



Population Density, Reproduction Cycle and Nutritional Value of *Sphaerechinus granularis* (Echinodermata: Echinoidea) in an Oceanic Insular Ecosystem

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Sea urchins play an important role in island coastal ecosystems as keystone species and as potential resources for harvesting and aquaculture. In Southern Europe, the most valuable and harvested species is the European purple sea urchin *Paracentrotus lividus*, but there is a growing interest to explore other edible species like the blunt sea urchin *Sphaerechinus granularis*. The echinoderms biodiversity of Madeira archipelago was previously described, but their local habitat, population densities and size distribution, important to establish their harvest and aquaculture potential, are poorly known. The present study aimed to evaluate *S. granularis* population in the oligotrophic and narrow insular shelf of Madeira archipelago, mainly regarding its habitat, densities, reproduction season and roe marketable characteristics. *Sphaerechinus granularis* was found in low densities (0.01 ind./m²) from shallow to deep reefs (5–30 m). The highest densities occurred in shallow waters and breakwaters in association to encrusting algae, turf and erect brown macroalgae. Here, the low primary productivity ecosystem influenced the long reproductive season of the local population. This population showed a relatively high gonadosomatic index (maximum GI of 8), high gonad weight (5.00–12.19 g) with high proportion of bright yellow and orange gonads (37.5–60%) between February and November. Additionally, the high level of total carotenoids, the high PUFA (particularly EPA) content, and the ratio n-6/n-3 close to one support the high nutritional value of *S. granularis* roe. The trend of incremental GI between September and November and the increasing content of PUFA in autumn suggests this could be the preferential harvesting season. This information is key to evaluate a sustainable exploitation of this living marine resource.

Keywords: Macaronesia region, gonad yield, gonad color, carotenoids, fatty acids, *Sphaerechinus granularis*, Madeira archipelago

INTRODUCTION

Sea urchins are major components of marine communities including 900 known species (Pearse, 2006). They are keystone species of the shallow coastal ecosystems (Steneck, 2013), playing a pivotal role in the community structure of subtidal bottoms, including seagrass meadows and rocky reefs in the islands of the Macaronesia biogeographic region (Hernández et al., 2008; Sangil et al., 2018). By grazing upon the seafloor, sea urchins are strong interactors and drivers of community structure controlling algal biomass (Pearse, 2006; Steneck, 2013).

Approximately 40 edible species are exploited worldwide for their gonads (or roe), a prized delicacy appreciated in many countries like Japan, Australia, New Zealand, United States, France, Italy and Spain (Hammer et al., 2013; Sun and Chiang, 2015). Globally, it is estimated that approximately 70,000 tons of sea urchins are harvested each year (Andrew et al., 2002; Sun and Chiang, 2015; Stefansson et al., 2017), and several species are over-exploited in many regions of the world (Andrew et al., 2002; González-Irusta et al., 2010). In the Northeast Atlantic coast, the most harvested and the most profitable species is the European purple sea urchin *Paracentrotus lividus* (Lamarck 1816), but other species such as *Strongylocentrotus droebachiensis* (Müller 1776), *Psammechinus miliaris* (Müller 1776) and *Sphaerechinus granularis* (Lamarck 1816) are also consumed (Andrew et al., 2002; Monfort, 2002; Martínez-Pita et al., 2008). This fishery is conducted both by professional and recreational fishermen collecting the sea urchins by hand with or without SCUBA diving gear. Due to the high level of illegal captures it is very difficult to quantify sea urchins' captures in Europe (Pais et al., 2007, 2012; Bertocci et al., 2014). Nevertheless, the available data show that only in Spain approximately 700 tons are captured each year, with a market value of about one and a half million euros (González-Irusta et al., 2010; Fernández-Boán et al., 2012).

The blunt sea urchin *S. granularis* is a regular sea urchin characterized by a test covered by massive and short white or purple spikes with occasional white tips displayed regularly through the spikes (Guillou and Lumingas, 1998). The species is commonly found in the Mediterranean (Vafidis et al., 2020) and in the northeast Atlantic between the English Channel and the Gulf of Guinea (Guillou and Michel, 1993), including Madeira and other archipelagos of the Macaronesia biogeographic region (Alves et al., 2001; Riera et al., 2012; Madeira et al., 2019). *Sphaerechinus granularis* populations are characterized by low densities, although it can occur locally in high densities (e.g., in southern Brittany – Guillou and Michel, 1993, western Mediterranean – Sartoretto and Francour, 1997 and in the Bay of Algiers (Algeria) – Soualili et al., 1999). The species can be found from the intertidal zone to depths up to 130 m (Guillou and Michel, 1993), in both calm and exposed areas (Martínez-Pita et al., 2008) and is frequently associated with rhodoliths beds (aggregations of free-living coralline algae that cover extensive benthic areas of subtidal environments worldwide) (Guillou et al., 2002), although it may occur in sandy bottoms (Gaspar et al., 2001).

Sphaerechinus granularis is a grazer consuming mostly encrusting coralline algae (Sartoretto and Francour, 1997).

However, in meadows of *Posidonia oceanica*, it consumes dead leaves (and their algal epiphytes), rhizomes and roots when they are accessible (Verlaque, 1981).

Studies on *S. granularis* growth conducted in the Bay of Brest (France) showed that this species has a fast growth rate during the first years of life (Lumingas and Guillou, 1994; Jordana et al., 1997). The longevity estimations conducted by Guillou and Michel (1993) showed that the *S. granularis* population of Brittany can attain 5 years old, with higher growth rates and shorter longevity when compared with *P. lividus*. *Sphaerechinus granularis* urchins eight to 10 months old can attain 15 mm of test diameter (TD) and 4 years old urchins have a mean TD of 100 mm (Guillou and Michel, 1993), whereas *P. lividus* only attains 50 mm TD in Northeast Atlantic coast and 45–65 mm in culture (Grosjean et al., 1998; Boudouresque and Verlaque, 2020).

Several studies showed that, concomitantly with other echinoids, the reproductive cycle of *S. granularis* follows a latitudinal gradient. The population inhabiting the English Channel presents a major spawning event in the early spring (Guillou and Michel, 1993), whereas in southern of Spain (western Mediterranean), the local population showed a single spawning event in summer months (Martínez-Pita et al., 2008). The recent study by Vafidis et al. (2020) corroborates the single annual reproductive season, with the *S. granularis* population of Aegean Sea (eastern Mediterranean) spawning during spring.

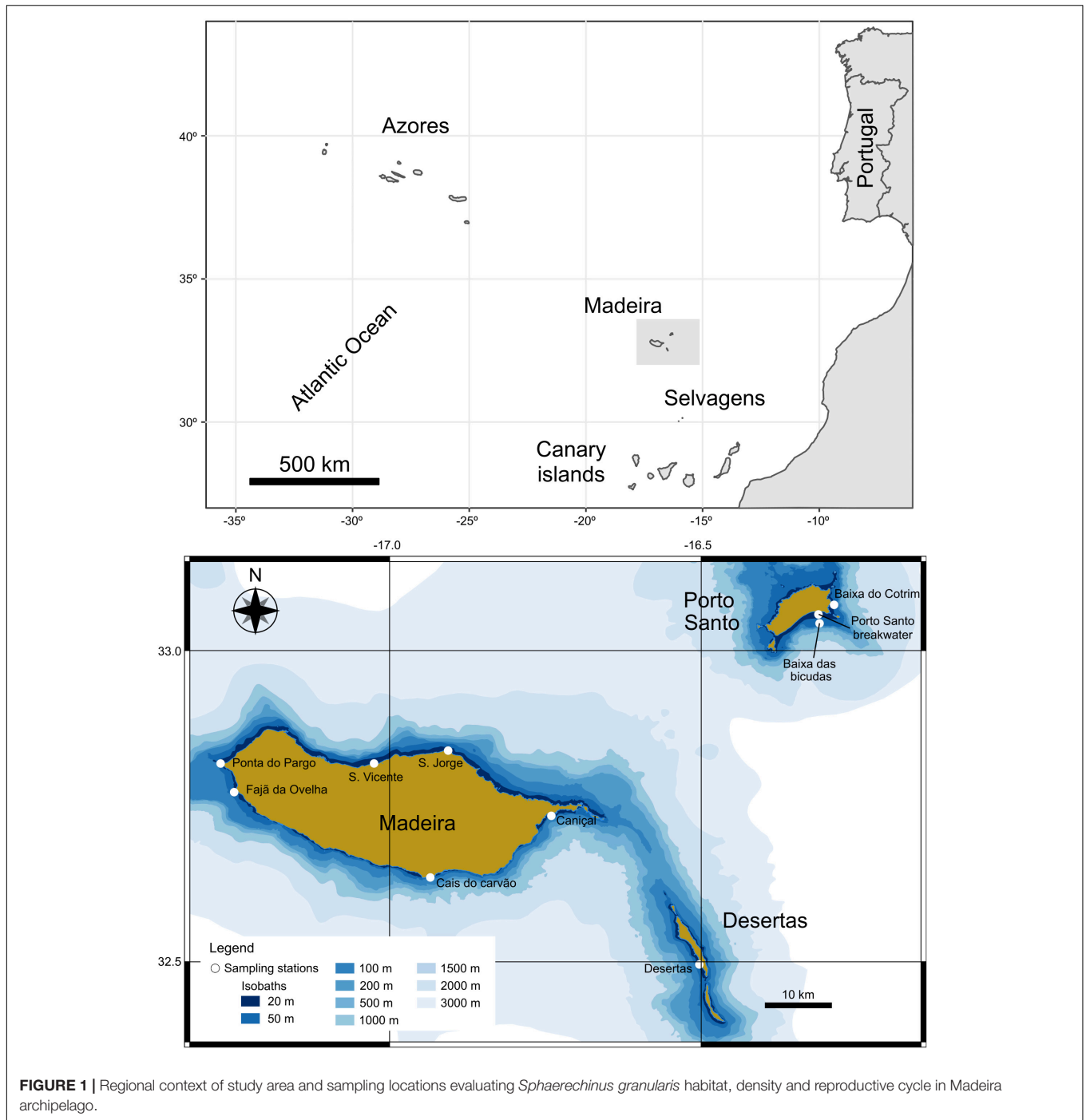
The unsustainable harvesting pressure on *P. lividus* in several areas of the Northeast Atlantic and Mediterranean (Farina et al., 2020), associated with its slow growth and low gonad yield have been driving the research for alternative species to supply the market demand on sea urchin roe and aquaculture production. *Sphaerechinus granularis* has been identified as an emergent species in Brittany (Guillou and Michel, 1993) and Mediterranean regions (Soualili et al., 1999; Martínez-Pita et al., 2010). However, the lack of knowledge on the biology (e.g., life cycle) and ecology of *S. granularis* has been referred to several regions (Guillou and Michel, 1993) and in Madeira archipelago that is no exception. There are no official records about the species harvesting, but anecdotal evidence indicates it is captured as an alternative to *P. lividus*.

