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Some biological aspects of juveniles of the rough ray, *Raja radula* Delaroche, 1809 in Eastern Sicily (central Mediterranean Sea)

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ABSTRACT

Several biological aspects of juvenile specimens of *Raja radula* were investigated from February to May 2017 in the central Mediterranean Sea. Diet, sex ratio, disc width-weight relationships, and size-frequency distribution were analyzed. Stomach content analysis of 127 collected specimens showed that juveniles of *R. radula* actively feed on small benthic crustaceans, particularly on amphipods and isopods (*Idotea balthica*) (%IRI values of 62.74% and 12.39%, respectively). Although the Levin's index value ($Bi = 0.44$) indicated that juveniles of *R. radula* are a moderately stenophagous feeders, active mostly on crustaceans, the analysis of the prey-specific (Pi) biomass of the main preys vs. the frequency of their occurrence (%F) showed no clear dominance. The analysis of the sex ratio showed no significant difference in sex distribution. However, there were significant differences in mean size between sexes: females were, on average, larger than males, and also the b value (slope of the curve) of the disc width-weight relationships was higher in females.

1. Introduction

In Italian seas, skates (Rajidae) are represented by 16 species, distributed in 4 genera: *Dipturus*, *Leucoraja*, *Raja,* and *Rostroraja*. Among these, the genus *Raja* is the most diverse, with 8 species recorded from Italy (Vacchi and Serena, 2010). Although most of these species have low or no commercial value (Silva et al., 2012), some such as *Raja asterias* Delaroche, 1809, *Raja clavata* Linnaeus, 1758 and *Raja miraletus* Linnaeus, 1758 are important for conservation and fishery (Minervini et al., 1985; Zorzi et al., 2001; Ragonese et al., 2003; Enever et al., 2009). However, due to their low fecundity and growth rates, skates, as the other elasmobranch species, are particularly vulnerable to overexploitation (Ragonese et al., 2003).

Raja radula Delaroche, 1809 is an endemic species of the Mediterranean Sea (Serena, 2005), as well as *Leucoraja melitensis* (Clark, 1926), *R. asterias* and *Raja polystigma* Regan, 1923. Furthermore, *R. radula* is listed as "endangered" in the IUCN Red List (Mancusi et al., 2018). In Italian seas, this species, known as "rough ray", seems uncommon in the central (with the exception of Sardinia) and the northern areas, while it is common in the southern areas, especially in Sicilian seas. Here, it is usually caught mostly in trawls and trammel nets and considered as a bycatch or discards (Relini et al., 2000; Tiralongo et al., 2018). This species is a relatively small skate that inhabits sandy (or mixed) and muddy bottoms, from shallow waters of 2–350 m (Mancusi et al., 2018; Tiralongo et al., 2018). Despite its conservation importance, few quantitative studies have been conducted on the feeding habits of this species (Capapé and Azouz, 1975; Abdel-Aziz, 1986; Consalvo et al., 2010; Kadri et al., 2013) and little is known about other aspects of its biology (Kadri et al., 2014), such as the sex ratio, the length-weight relationships, age and growth.

This study provides new additional biological information on *R. radula* juveniles from the central Mediterranean Sea including: (i) diet composition and feeding habits; (ii) disc width-weight relationships; (iii) size-frequency distribution and (iv) sex ratio. These data increase the knowledge on the biology and ecology of this species.

