Journal of *Marine Science and Engineering*

MDPI

Article

Revealing the Shamefaced Crab *Calappa granulata* (Crustacea: Brachyura) from the Adriatic Sea, Northern Basin of the Mediterranean

Mirela Petrić [1,*], Marina Mihaljević [2], Jure Brčić [1] and Željka Trumbić [1]

[1] Department of Marine Studies, University of Split, Ruđera Boškovića 37, 21000 Split, Croatia
[2] Austevoll Research Station, Institute of Marine Research, NO-5392 Storebø, Norway
* Correspondence: mpetric@unist.hr

Abstract: This study presents the first data on morphometry, length–weight relationship, diet, reproductive biology, epibionts and genetic identity of the shamefaced crab *Calappa granulata* from the central eastern Adriatic Sea. A total of 92 crabs were collected during 2011, 2014 and 2015, of which 64 were females and 28 were males. Overall, 11 morphometric characteristics were measured. Carapace length of sampled individuals ranged from 48.46 to 76.09 mm, and body weight from 47.06 to 221.39 g. The length–weight relationship showed negative allometry for males and isometric growth for females. Analysis of the stomach content revealed the crab's preference for crustaceans (20.28%) and cephalopods (10.58%), less for fish (3.4%) and shellfish (0.28%). Size at first sexual maturity ($CL_{50\%}$) of 59.25 and 66.92 mm was estimated for males and females, respectively. Epibiotic serpulid polychaetes were recorded on the crab exoskeleton with an overall prevalence of 29.3%. Analyses of a partial sequence of mtCOI showed high haplotype (Hd = 0.964) and low nucleotide diversity (π = 0.00598). Phylogenetic inference and estimation of population differentiation (F_{ST} = 0.013, p = 0.271) with publicly available Mediterranean sequences currently imply one homogenous population unit. To the best of our knowledge, these are the first nucleotide sequences of *C. granulata* from the Adriatic Sea made publicly available.

Keywords: shamefaced crab; morphometry; diet; reproduction; epibiosis; DNA barcoding

check for updates

Citation: Petrić, M.; Mihaljević, M.; Brčić, J.; Trumbić, Ž. Revealing the Shamefaced Crab *Calappa granulata* (Crustacea: Brachyura) from the Adriatic Sea, Northern Basin of the Mediterranean. *J. Mar. Sci. Eng.* **2022**, *10*, 1964. https://doi.org/10.3390/jmse10121964

Academic Editor: Dariusz Kucharczyk

Received: 31 October 2022
Accepted: 6 December 2022
Published: 9 December 2022

1. Introduction

The shamefaced crab *Calappa granulata* (Linnaeus, 1758) belongs to the family Calappidae, known as 'box crabs', a group of easily distinguished brachyuran crustaceans with broad and convex carapace, short walking legs that can be folded under the carapace and large claws held close to the body. Box crabs have widespread distribution with preference for tropical and subtropical waters and are typically found on inshore soft bottoms [1]. Species of the genus *Calappa* Weber, 1795 are most commonly found in the Atlantic, Indian and Pacific Oceans, where they occur at depths from 0 to 1000 m [2]. They usually occur on sandy and muddy bottoms that allow them to bury themselves backwards very quickly using their chelipeds [3].

The sublittoral species *C. granulata* has been recorded in the Mediterranean Sea and in the Eastern Atlantic from Portugal to Mauritania, including Azores, Madeira and the Cape Verde Islands [1,4], inhabiting sandy mud and muddy bottoms at depths between 13 to 700 m [4]. Although very rarely, *C. granulata* is found in the middle and south part of the Eastern Adriatic [5–8] and recently has been reported from the northern parts of the Adriatic Sea [9–11].

Life history traits of *C. granulata* are poorly known, which is not surprising considering its possible cryptic lifestyle [8] linked to burying behavior that serves many advantages, such as avoiding predators [12,13], maintaining position in unstable and high-energy environments [14,15] and preventing desiccation [15,16]. According to Bellwood [17],

biological and ecological data for a majority of brachyuran species are limited because crabs are hardly ever observed in their natural habitat with studied specimens obtained from trawl fisheries. To date, there are only a few published papers on this species. In addition to records indicating its distribution in the Ionian Sea [18], the Strait of Sicily [19], the Sea of Marmara [20], the Aegean Sea [21] and the Adriatic Sea [7–11], most information is given about its morphological description [1], larval development that includes description of the prezoeal stage [22], morphology of the megalopa and first crab instar [23] as well as morphology of the first zoeal stage [24]. Olianas et al. [25] give structural–functional characterization of the *C. granulata* hemocyanin. Equally, genetic structure, diversity and connectivity patterns have not been well studied in this species. Innocenti et al. [26] reported high haplotype and low nucleotide diversity of *C. granulata* in the Mediterranean basin (Tyrrhenian and Ionian Sea); however, the primary objective of this study was to clarify species status of *C. tuerkayana*. Using morphological and genetic approaches in the analyses of historical and recent samples, the authors have concluded that *C. tuerkayana* is in fact a juvenile stage of *C. granulata*, and not a separate species as indicated in the literature.

Due to paucity of data for *C. granulata*, our aim was to expand the knowledge base on this species by providing observations on its morphometry, length–weight relationships, diet, reproduction and genetic identity to facilitate future work and population analyses throughout the species distribution area.

2. Materials and Methods

2.1. Sampling

Specimens of *Calappa granulata* used in this study were obtained from commercial trawlers operating in the central eastern Adriatic Sea at two locations (Figure 1). Samples were collected at depths between 70 and 90 m, in October 2011, October and December 2014, and May 2015. A total of 92 individuals (64 females and 28 males) were collected with commercial bottom trawl fitted with a 48 mm mesh size codend, towed at a speed of 2.7–2.9 knots. Crabs were frozen on board and transported to the laboratory for further analysis. A subsample of crab fresh gonadal tissue was fixed in 7% formalin for histological processing.

Figure 1. Map of *Calappa granulata* sample localities (yellow dots) in the central eastern Adriatic Sea.

2.2. Morphometry and Length–Weight Relationships

In the laboratory, individuals were thawed and sorted by sex. Morphometric measurements were taken with a digital caliper, with an accuracy of 0.01 mm, and body weight (BW) was recorded to the nearest 0.01 g. In addition, the following morphometric measurements were taken separately for each sex: carapace length (CL, distance across the carapace along

the median line from the anterior to the posterior margin of the carapace), full carapace width (FCW, distance across the carapace from tip of the lateral spines), short carapace width (SCW, distance across the carapace from the anterior base of the lateral spines), abdominal width (AW, distance between the margins of the fourth abdominal segment in females and the fifth in males), thoracic width (TW, width is the distance across the thoracic sternum between the notches at the bases of the second periopods), propodus length (PL, maximum distance from the tip of the propodus to the articulation with the carpus), chela height (CD, maximum distance between the dorsal and ventral margins of the propodus), dactilus length (DL, distance from the tip of the dactylus to the articulation with the propodus) and gonopod length (GL, length of the first pleopod in males). Propodus length, chela height and dactilus length were measured on the left and right sides. Based on the characteristic abdomen shape (narrow in males and wide in females), as well as the appearance of the first pairs of pleopods (modified to gonopods in males), sex determination was performed.

The relationships between different morphometric characters as dependent (AW, FCW, SCW, TW, GL, CD, DL, PL) and carapace length (CL) as the independent variable were modelled using a simple linear regression for each sex separately. In linear regression formula ($Y = a + b \times CL$, a = intercept, b = slope), the slope denotes the mean change in dependent variable for one unit increase in independent variable (here carapace length). A slope of 1 therefore indicates isometric growth, slope > 1 positive allometry and slope < 1 negative allometry [27]. The relationship between carapace length (CL) and body weight (BW) was modelled for each sex using the following formula: $BW = a \times CL^b$, where the allometry coefficient is expressed by the exponent b. The length–weight relationship reflects an isometric growth when b = 3, negative allometry when b < 3 and positive allometry when b > 3 [28]. The parameters a and b of this relationship were determined using nonlinear least-squares estimates via nls() function for R software [29]. The bootstrap method was employed to estimate Efron's 95% confidence intervals [30] for all models and their coefficients. The lack of overlap of 95% confidence intervals was taken as an indication of statistical significance of differences between compared groups/parameters/target values. The analysis was performed using the dplyr [31], purr [32] and rsample [33] packages for R software. All plots were produced using the ggplot2 package [34].

2.3. Reproductive Biology

Each crab was dissected, and the stage of gonadal development determined after Silva Castiglioni and Negreiros-Fransozo [35], taking color, shape and volume of reproductive organs into account. Crabs were assigned as: immature (IM), rudimentary (RU), developing (DI), developed (DE) and spent (SP) individuals. Individuals with immature and rudimentary gonads were further classified as juvenile, and those with developing, developed and spent gonads as mature. To test whether the male to female ratio significantly differs from one, a simple Chi-square test was used.

For the estimation of size at first sexual maturity, the crab carapace length (CL) at which 50% of the population was sexually mature was used. Maturity ogives were made for both sexes separately, using percentage of mature individuals (developed and spent individuals) distributed within the 4 mm size classes. For the estimation of crab size at which 25% ($CL_{25\%}$), 50% ($CL_{50\%}$) and 75% ($CL_{75\%}$) of the specimens were mature, a classic generalized linear model with logit link function was applied. Confidence intervals were determined by model bootstrapping.

For histological analysis of the gonadal tissue of 6 mature individuals (3 females and 3 males), sections were fixed in 7% formalin and processed for routine histological preparation. The tissue was dehydrated in a series of increasing ethanol concentrations (from 70% to 100%), cleared in xylene and embedded in paraffin. Histological sections were cut 5 µm thick, stained with hematoxylin–eosin, mounted permanently on slides and inspected with an Olympus BX51 microscope.

2.4. Diet

Crab stomach content was examined under a dissecting microscope Olympus SZX10 and according to the prey remains (i.e., fish otoliths and bones, crustacean exoskeleton and cephalopod beaks). Three major prey categories were identified: crustacean prey, fish prey and cephalopod prey. Stomach contents that were unidentifiable due to the advanced state of digestion were considered as non-identified organic matter.

The degree of fulness (DF) of each stomach was determined by visual assessment of the amount of food in the stomach according to Williams [36] as: class 1 = 0% DF (empty), class 2 $\leq$ 5% DF (partially empty), class 3 = 5 to 35% DF (empty/intermediately full), class 4 = 35 to 65% DF (intermediately full), class 5 = 65 to 95% DF (intermediately full/full) and class 6 $\geq$ 95% DF (full). The percentage frequency of prey occurrence (%FO) was calculated after Hyslop [37] using the equation: %FO = nx/Nh $\times$ 100, where nx is the number of stomachs containing specific prey type and Nh is the number of stomachs with food. The Chi-square test was used for overall statistical diet comparisons regarding sex, season, maturity stage and size (crabs were grouped into 4 mm carapace length size classes). Due to the small sample size of stomachs with food (N = 2), winter was excluded from the analysis.

