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## Food sources, digestive efficiency and resource allocation in the sea cucumber *Holothuria forskali* (Echinodermata: Holothuroidea): Insights from pigments and fatty acids

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2  
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9 4 Running title: Pigments and fatty acids in *Holothuria forskali*  
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3 32 1. Abstract  
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8 Various research projects in Europe and North Africa have recently intended to breed  
9 35 temperate holothurians to alleviate fishing pressure on natural populations. However, to date  
10 36 little is known about the nutritional requirements of East Atlantic and Mediterranean species.  
11 37 In this study, we propose a “natural populations” oriented approach to characterise food  
12 38 sources, digestive efficiency and resources allocation based on the composition of pigments  
13 39 and fatty acids (FA) in gut contents and tissues (muscles, gonads and digestive tract walls) of  
14 40 wild individuals of the species *Holothuria (Panningothuria) forskali* (Delle Chiaje, 1823)  
15 41 sampled in Brittany (France). Our study reveals that neither green nor red algae enter the diet  
16 42 of *H. forskali* in spring, and that the only fresh vegetal material found in gut contents is brown  
17 43 algae (very likely diatoms). The high nutritional quality of gut contents however contrasts  
18 44 with the detrital nature of the ingested food sources, suggesting that a trophic upgrading of  
19 45 organic matter occurs before digestion. In addition, unusual FA (i.e. only present in a few  
20 46 groups of living species) such as long-chain monounsaturated FA (especially the FA 23:1 $\omega$ 9)  
21 47 were found in large proportions in muscles and gonads and their effect on sea cucumber  
22 48 fitness needs further investigation.  
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35 50 Keywords: sea cucumbers, echinoderms, *Holothuria forskali*, gut contents, pigments, fatty  
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## 53 2. Introduction

54 Sea cucumbers have been captured since hundreds of years in the central Indo-Pacific  
55 but the depletion of tropical populations and the increasing market demand for human  
56 consumption, especially from Asia, have recently forced the catch of new target species from  
57 the Mediterranean Sea and the Northeastern Atlantic Ocean (Eriksson et al. 2015). As a  
58 consequence, various populations are already threatened, as indicated by the diminution of  
59 their abundance, their genetic diversity, the loss of biggest individuals and even “local  
60 extinction” in some places (González-Wangüemert et al. 2018). To satisfy the Asian demand  
61 and reduce the pressure on natural populations, various research projects have been launched  
62 to initiate the breeding of temperate holothurians. Artificial reproduction and larval rearing is  
63 beginning to be managed for various Mediterranean species such as *Holothuria arguinensis*  
64 (Domínguez-Godino et al. 2015, Domínguez-Godino and González-Wangüemert 2019), *H.*  
65 *mammata* (Domínguez-Godino et al. 2018), *H. tubulosa* (Rakaj et al. 2018), *H. polii* (Rakaj et  
66 al. 2019) and one species spread both around the Mediterranean basin and in the Northeastern  
67 Atlantic Ocean: *Holothuria forskali* (Léonet et al. 2009, Santos et al. 2015, Laguerre et al.  
68 2020).

69 However, to date little is known about the nutritional requirements of temperate  
70 holothurians that are considered to date as detritivorous deposit-feeders (Roberts et al. 2000).  
71 Nutrition of *H. forskali* has essentially been studied through histological observations and  
72 sand progression in the digestive tract (Stott 1957, Massin and Jangoux 1976, Roberts et al.  
73 2000), digestive enzymes identification (Féral 1989, Roberts et al. 2000) and stomach  
74 contents analyses (Roberts et al. 2000, Mezali and Soualili 2013, Belbachir et al. 2014,  
75 Belbachir and Mezali 2018). The digestive tract is constituted by the pharynx, crop, stomach,  
76 intestine, rectum and the cloaca (Stott 1957, Féral and Massin 1982) that were attributed to  
77 specific digestive functions (Massin and Jangoux 1976): storage (pharynx and crop), digestion  
78 (stomach and intestine) and elimination (second descending segment of the intestine, rectum  
79 and cloaca). *H. forskali* ingests sediment at a feeding rate of 0.6 to 10 % of its dry body  
80 weight per hour with a gut residence time of 10 h (Roberts et al. 2000). Most hydrolytic  
81 enzyme activity occurs in the anterior intestine (first descending and ascending segments of  
82 the intestine), although the same types of enzymes also occur in the posterior intestine  
83 (second descending segment). It possesses the majority of common enzymes to digest lipids  
84 and glycosidic compounds but a limited ability to digest proteins, as revealed by weak  
85 endopeptidase activity (Féral 1989, Roberts et al. 2000). Its ability to select organic-rich

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3 86 patches of sediment has first been suggested through histological analysis (Bouland et al.  
4 87 1982) and then demonstrated on natural populations that favour the ingestion of 40-60  $\mu\text{m}$   
5 88 sand grain size (Mezali and Soualili 2013) and organic-rich particles (Belbachir et al. 2014).  
6 89 The digestive tract is mostly filled with sand grains although the species is most generally  
7 90 observed on rocky substrates (Massin and Jangoux 1976, Tuwo and Conand 1992, pers.  
8 91 observations). Out of the sand, the gut content is dominated by sponge spicules, diatoms,  
9 92 cyanophyceae, macrophytes, foraminifera, crustaceans and seagrass fragments, with large site  
10 93 variability (Belbachir and Mezali 2018, Badou pers. observations). Detritus and fungal  
11 94 hyphae were also recorded (Roberts et al. 2000).

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19 95 Yet, the ingestion of coarse fragments of macrophytes has been suggested to be the  
20 96 result of a not so efficient feeding selectivity. The concentration of certain items in the gut of  
21 97 holothurians may thus be the result of particles physical properties (such as specific gravity,  
22 98 density or microtopography of the surface) rather than selection of particles that are organic-  
23 99 rich or within a specific size range (Roberts et al. 2000). More generally, deposit-feeding  
24 100 holothurians process large quantities of nutrient poor sediments and ingested food items may  
25 101 not all be assimilated. Other studies than those previously conducted are thus required to  
26 102 understand nutritional requirements of *H. forskali* for a rearing purpose. Feeding requirements  
27 103 in marine species have generally been assessed using feeding trials and calculating optimal  
28 104 growth rate (e. g. Kanazawa et al. 1979, Domínguez-Godino et al. 2020) and/or maximal  
29 105 hydrolytic activity (Van Wormhoudt et al. 1980) related to the inclusion of a given amount of  
30 106 a specific compound in the food. In this study, we propose a “natural populations” oriented  
31 107 approach to characterise food sources, digestive efficiency and resources allocation based on  
32 108 differences in the composition of pigments and fatty acids (FA), that are reliable biomarkers  
33 109 in ecological studies (Madji et al. 2018), between gut fractions, faeces and tissues of *H.*  
34 110 *forskali*. This work aims at improving the basic knowledge of *H. forskali* nutrition in the wild  
35 111 to facilitate the development of a commercial food for its rearing in captivity. We  
36 112 hypothesised that (i) some food items are preferentially digested during gut transit and others  
37 113 remain untransformed; (ii) specific elements are preferentially retained and/or converted for  
38 114 physiological functions such as growth, tissues regeneration or reproduction.

### 115 3. Materials and methods

116

#### 117 3. 1. Sample collection of wild individuals

118 Ten sea cucumbers of the species *Holothuria (Panningothuria) forskali* Delle Chiaje,  
119 1823 were sampled on May 2, 2019 east of the Glenan Islands (Brittany – France; 47.710°N,  
120 3.948°E) at 8-11 m deep in the early afternoon (bottom water temperature  $\approx 16^{\circ}\text{C}$ ). The  
121 sampling area was characterised by a rocky substrate where *Laminaria sp.* (Phaeophyceae -  
122 Laminariales) forests develop along with various species of red algae. Patches of sediment  
123 dominated by sand and shell fragments accumulated in the depressions. Sea cucumbers were  
124 immediately dissected in the field and gut content was divided in three parts (Fig. 1)  
125 corresponding to specific digestive functions as proposed by Massin and Jangoux (1976):  
126 storage (foregut from the upper part of the digestive tract to 2 cm above the attachment point  
127 of the *rete mirabile*), digestion (midgut corresponding to the first descending and ascending  
128 segments of the intestine and sometimes referred in the literature as anterior intestine) and  
129 elimination (hindgut from the second descending segment of the intestine to the rectum). The  
130 midgut was carefully emptied to avoid remains of food and its first half was isolated from the  
131 *rete mirabile* and conserved for further analysis. Gonads and muscles isolated from muscular  
132 bands were also sampled. Histological examination of the gonads indicated that they were at  
133 the end of the gametogenesis and that collected individuals (six females and four males) were  
134 about to spawn (Tuwo and Conand 1992, Santos et al. 2015). Blades of brown (*Laminaria*  
135 *sp.*) and red (*Cryptopleura ramosa*) macroalgae were collected by hand and sediment was  
136 sampled in the depressions using a 50 mL syringe connected to a 5 mm inner diameter plastic  
137 tube. Surface water was also collected and vacuum-filtered through pre-combusted (5 h at  
138  $450^{\circ}\text{C}$ ) and pre-weighted glass fibre filters (Whatman GF/F  $0.7\ \mu\text{m}$ ).

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#### 140 3. 2. Collected of feed and faeces of farmed individuals

141 We collected food sources and corresponding faeces of holothurians opportunistically  
142 in May-June 2019 at the experimental sites where the different partners of the HOLOFARM  
143 research project maintain livestock. The Concarneau marine station maintains broodstock in  
144 500 L tanks with 5 cm of coarse sand on the bottom to allow microbial development, and  
145 feeds them with a combination of Shellfish Diet 1800<sup>®</sup> (Reed Mariculture) with a paste  
146 mixture of Grower Fertil<sup>®</sup> (Le Gouessant) shrimp pellets and fresh mussels. The Beg Meil

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3 147 station of Agrocampus-Ouest maintains 2-4 cm juveniles in 25 L plastic tanks cleaned daily,  
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5 148 and feeds them with a mixture of microalgae produced on site (the two Bacillariophyceae  
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7 149 *Chatoceros sp.* and *Skeletonema sp.* and the Prymnesiophyceae *Isochrysis sp.*). Finally, the  
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9 150 AquaB Company in Plobannalec-Lesconil maintains 2-4 cm juveniles in 50 cm diameter  
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11 151 polyamide sieves (20  $\mu\text{m}$  mesh size) placed in cylindroconical tanks with a circulating filtered  
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13 152 seawater system, and feeds them with decomposed green macroalgae (*Ulva sp.* or  
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15 153 *Enteromorpha sp.*). Green seaweeds are gathered on nearby beaches, left to decompose  
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17 154 during a few weeks in aerated cylindroconical tanks and the mixture is filtered before feeding  
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19 155 events to collect particles in the size 50-300  $\mu\text{m}$ . All experimental stations constantly renew  
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21 156 the water in culture tanks with filtered seawater pumped nearby, maintaining water  
22  
23 157 parameters similar to that of the ocean. The list of collected samples and major associated  
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25 158 weaknesses is presented in Table 1. All samples were kept at  $-25^{\circ}\text{C}$  and analysed within 2  
26  
27 159 months after recollection.