2. Material and methods

A total of 127 specimens of *R. radula* juveniles were collected from fishermen operating trammel nets in the south-east coast of Sicily

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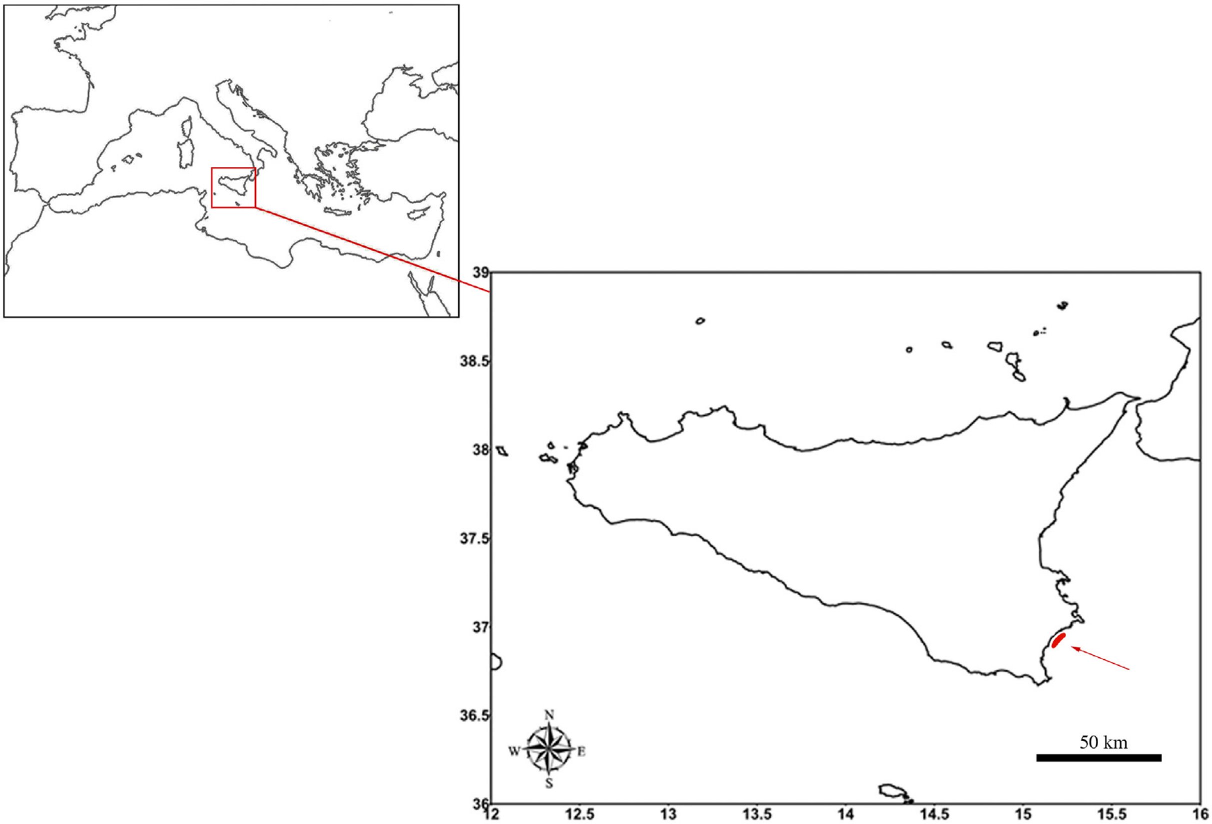

Fig. 1. Study area (in red and indicated by the red arrow) in the Ionian coast of Sicily (central Mediterranean Sea). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Avola, Ionian Sea) (Fig. 1). In order to better represent the population, specimens were randomly selected from different fishing vessels. About 32 specimens per month were collected between February and May 2017, at 2–12 m depth, during the fishing season of *Sepia officinalis* Linnaeus, 1758 (Tiralongo et al., 2018). Trammel nets were deployed overnight (from 6 pm to 4 am) for about 10 h, on sandy and mixed bottom (sand and rocks), close to *Posidonia oceanica* meadows.