2.5. Epibiosis

During examination of *C. granulata* specimens, serpulid polychaete tubes were observed on the crab exoskeleton. The number of these macro-epibionts per host and the site of occurrence on the host body (carapace, chelipeds and frontal between the eyes) were recorded. Parasite prevalence (P), mean intensity (I) and mean abundance (A) of infection were calculated and defined according to Margolis et al. [38] and Bush et al. [39]. Sterne's exact 95% confidence limits were calculated for prevalence, bootstrap 95% confidence limits (number of bootstrap replications = 2000) for mean abundances, variance to mean ratio (var/mean ratio) as a measure of overdispersion and exponent of the negative binomial (k) for the parasite skewness, using Quantitative Parasitology 3.0 software (Budapest, Hungry) [40]. For statistical analysis of differences in the prevalence, mean abundance and mean intensity between the sexes, maturity stages and season, Fisher's exact test and bootstrap *t*-tests were used according to Rózsa et al. [41]. Spearman's correlation coefficient R was applied to determine the degree of association between serpulid abundance and carapace length. A p value of less than 0.05 was considered statistically significant.

2.6. Molecular Identification

Fresh muscle leg tissue was sampled from 11 individuals caught in the spring of 2015, preserved in absolute ethanol and stored at +4 °C. DNA was extracted following a modified protocol from Turtinen and Juran [42]. Briefly, a small piece of each muscle tissue was digested in a solution of cell lysis buffer (0.2% SDS, 0.01 M TrisBase, 0.01 M EDTA, 0.15 M NaCl) and proteinase K (0.2 mg/mL) overnight at 55 °C. Proteins were salted out at 13,000 rpm for 10 min in 2.2 M NaCl concentration. After isopropanol precipitation, washing in 75% ethanol and drying, the DNA pellet was resuspended in TE buffer (0.01 M Tris-HCL, 0.0125 M EDTA, pH = 8).

A region of cytochrome oxidase subunit I mitochondrial gene (mtCOI, 650bp long) was amplified using primer pair LCO1490: 5'GGTCAACAAATCATAAAGATATTGG3' and HC02198: 5'TAAACTTCAGGGTGACCAAAAAATCA3' [43]. PCR reactions were performed in a final volume of 25 µL and consisted of 1X PCR buffer, 3 mM MgCl2, 0.2 mM dNTP, 0.4 µM of each primer, 1 U of Taq polymerase (Sigma Aldrich, St. Louis, MO, USA), 50 ng of DNA template and DNAse/RNAse free water. PCR conditions were set as follows: 3 min at 94 °C; 5 cycles of 94 °C for 30 sec, 45 °C for 90 sec, 72 °C for 60 sec; 30 cycles of 94 °C for 30 sec, 50 °C for 90 sec, 72 °C for 30 sec; with a final extension of 7 min at 72 °C. All amplifications were performed using MyCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA). Successful PCR products were screened using 1% agarose gel electrophoresis and sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) on an ABI 3100 automated sequencer, as provided by the

Macrogen Europe sequencing service. The obtained DNA sequences were processed and aligned in the software package Geneious 8.1.5 (Auckland, New Zealand) [44]. Sequences were deposited to GenBank under Accession numbers OM653503-OM653513.

For phylogenetic analyses, sequences acquired in this study were combined with mtCOI sequences of other *Calappa* species retrieved from GenBank https://www.ncbi.nlm.nih.gov/genbank, accessed on 16 June 2022) (Accession numbers in Supplementary Table S1). *Mursia africana* MZ434771 was used as the outgroup species. Bayesian phylogenetic inference was performed using MrBayes 3.2.6 [45]. The best-fit evolutionary model was determined using the IQ-TREE web server with auto model selection [46–49]. According to the Bayesian information criterion, the selected model was GTR+F+I+G4.

To measure genetic diversity, number of haplotypes (H), haplotype diversity (Hd), number of polymorphic sites (S), nucleotide diversity (π) and average number of nucleotide differences (k) were estimated using DnaSp 6.12.3.0 (Barcelona, Spain) [50,51]. Arlequin 3.5.2.2. [52] was used to estimate pairwise genetic differentiation between groups (F_{ST}) and the significance was tested by 10,000 permutations. Tajima's D [53] was also calculated using Arlequin 3.5.2.2. (Lausanne, Switzerland) [52]. The haplotype network was constructed using the median-joining distance method in PopART 1.7 (Dunedin, Otago, New Zealand) [54].

3. Results

3.1. Morphometry and Length–Weight Relationship

Minimum, maximum and mean values with lower and upper limits of 95% confidence intervals for morphometric parameters for all specimens of *C. granulata* caught in the central eastern Adriatic Sea are shown in Table 1, while Table 2. presents the same values for each sex separately. Body weight (BW) of shamefaced crabs ranged from 47.06 to 192.61 g in males, with a mean value of 134.98 (95% CI: 124.91–144.9) and 54.26 to 221.39 g in females, with a mean value of 123 (95% CI: 114.48–131.97). Carapace length (CL) of males ranged from 48.46 to 72.94 mm and from 51.31 to 76.09 mm in females, showing no statistical difference in mean values as indicated by overlapping 95% confidence intervals of the means (Table 2).

Table 1. Minimum (min), maximum (max) and mean values with lower and upper limits of 95% confidence intervals [95% CI] for morphometric characters (mm) of *Calappa granulata* (N = 92) from the central eastern Adriatic Sea.

Morphometric Character *	Total Sample		
	Min	Max	Mean [95% CI]
AW	7.14	19.77	14.49 [13.86, 15.1]
CL	48.46	76.09	65.92 [64.84, 66.97]
FCW	63.51	104.89	88.85 [87.09, 90.56]
SCW	60.06	101.22	84.44 [82.71, 86.09]
TW	13.13	24.54	20.36 [19.92, 20.81]
CD.D	32.41	54.76	45.8 [45.03, 46.56]
CD.L	31.89	54.53	45.73 [44.89, 46.53]
DL.D	21.69	38.79	31.84 [31.12, 32.46]
DL.L	19.94	35.51	30.25 [29.64, 30.88]
PL.D	35.52	58.5	49.76 [48.76, 50.75]
PL.L	35.4	58.72	49.56 [48.53, 50.63]

* The abbreviations of all morphometric measurements can be found in Section 2.2. *Morphometry and Length–Weight Relationships.*

In males, the left and right sides of propodus length, chela height and dactilus length did not significantly differ, unlike in females where statistical difference was observed for dactilus length between left and right side (Table 2). Between sexes, abdominal and thoracic widths were significantly larger in females, while the length of left and right propoduses was larger in males (Table 2). Gonopod length in males ranged from 15.28 to 24.1 mm, with

a mean value of 21.42 mm. The relationships between different morphometric characters and carapace length, i.e., the rate of their relative growth, were described using simple linear regression (Figure 2). The parameters with corresponding 95% confidence intervals are given in Table 3. In addition to its substantially larger size in females, abdominal width showed increased relative growth with respect to carapace length in females compared to males, with a significantly larger regression line slope (Table 3). Thoracic width also showed slightly increased relative growth in females, while other morphometric characters showed similar growth in both sexes (Figure 2).

Table 2. Minimum (min), maximum (max) and mean values with lower and upper limits of 95% confidence intervals [95% CI] for morphometric characters (mm) of males (N = 28) and females (N = 64) of *Calappa granulata* from the central eastern Adriatic Sea.

Morphometric Character *	Females			Males		
	Min	Max	Mean [95% CI]	Min	Max	Mean [95% CI]
AW	10.79	19.77	16.07 [15.55, 16.52]	7.14	11.98	10.92 [10.53, 11.22]
CL	51.31	76.09	65.56 [64.11, 66.91]	48.46	72.94	66.74 [64.81, 68.31]
FCW	66.59	104.89	87.67 [85.53, 89.75]	63.51	102.05	91.51 [89.04, 94.1]
SCW	64.07	101.22	83.44 [81.41, 85.46]	60.06	96.21	86.67 [83.95, 89]
TW	15.63	24.54	20.87 [20.25, 21.38]	13.13	21.47	19.21 [18.55, 19.71]
CD.D	34.93	54.76	45.9 [44.89, 46.91]	32.41	50.95	45.57 [44.29, 46.84]
CD.L	31.89	54.53	45.68 [44.64, 46.65]	32.08	50.93	45.84 [44.57, 46.95]
DL.D	22.61	38.79	31.69 [30.88, 32.49]	21.69	36.69	32.17 [31.06, 33.15]
DL.L	19.94	35.16	29.77 [28.97, 30.51]	19.99	35.51	31.32 [30.18, 32.37]
PL.D	36.72	57.94	48.76 [47.66, 49.93]	35.52	58.5	52.01 [50.11, 53.54]
PL.L	35.4	58.58	48.39 [47.25, 49.44]	35.44	58.72	52.2 [50.41, 53.79]

* The abbreviations of all morphometric measurements can be found in Section 2.2. *Morphometry and Length–Weight Relationships.*

Length–weight relationships showed negative allometry for males (b = 2.31, 95% CI: 2.04–2.54) and isometric growth for females (b = 3.14, 95% CI: 2.96–3.33) (Figure 3). As their respective 95% confidence intervals for parameter b did not overlap, females and males of shamefaced crab in the central Adriatic Sea demonstrated a different length–weight relationship, expressed by the following equations: $BW = 0.0002381 \times CL^{3.139033}$ (N = 64) for females and $BW = 0.0083295 \times CL^{2.305820}$ (N = 28) for males.

3.2. Reproductive Biology

During this study, 28 (30.43%) male and 64 (69.57%) female specimens of *Calappa granulata* were analyzed. The sex ratio did differ statistically from the expected 1:1 ($\chi^2 = 7.324$; $p = 0.007$) and was 0.44:1 in favor of females. In the total sample, the highest portion represented developed individuals (34.80%), followed by developing (28.30%), spent (16.30%), rudimentary (13.00%) and immature ones (7.61%). Ovigerous females were not found. Only one immature male was found (CL = 48.46 mm; BW = 47.06 g). No males in the rudimentary stage were recorded. Data on carapace length and body weight range of individuals in different maturity stage of both sexes are presented in Table 4. Smallest fully matured developed males and females had CL of 61.21 mm and 67.35 mm, respectively (Figure 4). Mature individuals were recorded in all samples, with a relative proportion of 85.37% in spring and 71.74% in autumn.

According to estimation of size at first maturity ($CL_{50\%}$), males mature at smaller carapace length than females (Figure 5). For males, $CL_{50\%}$ was estimated at 60.33 mm (95% confidence interval (CI): 54.81–64.95) and at 67.95 mm (95% CI: 66.95–69.50) for females. $CL_{25\%}$ and $CL_{75\%}$ were estimated respectively at 55.71 (95% CI: 50.34–61.42) and 64.94 (95% CI: 58.72–68.84) mm for males, and 66.40 (95% CI: 65.58–67.48) and 69.50 (95% CI: 67.07–71.84) mm for females.