### 28 161 3. 2. Sample processing

29  
30 162 Organic matter of wild *H. forskali* gut contents and nearby sediment was quantified  
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32 163 gravimetrically after combustion of freeze-dried material in a muffle furnace (4 h at  $450^{\circ}\text{C}$ ).  
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34 164 Pigments were analysed by high performance liquid chromatography (HPLC) according to  
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36 165 Brotas and Plante-Cuny (2003). Sub-samples of freeze-dried material (10-15 mg for sediment,  
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38 166 gut contents and faeces and 5-10 mg for potential food sources) were incubated with 2 mL of  
39  
40 167 methanol (buffered with 2% ammonium acetate) during 15 min, at  $-25^{\circ}\text{C}$  in the dark. Extracts  
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42 168 were then filtered with 0.2  $\mu\text{m}$  PTFE syringe filters and analysed within 16 h using an Agilent  
43  
44 169 1260 Infinity HPLC composed of a quaternary pump (VL 400 bar), a UV-VIS photodiode  
45  
46 170 array detector (DAD 1260 VL, 250–900 nm) and a 100  $\mu\text{l}$  automatic sample injector  
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48 171 refrigerated at  $4^{\circ}\text{C}$  in the dark. Chromatographic separation was carried out using a C18  
49  
50 172 column for reverse phase chromatography (Supelcosil, 25 cm long, 4.6 mm inner diameter).  
51  
52 173 The solvents used were A: 0.5 M ammonium acetate in methanol and water (85:15, v:v), B:  
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54 174 acetonitrile and water (90:10, v:v), and C: 100% ethyl acetate. The solvent gradient was set  
55  
56 175 according to Brotas and Plante-Cuny (2003), with a  $0.5\text{ mL min}^{-1}$  flow rate. Identification and  
57  
58 176 calibration of the HPLC peaks were performed with antheraxanthin,  $\beta\beta$ -carotene,  
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60 177 canthaxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyll *c2*, diatoxanthin, diadinoxanthin,  
178 fucoxanthin and pheophytin *a* standards. We identified all detected peaks by their absorption  
179 spectra and relative retention times using the Agilent OpenLab software. Quantification was

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3 180 performed using standard calibration curves built with repeated injections of standards over a  
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5 181 range of dilutions. Carotenoids and chlorophyll *b* and *c* were quantified at 470 nm,  
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7 182 chlorophyll *a* and their derivatives as well as pheopigments were quantified at 665 nm. The  
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9 183 relative abundance of each pigment (%) was calculated from its respective concentration in  
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11 184 the sample ( $\mu\text{g mg}^{-1}$ ).

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13 185 Lipids were extracted following a slightly modified protocol of Bligh and Dyer  
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15 186 (1959), as described in Meziane et al. (2007). Sub-samples of freeze-dried material (100-300  
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17 187 mg for sediment, gut contents and wild population faeces, 30-100 mg for potential food  
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19 188 sources, muscles and aquacultured sea cucumber faeces and 10-30 mg for digestive tracts and  
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21 189 gonads) were incubated with 4 mL of a water:methanol:chloroform mixture (1:2:1, v:v:v) and  
22  
23 190 sonicated during 20 min at room temperature. Chloroform and water were added to the  
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25 191 mixture to reach equal proportions of the three solvents and allow the formation of an  
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27 192 aqueous-organic bilayer system that was separated by centrifugation (3000 rpm, 5 min). The  
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29 193 heavy phase was retrieved and the operation was repeated a second time after completion with  
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31 194 2 mL of chloroform to retrieve as much lipids as possible. In addition, 15-30  $\mu\text{g}$  of tricosanoic  
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33 195 acid (23:0) provided by Sigma-Aldrich was added to every sample prior to extraction and  
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35 196 used as internal standard. The lipid fraction, contained in the chloroform, was evaporated  
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37 197 under nitrogen ( $\text{N}_2$ ) flux and dried lipid extracts were saponified using a methanol:sodium  
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39 198 hydroxide (2 N) mixture (2:1, v:v) during 1 h 30 min, at 90 °C. Finally, fatty acid esters were  
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41 199 methylated into fatty acid methyl esters (FAME) using boron trifluoride-methanol ( $\text{BF}_3\text{-CH}_3\text{-}$   
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43 200  $\text{OH}$ ) and stored at -25 °C. We analysed FAME by gas chromatography (Agilent, 7890B GC)  
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45 201 associated with triple quadrupole mass spectrometry (Agilent, 5977B MSD) equipped with an  
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47 202 Agilent HP-5ms UI non-polar capillary column (30 m length  $\times$  0.25 mm inner diameter  $\times$   
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49 203 0.25  $\mu\text{m}$  inner diameter) and using helium as gas carrier (1.5 mL  $\text{min}^{-1}$ ). The oven  
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51 204 temperature was set at 60 °C and held for 1 min, raised at 40 °C  $\text{min}^{-1}$  to 170 °C and held for  
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53 205 1 min, increased to 195 °C at 3 °C  $\text{min}^{-1}$  and held for 15 min, raised at 3 °C  $\text{min}^{-1}$  to 220 °C  
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55 206 and held for 20 min and finally increased to 240 °C at 3 °C  $\text{min}^{-1}$  and held for 12 min. The  
56  
57 207 two plateaux at 195 and 220 °C corresponded to the elution zones of C16 and C18 fatty acids,  
58  
59 208 respectively, and temperature was held constant to improve peak delineation. After column  
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209 separation, the gas carrier containing FA was split into two fractions. The largest fraction  
210 (~80%) was sent to a flame ionisation detector (FID) set at 250 °C for peaks quantification  
211 and the remaining part was sent to the MSD for identification. Mass spectra were acquired in  
212 electron ionisation (EI) mode at 70 eV between 35-600 m/z at a scan rate of 1.3 scan  $\text{s}^{-1}$ . All



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3 213 peaks in the FID chromatograms were integrated with a workflow on Agilent masshunter  
4 214 quantitative analysis software and a routine was developed with R version 3.5.3 (R Core  
5 215 Team 2019) to attribute to each compound the same unique identifier in every chromatogram  
6 216 (if present). Peaks corresponding to FA were then identified by comparison of MS spectrum  
7 217 with the NIST mass spectral library (version 2.3) and comparison of GC retention time with a  
8 218 commercial standard (Supelco 37 component FAME mix; Sigma-Aldrich). The FA 18:1 $\omega$ 9  
9 219 co-eluted with the FA 18:3 $\omega$ 3 and the FA 16:0iso co-eluted with the FA 16:4 $\omega$ 3, which was  
10 220 taken into account for data interpretation. Results were reported in % of total FA and/or in mg  
11 221 g<sup>-1</sup> of sample dry weight.  
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### 21 223 3. 3. Statistical analysis

22 224 We performed univariate multiple comparisons using the non-parametric Van der  
23 225 Waerden test (R package “agricolae”) due to the low amount of samples to be compared (4 to  
24 226 10). Potential sexual difference in FA proportions of sea cucumber gut contents and tissues  
25 227 was assessed using permutational analysis of variance (PERMANOVA) on Bray-Curtis  
26 228 dissimilarity matrices. Statistical analysis and graphical representations were performed using  
27 229 R 3.5.3 (R Core Team 2019) and type I error ( $\alpha$ ) was set to 5%.  
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## 37 231 4. Results

### 38 232 39 233 4. 1. Organic matter (OM)

40 234 The highest proportion of OM was measured in sea cucumber foreguts (14.1  $\pm$  4.5 %) and the lowest proportion was found in nearby sediment (6.7  $\pm$  2.7 %; Fig. 2). The Van der  
41 235 Waerden test revealed a significant decrease of OM during gut transit with, on average, a one-  
42 236 third reduction in OM content between foregut and faeces (14.1  $\pm$  4.5 % in foregut vs. 9.1  $\pm$   
43 237 2.5 % in faeces; Fig. 2).  
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### 53 239 54 240 4. 2. Pigments

55 241 A total of 62 pigments have been separated by the HPLC system in all samples. Yet,  
56 242 most of compounds could not be precisely identified as the corresponding purified standard  
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3 243 was not available. Most minor pigments were derivatives of major ones, and so they were  
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5 244 combined to their closest relative compound. Major compounds and all their derivatives were  
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7 245 summed and a selective list of pigments (plus derivatives) useful to assess the food sources of  
8  
9 246 *H. forskali* was constructed (Table 2). Globally, out of chlorophyll *a*, pheophorbide *a* and  
10  
11 247 pheophytin *a*, that are found in all decomposing vegetal sources, gut contents and faeces of  
12  
13 248 wild sea cucumbers were dominated by chlorophyll *c* (14-20 %), fucoxanthin (3-5 %) and  
14  
15 249 neoxanthin (2-3 %) with proportions relatively similar to that of sediment (Table 2).  
16  
17 250 Chlorophyll *c* and fucoxanthin were major pigments of suspended particulate matter (SPM)  
18  
19 251 and brown algae (diatoms and the macroalgae *Laminaria sp.*) and were also detected in faeces  
20  
21 252 of aquacultured sea cucumbers fed with diatoms. Neoxanthin was found in SPM and green  
22  
23 253 macroalgae (*Ulva sp.* and *Enteromorpha sp.*), but it was absent from diatoms and faeces of  
24  
25 254 aquacultured individuals fed diatoms. Finally, lutein was a major pigment in red macroalgae  
26  
27 255 (*Cryptopleura ramosa*) and in both fresh and decomposed green macroalgae (20-54 %) but it  
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29 256 was almost absent from any other kind of sample (0-1.2 %), except faeces of aquacultured  
30  
31 257 individuals fed with decomposed green algae where it was a major compound (11-14 %).  
32  
33 258 Total concentration of pigments was 4-5 times higher in gut contents and faeces of wild  
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35 259 animals compared to nearby sediment but no significant difference (Van der Waerden test;  $p$   
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37 260 = 0.662) was revealed between the three gut contents and the faeces (Fig. 3).