The aim of the present work was to characterize the habitat, population density, size structure and reproductive biology of *S. granularis* under the oligotrophic, relatively stable seawater temperature, and narrow insular shelf ecosystem of Madeira archipelago. Additionally, it was conducted a first assessment of gonads' nutritional quality and roe color characteristics along the year. This information will be useful to identify a potential harvesting season and to a for a future analysis of environmental and economic sustainability of this low-mobility resource in the archipelago.

MATERIALS AND METHODS

Study Area

The Madeira archipelago is located in the eastern North Atlantic Ocean about 700 km west of Casablanca (Morocco) and comprises the islands of Madeira (742 km²), Porto Santo



(43 km²), Desertas (14 km²) and Selvagens (3 km²) (Figure 1). The archipelago is surrounded by oligotrophic water masses (Campuzano et al., 2010), and stable seawater temperature year-round (average ranges from 18 to 24°C) (Caldeira et al., 2002). The major coastal marine environments are rocky shores characterized by pebble beaches, basalt platforms and areas armored by artificial breakwaters (Ribeiro, 2008). Madeira's intertidal and subtidal domain comprises a rocky substrate (boulders, vertical walls, platforms, and outcroppings) down to

depths of 15–20 m and sandy bottoms, which form a major part of this domain. The soft bottoms are formed by sediments ranging from coarse gravel to medium silt according to the classification of Wentworth (1922) (Instituto Hidrográfico, 2003, 2007, 2008).

To evaluate where *S. granularis* occur and estimate its abundance and density within the Madeira archipelago, several locations from Madeira, Desertas and Porto Santo islands were surveyed with SCUBA diving (Figure 1). These locations comprised the typical rocky reefs made of boulders (medium

to big boulders), rock platforms, outcrops and rhodoliths beds. Only two locations at the South coast of Madeira (Cais do Carvão and Caniçal 1) were monitored seasonally (winter, spring, summer, and autumn) due to their easy access and protection from the prevailing weather conditions. Four locations—Caniçal 2, breakwater of port of Porto Santo Island (hereafter Porto Santo breakwater), Baixa das Bicudas and Baixa do Cotrim—were surveyed in two seasons (summer and autumn), whereas the remaining were only surveyed in summer, due to logistical limitations. For each location where *S. granularis* was detected, the diversity indices were calculated using Hill numbers according to the framework described by Jost (2006).

Density Data Collection

During the year 2017, surveys to estimate the density of *S. granularis* across the entire study area were conducted using underwater visual census (UVC's) along strip transects of 25 m × 2 m, inside which all sea urchin specimens, and macrofaunal organisms that could be discerned as individuals (starfish, crabs, etc.) were counted. Moreover, to characterize the general habitat of the species in terms of sessile cover (macroalgae and macrofauna) 100 photographs were taken at each location, which were later analyzed to describe the habitat. All conspicuous sessile species observed were registered from imagery analysis and an abundance category approach used, with each species being classified according to DAFOR scale (D = Dominant: 51–100%; A = Abundant: 31–50%; F = Frequent: 16–30%; O = Occasional: 6–15%; R = Rare < 5%) (Tansley, 1993).

Biological Data Collection

Samples of sea urchins ($n = 10$) were collected monthly by SCUBA divers from subtidal rocky reef bottoms of Cais do Carvão (Figure 1). This sampling effort was not possible to be replicated in other locations during the study period, mostly due to logistical and team constraints. Therefore, additional samples (usually $n = 10$) were collected in other two locations, namely at Caniçal in every season and at Porto Santo in two seasons (summer and autumn). The specimens collected were transported in a cooler filled with seawater to the Funchal Marine Biology Station, where they were weighed to the nearest 0.01 g (body weight—BW) and the test diameter (TD) measured to the nearest 0.05 mm with a caliper. The individuals were then dissected by a circular incision around the peristomal membrane, and the gonads removed and weighed to the nearest 0.1 mg (gonad total weight—GTW). The gonad color was evaluated according to a semi-quantitative scale of four grades (Pearce et al., 2002): 1- bright yellow or orange; 2- yellow pale or orange (mustard); 3- brownish yellow, brownish orange, brownish red; 4- any other color. One gonad was fixed in 4% formalin and stored in ethanol 70% for histologic analysis of the maturation status and the remaining four were stored at -20°C to further biochemical analysis.

Histologic Analysis

The gonads of the urchins collected in Cais do Carvão, Caniçal and Porto Santo breakwater were dehydrated in baths of increasing ethanol concentration and xylol, embedded in paraffin

and sectioned at 5 μm for histologic analysis. The sections were stained with haematoxylin periodic acid Schiff reagent and analyzed under a light microscope (Leica® DM 2000 LED) equipped with a Leica® MC170 5MP HD Camera and the LAS V4.4.0 software at magnification 50 × and 100 ×. Gonad microscopic maturity stages were assessed using the four stages scale defined by Walker et al. (2013) (Maturity scale description and Figure 1 in Supplementary Material).

Biological Indices

The sex ratio was determined by Equation 1, where N_{males} and $N_{females}$ are the number of *S. granularis* males and females collected, respectively:

$$\text{Sex ratio} = \frac{N_{males}}{N_{males} + N_{females}} \quad (1)$$

The gonad index (GI) was determined individually as described in Equation 2. The GI monthly averages were determined by sex and by sampling site.

$$\text{GI} = 100 \times \frac{\text{GTW}}{\text{BW}} \quad (2)$$

The maturity index (MI) was determined monthly as the mean of maturity stages frequencies through Equation 3, where n_{stg} is the number of sea urchins assigned to each maturity stage at each month, stg is the numerical value assigned to each maturity stage (1—stage I; 2—stage II; 3—stage III and 4—stage IV), N_{month} is the number of sea urchins sampled monthly.

$$\text{MI}_{\text{month}} = \frac{\sum n_{stg} \times stg}{N_{\text{month}}} \quad (3)$$

Chemical Analysis

The gonad samples were freeze-dried, pooled by sex and by season, ground, homogenized and stored at -20°C for further analyses. Prior to chemical analysis, residual moisture was determined using a Gibertini-Eurotherm dry weight balance, and the results were expressed in percentage of dry mater (% DM). All chemical analyses were conducted at least in duplicates.

Crude protein was assessed by the total nitrogen content obtained with an elemental analyzer TruSpec 630-200-200. The total nitrogen content was converted to total protein equivalents using the following relationship: percentage crude protein = percentage nitrogen × 6.25 (AOAC, 1990).

Total lipid content was determined gravimetrically. To 100 mg of freeze-dried sample was added 3 mL of a chloroform/methanol (1:2, v/v) mixture, containing 0.01% (w/v) BHT (butylated hydroxytoluene), and 400 μL of a saturated solution of potassium chloride. Then, 2 mL of chloroform and 2 mL of distilled water were added, and the mixture placed under agitation for 20 min. The organic phase was collected, and the solvent removed using a rotary evaporator at 40°C .

The fatty acids (FA) profile was analyzed by converting the FA in methyl esters (FAME) by adding methanol/acetyl chloride (19:1, v/v), during 1 h at 80°C , and adding *n*-heptane as extraction solvent (Fernandes et al., 2016). FAME were

analyzed by gas chromatography-mass spectrometry following the conditions described in Nogueira et al. (2017) and identified by comparing the chromatographic retention times and mass spectra fragmentation with commercial standards (Supelco 37 component FAMES and mix bacterial acid methyl esters CP mix) and by consulting the NIST spectral library. The identified FA were quantified by comparison with the retention area of Nonadecanoic acid (C19:0, 4 mg/g DM) used as internal standard due to its absence in the samples. Each sample was injected in duplicate, and the replicates had a variation lower than 5%.

Total carotenoid extraction in urchins gonads followed Rocha et al. (2019), where freeze-dried gonads (c.a. 0.5 g) were extracted with acetone (5:1, v/w). After centrifugation at $3,500 \times G$ for 10 min, at 8°C, the supernatant was collected.

The extraction procedure was repeated twice to obtain a colorless supernatant. The resulting supernatant was evaporated to dryness under a constant stream of nitrogen, re-dissolved in *n*-hexane and filtered with a 0.2 µm syringe filter for further analysis. Total carotenoid content was determined by UV-Visible spectrophotometry (Shimadzu UV-1603, Japan), using the absorption maximum (471–477 nm) in *n*-hexane and the extinction coefficient of 2,500 L/mol.cm.