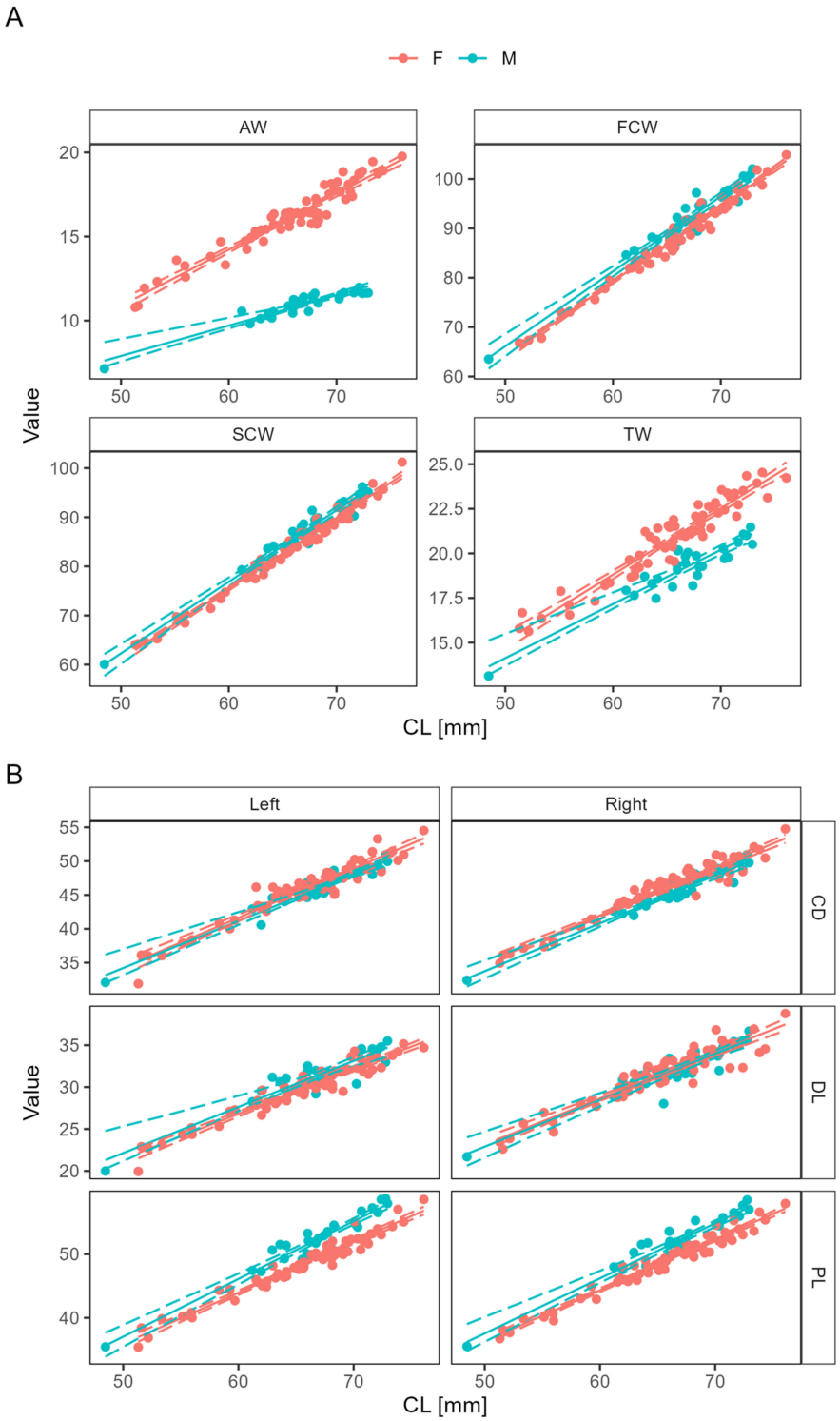

Figure 2. Linear regression models with 95% confidence intervals between different morphometric characters and carapace length of *Calappa granulata* males (M) and females (F) collected from the central eastern Adriatic Sea: (**A**) no left/right sided characters; (**B**) left/right sided characters.

Table 3. Parameters (intercept and slope) of linear regression equations with 95% confidence intervals between different morphometric characters and carapace length of *Calappa granulata* males and females collected from the central eastern Adriatic Sea.

Sex	Morphometric Character *	Intercept	95% CI [Lower, Upper Limit]	Slope	95% CI [Lower, Upper Limit]	R^2	Allometry
Females	FCW	−12.21	[−15.30, −9.36]	1.52	[1.48, 1.57]	0.98	+
	SCW	−10.78	[−13.88, −7.69]	1.44	[1.39, 1.48]	0.98	+
	AW	−5.81	[−7.25, −4.35]	0.33	[0.31, 0.36]	0.93	−
	TW	−3.48	[−5.37, −1.82]	0.37	[0.35, 0.4]	0.92	−
	PL.L	−4.14	[−7.02, −0.68]	0.80	[0.75, 0.85]	0.96	−
	PL.D	−3.62	[−6.05, −1.02]	0.80	[0.76, 0.84]	0.96	−
	CD.L	−2.12	[−6.55, 2.53]	0.73	[0.66, 0.8]	0.92	−
	CD.D	−0.53	[−3.08, 2.97]	0.71	[0.65, 0.75]	0.94	−
	DL.L	−5.33	[−8.03, −2.74]	0.54	[0.5, 0.58]	0.93	−
	DL.D	−4.36	[−7.42, −0.77]	0.55	[0.5, 0.6]	0.89	−
Males	FCW	−9.75	[−17.06, −1.95]	1.52	[1.4, 1.62]	0.94	+
	SCW	−10.55	[−18.93, −3.48]	1.46	[1.35, 1.58]	0.96	+
	AW	−1.14	[−2.46, 2.73]	0.18	[0.12, 0.2]	0.88	−
	TW	−1.04	[−2.71, 4.22]	0.30	[0.23, 0.33]	0.85	−
	PL.L	−8.26	[−13.75, −1.38]	0.91	[0.8, 0.98]	0.93	−
	PL.D	−5.67	[−10.04, 4.14]	0.86	[0.72, 0.93]	0.92	−
	CD.L	−0.50	[−4.23, 10.33]	0.69	[0.53, 0.75]	0.93	−
	CD.D	−1.58	[−5.43, 4.17]	0.71	[0.62, 0.77]	0.94	−
	DL.L	−5.34	[−9.21, 6.89]	0.55	[0.37, 0.61]	0.84	−
	DL.D	−4.82	[−9.36, 2.15]	0.55	[0.45, 0.62]	0.84	−

* The abbreviations of all morphometric measurements can be found in Section 2.2. *Morphometry and Length–Weight Relationships.*

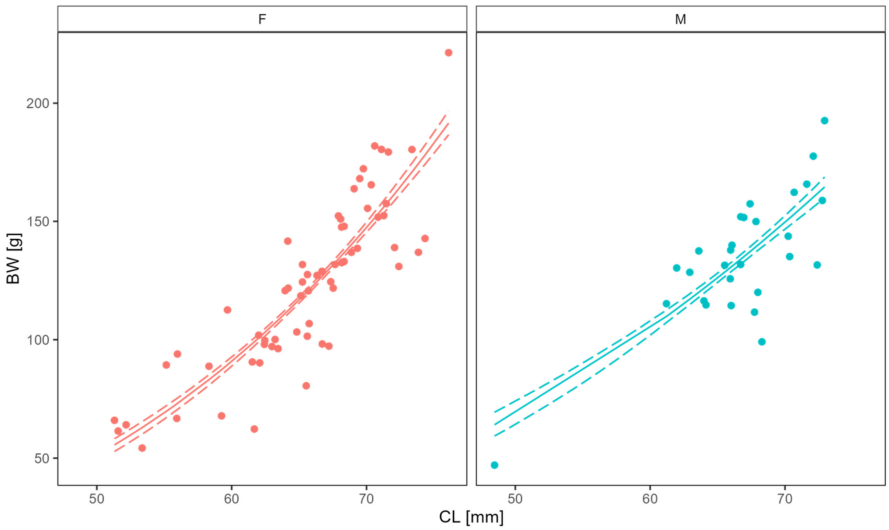

Figure 3. Length-weight relationships of *Calappa granulata* males (M) and females (F) collected from the central eastern Adriatic Sea (CL–carapace length, BW–body weight).

A total of six individuals, three males and three females, with mature developed gonads were processed for histological examination. Histological analysis of the testicular tissue revealed numerous seminiferous tubules surrounded by a thin layer of interstitial connective tissue (ct) and an inner layer of columnar epithelium (Figure 6A). Seminiferous tubules contained cells at different stages of spermatogenesis. Small clusters of spermatogonia (sg) were found in the germinative zone in the basal lamina of the seminiferous tubules, and clusters of spermatocytes (spc) were evident in the peripheral lining of seminiferous tissue (Figure 6C,D). Most tubules had large lumen (lu) with few residual spermatozoa (spz) indicating that mature spermatozoa were released towards the vasa deferentia (Fig-

ure 6A,B). A cross section of middle vas deferens showed numerous spermatophores (sp) that encapsulate mature spermatozoa (Figure 6E). The membrane of vas deferens has columnar epithelium (ce) beneath the thin layer of connective tissue (Figure 6E). Oval spermatophores were embedded in the homogenous eosinophilic matrix with some granular material (gm) (Figure 6F). All spermatophores contained one or more agranular substances (as) (Figure 6F).

Table 4. Number of individuals (N), range (min—max) and mean values with standard deviation (SD) of carapace length and body weight for each maturity stage of *Calappa granulata* collected from the central eastern Adriatic Sea.

	Males			>Females		
Maturity Stage	**N**	**Min—Max**	**Mean ± SD**	**N**	**Min—Max**	**Mean ± SD**
			Carapace Length (mm)			
Immature	1	48.46	/	6	51.31–61.68	54.89 ± 4.44
Rudimentary	0	/	/	12	55.15–67.21	61.35 ± 4.62
Developing	5	63.99–67.99	65.88 ± 1.91	21	59.69–70.61	64.84 ± 2.58
Developed	19	61.21–72.94	67.22 ± 3.41	13	67.35–71.28	69.23 ± 1.35
Spent	3	68.29–72.77	71.15 ± 2.48	12	67.53–76.09	71.65 ± 2.63
			Body Weight (g)			
Immature	1	47.06	/	6	54.26–67.83	62.65 ± 4.73
Rudimentary	0	/	/	12	66.77–106.79	90.66 ± 11.38
Developing	5	111.61–131.41	118.81 ± 7.66	21	90.22–181.9	120.44 ± 21.54
Developed	19	114.40–192.61	144.68 ± 20.31	13	124.46–180.43	153.47 ± 16.55
Spent	3	99.10–158.83	129.83 ± 29.90	12	121.77–221.39	152.98 ± 28.33

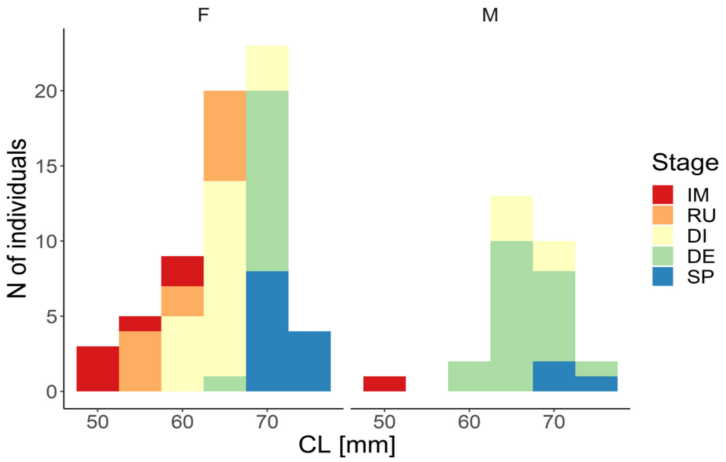

Figure 4. Distribution of female (F) and male (M) *Calappa granulata* maturity stages (IM—immature, RU—rudimentary, DI—developing, DE—developed, SP—spent) presented in 4 mm carapace length (CL) size classes. The specimens were caught in the central eastern Adriatic Sea.

