males and females of both *Diplodus* species (*D. vulgaris,* ANCOVA:F (2, 261) = 2.788 p=0.096; *D. sargus* ANCOVA:F (2, 127) = 1.049, p=0.308).
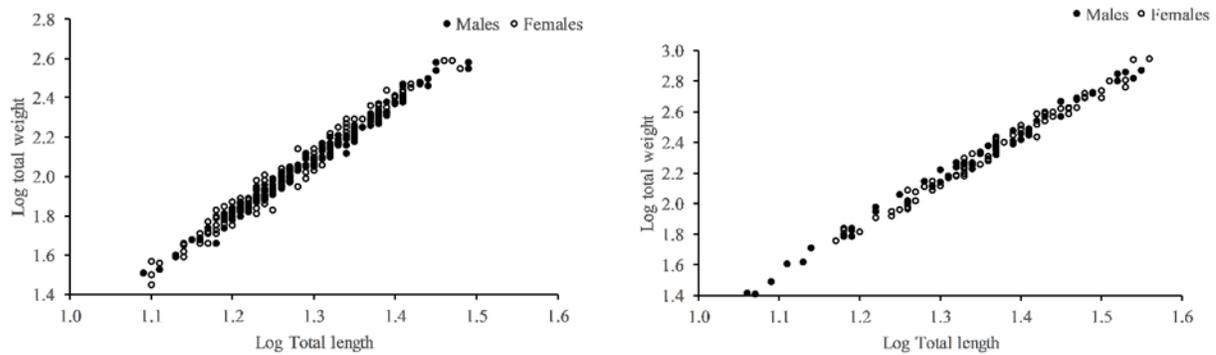
Figure 2. Length weight relationship for A) *D. vulgaris* B) *D. sargus*

Table 1. Parameters of the length-weight relationship, of *D. vulgaris*, and *D. sargus* in different regions.

Authors	Location	N	a	b	sex	R^2	Growth
D. vulgaris							
Current study	Maltese Islands	1204	0.024	2.858	F	0.98	A-
			0.032	2.762	M	0.98	A-
			0.016	3.010	All	0.99	I
Dulčić et al., 2011	E/M Adriatic, Croatia,	3729	0.012	3.068	All		I
			0.013	3.059	F		I
			0.013	3.055	M		I
Maci et al., 2009	Adriatic Sea, Italy	147	0.020	3.221	All	0.99	A+
İlkyaz et al., 2008	Turkey, C Aegean Sea	242	0.004	3.530	All	0.96	-
			0.003	3.590	F	0.96	-
			0.005	3.460	M	0.96	-
Karakulak et al., 2006	Turkey, N Aegean Sea	93	0.086	2.431	All	0.65	A-
			0.008	3.214	F	0.95	A+
			0.013	3.077	M	0.97	A+
Morey et al., 2003	W Medit.	328	0.015	3.006	All	0.99	I
Hadj Taieb et al., 2012	Gulf of Gabès, Tunisia.	916	0.019	2.932	All	0.98	A-
			0.019	2.916	F	0.97	A-
			0.018	2.943	M	0.98	A-
Abdalla et al, 2016	Libya, Benghazi coast	290	0.038	2.722	All	0.98	-
			0.016	3.016	F	0.99	-
			0.036	2.741	M	0.98	-
Moutopoulos et al., 2013	Ionian Sea, Greece	345	0.012	3.070	All	0.98	A+
D. sargus							
Current study	Maltese Islands	346	0.015	3.051	F	0.98	I

			0.022	2.947	M	0.98	I
			0.015	3.052	All	0.99	A+
Benchalel & Kara, 2013	Algeria	241	0.027	2.945	F	0.98	I
			0.019	3.009	M	0.98	I
			0.022	2.987	All	0.98	I
Man-Wai & Quignard, 1982	Gulf of Lion, France	270	0.010	3.123	All	0.99	A+
Morey et al., 2003	W Medit.	75	0.011	3.132	All	0.99	A+
Al-Beak et al., 2015	North Siani	991	0.011	3.165	All	0.98	A+
Maci et al., 2009	Adriatic Sea, Italy	150	0.019	3.313	All	0.98	A+
Moutopoulos et al., 2013	Korinthiakos Gulf, Greece	1055	0.014	3.056	All	0.97	A+
Balık & Emre, 2016	Beymelek Lagoon	355	0.014	3.103	All	0.98	A+

Condition factor (K) and relative condition (Kn)

The mean condition factor (K) was highest for *D. vulgaris* (M= 3.18, F= 2.43) and 1.85 and 1.59 in *D. sargus* males and females respectively. Relative condition factor (Kn) in *D. vulgaris* males was 0.99 and 1.01 in females and 0.97 in males and 1.00 in females of *D. sargus*.