Statistical Analysis

Mean density of the species *S. granularis* was calculated for each location surveyed. Differences among locations were tested between the five locations in which the species was present. Because the obtained data did not comply with the

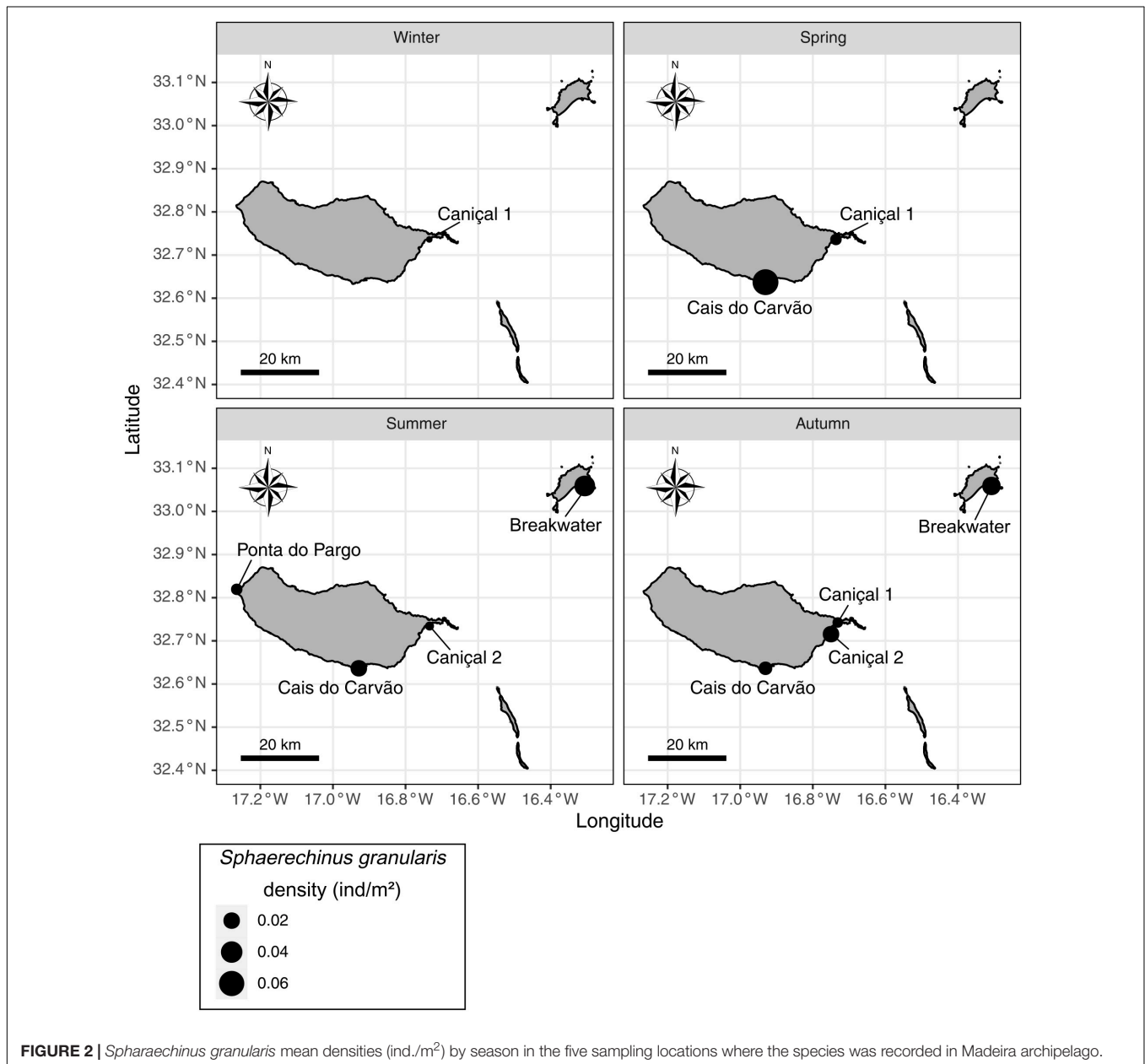


FIGURE 2 | *Sphaerechinus granularis* mean densities (ind./m²) by season in the five sampling locations where the species was recorded in Madeira archipelago.

assumptions of normality and homogeneity of variances, the non-parametric Kruskal–Wallis test was selected. Whenever significant differences were found, a pairwise Wilcoxon rank sum test was performed between group levels with corrections for multiple testing.

A two-way ANOVA was applied to test for differences in species abundance among seasons (four levels) and location (two levels: Cais do Carvão and Caniçal 1). As previously, violation of residuals normality occurred and therefore the Aligned Rank Transform for non-parametric factorial analyses (Wobbrock et al., 2011) was selected.

The biometric data used to determine the biological indices and reproductive biology was analyzed independently of sampling site. A two-way ANOVA was performed to assess the effect of the factors season and sex on the biometric parameters (TD and BW), reproductive variables (GTW, GI and MI), and protein (TP), lipid (TL), carotenoid (TC) and FA profile of sea urchin gonads. If ANOVA detected significant effects on single factors and/or factors' interaction, individual means were compared using *post hoc* pairwise Tukey's test. Whenever the assumptions of normality and homogeneity failed, a non-parametric factorial analysis was conducted using the aligned rank transform (ART) method. Whenever ART analysis showed significant differences, individual means were compared using the pairwise Wilcoxon test with a Bonferroni correction to the *p*-value. Differences in the maturity stages and color index distributions were analyzed by chi-square (χ^2) test of independence. In all cases, significant differences were considered when $p < 0.05$. All statistical tests were performed in R environment (R Core Team, 2020).

RESULTS

Sphaerechinus granularis Densities

The sea urchin *S. granularis* was found in five out of the eleven locations surveyed in this study: Cais do Carvão (five transects by season), Caniçal 1 (five transects by season, except in summer with only two), Caniçal 2 (18 transects in summer), Ponta do Pargo (six transects in summer) and Porto Santo breakwater (five transects in summer and six in autumn). In all these sampling locations, *S. granularis* occurred in low densities, with the maximum number of individuals (0.16 ind./m²) recorded at Cais do Carvão during spring (Figure 2). Regarding mean densities, the highest value was also recorded at Cais do Carvão in spring (0.064 ± 0.071 ind./m²). Porto Santo breakwater showed the second and the third higher density values in summer and autumn, respectively (Figure 2). The lowest values were recorded at Caniçal 1 (0 ind./m²) and Ponta do Pargo (0.007 ± 0.010 ind./m²) both in summer (Figure 2). Significant differences were observed in mean densities between locations ($H_4 = 1.45$, $p = 0.014$). Pairwise comparisons showed significant differences only between Porto Santo breakwater and Caniçal 1 and between Porto Santo breakwater and Caniçal 2 ($p = 0.022$). The results from the two-way ANOVA indicated significant differences on the species densities between locations ($F_1 = 8.64$, $p = 0.0064$), but no significant differences between seasons

($F_3 = 1.42$, $p = 0.26$), nor for the factors interaction ($F_{1,3} = 2.75$, $p = 0.06$).

Habitat Characterization and Diversity Indices

A list of the dominant sessile macrobenthic flora and fauna is presented in Table 1. The substrate in the five locations where the sea urchin was found was mostly rocky reefs, formed by rock boulders (generally > 1 m)—Cais do Carvão, Caniçal 1, and Porto Santo breakwater, but also rock platform—Ponta do Pargo and rhodoliths beds—Caniçal 2. The subtidal reefs where *S. granularis* inhabits (5–30 m depth) are generally characterized by encrusting algal communities and macroalgae mainly formed by turf and erect algae (e.g., *Asparagopsis taxiformis*, *Dasycladus vermicularis*, *Dictyota* sp., *Stypopodium zonale*), whereas sessile macrofauna cover is mostly composed by sponges [e.g., *Batzella inops*, *Callyspongia* (*Callyspongia*) *burtoni*, *Cliona* sp., *Ircinia* sp., *Phorbas fictitius*], hydrozoans (*Pennaria disticha* and *Macrorhynchia philippina*) and serpulids. Intermediate and deeper rocks (10–30 m) are mainly covered by sessile invertebrates, primarily barnacles and bryozoans as *Reptadonella violacea* and *Schizoporella dunkeri* and sponges (e.g., *Aplysina aerophoba*, *B. inops*, *Chondrosia reniformes*, *P. fictius*, *Petrosia ficiformes*). Algae cover is mostly crustose coralline algae (*Lithophyllum* sp.) or rocks deprived of algae—barren rocks—where the long spine sea urchin *Diadema africanum* is usually abundant.

Diversity indices for the mobile macrofauna associated to the *S. granularis* habitat at the five locations are presented in Table 2. The total species richness ranged between 10 and 16, the lowest value being recorded at Caniçal 1 and the highest at Cais do Carvão. For Shannon's diversity index the highest value was recorded at Porto Santo breakwater and the minimum at Caniçal 1 (Table 2) and the same happening for the Simpson diversity index. Many of the macrofauna species detected inside locations and among locations showed low abundance and frequency of occurrence (e.g., the gastropods *Bittium* sp., *Bursa* sp., *Felimare picta*, *Umbraculum umbraculum*, and the echinoderms *Ophidiaster ophidianus*, *Ophioderma longicauda*), whereas the most abundant and frequently found species for most of the locations was the sea urchin *D. africanum*. nr most of the subtidal effect, besides *S. granularis*, three more species of sea urchins were registered at these locations, namely *Arbacia lixula*, *D. africanum* and *P. lividus* (mean densities by season in Supplementary Table 1). The latter species was also recorded with low densities within most of the reefs surveyed, whereas *A. lixula* registered a total mean density three times higher than *S. granularis* in Porto Santo breakwater and Cais do Carvão reefs. *D. africanum* presented much higher densities for most of the locations, with a maximum of 5.6 ind./m² recorded at Baixa das bicudas in autumn, a location where *S. granularis* was not detected.

