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# Isotopic Niche and Trophic Position of the Invasive Portunid *Portunus segnis* Forskål, (1775) in Elounda Bay (Crete Island, Eastern Mediterranean)

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**Abstract:** There is a growing recognition that an advanced understanding of the trophic characteristics of an invasive consumer can provide important information on its ecological impact. In recent years, the blue swimming crab *Portunus segnis*, one of the earliest Lessepsian invaders, has considerably expanded its distribution range in the Mediterranean Sea, yet, its trophic habits in invaded areas remain scarcely investigated. In this study, we used carbon and nitrogen stable isotopes analysis (SIA) to determine the trophic position and isotopic niche of the crab compared with other representatives of the flora and fauna occurring in Elounda Bay (Crete). *P. segnis* showed a trophic position of 3.9, higher than the values determined by SIA or conventional gut content analysis in other areas of the Mediterranean Sea or in the native range. Crab specimens showed a high inter-individual variability in both $\delta^{13}$C and $\delta^{15}$N values; further analysis indicated negligible differences in the isotopic niche of adult males and females. Conversely, $\delta^{15}$N values were significantly related to the size of the specimens, ultimately suggesting an ontogenetic dietary shift. This study represents the first attempt to characterize the trophic habits of the blue swimming crab in the context of an invaded food web and may contribute to the implementation of long-term management strategies of control and mitigation of its ecological impact.

**Keywords:** non-indigenous species; blue swimming crab; trophic ecology; stable isotopes; trophic level; diet plasticity

## 1. Introduction

The Mediterranean Sea is recognized worldwide as a biodiversity hotspot as well as one of the marine regions most affected by biological invasions [1–3]. A number of anthropogenic factors including shipping, aquaculture trade, and artificial corridors interact and intensify the introduction of non-indigenous species (NIS hereafter) in the basin, enforcing the necessity of quantitatively assessing the impact of NIS to prioritize appropriate measures of control, management, and mitigation [4,5].

Noticeably, so far the impacts of bioinvaders have been primarily evaluated by relying on the qualitative evidence-based knowledge of experts, rather than on quantitative evidence itself [6,7]. In addition, when quantitative evidence is available, its generality is largely limited due to the spatial and temporal context-dependency of the observed effects [8–10]. Indeed, NIS effects on biodiversity and ecosystem functions necessarily imply alterations in the structure of invaded food webs, a "rewiring" of trophic interactions directly through predation or indirectly by competition [11–14]. This ultimately entails that an advanced assessment of an invader's ecological impact should include the determination of its position and trophic characteristics in the context of the recipient food web [15–18]. This is particularly true for introduced marine crustaceans, since they can exert strong top-down controls, indirectly affecting non-prey species through, for example, cascading effects [19–21].

In this study, we focused on the portunid blue swimming crab *Portunus segnis* (Forskål, 1775). The species is one of the earliest Lessepsian crustacean invaders [22] recorded in Egyptian and Palestinian waters 30 years after the completion of the Suez Canal [23]. In the following years, it has successfully colonized the Levantine basin from Egypt to Turkey [24]; the Aegean Sea; eastern Sicily and the northern Tyrrhenian Sea [25]; and more recently, Tunisian, Maltese, Libyan, and Cypriot coastal waters [26–29]. Figure 1 and Table S1 in the Supplementary Materials provide an updated review of the crab's occurrence in invaded Mediterranean areas.

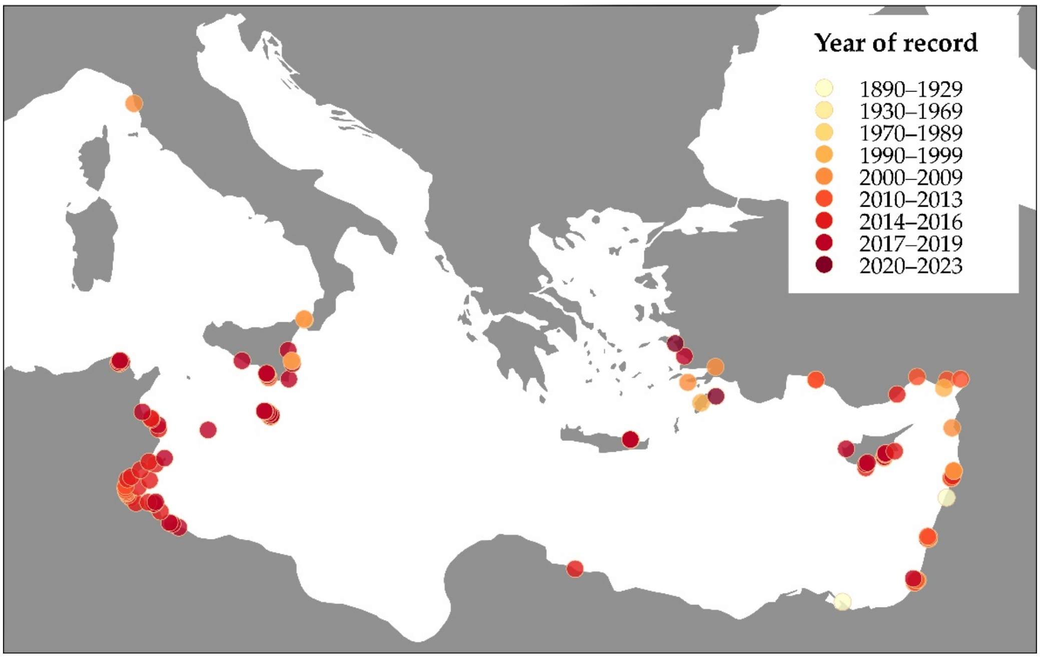


**Figure 1.** Updated distribution map of *Portunus segnis* in the Mediterranean Sea as of 17 September 2022. The list of occurrences is included in Table S1 in the Supplementary Materials, while the methodology adopted to collate them is described in Appendix A.