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#### 262 4. 3. Fatty acids (FA)

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39 263 We identified 37 fatty acids with the GC-FID/MSD system in all samples. As for  
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41 264 pigments, only a selective list of FA that we considered as meaningful to assess the food  
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43 265 sources of *H. forskali* has been provided (Table 3). Yet, we reported in Table 3 the sum of  
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45 266 major classes of FA that may largely inform on the general composition of samples. The  
46  
47 267 complete list of FA was provided for wild individual tissues (Table 4). Sexual comparisons  
48  
49 268 revealed no difference between male and female gut contents FA composition  
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51 269 (PERMANOVA;  $F = 0.375$ ,  $p = 0.848$  for foregut;  $F = 0.834$ ,  $p = 0.458$  for midgut; and  $F =$   
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53 270  $0.687$ ,  $p = 0.632$  for hindgut), no difference between digestive tracts (PERMANOVA;  $F =$   
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55 271  $0.238$ ,  $p = 0.840$ ), no difference between muscles (PERMANOVA;  $F = 1.045$ ;  $p = 0.366$ ) and  
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57 272 a significant difference between gonads (PERMANOVA;  $F = 8.667$ ;  $p = 0.007$ ).  
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59 273 Consequently, FA compositions of both gender gut contents, digestive tracts and muscles  
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274 were pooled, and those of gonads were displayed according to sex (Table 3 and 4).

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3 275 Unlike for pigments composition, sediment and foregut contents differed in their FA  
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5 276 composition with higher proportions of FA 20:4 $\omega$ 6 (arachidonic acid; ARA), 20:5 $\omega$ 3  
6  
7 277 (eicosapentaenoic acid; EPA) and presence of the FA 23:1 $\omega$ 9 in the foregut and lower  
8  
9 278 proportions of FA 16:0 and saturated FA (SFA) in the sediment (Table 3). In contrast, freshly  
10  
11 279 released faeces poorly differed to nearby sediments, with only FA 23:1 $\omega$ 9 and 24:1 $\omega$ 9  
12  
13 280 significantly higher and the sum of branched FA (BrFA) significantly lower in faeces (Table  
14  
15 281 3). Highly unsaturated FA (HUFA) and long-chain monounsaturated FA ( $\geq$  20-carbon chain  
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17 282 length; LC-MUFA) were present in low proportions in most potential food sources, while  
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19 283 they were relatively abundant in *H. forskali* tissues (Table 3 and 4). Total concentration of FA  
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21 284 strongly differed among tissues with higher concentrations in female gonads (Table 4).  
22  
23 285 Muscles were characterised by especially high levels of ARA and FA 23:1 $\omega$ 9 compared to  
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25 286 other tissues (Table 4) and different  $\omega$ 3/ $\omega$ 6 and eicosanoid ratios (Fig. 4). Male gonads were  
26  
27 287 significantly richer in ARA, EPA and FA 20:1 $\omega$ 9 compared to female gonads and poorer in  
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29 288 FA 16:0 and BrFA (Table 4).

## 30 290 5. Discussion

### 31 291 32 33 34 292 5. 1. Food sources

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37 293 The similarity between the pigment composition of sediment and foregut content of  
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39 294 wild *H. forskali* suggests that the species gathers its vegetal food sources in the sediment.  
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41 295 However, higher organic content, total pigment concentration and total FA concentration in  
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43 296 gut contents and faeces vs. the sediment shows that the species has the ability to select its  
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45 297 food particles. Many authors already suggested that deposit-feeding holothurians are selective  
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47 298 feeders, although it is not evident whether this selection is due to chemosensory apical buds  
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49 299 (Bouland et al. 1982) or to the physical properties of ingested particles (Roberts et al. 2000).  
50  
51 300 According to Roberts et al. (2000), ingestion of coarse fragments of macrophytes is explained  
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53 301 by the low feeding selectivity of holothurians and cannot be considered as herbivory. Yet,  
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55 302 Belbachir and Mezali (2018) reported the highest Ivlev selectivity index in *H. forskali* for  
56  
57 303 fresh *Posidonia sp.* fragments. The *Laminaria sp.* forests in which our samples were taken are  
58  
59 304 very different habitats from the *Posidonia* seagrasses where Belbachir and Mezali (2018)  
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305 conducted their study, suggesting that *Posidonia sp.* fragments are not essentials in the diet of  
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306 *H. forskali*. Instead, high proportions of fucoxanthin and chlorophyll *c* in gut contents of wild

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3 307 individuals indicate that diatoms and/or brown macroalgae are important food sources for *H.*  
4 308 *forskali* in our study (Schmid et al. 2005), while neoxanthin may originate from decomposing  
5 309 pelagic green microalgae as this carotenoid is present in high amounts in suspended  
6 310 particulate matter ( $16.7 \pm 0.8$  %) and found almost exclusively in Prasinophyceae and  
7 311 Chlorophyceae (Mackey et al. 1996, Brotas and Plante-Cuny 2003).

12 312 Conversely, low proportions of lutein in gut contents of wild *H. forskali* indicate that  
13 313 the species does not feed on the red algae *Cryptopleura ramosa* found in close proximity and  
14 314 exhibiting high levels of this carotenoid ( $37.0 \pm 0.4$  %). More largely, all groups of red algae  
15 315 exhibit high contents of antheraxanthin, lutein or zeaxanthin (Schubert et al. 2006). These  
16 316 carotenoids were not detected or below 1 % in *H. forskali* gut contents. Lutein is also an  
17 317 important component of green macroalgae such as *Ulva sp.* that may reach the sea floor in  
18 318 this area once in decomposition but that is not ingested by *H. forskali*. High levels of lutein in  
19 319 the faeces of sea cucumber juveniles fed with decomposed green algae confirm that lutein can  
20 320 be used as a reliable tracer of green algae in the diet of *H. forskali*, and that pigment  
21 321 composition of farmed animal faeces roughly resembled that of their diet. Finally, low  
22 322 proportions of violaxanthin and FA 18:4 $\omega$ 3 in gut contents reveal that brown macroalgae are  
23 323 barely ingested, as they usually exhibit high levels of these compounds (Fleurence et al. 1994,  
24 324 Schmid et al. 2005). Thus, the vegetal food sources ingested by *H. forskali* in our study are  
25 325 mainly diatoms.

36 326 In contrast to pigments, fatty acids were not only more concentrated in foregut  
37 327 contents compared to the sediment but also differed in composition, suggesting that other  
38 328 food sources not containing pigments or exhibiting similar pigment compositions than  
39 329 unsorted sediment enter the diet of *H. forskali*. Higher proportions of the FA 20:4 $\omega$ 6, the sum  
40 330 of highly unsaturated FA (HUFA  $\geq 20$  carbon chain-length and 2 double bounds), and lower  
41 331 proportions of the FA 16:0 in the foregut content compared to the sediment indicate that the  
42 332 sea cucumbers ingest particles with especially high nutritional value. Actually, HUFA are  
43 333 precious compounds with roles in membrane properties and immune response (Arts and  
44 334 Kohler 2008, Twining et al. 2016). They are usually rapidly degraded in decaying organic  
45 335 matter (Wakeham 1995, Vivier et al. 2019). Such elevated proportions of HUFA in the  
46 336 foregut content of *H. forskali* suggest that ingested particles are upgraded regarding their  
47 337 nutritional value before transiting in the gut (i.e. in the nearby sediment or in the oesophagus).  
48 338 Trophic upgrading and enhancement of PUFA relative proportions throughout the first levels  
49 339 of the food web is usual in the marine realm (Desvillettes and Bec 2009), and can be mediated

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3 340 by heterotrophic protists, also named “non-pigmented” protists in the old scientific literature.  
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5 341 Other authors mentioned elevated levels of HUFA in the gut content of holothurians. Mfilinge  
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7 342 and Tsuchiya (2016) reported 21.8 % of HUFA in the foregut content of *H. leucospilota* and  
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9 343 31.4 % in *H. atra*. Both species were assumed to feed on the nearby sediment that exhibited  
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11 344 only 9.5 and 16.8 % of HUFA, respectively. High levels of HUFA in the foregut were  
12  
13 345 attributed to the specific selection of algae and bacteria rich detrital particles (Mfilinge and  
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15 346 Tsuchiya 2016). Although microalgae have long been considered the major *de novo* producer  
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17 347 of PUFA in marine food webs (Nichols 2003), diatoms, that are the dominant microalgae  
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19 348 consumed by *H. forskali* in our study, are usually poor in FA 20:4 $\omega$ 6 (Dalsgaard et al. 2003,  
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21 349 Lang et al. 2011), suggesting that the HUFA measured in the foregut content of *H. forskali*  
22  
23 350 may rather originate from bacteria (Nichols 2003), heterotrophic protists (Desvillettes and Bec  
24  
25 351 2009) and/or their symbiotic relationship (Gast et al. 2009). Ginger et al. (2000) also  
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27 352 measured high amounts of HUFA in the gut contents of holothurians, but they attributed these  
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29 353 elevated levels to the lysis of gut walls, caused by stress during animals recovery. Yet, Ginger  
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31 354 et al. (2000) worked on abyssal holothurians and change in barometric pressure during the  
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33 355 ascent phase may have caused a sufficient stress to cause cell wall lysis, which may not have  
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35 356 occurred in our study as sea cucumbers were gathered only at a few meters deep. In addition,  
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37 357 Féral (1985) measured higher lipid levels in the anterior intestine wall compared to the  
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39 358 oesophagus of *Leptosynapta galliennei* ( $51.9 \pm 3.4 \mu\text{g mg}^{-1}$  vs.  $7.6 \pm 3.5 \mu\text{g mg}^{-1}$ ,  
40  
41 359 respectively). Assuming similar differences in *H. forskali*, cell wall lysis would be expected to  
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43 360 cause higher lipid contents in the midgut compared to the foregut content. The opposite was  
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45 361 measured in our study, confirming that cell wall lysis caused by the sampling stress was  
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47 362 minor. In an aquaculture perspective, further investigations would be required to determine  
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49 363 whether the nutritional upgrading of detrital organic matter takes place in the sediment and/or  
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51 364 in the foregut and which organisms mediate the biosynthesis pathways of HUFA. Actually, if  
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53 365 possible, feeding the microbiote of *H. forskali* instead of providing feeds of high nutritional  
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55 366 value may be of interest in a sustainable aquaculture perspective.