Size Distribution and Reproduction Cycle

The TD and BW distributions of the collect urchins ranged between 40 and 90 mm (Figure 3A) and 40 g and 289 g (Figure 3B), respectively, indicate that only the adult component

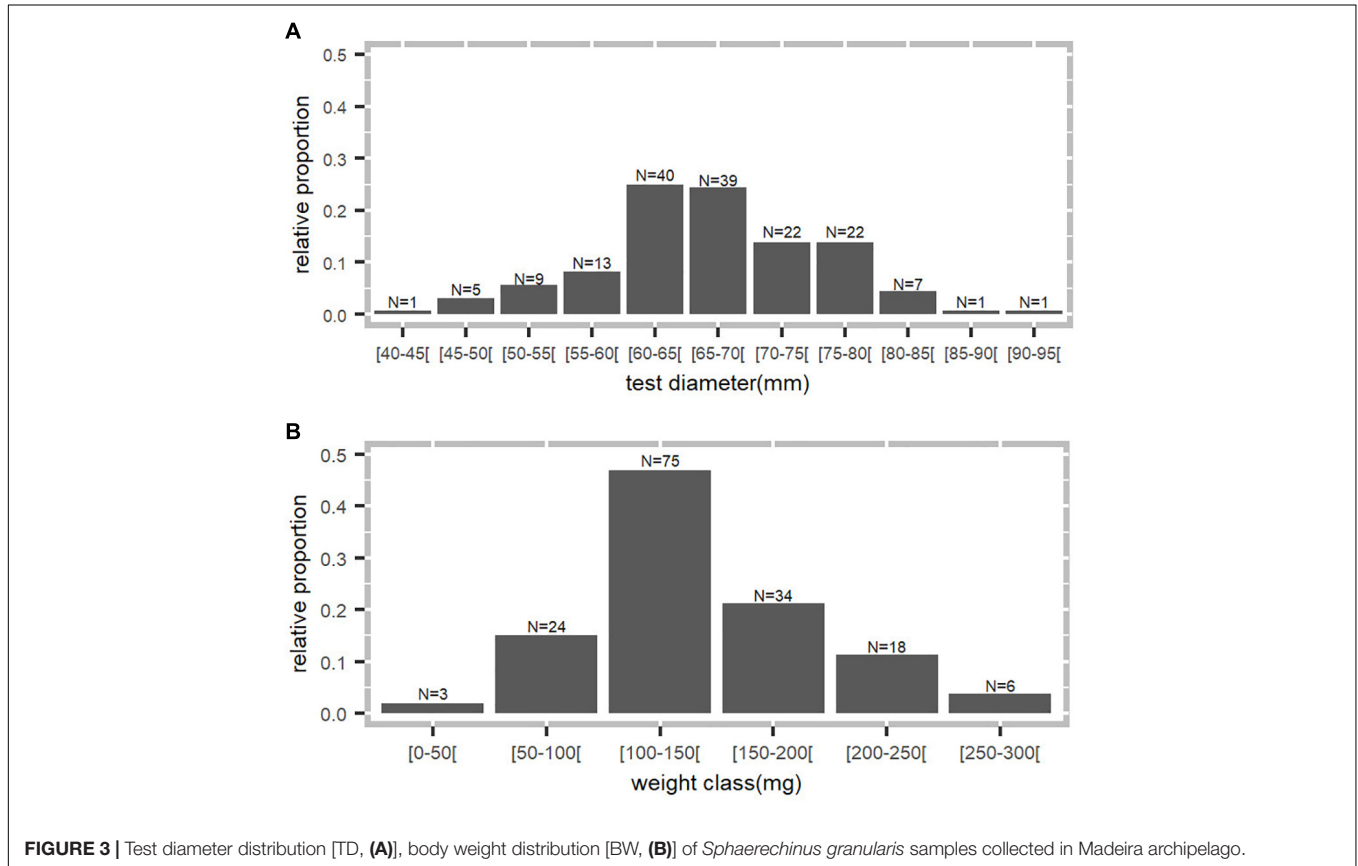
TABLE 1 | List of the main sessile macrobenthic fauna and flora associated to the five locations where *Sphaerechinus granularis* was observed in Madeira archipelago.

Depth interval (m)	5–12	5–12	15–25	5–10	20–25
Locations	Cais do Carvão	Canical 1	Canical 2	Porto Santo breakwater	Ponta Pargo
Macroalgae					
<i>Asparagopsis taxiformis</i> (Delile) Trevisan, 1845	A	F	O	F	F
<i>Caulerpa webbiana</i> Montagne, 1837	O		O	O	O
<i>Colpomenia sinuosa</i> (Mertens ex Roth) Derbès and Solier, 1851	O				
<i>Codium</i> sp.	O				
<i>Dasycladus vermicularis</i> (Scopoli) Krasser, 1898	F				
<i>Dictyota</i> sp.	A	F	O	O	
<i>Halopteris filicina</i> (Grateloup) Kützing, 1843	F			F	
<i>Jania</i> sp.	O				
<i>Lithophyllum</i> sp.	A	F	F	A	O
<i>Lobophora</i> sp.	O	O	O		F
<i>Liagora</i> sp.	O			F	
<i>Padina pavonica</i> (Linnaeus) Thivy, 1960	O			O	F
<i>Peyssonelia</i> sp.	A			A	O
<i>Sargassum</i> sp.	F			F	O
<i>Styopodium zonale</i> (J.V.L. amouroux) Papenfuss, 1940	F				
<i>Ulva</i> sp.	O				
Sponge					
<i>Aplysina aerophoba</i> (Nardo, 1833)	O	O	O	F	
<i>Aplysilla sulfurea</i> Schulze 1878	O				
<i>Batzella inops</i> (Topsent, 1891)	A	F	F	F	
<i>Callyspongia (Callyspongia) burtoni</i> Van Soest and Hooper, 2020	F		F		
<i>Chalinula parasimulans</i> (Lévi, 1959)	O	O	O		
<i>Chondrosia reniformis</i> Nardo, 1847	O	F			
<i>Cliona</i> spp.	F				
<i>Ircinia</i> sp.	O			F	
<i>Phorbas fictitius</i> (Bowerbank, 1866)	A	O	O	F	
<i>Petrosia (petrosia) ficiformis</i> (Poiret, 1789)	O		O		
<i>n.i. 1</i>	O		O		
<i>n.i. 2</i>	F		O		
<i>n.i. 3</i>		O			
Bryozoa					
<i>Favosipora purpurea</i> , Souto et al., 2015		O			
<i>Reptadeonella violacea</i> (Johnston, 1847)	O	F	F	F	A
<i>Schizobrachiella sanguinea</i> (Norman, 1868)	O				
<i>Rhynchozoon papuliferum</i> , Souto et al., 2015	O				
<i>Schizoporella dunkeri</i> (Reuss, 1848)	F	F	F	F	F
<i>Scrupocellaria</i> sp.	O				
Anthozoa					
<i>Madracis</i> sp.			O		
Hydrozoa					
<i>Antennella</i> sp.	F	F	O	O	O
<i>Aglaophenia pluma</i> (Linnaeus, 1758)				A	
<i>Macrorhynchia philippina</i> Kirchenpauer, 1872	F				
<i>Pennaria disticha</i> Goldfuss, 1820	O	O		O	
<i>n.i. 1</i>	O		O		
Crustacea					
<i>Balanus trigonus</i> Darwin, 1854	A	A	F	F	A
Gastropoda					
<i>Vermetus</i> sp.	A	A	F	O	F
Polychaets					
<i>Serpulidae</i> Rafinesque, 1815	A	A	F	F	O

A, abundant; F, frequent; O, occasional.

TABLE 2 | Diversity indices in each of the five locations where *Sphaerechinus granularis* was observed during the present study in Madeira archipelago.

Locations	Species richness	Shannon diversity	Simpson diversity	Shannon's evenness	Simpson's evenness
Cais do Carvão	16	2.05	1.35	0.13	0.08
Canical 1	10	1.26	1.09	0.13	0.11
Porto Santo breakwater	13	6.30	4.94	0.49	0.38
Canical 2	15	2.66	1.79	0.18	0.12
Ponta do Pargo	13	2.07	1.40	0.16	0.11