In EU-Mediterranean countries besides Italy, Cyprus, and Malta, *P. segnis* has been reported only in Greek waters in the islands of Rhodes and Crete (Figure 1). In Crete in particular, the species was recorded only in Elounda Bay in 2018 [30]; currently, the population is established and raising interest as a shellfish product [see interview with one of the authors (C.D.) at https://imbbc.hcmr.gr/2021/01/26/eksafanisthkan-ta-mple-kabouria accessed on 14 November 2022]. Indeed, in the Persian Gulf and other native habitats, *P. segnis* is an important fishery resource [31,32], while in the Mediterranean Sea it has become of economic value in Egypt, Turkey and, more recently, in Tunisia [24,33,34]. Furthermore, the General Fisheries Commission for the Mediterranean (GFCM) has recently presented the Recommendation GFCM/42/2018/7 to establish a regional research program to fill scientific and research gaps for a sustainable exploitation of *P. segnis* and of the Atlantic blue crab *Callinectes sapidus*. A number of studies have focused on the occurrence (see references in Table S1), morphometry, population structure, proximate composition, and biochemistry of *P. segnis* [25,35–38]. To date, however, information on the trophic habits of the species and its functional role in invaded food webs are still scant, and exclusively from Tunisian coastal waters [33,39].

The first aim of the present study was to provide an assessment of the trophic position and intra-specific variation in the trophic habits of *P. segnis* from Elounda Bay. This bay represents an important nursery area for a diverse assemblage of fish and cephalopods in the spring and early summer months [40,41]; thus, a thorough assessment of the trophic role of *P. segnis* may make available important information for the conservation and protection of local marine biological resources. In recent years, the analysis of carbon and nitrogen stable isotopes (SIA) has provided a remarkable contribution to the investigation of the impacts of invasive species via an elucidation of their role in recipient food webs [42,43]. Here, SIA was used to assess the trophic position of the crab in late spring and early summer, as compared with other native and non-indigenous fish and invertebrates occurring in the bay; in addition, individual-scale isotopic information was used to investigate the isotopic niche of the crab, and to test for sex- or ontogenetic-related variations in the trophic habits of individuals.

## 2. Materials and Methods

### 2.1. Study Site

Elounda Bay is a shallow, semi-enclosed embayment located along the north-eastern coast of the Island of Crete (35.279201° N, 25.730313° E; Figure 1). It has a total surface area of approximately 6.5 $km^2$ and a depth ranging between 2 and 9 m, and it is connected to the outer Mirabello Bay through two natural inlets and one artificial inlet located in the northern and southern area of the basin, respectively.

The abiotic parameters characterizing the water column of the bay are described in detail in [44]. In brief, water temperatures show a strong seasonality, ranging between 14 °C in winter to 29 °C in summer; salinity variations are less pronounced, fluctuating between 39 and 42 PSU in winter and summer, respectively. Nutrients and organic matter concentrations indicate oligotrophic to mesotrophic conditions, indirectly suggesting that anthropogenic impacts are mainly related to the intense touristic activities concentrated in the summer months [45]. Most of the coastline of the embayment is rocky, while its bottoms below 2 m depth are covered by fine siltous sediment that is colonized by monospecific meadows of *Caulerpa prolifera* during the summer months; in addition, the seagrass *Cymodocea nodosa* occurs in the shallowest areas in the southern sector of the basin [41,46]. Macrophytal beds are associated with a diverse and abundant macrofaunal assemblage of benthic crustaceans, mollusks, and polychaetes, supporting a variety of fish and cephalopods including species of commercial interest such as the bogue *Boops boops*, the pickerel *Spicara smaris*, the red mullet *Mullus barbatus*, and the cuttlefish *Sepia officinalis* [40,46]. *Portunus segnis* was first recorded in Elounda Bay between September and October 2018 by visual observations made by local fishermen; its occurrence was further confirmed in June 2019, see also Table S1 [30]. Currently, the species is established and has reached a relatively high abundance, to the point that it is considered by the local community of fishermen to be of potential economic interest [see interview to one of the authors (C.D.) at https://imbbc.hcmr.gr/2021/01/26/eksafanisthkan-ta-mple-kabouria accessed on 14 November 2022].

### 2.2. Sample Collection and Laboratory Procedures

Sample collection occurred in the southern sector of the bay and in two consecutive days, 25 May and 26 May 2021 (Figure 2). At each sampling occasion, five crab traps were deployed at five different locations at depths ranging between 2 and 5 m (25 May) and between 3 and 4 m (26 May), and a total of ten different sampling sites were covered over the two sampling dates (Figure 2). The crab traps baited with fish carcasses had a cylindrical shape with a length of 60 cm and a diameter of 30 cm. The structure was made of steel spring wire covered by a 1 cm mesh net, enabling the trap to be folded for transport and ensuring an adequate stiffness when unfolded into a working position. Traps had at their ends two 12 cm wide funnel-shaped entrances made of netting, and a net pocket located in a central position for the bait. Traps were collected by SCUBA divers after 24 h from deployment. At each sampling occasion, potential bivalve prey was hand-collected by snorkeling. Additionally, specimens of the bivalve *Pinctada radiata* were collected in an embayment in the eastern area of the bay, where this species was present in high abundance at that time (Figure 1; Skouradakis, personal observation). Two weeks after the traps' deployment, additional *P. segnis* adults were collected from fishermen operating gillnets in the central sector of the bay.