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## 5. 2. Digestive efficiency

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55 369 The significant but weak differences in OM between foregut contents and faeces  
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57 370 suggest that *H. forskali* have to process large quantities of sediment to cover its nutritional  
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59 371 needs. In the midgut (the largest compartment) of *H. forskali* found on rocky substrate in the  
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372 Glenan Islands, the OM content was slightly above that of the complete gut measured in

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3 373 Algerian Posidonia meadows ( $10.9 \pm 2.6$  % in our study,  $8.6 \pm 6.1$  % measured by Mezali and  
4 374 Soualili 2013 in the Sidi-fredj peninsula and  $\sim 8.3 \pm 2.7$  % found by Belbachir et al. 2014 in  
5 375 the Stidia region). The one-third reduction in OM content during gut transit ( $14.1 \pm 4.5$  % OM  
6 376 in foregut vs.  $9.1 \pm 2.5$  % OM in faeces) is similar to previous observations by Hammond  
7 377 (1983) where one-third to one-half of the organic carbon was lost during gut passage in three  
8 378 species of holothurians and two species of echinoids from shallow tropical waters (Discovery  
9 379 Bay, Jamaica). Yet, not all compounds were similarly digested as total concentration of  
10 380 pigments did not significantly vary during gut transit, while total FA experienced more than  
11 381 three-fold reduction between foregut and hindgut ( $2.6 \pm 1.3$  mg g<sup>-1</sup> in the foregut vs.  $0.7 \pm 0.5$   
12 382 mg g<sup>-1</sup> in the hindgut), which confirms our hypothesis (i.e. that some food items are  
13 383 preferentially digested during gut transit and others remain untransformed).

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23 384 The absence of significant changes in relative proportions and total concentrations of  
24 385 pigments during gut transit indicates that *H. forskali* poorly utilises such resources, and  
25 386 confirms that pigments are good biomarkers of diet. In addition, these results suggest that  
26 387 living diatoms, that are the main pigmented food sources consumed by *H. forskali* in our  
27 388 study, are not digested during gut transit. Fresh pelagic microalgae were administered to  
28 389 farmed juveniles. Given that *H. forskali* is a deposit feeding species, most of ingested cells  
29 390 were probably dead algae, which induced lower relative proportions of chlorophyll *a*,  
30 391 chlorophyll *c* and fucoxanthin in juveniles faeces compared to their living food sources, along  
31 392 with the almost exclusive presence of pheophorbide *a* in faeces, a degradation product of  
32 393 chlorophylls (Roy et al. 2011), that was absent from the fresh cultured microalgae. Inefficient  
33 394 digestion of living diatoms was also confirmed by higher relative proportions of C<sub>16</sub> PUFA  
34 395 and the FA 16:1 $\omega$ 7, both biomarkers of diatoms (Dalsgaard et al. 2003, Brett et al. 2009,  
35 396 Kelly and Schiebling 2012) in the hindgut compared to the foregut contents of wild *H.*  
36 397 *forskali*. Yet, inefficient digestion of living diatoms raises the question of reason for their  
37 398 ingestion, that may be due to inefficient feeding selectivity, or to the feeding on compounds  
38 399 that do not require cell lysis. The most abundant compounds that are released by microalgae  
39 400 in the adjacent environment are exopolymer secretions, mostly on the form of polysaccharides  
40 401 (Decho 1990, Mühlenbruch et al. 2018). Such compounds may provide a source of carbon for  
41 402 deposit feeders (Roberts et al. 2000), as previously evidenced in various echinoderms (e.g. in  
42 403 the holothurian *Isostichopus badionotus* studied by Baird and Thistle 1986 and in the  
43 404 brittlestar *Amphipholis gracillima* studied by Hoskins et al. 2003). In stressing conditions (as  
44 405 is most probably the digestive tract of holothurians for diatoms), algal cells are known to

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3 406 increase their exudation rate (Mühlenbruch et al. 2018) especially in terms of carbohydrates  
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5 407 when they are exposed for instance to changing environmental conditions such as pH and  
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7 408 surface roughness (Riera et al. 2018). Ingest and force diatoms to release nutritive compounds  
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9 409 may thus be a very efficient way to obtain energy for a deposit-feeding organism such as *H.*  
10 410 *forskali*.

11  
12 411 In contrast to diatom biomarkers, HUFA proportions significantly decreased during  
13 412 gut transit ( $31.8 \pm 9.2$  % in the foregut vs.  $19.8 \pm 4.2$  % in the hindgut), confirming that these  
14 413 compounds (with a high nutritional value) are those that are preferentially assimilated by the  
15 414 sea cucumbers, while increase in proportions of the FA 16:0 and the sum of SFA indicates  
16 415 that remaining compounds are in a more advanced state of degradation with lower nutritional  
17 416 value. The almost absence of significant differences between the FA profiles of nearby  
18 417 sediment and sea cucumber faeces indicates that *H. forskali* have little influence on sediment  
19 418 composition, except concerning the proportion of branched FA, indicative of bacteria  
20 419 (Kaneda 1991, Kelly and Schiebling 2012). Actually, lower BrFA proportions in faeces ( $2.0 \pm$   
21 420  $0.3$  %) compared to nearby sediment ( $4.6 \pm 1.2$  %), along with similar total FA  
22 421 concentrations, suggest that *H. forskali* has the ability to reduce bacterial loads on the sea  
23 422 floor. Thus, as other species of sea cucumbers, *H. forskali* may be an interesting candidate for  
24 423 integrated multitrophic aquaculture (Zamora et al. 2016), as already proposed by MacDonald  
25 424 et al. (2003).

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### 38 39 426 5. 3. Resources allocation

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41 427 High contents of FA in the digestive tract of *H. forskali* are in accordance with  
42 428 histological observations of Féral (1985) where the author measured especially high levels of  
43 429 lipids in the anterior intestine walls of *Leptosynapta galliennii* (i.e. lipid droplets). Féral and  
44 430 Massin (1982) suggested that the digestive tract may serve as a storage organ and Allen  
45 431 (1968) measured highest incorporation rates of radioacetate in lipids of the gut isolated from  
46 432 the *rete mirabile* of *H. forskali*. All these results suggest that the fatty acid composition of  
47 433 holothuroids digestive tract deserves particular attention. The energy storage role of this organ  
48 434 has however been dismissed by Féral (1985) who did not measure any decay in the anterior  
49 435 intestine lipid content of adult *L. galliennii* before and after two weeks of starvation.  
50 436 Similarly, no seasonal difference was measured in the digestive tract lipid content of  
51 437 *Molpadia violacea* by Féral and Magniez (1985) whereas a storage role of the digestive tract  
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3 438 would probably provoke a notable depletion of stocks during winter. Yet, at the larval stage  
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5 439 Peters-Didier and Sewell (2019) evidenced an accumulation of neutral lipids in the stomach  
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7 440 epithelium of 14 days post-fertilisation larvae of *Australostichopus mollis*. Lipid droplets ~ 10  
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9 441  $\mu\text{m}$  in diameter suspended in the larval blastocoel were further transported to the hyaline  
10  
11 442 spheres (an enigmatic structure present in the late auricularia stage of sea cucumbers) to fuel  
12  
13 443 the process of metamorphosis. Whether this accumulation of lipids in the stomach epithelium  
14  
15 444 has also a role at the adult stage is still to be investigated but high incorporation rates of  
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17 445 radioacetate in the gut of *H. forskali* measured by Allen (1968) suggest that this organ plays a  
18  
19 446 major role in lipid biosynthesis. Finally, the closest similarity between  $\omega 3/\omega 6$  FA and  
20  
21 447 eicosanoid ratios between the digestive tract and the gonads compared to that of muscles  
22  
23 448 suggests that the anterior intestine could exchange functional lipids with reproductive organs.

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25 449 The absence of sexual difference in the FA composition of gut contents, muscles and  
26  
27 450 digestive tract of wild *H. forskali* suggests that food resources and digestive efficiency are not  
28  
29 451 sex-dependant in this species. Sexual difference in the FA composition of gonads may thus  
30  
31 452 originate from different incorporation strategies and/or conversion of FA rather than feeding.  
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33 453 In an aquaculture perspective, a lot of studies on FA composition of gonads have been  
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35 454 conducted on fishes, where docosahexaenoic acid (22:6 $\omega 3$ ; DHA) is among the most  
36  
37 455 abundant constituent of testes (Bell et al. 1997, Jeong et al. 2002, Beirão et al. 2015). This FA  
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39 456 is expected to confer spermatozooids a high membrane fluidity (Stillwell and Wassal 1997)  
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41 457 that is necessary for fecundation. It has been demonstrated experimentally that the inclusion  
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43 458 of DHA in *Solea senegalensis* diet may improve sperm quality (Beirão et al. 2015). Yet, in  
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45 459 our study DHA was a minor component of gonads, both in males and females. This result is  
46  
47 460 consistent with previous works on other sea cucumber species, such as *Apostichopus*  
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49 461 *japonicus* (< 5 % DHA in gonads; Kasai 2003), *Cucumaria frondosa* (< 2 % DHA in gonads  
50  
51 462 of wild individuals and individuals fed diatoms; Gianasi et al. 2017), *Holothuria tubulosa* or  
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53 463 *H. polii* (< 2 % DHA in gonads; Biandolino et al. 2019) and in sea urchins (< 3 % DHA in  
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55 464 gonads; Martínez-Pita et al. 2010, Díaz de Vivar et al. 2019), suggesting that DHA is not as  
56  
57 465 crucial for echinoderm reproduction as it is for fishes. Instead, membrane fluidity in  
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59 466 echinoderms may be provided by non-methylene interrupted FA as suggested for the sea  
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467 urchin *Paracentrotus lividus* (Carboni et al. 2013). Such FA could not be properly identified  
468  
469 in our study but they may happen within the unidentified HUFA and/or co-elute with others  
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471 on the non-polar HP-5ms column. In *H. forskali*, male and female gonads differ by higher  
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473 proportions of eicosapentaenoic acid (20:5 $\omega 3$ ; EPA) and arachidonic acid (20:4 $\omega 6$ ; ARA) in



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3 471 males. However, the twice as high total concentration of fatty acids in female compared to  
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5 472 male gonads ( $78.0 \pm 24.9 \text{ mg g}^{-1}$  in female vs.  $36.8 \pm 3.7 \text{ mg g}^{-1}$  in male gonads) may be due  
6  
7 473 to the storage of lipids for further use as an energy source by eggs, rather than for functional  
8  
9 474 purpose. Maternal provisioning is actually a strong determinant of larval development in  
10  
11 475 echinoderms as embryos and larvae initiate their development using nutrients provided in the  
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13 476 eggs (Prowse et al. 2007). The lipid requirement of eggs being higher than for spermatozooids  
14  
15 477 (that require energy only until fecundation), FA may not be as strictly selected, thus  
16  
17 478 explaining lower HUFA proportions in female gonads compared to that of males ( $23.2 \pm 3.2$   
18  
19 480 % in female vs.  $36.4 \pm 7.0$  in male gonads). Exploring the provisioning of essential  
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21 481 compounds (i.e. lipids) during gametogenesis will be necessary for aquaculture purpose as  
22  
23 broodstock conditioning may affect larval survival and development.