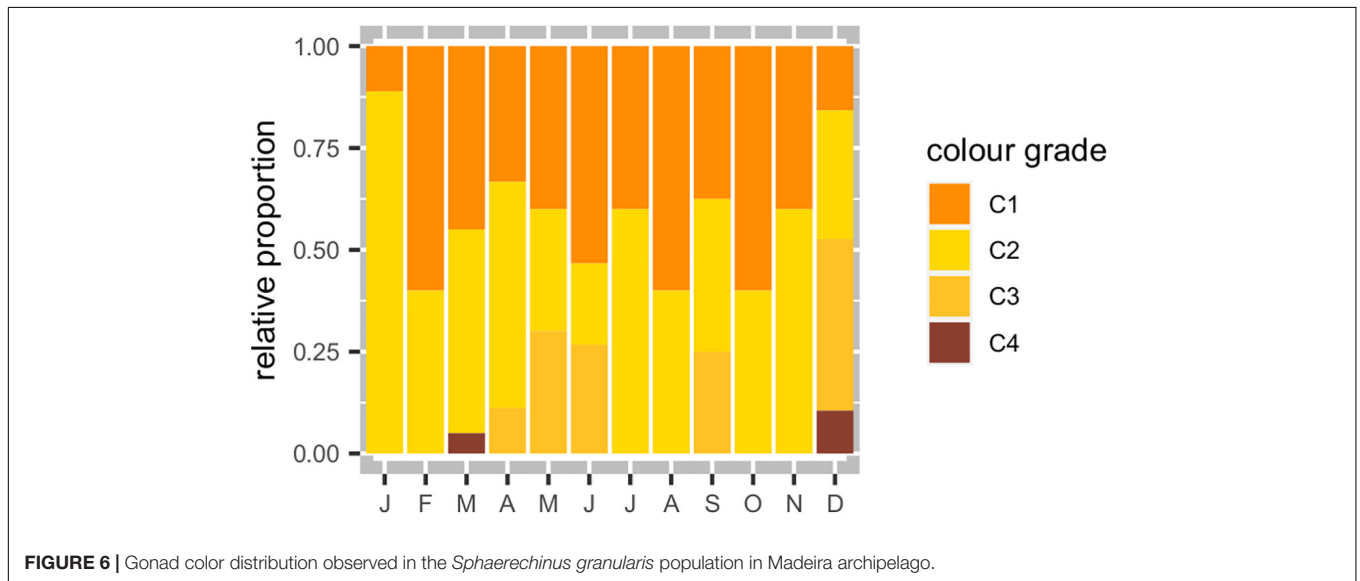
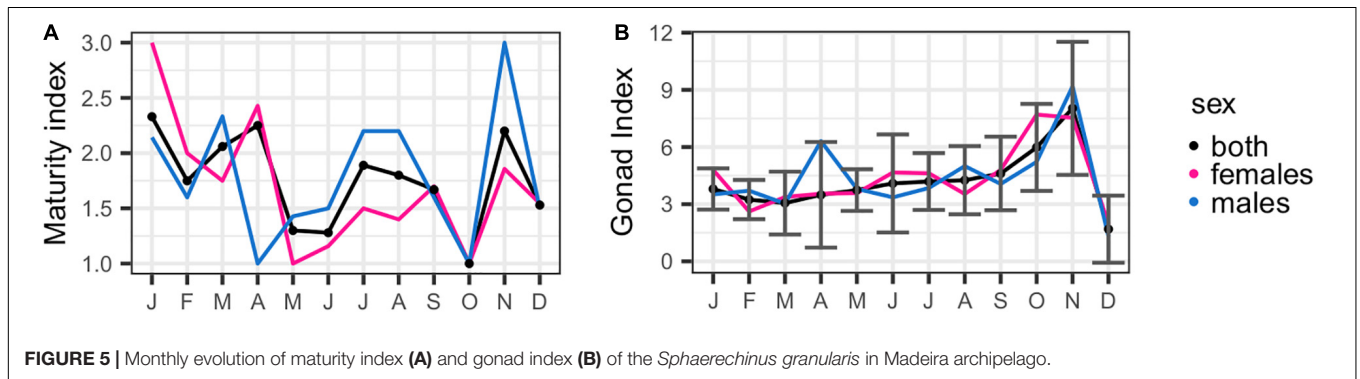
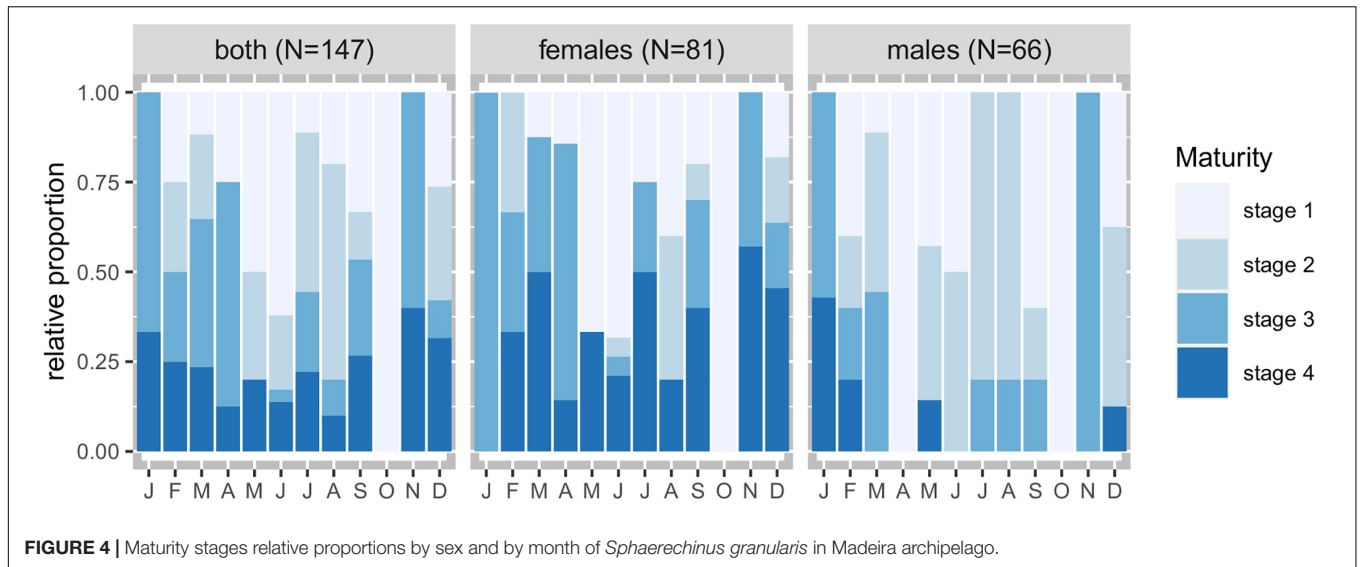
of the population was analyzed. The urchins mean TD was 65.82 ± 9.73 mm and mean BW 138.89 ± 52.56 g. It was observed a significant effect of season in the two parameters, with the urchins collected during spring being heavier ($BW = 156.38 \pm 54.83$, $N = 47$) and larger ($TD = 68.85 \pm 8.89$ mm) than those collected in winter ($BW = 121.65 \pm 52.37$ g, $TD = 62.39 \pm 9.34$ mm, $N = 30$) and in summer ($BW = 122.72 \pm 33.51$, $TD = 64.50 \pm 5.93$, $N = 34$). The mean BW and TD of the urchins collected in autumn was 149.94 ± 47.54 g and 67.41 ± 7.87 mm ($N = 36$). The two-way ANOVA output on the effect of season and sea urchin sex in the biometric parameters and biological indices is presented in **Supplementary Table 2**.

Mature urchins were identified all year around, except for October (**Figure 4**). The proportion of immature and maturing individuals increased between May and September with a strong association between the immature individuals and spring ($\chi^2 = 25.69$, $p = 0.002$). Mature females appeared in similar

proportions across all seasons, whereas mature males were strongly associated with winter ($\chi^2 = 20.95$, $p = 0.013$). The evolution of the maturity index through the year (**Figure 5A**) indicated the occurrence of two maturity peaks, one in April and another in November.

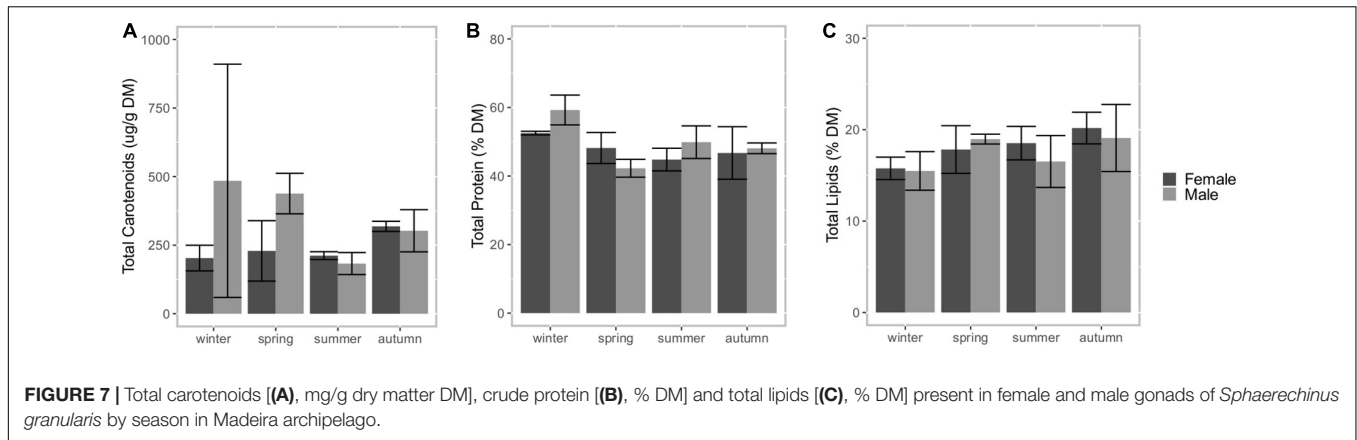
Gonad Size, Color, and Nutritional Value

The average GI (**Figure 5B**) was 3.84 ± 2.54 and increased steadily from March to November, when attained its maximum (8.03 ± 3.49 , $N = 10$), in synchrony with the maturation cycle. The GI decreased sharply between November and December, when it reached a minimum (1.69 ± 1.76 , $N = 19$). Most of the sea urchins' gonads were classified with a color grade between 1 and 2 (**Figure 6**). Gonad color distribution was influenced by season of capture ($\chi^2 = 19.02$, $p = 0.025$), with color grades 3 and 4 associated with autumn and winter. No statistically significant differences associated to the gonad sex were identified in the color grade distribution.



The gonads of *S. granularis* presented a relatively high content of total carotenoids (average $296.36 \pm 213.24 \mu\text{g/g DM}$), significantly affected by the interaction of factors season and sex

($F = 4.291, p = 0.012$). The total carotenoid content was lower in summer (average $195.41 \pm 34.15 \mu\text{g/g DM}$) with identical concentration in both males and females (Figure 7A), followed



by autumn (average $310.66 \pm 52.41 \mu\text{g/g DM}$). Differences in the total content of carotenoids between sexes were registered in spring and winter, being higher in females (Figure 7A).

The total protein content of *S. granularis* gonads was, on average, 49.76% DM. The season ($F = 12.51$, $p = 0.001$) and its interaction with sea urchin sex ($F = 4.472$, $p = 0.009$) significantly affected the total protein content of gonads. During winter, males were richer in protein than females, and females presented a significantly higher protein content in spring and summer. In autumn no significant differences were recorded between sexes, with the mean protein content of the gonads being $47.40 \pm 5.18\%$ DM (Figure 7B).

