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Seagrass debris as potential food source to enhance *Holothuria arguinensis*' growth in aquaculture

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Abstract

Holothuria arguinensis aquaculture started to be developed in 2014, being the first sea cucumber species from Europe. However, some aspects of its aquaculture biotechnology, such diets, need to be assessed. This work aimed to evaluate seagrass debris of *Zostera noltii* and *Cymodocea nodosa* as food source for broodstock maintenance in tanks, during breeding periods. The given feed rations per tank were calculated as the 30% of the total sea cucumber biomass in each tank and reviewed each week. Then, feed rations of seagrass and sediment were calculated from this value, according to the following percentages: 40% sediment, 15% *Z. noltii*, 40% *Z. noltii*, 15% *C. nodosa* and 40% *C. nodosa*. *H. arguinensis* growth, feeding rate and nutritional value were assessed under these diets. *H. arguinensis* fed with 40% of *Z. noltii* showed the highest growth (specific growth rate = 0.09 ± 0.06%/day, absolute growth rate = 0.11 ± 0.07 g/day) increasing their final weight in 5.86 ± 3.57% in 57 days. However, the individuals fed with *C. nodosa* showed a negative growth. *H. arguinensis* showed a reduction in its feeding rate as the organic matter content in the diets increased. *H. arguinensis* did not show any important change on proximate composition, protein, lipid, mineral contents and fatty acids profile among the feeding groups, or in comparison with the individuals collected from wild habitat. Therefore, *H. arguinensis* could be fed with *Z. noltii* debris during tanks maintenance along breeding period, ensuring its growth and maintaining its nutritional profile.

KEYWORDS
aquaculture, growth, *Holothuria arguinensis*, sea cucumber, seagrass

1 | INTRODUCTION

In the last decades, it was recorded the overexploitation of the traditional fishing grounds of sea cucumbers along the Pacific and Indian Oceans due to the increasing demand of bêche-de-mer (dried sea cucumber) by the Asiatic market (Conand, 2004; Purcell et al., 2013; Purcell, Samyn, & Conand, 2012). Since the demand of this product exceeds its supply, there has been a continuing search of new target species from other geographical regions, being harvested more than 66 species nowadays, whose declared capture is about 93.7 million tons (FAO, 2014; Purcell, 2010; Purcell et al., 2013, 2012). Additionally, the aquaculture development of the depleted species has become an important source to supply the high demand from Asiatic markets and a profitable industry with a total production of 90.4 million tonnes (US$ 144.4 billion; FAO, 2014; Purcell et al., 2013).

Sea cucumber commercial fisheries in the Mediterranean Sea started in 1996 in Turkey, mainly focused on *Parastichopus regalis*

as 'by catch' and later *Holothuria polii*, *H. tubulosa* and *H. mammata*; however, the target species have been changing along the last years (González-Wangüemert, Aydin, & Conand, 2014; González-Wangüemert, Domínguez-Godino, & Cánovas, 2018; M. Aydin, personal Communication). Due to the heavy fishing pressure, the first insight of their overexploitation has been found, with reduction in the largest and heaviest individuals, loss of genetic diversity and reduction in catches (González-Wangüemert et al., 2014; González-Wangüemert, Valente, & Aydin, 2015, 2018). Similar overexploitation insight has been found on *P. regalis* fisheries in Catalonia (NE Spain; Maggi & González-Wangüemert, 2015). Additionally to the commercial fisheries, there is a high incidence of illegal fishing of sea cucumbers in the Mediterranean and NE Atlantic waters (Domínguez-Godino & González-Wangüemert, 2018a; González-Wangüemert et al., 2018). *H. arguinensis* is the better-reported case of illegal fishing along all its geographical distribution, finding its first insight of overexploitation at Ria Formosa (S Portugal; González-Wangüemert et al., 2018). The incidence of illegal fishing on *H. arguinensis* is driven by the high prices that this species reaches in the market, which can be up to 350 €/kg (Domínguez-Godino & González-Wangüemert, 2018a; González-Wangüemert et al., 2018).

Under this scenario of overexploitation, illegal fisheries, high demand and high economic value of the North-Eastern Atlantic and Mediterranean Sea cucumber species, there has been a development of the aquaculture for some of new target species, which includes, for example, *H. arguinensis* (Domínguez-Godino, Slater, Hannon, & González-Wangüemert, 2015), *H. mammata* (Domínguez-Godino & González-Wangüemert, 2018b), *H. polii* and *H. tubulosa* (Rakaj et al., 2017, 2018). However, only for *H. arguinensis* has been developed a complete biotechnological process aiming its aquaculture production. In 2014, it was successfully induced to spawn, their embryonic and larval development described, and the first juveniles obtained (Domínguez-Godino et al., 2015). From that year, the juveniles' production has exponentially grown (Domínguez-Godino & González-Wangüemert, 2017a). For this species, the optimal stocking density and critical biomass have been also determined and its co-culture with the green macroalgae *Ulva lactuca* and the purple sea urchin *Paracentrotus lividus* assessed (in review a; Domínguez-Godino & González-Wangüemert, 2017b). Additionally, the genetic diversity of broodstock and of the different larvae stages has been studied (González-Wangüemert & Domínguez-Godino, 2017). In spite of all this information, there is an urgent need to assess artificial diets that ensure and enhance the *H. arguinensis'* growth, considering the total lack of knowledge on this subject. Several studies have been focused on other commercial sea cucumber species, mainly on juveniles of *Apostichopus japonicus* and *Holothuria scabra*, using dried powder of macroalgae (*Chaetomorpha linum*, *Laminaria japonica*, *Sargassum thunbergii*, *S. Polycystum and U. lactuca*), dried powered of seagrass (*Zostera marina*) and sea mud, with significant growth being obtained with all diets (Battaglene, Seymour, & Ramofafia, 1999; Liu, Zhou, Yang, & Ru, 2013; Liu, Dong, Tian, Wang, & Gao, 2010; Song, Xu, Zhou, Lin, & Yang, 2017; Xia et al., 2012; Zhu et al., 2007). For *A. japonicus* were initially formulated feeding diets based on *S.thunbergii* and *S. polycystum*, macroalgae that seems to enhance growth of this sea cucumber species in comparison with the other assessed macroalgae and seagrass species (Liu et al., 2013, 2010; Song et al., 2017; Xia et al., 2012; Zhu et al., 2007). However, these diets depend on the wild production of the macroalgae species, and therefore, it was reaching their overexploitation when the aquaculture production of *A. japonicus* grew, becoming a very expensive formulated feeding (Xia et al., 2012). On this way, there was a need to assess new artificial feeding diets based on other macroalgae and seagrass species inhabiting with *A. japonicus*.