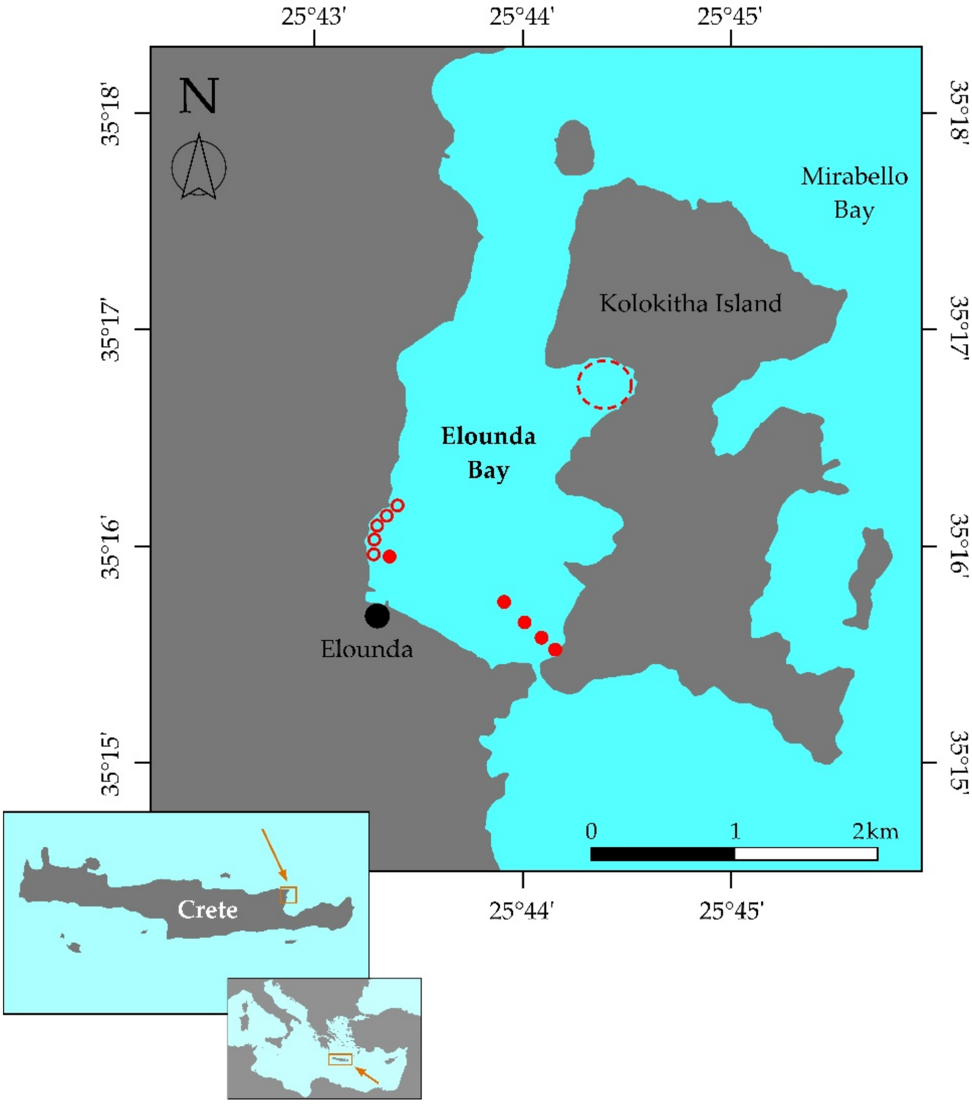


**Figure 2.** Map showing the sampling locations in Elounda Bay. Full circles: crab traps deployed on 25 May; empty circles: crab traps deployed on 26 May. The area identified by the dashed circle shows the location where specimens of the bivalve *Pinctada radiata* were sampled.

All collected specimens were transferred to the laboratory in refrigerated containers (4 °C), where they were identified to the lowest taxonomic level, enumerated, and had their individual carapace width (for brachyurans), shell length (for molluscs), and total standard length (for fish) measured to the nearest mm. Subsequently, *P. segnis* individuals were sexed by inspecting the shape of the abdomen apron and dissected to assess their gonadal development and maturity stage, according to the criteria reported in [47,48].

### 2.3. Stable Isotope Analysis (SIA)

For stable isotope analysis, claw muscles were removed with a scalpel from the brachyurans and individually dried (60 °C, >1 week). For mollusks, the foot was removed and dried, while for fish specimens a portion of the dorsal muscle was dissected. After drying, all collected samples were pulverized using a mortar and pestle. For each specimen, subsamples (0.82 ± 0.03 mg, mean ± SD) of powdered tissue were pressed into Ultra-Pure tin capsules (Costech Analytical Technologies, Valencia, CA, USA) and analyzed using an Elemental Analyzer connected with an Isotope Ratio Mass Spectrometer (Thermo Scientific Flash EA 1112 and Delta Plus XP, respectively, Waltham, MA, USA). Isotopic notations for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were expressed in relation to international standards using the conventional δ unit notation as parts per mil deviations from international standards:

$$\delta^{13}C \text{ or } \delta^{15}N \;=\left[\left(R_{sample}/R_{standard}\right)-1\right]\times 1000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Pee Dee belemnite (PDB) limestone carbonate and atmospheric nitrogen ($N_2$) were used as standards for carbon and nitrogen isotope ratios. Analytical precision based on the standard deviation of replicates of internal secondary isotopic standards (International Atomic Energy Agency IAEA-NO-3 for $\delta^{15}N$ and IAEA-CH-6 for $\delta^{13}C$) was 0.2‰ for both $\delta^{15}N$ and $\delta^{13}C$.

### 2.4. Data Analysis

Values in the text are expressed as mean ± 1SD. For parametric statistical analysis (i.e., *t*-test with separate variance estimates, Pearson correlation), data were preliminarily tested for their conformity to assumptions of variance homogeneity (Cochran's C test) and normality (Shapiro-Wilks test), and were transformed when required.

In general, fish taxa showed C:N ratios < 3.5, whereas invertebrates were characterized by variable C:N ratios, in most cases > 3.5 (see Table A1 in Appendix B), which testified to a considerable contribution of lipids in the tissues carbon pool [49]. Lipids are depleted in ${}^{13}C$ compared to proteins and carbohydrates and can significantly bias $\delta^{13}C$ estimations [50]. Accordingly, for samples with a C:N ratio > 3.5, $\delta^{13}C$, values were corrected for lipid content using tissue C:N ratios following the algorithm proposed in [49], and lipid-corrected $\delta^{13}C$ values were used in further analyses.

One-way Permutational Analysis of Variance (PERMANOVA hereafter) [51] was run on an Euclidean distance matrix calculated on individual CN isotopic signatures with 9999 permutations of residuals within a reduced model to test for dissimilarities in $\delta^{13}C$ and $\delta^{15}N$ values among taxa, and to test for sex-related differences in isotopic values in *P. segnis*.

The trophic position of the crab and, for comparative purposes, of other invertebrate and fish taxa sampled during the study were estimated implementing a one-baseline, two-discrimination factor Bayesian model run with two parallel chains and 40,000 adaptive iterations. The choices of appropriate baseline species and Trophic Enrichment Factors (TEF hereafter) were key steps in ensuring robust trophic position estimations [52]. Suspension feeding bivalves have long been recognized as reliable baseline references in studies of freshwater [53,54] and, more recently, marine food webs [55,56], since they have a relatively long biological cycle, a low metabolic rate, and are able to integrate highly variable isotope values among primary producers [53,57]. Here we used the cumulated $\delta^{13}C$ and $\delta^{15}N$ values of the two suspensivore bivalves *Modiolus barbatus* and *Pinctada radiata* as baseline, assuming a trophic level $\lambda = 2$ after a PERMANOVA indicated negligible inter-specific differences (see Results). Regarding the choice of an appropriate TEF, species- or taxon-specific values were generally suggested [52]. Here we adopted TEFs for carbon and nitrogen of $\Delta^{13}C = 0.27 \pm 2.44$ and $\Delta^{15}N = 2.57 \pm 1.72$ (mean ± 1SD, n = 27 and 65, respectively), calculated as the average of the enrichment factors of marine crustaceans feeding on animal items reported in [58] and more consistent with other literature synthesis focused on crustaceans [59,60].