Each specimen was weighed and measured (straight disc width) and the sex identified. The previous studies conducted in nearby areas, such as Tunisian (Mejri et al., 2004) and Sicilian waters (Consalvo et al., 2010), reported that males and females of *R. radula* become adult (i.e. 100% of the specimens become mature) above 320 and 340 mm disc width, respectively. These reference values were applied to classify specimens as adult or juvenile depending on whether they were above or below such sizes. Weight and disc width measures were used for the length-weight relationships following the formula: $W = aL^b$, where W is the weight in grams (g), L is the disc width (DW) in millimeters (mm), a is the intercept and b is the slope. Disc-width frequency distributions were constructed for both sexes. Chi-square test was used to verify if there was a significant difference ($\alpha = 0.05$) between the observed and the expected sex ratio (M:F) of the whole sample. To test if the regressions of the weight on length were significantly different ($\alpha = 0.01$) for the two sexes, an analysis of covariance (ANCOVA) was employed. We also analyzed the regression between the length and the weight for both sexes on a log-scale to derive the following equation: log(W) = log (alpha) + beta * log(DW) and the related exponential parameters.

The stomach was removed from each freshly caught fish and its content analyzed. All prey items in the stomachs were transferred into a petri dish and counted, weighed (after being washed in clean seawater and dried with blotter paper) to the nearest 0.01 g and identified under a stereomicroscope to the lowest taxonomic level possible.

The frequency of occurrence (%F), percentage weight (%W), percentage abundance (%N) and the Index of Relative Importance (% IRI) were calculated for each prey category (Hureau, 1970; Hyslop, 1980; Carrassòn et al., 1997). The vacuity index (percentage of empty stomachs) was also calculated.

According to the value of their percentage abundance (%N), preys were grouped into three categories (N'Da, 1992): dominant ($N > 50\%$), secondary ($10\% < N < 50\%$) and accidental ($N < 10\%$).

The feeding strategy of *R. radula* was investigated by plotting the prey-specific biomass (P*i*) against their frequency of occurrence (%F) (Amundsen et al., 1996):

$$Pi = \frac{SWi}{SWt_i} \times 100$$

where P*i* is the prey-specific biomass of prey *i*, SW_i the stomach content biomass of prey *i*, and SWt_i the total stomach content biomass in those predators with prey *i* in the stomach.

Standardized Levins' index (B*i*) was used to evaluate the breadth of the diet (Krebs, 1989):

$$B = \frac{1}{\sum p_j^2}$$

$$Bi = \frac{B - 1}{B_{max} - 1}$$

where p_j is the relative frequency specimens in the j^{th} prey item and B_{max} is the total number of prey item categories found (not identified categories were excluded from the analysis). B*i* is comprised between 0 (narrow trophic niche) and 1 (wide trophic niche); if $Bi < 0.40$ the species is considered a specialist, if $0.40 < Bi < 0.60$ is considered an "intermediate", if $Bi > 0.60$ is considered a generalist (Novakowski et al., 2008).