H. arguinensis is generally associated with macroalgal beds, sandy bottoms and seagrass meadows of *Z. noltii* and *Cymodocea nodosa* in the Ria Formosa (Southern Portugal; Domínguez-Godino and González-Wangüemert, in review b; González-Wangüemert & Borrero-Pérez, 2012; González-Wangüemert et al., 2013; Navarro, García-Sanz, & Tuya, 2014; Siegenthaler, Cánovas, & González-Wangüemert, 2015). *H. arguinensis* as a deposit feeder assimilates the organic matter, such as bacteria, microalgae and detritus (from macroalgae and seagrass) from the ingested sediment (Uthicke, 1999). Large amounts of seagrass debris can be found along the shores of the Ria Formosa, which could be used to produce formulated feed for *H. arguinensis*. Therefore, the aim of this work was to assess the growth, feeding rate, proximate composition, mineral content and fatty acids profile of *H. arguinensis* adults under a tank-based experiment, using sediment (as control), dry powder of *Z. noltii* and *C. nodosa* as food sources. This is the first work assessing artificial diets performed in any North-Eastern Atlantic and Mediterranean Sea cucumber species to improve growth performance under aquaculture conditions. Results from this study will allow to establish the baseline for the development and improvement of artificial diets for *H. arguinensis* commercial production.

2 | MATERIALS AND METHODS

2.1 | Experimental animals and rearing conditions

Adults of *H. arguinensis* (n = 50; 213.19 g ± 9.57, SE) were collected from Ria Formosa at Praia de Faro (37°0′33.92″N, 7°59′44.99″W; Faro, Portugal) in April 2014. The individuals were transferred in cold boxes to the Ramalhete Marine Station and allocated into a 650-L tank. Adults were acclimated to tank conditions for a period of a week before the start of the experiment. To ensure feed during the acclimation period, the bottom of the tank was filled with natural sediment from the collection site. Flowing filtered seawater at ambient temperature, continuous aeration and natural photoperiod were used during acclimation and experimental period. Forty-eight hours before the beginning of experiment, the animals were allocated into bare tanks to allow that the gut contents were totally evacuated.

2.2 | Experimental feed treatments

Decaying debris of the two most common seagrasses from the Natural Park Ria Formosa, *C. nodosa* and *Z. noltii*, and sediment was

used to feed *H. arguinensis'* adults every week, for two months. Decaying leaves of the seagrass debris of both species were collected from the shores at Ria Formosa, cleaned from external contaminants, such as other plants, algae, animals and/or plastics, and oven dried at 60°C until constant weight was obtained. Then, the seagrasses were ground to obtain a fine powder. Sediment was collected at Praia de Faro sieved and oven dried at 60°C. The given feed rations per tank were calculated as the 30% of the total sea cucumber biomass in each tank and reviewed each week (Zamora & Jeffs, 2011). Then, feed rations of seagrass and sediment were calculated from this value, according to the following percentages: 40% Sediment (S40), 15% *Zostera noltii* (Z15), 40% *Zostera noltii* (Z40), 15% *Cymodocea nodosa* (15C) and 40% *Cymodocea nodosa* (40C). The remained percentage (60% or 85%) was reached by addition of dry sediment. The weekly feed ration was divided into two equal portions and allocated into the tanks every two/three days. Previously to give the feed ration, water flow was cut for 15 min to allow feed deposition, which was not siphoned over the experiment.

2.3 | Experimental design and samples collection

The experiment was performed in a flow-through tank system, with seawater at ambient temperature and natural photoperiod. A total of 15 circular tanks (*n* = 3 per treatment) were used, with an area of 0.2 m² each. The bottom of the tanks was filled with a thin layer (2–3 cm) of sediment from Praia de Faro. Previously to register the weight of sea cucumbers, the posterior part of the animals was gently squeezed, to allow expulsion of internal water and external water was dried (Sewell, 1990). Animals were grouped on 15 sets with three individuals each, showing similar mean wet weight among groups (Table 1; Kruskal–Wallis test, χ^2 = 0.541, *p* = 1). Each group was allocated randomly into an experimental tank. The weight of the sea cucumbers was recorded until the end of the experiment (8 weeks), as previously explained. Later, sea cucumbers were sacrificed by cold shock (Domínguez-Godino & González-Wangüemert, 2018a), dissected and the internal organs were removed. Then, gutted weight was registered and individuals stored at −20°C. Initial gutted weight was estimated by using the

equation obtained from final wet weight–final gutted weight relationship (Domínguez-Godino & González-Wangüemert, 2018a; González-Wangüemert, Valente, Henriques, Domínguez-Godino, & Serrão, 2016). In addition, three individuals were collected from the wild population previously to start the experiment (T0) and another three individuals at the end of the experiment (Tf). These individuals were dissected and stored as it was previously explained. These two groups of animals were collected to establish the nutritional profile of wild populations and to compare and assess possible changes on the biochemical composition produced by the different diets on the individuals from each experimental treatment.

Faeces produced by the sea cucumbers were collected two times per week using a spoon directly from the bottom of the experimental tanks. At the laboratory, the faeces were oven dried at 60°C for 48 hr and weighted in order to estimate feeding rate since the amount of sediment ingested is approximately similar as the amount defecated (Domínguez-Godino & González-Wangüemert, 2018a; Hammond, 1983; Yamanouti, 1939). Sea cucumbers were fed two times per week dividing into two ratios the feed amount for each diet. Seawater temperature was measured every day using the temperature data loggers (*n* = 4, allocated randomly).

2.4 | Biochemical composition analysis

The moisture content of sea cucumbers was determined by drying fresh samples at 50°C until to reach a constant weight. Sea cucumber bodies were freeze-dried for 48 hr. The dry samples were ground to powder, pooled together for each treatment and stored at −20°C for analyses. The ash content was obtained by incineration of 0.1 g (±0.01) of sample in a muffle furnace at 525°C for 8 hr (Gressler et al., 2010). The crude protein content (N × 6.25) was determined by the macro-Kjeldahl method (Kjeldahl, 1883).