Age

Of the 1524 otoliths processed (*D. vulgaris*, N= 1191; *D. sargus*, N= 333) 1423 otoliths were used for age estimation (*D. vulgaris*, N= 1122; *D. sargus* N=301) (Figure 3) since 102 were discarded as their age readability was not optimal. The percentages of otoliths with opaque rings at the margin peaked between May and September (Figure 4) for both species. The maximal growth rate in TL was at the end of the first year of life (11.66 cm *D. vulgaris*; 11.02cm *D. sargus*). This was followed by a steady decrease in the annual increments as age increased.
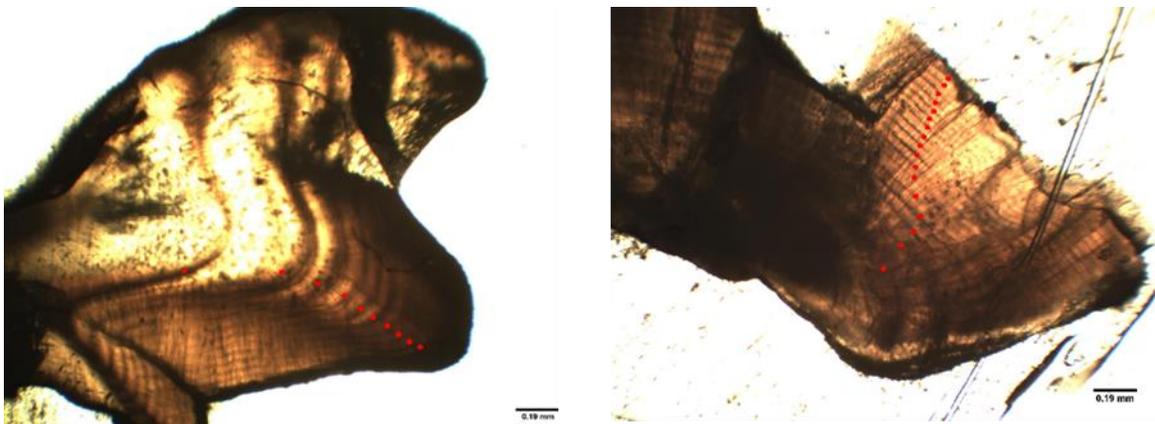
Figure 3. Example of sectioned otoliths. A) 10-year-old *D. vulgris,* B) 17-year-old *D. sargus.*

The age of the samples studied varied from 0+ to 16+ years for *D. vulgaris* and 0+ to 17+ years for *D. sargus*. The oldest female was 15+ years for *D. vulgaris* and 17+ years for *D. sargus* while the oldest male was 16+ years *D. vulgaris* and 11+ years for *D. sargus*. An overlap was observed in the length-at-age distributions of flanking age classes for both species mainly in the

larger sized individuals. Dominating the sample were 0+ for both *D. vulgaris* (47.59%) and *D. sargus* (26.16%).
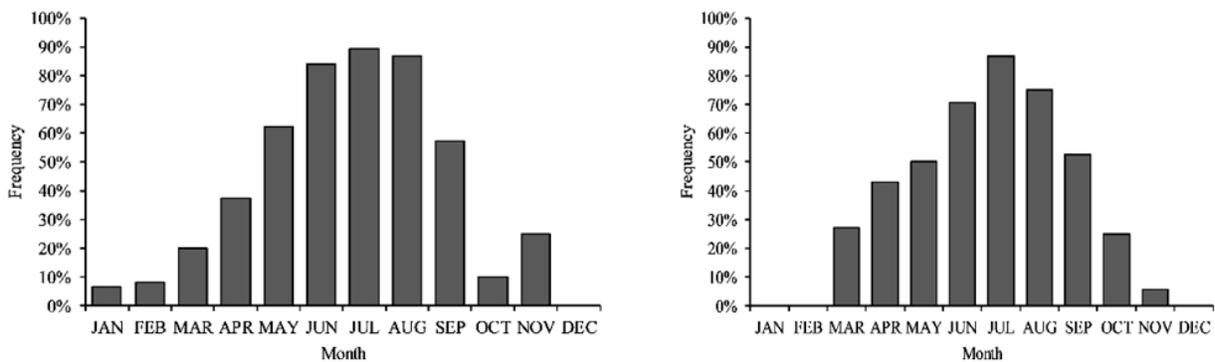
Figure 4. Monthly percentages of otoliths with opaque edge for A) *Diplodus vulgaris* and B) *Diplodus sargus*.