The mean lipidic content of *S. granularis* gonads was $17.51 \pm 2.64\%$ DM. The lipidic content was significantly affected by season ($F = 0.92$, $p = 0.003$) but not affected by sex ($F = 1.87$, $p = 0.179$) nor by factors' interaction ($F = 0.92$, $p = 0.442$). Sea urchin gonads collected during spring and autumn presented higher lipidic content (spring average $18.29 \pm 2.06\%$ DM; autumn average $19.63 \pm 2.72\%$ DM) followed by the gonads collected in summer ($17.38 \pm 2.58\%$) (Figure 7C).

Both saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) were equally abundant in the gonads of *S. granularis* (Table 3), with their content being mainly affected by season (SFA: $F = 3.44$, $p = 0.030$; PUFA: $F = 5.98$, $p = 0.002$; Table 3). The most abundant SFA was the palmitic acid (C16:0), whose content was unaffected by season and sex. The most abundant PUFA in the gonads was the eicosapentaenoic acid (EPA, C20:5n3), which was affected by season ($F = 4.69$, $p = 0.007$; Table 3) and the highest content observed in the autumn (Table 3). The second most abundant PUFA was the arachidonic acid (ARA, C20:4n6) and its content was independent of season and sex (Table 3). The most abundant monounsaturated fatty acids (MUFA) were the oleic acid (C18:1n9) and the gondoic acid (C20:1n9). The content of C18:1n9 in the gonads was also independent of season and sex, whereas C20:1n9 changed with season, being higher during autumn (Table 3). The content of other FA was affected by sex. For example, the content of myristic acid (C14:0) was higher in females during winter, spring and summer (Table 3). The FA pentadecanoic acid (C15:0), stearic acid (C18:0) and 10-non-adeceanoic acid (C19:1n9) were more

abundant in males, and these differences were more evident during spring (Table 3). The dihomo-linoleic acid (C20:2n6) was more abundant in males across all sampling seasons (Table 3).

DISCUSSION

For most of the subtidal bottoms surveyed in Madeira archipelago, *S. granularis* occurred at low densities (average 0.01 ind./m^2), from shallow to deep reefs (5–30 m). In effect the densities obtained were in average lower than those reported by Alves et al. (2001) who already referred average densities lower than $2 \pm 0.6 \text{ ind./m}^2$. The present results were much lower than those reported for the northwestern ($< 30 \text{ ind./m}^2$ to $< 2 \text{ ind./m}^2$ in Marseille Bay– Sartoretto and Francour, 1997) and for the southwestern ($1.4\text{--}4.9 \text{ ind./m}^2$ in Algeria—Soualili et al., 1999) Mediterranean coast. Both studies indicated that the species density was affected by sea urchin size, depth, and season, with higher densities found during summer and autumn in shallower grounds and composed by relatively small animals (diameter $< 58 \text{ mm}$) (Sartoretto and Francour, 1997). These observations can explain the high densities indicated for the region of southern Brittany (50 ind./m^2 —Guillou and Michel, 1993). In fact, the low densities of *S. granularis* registered within the subtidal habitats of Madeira archipelago can be driven by several factors. Among these, is the high abundance of the sea urchin, *D. africanum* (Alves et al., 2001; Ribeiro and Neves, 2020). In highly abundant aggregations, *D. africanum* displays an aggressive behavior competing for food or even predated other echinoids (Shulman, 1990), which can cause detrimental effects in the *S. granularis* population within most of the locations sampled. Additionally, the typical oligotrophic conditions that characterize Madeira archipelago (Longhurst et al., 1995), comprising nutrient-poor waters and low chlorophyll concentrations (Falkowski et al., 1998) can also contribute to *S. granularis* low densities. A similar situation was reported in the oligotrophic southern part of the Aegean Sea (Vafidis et al., 2020). Yet, studies addressing this issue are needed for the study region.

TABLE 3 | Fatty acids (mg/g DM) profile and ANOVA results of *Sphaerechinus granularis* from Madeira archipelago.

	Winter			Spring			Summer			Autumn			Two-way ANOVA		
	Females	Males	Both	Females	Males	Both	Females	Males	Both	Females	Males	Both	Sx	Ss	Sx x Ss
Saturated fatty acid															
C14:0	3.6 ± 0.3 ^a	3.0 ± 0.6 ^b	3.2 ± 0.6	3.9 ± 0.7 ^a	3.1 ± 0.1 ^b	3.6 ± 0.7	3.8 ± 0.5 ^a	3.1 ± 0.8 ^b	3.4 ± 0.8	3.5 ± 0.2	3.8 ± 0.9	3.7 ± 0.6	0.016	0.659	0.263
C15:0	1.3 ± 0.1	1.6 ± 0.4	1.5 ± 0.3	1.4 ± 0.1 ^b	1.7 ± 0.1 ^a	1.5 ± 0.2	1.4 ± 0.2	1.5 ± 0.7	1.4 ± 0.6	1.6 ± 0.2	1.9 ± 0.3	1.7 ± 0.3	0.031	0.089	0.355
C16:0	15.5 ± 2.0	13.5 ± 3.0	14.2 ± 2.8	15.9 ± 1.6	16.0 ± 0.9	15.9 ± 1.3	15.2 ± 3.1	13.5 ± 3.0	14.2 ± 3.1	16.3 ± 3.3	19.6 ± 3.6	17.9 ± 3.6	0.847	0.072	0.213
C18:0	3.3 ± 0.02	3.7 ± 0.6	3.5 ± 0.5	3.4 ± 0.8	3.6 ± 0.3	3.5 ± 0.6	3.0 ± 0.7	3.3 ± 0.3	3.2 ± 0.5	3.0 ± 0.3 ^b	4.3 ± 0.3 ^a	3.7 ± 0.8	0.002	0.162	0.001
C21:0	2.5 ± 0.0	2.5 ± 0.04	2.5 ± 0.04 ^a	2.5 ± 0.04	2.4 ± 0.1	2.4 ± 0.1 ^β	2.5 ± 0.01	2.5 ± 0.02	2.5 ± 0.02 ^{aβ}	2.4 ± 0.05 ^b	2.5 ± 0.02 ^a	2.4 ± 0.09 ^β	0.277	0.001	<0.001
¹ ΣSFA	28.0 ± 1.8	26.3 ± 4.7	26.9 ± 4.0 ^β	29.7 ± 3.8	29.4 ± 1.5	29.6 ± 3.0 ^β	28.1 ± 4.9	25.9 ± 4.7	26.8 ± 4.7 ^β	29.3 ± 0.1	35.2 ± 3.5	32.2 ± 4.7 ^a	0.752	0.027	0.160
Monosaturated fatty acid															
C16:1n7	2.1 ± 0.6	1.4 ± 0.7	1.6 ± 0.5 ^β	2.3 ± 0.4 ^b	2.9 ± 0.2 ^a	2.5 ± 0.5 ^a	3.4 ± 0.9 ^a	2.2 ± 0.7 ^b	2.7 ± 1.0 ^a	2.4 ± 0.4	2.2 ± 0.7	2.3 ± 0.5 ^{aβ}	0.059	<0.001	0.011
² C18:1n9	6.0 ± 2.7	4.7 ± 1.1	5.1 ± 1.8	5.5 ± 0.9	6.9 ± 0.6	6.1 ± 1.0	5.4 ± 1.2	5.9 ± 1.1	5.7 ± 1.1	6.4 ± 1.4	7.0 ± 1.3	6.7 ± 1.3	0.477	0.141	0.138
C19:1n9	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.04 ^b	1.0 ± 0.1 ^a	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.14	0.031	0.021	0.390
² C20:1n9	4.4 ± 0.5	4.6 ± 1.1	4.5 ± 0.9 ^β	4.8 ± 0.3	5.6 ± 0.1	5.1 ± 0.5 ^{aβ}	5.0 ± 1.2	4.4 ± 0.9	4.6 ± 1.0 ^β	6.0 ± 0.5	6.1 ± 1.1	6.0 ± 0.4 ^a	0.659	<0.001	0.215
C22:1n9	2.4 ± 0.1 ^a	1.7 ± 0.3 ^b	2.0 ± 0.4 ^a	3.0 ± 0.5 ^a	2.4 ± 0.1 ^b	2.8 ± 0.5 ^β	2.3 ± 0.3	1.7 ± 0.9	2.0 ± 0.7 ^a	3.5 ± 0.8	2.6 ± 0.3	3.0 ± 0.8 ^β	0.001	0.001	0.863
³ ΣMUFA	16.2 ± 3.9	13.7 ± 2.9	14.5 ± 3.3 ^a	17.1 ± 1.7	19.2 ± 0.7	17.9 ± 1.7 ^{aβ}	17.2 ± 3.7	15.6 ± 2.7	16.3 ± 3.1 ^β	19.7 ± 0.2	19.3 ± 1.4 ^a	19.5 ± 1.8	0.472	0.005	0.255
Polysaturated fatty acid															
C18:2n6	1.4 ± 0.1	1.4 ± 0.04	1.4 ± 0.4	1.6 ± 0.1	1.9 ± 0.04	1.7 ± 0.2	1.5 ± 0.3	1.6 ± 0.8	1.5 ± 0.6	1.3 ± 0.1	1.4 ± 0.04	1.3 ± 0.1	0.290	0.225	0.919
C18:3n3	1.9 ± 0.1 ^a	1.4 ± 0.3 ^b	1.5 ± 0.4 ^{aβ}	1.9 ± 0.2	1.8 ± 0.1	1.9 ± 0.2 ^a	1.5 ± 0.2	1.4 ± 0.6	1.4 ± 0.3 ^β	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1 ^{aβ}	0.001	0.001	0.008
C20:2n6	1.1 ± 0.1 ^b	1.6 ± 0.6 ^a	1.4 ± 0.5	1.2 ± 0.1 ^b	1.6 ± 0.01 ^a	1.3 ± 0.2	1.2 ± 0.3 ^b	1.6 ± 0.2 ^a	1.4 ± 0.3	1.2 ± 0.1 ^b	1.6 ± 0.1 ^a	1.4 ± 0.2	<0.001	0.822	0.744
C20:3n6	0.4 ± 0.1	0.3 ± 0.04	0.3 ± 0.1 ^β	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.03 ^a	0.4 ± 0.1	0.4 ± 0.05	0.4 ± 0.1 ^{aβ}	0.4 ± 0.01	0.4 ± 0.02 ^a	0.4 ± 0.01	0.030	0.009	0.139
C20:4n6 (ARA)	8.7 ± 0.1	11.0 ± 3.5	10.3 ± 3.0	9.6 ± 1.1	12.8 ± 0.7	10.9 ± 1.9	8.8 ± 1.9	10.5 ± 5.9	9.8 ± 4.6	10.6 ± 2.6	12.9 ± 3.0	11.7 ± 2.9	0.089	0.133	0.526
C20:5n3 (EPA)	10.1 ± 1.4	9.1 ± 2.8	9.5 ± 2.4	11.8 ± 1.1	11.5 ± 1.0	11.7 ± 1.0 ^a	9.8 ± 2.2	8.1 ± 0.6	8.9 ± 1.7 ^β	11.3 ± 3.3	13.2 ± 4.3	12.3 ± 3.6 ^{aβ}	0.589	0.007	0.661
C22:6n3 (DHA)	1.0 ± 0.4	0.9 ± 0.4	0.9 ± 0.3 ^β	1.7 ± 0.4	2.5 ± 0.4	2.0 ± 0.5 ^a	1.8 ± 0.5	1.6 ± 0.7	1.7 ± 0.6 ^a	1.1 ± 0.6	1.1 ± 0.6	1.1 ± 0.6 ^{aβ}	0.187	<0.001	0.192
⁴ Σ PUFA	26.2 ± 2.4	26.9 ± 4.1	26.7 ± 3.5 ^{aβ}	30.2 ± 0.7	34.6 ± 0.9	31.9 ± 2.4 ^a	26.9 ± 5.3	26.6 ± 6.7	26.7 ± 5.9 ^β	29.1 ± 0.02	33.9 ± 6.5	31.5 ± 6.3 ^{aβ}	0.226	0.002	0.308
EPA + DHA	11.1 ± 1.7	10.1 ± 2.7	10.4 ± 2.4 ^β	13.5 ± 1.0	13.9 ± 1.4	13.7 ± 1.1 ^a	11.6 ± 2.5	9.8 ± 0.9	10.5 ± 1.9 ^β	12.4 ± 0.3	14.3 ± 3.6	13.4 ± 3.1 ^{aβ}	0.615	<0.001	0.355
Σ n6	12.0 ± 0.2	14.7 ± 4.6	13.8 ± 3.9	13.3 ± 1.2 ^b	17.3 ± 0.7 ^a	14.9 ± 2.3	12.3 ± 2.4	14.4 ± 6.7	13.5 ± 5.6	13.9 ± 0.2	16.9 ± 3.0	15.4 ± 3.2	0.024	0.152	0.457
Σ n3	13.6 ± 2.0	11.8 ± 2.6	12.4 ± 2.5 ^β	16.3 ± 1.0	16.7 ± 1.4	16.5 ± 1.1 ^a	13.8 ± 2.9	11.7 ± 0.9	12.6 ± 2.2 ^β	14.8 ± 0.2	16.6 ± 3.8	15.7 ± 3.3 ^{aβ}	0.537	<0.001	0.322
(n6) / (n3)	0.9 ± 0.1	1.3 ± 0.6	1.2 ± 0.5	0.8 ± 0.1 ^b	1.0 ± 0.1 ^a	0.9 ± 0.2	0.9 ± 0.1	1.2 ± 0.6	1.1 ± 0.5	0.9 ± 0.02	1.03 ± 0.1	0.98 ± 0.1	0.046	0.768	0.589