To compare the isotopic niche areas of *P. segnis* males and females and to assess their overlap, we use standard ellipse area (SEA) as a measure of the mean core population isotopic niche [61]. In brief, the standard ellipse of a set of bivariate data is calculated from the variance and covariance of the x and y data and contains approximately 40% of the data. Accordingly, it represents the core niche area and is expected to be insensitive to sample size. Given the different number of sampled males and females (see Results), we used a sample size-corrected version of the standard ellipse area (SEAc hereafter) [61]. In order to compare the isotopic niche area between sexes, a Bayesian approach was used that calculated 100,000 posterior iterations of SEA ($SEA_B$) based on the data set. Pair-wise comparisons were subsequently performed by calculating the proportion of $SEA_B$ that differed between the two sexes and interpreting it as a direct proxy for the probability that one group was larger than the other [61].

Statistical analyses were performed in the R statistical environment [62]. Specifically, PERMANOVA was run in the *vegan* package [63], standard ellipse areas were calculated using *SIBER* [61,64], and trophic positions were estimated using *tRophicPosition* [65,66]. The function "pairwiseComparisons" available in SIBER was used to statistically compare in a Bayesian context the trophic position values estimated for the different taxa and for adult and immature *P. segnis* males and females.

## 3. Results

### 3.1. Composition of Collected Samples

A total of 92 specimens belonging to 12 animal species were collected (Table 1). Seven *Portunus segnis* individuals, five males and two females, were captured in crab traps. Males ranged in carapace width between 29 and 89 mm (78.8 ± 25.1 mm) while females measured between 97 and 99 mm, respectively; independently from sex, all captured specimens were sexually immature.

**Table 1.** List of native (N) and non-indigenous (NIS) invertebrate and fish taxa sampled during the study. Mean sizes (±1SD) and size ranges are reported in mm and refer to carapace length for *Diogenes* sp., carapace widths for the remaining arthropods, standard lengths (i.e., measured from the tip of the snout to the posterior end of the last vertebra) for fish, and shell lengths for mollusks. The number of analyzed specimens is reported in square brackets.

| Phylum | Species | Mean Size | Size Range | Status |
|---|---|---|---|---|
| Arthropoda | *Diogenes* sp. [3] | 11 ± 1.2 | 9–13 | N |
| | *Eriphia verrucosa* (Forskål, 1775) [3] | 56.1 ± 15.1 | 26–72 | N |
| | *Portunus segnis* (Forskål, 1775) [56] | 131.3 ± 3.4 | 29–175 | NIS |
| Chordata | *Boops boops* (Linnaeus, 1758) [2] | 125 ± 8 | 117–133 | N |
| | *Pagellus acarne* (Risso, 1827) [2] | 129.5 ± 15.5 | 114–145 | N |
| | *Stephanolepis diaspros* Fraser-Brunner, 1940 [2] | 130 ± 7 | 123–137 | NIS |
| | *Torquigener flavimaculosus* Hardy & Randall, 1983 [3] | 86.7 ± 6.1 | 77–98 | NIS |
| Mollusca | *Bolinus brandaris* (Linnaeus, 1758) [2] | 43 ± 1.4 | 42–44 | N |
| | *Cerithium vulgatum* Bruguière, 1792 [3] | 44.7 ± 1.5 | 42–47 | N |
| | *Hexaplex trunculus* (Linnaeus, 1758) [5] | 39.2 ± 3.2 | 28–45 | N |
| | *Modiolus barbatus* (Linnaeus, 1758) [5] | 36.2 ± 1.3 | 33–40 | N |
| | *Pinctada radiata* (Leach, 1814) [6] | 66.3 ± 2.4 | 57–74 | NIS |

Hermit crabs of the genus *Diogenes*, warty crabs (*Eriphia verrucosa*), and the gastropods *Bolinus brandaris*, *Cerithium vulgatum*, and *Hexaplex trunculus* were also found in crab traps (Table 1). Among fish, nine specimens were captured in total in crab traps, including bogues (*Boops boops*) and axillary sea breams (*Pagellus acarne*); in addition, two NIS were collected, i.e., the reticulated leatherjacket *Stephanolepis diaspros* and the yellowspotted puffer *Torquigener flavimaculosus*. Specimens of the bivalve *Modiolus barbatus* were collected by snorkeling along the western coast of the bay, while *Pinctada radiata* individuals were collected by hand from the dense beds formed by this non-indigenous bivalve at shallow depths (approximately 1 m) in the eastern sector of the bay (Figure 2).

Forty-nine *P. segnis* specimens were obtained from local fishermen. They were all mature adults (see an example in Figure 3) ranging in carapace widths between 106 and 175 mm (137.1 ± 17.6 mm, mean ± 1SD); they were predominantly males (40 males vs. 9 females); in addition, four out of nine females were ovigerous.

**Figure 3.** A female (**a**) and a male (**b**) *Portunus segnis* obtained from fishermen during the study. Please refer to the caliper for the size, since the two images are not to scale.

### 3.2. Trophic Positions and Isotopic Niche of Portunus Segnis

Among the species analyzed, the bivalves *Modiolus barbatus* and *Pictada radiata* were the most $^{13}$C- and $^{15}$N-depleted, with $\delta^{13}$C values comprised between −19.6 ± 0.6 and −19.9 ± 1.1 (mean ± 1SD), respectively, and $\delta^{15}$N values ranging between 2.4 ± 1.1 and 2.7 ± 0.3 (Figure 4). The isotopic values of the two species were not statistically different (PERMANOVA post-hoc test, t = 0.73, $p_{\text{(Monte Carlo)}} = 0.56$), indicating a negligible variation in nitrogen and carbon sources between bivalves from the eastern (*P. radiata*) and the western (*M. barbatus*) sectors of Elounda Bay.