Histologically, mature ovaries consisted of the following types of oocytes: oogonia (og), previtellogenic oocytes (pvo), vitellogenic oocytes (vo) and mature oocytes (mo) (Figure 7). The ovary is surrounded by a thick layer of connective tissue (ct) (Figure 7A). Vitellogenic oocytes were the predominant cell type, while oogonia could be seen in the central germinative zone next to the few previtellogenic oocytes (Figure 7B,C). Previtelogenic oocytes are larger than oogonia, have basophilic cytoplasm and oval nuclei (n) with distinct peripheral nuclei (nc). In vitellogenic oocytes, cytoplasm increase was noticed because of yolk granule (yg) formation and accumulation. A layer of follicular cells (fc) surrounds the vitellogenic and mature oocytes. Mature oocytes are characterized by numerous large yolk granules and lipid droplets (ld) that fill the entire cell, so the nucleus is either dispersed or not visible (Figure 7D).

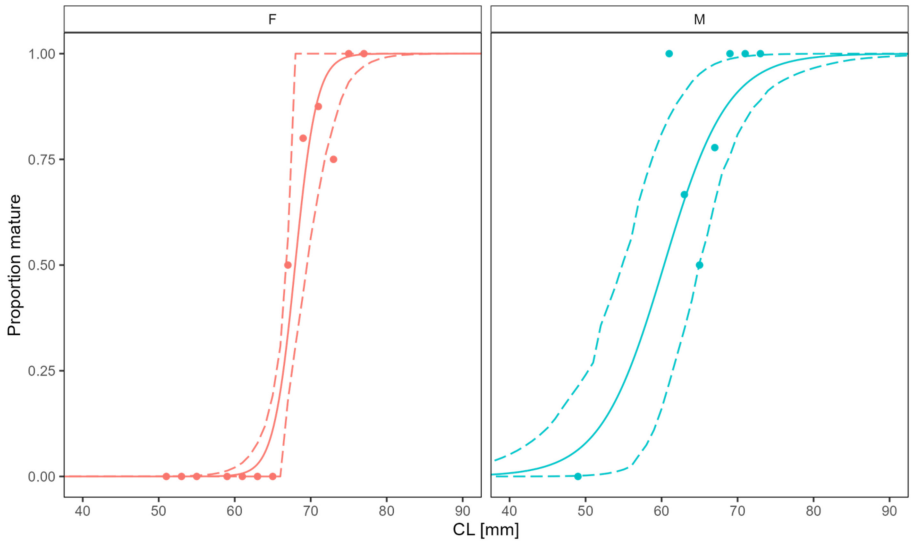

Figure 5. Maturity ogives for females (F) and males (M) of *Calappa granulata* collected from the central eastern Adriatic. The full line is the predicted maturity ogive over the size classes (carapace length) of the species, and the dotted lines show the bootstrapped 95% confidence intervals. Dots indicate observed proportions of mature crabs.

3.3. Diet

Stomach weight of *Calappa granulata* ranged from 0.33 to 2.42 g (mean = 1.13 ± 0.10 SE) in males and from 0.34 to 3.35 g (1.08 ± 0.07 SE) in females. Mean stomach weight for the total sample was 1.09 g (±0.06 SE).

From a total of 92 collected stomachs, 32 (34.78%) stomachs were empty and 60 (65.22%) contained food items and were used for further analysis. Non-identified organic matter was evident in five (8.33%) stomachs, all in females collected during autumn. Regarding the degree of fullness (DF) of all analyzed individuals, 34.78% were of class 1, 19.57% class 2, 18.48% class 3, 9.78% class 4, 11.96% class 5 and 5.43% of class 6. The results of χ^2 tests showed no significant differences in the degree of fullness between sexes ($\chi^2 = 3.054$; $p = 0.692$), seasons ($\chi^2 = 4.141$; $p = 0.529$), maturity stages ($\chi^2 = 22.325$; $p = 0.323$) and size ($\chi^2 = 9.224$; $p = 0.817$). During both seasons, autumn and spring, the most individuals had a degree of fullness of class 1, 39.13% and 29.27%, respectively, and the least individuals were of class 6, 2.17% and 4.88%, respectively.

The analysis of stomach content showed that *C. granulata* mainly preys on crustaceans (81.82%), then fish (30.91%) and cephalopods (25.45%) (Table 5). Bivalve remnants (1.81%) were detected in the stomach content of only one female. There was no statistical difference in diet composition between males and females ($\chi^2 = 0.016$; $p = 0.992$). Furthermore, the diet preference did not change with respect to crabs' maturity stages ($\chi^2 = 22.325$; $p = 0.323{,}992$) or across size categories (4 mm CL size classes, $\chi^2 = 32.451$; $p = 0.592$) (Table 5). Crustaceans were also a preferred crab diet item, followed by fish and cephalopods, across all seasons ($\chi^2 = 7.439$; $p = 0.574$).

3.4. Epibiosis

The overall prevalence, mean intensity and mean abundance of a serpulid tubeworm colonizing *Calappa granulata* (N = 92) were 29.3%, 3.81 and 1.12, respectively (Table 6). The epibionts were found dorsally on the carapace (46.62%), chelipeds (51.46%) and frontally between the eyes (1.94%). Most crabs had a low number of epibiotic polychaets and only one female (CL = 72.4 mm) collected from the central eastern Adriatic contained 19 of them. Data on the prevalence, mean intensity and mean abundance for the total sample, both sexes, season and maturity stages are presented in Table 6. Fisher's exact test showed no significant differences in serpulid prevalence between males and females ($p = 0.804$) and the bootstrap *t*-test showed no significant differences between the sexes in mean intensity

($t = -1.043$, $p = 0.301$) and in mean abundance ($t = -1.167$, $p = 0.256$). Serpulids were found on individuals of all maturity stages, with the highest prevalence of 33.3% recorded for spent, and the lowest prevalence of 14.3% for immature individuals. However, there was no evident statistical difference in prevalence across maturity stages (Fisher's exact test: $p = 0.913$). However, there was a significant difference in the prevalence of occurrence between seasons (Fisher's exact test: $p = 0.0375$), with more individuals bearing serpulids in autumn, but with similar mean intensity (bootstrap t-test: 0.4415) and mean abundance (bootstrap t-test: 0.066).

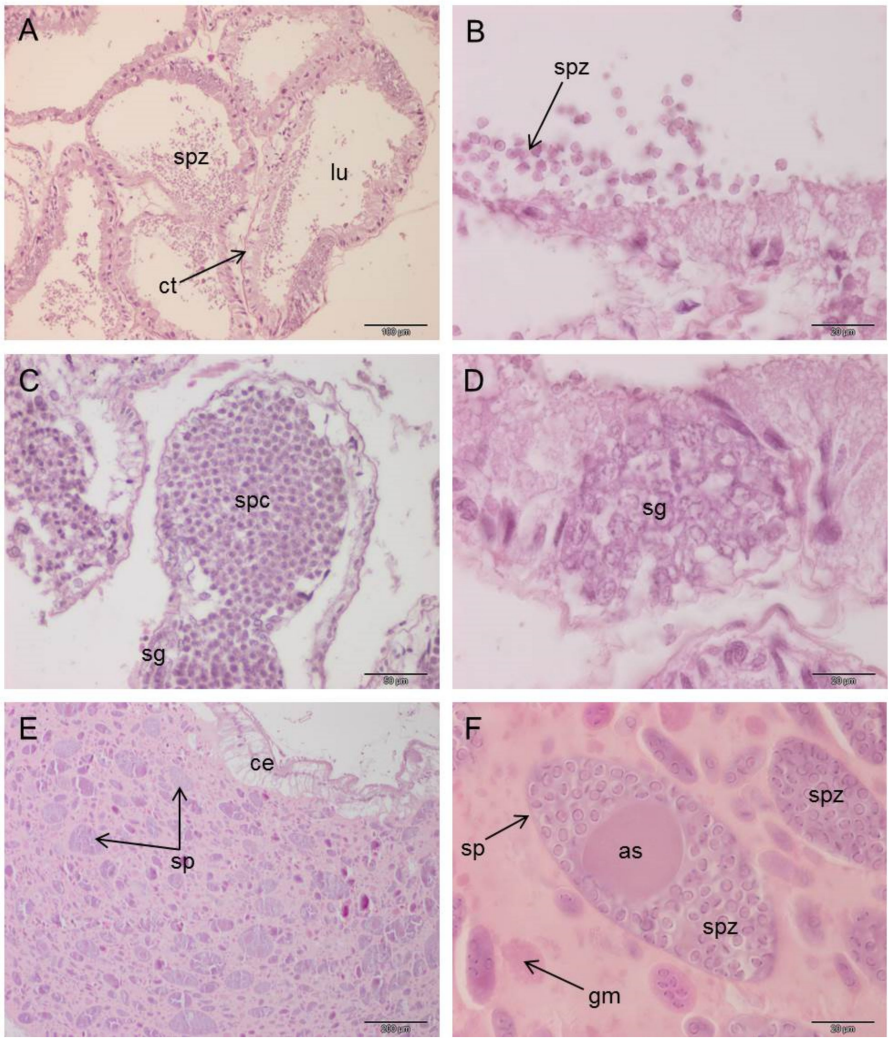

Figure 6. Histological cross sections of male *Calappa granulata* reproductive system: (**A**) developed testes showing seminiferous tubules surrounded by layer of connective tissue (ct) and with lumen (lu) containing few residual spermatozoa (spz); (**B**) detail of spermatozoa (spz) from the lumen of the seminiferous tubule; (**C**) seminiferous tubules with large clusters of spermatocytes (spc) and small cluster of spermatogonia (sg); (**D**) cluster of spermatogonia (sg) in the wall of seminiferous tissue; (**E**) section of anterior vas deferens with numerous spermatophores (sp) and membrane with columnar epithelium (ce) beneath the thin layer of connective tissue; (**F**) detail of spermatophore (sp) filled with spermatozoa (spz) and agranular substance (as).

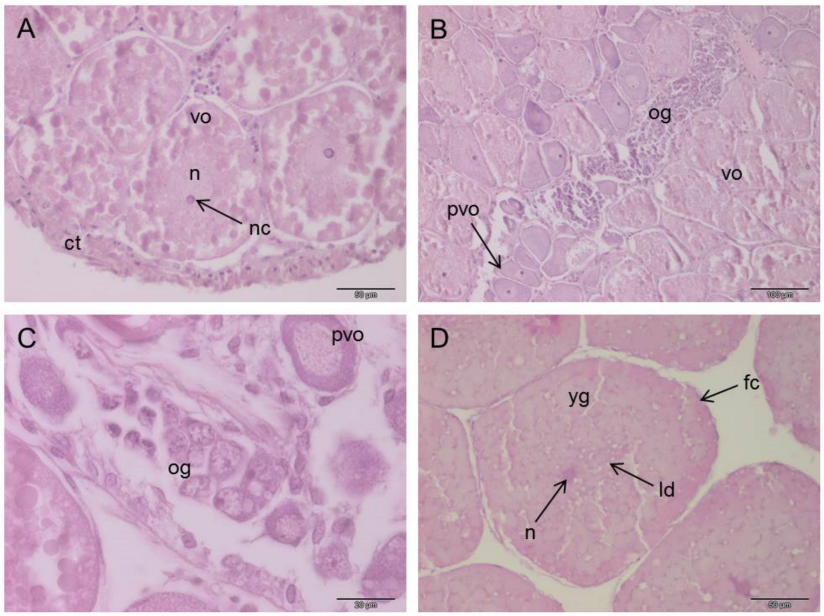

Figure 7. Histological cross sections of *Calappa granulata* ovary: (**A**) Developed ovary showing vitellogenic oocytes (vo), with oval nucleus (n) and distinct peripheral nucleolus (nc), surrounded by a thick layer of connective tissue (ct); (**B**) ovarian section through central germinative zone filled with oogonia (og), previtellogenic oocytes (pvo) and vitellogenic oocytes (vo).; (**C**) detail of ovary with oogonia (og) and previtellogenic oocyte (pvo); (**D**) mature oocyte surrounded by a thin layer of follicular cells (fc), showing numerous yolk granules (yg) and lipid droplets (ld) with nucleus (n) barely visible.

