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# Integrating data from cold-water coral habitats increase knowledge on age and growth studies: the case of *Helicolenus dactylopterus* (Delaroche, 1809) in the central Mediterranean

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The aim of this study is to provide new insights on the age and growth of *H. dactylopterus* in the north-western Ionian Sea (central Mediterranean) by using different data sets and comparing three different methods, one direct by otolith reading corroborated by two indirect methods, by means of back calculation and Length Frequency Distribution analysis (LFDa), in order to obtain robust data for use in analytical management models. A total of 1403 specimens of blackbelly rosefish were sampled from habitats explored on muddy bottoms in the depth range 106–721 m and 309 individuals caught in the cold-water coral habitat between 418 and 635 m in depth. The length-frequency distributions of blackbelly rosefish showed a polymodal pattern in both habitats explored, with medium-small individuals caught on muddy bottoms and larger ones in coral habitats and canyons. The maximum age observed in females was 13 years (315 mm TL), while in males it was 15 years (336 mm TL), and no significant difference was observed comparing age-length keys from otolith readings of females with the males. The analysis of the daily increments allowed us to validate the average length of the first year of age (approximately 90–100 mm LT) obtained from the three different methods used. No significant difference was highlighted comparing the average lengths of age class 1, calculated with the direct method and by counting the daily increments. The von Bertalanffy growth parameters were estimated for the sexes and for the whole population from otolith readings and for the entire population by LFD analysis and the back calculation method. The growth curves were comparable between the different methods and no significant difference was observed. The von Bertalanffy growth parameters estimated for the different methods were

comparable (otolith readings  $L_{\infty}$  494 mm,  $k=0.06$  years<sup>-1</sup>,  $t_0=-3.00$  years; back calculation method  $L_{\infty}$  487 mm,  $k=0.06$  years<sup>-1</sup>,  $t_0=-2.30$  years; LFDa  $L_{\infty}$  415 mm,  $k=0.07$  years<sup>-1</sup>,  $t_0=-2.23$  years), indicating a slow growth in accordance with what has already been observed in other areas of the Mediterranean.

#### KEYWORDS

*Helicolenus dactylopterus*, age and growth, back calculation, length frequency distribution analysis, cold-water coral habitats, Central Mediterranean

## 1 Introduction

Age and growth studies are fundamental components of fisheries management. Although age and growth data are generally considered together, each component provides exclusive information on individuals and population. Age refers to some quantitative description of the length of time that an organism has lived, whereas growth is the change in body or body part size between two points in time, and growth rate is a measure of change in some metric of fish size as a function of time (DeVries and Frie, 1996). Fish age estimations and growth studies using hard parts, such as otoliths, provide basic information for understanding population dynamics and stock assessments that are essential for responsible fisheries management (Panfili et al., 2002).

The blackbelly rosefish, *Helicolenus dactylopterus* (Delaroche, 1809) (Osteichthyes, Scorpaeniformes), is a benthic deep-water scorpionfish found at depths of 100–1000 m both on muddy bottoms and in heterogeneous habitats such as seamounts, canyons and cold-water coral habitats. It feeds on both benthic and pelagic organisms (mainly crustaceans and fishes) showing dietary variation with ontogenetic development and in relation to the habitat in which it lives (Capezzuto et al., 2020).

It is viviparous species, and larvae and offspring are pelagic (Massutí et al., 2001; Costello et al., 2005; D'Onghia et al., 2010, 2012; Sartor et al., 2017). It is widespread both in the eastern Atlantic and in the Mediterranean Sea (Whithehead et al., 1986), where it is both common and exploited. In the Mediterranean, this teleost is a commercial species with an important economic value targeted by long-lines and gillnets, but it also regularly appears in the by-catch of bottom trawls over a wide depth range, with adult specimens mostly collected during deep-sea fishing targeting crustaceans (Fisher et al., 1987; Relini et al., 1999).

Concerning age and growth, several studies published concern both the Atlantic and the Mediterranean. In the latter, studies on the growth of blackbelly rosefish were conducted by (Massutí et al., 2000, 2001) in Iberian Mediterranean coast and in the Alboran Sea respectively; by Mili et al. (2016) in the Northern Waters of Tunisia and in Italian waters by Peirano and Tunesi (1986) and Ragonese (1989) in the Ligurian Sea; Ragonese and Reale (1995) in the Strait of Sicily; by Ungaro and Marano (1995) and Romanelli et al. (1997)

in the Adriatic Sea and by D'Onghia et al. (1992, 1996) and by Sion et al. (2018, 2022) in the western Ionian Sea. A wide range of von Bertalanffy growth parameters were estimated by different authors in relation to different factors: the method used (otolith reading method and/or length frequency distribution analysis), the heterogeneity of the sample studied (fish of different size but also sampled with different types of gear), different environmental conditions, different latitudes and different fishing pressures. Despite many studies having been published, there is still no study to our knowledge focused on the validation and/or corroboration.

Poor-quality ageing data could result in inaccurate population status evaluation which in turn could lead stock collapse (Beamish and McFarlane, 1995). Age data discrepancies can be attributed to preparation methods (Smith et al., 2016), ageing standards (Carbonara and Follesa, 2019a; Hüsey et al., 2016), and sample strategies (fishing related or fishing independent data) (Coggins et al., 2013). Diverse degrees of fishing pressure (Carbonara et al., 2022) and geographical variances resulting from genetic or environmental factors (Carbonara et al., 2018; Isely et al., 1987) may explain differences in growth patterns between population, even within adjacent populations (Carbonara et al., 2022). Furthermore, otolith reader experience level may be another source of variation (Carbonara et al., 2019b). Moreover, fish distribution could be an additional source of bias. Indeed, large individuals of *H. dactylopterus* are often rare on muddy bottoms because they are removed by trawling. Whereas large individuals distributed in canyons and cold-water coral habitats, generally collected by bottom longline and only the presence in the sample of two population components (juvenile and adult) can provide an important contribution to resolving the existing disagreement on *H. dactylopterus* age and growth pattern. All these factors can compromise both precision and accuracy of age data and consequently the analysis of the level of population exploitation. For this reason, the aim of this study is to provide new insights into the age and growth of *H. dactylopterus* in the north-western Ionian Sea by using different data sets and comparing three different methods, a direct by otolith reading verified by means of back calculation and corroborated by Length Frequency Distribution analysis (LFDa), in order to obtain more robust data for use in