*Eriphia verrucosa* showed the highest $\delta^{15}$N values, more than 8‰ higher than that of bivalves (Figure 4) and equivalent with a trophic position of 4.2 (Table 2). The warty crab *E. verrucosa* was the crustacean with the highest trophic position, followed by *Diogenes* sp. (TP = 3.9) and *P. segnis, the latter* characterized by a mean trophic position of 3.8 with a 3.3–4.6 95% CI (Table 2). *Portunus* was isotopically similar to the gastropods *Bolinus brandaris*, *Hexaplex trunculus*, and *Cerithium vulgatum* (Figure 4), and was characterized by remarkably similar trophic position values (Table 2). With the exception of *Pagellus acarne*, other fish —*Stephanolepis diaspros*, *Torquigener flavimaculosus*, and, in particular, *Boops boops*— were characterized by isotopic values significantly more depleted than those of *P. segnis* (Figure 4; PERMANOVA post-hoc tests, min t = 2.67, $p_{\text{(Monte Carlo)}} = 0.004$ for the comparison *T. flavimaculosus* vs. *P. segnis*). The corresponding trophic positions were relatively low, ranging between 3.4 and 3.5 (*B. boops* and *T. flavimaculosus*, respectively; Table 2).

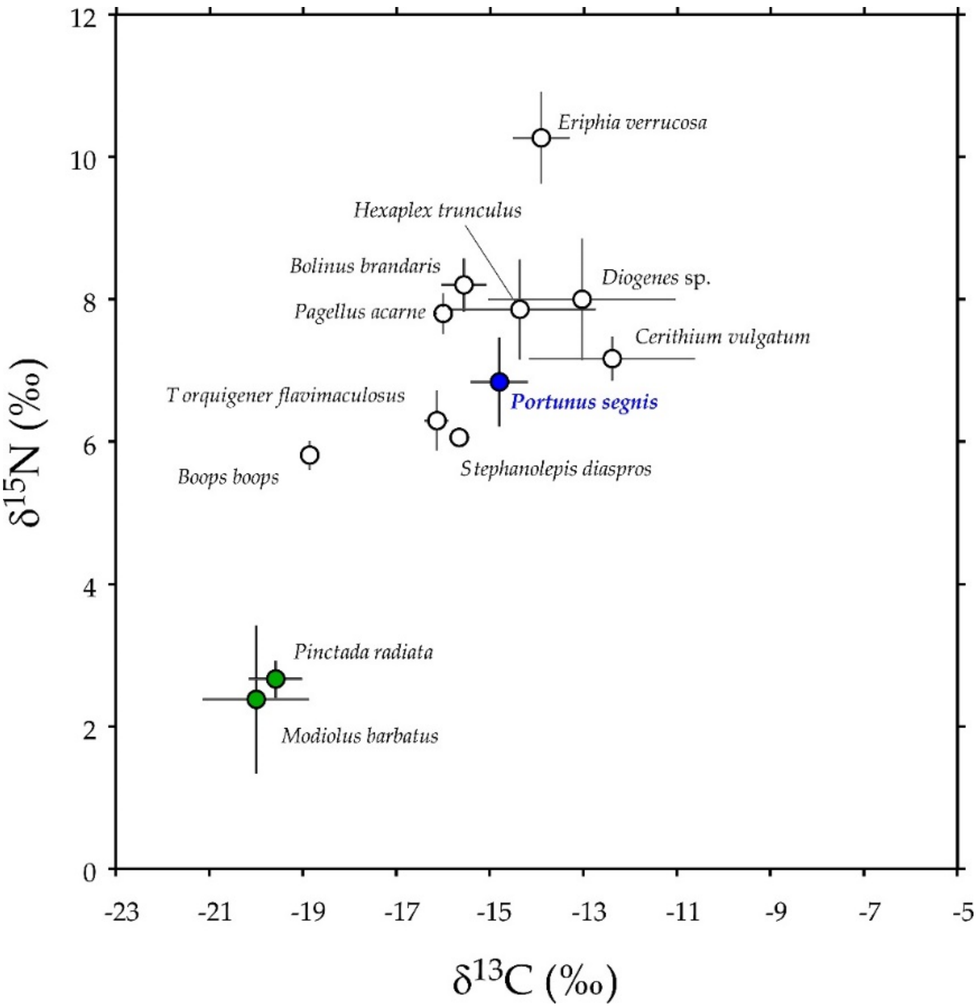
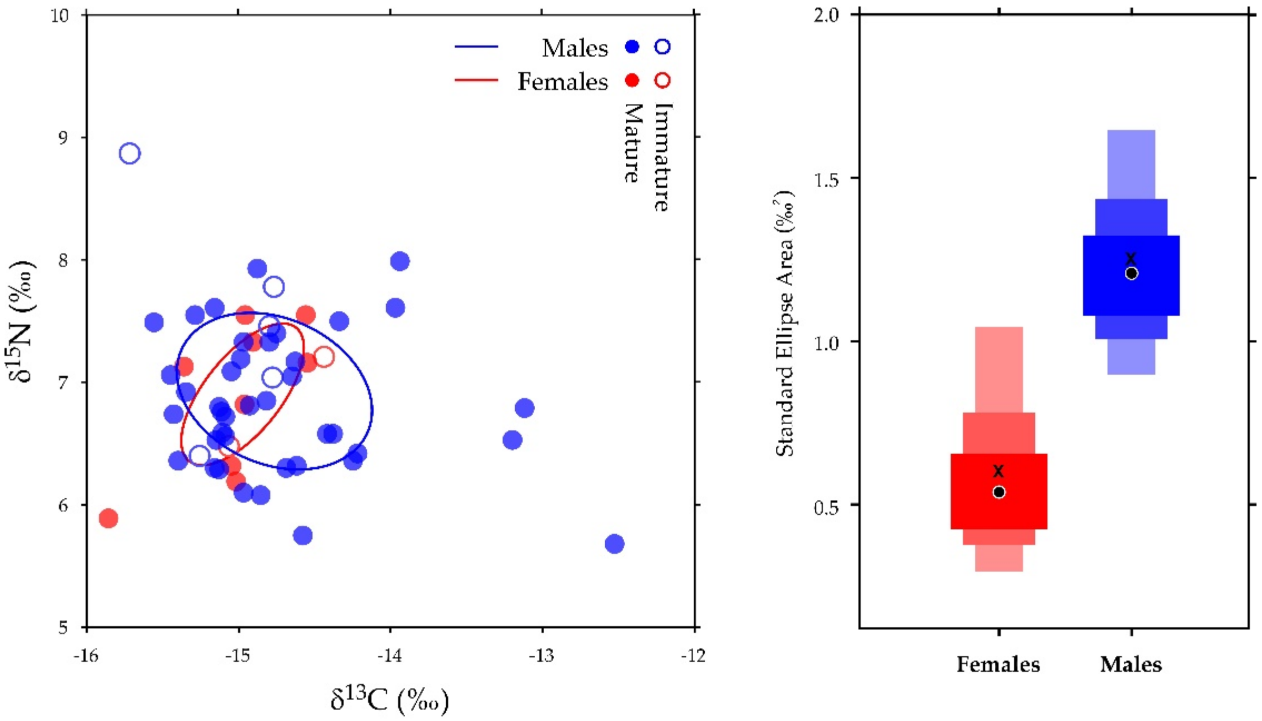