To evaluate whether the number of skate stomachs was sufficient to

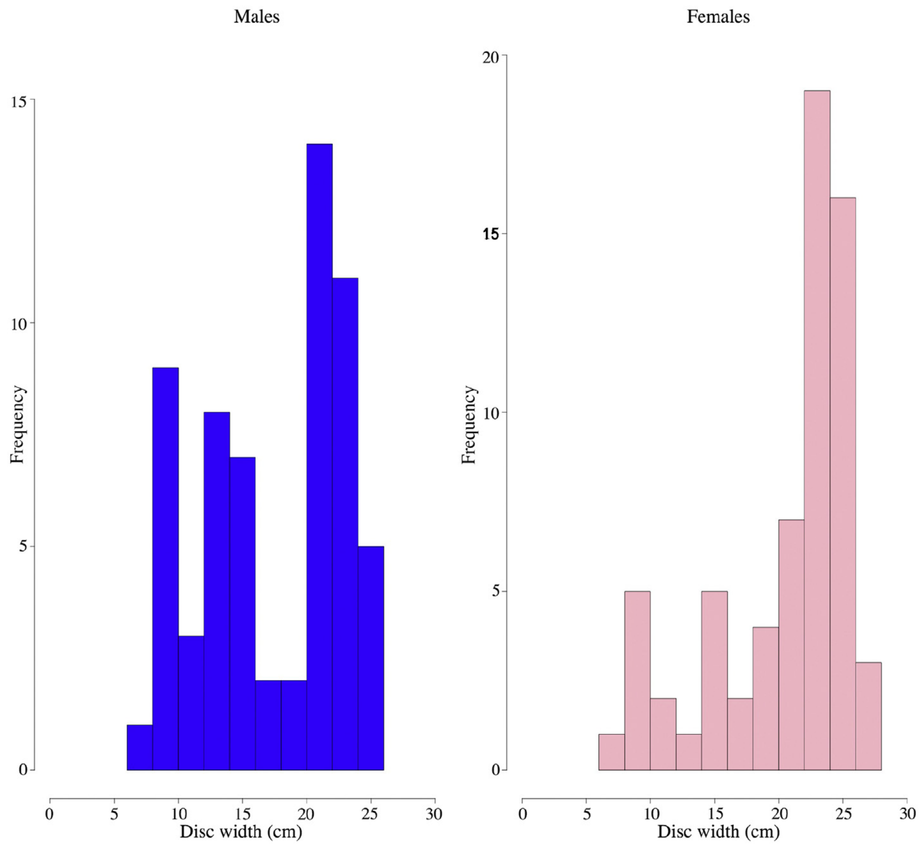

Fig. 2. Sex composition by size (disc width in cm) classes of *R. radula*.

describe the diet, accumulation prey curves (Ferry and Cailliet, 1996; Brown et al., 2012; Messina et al., 2016; Cazzolla Gatti, 2016, 2017) for three estimators (the expected number of prey groups in t pooled samples, given the reference sample – analytical – after 100 randomizations; the Chao 1 richness estimator; and the bootstrap estimator) were computed with EstimateS (Version 9.1.0, R. K. Colwell, http://viceroy.eeb.uconn.edu/estimates/). The estimated number of prey groups and associated SD were plotted against the cumulative number of individuals whose stomach was examined.

3. Results

Of the total 127 specimens examined, 62 (48.82%) males and 65 (51.18%) females, only 5, two males and three females, showed an empty stomach (vacuity index of 3.9%). The sex ratio (M:F = 0.9) was not significantly different from 1:1 ($p > 0.05$). Disc width (DW) sizes ranged between 7.3 and 25.1 cm for males and 7.2–27.1 cm for females (Fig. 2), with mean values of 17.26 and 20.63 cm, respectively (Table 1). Weights ranged between 10 and 341 g for males and 8–443 g for females, with mean values of 142 and 219 g, respectively (Table 1). The ANCOVA performed to compare the DW-W relationship of males vs. females showed a significant difference between sexes (SStot: 4143.74, df = 126, $p < 0.01$), and females were on average larger (20.63 ± 5.38 cm vs. 17.26 ± 5.62 cm) and heavier (219.06 ± 121.09 g vs. 141.92 ± 104.43 g) than males. We also calculated the parameters of the disc width-weight relationship by the exponentiation of the log-log regression as, for males: W = 0.21 + 18.5 * DW; and, for females: W = 0.19 + 19.86 * DW.

A total of 22 prey types were found during the stomach content analysis. These were divided into 3 main groups: Mollusca, Polychaeta, and Crustacea. Highly digested prey (%*N* = 1.07) were not considered for the analysis and were included into the "not identified" category. The values of %IRI clearly show that crustaceans were the main prey item in the diet of *Raja radula* (Table 2). The most frequently recorded prey types were amphipods (%F = 39.34), *Idotea balthica* (%F = 19.67) and *Portunus hastatus* (%F = 13.11). *Idotea balthica* (%W = 11.92) and *P. hastatus* (%W = 16.44) were also the most important preys by weight, while amphipods represented the most important preys in terms of percentage abundance (%*N* = 49.02). IRI percentage (%IRI) showed maximum values for amphipods (%IRI = 62.74) and *Idotea balthica* (%IRI = 12.39). From a total of 22 prey types, no dominant (%*N* > 50) ones were found, and only amphipods, with %N = 49.02, showed a value close to 50%, falling into the "secondary preys" category (10% < *N* < 50%). All the other prey types fall into the "accidental preys" category (*N* < 10%).