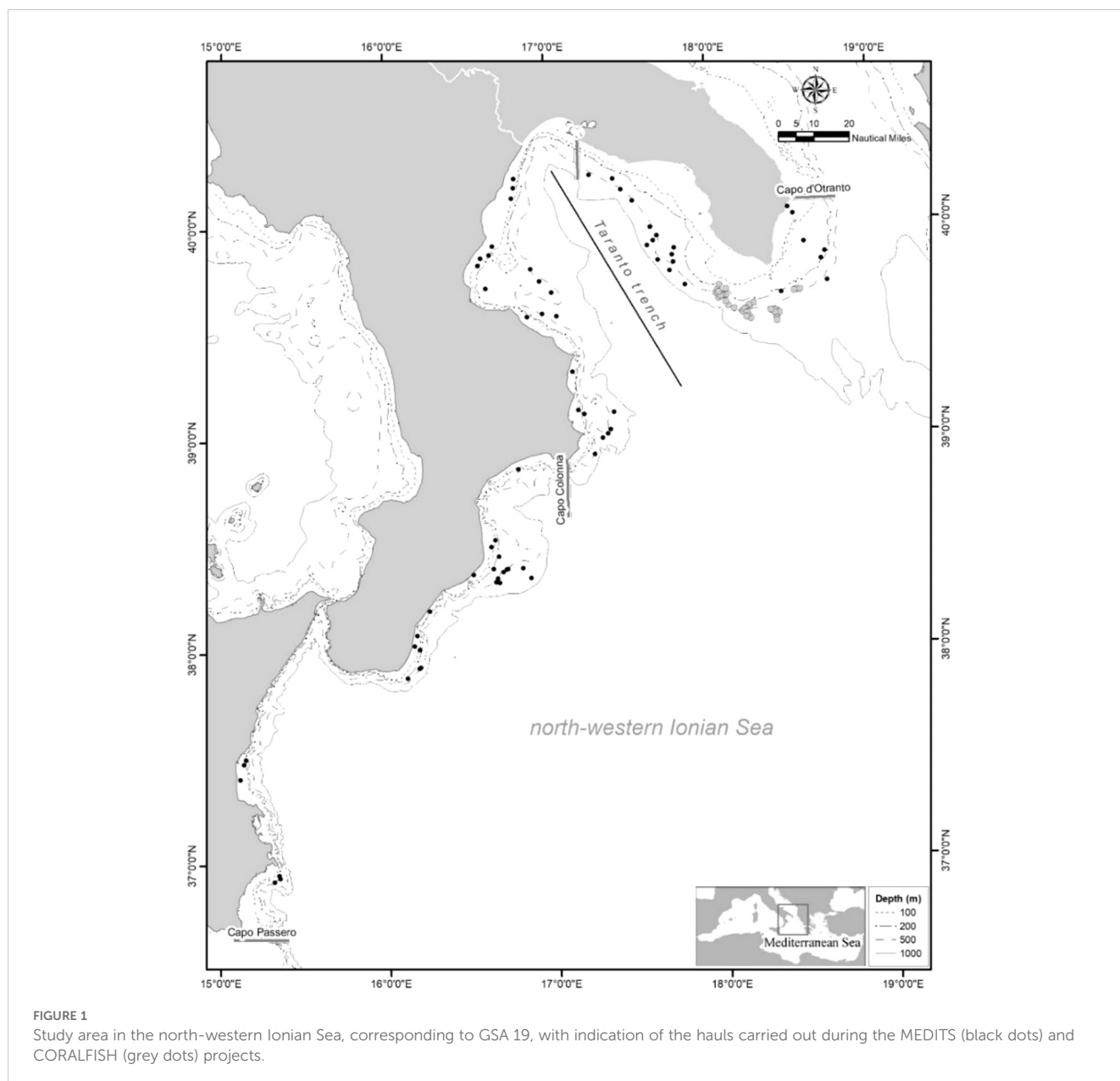


FIGURE 1

Study area in the north-western Ionian Sea, corresponding to GSA 19, with indication of the hauls carried out during the MEDITS (black dots) and CORALFISH (grey dots) projects.

analytical management models (Carbonara et al., 2023; ICES, 2020). Moreover, a validation of first annulus was carried out by Daily Growth Increments (Campana, 2001).

## 2 Materials and methods

### 2.1 Data collection

Data on *H. dactylopterus* have been collected in the north-western Ionian Sea, corresponding to Geographical Sub-Area (GSA) 19 (Figure 1) between Cape Otranto and Cape Passero, during two international research projects: the MEDITS experimental bottom trawl surveys (Spedicato et al., 2019) and CoralFISH bottom longline surveys (Grehan et al., 2017). Data from four MEDITS surveys (from 2013 to 2016) and three CoralFISH surveys (2010-2011) were used

(Table 1). In the first project the trawlable fishing grounds were explored, while the second involved areas where trawl fishing activity does not take place due to irregular and hard bottoms, giving the opportunity of sampling larger individuals, using the longline.

From the sampled specimens the Total Length (TL; in millimeters) to the nearest 1 mm, Total Weight (TW) to the nearest 0.1 g, sex, and maturity stages (AAVV, 2017) were measured. A total of 1403 specimens of *H. dactylopterus* were sampled from muddy bottoms in the depth range 106-721 m and 309 individuals were caught in the cold-water coral habitats between 418 and 635 m depth (Table 1).

### 2.2 Age analysis

The sagittal otoliths were extracted from a subsample of specimens captured during the trawl and the bottom longline

TABLE 1 Number of individuals and range of total length (mm), by sex, corresponding to each sampling period.