The mean total lengths of individuals allocated to every group were used to fit the Bertalanffy growth function displayed in Figures 5A and B. The calculated asymptotic length values were lower than the maximum observed length for both *D. vulgaris* and *D. sargus*. In both cases gaps were present between the ages of the oldest specimens, which were not used in the Bertalanffy model calculations. Asymptotic values of total length ($L\infty$) were higher for males than for females in *D. sargus* and the opposite for *D. vulgaris*. The calculated asymptotic weights (W_∞) of *D. vulgaris* and *D. sargus* were found to be higher in females for *D. vulgaris* (M= 298.62g, F= 332.106g, All = 316.04g) and higher in males for *D. sargus* (M= 597.78g, F= 508.46g, All = 526.80g). In order to determine overall growth performance, the growth performance index phi-prime (Φ') was estimated for males, females and the whole sample (Table 2).
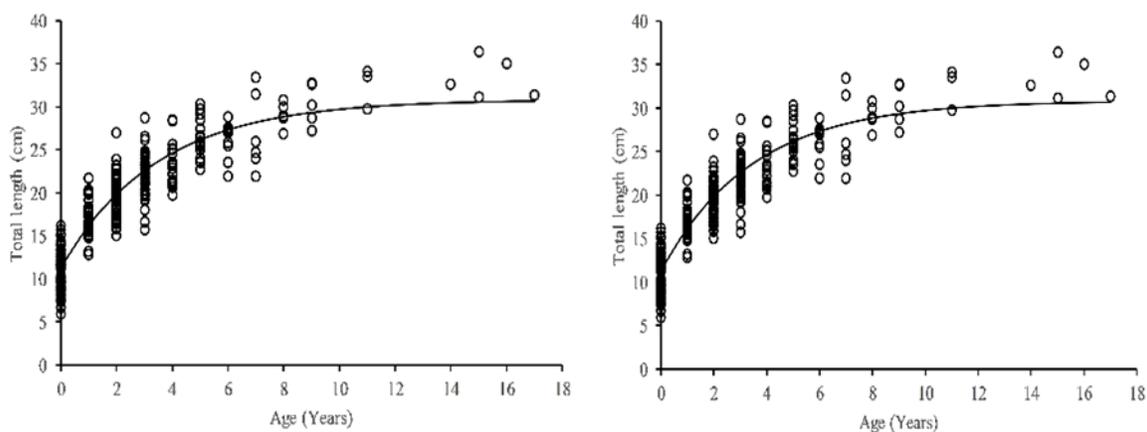
Figure 5. Length at age data with fitted VBGF curve: A) *D. vulgaris* = $L_t = 26.72 [1-e^{-0.275}(t + 2.271)]$. $r^2 = 0.89$; B) *D. sargus* = $L_t = 30.86 [1-e^{-0.286}(t + 1.610)]$. $r^2 = 0.93$.

Table 2. Bertalanffy growth parameters and growth performance index (Φ') for *D. vulgaris* and *D. sargus* from different regions (M=method; O=otoliths, S=scales, LF=length frequency).

Author & date	Location	M	Max age	N	L_∞	K	t_0	Sex	Φ'
D. vulgaris									
Current study	Maltese Islands	O	16	1121	28.1	0.21	-3.18	F	2.22
					27.4	0.24	-2.60	M	2.25
					26.7	0.28	-2.27	All	2.29
Mouine et al., 2010	Gulf of Tunis	O	11	492	39.0	0.10	-0.96	All	2.18
Pajuelo & Lorenzo, 2003	Canary Islands	O	9	519	39.7	0.23	-0.91	All	2.56
Dulčić et al., 2011	E/M Adriatic, Croatia	S	11	2953	52.0	0.10	-2.84	F	2.41
					56.0	0.08	-2.92	M	2.42
Gonçalves et al., 2003	S & SW Portugal	O	14	1076	28.6	0.36	-0.38	M	2.47
					27.7	0.39	-0.34	F	2.48
					27.7	0.40	-0.34	All	2.49
Mahmoud et al., 2010a	Abu Qir Bay, Egypt	S	6	616	31.3	0.26	-0.56	All	2.40
Girardin, 1978	Gulf of Lion, France	S	3	-	26.8	0.26	-0.61	All	2.26
Man Wai, 1985	Gulf of Lion, France	S	10	1315	37.8	0.18	-0.83	All	2.41
Beltrano et al., 2003	Strait of Sicily	O	-	603	33.5	0.17	-2.59	All	2.28
Mennes, 1985	Morocco, E Atlantic	LF	-	-	39.0	0.40		All	2.78
Abecasis et al., 2008	Portugal	O	14	1076	27.7	0.34	-0.34	F	2.42
					28.6	0.36	-0.38	M	2.47
					27.4	0.40	-0.77	All	2.48
Gordoa & Molí, 1997	Spain	O	6	201	28.8	0.39	-0.66	All	2.51
Soykan et al., 2015	Izmir bay, Turkey	O	3	709	28.0	0.25	-1.18	All	2.30
Hadj Taieb et al., 2013	Gulf of Gabès, Tunisia	O	8	955	25.4	0.18	-1.63	All	2.06
D. sargus									
Current Study	Maltese Islands	O	17	301	30.5	0.28	-1.56	F	2.41
					32.0	0.25	-2.18	M	2.40
					30.9	0.29	-1.61	All	2.44
Benchalel & Kara, 2013	Algeria	O	10	241	36.3	0.15	-0.49	All	2.31
Man-Wai & Quignard, 1982	Gulf of Lion, France	S	10	1312	41.7	0.25	-0.76	All	2.40