The plotted results of the main prey-specific biomass (P*i*) against the

Table 1
Sex distribution, disc width (cm), weight (g) and disc width-weight relationships parameters of *R. radula* in the Ionian Sea (Sicily, central Mediterranean Sea). Sex ratio (M:F) equal to 0.9 was not statistically different from 1:1 ($p > 0.05$).

Sex	N	Range DW (cm)	Mean DW (cm)	Range W (g)	Mean W (g)	a	b	r^2	*p*-Value
M	62	7.3–25.1	17.26	10–341	141.92	0.027	2.918	0.99	< 0.05
F	65	7.2–27.1	20.63	8–443	219.1	0.021	2.989	0.99	< 0.05
Combined	127	7.2–27.1	18.99	8–443	181.4	0.025	2.944	0.99	< 0.05

Table 2
Diet composition of 122 specimens of *R. radula* from the Ionian Sea (Sicily, central Mediterranean Sea). Values > 10% are in bold. %F = percentage frequency of occurrence; %N = percentage in number; %W = percentage in biomass; IRI = index of relative importance of prey items and its percentage (%IRI); n.i. = not identified.

Prey items	%F	%N	%W	IRI	%IRI
Mollusca					
Naticidae n.i.	1.64	0.89	1.12	3.3	0.10
Polychaeta					
Nereis sp.	4.92	1.07	1.39	12.1	0.35
Polychaetes n.i.	4.92	1.07	0.99	10.1	0.29
Crustacea					
Brachyura					
Goneplax rhomboides	4.1	0.89	4.9	23.7	0.69
Liocarcinus corrugatus	1.64	0.36	2.61	4.9	0.14
Liocarcinus navigator	1.64	0.36	0.98	2.2	0.06
Liocarcinus pusillus	7.38	1.96	8.09	74.2	2.15
Liocarcinus sp.	5.74	1.25	5.42	38.3	1.11
Portunus hastatus	**13.11**	3.39	**16.44**	260	7.54
Xantho poressa	9.84	2.32	6.06	82.4	2.39
Majidae n.i.	3.28	0.89	4.45	17.5	0.51
Brachyura n.i.	3.28	0.71	3.19	12.8	0.37
Isopoda					
Idotea balthica	**19.67**	9.8	**11.92**	427.2	**12.39**
Isopoda n.i.	6.56	1.6	3.32	32.3	0.94
AMPHIPODA					
Amphipoda n.i.	**39.34**	**49.02**	5.96	2163	**62.74**
Caridea					
Alpheus sp.	6.56	1.43	4.71	40.3	1.17
Crangon crangon	9.84	2.32	4.31	65.2	1.89
Hyppolytidae n.i.	0.82	0.18	0.02	0.2	0.01
Caridea n.i.	7.38	3.39	2.67	44.8	1.30
Dendrobranchiata					
Sicyonia carinata	5.74	1.25	6.94	47	1.36
Mysida					
Mysida n.i.	3.28	7.84	0.68	28	0.81
Ostracoda					
Ostracoda n.i.	4.92	6.95	0.41	36.2	1.05
Not identified	4.92	1.07	3.43	22.1	0.64

frequency of occurrence (%F) showed (Fig. 3) that there is no clear dominance of any prey. The value of the standardized Levins' index (B*i*) was 0.44, indicating a relatively narrow trophic niche.

Disc width-weight relationships for both sexes are reported in Table 1 and graphically represented in Fig. 4.

Analyzing the accumulation prey curves with the three estimators (the expected number of prey groups, the Chao 1 index, and the bootstrap estimator), we show that the number of sampled juvenile skate stomachs was sufficient to describe their diet (Fig. 5).