Project	Habitat	Sampling period	N	Indeterminate		Female		Male	
				N	TL (mm)	N	TL (mm)	N	TL (mm)
MEDITIS	bottom trawl	June 2013	649	512	32-123	82	87-267	55	107-300
MEDITIS	bottom trawl	September 2014	300	68	42-114	129	87-250	103	86-293
MEDITIS	bottom trawl	June-July 2015	230	125	22-150	70	88-259	35	100-268
MEDITIS	bottom trawl	June 2016	224	146	41-157	43	93-213	35	93-236
CoralFISH	bottom longline	May-June 2010	135	-	-	58	140-294	77	160-325
CoralFISH	bottom longline	September-October 2010	151	-	-	61	143-293	90	174-380
CoralFISH	bottom longline	December 2011	23	-	-	9	183-230	14	190-315

surveys. Both otoliths (right and left) were removed but the right one was read preferentially. All the readings were carried out in the posterior area on the distal side along the longitudinal axis joining the *sulcus* and the *nucleus*. For larger individuals, grinding was performed on the proximal surface to obtain the best reading. The *sagittae* were placed in a black dish with glycerine (30%) and alcohol (70%) and read by standard techniques (Morales-Nin, 1987). All otoliths showed the ring pattern common to teleost fish, opaque and transparent rings laid down around an opaque *nucleus*, corresponding to fast and slow growth phases (Williams and Bedford, 1974). The age and growth estimations were carried out by band count, with one opaque band followed by a transparent one assumed to be an *annulus* (Panfili et al., 2002). An age was assigned to each otolith according to the age estimation scheme proposed by Carbonara and Follesa (2019a) with a yearly resolution, assuming the birthday as January 1. In the *sagittae* the transparent bands were counted using a stereo microscope (Leica M165C, x 10

magnification), under reflected light against a dark background (Figure 2).

An age-length key for the females, males and the entire population from whole otolith readings was provided and for each age classes the growth rate was calculated as:  $Gr = (L_{i+1} - L_i) / (L_{i+1} + L_i) / 2$ ; where  $L_i$  is the total length of the *i*th annulus and  $L_{i+1}$  is the total length of the  $(i+1)^{th}$  annulus. The differences between females and males in otolith readings were assessed using the non-parametric Kolmogorov-Smirnov (KS) statistical test.

In addition, Daily Growth Increments (DGI) present in the otoliths were estimated by the direct method of *annuli* counting to validate the first year of age (Campana, 2001). The daily periodicity was made visible, after sanding and polishing, using a microscope (Leica DM LS2, x 800 magnification) with transmitted light (Figure 3).

Regarding the otolith readings, the precision, defined as the reproducibility (Chilton and Beamish, 1982), was measured from

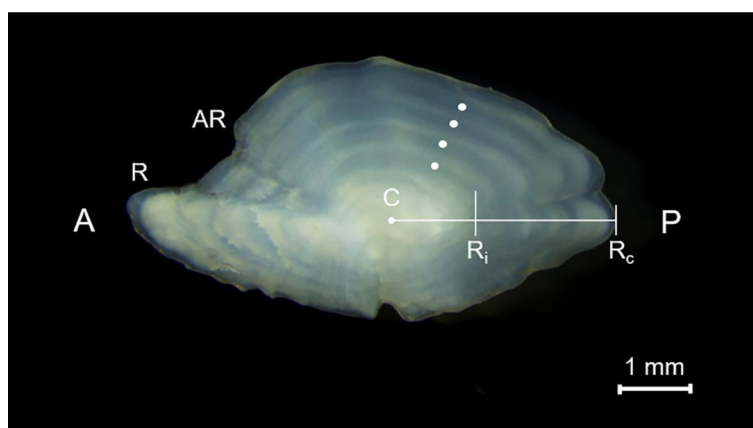


FIGURE 2

Right otolith of *H. dactylopterus*: evident opaque and transparent bands, 4-year-old (161 mm TL). A: Anterior part; P: Posterior part; R: Rostrum; AR: Anti Rostrum;  $R_i$ : Radius of the *i*th band (distance from the centre of the otolith to the outer margin of the *annulus*),  $R_c$  is the radius of the otolith at capture.



FIGURE 3  
Daily increments present in the otolith of *H. dactylopterus*.

the average percent error (APE), the percentage agreement (PA), and the coefficient of variation (CV). The formula used to calculate APE was the following (Beamish and Fournier, 1981):

$$APE_j(\%) = 100 \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} + X_j|}{X_j}$$

where  $X_{ij}$  is the  $i$ th age determination of the  $j$ th fish,  $X_j$  is the average age calculated for the  $j$ th fish, and  $R$  is the number of times each fish was aged.

CV and PA within one year (+/-1 yr) were proposed by Campana, 2001:

$$PA = \frac{\sum |n_{diff} \leq 1|}{n}$$

$$CV(\%) = 100 \frac{\sqrt{\sum_{i=1}^R \frac{(X_{ij} - X_j)^2}{R-1}}}{x_j}$$

where  $R$  is the number of times each fish is aged,  $X_{ij}$  the  $i$ (th) age determination of the  $j$ (th) fish,  $X_j$  is the mean age calculated for the  $j$ (th) fish, and  $n_{diff}$  is the difference in age determination between the readings of three readers.