The total lipid content was obtained using the modified protocol of Bligh and Dyer method (Bligh & Dyer, 1959). A mixture of methanol, chloroform and water (2:2:1) was added to 100 mg of dry powder of sea cucumber samples and diets (sediment, *C. nodosa* and *Z. noltii*), which were ground with an IKA Ultra-Turrax disperser. Once the samples were homogenized, different phases

TABLE 1 Mean values of initial gutted weight (g), final gutted weight (g), mean specific growth rate (%/day), mean absolute growth rate (g/day), mean percentage of weight change (%) and feeding rate (g ind/day) of adults of *H. arguinensis* for each experimental feeding treatment

	40S	15C	15Z	40C	40Z
Initial gutted weight (g)	109.66 ± 0.96	109.84 ± 1.33	108.2 ± 0.24	108.36 ± 0.09	108.61 ± 0.25
Final gutted weight (g)	112.36 ± 0.55	105.93 ± 5.06	111.99 ± 4.22	106.81 ± 4.18	114.95 ± 3.62
%WC (%)	2.49 ± 1.41	−3.41 ± 5.78	3.51 ± 3.85	−1.44 ± 3.77	5.86 ± 3.57
SGR (% per day)	0.04 ± 0.02	−0.07 ± 0.11	0.06 ± 0.06	−0.03 ± 0.06	0.09 ± 0.06
AGR (g/day)	0.05 ± 0.02	−0.07 ± 0.11	0.06 ± 0.07	−0.03 ± 0.07	0.11 ± 0.07
Feeding rate (g ind/day)	19.37 ± 1.62[a]	10.35 ± 1.08[b]	9.48 ± 0.78[b]	6.15 ± 0.63[c]	4.12 ± 0.37[c]

Note: Different letters within the same variable indicate significant differences, (Nemenyi post hoc, *p* < .05). Mean ± standard error.

were obtained by centrifugation, and 1 ml of the organic phase (chloroform) was transferred to a pre-weighted tube. The chloroform was totally evaporated in a block heater at 60°C overnight. Then, the tubes were cooled down for at least two hours in a desiccator. The tubes were weighted to estimate the total lipids by gravimetric determination.

2.5 | Fatty acid analysis

The fatty acid methyl ester (FAME) profile was determined using a modified protocol from Lepage and Roy (Lepage & Roy, 1984), described in Pereira et al., 2012. Samples (0.1 g) of each group (n = 3) were added to acetyl chloride and methanol (20:1, v/v) in reaction vessels and homogenized using an IKA Ultra-Turrax during 2 min. Then, 1 ml of hexane was added, and the samples were heated at 100°C during 1 hr in a water bath. After cooling down, 1 ml of distilled water was added, samples were centrifuged and the organic phase removed, while the remaining water was dried with anhydrous sodium sulphate. The extracts were injected in a Varian 450-GC/240-MS (Varian 450-GasChromatograph/240-MS IT Mass Spectrometer, Varian Inc.), equipped with a BR-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness, Bruker). FAMES were identified by comparing their retention time with those of the standards 37-FAME Mix (Supelco). Results were expressed as percentage of total fatty acids (FA).

2.6 | Mineral composition

For minerals determination, 100 mg of dried samples was burned in a muffle furnace at 525°C for 8 hr (Gressler et al., 2010). Then, samples were digested using hot nitric acid (HNO_3) and hydrogen peroxide. Nitric acid and the hydrogen peroxide were totally evaporated in a block heater at 110°C, and the precipitate diluted in 5% HNO_3. Minerals were analysed by Microwave Plasma-Atomic Emission Spectrometer (MP-AES; Agilent 4200 MP-AES, Agilent).

Different concentrations of working solutions of minerals were prepared from certified standard solutions. For analytical quality accuracy, results were corrected by subtracting a blank from the analysed standard concentrations. Quantification wavelengths and calibration curves were selected to obtain the highest signal ratio and the lowest interference for the target elements. Spiking-and-recovery readings were carried out to assess validity of the results. Samples were analysed in triplicate, and results were expressed as mg/g.

2.7 | Data and statistical analysis

The specific growth rate (SGR; Yuan et al., 2006), the absolute growth rate (AGR; Battaglene et al., 1999), the weight change (%WC; An, Dong, & Dong, 2007) and the feeding rate (FR; Hammond, 1983; Yamanouchi, 1939) were calculated as follows using the initial and final gutted weight:

$$\text{Specific Growth Rate }(\%/\text{day}) = 100 * (\text{LN}(\text{final weight}) - \text{LN}(\text{initial weight}))/\text{time}$$

$$\text{Absolute Growth Rate }(g/\text{day}) = (\text{Final weight} - \text{Initial weight})/\text{time}$$

$$\text{Weight Change }(\%) = 100 * (\text{final weight} - \text{initial weight})/\text{initial weight}$$

$$\text{Feeding Rate }(g\,\text{ind}/\text{day}) = (\text{dry weight faeces}/\text{no. sea cucumbers})/\text{time}$$

The mean values of the growth variables, feeding rate and the nutritional values obtained were compared among treatments using Kruskal–Wallis test (Kruskal & Wallis, 1952). The post hoc test Nemenyi was used as *post hoc* analyses, using the package PMCMR (Pohlert, 2014). Differences were considered significant if *p* < .05. The statistical analyses were performed in R statistic software (R Core Team, 2013).

3 | RESULTS

3.1 | Growth and feeding rates

The mean gutted weight of the individuals at the feeding treatments of 40S, 15Z and 40Z slightly increased by the end of the experiment, which corresponded to a percentage of weight change of 2.49% (±1.41), 3.51% (±3.85) and 5.86% (±3.57), respectively (Table 1; Figure 1a). However, the individuals at the feeding treatment of 15C and 40C showed a reduction in their final mean gutted weight in comparison with the initial mean gutted weight (Table 1), linked to a reduction in weight change of −3.41% (±5.78) and −1.44% (±3.77), respectively (Table 1; Figure 1a). No significant differences were found among the initial and final mean gutted weight, and for the mean weight change among feeding treatments (Table 1; Figure 1a; final mean gutted weight: Kruskal–Wallis test, $\chi^2 = 0.507$, *p* = .973; %WC: Mann–Whitney, *p* > .05). Positive values of mean SGR and AGR were obtained for the individuals fed with 40S, 15Z and 40Z (Table 1; Figure 1b, c), being the values slightly higher for the individuals at 40Z feeding treatment with 0.09%/d (±0.06; SGR) and 0.11 g/d (±0.07; AGR). No significant differences were found for the mean SGR and AGR among feeding treatments (Table 1; Figure 1b,c Mann–Whitney, *p* > .05).