24  
25 482 Finally, muscle FA composition strongly differed with that of gut contents, digestive  
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27 483 tract and gonads, confirming that specific elements are preferentially retained and/or  
28  
29 484 converted to be allocated to tissues, as hypothesised in the introduction. The preferential  
30  
31 485 accumulation of PUFA in consumers relative to their diet is common in marine food webs,  
32  
33 486 but the retention of long-chain MUFA is more unusual (Budge et al. 2006). The FA 23:1 $\omega$ 9 is  
34  
35 487 found in significant proportions only in sea cucumbers, although sometimes detected as traces  
36  
37 488 in other echinoderms, crustaceans and bivalves (Kaneniwa et al. 1986, Rezanka and Sigler  
38  
39 489 2008, Drazen et al. 2008). Kaneniwa et al. (1986) found higher proportions of the FA 23:1 $\omega$ 9  
40  
41 490 in phospholipids compared to neutral lipids of two species of sea cucumbers, suggesting that  
42  
43 491 this FA has a physiological role in holothurians. It most probably does not originate from diet,  
44  
45 492 as none of the potential food sources exhibited even trace amounts of this compound, and as  
46  
47 493 previously proposed by Kaneniwa et al. (1986). Yet, it was detected in gut contents since the  
48  
49 494 foregut, showing that the mechanism leading to its synthesis (most probably  $\alpha$ -oxidation of  
50  
51 495 the FA 24:1 $\omega$ 9; Kaneniwa et al. 1986) is activated at the earliest stage of the digestive process  
52  
53 496 and/or that it is synthesised by endosymbionts. The absence of FA 23:1 $\omega$ 9 in farmed juvenile  
54  
55 497 faeces indicates that the mechanism appears later in the development, that endosymbionts  
56  
57 498 could not colonise individuals breed in captivity and/or that precursors of this FA have to be  
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59 499 found in sediment microbial communities, that was absent in juvenile tanks. In an aquaculture  
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500 perspective, the role and origin of this FA will require further investigation.

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## 6. Conclusion

Our study reveals that neither green nor red algae enter the diet of *H. forskali* in the Glenan Islands in spring, and that the only fresh vegetal material found in gut contents is diatoms. These diatoms are however poorly digested during gut transit and the utility of their ingestion remains unclear. In addition, gut contents exhibit a high nutritional quality that contrasts with the detrital nature of ingested diet, and suggesting that a trophic upgrading of OM occurs before digestion. To date, the few attempts that were made to feed *H. forskali* have been focusing on macroalgae that are actually cheap, easy to obtain and achieving relatively good results. Yet, as they may not constitute the natural food resources of *H. forskali* their use could lead to unexpected difficulties (e.g. health issues, poor reproduction efficiency) and/or sub-optimal growth rate. Our study aims to open up a wider range of nutritional options for the rearing of *H. forskali* (e.g. microbial cultures, food industry discards, dejections of other animals in multitrophic systems). Finally, unusual fatty acids such as non-methylene interrupted FA or the FA 23:1 $\omega$ 9 may play a non-negligible physiological role in *H. forskali*. How these compounds are affected by nutrition in aquaculture and their effect on sea cucumber fitness needs further investigation for a sustainable production of holothurians.

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2 753 Table 1: List of collected samples and major weaknesses associated

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Collection site	Sea cucumbers diet	Samples	Major drawbacks
Glenan Islands (47.710°N, 3.948°E)	Natural	Potential food sources (brown and red macroalgae, suspended particulate matter, sediment), faeces, gut contents, muscles, gonads and digestive tract	No seasonal or spatial variability
Concarneau marine station (MNHN)	Shelfish Diet 1800® + Grower Fertil® + fresh mussels	Faeces and feed	Possible growth of microbial communities in the tank bottom sediment
Beg Meil marine station (Agrocampus-Ouest)	Microalgae produced on site ( <i>Chatoceros sp.</i> , <i>Skeletonema sp.</i> and <i>Isochrysis sp.</i> )	Faeces and feed	2-4 cm juveniles
AquaB company	Decomposed <i>Ulva sp.</i> or <i>Enteromorpha sp.</i>	Faeces, feed and fresh macroalgae	2-4 cm juveniles

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Table 3: Selected fatty acid proportions in samples (mean  $\pm$  SD). Letters in exponent indicate significant differences between nearby sediment, gut contents and faeces of wild individuals (Van der Waerden test,  $\alpha = 5\%$ ).

	n	16:0	$\Sigma$ SFA	16:1 $\omega$ 7	20:1 $\omega$ 9	22:1 $\omega$ 9	23:1 $\omega$ 9	24:1 $\omega$ 9	$\Sigma$ LC-MUFA	$\Sigma$ MUFA
<b>Food sources</b>										
<i>Laminaria</i> sp. (brown macroalgae)	2	21.1 $\pm$ 1.0	30.0 $\pm$ 1.6	3.1 $\pm$ 0.0	0.3 $\pm$ 0.0				0.5 $\pm$ 0.1	28.3 $\pm$ 0.8
<i>Cryptopleura ramosa</i> (red macroalgae)	2	61.9 $\pm$ 1.1	77.6 $\pm$ 1.0	3.6 $\pm$ 0.5	0.6 $\pm$ 0.1				0.6 $\pm$ 0.1	14.4 $\pm$ 0.2
<i>Ulva</i> sp. (green macroalgae)	2	44.8 $\pm$ 0.9	51.8 $\pm$ 0.7	4.2 $\pm$ 0.2					0.2 $\pm$ 0.0	33.1 $\pm$ 0.0
Decomposed <i>Ulva</i> sp.	2	16.6 $\pm$ 1.0	29.8 $\pm$ 0.3	17.9 $\pm$ 2.6	0.4 $\pm$ 0.1	0.1 $\pm$ 0.1		0.1 $\pm$ 0.1	1.3 $\pm$ 0.3	46.9 $\pm$ 0.1
<i>Enteromorpha</i> sp. (green macroalgae)	2	29.7 $\pm$ 0.8	36.4 $\pm$ 1.3	3.8 $\pm$ 0.4	0.2 $\pm$ 0.0				0.2 $\pm$ 0.0	32.4 $\pm$ 0.5
Decomposed <i>Enteromorpha</i> sp.	2	18.7 $\pm$ 2.1	34.1 $\pm$ 3.1	10.7 $\pm$ 0.9	0.6 $\pm$ 0.1	0.1 $\pm$ 0.0		0.2 $\pm$ 0.1	2.4 $\pm$ 0.2	38.8 $\pm$ 2.9
Cultured diatoms (brown microalgae)	2	27.4 $\pm$ 3.2	64.2 $\pm$ 3.4	12.4 $\pm$ 4.9						20.2 $\pm$ 1.8
Shelfish Diet 1800	2	14.9 $\pm$ 0.0	23.7 $\pm$ 0.1	9.5 $\pm$ 0.2	0.4 $\pm$ 0.0	0.9 $\pm$ 0.0			1.5 $\pm$ 0.0	31.4 $\pm$ 0.0
Grower Fertil + fresh mussels	2	19.6 $\pm$ 1.4	29.8 $\pm$ 1.6	10.0 $\pm$ 2.0	3.2 $\pm$ 0.1	0.3 $\pm$ 0.0		0.2 $\pm$ 0.0	4.9 $\pm$ 0.0	32.3 $\pm$ 0.1
Suspended particulate matter	2	33.7 $\pm$ 1.5	66.9 $\pm$ 4.2	7.0 $\pm$ 2.2	0.4 $\pm$ 0.1	0.6 $\pm$ 0.4			1.4 $\pm$ 0.5	22.8 $\pm$ 3.2
<b>Faeces of farmed individuals</b>										
Juveniles fed decomposed <i>Ulva</i> sp.	3	22.7 $\pm$ 6.0	47.7 $\pm$ 9.6	13.1 $\pm$ 2.2	0.3 $\pm$ 0.3				0.9 $\pm$ 0.5	34.5 $\pm$ 6.8
Juveniles fed decomposed <i>Enteromorpha</i> sp.	3	29.4 $\pm$ 6.4	53.9 $\pm$ 11.5	5.8 $\pm$ 2.2	0.2 $\pm$ 0.4				0.5 $\pm$ 0.8	30.0 $\pm$ 5.8
Juveniles fed fresh microalgae	3	31.8 $\pm$ 17.8	60.9 $\pm$ 28.1	9.6 $\pm$ 7.6	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.0 $\pm$ 0.1	0.0 $\pm$ 0.1	0.7 $\pm$ 0.7	22.2 $\pm$ 16.9
Adults fed Shelfish Diet 1800 + Grower Fertil + fresh mussels	3	29.1 $\pm$ 2.5	45.6 $\pm$ 1.1	9.4 $\pm$ 1.1	5.6 $\pm$ 1.7	0.3 $\pm$ 0.1	0.8 $\pm$ 1.3	0.2 $\pm$ 0.0	8.1 $\pm$ 0.6	33.4 $\pm$ 0.8
<b>Wild <i>H. forskali</i> samples</b>										
Nearby sediment	4	23.0 $\pm$ 2.9 <sup>a</sup>	42.0 $\pm$ 3.1 <sup>a</sup>	11.3 $\pm$ 4.7 <sup>bc</sup>	3.3 $\pm$ 2.3	0.5 $\pm$ 0.4		0.1 $\pm$ 0.2 <sup>c</sup>	5.0 $\pm$ 3.0 <sup>b</sup>	30.9 $\pm$ 2.7
Foregut content	10	12.0 $\pm$ 6.0 <sup>b</sup>	30.7 $\pm$ 7.4 <sup>b</sup>	9.9 $\pm$ 4.0 <sup>c</sup>	2.3 $\pm$ 1.1	0.4 $\pm$ 0.3	2.2 $\pm$ 0.6 <sup>a</sup>	1.7 $\pm$ 1.1 <sup>a</sup>	9.4 $\pm$ 3.2 <sup>a</sup>	29.1 $\pm$ 3.2
Midgut content	10	14.1 $\pm$ 3.7 <sup>b</sup>	33.5 $\pm$ 3.6 <sup>b</sup>	14.0 $\pm$ 3.7 <sup>ab</sup>	1.0 $\pm$ 0.5	0.7 $\pm$ 0.4	1.3 $\pm$ 0.6 <sup>b</sup>	1.2 $\pm$ 0.5 <sup>a</sup>	6.2 $\pm$ 1.6 <sup>b</sup>	30.7 $\pm$ 2.1
Hindgut content	10	21.3 $\pm$ 5.2 <sup>a</sup>	39.7 $\pm$ 6.0 <sup>a</sup>	17.1 $\pm$ 3.7 <sup>a</sup>	1.3 $\pm$ 0.5	0.3 $\pm$ 0.4	1.0 $\pm$ 0.7 <sup>b</sup>	0.5 $\pm$ 0.4 <sup>bc</sup>	4.2 $\pm$ 2.1 <sup>b</sup>	30.6 $\pm$ 3.2
Freshly released faeces	4	23.7 $\pm$ 0.9 <sup>a</sup>	40.6 $\pm$ 1.1 <sup>a</sup>	15.0 $\pm$ 1.8 <sup>ab</sup>	2.1 $\pm$ 0.6	0.2 $\pm$ 0.1	2.2 $\pm$ 1.0 <sup>a</sup>	0.9 $\pm$ 0.3 <sup>ab</sup>	6.4 $\pm$ 2.1 <sup>ab</sup>	31.9 $\pm$ 2.8