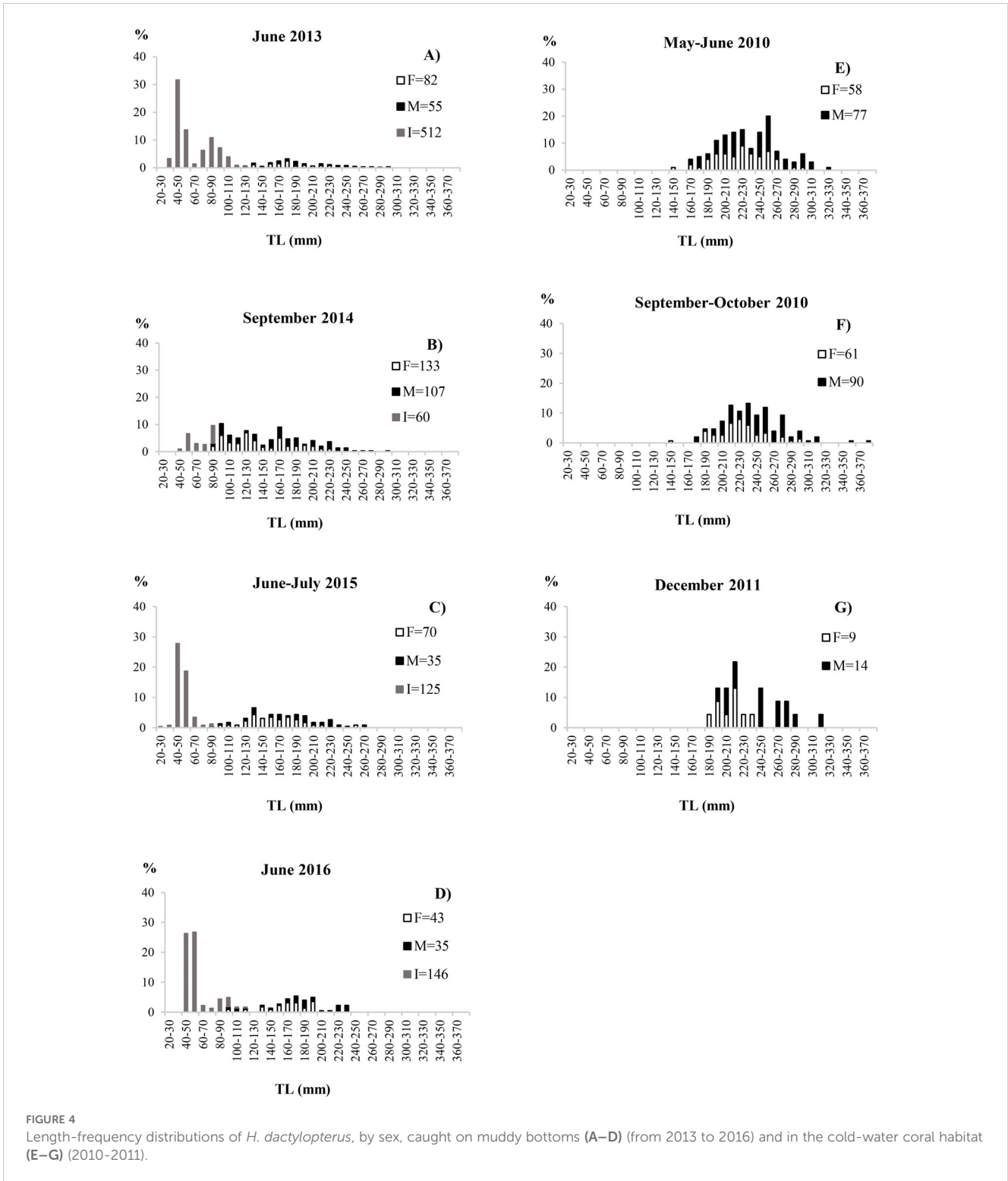
The direct method was corroborated with the back calculation method, in particular, the formula of Fraser-Lee (Francis, 1990) was used:

$$L_i = c + (L_c - c) \frac{R_i}{R_c}$$

where  $L_i$  is the total length (mm) of the fish at the time of *annulus* “ $i$ ” formation, and  $L_c$  is the total length (mm) of the fish at the time of otolith removed.  $R_i$  is otolith *radius* (mm) from the centre of the otolith to the outer margin of the transparent ring *annulus*, and  $R_c$  is the *radius* of the otolith at capture (mm), the distance from the centre of the otolith to the periphery;  $c$  is the intercept obtained from the linear regression of the otolith *radius versus* the total length. All measurements were taken in the posterior area on the distal side along the longitudinal axis joining the *sulcus* and the *nucleus*, the centre of the otolith. They were taken from the right otolith of only *annuli* that were clearly defined according to the criteria proposed by ICES, 2019. The mean values for age classes coming from the otoliths reading and back calculation method were tested using the Kolmogorov-Smirnov (KS) statistical test.

The Length Frequency Distribution analysis (LFD) was based on data collected in MEDITS 2010 survey. The Bhattacharya method, incorporated in FiSAT software (Gayani et al., 2006), was used to discriminate the normal distribution assuming that each mode in the overall size-frequency distribution represented a cohort. The separation index among different cohorts was taken into account and values < 2 indicated a large overlap between cohorts, which was considered unacceptable.

Furthermore, the von Bertalanffy growth equation was fitted to the length-at-age data obtained, for males, females and whole



population from otolith readings and for entire population from LFD analysis and the back calculation method, using FiSAT computer software (Gayani et al., 2006). The growth curves ( $L_{\infty}$ ,  $k$  and  $t_0$ ) were compared using the Chen test (Chen et al., 1992).

Finally, the growth performance index ( $\phi' = \log K + 2 \log TL_{\infty}$ ) (Munro and Pauly, 1983) was also estimated to compare the results of the present study with those obtained in different areas of the Mediterranean Sea and in the Atlantic Ocean.

## 2 Results

The length-frequency distributions of blackbelly rosefish showed a polymodal pattern in both habitats explored, with sizes between 22 and 300 mm TL and total weight from 0.60 to 462.6 g from muddy bottoms and between 140 and 380 mm TL and total weight from 44.30 to 700.00 g taken in the cold-water coral habitat (Figure 4). In muddy habitats most individuals were smaller than 100 mm TL and sexually indeterminate. In both surveys the males

TABLE 2 Age-length key for the entire population of *H. dactylopterus* in the north-western Ionian Sea, from whole otolith readings, with number of fish (N), mean length (TL, mm), standard deviation (s.d.) and Growth rate (Gr).

TL (mm)	Age class (years)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
330-340																2
320-330															1	
310-320														1	3	1
300-310												1	2	5		
290-300									1		2	6	8	8		
280-290										3	7	9	4	2		
270-280									3	9	21	12	2			
260-270									12	16	16	4				
250-260								2	7	14	10	1				
240-250								1	16	12	2	1				
230-240							1	10	13	4	2					
220-230							4	26	7	5						
210-220							12	19	11	3						
200-210						8	13	13	2							
190-200					3	9	18	9								
180-190					8	22	5	1								
170-180					16	13	1									
160-170				6	27	1										
150-160			2	15	12											
140-150			4	14	3											
130-140		1	12	13	1											
120-130		4	14	2												
110-120		15	7													
100-110	4	28	3													
90-100	16	25														
80-90	21	5														
70-80	26															
60-70	12															
50-60	14															
40-50	9															
N	102	78	42	50	70	53	54	81	72	66	60	34	16	16	4	3
Mean TL (mm)	74	103	127	147	167	186	203	217	241	254	268	278	290	297	318	328
s.d.	16.01	9.81	11.41	10.59	12.09	9.49	12.55	14.05	19.38	17.14	12.22	12.62	7.50	8.69	4.11	11.59
Growth rate (Gr)	-	0.33	0.21	0.15	0.13	0.11	0.09	0.07	0.10	0.05	0.06	0.04	0.04	0.03	0.07	0.03