4. Discussion

The low percentage (3.9%) of empty stomachs (5 out of 127) of juvenile specimens of *R. radula*, all caught during nighttime hours, indicates that they actively feed during the night while, in the nearby area of Portopalo di Capo Passero, Consalvo et al. (2010) reported a daytime feeding habits in May–July 2005. Therefore, we can consider the juvenile of *R. radula* a very active feeder in the daytime and in both cold and warm months. Furthermore, especially in elasmobranchs, juveniles show a higher metabolic rate than adults, and this feature results in a higher feeding rate. Moreover, Wetherbee and Cortés (2012) defined all batoids as continuous feeders.

With a relative abundance of > 95%, crustaceans were the dominant preys; other preys were represented by incidental species (N'Da, 1992). These results were similar to those found by other authors in juveniles of *R. radula* and of other species of the genus *Raja* (Ebert et al., 1991; Ellis et al., 1996; Consalvo et al., 2010; Kadri et al., 2013). However, in our study, the main preys among crustaceans were represented by amphipods, isopods (*I. balthica* in particular) and the portunid crab *Portunus hastatus*. Differently, in the nearby area of Portopalo di Capo Passero, about 25 km further south from Avola, the previous study on *R. radula* of Consalvo et al. (2010) reported *Liocarcinus pusillus* as the main prey, with a %IRI of 45.25. In our study, this species showed, instead, a very low %IRI of 2.15. These data support the hypothesis that, although *R. radula* juveniles mainly feed on benthic crustaceans, their feeding habits are connected to spatial and temporal variations of their preys' presence and abundance.

The plot of the prey-specific biomass (P*i*) and the frequency of occurrence (%F) did not show a clear evidence of any prey dominance. However, the high percentage of amphipods found in our study is similar to those recorded in Sardinian waters in juvenile specimens of *Raja brachyura* and *Raja miraletus* (Follesa et al., 2010) and in the Ligurian Sea for *Raja asterias* (Cuoco et al., 2005). This result highlights the importance of amphipods (but also of other small crustacean species) in the diet of juvenile skates (Capapé and Azouz, 1975; Capapé, 1977; Muto et al., 2001).

The Levin's index was relatively low (0.44), suggesting a moderately

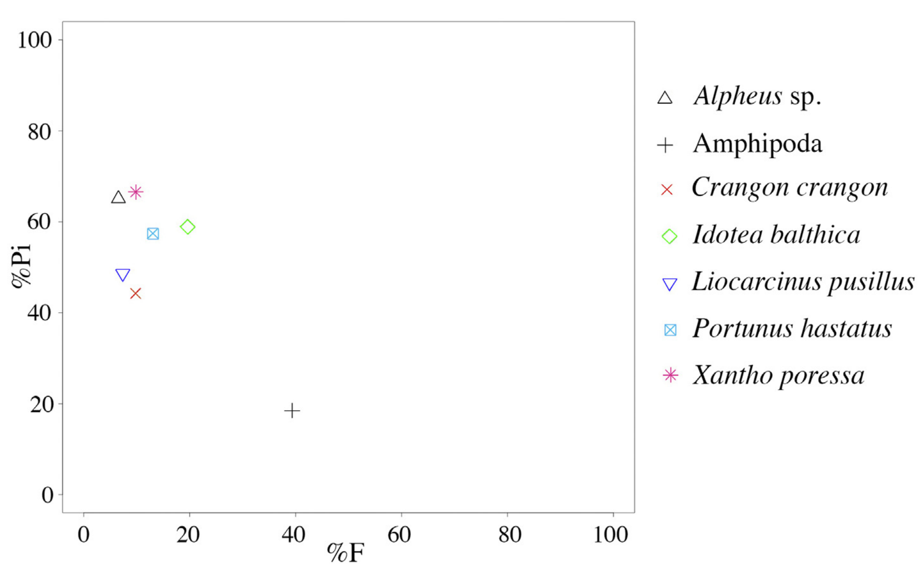

Fig. 3. Prey-specific biomass (P*i*) plotted against the frequency of occurrence (%F) of the main prey items for *R. radula* from the Ionian coast of Sicily (central Mediterranean Sea).