The feeding rate for the individuals fed with 40S diet was 19.37 g ind/day (±1.62); this value was significantly higher than the ones obtained from the other feeding treatments (Table 1; Figure 1d; Kruskal–Wallis test, *p* < .05). The individuals fed with 15Z and 15C showed similar medium values of feeding rates (Table 1; Figure 1d; Kruskal–Wallis test, *p* > .05), which were significantly different to the rates obtained for the other feeding treatments (Table 1; Figure 1d; Kruskal–Wallis test, *p* < .05). The individuals fed with 40Z diet showed the lowest feeding rate, which was significantly different to the obtained ones by the individuals fed with 15Z and 15C diets, and the ones under control conditions (40S) (Table 1; Figure 1d; Kruskal–Wallis test, *p* < .05), but not to the feeding rate obtained for 40C diet (Table 1; Figure 1d; Kruskal–Wallis test, *p* > .05).
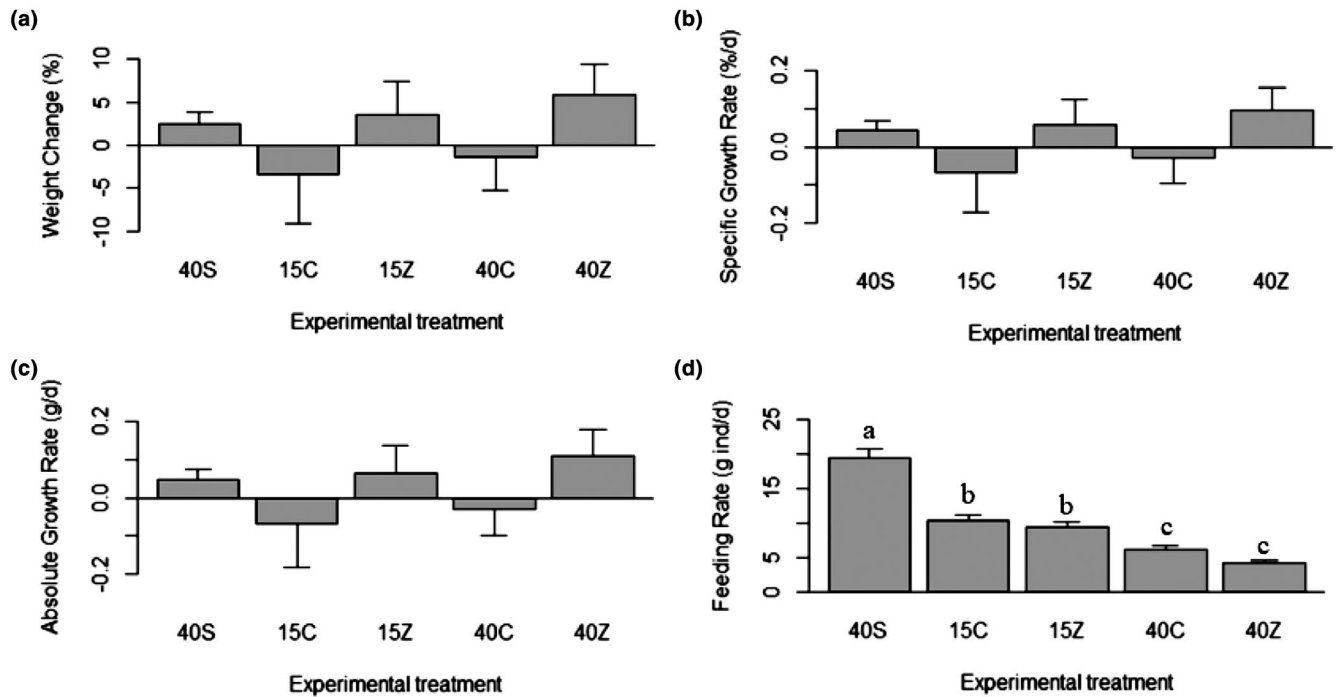
FIGURE 1 (a) Percentage of wet weight change (%), (b) specific growth rate (%/day), (c) absolute growth rate (g/day) and (d) feeding rate (g ind^{-1} day^{-1}) of *H. arguinensis* individuals over eight weeks' experiment for each feeding treatment. Values are given as mean ± *SE* (*n* = 3). Different letters indicate significant differences (Nemenyi post hoc, *p* < .05)

The seawater temperature showed a mean value of 22.22°C (±0.21, *SD*) during the experimental period, although it dropped to 18°C (± 1, *SD*) in two periods (since 24 April 2014 to 27 April 2014, and since 21 May 2017 to 25 May 2014; Figure 2). The salinity along the experiment reached a mean value of 36.66 ppm (±0.08, *SD*), being stable for the whole experimental duration (Figure 2).

3.2 | Biochemical composition of *H. arguinensis*

Animals from all the different diet treatments showed similar moisture content (%), with a range of variation of 3% (Table 2). Individuals fed with 40S had the highest moisture content, and however, it was only significantly higher than the obtained values for those fed with 40Z and for individuals collected at the

experiment setup (T0), which were the ones with the lowest values of moisture content (Table 2, Kruskal–Wallis test, *p* < .05). The individuals fed with 15Z showed the highest ash content, which was significantly higher than the individuals fed with 15C and the individuals collected at T0, groups that again showed the lowest values of ash content (Table 2, Kruskal–Wallis test, *p* < .05). The highest content of protein was obtained for the individuals fed with 15C, and however, no significant differences were found among any feeding treatment and/or group of collected sea cucumbers (T0 and TF; Table 2, Kruskal–Wallis test, *p* > .05). The highest total lipids content was presented by the individuals collected at T0, which was only significantly higher than the total lipids levels obtained for the individuals fed with 15Z (Table 2, Kruskal–Wallis test, *p* < .05), group that showed the lowest value.

The individuals fed with 40S showed a lower level of protein and ash than the individuals fed with the diets based on seagrass species, except for the individuals fed with 15C in ash content (Table 2). However, the individuals fed with 40S presented higher level of lipids and moisture content in comparison with the other groups (Table 2).

The individuals collected at T0 showed higher amount of ash, protein and lipids content than those collected at the end of the experiment (TF). However, for most of the groups fed with the different diets, the amount of ash and protein was higher and/or similar than the individuals collected at T0 (Table 2). A lower lipids content was registered for all fed groups in comparison with T0 (Table 2).
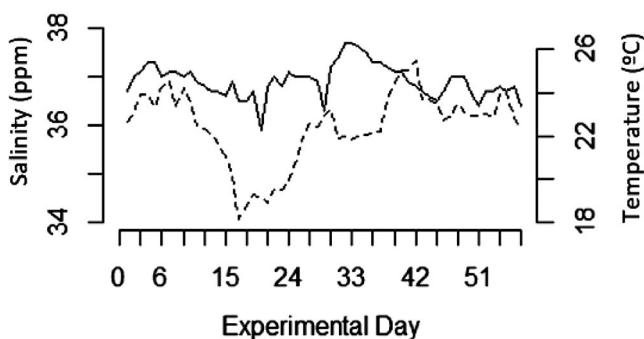

FIGURE 2 Seawater temperature (dashed line, °C) and salinity (straight line, ppm) registers from the experiment

TABLE 2 Fatty acid profiles (% total fatty acids) and biochemical compositions of the two groups of *H. arguinensis* collected at the setup (T0) and end of the experiment (TF), and the five different groups under feeding treatments (40S, 15C, 15Z, 40C and 40Z), and the three feed ingredients used in the experimental diets (Sediment, *Zostera noltii* and *Cymodocea nodosa*)