were larger than females (Table 1; Figure 4). Age estimation, through otolith reading, was carried out on a total of 834 specimens, of which 4% (N=33) were unreadable. Otoliths showed the characteristic pattern of teleosts, with transparent and

opaque rings laid down around an opaque nucleus due to slow and fast growth phases. Analysis of 801 otoliths produced estimates from 0 to 15 years, with sizes between 42 and 336-mm TL (Table 2). In particular, the *sagittae* were however read separately by sex, 350

otoliths of the females gave estimates from 0 to 13 years (Supplementary Table S1), with sizes between 64 and 315-mm TL while for the males the age estimated was from 0 to 15 years (Supplementary Table S2), with sizes between 57 and 336-mm TL. Most of the samples belonged to the age class 0 and 1, representing about 13% (N=102) and 10% (N=78) respectively, however a large length range was recorded for each age group. A higher increase in the Growth rate (Gr), for both sexes and for the entire population, was observed in the first two age classes, while it slows down starting from the third year. Regarding the ageing precision CV and APE values were 5.14 and 4% respectively and the PA was of 82.6%, suggesting a good agreement between readers. No significant difference was found when comparing age keys from otolith readings of females with those of males (Kolmogorov-Smirnov test ( $D=0.17$ ;  $p>0.05$ ), the estimation of growth parameters using the von Bertalanffy model was, however, carried out by sex and for the entire sampled population.

Age validation of the first annulus was achieved by analysis of Daily Growth Increments (DGI). From the 61 otoliths prepared for DGI interpretation, 32 (52%) were read, while for the rest polishing and breakage problems hampered the reading. On the assumption that these increments were deposited daily, the age range determined was from 191 to 431 days old for specimens between 42 and 113mm TL (Table 3). A linear regression between TL and DGI (Figure 5) was used to validate the first annulus; the variance explained by the regression ( $r^2$ ) was 0.95, showing a very good correlation between the two variables. From the analysis of the daily rings, it was observed that the specimens, belonging to age class 1 (starting from 352 days), had TL between 85 and 113 mm and an

TABLE 3 Total lengths (TL) and number of daily increments (DGI) identified in *H. dactylopterus* individuals caught in the north-western Ionian Sea.

TL (mm)	DGI	TL (mm)	DGI
42	191	85	352
48	207	85	383
50	228	85	372
52	233	85	386
56	245	87	389
58	247	88	395
58	243	88	392
62	287	88	395
63	267	93	397
66	291	95	402
67	287	97	401
70	298	98	394
72	327	101	412
73	305	107	421
75	339	108	427
77	343	113	431

average TL value of 94 mm  $\pm$  9.7 s.d. Comparing, the average lengths of age class 1, calculated both with the direct method (103 mm  $\pm$  9.81 s.d.) and by counting the daily increments (94 mm  $\pm$  9.7 s.d.) there was no significant difference (Student's t-test,  $t=1.69$ ,  $p>0.05$ ).

Fish Total Length (TL) and otolith radius (R) were highly correlated (Figure 6); the relationship estimated using a linear model was  $TL = 7.3579R - 17.446$  ( $r^2 = 0.96$ ). The intercept value and the ray measurements were carried out on 746 structures and a length-age key (Table 4) was constructed through the back calculation procedure using the Fraser-Lee formula (1920). The Length Frequency Distribution analysis produced 8 modes (from 0 to 7) (Figure 7); all modal components were identified with Separation Indices (S.I.) largely greater than 2 (Gayani et al., 2006). The age-length keys obtained from otolith reading and the back calculation method, in which the number of age classes was comparable (Table 5), were tested using the Kolmogorov-Smirnov test showing no significant differences ( $D=0.18$ ;  $p>0.05$ ). The growth curves were comparable between the different methods ( $F_{critic} = 2.42 > F_{observed} = 1.79$ ;  $p>0.05$ ) and no significant difference was observed. The growth parameters calculated are presented in Table 6 and the growth curves obtained with different methods are shown in Figure 8.

## 4 Discussion

This study contributes to a better understanding of the of age and growth estimation of *H. dactylopterus* in the north-western Ionian Sea by comparing three different methods, a direct method by otolith reading verified by means of back calculation and corroborated by the Length Frequency Distribution analysis (LFDa). The three methods provided consistent results confirming the same growth pattern. Furthermore, the analysis of the daily increments allowed the validation of the first annulus to which one year of age was assigned using the direct method (reading of the otoliths).

The use of different sampling tools, in different habitats, allowed the capture of a wide range of sizes. In the north-western Ionian Sea, as in other areas of the Mediterranean, the smaller individuals of *H. dactylopterus* are distributed in shallower waters and, as they grow, move towards greater depths (D'Onghia et al., 1992). In habitats with muddy bottoms, however, the use of the trawl net allowed the capture, mainly, of small-medium sized individuals; on these substrates, fishing activity is widespread even at great depths, significantly affecting the structure of fish stocks and reducing the adult fraction. The size of the specimens captured was greater in coral habitats than in habitats with muddy substrates, confirming the peculiarity of coral habitats as refuge areas linked to sensitive phases of the life cycle (reproduction, nursery site and feeding) (D'Onghia, 2019). For this species there seems to be a strong association with structurally very complex seabeds such as those with cold-water corals or in canyons (D'Onghia et al., 2012, 2015; Mytilineou et al., 2014). Carbonara et al. (2020) found double biomass of *H. dactylopterus* in areas associated with bamboo coral (*Isidella elongata*) compared to areas of deep mud without any