Data are expressed as mean ± SD (N = 2).

The superscripts α and β indicate statistically significant differences in the fatty acid content associated to the factor season.

The superscripts a and b indicate statistically significant differences in the fatty acid content associated to the factor sea urchin sex ($p < 0.05$). Two-way ANOVA output values in bold indicate statistical significant differences among groups.

¹ ΣSFA include the minor FA C12, C13, C17, C20, C22, C23 and C24.

² Includes the forms cis and trans.

³ ΣMUFA include the minor forms of C14:1n5, C16:1n9 and C24:1n9.

⁴ ΣPUFA include the minor forms of C16:2n6, C18:3n6, C18:3n3, C20:4n3 and C20:3n6.

The habitat of this species consists of a subtidal rocky reefs' ecosystem described for these oceanic islands (e.g., Bianchi et al., 1998; Alves et al., 2001, 2019; Friedlander et al., 2017; Sangil et al., 2018; Neves and Ribeiro, 2019; Ribeiro and Neves, 2020). The five locations where the species was observed revealed similarity in species composition for macroflora and macrofauna, especially among the most abundant species (Table 1), nonetheless the highest densities of *S. granularis* occurred in locations presenting higher diversity indices (Table 2). Recently, Neves et al. (2019) referred that *S. granularis* inhabits the "Frondose algal communities" (EUNIS code MB1.15/A3.15) and "Faunal communities on moderate energy infralittoral rock" (EUNIS code MB1.24/A3.24). Our findings showed that among the major groups of macrobenthic mobile fauna associated with the surveyed subtidal communities are the echinoderms, with the sea urchin *D. africanum* as the most abundant as mentioned previously. In fact, this species has been pointed to effectively reduce algal abundance and determine their community structure within Madeira archipelago rocky reefs (Alves et al., 2003; Tuya et al., 2004) and responsible for the variability pattern of those benthic communities (Alves et al., 2019).

Sphaerechinus granularis is frequently associated to rhodoliths beds (Guillou et al., 2002). Nonetheless, according to this study and other surveys performed in rhodoliths beds within Madeira archipelago (Neves et al., 2021) the species is not abundant within this habitat, particularly when compared to rocky reefs. Data obtained from an ongoing habitat-mapping project revealed high abundance of the sea urchin *D. africanum* within rhodoliths beds as in rocky reefs (a maximum of 13.4 ind./m² was recorded in intermediate and deep strata) (Ribeiro and Neves, 2020). As previously mentioned, the *D. africanum* voracious and aggressive behavior can restrict *S. granularis* to the vicinity of rhodoliths beds and deeper reefs, as shown in the neighbor Canaries archipelago (Tuya et al., 2001, 2007). Moreover, observations made by the authors (CR and PN) in ongoing monitoring surveys (Neves et al., 2018, 2019; Ribeiro and Neves, 2020), suggests this habitat shift of *S. granularis* to shallow reefs, especially in some natural rock breakwaters (e.g., breakwater of marina of Quinta do Lorde—Caniçal, of Porto Santo harbor and Fortaleza de Santiago-Funchal). Our results and additional observations allow to hypothesize that increasing load of nutrients from terrestrial sources/activities in these locations of higher anthropogenic pressure can provide more trophic resources (e.g., increasing macroalgal coverage) to the species, as have been shown elsewhere (Jordana et al., 1997).

Most of *S. granularis* urchins were collected from 5 to 12 m depth stratum of Cais do Carvão, Porto Santo breakwater and Caniçal. The diameter (average 65 mm) and weight distribution (average 139 g) were unimodal and showed that these depths are occupied mainly by adults, using as reference the presence of mature gonads for urchins with mean diameter > 40 mm (Guillou and Michel, 1993). The seasonal differences found in the size and weight distributions are related with the contribution of sample collected in Porto Santo breakwater which included urchins significantly larger and heavier than those collected in Cais do Carvão and Caniçal locations.

Despite the limitations related to the small sample size ($N \approx 10$ urchins per month), the analysis of the maturity stages

distribution throughout the year pointed to a single annual gametogenic cycle for *S. granularis* in line with the observations made by Spirlet et al. (1998) for other echinoid species. Our data indicate that, in Madeira archipelago, the reproductive season occurred between November and April with a notable synchrony between females and males. The evolution of the GI, increasing from March until a maximum in November, supports this extended reproductive cycle. The maturity index indicated two spawning peaks, one in April (preceded by relatively high values since January) and another stronger peak in November. These results show a delay of the reproductive season in relation to other regions, indicating that *S. granularis* could be a winter spawner. In the Mediterranean Sea, the study conducted by Martínez-Pita et al. (2008) indicate that reproduction season occurred during summer-autumn, starting with a spawning peak in June in southern location of the study area and ending with another and longer spawning peak in November in the northern site of the study area. Vafidis et al. (2020) defined a shorter reproductive season with a single spawning event during spring for the *S. granularis* population of the Aegean Sea (eastern region of the Mediterranean Sea) in line with the studies conducted by Guillou and Michel (1993) and Guillou and Lumingas (1998). These differences in the energy allocation for reproduction may be related with population densities, local productivity, and hydrodynamics. Larger populations within regions of high primary productivity tend to show relatively lower investment in reproduction (Lozano et al., 1995). In the sea urchins, as in other invertebrates, gametogenesis and spawning occur in synchrony with the best environmental conditions in terms of seawater temperature and local primary productivity (Soualili and Guillou, 2009; Amri et al., 2017). In fact, a winter reproduction season is also observed in the local populations of other sea urchin species as *P. lividus* (Lourenço, unpublished data) and other intertidal species as the gastropod *Patella candei* (Henriques et al., 2012) and *P. aspera* (Sousa et al., 2017). Both echinoderms and gastropods have in common a planktotrophic larval phase whose feeding success depends on phytoplankton blooms that in Madeira archipelago occurring from late autumn to early spring (Caldeira et al., 2002; Kaufmann et al., 2015).