n.d. not determined

	n	Σ C16PUFA	18:4ω3	20:4ω6 (ARA)	20:5ω3 (EPA)	22:6ω3 (DHA)	Σ HUFA	Σ PUFA	Σ BrFA	Total concentration (mg g <sup>-1</sup> )	
<b>Food sources</b>											
<i>Laminaria</i> sp. (brown macroalgae)	2	0.2 ± 0.0	16.4 ± 1.1	9.9 ± 0.4	10.8 ± 0.9	0.0 ± 0.0	21.0 ± 1.3	41.3 ± 2.3	0.1 ± 0.1	10.5 ± 2.4	2.4
<i>Cryptopleura ramosa</i> (red macroalgae)	2		0.6 ± 0.0	3.5 ± 0.1	1.8 ± 0.3	0.7 ± 0.3	5.9 ± 0.7	7.7 ± 0.7	0.4 ± 0.0	3.6 ± 0.0	0.0
<i>Ulva</i> sp. (green macroalgae)	2		7.6 ± 0.5		0.4 ± 0.0		0.4 ± 0.0	14.1 ± 0.8	1.0 ± 0.1	4.1 ± 0.7	0.7
Decomposed <i>Ulva</i> sp.	2	1.3 ± 0.3	0.5 ± 0.2	1.6 ± 0.4	2.5 ± 0.7	0.8 ± 0.1	5.3 ± 1.1	11.4 ± 1.8	11.6 ± 2.2	5.5 ± 0.0	0.0
<i>Enteromorpha</i> sp. (green macroalgae)	2	1.6 ± 0.1	5.9 ± 0.4	0.8 ± 0.0	2.0 ± 0.0		3.0 ± 0.0	29.6 ± 0.8	0.6 ± 0.1	9.6 ± 0.9	0.9
Decomposed <i>Enteromorpha</i> sp.	2	0.9 ± 0.1	0.3 ± 0.2	1.0 ± 0.4	1.4 ± 0.4	0.9 ± 0.2	4.0 ± 0.8	8.8 ± 1.3	18.3 ± 1.2	3.9 ± 1.3	1.3
Cultured diatoms (brown microalgae)	2	3.8 ± 1.9	3.8 ± 2.0	0.4 ± 0.6	2.3 ± 1.4	0.5 ± 0.7	3.2 ± 1.3	12.6 ± 0.6	0.8 ± 1.1	n.d.	
Shelfish Diet 1800	2	4.7 ± 0.2	12.9 ± 0.2	0.4 ± 0.0	10.4 ± 0.1	7.7 ± 0.2	20.2 ± 0.4	42.1 ± 0.2	0.6 ± 0.0	18.2 ± 1.0	1.0
Grower Fertil + fresh mussels	2	1.1 ± 0.2	1.9 ± 0.4	1.4 ± 0.2	16.3 ± 4.0	3.7 ± 5.3	22.3 ± 0.8	36.6 ± 2.1	0.5 ± 0.1	66.2 ± 1.3	1.3
Suspended particulate matter	2	0.9 ± 0.3	1.8 ± 0.3	0.0 ± 0.0	1.9 ± 0.7	1.3 ± 0.8	3.2 ± 1.5	8.3 ± 1.2	2.0 ± 0.1	6.5 ± 4.7	4.7
<b>Faeces of aquacultured individuals</b>											
Juveniles fed decomposed <i>Ulva</i> sp.	3	1.5 ± 0.2	0.7 ± 0.6	0.6 ± 0.3	1.0 ± 0.7	0.2 ± 0.4	2.0 ± 1.7	7.9 ± 1.7	9.8 ± 1.2	1.2 ± 0.3	0.3
Juveniles fed decomposed <i>Enteromorpha</i> sp.	3	0.5 ± 0.4	0.6 ± 0.5	0.2 ± 0.4	0.5 ± 0.9	0.3 ± 0.5	1.0 ± 1.8	5.5 ± 2.9	10.6 ± 3.2	1.0 ± 0.5	0.5
Juveniles fed fresh microalgae	3	1.9 ± 1.7	0.5 ± 0.5	1.0 ± 0.9	2.7 ± 2.3	3.5 ± 3.3	7.8 ± 7.0	11.8 ± 10.4	4.7 ± 0.5	15.8 ± 21.8	
Adults fed Shelfish Diet 1800 + Grower Fertil + fresh mussels	3	1.3 ± 0.2	1.2 ± 0.2	0.6 ± 0.3	3.3 ± 1.2	2.4 ± 0.7	9.4 ± 0.6	18.8 ± 0.4	1.7 ± 0.8	8.1 ± 8.5	8.5
<b>Wild <i>H. forskali</i> samples</b>											
Nearby sediment	4	1.8 ± 1.0 <sup>b</sup>	1.1 ± 0.6	3.7 ± 1.2 <sup>c</sup>	9.2 ± 3.9	2.6 ± 1.0	17.1 ± 6.9 <sup>c</sup>	21.0 ± 6.5 <sup>c</sup>	4.5 ± 1.2 <sup>a</sup>	0.3 ± 0.1 <sup>d</sup>	0.1 <sup>d</sup>
Foregut content	10	1.7 ± 0.8 <sup>b</sup>	1.3 ± 0.3	8.6 ± 2.7 <sup>a</sup>	15.0 ± 5.3	5.1 ± 4.9	31.8 ± 9.2 <sup>a</sup>	34.9 ± 8.7 <sup>a</sup>	3.1 ± 1.0 <sup>b</sup>	2.6 ± 1.3 <sup>a</sup>	1.3 <sup>a</sup>
Midgut content	10	2.5 ± 0.7 <sup>ab</sup>	1.6 ± 0.2	6.3 ± 1.7 <sup>b</sup>	13.2 ± 2.6	3.2 ± 0.9	24.7 ± 5.0 <sup>ab</sup>	29.5 ± 4.7 <sup>ab</sup>	4.1 ± 1.3 <sup>a</sup>	1.1 ± 0.4 <sup>b</sup>	0.4 <sup>b</sup>
Hindgut content	10	2.9 ± 0.5 <sup>a</sup>	1.5 ± 0.2	4.6 ± 1.4 <sup>c</sup>	11.1 ± 2.2	2.7 ± 0.9	19.8 ± 4.2 <sup>bc</sup>	24.9 ± 4.2 <sup>bc</sup>	2.7 ± 0.9 <sup>bc</sup>	0.7 ± 0.5 <sup>c</sup>	0.5 <sup>c</sup>
Freshly released faeces	4	2.7 ± 0.5 <sup>ab</sup>	1.6 ± 0.4	5.0 ± 1.1 <sup>bc</sup>	10.1 ± 1.7	2.0 ± 0.7	18.3 ± 2.4 <sup>bc</sup>	23.5 ± 2.4 <sup>bc</sup>	2.0 ± 0.3 <sup>c</sup>	0.4 ± 0.2 <sup>cd</sup>	0.2 <sup>cd</sup>

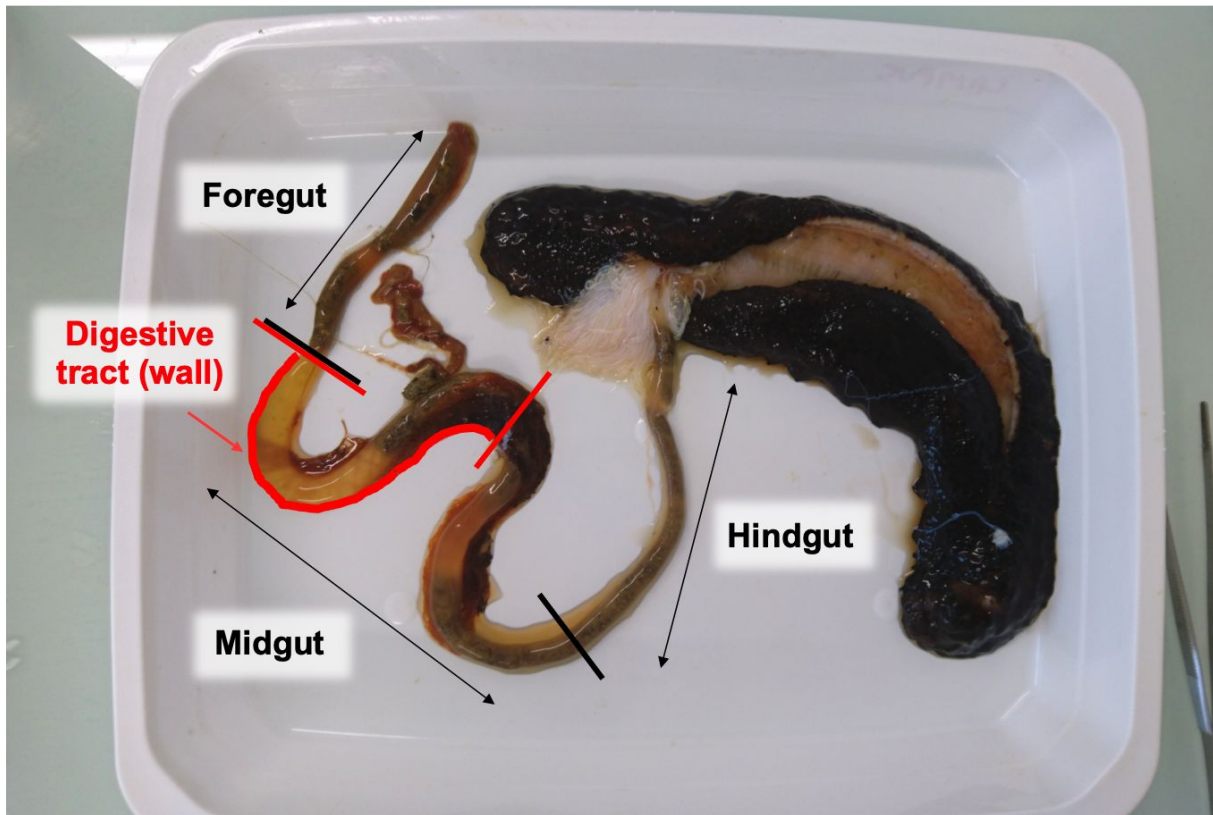
n.d. not determined

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768 Table 4: Fatty acid proportions of wild *H. forskali* tissues (mean  $\pm$  SD). Letters in exponent  
 769 indicate significant differences between groups (Van der Waerden test,  $\alpha = 5\%$ ). Note that  
 770 traces of 18:3 $\omega$ 3 may have co-eluted with 18:1 $\omega$ 9 and traces of 16:0iso may have co-eluted  
 771 with 16:4 $\omega$ 3. LC-MUFA Long-Chain MUFA ; HUFA Highly Unsaturated FA  
 772