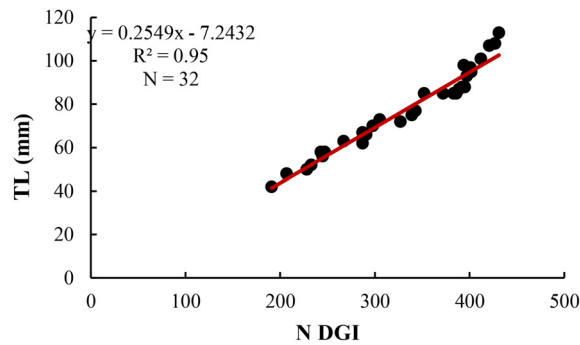


FIGURE 5  
Regression line between total number of daily increments (DGI) and corresponding Total Length (TL) in specimens of *H. dactylopterus*.

three-dimensional structure. Coral and/or canyon habitats act as source areas in which individuals, thanks to the protection offered by the habitat, can reach larger dimensions and carry out reproduction, generating new individuals who, thanks to the spill-over effect, can renew their populations in sink areas present on muddy seabeds, where organisms are continuously removed due to fishing activities (D'Onghia et al., 2016; Sion et al., 2019; Carbonara et al., 2020). However, the differences in size observed in the survey areas could be due both to the method of capture (different gear used) but also to the different fishing pressure (Massutí et al., 2000).

The length-frequency distributions of the two habitats explored showed sexual dimorphism with males larger than females, confirming what has been observed for this teleost in other areas of the Mediterranean basin (Massutí et al., 2000, 2001; Anastasopoulou et al., 2017; Sequeira et al., 2009; Mili et al., 2016). The reproductive effort, considering the bioenergetic constraints of the energy budget of the organisms, implies reduced growth rates of females (Gunderson, 1997). However, this is not always the case, in fact in other deep-sea fishes, such as the Gadiforms, the females are larger than the males. The maximum age observed in females was 13 years (315 mm TL), while in males it was 15 years (336 mm TL), but no significant difference was observed comparing age-length keys from otolith readings of females with the males (Tables 3, 4). The maximum length sampled in our sample was equal to a male of 380 mm TL,

consequently one could think of an age of approximately 25 years old, calculated using the von Bertalanffy growth parameters obtained in this study, an age comparable to other areas of the Mediterranean Sea.

The problems encountered were essentially due to the otoliths of adult individuals, in which there is the difficulty in clearly distinguishing all the growth rings, even by making thin sections of the otoliths. As the greater deposition of calcium carbonate makes the separation of the first rings less evident, but also because the presence of false rings due to the slowdown in the growth speed makes the rings thinner and closer together, making the count less certain. This is confirmation of why numerous works have been published on estimating the age and growth of blackberry rosefish, but there are still numerous controversies linked mainly to the maximum age detected (Abecasis et al., 2006). However, the alternation of transparent and opaque rings in the otoliths of *H. dactylopterus* indicates that, despite being a bathyal species, its growth shows aspects of seasonality that are certainly not linked to the thermal regimes that remain almost constant in the deep environment. For this species, there seems to be a strong link with the seasonal fluctuations in primary productivity that occur in surface waters, and which have repercussions in terms of secondary productivity in the deep environment (Massutí et al., 1995). The deposition of the rings discussed by Massutí et al. (2000) is confirmed in the present study, in fact the first 4–8 pairs of rings

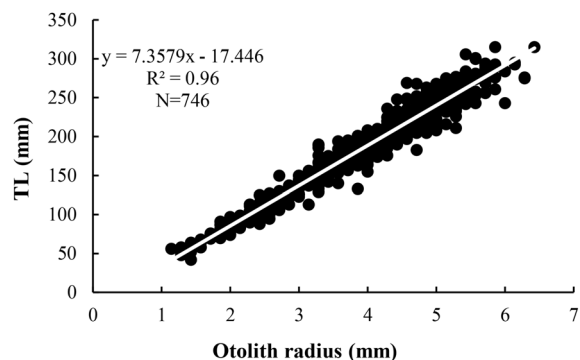


FIGURE 6  
Regression line between otolith radius and corresponding Total Length (TL) in specimens of *H. dactylopterus*.

TABLE 4 Back calculated lengths at-age (TL) for whole population of *H. dactylopterus* caught in the north-western Ionian Sea.

Age group (years)	N	TL 0	TL 1	TL 2	TL 3	TL 4	TL 5	TL 6	TL 7	TL 8	TL 9	TL 10	TL 11	TL 12	TL 13	TL 14
0	67	69														
1	78	69	86													
2	47	69	86	110												
3	50	69	86	110	132											
4	70	69	86	111	133	154										
5	53	69	87	111	133	154	172									
6	55	69	87	109	157	152	172	191								
7	80	69	87	110	132	152	171	188	206							
8	72	69	87	111	134	155	175	193	210	228						
9	64	69	86	110	132	153	171	189	206	223	241					
10	55	69	85	110	133	154	172	189	205	222	238	255				
11	32	69	84	107	128	148	166	184	201	218	234	250	266			
12	12	69	82	106	133	153	173	190	206	222	236	250	266	280		
13	9	69	84	105	128	145	163	180	196	212	227	243	257	271	287	
14	2	69	86	110	130	154	174	190	210	226	237	253	265	281	293	315
N	746															
mean TL (mm)		70	86	109	134	152	171	188	205	222	236	250	263	277	290	305
s.d		7.55	1.59	2.08	7.50	3.12	3.62	3.90	4.76	5.13	4.68	4.44	4.50	5.64	4.50	-
Growth rate (Gr)			0.21	0.24	0.20	0.13	0.12	0.10	0.09	0.08	0.06	0.06	0.05	0.05	0.04	0.05

were wider than the following rings, which were laid down with decreasing thickness, while in adult fish the outer rings decreased in width, becoming irregular and equally wide.