Most sea urchin species are harvested for their gonads, or roe as commercially referred. High quality roe usually consists of large golden orange to yellow gonads with a distinct sweet ocean taste commercially identified as *uni* (Sun and Chiang, 2015). *Sphaerechinus granularis* adults attained particularly large body diameter and gonads when compared with *P. lividus*, a species of high commercial interest and the most harvested sharing the same habitat. The average GI (3.84 ± 2.54) of the local population of *S. granularis* is within the range observed by Guillou and Lumingas (1999) for the Bay of Brest (Brittany) and by Martínez-Pita et al. (2008) in the western Mediterranean Sea and is higher than those observed in the eastern Mediterranean (Aegean Sea), where the GI averaged approximately 2.0 (Vafidis et al., 2020). This difference between the Madeira archipelago and the eastern Mediterranean populations could be related to differences in the seawater temperature regimes. During the survey period, the local seawater temperature (measured in Funchal harbor) ranged between 18 °C (in March) and 24 °C (in September), whereas sea-bottom temperature recorded in the Aegean Sea

ranged between 13°C in March and 27°C in July. The relative stability in seawater temperature at Madeira among seasons could benefit the gonadal growth. Nevertheless, the effect of local temperature trends in sea urchins GI is not clear (González-Irusta et al., 2010; Machado et al., 2019). Additionally, higher roe production yield could be obtained between September and November when gonads weight and GI attain its maximum (GI = 8.02 ± 3.49 ; GTW = 11.34 ± 7.34 g).

Most of the gonads observed were classified with color grade I (bright yellow or orange) and II (pale yellow or orange), ranging between 11 and 60% and between 20 and 89%, respectively, and independently of season. This intense orange color was consistent with the higher content of total carotenoids in their gonads. In fact, *S. granularis* gonads presented a particularly high carotenoid content, comparable to *S. droebachiensis* from Norway (Hagen et al., 2008). Both populations present higher carotenoid content in relation to those of *P. lividus* from the northwestern Portuguese coast (Rocha et al., 2019), *S. droebachiensis* from Newfoundland (Liyana-Pathirana et al., 2002), and *P. miliaris* from the west coast of Scotland (Symonds et al., 2007). The relatively high content of carotenoids in the *S. granularis* gonads may be related with the dominance of brown and red macroalgae rich in β -carotene (e.g., *Dictyota* sp., *Halopteris filicina*, *Peyssonelia* sp. and *Sargassum* sp. in **Table 1**) in the diet of local population that use this molecule to synthesize the echinenone, the predominant carotenoid of sea urchin gonads (Symonds et al., 2007). The total carotenoid content varied significantly with season, being lower during summer, following the GI trend, when gonads are at their lowest level of stored reserves and start to recover from spawning. The high variability of total carotenoid content in male gonads may be related with the pooled samples as they included different maturity stages with various levels of stored carotenoids. According to Hagen et al. (2008) male and female urchins exhibit different patterns of carotenoid variation. Throughout the annual reproductive cycle, female carotenoid levels were generally lower and less variable than male carotenoids levels. Males do not lose carotenoids during spawning, and their concentration of carotenoids vary widely as a passive result of seasonal variation of gonad size and structure.

To our knowledge, this study constitutes the first analysis of the nutritional characteristics of *S. granularis* gonads. Both lipidic and protein content were in line to studies conducted in species partially sharing the same subtidal rocky habitat, namely *P. lividus*, in Madeira archipelago and in other regions like the Mediterranean Sea (Siliani et al., 2016) and the Northeast Atlantic coast (Montero-Torreiro and Garcia-Martinez, 2003). This consistency within the content of the major macronutrients in the sea urchin gonads between species and across regions could be related with the fact that sea urchins feed intake is mainly controlled by the dietary protein level. In sea urchins, the feed intake increases when the dietary protein level is low and decreases when the dietary protein level is high based in a dietary protein intake threshold (Heflin et al., 2016; Lourenço et al., 2020). On the other hand, the ratio between gonad lipidic and protein content is influenced by season as a response to the reproductive cycle. The lipidic content of the local population of *S. granularis* was on average $17.51 \pm 2.64\%$ DM independently

of the sea urchins' sex, but with variations across seasons. *Sphaerechinus granularis* gonads collected in winter presented lower lipidic content. The pattern of seasonal variation of the gonad lipidic content is complementary with the gonad protein content. Particularly in males, the lower lipidic content in winter matched with the highest protein content. This high protein content of males gonads is associated with the relatively high carotenoid content of their gonads, due to the binding relationship between carotenoids and protein in the sea urchins storage tissues (Pilbrow et al., 2014).

The FA profile of the *S. granularis* was highly influenced by sex, but also by season, as a proxy of seasonal changes in the gonad structure, and seasonal variations of diet. Particularly, the content of SFA C14:0, C15:0 and C18:0 in the gonad were affected by sex, in line with the results obtained by Martínez-Pita et al. (2010) for *P. lividus*. Females were richer in C14:0 while males were richer in C15:0 and C18:0. In contrast, the content of most abundant FA (C16:0) was not affected by season nor by sex, indicating the strong herbivorous behavior of this species (Kelly et al., 2008) as reported for other species such as *P. lividus*, *A. lixula* (Martínez-Pita et al., 2010), *Spatangus purpureus* and *Echinus melo* (Angioni and Addis, 2014). The MUFAs content was affected by season, particularly the C16:1n7 and the C20:1n9 suggesting that these two fatty acids are associated to the seasonal variation of food sources. The most abundant MUFA was C20:1n9 in line with the profile presented for the *S. granularis* of Sardinian Sea (Mediterranean basin) (Angioni and Addis, 2014). Both C19:1n9 (males with higher content) and C22:1n9 (females with higher content) were affected by the sea urchin sex suggesting it could be associated to gonads structural differences. The structural role of C22:1n9 in the sea urchins gonads is also evident in *P. lividus*, though with males presenting higher content of this fatty acid (Martínez-Pita et al., 2010). The seasonal and sex related variation of C22:1n9 was already reported in other studies (Rocha et al., 2019). The C18:3n3 and C20:3n6 were both affected by sex and season, also revealing a structural role in the gonad. The C18:2n6 and particularly ARA are very abundant in the gonads of *S. granularis* independently of season or gonad sex. The C18:2n6 and C18:3n3 are both involved in the "Δ8 metabolic pathway" that leads to the bio-synthesis of ARA and EPA, respectively (Kabeya et al., 2017). EPA influences many physiological processes such as reproduction, immunity efficiency and osmoregulation (Bret and Müller-Navarra, 1997). The absence of the PUFA C18:4n3 and C16:4 from the FAs profile in the gonads of the local population of *S. granularis* reveals that green algae and seagrasses do not have a predominant role in the diet of the species local population (Hughes et al., 2005) unlike the evidences observed by Angioni and Addis (2014) in the population of Sardinia Sea (Western Mediterranean). Moreover, the low gonadal content of C22:6n3 (DHA) reveals that the carnivory level of this species was also very low. The C22:6n3 (DHA) is an indicator of carnivory, as it is associated to filter feeding preys that in their turn feed on dinoflagellates (Cook et al., 2000; Hughes et al., 2005). The low level of DHA together with the absence of C18:4n3 and C16:4 indicates that *S. granularis* population of Madeira archipelago is closely associated with brown algae coverage, probably *Dictyota* sp., the most abundant brown macroalgae in the region (Graeve et al., 2002).

Despite the significant differences among females and males, the ratio $n-6/n-3$ was in all cases close to 1, denoting the balance between the more abundant PUFA, EPA, ARA and LA.

CONCLUSION

In the Madeira archipelago, the blunt sea urchin, *S. granularis* occurred in low densities in shallow subtidal rocky reefs and in breakwaters. In these ecosystems, the species is associated with encrusting algae and turf and erect macroalgae communities, on which the species grazes upon, mainly over brown macroalgae. Here, the *S. granularis* presents a long reproductive season occurring from late autumn to early spring, being considered a winter spawner. This reproductive strategy is associated with the southern limit of species distribution and with the low level of primary productivity and stable seawater temperature of this oceanic insular ecosystem. The species presents a relatively high gonad yield and proportion of sea urchins of bright orange color in line with roe market demand. Additionally, the high level of total carotenoids, the high PUFA (particularly EPA) content, and ratio $n-6/n-3$ close to one supports the high nutritional value of *S. granularis* roe.

The increasing interest in sea urchin roe in many regions of Mediterranean and southern Europe (James et al., 2016), the willingness of the local population to harvest and consume seafood and the growing interest of the local touristic sector for endogenous resources, foresees the increasing pressure to explore *S. granularis* and other edible species (e.g., *P. lividus*) directly to market or to sea urchin gonad enhancement programs. The consistency of high proportions of gonads with highly marketable color and the single annual GI peak, indicates that *S. granularis* has potential to be included in the edible sea urchins. Nevertheless, the species reduced densities demand for a precautionary approach when projecting any future exploitation of their roe. Further studies on the use of *S. granularis* as marine resource should focus in its full-cycle aquaculture in parallel with studies on the potential market for sea urchin roe at the regional level.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request.

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AUTHOR CONTRIBUTIONS

SL contributed to this study with conceptualization, methodology, formal analysis, original draft writing, review, and editing. RJ contributed with methodology, data collection and curation, original draft review, and editing. PN contributed with methodology, data collection and curation, formal analysis, original draft writing, review, and editing. AG and NC collected and analyzed chemical data and original draft writing, review, and editing. CA contributed with resources allocation, funding acquisition, and original draft review and editing. CR contributed to this study with methodology selection, data collection and curation, formal analysis, original draft writing, review, and editing. All authors contributed significantly to the content of this manuscript and the work that supported and agreed with its final content before submission.

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SUPPLEMENTARY MATERIAL

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