Biochemical composition	Sea cucumber T0	Sea cucumber TF	Sea cucumber 40S	Sea cucumber 15C	Sea cucumber 15Z	Sea cucumber 40C	Sea cucumber 40Z	Diet sediment	Diet Zostera noltii	Diet Cymodocea nodosa
Moisture (%)	84.09 ± 0.17[ab]	86.02 ± 0.18[abc]	86.63 ± 0.28[bc]	85.88 ± 0.26[abc]	84.79 ± 0.41[abc]	84.92 ± 0.4[abc]	83.89 ± 0.4[ab]	–	–	–
Ash (mg/g)	429.88 ± 1.13[ab]	391.83 ± 5.34[a]	422.17 ± 7.56[ab]	391.48 ± 1.87[a]	467.93 ± 8.54[ab]	448.2 ± 17.43[ab]	444.76 ± 3.26[ab]	987.06 ± 0.16	301.98 ± 2.57	452.62 ± 5.29
Protein (mg/g)	0.75 ± 0.06[a]	0.65 ± 0.04[a]	0.68 ± 0.01[a]	0.93 ± 0.13[a]	0.74 ± 0.02[a]	0.74 ± 0.05[a]	0.86 ± 0.17[a]	0.01 ± 0.01	0.17 ± 0.02	0.15 ± 0.01
Lipids (%)	4.72 ± 0.47[a]	3.03 ± 0.19[ab]	4.12 ± 0.08[ab]	3.6 ± 0.19[ab]	2.06 ± 0.76[b]	3.5 ± 0.05[b]	1.88 ± 0.45[b]	0.44 ± 0.05	1.12 ± 0.04	0.71 ± 0.12
FAME										
C10:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.15 ± 0.05	n.d.	0.22 ± 0.17
C11:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C12:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.46 ± 0.09	1.11 ± 0.41
C13:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C14:0	1.27 ± 0.39	0.717 ± 0.21	0.41 ± 0.08	0.41 ± 0.04	0.67 ± 0.09	0.4 ± 0.12	0.51 ± 0.23	0.4 ± 0.05	1.33 ± 0.25	2.44 ± 0.42
C15:0	0.52 ± 0.14	0.38 ± 0.11	0.18 ± 0.02	0.24 ± 0.02	0.47 ± 0.03	0.26 ± 0.07	0.31 ± 0.11	0.41 ± 0.05	1.14 ± 0.11	1.24 ± 0.25
C16:0	5.66 ± 1.15	3.22 ± 0.12	1.37 ± 0.22	1.35 ± 0.14	2.05 ± 0.18	1.24 ± 0.34	1.08 ± 0.41	2.39 ± 0.24	24.12 ± 1.62	36.67 ± 3.42
C17:0	0.56 ± 0.22	0.39 ± 0.08	0.35 ± 0.03	0.24 ± 0.05	0.4 ± 0.06	0.27 ± 0.1	0.32 ± 0.15	n.d.	2.95 ± 0.2	0.72 ± 0.03
C18:0	5.06 ± 1.52	3.11 ± 0.21	1.84 ± 0.13	1.4 ± 0.16	1.82 ± 0.07	1.68 ± 0.2	1.17 ± 0.23	1.04 ± 0.13	5.66 ± 0.5	4.09 ± 0.21
C19:0	0.56 ± 0.11	0.36 ± 0.06	0.49 ± 0.01	0.53 ± 0.05	0.7 ± 0.01	0.67 ± 0.2	0.63 ± 0.18	n.d.	n.d.	n.d.
C20:0	1.66 ± 0.43	1.26 ± 0.17	1.59 ± 0.07	1.37 ± 0.12	1.53 ± 0.07	1.53 ± 0.14	1.27 ± 0.24	n.d.	2.89 ± 0.57	0.99 ± 0.19
C21:0	1.1 ± 0.27	0.73 ± 0.15	1.44 ± 0.13	1.24 ± 0.11	1.35 ± 0.03	1.35 ± 0.2	1.61 ± 0.03	n.d.	1.12 ± 0.09	8.52 ± 1.23
C22:0	1.12 ± 0.29	0.99 ± 0.25	1.19 ± 0.02	0.85 ± 0.12	1.23 ± 0.29	1.12 ± 0.46	1.01 ± 0.48	n.d.	13.28 ± 1.31	2.3 ± 0.99
C23:0	1.25 ± 0.45	0.82 ± 1.18	1.26 ± 0.23	1.951 ± 0.36	1.33 ± 0.49	1.2 ± 1.16	3.52 ± 0.78	4.12 ± 0.42	11.76 ± 0.44	14.94 ± 0.72
C24:0	1.52 ± 0.37	10.16 ± 9.24	1.18 ± 0.72	1.84 ± 1.02	0.45 ± 0.09	1.21 ± 0.35	n.d.	4.58 ± 6.05	11.91 ± 1.42	7.88 ± 1.25
C26:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C28:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ΣSFA	22.87 ± 1.89	29.74 ± 3.34	17.05 ± 1.02	17.28 ± 1.07	18.95 ± 1.12	17.06 ± 0.98	17.12 ± 1.36	13.1 ± 1.48	76.65 ± 6.83	81.07 ± 9.17
C14:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C15:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	5.7 ± 0.48	n.d.	n.d.
C16:1	13.29 ± 3.41	8.56 ± 0.36	1.73 ± 0.35	0.95 ± 0.23	1.57 ± 0.34	1.04 ± 0.01	0.75 ± 0.34	0.89 ± 0.2	3.6 ± 0.24	2.69 ± 0.29
C17:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	64.04 ± 4.04	n.d.	n.d.
C18:1n9 c	0.67 ± 0.17	0.41 ± 0.04	0.35 ± 0.02	0.4 ± 0.07	0.59 ± 0.01	0.42 ± 0.08	0.45 ± 0.19	n.d.	3.38 ± 0.17	2.99 ± 1.07
C18:1n9 t	1.2 ± 0.38	0.69 ± 0.08	0.47 ± 0.04	0.41 ± 0.1	0.58 ± 0.02	0.46 ± 0.08	0.43 ± 0.27	n.d.	2.12 ± 0.07	0.97 ± 0.15
C20:1n9	5.05 ± 0.81	4.59 ± 0.47	5.99 ± 0.03	6.34 ± 0.46	6.87 ± 0.16	6.51 ± 0.21	5.67 ± 0.36	n.d.	n.d.	n.d.