Fatty acids (%)	Muscle (n = 10)	Digestive tract (n = 10)	Female gonad (n = 6)	Male gonad (n = 4)
<i>Saturated</i>				
12:0	0.3 $\pm$ 0.1 <sup>a</sup>	0.1 $\pm$ 0.1 <sup>b</sup>	0.1 $\pm$ 0.0 <sup>b</sup>	0.1 $\pm$ 0.1 <sup>b</sup>
14:0	2.5 $\pm$ 0.4 <sup>c</sup>	3.9 $\pm$ 0.8 <sup>b</sup>	5.3 $\pm$ 0.5 <sup>a</sup>	2.2 $\pm$ 1.5 <sup>c</sup>
15:0	0.7 $\pm$ 0.2 <sup>b</sup>	0.7 $\pm$ 0.2 <sup>b</sup>	1.3 $\pm$ 0.2 <sup>a</sup>	0.5 $\pm$ 0.3 <sup>b</sup>
16:0	5.3 $\pm$ 0.9 <sup>b</sup>	6.0 $\pm$ 1.1 <sup>b</sup>	8.9 $\pm$ 0.9 <sup>a</sup>	5.0 $\pm$ 1.7 <sup>b</sup>
17:0	0.7 $\pm$ 0.1 <sup>c</sup>	0.9 $\pm$ 0.1 <sup>b</sup>	1.1 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.5 <sup>a</sup>
18:0	4.6 $\pm$ 0.4 <sup>c</sup>	7.2 $\pm$ 0.7 <sup>b</sup>	6.5 $\pm$ 0.8 <sup>b</sup>	9.4 $\pm$ 1.9 <sup>a</sup>
19:0	1.2 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.3 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.1 <sup>a</sup>
20:0	2.9 $\pm$ 0.2 <sup>ab</sup>	2.8 $\pm$ 0.4 <sup>b</sup>	2.3 $\pm$ 0.2 <sup>c</sup>	3.8 $\pm$ 1.2 <sup>a</sup>
21:0	1.8 $\pm$ 0.3 <sup>a</sup>	1.4 $\pm$ 0.3 <sup>b</sup>	0.9 $\pm$ 0.1 <sup>c</sup>	1.2 $\pm$ 0.1 <sup>b</sup>
22:0	1.8 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.4 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>b</sup>	1.1 $\pm$ 0.1 <sup>b</sup>
<b><math>\Sigma</math>SFA</b>	<b>21.8 <math>\pm</math> 1.5</b>	<b>26.2 <math>\pm</math> 2.4</b>	<b>28.7 <math>\pm</math> 1.0</b>	<b>26.4 <math>\pm</math> 0.9</b>
<i>Monounsaturated</i>				
16:1 $\omega$ 9	0.3 $\pm$ 0.1 <sup>b</sup>	0.3 $\pm$ 0.1 <sup>b</sup>	0.7 $\pm$ 0.2 <sup>a</sup>	0.1 $\pm$ 0.2 <sup>c</sup>
16:1 $\omega$ 7	3.9 $\pm$ 0.5 <sup>b</sup>	8.3 $\pm$ 1.8 <sup>a</sup>	9.5 $\pm$ 1.1 <sup>a</sup>	4.4 $\pm$ 2.7 <sup>b</sup>
16:1 $\omega$ 5	0.4 $\pm$ 0.1 <sup>bc</sup>	0.4 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.2 <sup>a</sup>	0.3 $\pm$ 0.1 <sup>c</sup>
18:1 $\omega$ 9	2.2 $\pm$ 0.4 <sup>c</sup>	2.6 $\pm$ 0.3 <sup>b</sup>	3.9 $\pm$ 0.5 <sup>a</sup>	2.2 $\pm$ 0.7 <sup>c</sup>
18:1 $\omega$ 7	3.7 $\pm$ 0.4 <sup>c</sup>	5.7 $\pm$ 0.4 <sup>b</sup>	6.4 $\pm$ 0.7 <sup>ab</sup>	7.9 $\pm$ 2.9 <sup>a</sup>
20:1 $\omega$ 9	7.3 $\pm$ 1.0 <sup>a</sup>	4.5 $\pm$ 0.4 <sup>bc</sup>	3.9 $\pm$ 0.8 <sup>c</sup>	5.5 $\pm$ 1.0 <sup>b</sup>
20:1 $\omega$ 7	1.3 $\pm$ 0.1 <sup>b</sup>	1.6 $\pm$ 0.2 <sup>a</sup>	1.4 $\pm$ 0.1 <sup>b</sup>	2.0 $\pm$ 0.3 <sup>a</sup>
22:1 $\omega$ 9	1.2 $\pm$ 0.2 <sup>a</sup>	0.6 $\pm$ 0.1 <sup>c</sup>	0.8 $\pm$ 0.2 <sup>a</sup>	0.6 $\pm$ 0.2 <sup>bc</sup>
22:1 $\omega$ 7	1.8 $\pm$ 0.2 <sup>a</sup>	1.8 $\pm$ 0.3 <sup>a</sup>	1.4 $\pm$ 0.3 <sup>b</sup>	1.5 $\pm$ 0.4 <sup>ab</sup>
23:1 $\omega$ 9	7.7 $\pm$ 1.5 <sup>a</sup>	3.5 $\pm$ 0.6 <sup>b</sup>	2.1 $\pm$ 0.9 <sup>c</sup>	3.6 $\pm$ 1.3 <sup>bc</sup>
24:1 $\omega$ 9	3.5 $\pm$ 0.7 <sup>a</sup>	1.4 $\pm$ 0.2 <sup>b</sup>	1.4 $\pm$ 0.3 <sup>b</sup>	1.8 $\pm$ 0.3 <sup>b</sup>
<b><math>\Sigma</math>LC-MUFA</b>	<b>22.9 <math>\pm</math> 3.3<sup>a</sup></b>	<b>13.3 <math>\pm</math> 1.4<sup>b</sup></b>	<b>11.0 <math>\pm</math> 2.1<sup>c</sup></b>	<b>15.0 <math>\pm</math> 2.8<sup>b</sup></b>
<b><math>\Sigma</math>MUFA</b>	<b>33.5 <math>\pm</math> 2.1<sup>a</sup></b>	<b>30.7 <math>\pm</math> 1.3<sup>b</sup></b>	<b>32.4 <math>\pm</math> 1.7<sup>ab</sup></b>	<b>29.9 <math>\pm</math> 3.8<sup>b</sup></b>
<i>Polyunsaturated</i>				
16:4 $\omega$ 3	1.1 $\pm$ 0.1 <sup>c</sup>	1.6 $\pm$ 0.3 <sup>b</sup>	2.4 $\pm$ 0.4 <sup>a</sup>	1.1 $\pm$ 0.6 <sup>c</sup>
16:3 $\omega$ 4	0.5 $\pm$ 0.1 <sup>c</sup>	0.7 $\pm$ 0.2 <sup>b</sup>	1.1 $\pm$ 0.4 <sup>a</sup>	0.4 $\pm$ 0.2 <sup>c</sup>
16:2 $\omega$ 4	0.4 $\pm$ 0.1 <sup>b</sup>	0.7 $\pm$ 0.3 <sup>a</sup>	0.8 $\pm$ 0.2 <sup>a</sup>	0.4 $\pm$ 0.3 <sup>b</sup>
18:4 $\omega$ 3	1.3 $\pm$ 0.3	1.7 $\pm$ 0.3	1.8 $\pm$ 0.4	1.4 $\pm$ 0.7
18:2 $\omega$ 6	0.8 $\pm$ 0.2 <sup>c</sup>	0.9 $\pm$ 0.1 <sup>b</sup>	1.3 $\pm$ 0.2 <sup>a</sup>	0.7 $\pm$ 0.1 <sup>c</sup>
20:4 $\omega$ 6	19.5 $\pm$ 0.9 <sup>a</sup>	11.0 $\pm$ 1.9 <sup>b</sup>	7.4 $\pm$ 1.1 <sup>c</sup>	12.8 $\pm$ 2.5 <sup>b</sup>
20:5 $\omega$ 3	11.9 $\pm$ 1.5 <sup>b</sup>	15.9 $\pm$ 1.8 <sup>a</sup>	11.5 $\pm$ 1.8 <sup>b</sup>	18.5 $\pm$ 4.0 <sup>a</sup>
20:2 $\omega$ 6	0.8 $\pm$ 0.2 <sup>b</sup>	1.3 $\pm$ 0.3 <sup>a</sup>	1.0 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.6 <sup>a</sup>
Unidentified HUFA	1.3 $\pm$ 0.2	1.0 $\pm$ 0.2	0.8 $\pm$ 0.1	0.9 $\pm$ 0.1
22:5 $\omega$ 6	1.1 $\pm$ 0.1 <sup>a</sup>	0.7 $\pm$ 0.2 <sup>b</sup>	0.5 $\pm$ 0.1 <sup>c</sup>	0.8 $\pm$ 0.2 <sup>b</sup>
22:6 $\omega$ 3	2.0 $\pm$ 0.4 <sup>b</sup>	2.7 $\pm$ 0.5 <sup>a</sup>	2.0 $\pm$ 0.6 <sup>b</sup>	2.1 $\pm$ 0.6 <sup>ab</sup>
<b><math>\Sigma</math>C16PUFA</b>	<b>0.9 <math>\pm</math> 0.2<sup>b</sup></b>	<b>1.4 <math>\pm</math> 0.4<sup>a</sup></b>	<b>1.9 <math>\pm</math> 0.6<sup>a</sup></b>	<b>0.8 <math>\pm</math> 0.5<sup>b</sup></b>
<b><math>\Sigma</math>HUFA</b>	<b>36.6 <math>\pm</math> 2.0<sup>a</sup></b>	<b>32.6 <math>\pm</math> 4.4<sup>a</sup></b>	<b>23.2 <math>\pm</math> 3.2<sup>b</sup></b>	<b>36.4 <math>\pm</math> 7.0<sup>a</sup></b>
<b><math>\Sigma</math>PUFA</b>	<b>38.9 <math>\pm</math> 1.8<sup>a</sup></b>	<b>36.5 <math>\pm</math> 3.8<sup>a</sup></b>	<b>28.7 <math>\pm</math> 3.3<sup>b</sup></b>	<b>39.2 <math>\pm</math> 5.5<sup>a</sup></b>
<i>Branched</i>				
14:0iso	0.5 $\pm$ 0.1 <sup>bc</sup>	0.6 $\pm$ 0.1 <sup>b</sup>	1.0 $\pm$ 0.2 <sup>a</sup>	0.3 $\pm$ 0.2 <sup>c</sup>
15:0iso	1.7 $\pm$ 0.4 <sup>bc</sup>	2.1 $\pm$ 0.4 <sup>b</sup>	3.6 $\pm$ 0.6 <sup>a</sup>	1.2 $\pm$ 0.8 <sup>c</sup>
15:0anteiso	0.7 $\pm$ 0.2 <sup>bc</sup>	0.8 $\pm$ 0.2 <sup>b</sup>	1.4 $\pm$ 0.3 <sup>a</sup>	0.5 $\pm$ 0.3 <sup>c</sup>
17:0iso	0.8 $\pm$ 0.1 <sup>c</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	1.6 $\pm$ 0.2 <sup>a</sup>	0.9 $\pm$ 0.3 <sup>c</sup>
17:0anteiso	0.4 $\pm$ 0.1 <sup>b</sup>	0.4 $\pm$ 0.0 <sup>b</sup>	0.6 $\pm$ 0.1 <sup>a</sup>	0.3 $\pm$ 0.1 <sup>b</sup>
<b><math>\Sigma</math>BrFA</b>	<b>4.1 <math>\pm</math> 0.8<sup>bc</sup></b>	<b>4.9 <math>\pm</math> 0.9<sup>b</sup></b>	<b>8.3 <math>\pm</math> 1.3<sup>a</sup></b>	<b>3.2 <math>\pm</math> 1.8<sup>c</sup></b>
<b><math>\Sigma</math>FA (mg g<sup>-1</sup>)</b>	<b>5.2 <math>\pm</math> 1.8<sup>c</sup></b>	<b>40.1 <math>\pm</math> 10.1<sup>b</sup></b>	<b>78.0 <math>\pm</math> 24.9<sup>a</sup></b>	<b>36.8 <math>\pm</math> 3.7<sup>b</sup></b>