Counts of daily increments provided the validation of the first annulus. In species with a clearly interpretable otolith microstructure, daily increment counts can often be used to confirm the identity of the first annulus (Campana, 2001). The analysis of the daily increments allowed us to validate the average

length of the first year of age (approximately 90-100 mm LT) obtained from the three different methods used. No significant difference was highlighted comparing the average lengths of age class 1, calculated with the direct method and by counting the daily increments. Therefore, the analysis of the daily increments, allowed the validation of the first annulus to which one year of age was assigned using the direct method (reading of the otoliths). Our results are comparable with those found by other authors for the

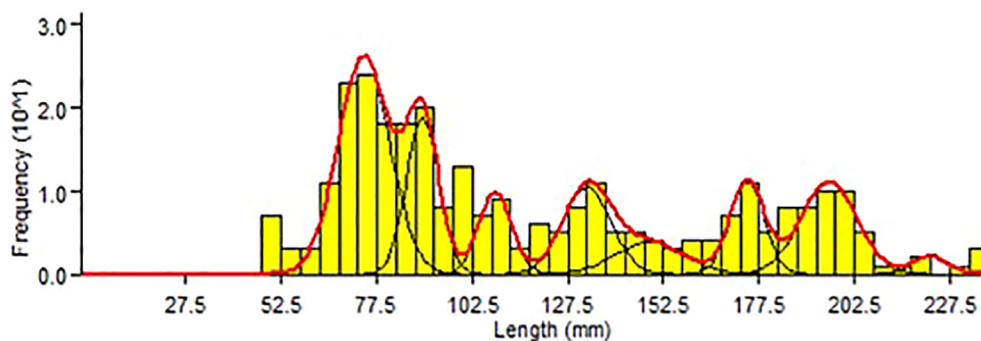


FIGURE 7 Length Frequency Distribution analysis (LFDa) calculated for individuals of *H. dactylopterus* caught during the MEDITS 2010 survey.

TABLE 5 The age-length key estimated, by three different methods, for *H. dactylopterus* caught in the north-western Ionian Sea.

	Otolith readings	Back calculation method	LFDa
Age class (year)	Mean TL (mm) ± s.d.	Mean TL (mm) ± s.d.	Mean TL (mm) ± s.d.
0	74 ± 16.0	70 ± 7.55	74 ± 6.77
1	103 ± 9.8	86 ± 1.59	89 ± 4.23
2	127 ± 11.4	109 ± 2.08	109 ± 4.29
3	147 ± 10.6	134 ± 7.50	133 ± 6.30
4	167 ± 12.1	152 ± 3.12	149 ± 8.92
5	186 ± 9.5	171 ± 3.62	174 ± 4.44
6	203 ± 12.6	188 ± 3.90	196 ± 7.37
7	217 ± 14.1	205 ± 4.76	221 ± 3.66
8	241 ± 19.4	222 ± 5.13	-
9	254 ± 17.1	236 ± 4.68	-
10	268 ± 12.2	250 ± 4.44	-
11	278 ± 12.6	263 ± 4.50	-
12	290 ± 7.5	277 ± 5.64	-
13	297 ± 8.7	290 ± 4.50	-
14	318 ± 4.1	315	-
15	328 ± 11.6		

s.d., standard deviation.

TABLE 6 Von Bertalanffy growth parameters ( $L_{\infty}$ ,  $k$ ,  $t_0$ ) and growth performance indexes ( $\phi'$ ) for *H. dactylopterus* reported in different study areas.

Author	Area	Sex	Method	$L_{\infty}$ (mm)	$k$ ( $\text{year}^{-1}$ )	$t_0$ (year)	TL range (mm)	Age classes (year)	$\phi'$
Abecasis et al. (2006)	Azores	F	Whole otoliths	565	0.06	-1.13	130-410	2-15	4.28
		M	Whole otoliths	590	0.07	-0.21	140-460	3-16	4.39
		F	Sliced otoliths	570	0.05	-2.28	140-420	2-28	4.21
		M	Sliced otoliths	548	0.06	-2.29	120-470	2-32	4.26
Krug et al. (1998)	Azores	F+M	Whole otoliths	505	0.14	-0.23	140-470	1-16	4.55
		F+M	MULTIFAN	505	0.16	-0.46	140-470	1-16	4.61
Esteves et al. (1997)	Azores	F	Whole otoliths	547	0.10	-1.16	140-470	3-12	4.48
		M	Whole otoliths	502	0.16	0.05	150-470	3-14	4.61
		F	Back-calculation	526	0.11	-0.24	160-470	3-12	4.48
		M	Back-calculation	574	0.11	-0.32	170-470	3-14	4.56
		F	LFDa	560	0.15	1.08	180-470	3-12	4.67
		M	LFDa	653	0.13	0.71	190-470	3-14	4.74
Isidro (1987)	Azores	F	Whole otoliths	389	0.18	-0.42	160-410	3-12	4.43
		M	Whole otoliths	449	0.11	-1.83	160-380	3-16	4.34
Sequeira et al. (2009)	Continental Portuguese slope	F+M	Whole otoliths	455	0.05	-4.01	52-420	0-30	4.01
		F	Whole otoliths	453	0.05	-4.17	52-420	0-30	4.17

(Continued)

TABLE 6 Continued

Author	Area	Sex	Method	$L_{\infty}$ (mm)	$k$ (year <sup>-1</sup> )	$t_0$ (year)	TL range (mm)	Age classes (year)	$\phi'$
		M	Whole otoliths	433	0.05	-3.68	58-350	0-25	3.97
Allain and Lorange (2000)	British Isles (NE Atlantic)	F+M	Whole and sliced otoliths	290	0.10	-2.79	60-320	1-17	3.92
Mamie et al. (2007)	North Sea	F+M	LFDa	282	0.11	-2.10	50-260	-	3.94
Kelly et al. (1999)	Rockall Trough	F	Whole and sliced otoliths	310	0.04	-3.00	100-380	1-37	3.58
		M	Whole and sliced otoliths	370	0.06	-4.00	100-380	1-43	3.91
Massuti et al. (2000)	Alboran Sea	F+M	Whole otoliths	300	0.10	-2.86	80-290	1-30	3.95
		F	Whole otoliths	271	0.12	-2.65	80-280	1-26	3.95
		M	Whole otoliths	325	0.09	-3.31	80-290	1-30	3.97
	Balearic Islands	F+M	Whole otoliths	299	0.13	-1.75	70-300	1-22	4.07
		F	Whole otoliths	270	0.16	-1.62	70-280	1-22	4.07
		M	Whole otoliths	325	0.10	-2.62	80-300	1-21	4.02
Mili et al. (2016)	Northern waters of Tunisia	F+M	Whole otoliths	372	0.14	-1.67	80-305	0-9	4.29
Peirano and Tunesi (1986)	Ligurian Sea	F+M	Whole otoliths	707	0.05	-0.41	40-280	0-9	4.40
Ragonese and Reale (1995)	Canal of Sicily	F+M	Whole otoliths	392	0.13	-1.46	40-340	0-10	4.30
Consoli et al. (2010)	Tyrrhenian Sea	F+M	Whole otoliths	261	0.14	-1.92	63-270	0-21	3.98
Ungaro and Marano (1995)	Southern Adriatic	F+M	Whole otoliths	299	0.19	-0.85	-	-	4.23
Romanelli et al. (1997)	Southern Adriatic	F+M	Whole otoliths	-	-	-	50-340	1-10	-
D'Onghia et al. (1992)	NW Ionian Sea	F+M	LFDa	300	0.20	-1.41	40-290	-	4.26
D'Onghia et al. (1996)		F+M	Whole otoliths	307	0.16	-0.93	35-235	0-7	4.18
Present study	NW Ionian Sea	F	Whole otoliths	456	0.07	-2.80	64-315	0-13	4.16
		M	Whole otoliths	486	0.06	-2.76	57-336	0-15	4.15
		F+M	Whole otoliths	494	0.06	-3.00	42-336	0-15	4.17
		F+M	Back calculation	487	0.06	-2.30	62-315	0-14	4.15
		F+M	LFDa	415	0.07	-2.23	50-230	0-7	4.08