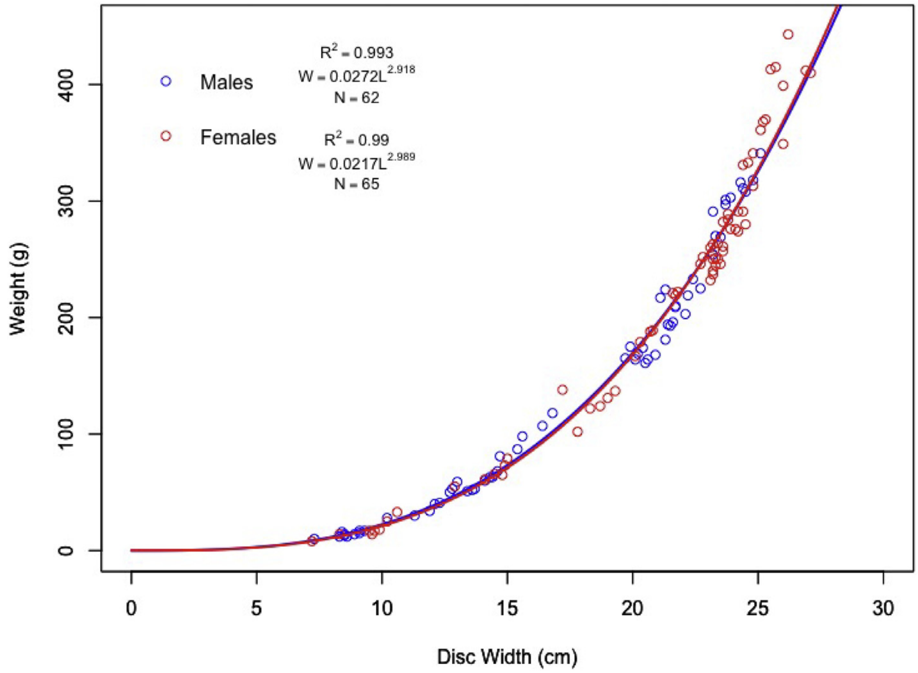

Fig. 4. Disc width-weight relationships of *R. radula* in the Ionian Sea (Sicily, central Mediterranean Sea).