Table 5. Percentage frequency of prey occurrence (% FO) from stomach contents of *Calappa granulata* from the central eastern Adriatic Sea.

	% FO		
	Crustacea	**Fish**	**Cephalopoda**
		Sex	
Males	94.12	35.29	29.41
Females	80.56	30.56	25.00
Total	81.82	30.91	25.45
		Season	
Autumn	84.00	12.00	20.00
Spring	78.57	46.43	25.00
		Maturity Stage	
Immature	80.00	40	40
Rudimentary	80.00	/	40
Developing	69.23	30.77	30.77
Developed	88.89	33.33	33.33
Spent	100.00	45.45	/

The mean carapace length of infected and non-infected crabs (66.69 ± 0.98 SE and 65.46 ± 0.70 SE, respectively) did not significantly differ ($t = -0{,}98$; $p = 0.330$). There was a weak positive relationship between crab size and serpulid abundances, but it was not statistically significant (Spearman's R = 0.213, $p = 0.287$).

Table 6. Total mean prevalence (%) with Stern's exact 95% confidence limits (CI), mean intensity and mean abundances with bootstrap 95% confidence limits (CI) and variance to mean ratio of serpulid polychaete colonizing *Calappa granulata* exoskeleton from the central eastern Adriatic Sea.

	N of Host		Prevalence (%) (CI)	Intensity (CI)	Abundance (CI)	Var/Mean Ratio
	Total	**Infected**		**Sex**		
Males	28	9	32.1 (0.17–0.52)	4.89 (3.00–6.22)	1.57 (0.71– 2.79)	4.78
Females	64	18	28.1 (0.18–0.41)	3.28 (1.89–6.61)	0.92 (0.47–1.98)	8.28
Total	92	27	29.3 (0.20–0.39)	3.81 (2.70–5.74)	1.12 (0.70–1.87)	6.79
				Season		
Autumn	49	19	41.3 (0.28–0.57)	4.16 (2.79–6.68)	1.72 (0.96–3.07)	6.95
Spring	19	8	19.5 (0.09–0.34)	3.00 (1.38–5.38)	0.59 (0.20–1.32)	5.12
				Maturity Stage		
Immature	7	1	14.3 (/)	1.00 (/)	0.14 (/)	1.00
Rudimentary	13	3	23.1 (0.07–0.52)	1.00 (/)	0.23 (0.00–0.38)	0.83
Developing	23	7	30.4 (0.15–0.52)	3.86 (1.86–6.14)	1.17 (0.43–2.48)	5.16
Developed	34	11	32.4 (0.19–0.50)	3.64 (2.27–5.18)	1.18 (0.56–2.06)	4.30
Spent	15	5	33.3 (0.14–0.60)	6.40 (2.40–15.40)	2.13 (0.53–6.47)	11.91

3.5. Molecular Identification

Partial sequences for the mtCOI were obtained for all 11 analyzed specimens, quality trimmed and aligned. According to BLAST results with the Genbank nr database, they were all identified as *Calappa granulata* (average 99.1% identity). An alignment of 547 bp was obtained and analyzed. A total of 11 variable polymorphic sites were detected, of which six were singleton and five were parsimony sites. Overall, nine haplotypes were identified with a high haplotype diversity (Hd) of 0.964 and a low nucleotide diversity (π) of 0.00598. Seven haplotypes were represented by a single individual. The estimated average number of nucleotide differences (k) was 3.273. Tajima's D was −0.55324 (not significant, $p > 0.10$). By combining *C. granulata* sequences from this study (Adriatic Sea) with those publicly available from Genbank (Supplementary Table S1) from the Mediterranean Sea and one sequence from the Atlantic Ocean (24 sequences, 512 bp long) into a joint alignment, 17 haplotypes were identified, also with a high haplotype diversity of 0.96. Nucleotide diversity was 0.00686 with an estimated k value of 3.511. Of 20 detected variable polymorphic sites, 12 were singletons and eight were parsimony sites. Tajima's D was −1.25602 (not significant, $p > 0.10$). In this case, 13 haplotypes were represented by a single individual (Figure 8). A relatively low and not significant F_{ST} value (0.013, $p = 0.271$) was estimated between Adriatic and Mediterranean samples. The lack of genetic structure was further supported by Bayesian phylogenetic inference. A phylogenetic tree based on mtCOI sequences of multiple species of genus *Calappa*, including sequences from this study, showed a well-supported joint clade composed of *C. granulata* from the Atlantic, Mediterranean, and Adriatic areas (Figure 9), also confirming this species' correct identification. A sister species to this clade seems to be *C. pelli*. Strong nodal support was also found for the *C. exanthematosa*, *C. galloides C. pokipoki* grouped clade with a close relationship to the main *C. granulata* and *C. pelli* clade.

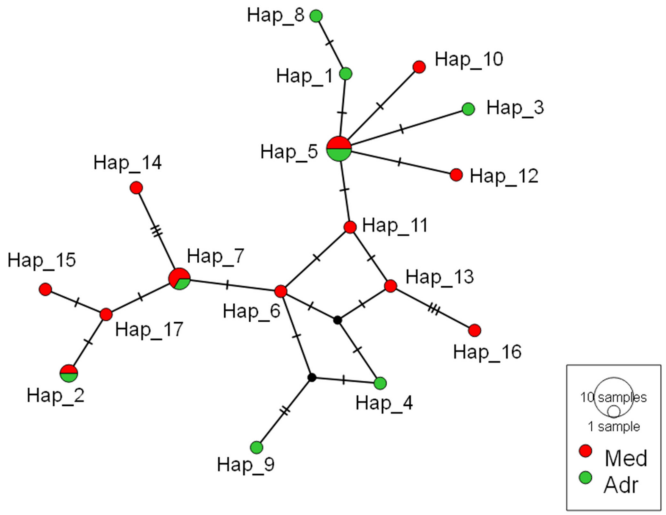

Figure 8. Haplotype network of mtCOI sequences of *Calappa granulata* from the Mediterranean and Adriatic Sea (24 sequences, alignment 512 bp long) constructed using the median-joining distance method. The size of each circle is proportional to the number of sequences belonging to each haplotype and short lines depict the number of mutations between haplotypes. Black spots represent missing haplotypes.

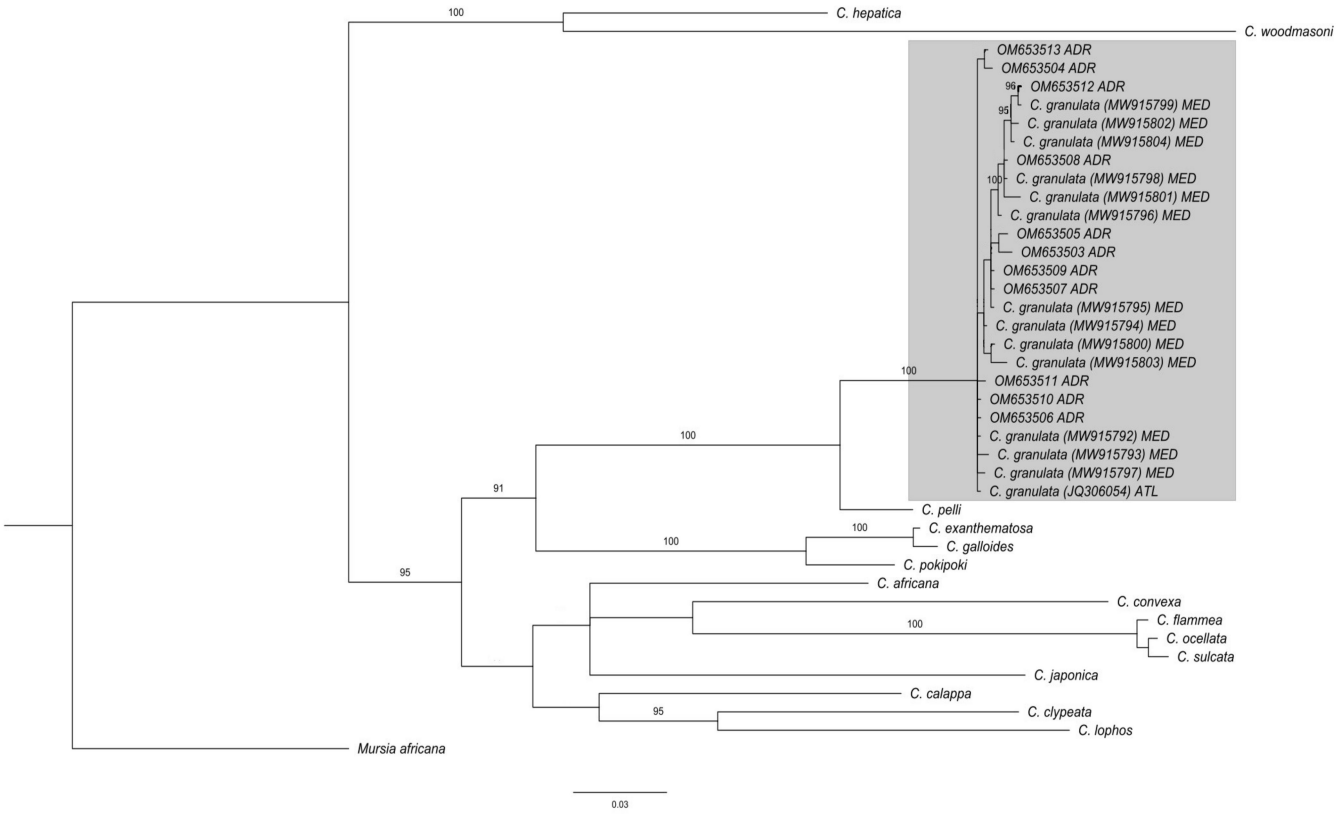

Figure 9. Bayesian phylogenetic tree based on the analysis of mtCOI sequences for the genus *Calappa*. BI posterior probability values above 90% are shown above the branches. Colored part distinguishes *Calappagranulata* clade from geographical area: ADR—Adriatic Sea, MED—Mediterranean Sea, ATL—Atlantic Ocean. GenBank accession numbers for *C. granulata* are shown after the species name. *Mursia africana* is used as outgroup. Scale bar indicates number of nucleotide substitutions per site.

4. Discussion

This study gives first insight into bio-ecological characteristics of the shamefaced crab *Calappa granulata* from the Adriatic Sea. From a total of 92 crabs collected, one of the largest samples ever described, only 28 were males, so there was a rather evident statistical difference in crab sex ratio (0.44:1) in favor of females. This difference could be due to the different behavior of males that perhaps dig deeper into sediment and therefore are inaccessible to the fishing gear or possibly do not share the same habitat as females. However, unlike these investigated crabs from the central part of the Adriatic, findings from the northernmost part report all adult male individuals [9–11]. In Atlantic waters (northeastern Venezuela), Alio et al. [55] also found the sex ratio being shifted towards males in *C. sulcata*, *C. flammea* and *C. nitida*. However, these *Calappa* species are of great commercial interest and these findings come from obtained data catches of the industrial trawl vessels, from 1994 to 2000.