Gordoa & Molí, 1997	Spain	O	10	184	41.7	0.25	-0.76	All	2.63
Al-Beak et al., 2015	North Siani		5	991	40.7	0.25	-0.28	All	2.62
Mahmoud et al., 2010b	Abu Qir bay	O	6		31.4	0.262	-0.73	All	2.41
Martinez-Pastor & Villegas-Cuadros, 1996	Cantabrian Sea, Spain	-			57.59	0.10	-5.33	F	2.52
					52.9	0.13	-3.73	M	-
					48.8	0.18	-0.58	All	
Abecasis *et al.*, 2008	Portugal	O	18	737	40.9	0.18	-1.28	All	2.48
Balık & Emre, 2016	Beymelek Lagoon	S	3	355	39.9	0.27	-1.75	All	2.63

Discussion

This study provides the first published data on length and age structure for *D. vulgaris* and *D. sargus* from the Maltese waters. It contributes additional data with larger sample size for *D. vulgaris* for the central Mediterranean, while also providing the first data in the central Mediterranean for *D. sargus* (Table 2).

The results obtained for condition factor (K) and relative condition factor Kn from Maltese coastal waters showed differences between the species. K values for *D. vulgaris* were much higher (F= 2.43; M= 3.18) than those for *D. sargus* (F= 1.59; M= 1.85) however, such great differences were not reflected in the Kn values (*D. vulgaris*, F= 1.01; M= 0.99; *D. sargus*, F= 1.00, M =0.97) where in both cases, females were in a better condition than males with a Kn value >1 indicating a good general condition of the fish (Le Cren, 1951; Din *et al*., 2015). Kn fluctuated throughout the year for both *D. vulgaris* and *D. sargus* (Figure 1). K values for *D. sargus* were similar to other findings in the Central Mediterranean by Mouine et al. (2007).

The b values for both *Diplodus* species were found to be within the expected range of 2.5-3.5 (Froese, 2006). These results can be regarded as mean values for the species since the fish were sampled during the whole year throughout the data collection period. The comparison of b values for the species studied in this study and previously published work in other locations suggest inter-regional differences (Table 1). Differences in b-values may be attributed to: Seasonality; Effects of different area; Variations in the number of specimens examined; Differences in the documented length series of the species sampled; Area/season effect; Availability of food sources; Rate of food consumption; Gonadal maturation or spawning period (Weatherley et al., 1987; Wootton, 1999; Froese, 2006; Moutopoulos et al., 2011). In this study, *D. vulgaris* and *D. sargus* showed isometric growth and a positive allometric growth respectively for the whole population.

The Bertalanffy parameters calculated in this study show that both the common two-banded seabream and the white seabream are relatively slow growing and long-lived species in the Central Mediterranean. The new maximum recorded age for *D. vulgaris* of 16+ years and the almost maximal recorder age for *D. sargus* at 17+ years indicate that the environmental conditions around

the Maltese Islands are favourable for the growth of these species. The Bertalanffy model explained 89% and 93% of the growth pattern of *D. vulgaris* and *D. sargus* respectively as shown by the determination coefficient from each curve fitting. Such findings are similar to results by Gordoa & Moli (1997). An overlap was observed between the different age groups in every species which is a common phenomenon in fish due to the presence of differences in growth rates among individuals within the age groups (Mahmoud, 2010a). In all of the publications in which the von Bertalannfy is applied for both species, including this study, the estimations of t_0 are negative suggesting that early stage growth is not accurately described by this model (Sparre & Venema, 1992; Gordoa & Molí, 1997). Growth in Sparid fish is generally rapid during the immature stage and decreases substantially at adult stage (Kallianiotis et al., 2005). Such differences may be accounted for by changes in environmental conditions between habitat of juveniles and adults.