774 Fig. 1: Dissection and zonation of the digestive tract of *Holothuria (Panningothuria) forskali*  
775 Delle Chiaje, 1823



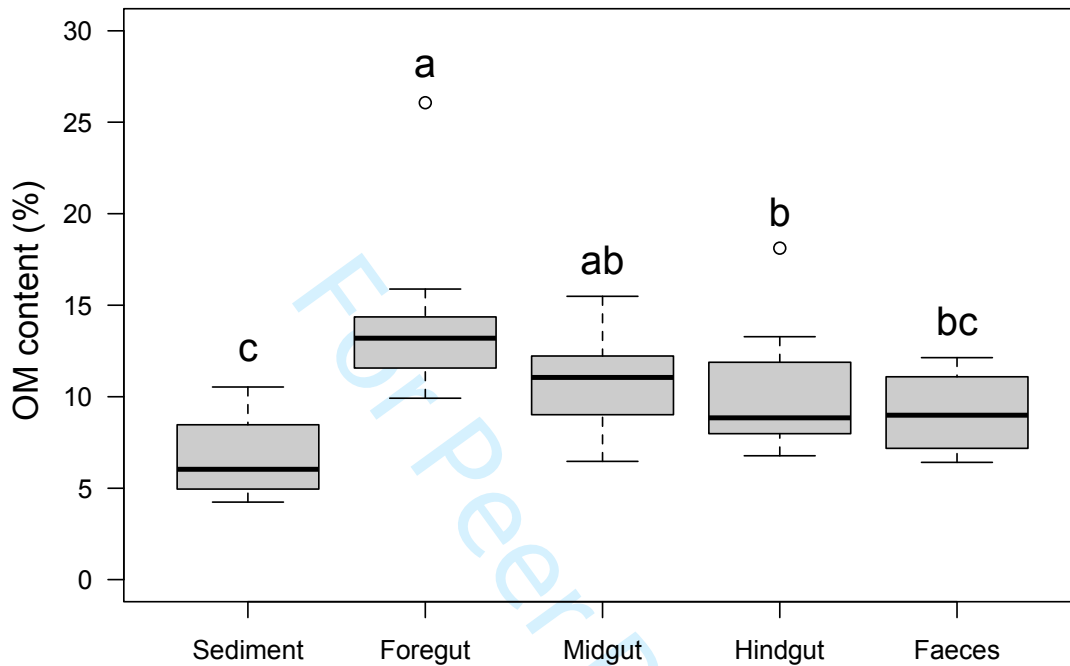
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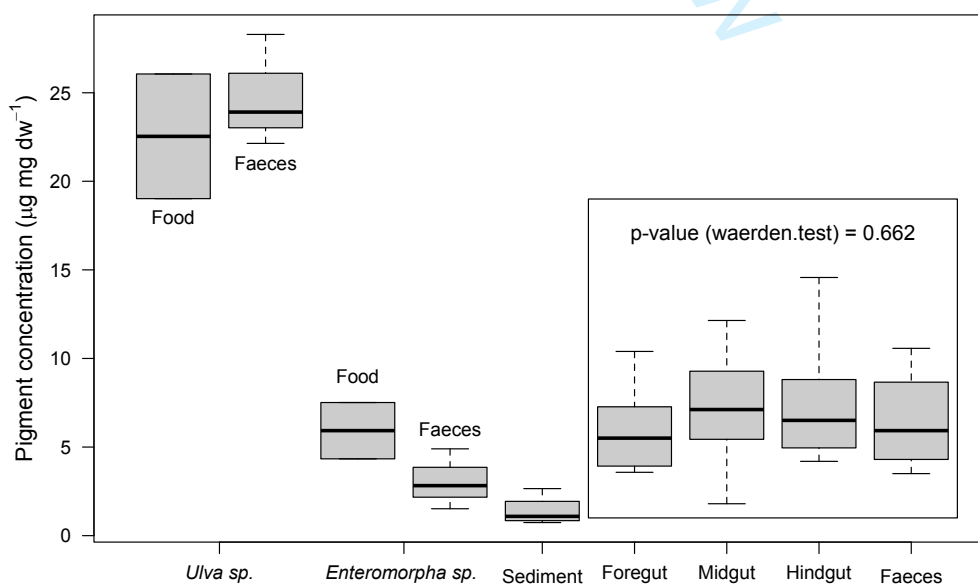
Review

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3 779 Fig. 2: Boxplot of the organic matter content (%) in wild *H. forskali* samples and nearby  
4 780 sediment. Broad lines indicate median, box edges refer to 1<sup>st</sup> and 3<sup>rd</sup> quartiles and circles  
5 781 indicate outliers. Letters indicate significant differences between samples (Van der Waerden  
6 782 test,  $\alpha = 5\%$ )



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784 Fig. 3: Total pigment concentration in aquaculture and wild samples ( $\mu\text{g mg dw}^{-1}$ ). Broad  
785 lines indicate median and box edges refer to 1<sup>st</sup> and 3<sup>rd</sup> quartiles.

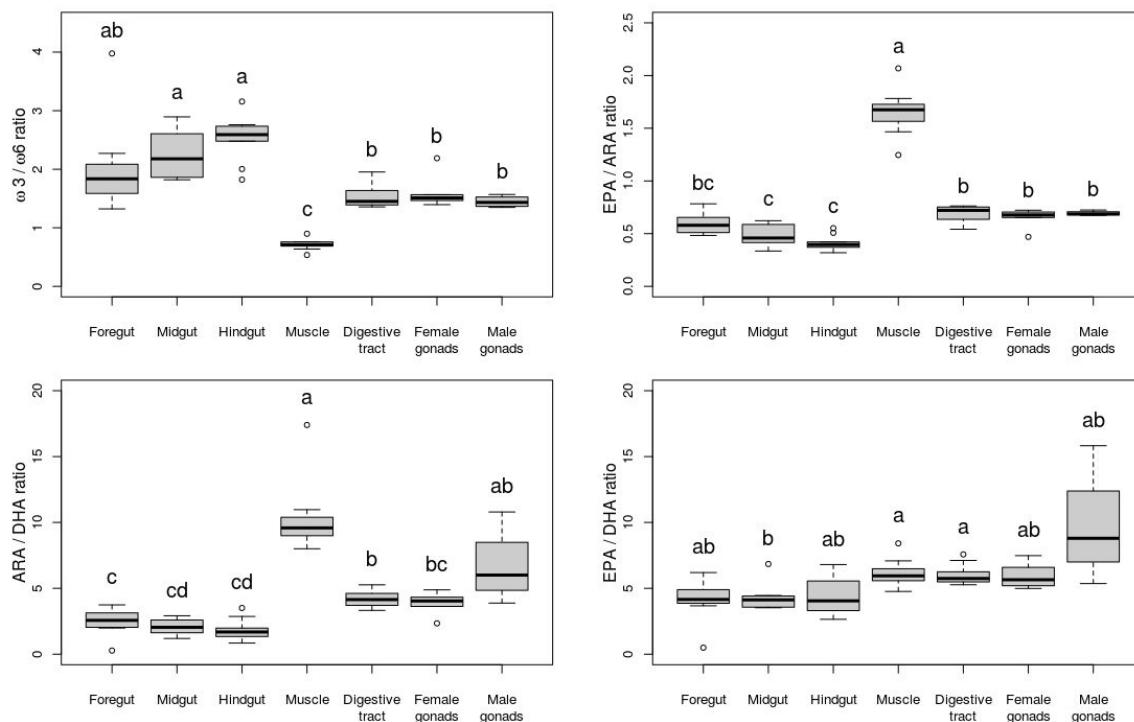


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788 Fig. 4: Eicosanoid ratios in gut content and tissues of wild *H. forskali* samples. Letters  
 789 indicate significant differences between samples (Van der Waerden test,  $\alpha = 5\%$ ). Broad lines  
 790 indicate median, box edges refer to 1<sup>st</sup> and 3<sup>rd</sup> quartiles and circles indicate outliers.



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view