**Figure 4.** Isotopic bi-plot of mean $\delta^{13}$C and $\delta^{15}$N values of invertebrate and fish taxa sampled in Elounda Bay. Bars = 1SD. *Portunus segnis* is highlighted in blue, while bivalves (*Modiolus barbatus* and *Pinctada radiata)* are in green.

**Table 2.** Mean trophic positions of *Portunus segnis* and other invertebrate and fish taxa captured in Elounda Bay. Standard deviations, median, and 95% CI are also reported.

| Species | Mean | SD | 2.5% | 50% | 97.5% |
|---|---|---|---|---|---|
| *Portunus segnis* | 3.85 | 0.31 | 3.36 | 3.81 | 4.58 |
| *Bolinus brandaris* | 3.92 | 0.4 | 3.38 | 3.84 | 5.01 |
| *Boops boops* | 3.42 | 0.3 | 2.89 | 3.39 | 4.11 |
| *Cerithium vulgatum* | 3.89 | 0.3 | 3.4 | 3.86 | 4.58 |
| *Diogenes* sp. | 3.92 | 0.41 | 3.32 | 3.84 | 5.03 |
| *Eriphia verrucosa* | 4.16 | 0.42 | 3.58 | 4.07 | 5.29 |
| *Hexaplex trunculus* | 3.91 | 0.38 | 3.37 | 3.84 | 4.91 |
| *Pagellus acarne* | 3.9 | 0.48 | 3.27 | 3.81 | 5.39 |
| *Stephanolepis diaspros* | 3.49 | 0.32 | 2.96 | 3.45 | 4.29 |
| *Torquigener flavimaculosus* | 3.54 | 0.32 | 3.01 | 3.5 | 4.31 |

*Portunus segnis* was characterized by a considerable inter-individual isotopic variability, with $\delta^{13}$C values ranging between −15.9 and −12.5 and $\delta^{15}$N values between 5.7 and 8.9 (Figure 5). Such variability was not related to sex: PERMANOVA procedures did not highlight any significant isotopic difference between males and females (Pseudo-$F_{1,54}$ = 0.54, $p_{\text{(Monte Carlo)}}$ = 0.58), and, among females, there was no difference between reproductive conditions (Pseudo-$F_{1,9}$ = 0.62, $p_{\text{(Monte Carlo)}}$ = 0.51). Negligible sex-related differences were observed in trophic position values ($p = 0.47$). Only the analysis of isotopic niches indicated that the standard ellipse area (SEAc) of females was significantly smaller (0.61 vs. 1.25 ‰$^2$, $p = 0.02$) and almost completely overlapping with that of the males (Figure 5; 91% overlap).



(a) (b)

**Figure 5.** (**a**) Isotopic bi-plot of *Portunus segnis* $\delta^{13}$C and $\delta^{15}$N values. Circles indicate the sample size-corrected standard ellipse area (SEAc) for females and males; (**b**) Density plots of the posterior Bayesian estimates of the standard ellipse area (SEA$_B$) for females and males. Shaded density plots represent 50, 75, and 95% credible intervals in decreasing order of size, with SEA$_B$ mode indicated by a black circle and SEAc by a black cross.

In contrast with sex, the individual size of *P. segnis* specimens significantly co-varied with their isotopic values. Specifically, while $\delta^{13}$C showed no relationship with the size of *P. segnis* individuals, for mature individuals (>100 mm in carapace widths) a significant positive correlation was observed between size and $\delta^{15}$N values for both males and females (Figure 6), with negligible differences in the slopes of the two regression models (ANCOVA, $F_{1,44} = 0.28$, $p = 0.59$). Conversely, for immature individuals (<100 mm in carapace widths), size co-varied negatively with $\delta^{15}$N (Figure 6).