(Continues)

TABLE 2 (Continued)

Biochemical composition	Sea cucumber T0	Sea cucumber TF	Sea cucumber 40S	Sea cucumber 15C	Sea cucumber 15Z	Sea cucumber 40C	Sea cucumber 40Z	Diet sediment	Diet Zostera noltii	Diet Cymodocea nodosa
C22:1n9	0.59 ± 0.13	0.65 ± 0.49	0.8 ± 0.46	1.04 ± 0.11	0.66 ± 0.39	0.53 ± 0.3	0.93 ± 0.45	n.d.	n.d.	n.d.
C23:1	5.1 ± 1.73	3.83 ± 1.68	7.52 ± 0.39	12.43 ± 2.36	6.47 ± 1.43	10.34 ± 1.77	15.16 ± 3.97	13.91 ± 8.46	n.d.	n.d.
C24:1	3.86 ± 0.95	3.86 ± 1.44	4.32 ± 0.72	4.46 ± 0.77	3.32 ± 0.5	3.84 ± 0.74	4.71 ± 1.27	0.58 ± 0.47	2.8 ± 1.59	3.12 ± 1.88
∑MUFA	33.54 ± 1.89	30.34 ± 3.34	31.9 ± 1.02	39.4 ± 1.07	31.72 ± 1.12	35.99 ± 0.99	42.04 ± 1.36	85.16 ± 1.48	11.92 ± 6.83	9.78 ± 9.17
C16:3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C16:2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C18:3n6	0.29 ± 0.11	0.16 ± 0.07	0.21 ± 0.04	n.d.	n.d.	n.d.	n.d.	0.51 ± 0.06	0.92 ± 0.2	1.27 ± 0.28
C18:2n6	0.45 ± 0.33	0.26 ± 0.04	0.29 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	7.97 ± 1.09	5.12 ± 1.06
C20:4n6	14.13 ± 1.06	18.45 ± 4.15	27.22 ± 1.12	27.65 ± 1.45	31.41 ± 0.7	29.92 ± 1.27	27.54 ± 2.74	n.d.	n.d.	n.d.
C20:5n3	21.42 ± 4.03	12.81 ± 1.85	11.29 ± 0.03	9.73 ± 0.4	10.2 ± 0.61	10.56 ± 0.36	8.73 ± 0.87	1.24 ± 0.23	2.54 ± 0.55	2.788 ± 0.35
C20:3n3/C20:3n6	2.7 ± 0.74	2.13 ± 0.37	3.34 ± 0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C20:2n6	0.59 ± 0.25	0.36 ± 0.04	0.42 ± 0.04	0.38 ± 0.03	0.44 ± 0.07	0.39 ± 0.05	0.36 ± 0.06	n.d.	n.d.	n.d.
C22:6n3	0.66 ± 0.1	0.26 ± 0.03	0.26 ± 0.04	0.24 ± 0.04	0.28 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.
C22:2	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.14	n.d.	n.d.	n.d.	n.d.	n.d.
∑PUFA	43.58 ± 4.7	39.92 ± 3.85	51.05 ± 4.21	43.31 ± 6.16	49.33 ± 4.21	46.95 ± 5.49	40.85 ± 7.17	1.74 ± 20.01	11.42 ± 1.59	9.15 ± 1.39
∑(n-3)	24.89	17.55	17.38	15.09	16.58	16.42	13.19	1.26	2.54	2.78
∑(n-6)	15.65	19.51	28.63	28.23	32.11	30.53	28.08	0.51	8.88	6.38
∑(n-3)/∑(n-6)	1.59	0.89	0.61	0.53	0.52	0.54	0.49	2.47	0.28	0.44
PUFA/SFA	1.91	1.34	2.99	2.51	2.6	2.75	2.39	0.13	0.15	0.11

Note: Different letters within the same variable indicate significant differences, (Nemenyi post hoc, $p < .05$). Mean ± standard error.

3.3 | Mineral content of *H. arguinensis*

H. arguinensis showed different proportions among analysed minerals, but any evident pattern linked with diets. *H. arguinensis* showed similar Ca, Mg and K content in all the individuals fed with the different feeding treatments (Table 3). The individuals *arguinensis* fed with 40C showed the highest Na content, being only significantly different to the individuals collected at the experiment setup (T0) which had the lowest Na content (Table 3, Kruskal–Wallis test, $p < .05$). The individuals collected at the end of the experiment (TF) showed the highest Fe, Cr and Mn content, being only significantly different to the individuals fed with 40Z for the Fe and Cr content and to animals fed with 40C for the Mn content; these last groups showed the lowest Fe and Mn content for 40Z and 40C, respectively (Table 3, Kruskal–Wallis test, $p < .05$). The individuals of *H. arguinensis* collected at the experiment setup (T0) showed the highest Zn and Cu content, being only significantly different to the individuals fed with 40C (Table 3, Kruskal–Wallis test, $p < .05$). The toxic minerals Ni, Pb and Cd were not identified in any of the groups (Table 3).

3.4 | Fatty acid (FA) profiles of *H. arguinensis*

The FA profiles of all *H. arguinensis* groups were mainly composed of arachidonic acid (ARA; C20:4n-6), 14-tricosenoic acid (C23:1), eicosapentaenoic acid (EPA; C20:5n-3), cis-11-eicosenoic acid (C20:1n9) and nervonic acid (C24:1; Table 2), which represent more than 56% of the FA content for the animals fed with the different diets, and 43% and 49% for those collected at T0 and TF, respectively. Similar amount of cis-11-eicosenoic acid (C20:1n9) and nervonic acid (C24:1) was found for all the different groups (Table 2). A slightly higher amount of eicosapentaenoic acid (EPA; C20:5n-3) was found in the individuals collected from the Ria Formosa at TF (12%) than the individuals fed with the different diets (6%–12%; Table 2). However, individuals fed with the different diets showed a significant higher amount of arachidonic acid (ARA; C20:4n-6) and 14-tricosenoic acid (C23:1; >27% and >6%, respectively) than the individuals collected at TF (18% and 3%, respectively; Table 2). Higher amount of PUFA and MUFA (>30%–51%) were found in all the groups than SFA (17%–29%; Table 2). The individuals fed with the different diets showed higher amount of PUFA and MUFA than the individuals collected from the Ria Formosa (T0 and TF), which showed higher amount of SFA (Table 2). Therefore, these last two groups showed lower ratio PUFA/SFA (Table 2). The individuals fed with the different diets presented higher level of omega-6 FA, due mainly to ARA, than the individuals collected from the Ria Formosa (T0 and TF). However, similar amounts of omega-3 FA were found for all the groups (Table 2). So, higher ratio omega-3/omega-6 was found for these last two groups (T0 and TF, Table 2).

The individuals fed with the different diets showed similar SFA (Table 2). The individuals fed with 40S showed lower level of MUFA than the individuals fed with the two seagrass species, however, the 40S individuals showed higher level of MUFA, therefore, the 40S individual showed higher ratio PUFA/SFA (Table 2). The individuals fed with 40S showed higher level of omega-3 FA and lower/similar levels of omega-6 FA than the other feed groups, and therefore, higher ratio omega-3/omega-6 was obtained for the 40S individuals (Table 2).