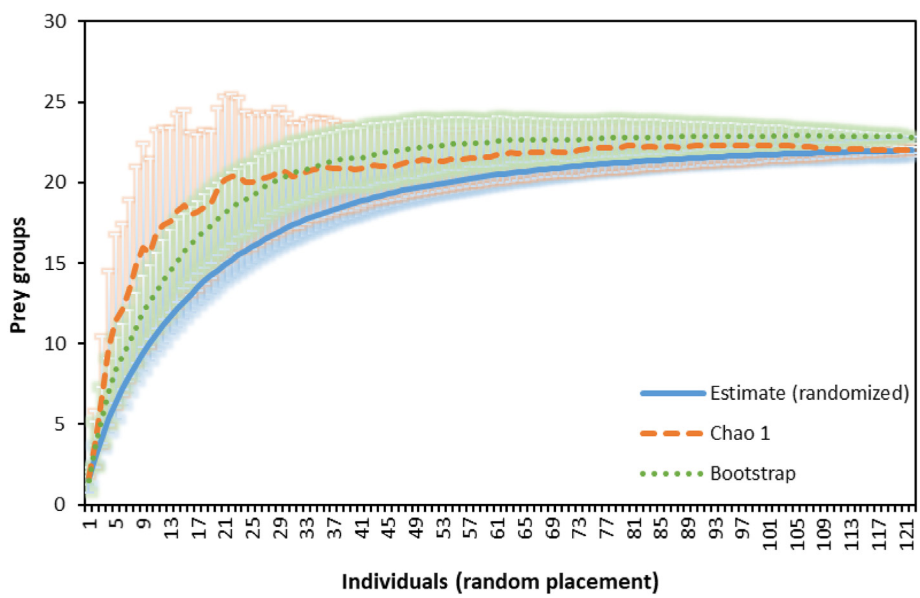

Fig. 5. Accumulation curves for a randomized (n. 100) estimate, the Chao 1 index and a bootstrap estimator (SD as shadowed areas). The saturation points are located – for all the 3 estimators – between 50 and 60 randomized individuals (juveniles) of *R. radula* at the level of the collected n. 22 prey groups.

stenophagous diet (i.e. juveniles of *R. radula* are demersal carnivores that feed on invertebrate prey). Stomach content analysis did not show the presence of fish species, in agreement with other studies (Ebert et al., 1991; Ellis et al., 1996; Consalvo et al., 2010; Kadri et al., 2013). Indeed, only the largest specimens feed also on (and, in some cases, prefer) fishes. This further supports an ontogenetic shift in diet (Follesa et al., 2010; Kadri et al., 2013) that, through niche partitioning (Schellekens et al., 2010; Cazzolla Gatti, 2011) and emergence (Cazzolla Gatti et al., 2017b, 2018), could increase marine biodiversity.

The proportion between males and females was equal to 1:1, indicating that there is no sexual segregation in juveniles. The disc width-weight relationships showed a slightly negative allometric growth in both sexes. Female specimens were, on average, larger than males.

Taking into consideration the number of juveniles caught in the area, the species could be considered relatively common, as in the nearby central Mediterranean area of the Sicilian (Consalvo et al., 2010) and Tunisian coasts (Kadri et al., 2013), but it seems uncommon in most other Mediterranean zones (Mancusi et al., 2018). The virtual absence of adult specimens, emerged from the size distribution analysis, suggests that juveniles of the species use the study area, consisting of sandy and mixed bottoms of shallow waters close to the *Posidonia oceanica* meadows, as a feeding ground. Indeed, although the fishing gear utilized for sampling (trammel net) is able to collect bigger sized skates (Tiralongo et al., 2018), no adult specimens were found in the present study.

In summary, our results suggest that juveniles of *R. radula* are very active feeders and selectively feed on benthic crustaceans. However, although amphipods are significantly selected, there is no clear dominance of any prey preference. This suggests that *R. radula* is able to exploit the spatial-temporal changes in abundance of several crustacean species.

The estimated number of prey groups plotted against the cumulative number of individuals whose stomach content was examined showed an evident saturation point between 50 and 60 randomized individuals (juveniles) of *R. radula* at the level of the collected n. 22 prey groups. This confirms that our sample size was well representative of the diversity of juvenile *R. radula*'s preys. Moreover, the fact that the Chao 1 curve, which is one of the most reliable estimators (Chao, 1984; Cazzolla Gatti et al., 2017a) of the absolute diversity of a sample (i.e. it calculates the estimated true species diversity of a sample), converges towards both the expected number of prey groups randomized 100 times and the bootstrap estimator represents a confirmation of the completeness of our sample. On the basis of this result, we provide the evidence that about 22 main prey groups represent the whole diet of *R. radula* juveniles.

In conclusion, our results improve the current knowledge on the biology and the ecology of *R. radula* in the central Mediterranean Sea, where the only data available from the Ionian Sea were based on the 43 specimens studied by Consalvo et al. (2010). However, still little is known about the spatial-temporal variations in *R. radula*'s diet, and, in general, of its biology and ecology, such as population structure, habitat selection, abundance, and distribution. Further studies from Sicilian and other Mediterranean areas would be relevant to provide additional information in order to protect this endemic and vulnerable species.

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