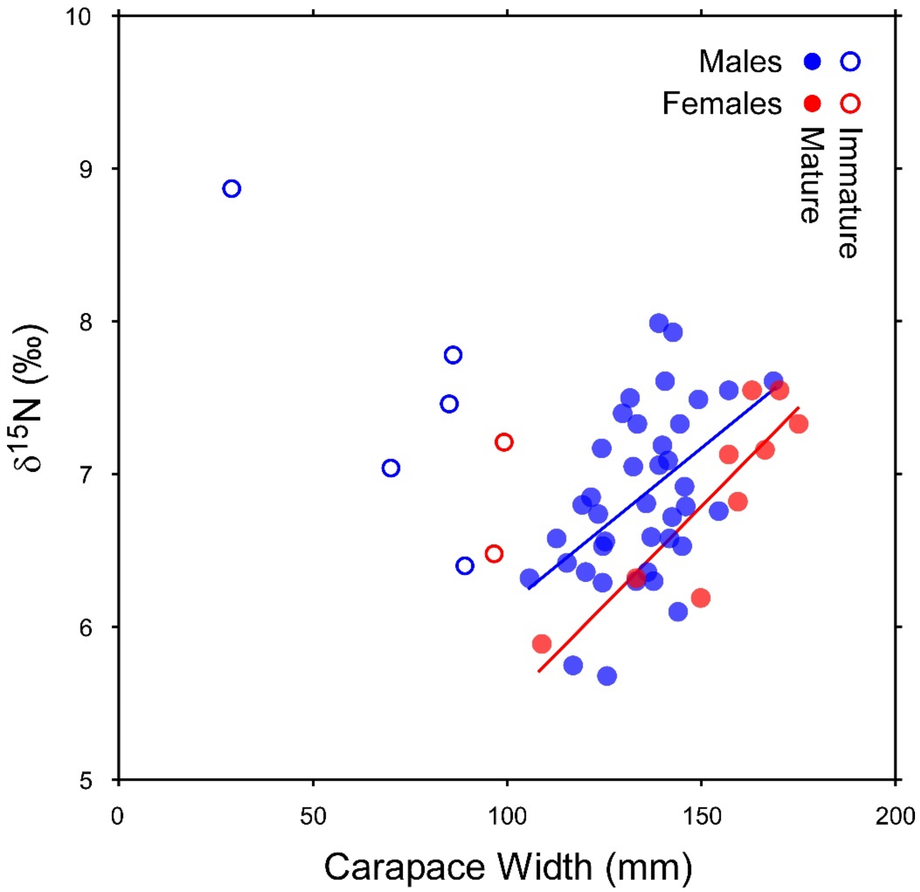


**Figure 6.** Relationships between the carapace width of *Portunus segnis* individuals and their respective $\delta^{15}N$ values. Continuous lines indicate best-fitting linear models for mature males (blue line) and females (red line).

## 4. Discussion

The analyses indicated that *Portunus segnis* in the Elounda Bay food web has a trophic position of 3.8, varying remarkably by almost one trophic level between 3.4 and 4.6 (Table 2). These results confirmed the predatory nature of the species, which is known to feed opportunistically on a wide set of invertebrates (e.g., bivalves, gastropods, crustaceans, polychaetes) and fish taxa [32,67]. In Mediterranean waters, only two studies have previously addressed the trophic habits of *P. segnis*, both performed in the Gulf of Gabès (Tunisia). Specifically, the isotopic investigation by Annabi and colleagues [39] determined a trophic position of 3.3, with individual values ranging between 2.8 and 3.7. Hamida and colleagues [33] performed stomach content analysis (SCA hereafter) on specimens collected in different seasons but did not estimate their trophic position. Here, using a Monte Carlo routine available in TrophLab (a standalone Microsoft Access routine [68]; downloadable from www.fishbase.org) and the qualitative information on ingested food items provided by the aforementioned study, a mean trophic position value of 3.5 was calculated, broadly confirming the isotopic estimations by Annabi and colleagues. Based only on this scant information, it is not possible to conclude if the trophic position of *P. segnis* in Elounda bay and in the Gulf of Gabès differs; indeed, SCA-based estimations from native areas provided in [39] ranges between 3.1 and 3.8, suggesting a considerable spatial variability. Thus, further studies are needed to clarify how local variations in prey identity, abundance, and trophic position, coupled with the feeding opportunism of *P. segnis*, reflect on the trophic habits of the species, and on the role the species plays in recipient food webs.

Noticeably, the trophic position of *P. segnis* was comparable with that of *Pagellus acarne* and of the hermit crab *Diogenes* sp., higher than those characterizing *Boops boops* and the two NIS *Stephanolepis diaspros* and *Torquigener flavimaculosus*, and remarkably lower that the trophic position estimated for the warty crab *Eriphia verrucosa* (Table 2). The high trophic position value that characterized *E. verrucosa* confirmed other observations [69] [TP = 3.97], and was related to the diet of the species focused on predatory gastropods (including *Bolinus brandaris* and *Hexaplex trunculus*, showing in the present study $\delta^{15}$N values higher or comparable with that of *P. segnis*), hermit crabs, and other brachyurans [70–73]. The trophic position values of fish species were consistent with other SCA-based estimations provided in [74] for *B. boops* (3.52), in [75] for *T. flavimaculosus* (3.47), and in [76] for *S. diaspros* (3.32, estimated using TrophLab on individual food items). Together with the similar values obtained from SIA and SCA analysis in *P. segnis* from the Gulf of Gabès, these values provide a general confirmation of the consistency of the two methodological approaches and of the robustness of the estimations provided in this study.

The negligible differences in isotopic values and trophic position between males and females together with the high overlap (>90%) of the respective isotopic niches generally suggests negligible sex-related differences in feeding habits in *P. segnis*. These findings contrast with the observations of Hamida and colleagues [77], showing a significant sex-related variation in diet, but are consistent with other studies from native areas [67,78]. It is worth mentioning that our observations were isotopic; to provide detailed information on prey choice using SIA imposes the use of mixing models [79] on isotopic data of all the potential prey of a consumer, and this was beyond the scope of the present investigation. On the other hand, SCA provides only a snapshot of the diet of an individual at the moment of collection, while SIA integrates over longer time periods. Further investigations performed using both SIA and SCA are needed to clarify if any sex-related differentiation in trophic habits characterize *P. segnis*; additional studies are also necessary to clarify the effect of reproductive state on the trophic habits of females. Hamida et al. found significant differences between ovigerous and non-ovigerous females, only partially confirming the findings of other studies in the Persian Gulf [67,80]. Here, there were no differences due to the reproductive state of females were observed, yet our results must be taken with cautions given the low sample numerosity (nine females in total, six ovigerous). Noticeably, we observed significant differences in SEAc values, indicating that, independently from the number of specimens analyzed, females have a smaller isotopic niche than males. The occurrence of sex- or reproductive state-related differences in isotopic niche width or other metrics deserves to be addressed in the future, since the issue is unexplored for *P. segnis* as well as for other portunids, while isotopic studies focused on marine brachyurans have provided non-univocal, even contrasting results [15,81–83].

Contrary to sex, the results indicated that the ontogenetic stage and size of adults exerted a significant role in determining the $\delta^{15}$N values of *P. segnis* specimens. Indeed, an evident break was observed between immature and mature individuals, and a size-related shift in adult mature specimens (Figure 6). Noticeably, in the present study immature individuals were all collected using crab traps, while adults were captured by fishermen using gillnets. This ultimately indicates that only the use of different sampling methods may allow for the collection of *P. segnis* specimens that span one order of magnitude in carapace width, and that are fully representative of the whole size range and ontogenetic stages characterizing the population under analysis.