MPA, Modal Progression analysis.

western Mediterranean (Massuti et al., 2001; Sequeira et al., 2009). In particular, Massuti et al. (2000) reported a mean TL of 83 mm TL for the first-year class, based on DGI analysis. The growth parameters estimated for the sex and pooled, according to the von Bertalanffy model, would indicate slow growth in agreement with what has already been observed in the northern Ionian (D'Onghia et al., 1992, 1996), in the Adriatic Sea (Romanelli et al., 1997), for the Balearic Islands (Massuti et al., 2000) and for the Tyrrhenian Sea (Consoli et al., 2010). In the Mediterranean, the

maximum ages observed were 21 years (270 mm TL) in the Tyrrhenian Sea (Consoli et al., 2010) and 10 years (340 mm TL) in the southern Adriatic (Romanelli et al., 1997). Moreover, this latter author reported an individual with a length of 410 mm TL with an estimated age of 25-30 years old, indicating that this species may grow to be 25 years old or more. These authors suggested that the maximum number of growth zones distinguishable on the whole otoliths is approximately 30, and that divergence on ages, estimated using whole or sectioned otoliths, usually occurs after 20-

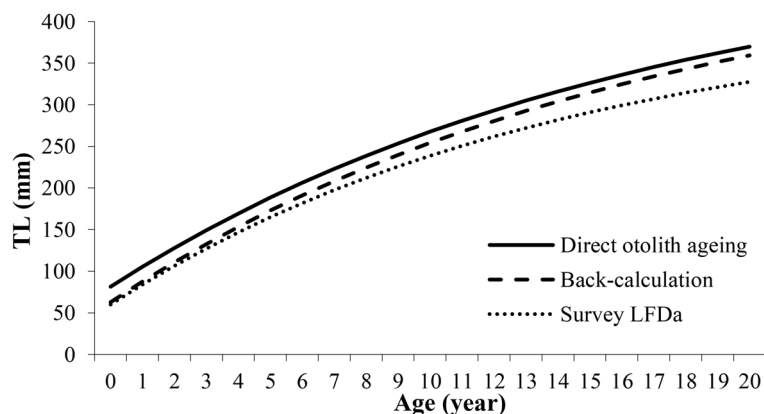


FIGURE 8

*H. dactylopterus* von Bertalanffy growth curves obtained from direct otolith readings (continuous line); back-calculation (dashed line) and Length Frequency Distribution analysis (dotted line).

25 years of age. A large variation of results can be observed in the von Bertalanffy growth parameters for *H. dactylopterus* between different authors (Table 6) and this may be a consequence of the method used (otolith reading or length-frequency analysis) and of the heterogeneity of the sample studied (fish of different size distribution caught with different types of sampling gear). In relation to the growth parameters obtained for this species in the Mediterranean basin (Table 6), a longevity of over 30 years can be hypothesized, indicating a long-lived species, with numerous age-classes in the population. Differences in growth parameters reported for Mediterranean and Atlantic populations, in terms of asymptotic length, can be related to different range sizes analyzed and environmental variability, such as differences in temperature, prey availability, nutrient levels, pollution, or strong regional genetic differentiation between Atlantic and Mediterranean populations (Atkinson and Sibly, 1997), together with different degrees of fishing pressure (Schindler et al., 2000; Carbonara et al., 2022). These are all factors that commonly represent an additional source of variation in growth characteristics.

Comparing the results of the present study with those obtained on a larger spatial scale, it is possible to confirm that the size of this teleost is generally larger in the Atlantic Ocean than in the Mediterranean Sea (Table 6).

The differences of maximum age estimates are very important for the management because the exploitation of the species is correlated to its life history (Jennings et al., 1998; Denney et al., 2002). Deep-water species like the blackbelly rosefish are vulnerable to overfishing because of their biological characteristics (long life, large size, late maturity and slow growth). The accuracy and precision of age and growth data have a negative impact on the assessment of the status of stocks and the application of management measures aimed at achieving sustainable exploitation of this species in the Mediterranean basin (Carbonara et al., 2019b). If an age overestimation occurs, the stock assessment will provide an erroneous scenario with a population composed of older individuals and, consequently, affected by lower fishing mortality,

while in the opposite case, fish would be younger with an overestimation of fishing mortality (Campana, 2001).

In the present study, age determination of large individuals contributes to better understanding of the growth pattern of *H. dactylopterus*, which is useful for future stock assessment by providing more robust scientific advice on the management of this Mediterranean stock. This agrees with Caddy (1993, 2008) who argued for the inclusion of habitat components in the fisheries management to recover and maintain overexploited or depleted fishery resources.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

## Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the study was carried out on dead animals caught in fishing activities.

## Author contributions

LS: Conceptualization, Data curation, Formal analysis, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LC: Data curation, Writing – original draft, Software, Methodology, Formal analysis. GG: Writing – original draft, Software, Methodology, Formal analysis, Data curation. GD: Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

PC: Writing – review & editing, Visualization, Validation, Supervision, Methodology.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1506180/full#supplementary-material>

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