4 | DISCUSSION

H. arguinensis is a sea cucumber species from the North-Eastern Atlantic and SW Mediterranean that it is being exploited to supply the high demand for sea cucumbers from the Asiatic markets. In parallel to its exploitation, *H. arguinensis'* aquaculture has started to be developed, being widely improved in the last years (Domínguez et al., 2015; Domínguez-Godino & González-Wangüemert, 2017a, 2018a, 2018c, in review a). Artificial diets for sea cucumber aquaculture are highly needed to ensure and improve growth rates. This is the first work in which different artificial diets were tested on *H. arguinensis* aquaculture, using the two more common species of seagrass (*Z. noltii* and *C. nodosa*) present in the same habitats where *H. arguinensis* can be found at Ria Formosa (Domínguez-Godino and González-Wangüemert, in review b; González-Wangüemert et al., 2013; Siegenthaler et al., 2015).

In this study, the specific growth rate (SGR), absolute growth rate (AGR) and weight change for the individuals fed with the two levels of *Z. noltii* were slightly higher than the control animals fed with sediment, and than those ones fed with *C. nodosa*, which showed negative values for these parameters. The improvement of growth rates when *H. arguinensis* was fed with *Z. noltii* compared with the control might be due to the lower organic content and nutritional values of the sediment. In fact, this trend could be reinforced with the observation of higher improvement of growth, when the individuals were fed with the 40% of *Z. noltii* (40Z feeding treatment). Similar results were obtained for *A. japonicus*, which showed significantly higher SGR and higher final wet weight for the individuals fed with *Z. marina* debris in comparison with the ones fed just with sediment (control; Liu et al., 2013). However, unexpected negative values of growth were obtained for the individuals fed with the two levels of *C. nodosa*. This result could be explained by the different microbial and microphytobenthic community linked to these seagrass species under decomposition. In fact, *H. arguinensis* along Ria Formosa is mainly associated with *Z. noltii* meadows, where higher densities can be found (authors observation). Therefore, *C. nodosa* under our experimental conditions was an unsuitable food source for *H. arguinensis*, which had harmful effect on the target sea cucumber species with a negative growth. In addition, it is important to take in account that deposit-feeder sea cucumbers have low cellulase activity in their guts (Yingst, 1976), and therefore, they could not digest seagrass debris which is rich in cellulose content (*Z. noltii* 28% and *C. nodosa* 45% cellulose, Milović et al., 2017; Sèbe, Pardon, Pichavant, Grelier, & De Jéso, 2004). Also, seagrass decomposition rate is negatively correlated to the total fibre content (Godshalk &

TABLE 3 Mineral composition (mg/g) of the two groups of *H. arguinensis* collected at the setup (T0) and end of the experiment (TF) and the five different groups under feeding treatments (40S, 15C, 15Z, 40C and 40Z)

Mineral	Symbol	T0	TF	40S	15C	15Z	40C	40Z
Macroelements								
Calcium	Ca	64.456 ± 2.139[a]	63.247 ± 1.08[a]	62.924 ± 2.617[a]	64.593 ± 3.885[a]	68.329 ± 8.8[a]	62.853 ± 2.93[a]	66.141 ± 6.533[a]
Magnesium	Mg	9.952 ± 0.426[a]	10.259 ± 0.066[a]	10.364 ± 0.187[a]	11.058 ± 0.332[a]	10.448 ± 0.659[a]	11.269 ± 0.145[a]	10.621 ± 0.462[a]
Sodium	Na	39.747 ± 1.323[a]	43.819 ± 0.802[ab]	45.682 ± 1.213[ab]	46.551 ± 1.048[ab]	41.587 ± 1.459[ab]	48.4 ± 0.583[b]	43.196 ± 1.523[ab]
Potassium	K	14.126 ± 0.97[a]	14.673 ± 0.241[a]	14.178 ± 0.404[a]	14.449 ± 0.428[a]	12.955 ± 0.861[a]	14.428 ± 0.42[a]	12.956 ± 0.291[a]
Microelements								
Iron	Fe	0.177 ± 0.016[ab]	0.247 ± 0.015[a]	0.135 ± 0.009[ab]	0.112 ± 0.006[ab]	0.151 ± 0.007[ab]	0.111 ± 0.007[ab]	0.109 ± 0.01[b]
Manganese	Mn	0.01 ± 0.001[ab]	0.039 ± 0.001[a]	0.0121 ± 0.001[ab]	0.011 ± 0.001[ab]	0.012 ± 0.001[ab]	0.008 ± 0.001[b]	0.009 ± 0.001[ab]
Zinc	Zn	0.037 ± 0.002[a]	0.027 ± 0.001[ab]	0.021 ± 0.001[ab]	0.021 ± 0.003[ab]	0.015 ± 0.001[ab]	0.025 ± 0.002[b]	0.016 ± 0.001[ab]
Toxic elements								
Chromium	Cr	0.012 ± 0.001[ab]	0.013 ± 0.001[a]	0.008 ± 0.001[ab]	0.004 ± 0.001[ab]	0.007 ± 0.001[ab]	0.006 ± 0.001[ab]	0.004 ± 0.001[b]
Nickel	Ni	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Copper	Cu	0.028 ± 0.001[ab]	0.007 ± 0.001[a]	0.005 ± 0.001[ab]	0.005 ± 0.001[ab]	0.004 ± 0.001[ab]	0.006 ± 0.001[b]	0.005 ± 0.001[ab]
Lead	Pb	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Cadmium	Cd	n.d	n.d	n.d	n.d	n.d	n.d	n.d

Note: Different letters within the same variable indicate significant differences, (Nemenyi post hoc, $p < .05$). Mean ± standard error.

Wetzel, 1978); on this way, considering only the cellulose content of both species (Milović et al., 2017; Sèbe et al., 2004), *C. nodosa* would decompose slower than *Z. noltii*, accumulating on the sediment and not being available as food resource for sea cucumbers.

Land-based intensive culture systems in China use as optimal feed the macroalgae *Sargasum polycystum* or *S. thunbergii* for the commercial production of *A. japonicus* (Zhu et al., 2007). However, the use of these macroalgae relies on wild populations, therefore, with the increase in *A. japonicus* aquaculture production, there is a need to find other suitable food sources for artificial fed. Some studies have been done testing other macroalgae species, along with the seagrass *Z. marina*. For *A. japonicus* individuals (~4 g) fed with *Z. marina*, *U. lactuca*, fresh *Laminaria japonica* or boiled *L. japonica*, similar SGR values were registered, being also significantly higher than those obtained on sea cucumbers fed with *S. polycystum* or *S. thunbergii* (Xia et al., 2012). However, larger individuals of *A. japonicus* (~31 g) showed higher SGR, apparent digestibility rate and food conversion efficiency when were fed with *S. thunbergii*, when compared with animals fed with *Chatomorpha linum*, *Z. marina* and with a mix of these last two species (Song et al., 2017). Additionally, in this last study, it was found that there was a reduction on the apparent digestibility rate and food conversion efficiency as the proportion of *Z. marina* increased, which could be related to the low cellulase activity of sea cucumber´s guts according to the authors. The different results obtained on the same species might be due to the different nutrition requirements linked with the weight/length of individuals (Yanagisawa, 1998; Yingst, 1976).