In large-sized crustaceans, gonadal maturation induces a strong metabolic and physiological trade-off between investment in somatic or reproductive tissues reflecting significantly in their trophic habits and literature cited therein [84]. In portunids in particular, juveniles pass through frequent molting stages during growth. Calcium is required for shell formation, therefore, juvenile crabs would prefer shelled mollusks and crustaceans [85,86]. This is confirmed by studies on *P. segnis* from Persian waters and on the congener *P. pelagicus* from the Indian Ocean: significant dietary shifts have been observed between immature juveniles < 90–100 mm and mature adults [32,67,87–89]. For adults, no information on size-related shifts are available for *P. segnis* beside the present study; for *P. pelagicus* significant differences were highlighted among adult size groups, indicating that as crabs increase in size they become more active and efficient in capturing larger and more mobile prey such as fish and decapods, including conspecifics [89]. Even though additional SIA and SCA studies are necessary, in particular focusing on immature stages of the crab, our findings clearly suggest that *P. segnis*, depending on its ontogenetic stage and size once it reaches the adult stage, varies considerably its role within the food web of Elounda Bay. Accordingly, future strategies of commercial exploitation of *P. segnis* should take into consideration the possibility of controlling and mitigating the species' impacts on different components of the native community through the promotion of appropriate sampling gears that target specific life stages and size classes of the population, as preliminarily demonstrated in the present study.

## 5. Conclusions

The present investigation provided original data on the trophic position of the swimming blue crab *P. segnis* in Elounda Bay as compared with other invertebrate and fish taxa occurring in the embayment, further suggesting previously unexplored ontogenetic and size-related variations in the trophic habits of the species. It is worth emphasizing the preliminary nature of the study: analyses were carried out only in late spring and focused on a reduced set of species; as such, they were not necessarily representative of the complexity of the food web characterizing the area. Nonetheless, by adopting a robust SIA-based methodological approach to investigate the trophic ecology of *P. segnis*, the study presented evidence of a number of ecological issues that may be further investigated in the future by more extensive studies.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su142215202/s1, Table S1: "occurrences of *Portunus segnis* in the Mediterranean Sea as of 17 September 2022". References [90–113] are cited in the Supplementary Materials.

**Author Contributions:** Conceptualization, G.M. and S.V.; methodology, G.M., S.V., T.D., G.S., P.K. (Panagiotis Kasapidis), P.K. (Panayota Koulouri) and C.D.; formal analysis, G.M., M.T.G. and R.B.; investigation, G.M., T.D., G.S., P.K.; data curation, G.M. and M.T.G.; writing—original draft preparation, G.M. and C.D.M.; writing—review and editing, all authors; funding acquisition, G.M. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** No ethical issues related with the use of animals in the performed analyses were involved.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data on trophic enrichments factors used in this study are openly available in FigShare at https://doi.org/10.6084/m9.figshare.21206663.v1 accessed on 14 November 2022; while the occurrence data used to produce Figure 1 are included in the Supplementary Materials. Isotopic data are available upon request from the corresponding author. The data are not publicly available due to ongoing comparative analyses.

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## Appendix A. Compilation of *Portunus segnis* Occurrences in the Mediterranean Sea

The general procedure is described in detail in Mancinelli et al. [114] for the Atlantic blue crab *Callinectes sapidus*. In brief, between 16–19 September 2022, the online databases ISI Web of Science and Scopus were searched for publications by a multiple search criterion using the terms "*Portunus segnis*" or "*Portunus pelagicus*" and "swimming blue crab" in conjunction with "Mediterranean". The results were complemented with those obtained from queries on Google Scholar (https://scholar.google.com/ accessed on 14 November 2022) using identical keywords and subsequently saved using the freeware Harzings's Publish or Perish ver. 7.27.284953. Only the studies providing the coordinates of the record location or, alternatively, a univocal indication on a map (in this case, maps were digitized, georeferenced, and the coordinates of the location recorded) were selected. Figures were digitized after a fivefold enlargement and converted to numerical form using the graph capture freeware WebPlotDigitizer (ver. 4.5; https://automeris.io/WebPlotDigitizer/ accessed on 14 November 2022). For those studies where sampling locations had no coordinates but were reported in maps, all contextual information was used to locate the geographic area of the study in Google Earth. Maps were extracted using the freeware GIMP (ver. 2.10.20; https://www.gimp.org/ accessed on 14 November 2022), overlaid to the study area in Google Earth, and adjusted to match the background. Subsequently, the sampling area was georeferenced using place marks; the coordinates of the area were used to estimate the latitude and longitude in decimal degrees of its centroid using the centroid function in the R package *geosphere* [115]. In addition, occurrences of *Portunus segnis* in the Mediterranean Sea were retrieved from the Global Biodiversity Information Facility (GBIF, www.gbif.org accessed on 14 November 2022), the Ocean Biogeographic Information System (OBIS, www.obis.org accessed on 14 November 2022), and the citizen science initiative iNaturalist (https://www.inaturalist.org/ accessed on 14 November 2022). After removing duplicates, the final version of the dataset included 115 unique records of *P. segnis* (Table S1 in the Supplementary Materials).

## Appendix B.

**Table A1.** C:N Ratios (Means ± 1SD, and Min-Max Range) Characterizing the Tissues of the Taxa Collected in the Present Study.

| Phylum | Species | C:N | C:N Range |
|---|---|---|---|
| Arthropoda | *Diogenes* sp. | 5.1 ± 0.6 | 4.4–5.6 |
| | *Eriphia verrucosa* | 3.4 ± 0.1 | 3.3–3.4 |
| | *Portunus segnis* | 3.4 ± 0.2 | 2.5–4.1 |
| Chordata | *Boops boops* | 3.5 ± 0.2 | 3.4–3.7 |
| | *Pagellus acarne* | 3.2 ± 0.1 | 3.1–3.3 |
| | *Stephanolepis diaspros* | 2.9 ± 0.1 | 3–3.1 |
| | *Torquigener flavimaculosus* | 3.1 ± 0.1 | 3.1–3.2 |
| Mollusca | *Bolinus brandaris* | 3.5 ± 0.2 | 3.3–3.6 |
| | *Cerithium vulgatum* | 4.1 ± 0.3 | 3.7–4.2 |
| | *Hexaplex trunculus* | 3.7 ± 0.1 | 3.5–3.8 |
| | *Modiolus barbatus* | 4.9 ± 0.6 | 4.3–5.7 |
| | *Pinctada radiata* | 4.3 ± 0.1 | 4.2–4.4 |

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