The phi-prime growth performance index (Φ') is regarded as a suitable and sound instrument for contrasting growth parameters from diverse sets of data (Bellido *et al*., 2000) and rounding the growth parameters of an individual species (Sparre & Venema, 1992). The growth rates represented by K are lower in *D. vulgaris* than in *D. sargus* indicating a slower growth rate in the former species. The calculated asymptotic lengths for both species show similarity with other research. In the case of *D. vulgaris*, the value of the calculated asymptotic length was higher for females than males unlike previous studies in the Eastern Middle Adriatic, and the North East Atlantic Ocean that calculated the asymptotic length for males and females separately (Gonçalves et al., 2003; Abecasis et al., 2008; Dulčić et al., 2011) which may have been due to the different length frequencies of the samples. The overall L_∞ was also lower than the total length of the largest specimen collected (29.6cm). As no individuals were collected at ages 12+ and 14+, the largest specimens were not used in the Bertalanffy model calculations. This may have contributed to the much lower L_∞ than that reported by Mouine et al. (2010) in the Gulf of Tunis in the central Mediterranean and by Beltrano et al. (2003) in the Strait of Sicily. Results for the overall L_∞ in Maltese waters were however only slightly larger than what was reported by Hadj Taieb et al. (2013) in the Gulf of Gabes, Central Mediterranean and very similar to studies in Portugal, the North Western Atlantic by Goncalves et al. (2003) and Abecasis et al. (2008) and by Soykan et al. (2015) in, Turkey, Western Mediterranean.

The value of the fitted asymptotic length (30.5 – 32.0cm) for *D. sargus* was also lower than the total length of the largest specimen collected (35.0cm) due to the same reasons described for *D. vulgaris*. L_∞ results were similar to studies in Abu Qir bay by Mahmoud et al. (2010b) but lower than all other studies in the Mediterranean and North Western Atlantic. A longer L_∞ for males than females was observed, contrary to the study by Martinez-Pastor & Villegas-Cuadros (1996) in the Cantabrian Sea. On the other hand, the K values were similar to the studies in Spain, Gulf of Lion, Turkey, North Sinai and Abu Qir bay, Egypt (Gordoa & Molí, 1997; Mahmoud et al., 2010b; Al-Beak et al., 2015; Balık & Emre, 2016; Man-Wai and Quignard, 1982) and higher than those in Algeria (Benchalel & Kara, 2013) indicating a slower growth in this area. In each of the *Diplodus* species, the calculated values for Φ' were however fairly similar indicating that such values are characteristic of the species (Mouine et al., 2010).

Fisheries management involves the setting of a minimum legal size to allow each individual to reproduce at least once (Türkmen & Akyurt, 2003). However, recent studies also suggest that preservation of old and larger fish may offer benefits for replacing marine fish stocks and enhance fishing quality (Arlinghaus et al., 2010). Conserving adult fish and large individuals to maintain

reproductive potential is central in fisheries management and can be attained through the use of more selective gear (Mullon et al., 2012). Management of recreational fisheries is also necessary since with the human population increase and superior technical equipment, the amount of recreational utilization of coastal marine resources has intensified (Birkeland & Dayton, 2005). The largest and oldest specimens of both species were all captured by recreational fishermen, mostly in fishing spots that were difficult to access, therefore management options also need to include this type of fishery. Such options could include developing tackle and equipment that are more selective, since some of the latter such as nets or angling make selection of fish before catch difficult, resulting in fish injuries during catch and release (Birkeland & Dayton, 2005), establishing marine protected area networks (Mullon et al., 2012) representing different habitats, with season closures that coincide with the spawning season and encouraging the removal of medium sized adults rather than immature fish or large adults.

The presently determined population parameters of these two species around the Maltese Islands will be useful in future fisheries studies around the Maltese Islands and the central Mediterranean and should consent a better insight of the long-term variations in stock sizes. Local conservation management is however required to safeguard and protect the local stock from overexploitation and future genetic studies on both species to investigate whether geographical distance between the Maltese Islands and other regions of the Mediterranean are resulting in any genetic divergence and isolation of the stocks are suggested.

Acknowledgment

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