Dulčić and Tutman [9] reported the first finding of one male *C. granulata* near Umag (the western coast of the Istrian peninsula) with CL of 75.0 mm, caught with gillnet at about 25 m deep, concluding that the record of this species in the most northern Adriatic indicates its broad distribution. Recently, Bettoso et al. [10] analyzed the morphometry of two adult males, with CL of 73.7 and 77.5 mm, caught in the northernmost part of the Adriatic (near Funtana and Piran) with different fishing gears, i.e., trammel net (at 20 m depth) and bottom-set gillnet. In addition, Dulčić et al. [11] reported six adult males, with CL ranging from 74.0 to 80.0 mm, caught near the waters of Savudrija, Poreč and Vrsar (north Adriatic) by trawl net and trammel net, at about 20–25 m depth. The CL of male *C. granulata* investigated in this study ranged from 48.46 to 72.94 mm, while CL of females ranged from 51.31 to 76.09 mm. It appears that the males caught in the northern Adriatic are somewhat larger than in our study, but due to the small sample size and the use of different fishing gear, it is difficult to make any firm conclusions. Our samples were collected at depths between 70 and 90 m, so much deeper than reported in the northern Adriatic [10,11]. Perhaps these size differences indicate a possible size–depth relationship in *C. granulata*. According to Bettoso et al. [10], this northward expansion of *C. granulata* could be due to climate change and global warming that are shifting benthic community structure.

Growth is an important part of species life history and an understanding of size, weight and reproduction relationships is much needed for population dynamic studies. Our results of calculated morphometric regressions showed significant differences for only a few parameters: abdominal and thoracic width was larger in females, and propodus length was larger in males. These sex-specific differences reflect already noted distinct sexual dimorphism, i.e., in females the abdomen is substantially wider for accommodation of fertilized eggs. Crustacean helipeds are secondary sexual characteristics and male crabs use their larger chelipeds to embrace and protect females during pre-and postcopulatory behavior (see references in [56]). Furthermore, a statistically significant difference was observed in female chelipeds, with the right cheliped being larger, also reported in many brachyuran crabs [57,58]. Analysis of the length–weight relationship of Adriatic *C. granulata* showed negative allometry for males and isometric growth for females. Growth in brachyuran crabs is generally isometric; however, differences in growth between juveniles and adults have been reported [58]. In our sample we only had individuals with carapace lengths larger than 48 mm, which clearly indicates the lack of smaller juvenile individuals and also a small number of males, which had a strong impact on the analysis results. Furthermore, our data were collected throughout the years and pooled for the study period; therefore, it could be affected or biased by seasonal variations and their effects on the growth of the species. Therefore, future studies should aim to obtain a larger sample including all size ranges in order to better understand relationships of morphometric traits with sexual maturity, reproduction and seasonality.

To understand a species' life cycle, the knowledge of its reproductive biology and strategy is essential. Unfortunately, we were not able to investigate the complete temporal or seasonal gonad maturation cycle of investigated species, but we provide the first insight

into some of *C. granulata*'s reproductive aspects. Our results show that males mature at smaller carapace length (CL) than females. Size at first sexual maturity ($CL_{50\%}$) of 59.25 and 66.92 mm was estimated for males and females, respectively. The smallest developed male measured 61.21 mm and female 67.35 mm CL, indicating that both sexes achieve sexual maturity at the comparable size. In winter, only five mature individuals were sampled. In both spring and autumn samples, individuals of all stages of gonadal development (from immature to spent) have been found in similar frequencies. However, it is important to emphasize that no ovigerous females were found during sampling. In brachyuran crabs, continuous reproduction is indicated by the year-round presence of ovigerous and mature females with similar monthly frequencies, unlike seasonal continuous reproduction when these females are restricted to a determined season [59]. However, because our collected material was not representative of all year seasons, i.e., lacking samples from summer and a good sample size from winter, we can only speculate about *C. granulata* reproduction patterns. At the moment, it seems that *C. granulata* in the Adriatic Sea has seasonal continuous reproduction, and that the onset of spawning is during the warmer period of the year when environmental conditions, namely temperature and food availability, are more favorable. This hypothesis is supported by the only published data from Zariquiey Alvarez [1] reporting ovigerous females of *C. granulata* in June, August and September. To further elucidate the shamefaced crab reproductive pattern, a monthly year-round periodical sampling of the investigated population is needed to assess the frequency of all maturation stages of gonads across all seasons. Further, it would be of great importance to validate macroscopically observed gonadal stages using histological analysis as well as to combine gonad development and molt cycle data as suggested by Reigada and Negreiros-Fransozo [60].

The findings of the present study provide a preliminary insight into the feeding habits of *C. granulata* by examining general dietary categories. Brachyurans are known to prey on slow benthic macroinvertebrates, such as crustaceans and bivalves [61], and crabs of the genus *Calappa* usually use their right cheliped to crush the shell of their prey, mainly mollusks [62]. According to Števčić [3], *C. granulata* feeds on molluscs and hermit crabs that use gastropod shells for protection. However, our results indicate that *C. granulata* is an omnivorous species, with crustaceans being a preferent food component, followed by fish and cephalopods. Other calappid crabs are also known for their omnivorous nature, such as *Acanthocarpus alexandri* [63], *Hepatus pudibundus* [64], *Matuta lunaris* [3] and *Matuta victor* [65]. In accordance with the crab's predatory nature, the presence of hard remains such as crustacean exoskeleton, fish otoliths and bones, was not surprising; however, observation of cephalopod beaks and sucker rings in the crab stomach was. Cephalopods are not a typical diet component of brachyurans and this finding could indicate their scavenging activity or perhaps predation within the trawl sac. Therefore, cephalopods would represent a diet component of opportunity. Unidentified cephalopods have been reported in the diet of deep-water brachyuran crabs *Paromola cuvieri* and *Geryon longipes* [66] and squid in the diet of brachyuran snow crab *Chionoecetes opilio* [67], however always in a small percentage, indicating this is a rare diet component.

The results of this study showed no statistical difference in diet preference between the crab sizes and sex, suggesting that individuals share the same habitat. However, it is important to emphasize that the smallest sampled individual was an immature male of 48.46 mm CL. It would be of interest to explore the feeding habits of smaller immature individuals to establish possible ontogenic habitat shifts. Bueno and Bond-Buckup [68] did observe variation in prey choice between juvenile and adult anomurans *Aegla platensis* and *A. ligulata*, but with no statistically significant differences. Stevens et al. [69] reported that the ontogenetic switch in food preferences is probably a general phenomenon for *Cancer magister* linked with the functional morphology of the mouth parts and chelae size. Choy [61] also indicated that change in the cheliped strength and foraging behavior could be the reason for the observed difference in the diet preferences of juvenile and adult *Lio-carcinus puber*. Supporting this hypothesis are the findings of Perez and Bellwood [70] that

reported considerable dietary changes during the ontogeny of *Matuta lunaris* in sampled individuals of all size classes sharing the same habitat.

Our results also showed no statistical difference in prey preference regarding seasons. The basis of traditional visual dietary analysis is taxonomic identification that requires expert knowledge and mostly relies on hard structures, while soft-bodied or highly digested prey items can be under-represented or not detected at all. Furthermore, frequency of occurrence data obtained by this classical methodological approach can be strongly affected by level of detail during stomach content identification [67]. Recently, DNA metabarcoding has been successfully applied to dietary studies of economically important lobsters *Panulirus cygnus* [71], *Jasus edwardsii* [72] and *Metanephrops challengeri* [73]. Given the cryptic nature of investigated crab, a better understanding of its trophic interactions could be achieved by using DNA metabarcoding in future studies [74,75].

Crustaceans are known to be colonized by sessile benthic organisms [76], especially in soft bottom habitats [77], because their hard carapace represents a very suitable surface to settle on [78–84]. In this study, we found macroepibiont serpulid Polychaeta settled on the *Calappa granulata* exoskeleton. There are no studies of epibiotic polychaetes on *C. granulata* reported yet, although their occurrence has already been documented for other brachyurans, such as *Carcinus maenas* [85], *Bathynectes piperitus* [79], *Callinectes ornatus* and *Callinectes danae* [80], *Hyasthenus diacanthus* and *Lophozozymus pictor* [86], *Cancer gracilis* and *C. magister* [83] and *Hyas araneus* [84]. Most of the above-mentioned studies are lacking quantitative information about epibiotic polychaete prevalence and intensity. It is interesting that Becker [86] found bacteria and diatoms among epibionts of *Calappa philargius* from the Gulf of Thailand, however no polychaetes. On the investigated shamefaced crab's body, most epibiotic serpulids were found dorsally on chelipeds and carapace, likely because those body parts are exposed when the crab is buried in soft sediment. Our results showed that the rate of prevalence, intensity and abundance increase with carapace length and maturity; however, no statistically significant relationship was observed between serpulid prevalence and crab maturity stages. A similar finding has been reported for serpulid epibionts on *H. araneus* by Dvoretsky [84], who explained that older and larger crabs are exposed to a higher number of larvae which eventually grow on crabs that reached their terminal molt and because larvae prefer carapace, a surface covered with bacterial film. It appears, due to the low infestation numbers, that serpulids do not harm nor benefit the shamefaced crab. As McGaw [83] suggested, there is probably no physiological cost to the crabs by carrying an extra load of Polychaeta on the carapace because they do not reach high densities.

Epibiosis correlates very well with crustacean molting cycle. Molting frequency decreases as crab age, and when epibionts cover the major part of the carapace, it is evidence of terminal molt [78]. So, due to the strong relationship between the epibiont and its host, further investigations on epibiont distribution and occurrence pattern could be useful to gain valuable information on the crab growth and molt cycle.

Here we report 11 new mtCOI sequences for *Calappa granulata* from the eastern Adriatic Sea, the first sequences contributed from this geographical location. We have observed large haplotype and low nucleotide diversity, similar to what has been found for this species in the Mediterranean [26]. Genetic heterogeneity is commonly considered a sign of a healthy population, although this case requires further investigation. Tajima's D negative value indicates possible signs of population expansion of *C. granulata* through the Mediterranean; however, it was not significantly different from neutrality, which is in agreement with the non-star shaped appearance of the haplotype network. The haplotype network showed a dispersed topology with no most common haplotype identified, the possible existence of missing haplotypes and no segregation according to Adriatic or Mediterranean geographical origin. Lack of differentiation was also confirmed by the low F_{ST} value (0.013, $p = 0.271$). Genetic diversity and differentiation need to be further investigated to obtain a clear picture of *C. granulata* genetic structure and patterns of spatial and temporal connectivity. This needs to include larger samples from different areas across

different sampling times, especially for widespread marine species with larval dispersals [87]. Nevertheless, genetic homogeneity within the Mediterranean basin has been previously demonstrated for crab species, such as *Pachygrapsus marmoratus* [87] or *Palaemon serratus* [88], with geo-oceanographic barriers between Atlantic and Mediterranean waters (Gibraltar Strait, Almeria-Oran Front), or Mediterranean and Aegean and Black seas restricting gene flow and larvae dispersal, causing population sub-division. To what extent this applies to *C. granulata* remains to be seen. The phylogeny and species delineation within the genus *Calappa* has not yet been completely resolved [26,89,90]. Innocenti et al. [26] present morphological and genetic evidence (*p*-distances of 0.49% using the mtCOI marker, with a threshold for interspecies differentiation being 2%) that the previous *C. tuerkayana* is a juvenile stage of *C. granulata*. Using Bayesian phylogenetic inference, we reconstruct a single well-supported clade of Adriatic and Mediterranean sequences from Innocenti et al. [26], supporting the identity of *C. granulata* in the central eastern Adriatic Sea. In an era of rapidly declining biodiversity, it is important to constantly populate DNA barcoding databases with new sequencing data to facilitate molecular identification and genetic diversity analyses, especially for not-well-investigated species. Decapods contain over 17,000 species, constituting important trophic members of benthic communities with some supporting extremely valuable seafood and marine industries [91]. Understanding and monitoring their life history traits and populations represent a key aspect in conservation and management plans.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse10121964/s1, Table S1: List of GenBank Accession Numbers of *Calappa* genus sequences used in this study.