The feeding rates obtained for the *H. arguinensis* individuals at the different feeding treatments showed a significant reduction, as the organic matter content increased on the supplied food. Additionally, when the same percentage of the seagrass species (15% and 40%) were used in the diets, lower feeding rates were registered on animals under *Z. marina* diets, corresponding also with a different organic matter content (301.98 and 452.62 mg/g for *Z. marina* and *C. nodosa*, respectively). This result is in accordance with sea cucumber ability to modulate their feeding activity/rate and absorption efficiency, as compensatory response to sediment quality and quantity (Roberts, Gebruk, Levin, & Manship, 2003). Similar compensatory response has been found for *A. japonicus* (Yuan et al., 2006) and *Australostichopus mollis* (Zamora & Jeffs, 2012). Generally, *A. japonicus* feeds on sediments containing organic matter, which includes microorganisms and the detritus of plants or animals (Song et al., 2017). According to that study, a mixture of *Z. marina* debris and seafloor muddy sediments with an organic content of 19.6% (ES40) could lead to a better growth on *A. japonicus* (Song et al., 2017).

The use of two different seagrass species debris at different proportions on diets has not changed the biochemical composition, minerals and fatty acids content of *H. arguinensis'* body wall. Similar results of lack of changes on biochemical composition and fatty acids of *A. japonicus* fed with different artificial diets, composed of seaweeds and/or terrestrial plant, were obtained previously (Seo, Shin, & Lee, 2011; Xia et al., 2012). However, Wen et al. (2016) found changes on *A. japonicus* FAs content among the different groups fed

with prepared diets: FAs were detected in high quantities in the body wall of animals fed with a single macroalgae species. However, *A. japonicus'* individuals fed with a mixture of different macroalgae and benthic matter showed a significant decrease of FAs level (mainly ARA; Wen et al., 2016). These individuals showed similar or elevated concentrations of EPA and docosahexaenoic acid (22:6n-3, DHA) and consequently enhanced n-3/n-6 PUFAs ratios than the ones fed with single macroalgae (Wen et al., 2016). According to the authors, these results could be due to the ingestion of diatoms and dinoflagellates which are rich in EPA and DHA, respectively, and which are present in the benthic matter (Budge, Parrish, & McKenzie, 2001; Meziane & Tsuchiya, 2000; Wen et al., 2016; Wong, Gao, Cheung, & Shin, 2008). In addition to the growth and feeding rates, the FAs profile confirms that *H. arguinensis* is not assimilating the different seagrass species since both presented high levels of SFA (>76% for *Z. noltii* and 81% for *C. nodosa*) and low levels of MUFA (<12%) and PUFA (<12%), in contrast to *H. arguinensis* profile which is showing high level of MUFA (>30%) and PUFA (>39%), with low level of SFA (<19%). *H. arguinensis* showed high level of ARA (>27%) and EPA (>8%), which were not detected in *Z. noltii* or found in low proportion (<2.7%) in *C. nodosa*. The high levels of ARA and EPA found on *H. arguinensis* could be explained by the ingestion of diatoms and other microalgae species living in the added sediment to the bottom of the tanks, species that are rich in these same FAs (Budge et al., 2001; Cañavate, 2018; Meziane & Tsuchiya, 2000; Wong et al., 2008). As it was suggested previously, the microphytobenthic community of the sediment would have taken advantages from the seagrass decomposition (release nutrients), which could favour the microphytobenthic growth. This hypothesis could be supported by the fact that *H. arguinensis´* individuals fed with the different diets showed slightly higher PUFA and FAs (mainly ARA) than the *H. arguinensis* collected at Praia de Faro (T0 and TF). The lower values obtained for *H. arguinensis* at TF in comparison with the ones at T0 in terms of protein, lipids and FAs could be due to the use of these components on gonad development and gamete maturation during the last period of *H. arguinensis´* gametogenic cycle (April, May and June; Marquet, Conand, Power, Canário, & González-Wangüemert, 2017), such as it has been reported on *H. scabra* from India (Krishnan, 1968).

H. arguinensis did not show any significant pattern on its biochemical composition, mineral and FAs along the duration of the experiment, since similar results were obtained for the individuals collected at T0 and TF, and the ones fed with the different diets. The absence of differences could be due to the short duration of the experiment and the low range of variation of seawater temperature during the experimental period. For example, moisture, carbohydrate, protein, lipid contents, total amino acids, ratios of essential amino acids, proportions of SFA and PUFA on *A. japonicus* showed significant seasonal variation (Gao, Xu, & Yang, 2011). *A. japonicus* is a temperate sea cucumber species importantly influenced by seawater temperature; in fact, this species enters in aestivation during the summer months, when it can lose 30%–50% of its body mass (Liu et al., 1996; Yang et al., 2005). Therefore, this aestivation period

is driving its changes on biochemical composition, amino acids and FAs (Gao et al., 2011). *H. arguinensis* could show changes on biochemical composition, amino acids and FAs along the year, because this species enters in a hibernation period during winter months with low and/or negative values of specific growth rates, which are positive and high during summer months (Domínguez-Godino & González-Wangüemert, 2018a; Olaya-Restrepo, Erzini, & González-Wangüemert, 2018). In addition, changes on lipids and protein on *H. arguinensis* could be found along the year, since it could be used for gametogenesis during winter (Marquet et al., 2017). Therefore, it could be expected to find differences on biochemical composition, amino acids and FAs among the main four seasons and not across each season and/or periods with similar environmental conditions (mainly seawater temperature and salinity), such as during our experimental period.

5 | CONCLUSION

Adults of *H. arguinensis* showed higher growth when were fed with *Z. noltii* debris than with *C. nodosa* and/or the sediment (control). However, this sea cucumber species showed negative growth when fed with *C. nodosa*, which could be due to different microphytobenthic communities growing in the sediment and/or the different cellulose content among both seagrass species. Therefore, *C. nodosa* would be an unsuitable food source. *H. arguinensis*, as other sea cucumbers species, modulates its feeding rate which increased as the organic matter decreased in the given food. *H. arguinensis* fed with the different seagrass species and proportions did not show significant differences on biochemical composition (proximate composition, protein, lipid and mineral contents, and fatty acids profile) and neither in comparison with the individuals collected from the natural populations (T0 and/or Tf). Therefore, *Z. Noltii* debris could be used as food source for *H. arguinensis* adults for its maintenance under tank conditions during the breeding periods.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR'S CONTRIBUTION

The authors contributed on the research and its publication along the study design, data analysis and paper drafting.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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