Author Contributions: Conceptualization, M.P.; methodology, M.P. and M.M.; formal analysis, M.P., Ž.T. and J.B.; investigation, M.P. and M.M.; writing—original draft preparation, M.P.; writing—review and editing, M.P., M.M. and Ž.T.; visualization, M.P., Ž.T. and J.B.; supervision, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank Tomislav Klarin and the crew of the fishing vessel "Tomiša" for their help in providing crab specimens. We would also like to thank anonymous reviewers whose valuable and helpful comments greatly improved the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zariquiey Alvarez, R. Crustáceos Decápodos Ibéricos. *Investig. Pesq.* **1968**, *32*, 1–510.
2. Rahtburn, M.J. *The Oxystomatous and Allied Crabs of America*; Smithsonian Institution United States National Museum: Washington, DC, USA, 1937; Bulletin 166; pp. 1–278.
3. Števčić, Z. Revision of Calappidae. *Aust. Mus. Mem.* **1983**, *18*, 165–171. [CrossRef]
4. Manning, R.B.; Holthuis, L.B. *West African Brachyuran Crabs (Crustacea: Decapoda)*; Smithsonian Contribution to Zoology: Washington, DC, USA, 1981; Number 306; pp. 1–379.
5. Manning, R.B.; Števčić, Z. Deacapod fauna of the Piran Gulf. *Quad. Lab. Tecnol. Pesca* **1982**, *3*, 285–304.
6. Števčić, Z. Checklist of the Adriatic decapod Crustacea. *Acta Adriat.* **1990**, *31*, 183–274.
7. Ungaro, N.; Marano, C.A.; Ceriola, L.; Martino, M. Distribution of demersal crustaceans in the southern Adriatic Sea. *Acta Adriat.* **2005**, *46*, 27–40.
8. Müller, C.H.G.; Schubart, C.D. Insights into the Crustacea Decapoda of the Adriatic Sea. Observations from four sampling locations along the Croatian coast. *Rostock. Meeresbiol. Beitr.* **2007**, *18*, 112–130.
9. Dulčić, J.; Tutman, P. Northernmost record of the shamefaced crab *Calappa granulata* (Linnaeus, 1767) (Brachyura, Calappidae) in the Mediterranean area. *Crustaceana* **2012**, *85*, 601–606. [CrossRef]

10. Bettoso, N.; Kirinčić, M.; Mavrič, B.; Lipej, L. On the rare and less known shamefaced crab *Calappa granulata* (Brachyura, Calappidae) in the northern Adriatic Sea. *Ann. Istrian Medit. Stud. (Hist. Nat.)* **2018**, *28*, 15–20.
11. Dulčić, J.; Tutman, P.; Dragičević, B. Additional records of *Calappa granulata* (Linnaeus, 1758) (Brachyura, Calappidae) in the Northern Adriatic Sea. *Crustaceana* **2019**, *92*, 757–761. [CrossRef]
12. Nye, P.A. Burrowing and burying by the crab *Macrophthalmus hirtipes*. *N. Z. J. Mar. Freshw. Res.* **1974**, *8*, 243–254. [CrossRef]
13. Barshaw, D.E.; Able, K.W. Deep burial as a refuge for lady crabs *Ovalipes ocellatus*: Comparisons with blue crabs *Callinectes sapidus*. *Mar. Ecol. Prog. Ser.* **1990**, *66*, 75–79. [CrossRef]
14. Trueman, E.R.; Ansell, A.D. The mechanisms of burrowing into soft substrata by marine animals. *Oceanogr. Mar. Biol. Annu. Rev.* **1969**, *7*, 315–366.
15. Perez, O.S. Studies on the Biology of the Indo-Pacific Sandy Shore Crab, *Matuta lunaris* Forskal (Brachyura: Calappidae). Master's Thesis, James Cook University, Douglas, Australia, 1986.
16. Warner, G.F. *The Biology of Crabs*; Elek Science: London, UK, 1977; 202p.
17. Bellwood, O. The occurrence, mechanics and significance of burying behaviour in crabs (Crustacea: Brachyura). *J. Nat. Hist.* **2002**, *36*, 1223–1238. [CrossRef]
18. Pastore, M. The genus *Calappa* in the Ionian Sea. *Oebalia* **1995**, *21*, 187–196.
19. Spanò, N.; Bono, G.; Ragonese, S. On the occurrence of the shamefaced crabs *Calappa granulata* and *C. rissoana* (Decapoda: Brachyura) in the Strait of Sicily (central Mediterranean Sea). *Vie Milieu* **2004**, *54*, 249–250.
20. Artüz, M.L. First record of *Calappa granulata* (Linnaeus, 1758) in the coastal waters of the Sea of Marmara, western Turkey. *Zoonatantia* **2006**, *1*, 72–73.
21. Balkis, H.; Kurun, A. The crab species found in the Edremit Bay (NE Aegean Sea). *J. Black Sea/Mediterr. Environ.* **2008**, *14*, 39–51.
22. González-Gordillo, J.I. Descripción de los estadios de prezoea en *Cycloes cristata* (Brulle, 1837) y *Calappa granulata* (Linnaeus, 1758) (Decapoda, Brachyura, Calappidae). *Bol. Inst. Esp. Oceanogr.* **1994**, *10*, 33–39.
23. Guerao, G.; Abelló, P.; Cartes, J. Morphology of the megalopa and first crab instar of the Shamefaced Crab *Calappa granulata* (Crustacea, Brachyura, Calappidae). *Misc. Zool.* **1998**, *21*, 37–47.
24. Guerao, G.; Abelló, P.; Torres, P. Morphology of the first zoea of the shamefaced crab *Calappa granulata* (Linnaeus, 1758) (Brachyura, Calappidae) obtained in the laboratory. *Graellsia* **1999**, *55*, 157–162. [CrossRef]
25. Olianas, A.; Sanna, M.T.; Messan, I.; Castagnola, M.; Masia, D.; Manconi, B.; Cau, A.; Giardina, B.; Pellegrini, M. The Hemocyanin of the Shamefaced Crab *Calappa granulata*: Structural-Functional Characterization. *J. Biochem.* **2006**, *139*, 957–966. [CrossRef]
26. Innocenti, G.; Fratini, S.; Tiralongo, F.; Natali, C.; Crocetta, F. The rare *Calappa tuerkayana* Pastore, 1995 is a juvenile stage of the common *Calappa granulata* (Linnaeus, 1758) (Brachyura: Calappidae). *Zool. Anz.* **2021**, *293*, 9–16. [CrossRef]
27. Williner, V.; Torres, M.V.; Carvalho, D.A.; König, N. Relative growth and morphological sexual maturity size of the freshwater crab *Trichodactylus borellianus* (Crustacea, Decapoda, Trichodactylidae) in the Middle Paraná River, Argentina. *Zookeys* **2014**, *457*, 159–170. [CrossRef]
28. Ricker, W.E. *Computation and Interpretation of Biological Statistics of Fish Populations*; The Blackburn Press: Caldwell, NJ, USA, 1975; Volume 191, pp. 1–382.
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019; Available online: https://www.R-project.org/ (accessed on 23 June 2022).
30. Efron, B. *The Jackknife, the Bootstrap and Other Resampling Plans*; SIAM Monograph No. 38; CBSM-NSF Regional Conference Series in Applied Mathematics; Society for Industrial and Applied Mathematics: Philadelphia, PA, USA, 1982.
31. Wickham, H.; François, R.; Henry, L.; Müller, K. dplyr: A Grammar of Data Manipulation. R Package Version 1.0.9. 2022. Available online: https://CRAN.R-project.org/package=dplyr (accessed on 23 June 2022).
32. Henry, L.; Wickham, H.; purrr: Functional Programming Tools. R Package Version 0.3. 2020. Available online: https://CRAN.R-project.org/package=purrr (accessed on 23 June 2022).
33. Silge, J.; Chow, F.; Kuhn, M.; Wickham, H. rsample: General Resampling Infrastructure. R Package Version 1.0.0. 2022. Available online: https://CRAN.R-project.org/package=rsample (accessed on 8 August 2022).
34. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*; Springer: New York, NY, USA, 2016; 213p.
35. Da Silva Castiglioni, D.; Negreiros-Fransozo, M.L. Physiologic Sexual Maturity of the Fiddler Crab *Uca rapax* (Smith, 1870) (Crustacea, Ocypodidae) from Two Mangroves in Ubatuba, Brazil. *Braz. Arch. Biol. Technol.* **2006**, *49*, 239–248. [CrossRef]
36. Williams, M.J. Methods for analysis of natural diet in portunid crabs (Crustacea: Decapoda: Portunidae). *J. Exp. Mar. Biol. Ecol.* **1981**, *52*, 103–113. [CrossRef]
37. Hyslop, E. Stomach content analysis—A review of methods and their application. *J. Fish Biol.* **1980**, *17*, 411–429. [CrossRef]
38. Margolis, L.; Esch, G.W.; Holmes, J.C.; Kuris, A.M.; Schad, G.A. The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J. Parasitol. Res.* **1982**, *68*, 131–133. [CrossRef]
39. Bush, A.O.; Lafferty, K.D.; Lotz, J.M.; Shostak, A.W. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol. Res.* **1997**, *83*, 575–583. [CrossRef]
40. Reiczigel, J.; Marozzi, M.; Fábián, I.; Rózsa, L. Biostatistics for parasitologists—A primer to Quantitative Parasitology. *Trends Parasitol.* **2019**, *35*, 277–281. [CrossRef]
41. Rózsa, L.; Reiczigel, J.; Majoros, G. Quantifying parasites in samples of hosts. *J. Parasitol.* **2000**, *86*, 228–232. [CrossRef]

42. Turtinen, L.W.; Juran, B.D. Protein salting-out method applied to genomic DNA isolation from fish whole blood. *Biotechniques* **1998**, *24*, 238–239.
43. Folmer, O.; Black, M.; Hoeh, W.; Lutz, R.; Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **1994**, *3*, 294–299. [PubMed]
44. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Cooper, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef] [PubMed]
45. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. [CrossRef] [PubMed]
46. Nguyen, L.-T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
47. Trifinopoulos, J.; Nguyen, L.-T.; von Haeseler, A.; Minh, B.Q. W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.* **2016**, *44*, W232–W235. [CrossRef]
48. Kalyaanamoorthy, S.; Minh, B.Q.; Wong, T.K.F.; von Haeseler, A.; Jermiin, L.S. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **2017**, *14*, 587–589. [CrossRef]
49. Hoang, D.T.; Chernomor, O.; von Haeseler, A.; Minh, B.Q.; Vinh, L.S. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **2018**, *35*, 518–522. [CrossRef]
50. Rozas, J.; Ferrer-Mata, A.; Sánchez-DelBarrio, J.C.; Guirao-Rico, S.; Librado, P.; Ramos-Onsins, S.E.; Sánchez-Gracia, A. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. *Mol. Biol. Evol.* **2017**, *34*, 3299–3302. [CrossRef]
51. Nei, M. *Molecular Evolutionary Genetics*; Columbia University Press: New York, NY, USA, 1987; 514p.
52. Excoffier, L.; Lischer, H.E.L. Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows. *Mol. Ecol. Resour.* **2010**, *10*, 564–567. [CrossRef]
53. Tajima, F. Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics* **1989**, *123*, 585–595. [CrossRef]
54. Leigh, J.W.; Bryant, D. Popart: Full-Feature Software for Haplotype Network Construction. *Methods Ecol. Evol.* **2015**, *6*, 1110–1116. [CrossRef]
55. Alió, J.J.; Marcano, L.A.; Altuve, D.E. Pesquería y parámetros biométricos de los cangrejos del género *Calappa* (Brachyura: Calappidae) en el oriente de Venezuela. *Rev. Biol. Trop. (Int. J. Trop. Biol.)* **2005**, *53*, 463–474. [CrossRef]
56. Hamasaki, K.; Dan, S. Seasonal Changes in the Sexual Size Dimorphisms of the Chelipeds and Pleons of the Porcellanid Crab *Petrolisthes japonicus*. *Zool. Stud.* **2021**, *60*, e18. [CrossRef]
57. Negreiros-Fransozo, M.L.; Fransozo, V. A morphometric study of the mud crab, *Panopeus austrobesus* Williams, 1983 (Decapoda: Brachyura) from a sub-tropical mangrove in South America. *Crustaceana* **2003**, *76*, 281–294. [CrossRef]
58. Fumis, P.B.; Fransozo, A.; Bertini, G.; Braga, A.A. Morphometry of the crab *Hexapanopeus schmitti* (decapoda: Xanthoidea) on the northern coast of state of Sao Paulo, Brazil. *Rev. Biol. Trop. (Int. J. Trop. Biol.)* **2007**, *55*, 163–170. [CrossRef]
59. Lima, G.V.; Soares, M.R.S.; Oshiro, L.M.Y. Reproductive biology of the sesarmid crab *Armases rubripes* (Decapoda, Brachyura) from an estuarine area of the Sahy River, Sepetiba Bay, Rio de Janeiro, Brazil. *Iheringia Sér. Zool.* **2006**, *96*, 47–52. [CrossRef]
60. Reigada, A.L.D.; Negreiros-Fransozo, M. Reproductive cycle of *Hepatus pudibundus* (Herbst, 1785) (Crustacea, Decapoda, Calappidae) in Ubatuba, SP, Brazil. *Rev. Brasil. Biol.* **2000**, *60*, 483–491. [CrossRef]
61. Choy, S. Natural diet and feeding habits of the crabs *Liocarcinus puber* and *L. holsatus* (Decapoda, Brachyura, Portunidae). *Mar. Ecol. Prog. Ser.* **1986**, *31*, 87–99. [CrossRef]
62. Shoup, J.B. Shell opening by crabs of the genus *Calappa*. *Science* **1968**, *160*, 887–888. [CrossRef]
63. Haefner, P.A. Morphometry, reproductive biology, and diet of *Acanthocarpus alexandri* Stimpson, 1871 (Decapoda, Brachyura) in the Middle Atlantic Bight. *J. Crustac. Biol.* **1981**, *1*, 348–357. [CrossRef]
64. Mantelatto, F.L.M.; Petracco, M. Natural diet of the crab *Hepatus pudibundus* (Brachyura: Calappidae) in Fortaleza Bay, Ubatuba (SP), Brazil. *J. Crustac. Biol.* **1997**, *17*, 440–446. [CrossRef]
65. Innocenti, G.; Stasolla, G.; Mendelson, M.; Galil, B.S. Aggressive, omnivorous, invasive: The Erythraean moon crab *Matuta victor* (Fabricius, 1781) (Crustacea: Decapoda: Matutidae) in the eastern Mediterranean sea. *J. Nat. Hist.* **2017**, *51*, 2133–2142. [CrossRef]
66. Cartes, J.E. Diets of deep-sea brachyuran crabs in the Western Mediterranean Sea. *Mar. Biol.* **1993**, *117*, 449–457. [CrossRef]
67. Squires, H.J.; Dawe, E.G. Stomach Contents of Snow Crab (*Chionoecetes opilio*, Decapoda, Brachyura) from the Northeast Newfoundland Shelf. *J. Northwest Atl. Fish. Sci.* **2003**, *32*, 27–38. [CrossRef]
68. Bueno, A.A.P.; Bond-Buckup, G. Natural Diet of *Aegla platensis* Schmitt and *Aegla ligulata* Bond-Buckup & Buckup (Crustacea, Decapoda, Aeglidae) from Brazil. *Acta Limnol. Bras.* **2004**, *16*, 115–127.
69. Stevens, B.G.; Armstrong, D.A.; Cusimano, R. Feeding Habits of the Dungeness Crab *Cancer magister* as Determined by the Index of Relative Importance. *Mar. Biol.* **1982**, *72*, 135–145. [CrossRef]
70. Perez, O.S.; Bellwood, D.R. Ontogenetic Changes in the Natural Diet of the Sandy Shore Crab, *Matuta lunaris* (Forskll) (Brachyura:Calappidae). *Aust. J. Mar. Freshw. Res.* **1988**, *39*, 193–199. [CrossRef]
71. O'Rorke, R.; Lavery, S.; Chow, S.; Takeyama, H.; Tsai, P.; Beckley, L.E.; Thompson, P.A.; Waite, A.M.; Jeffs, A.G. Determining the diet of larvae of western rock lobster (*Panulirus cygnus*) using high-throughput DNA sequencing techniques. *PLoS ONE* **2012**, *7*, e42757. [CrossRef]

72. O'Rorke, R.; Lavery, S.; Wang, M.; Nodder, S.D.; Jeffs, A.G. Determining the diet of larvae of the red rock lobster (*Jasus edwardsii*) using high-throughput DNA sequencing techniques. *Mar. Biol.* **2014**, *161*, 551–563. [CrossRef]
73. Van der Reis, A.L.; Laroche, O.; Jeffs, A.G.; Lavery, S.D. Preliminary analysis of New Zealand scampi (*Metanephrops challengeri*) diet using metabarcoding. *PeerJ* **2018**, *6*, e5641. [CrossRef]
74. Kress, W.J.; García-Robledo, C.; Uriarte, M.; Erickson, D.L. DNA barcodes for ecology, evolution, and conservation. *Trends Ecol. Evol.* **2015**, *30*, 25–35. [CrossRef]
75. Sousa, L.L.; Sofia Marques Silva, S.M.; Xavier, R. DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environ. DNA* **2019**, *1*, 199–214. [CrossRef]
76. Ross, D.M. Symbiotic relations. In *The Biology of Crustacea, Vol. 7 Behaviour and Ecology*; Vemberg, S.J., Vemberg, W.B., Eds.; Academic Press: New York, NY, USA, 1983; pp. 163–212.
77. Connell, J.H.; Keough, M.J. Disturbance and patch dynamics of subtidal marine animals on hard substrata. In *The Ecology of Natural Disturbance and Patch Dynamics*; Pickett, S.T.A., White, P.S., Eds.; Academic Press: San Diego, CA, USA, 1985; pp. 125–151.
78. Abello, P.; Villanueva, R.; Gili, J.M. Epibiosis in deep sea crab populations as an indicator of biological and behavioural characteristics of the host. *J. Mar. Biol. Assoc. U. K.* **1990**, *70*, 687–695. [CrossRef]
79. Gili, J.M.; Abello, P.; Villanueva, R. Epibionts and intermoult duration in the crab *Bathynectes piperitus*. *Mar. Ecol. Prog. Ser.* **1993**, *98*, 107–113. [CrossRef]
80. Negreiros-Fransozo, M.L.; Costa, T.M.; Fransozo, A. Epibiosis and molting in two species of *Callinectes* (Decapoda: Portunidae) from Brazil. *Rev. Biol. Trop.* **1995**, *43*, 257–264.
81. Abello, P.; Corbera, J. Epibiont bryozoans (Bryozoa, Ctenostomatida) of the crab *Goneplax rhomboides* (Brachyura, Goneplacidae) off the Ebro delta (western Mediterranean). *Misc. Zool.* **1996**, *19*, 43–52.
82. Dick, M.H.; Donaldson, W.E.; Vining, I.W. Epibionts of the tanner crab *Chionoecetes bairdi* in the region of Kodiak Island, Alaska. *J. Crustac. Biol.* **1998**, *18*, 519–528. [CrossRef]
83. McGaw, I.J. Eepibionts of sympatric species of cancer crabs in Barkley Sound, British Columbia. *J. Crustac. Biol.* **2006**, *26*, 85–93. [CrossRef]
84. Dvoretsky, A.G. Epibionts of the great spider crab, *Hyas araneus* (Linnaeus, 1758), in the Barents Sea. *Polar Biol.* **2012**, *35*, 625–631. [CrossRef]
85. Crothers, J.H. The biology of the shore crab *Carcinus maenas* (L.) 2. The life of the adult crab. *Field Stud.* **1968**, *2*, 579–614.
86. Becker, K. Epibionts on Carapaces of Some Malacostracans from the Gulf of Thailand. *J. Crustac. Biol.* **1996**, *16*, 92–104. [CrossRef]
87. Fratini, S.; Ragionieri, L.; Deli, T.; Harrer, A.; Marino, I.A.M.; Cannicci, S.; Zane, L.; Schubart, C.D. Unravelling population genetic structure with mitochondrial DNA in a notional panmictic coastal crab species: Sample size makes the difference. *BMC Evol. Biol.* **2016**, *16*, 150. [CrossRef] [PubMed]
88. Weiss, R.; Torrecilla, Z.; González-Ortegón, E.; González-Tizón, A.; Martínez-Lage, A.; Schubart, C. Genetic differentiation between Mediterranean and Atlantic populations of the common prawn *Palaemon serratus* (Crustacea: Palaemonidae) reveals uncommon phylogeographic break. *J. Mar. Biol. Assoc. U. K.* **2018**, *98*, 1425–1434. [CrossRef]
89. Holthuis, L.B. Nomenclatural notes on Mediterranean species of *Calappa* Weber, 1795 (Crustacea: Decapoda: Brachyura). *Zool. Verh.* **2001**, *334*, 99–102.
90. Marco-Herrero, E.; Abelló, P.; Drake, P.; García-Raso, J.E.; González-Gordillo, J.I.; Guerao, G.; Palero, F.; Cuesta, J.A. Annotated checklist of brachyuran crabs (Crustacea: Decapoda) of the Iberian Peninsula (SW Europe). *Sci. Mar.* **2015**, *79*, 243–256. [CrossRef]
91. Matzen da Silva, J.; Creer, S.; dos Santos, A.; Costa, A.C.; Cunha, M.R.; Costa, F.O.; Carvalho, G.R. Systematic and Evolutionary Insights Derived from mtDNA COI Barcode Diversity in the Decapoda (Crustacea: Malacostraca). *PLoS ONE* **2011**, *6*, e19449. [CrossRef]