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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1   Title: Food sources, digestive efficiency and resources allocation in the sea cucumber

2   *Holothuria forskali* (Echinodermata: Holothuroidea): insights from pigments and fatty acids

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4   Running title: Pigments and fatty acids in *Holothuria forskali*

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31   the corresponding author upon reasonable request.

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32   1. Abstract

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34   Various research projects in Europe and North Africa have recently intended to breed

35   temperate holothurians to alleviate fishing pressure on natural populations. However, to date

36   little is known about the nutritional requirements of East Atlantic and Mediterranean species.

37   In this study, we propose a "natural populations" oriented approach to characterise food

38   sources, digestive efficiency and resources allocation based on the composition of pigments

39   and fatty acids (FA) in gut contents and tissues (muscles, gonads and digestive tract walls) of

40   wild individuals of the species *Holothuria (Panningothuria) forskali* (Delle Chiaje, 1823)

41   sampled in Brittany (France). Our study reveals that neither green nor red algae enter the diet

42   of *H. forskali* in spring, and that the only fresh vegetal material found in gut contents is brown

43   algae (very likely diatoms). The high nutritional quality of gut contents however contrasts

44   with the detrital nature of the ingested food sources, suggesting that a trophic upgrading of

45   organic matter occurs before digestion. In addition, unusual FA (i.e. only present in a few

46   groups of living species) such as long-chain monounsaturated FA (especially the FA 23:1ω9)

47   were found in large proportions in muscles and gonads and their effect on sea cucumber

48   fitness needs further investigation.

49

50   Keywords: sea cucumbers, echinoderms, *Holothuria forskali*, gut contents, pigments, fatty

51   acids

52

2. Introduction

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

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

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

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

3. Materials and methods

3. 1. Sample collection of wild individuals

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

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

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

147 station of Agrocampus-Ouest maintains 2-4 cm juveniles in 25 L plastic tanks cleaned daily,

148 and feeds them with a mixture of microalgae produced on site (the two Bacillariophyceae

149 *Chatoceros sp.* and *Skeletonema sp.* and the Prymnesiophyceae *Isochrysis sp.*). Finally, the

150 AquaB Company in Plobannalec-Lesconil maintains 2-4 cm juveniles in 50 cm diameter

151 polyamide sieves (20 μm mesh size) placed in cylindroconical tanks with a circulating filtered

152 seawater system, and feeds them with decomposed green macroalgae (*Ulva sp.* or

153 *Enteromorpha sp.*). Green seaweeds are gathered on nearby beaches, left to decompose

154 during a few weeks in aerated cylindroconical tanks and the mixture is filtered before feeding

155 events to collect particles in the size 50-300 μm. All experimental stations constantly renew

156 the water in culture tanks with filtered seawater pumped nearby, maintaining water

157 parameters similar to that of the ocean. The list of collected samples and major associated

158 weaknesses is presented in Table 1. All samples were kept at -25°C and analysed within 2

159 months after recollection.

160

161   3. 2. Sample processing

162   Organic matter of wild *H. forskali* gut contents and nearby sediment was quantified

163 gravimetrically after combustion of freeze-dried material in a muffle furnace (4 h at 450°C).

164 Pigments were analysed by high performance liquid chromatography (HPLC) according to

165 Brotas and Plante-Cuny (2003). Sub-samples of freeze-dried material (10-15 mg for sediment,

166 gut contents and faeces and 5-10 mg for potential food sources) were incubated with 2 mL of

167 methanol (buffered with 2% ammonium acetate) during 15 min, at -25°C in the dark. Extracts

168 were then filtered with 0.2 μm PTFE syringe filters and analysed within 16 h using an Agilent

169 1260 Infinity HPLC composed of a quaternary pump (VL 400 bar), a UV–VIS photodiode

170 array detector (DAD 1260 VL, 250–900 nm) and a 100 μl automatic sample injector

171 refrigerated at 4°C in the dark. Chromatographic separation was carried out using a C18

172 column for reverse phase chromatography (Supelcosil, 25 cm long, 4.6 mm inner diameter).

173 The solvents used were A: 0.5 M ammonium acetate in methanol and water (85:15, v:v), B:

174 acetonitrile and water (90:10, v:v), and C: 100% ethyl acetate. The solvent gradient was set

175 according to Brotas and Plante-Cuny (2003), with a 0.5 mL min^{-1} flow rate. Identification and

176 calibration of the HPLC peaks were performed with antheraxanthin, ββ-carotene,

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

180 performed using standard calibration curves built with repeated injections of standards over a

181 range of dilutions. Carotenoids and chlorophyll *b* and *c* were quantified at 470 nm,

182 chlorophyll *a* and their derivatives as well as pheopigments were quantified at 665 nm. The

183 relative abundance of each pigment (%) was calculated from its respective concentration in

184 the sample ($\mu g \ mg^{-1}$).

185 Lipids were extracted following a slightly modified protocol of Bligh and Dyer

186 (1959), as described in Meziane et al. (2007). Sub-samples of freeze-dried material (100-300

187 mg for sediment, gut contents and wild population faeces, 30-100 mg for potential food

188 sources, muscles and aquacultured sea cucumber faeces and 10-30 mg for digestive tracts and

189 gonads) were incubated with 4 mL of a water:methanol:chloroform mixture (1:2:1, v:v:v) and

190 sonicated during 20 min at room temperature. Chloroform and water were added to the

191 mixture to reach equal proportions of the three solvents and allow the formation of an

192 aqueous-organic bilayer system that was separated by centrifugation (3000 rpm, 5 min). The

193 heavy phase was retrieved and the operation was repeated a second time after completion with

194 2 mL of chloroform to retrieve as much lipids as possible. In addition, 15-30 μg of tricosanoic

195 acid (23:0) provided by Sigma-Aldrich was added to every sample prior to extraction and

196 used as internal standard. The lipid fraction, contained in the chloroform, was evaporated

197 under nitrogen (N_2) flux and dried lipid extracts were saponified using a methanol:sodium

198 hydroxide (2 N) mixture (2:1, v:v) during 1 h 30 min, at 90 °C. Finally, fatty acid esters were

199 methylated into fatty acid methyl esters (FAME) using boron trifluoride-methanol (BF_3-CH_3-

200 OH) and stored at −25 °C. We analysed FAME by gas chromatography (Agilent, 7890B GC)

201 associated with triple quadrupole mass spectrometry (Agilent, 5977B MSD) equipped with an

202 Agilent HP-5ms UI non-polar capillary column (30 m length × 0.25 mm inner diameter ×

203 0.25 μm inner diameter) and using helium as gas carrier (1.5 mL min^{-1}). The oven

204 temperature was set at 60 °C and held for 1 min, raised at 40 °C min^{-1} to 170 °C and held for

205 1 min, increased to 195 °C at 3 °C min^{-1} and held for 15 min, raised at 3 °C min^{-1} to 220 °C

206 and held for 20 min and finally increased to 240 °C at 3 °C min^{-1} and held for 12 min. The

207 two plateaux at 195 and 220 °C corresponded to the elution zones of C16 and C18 fatty acids,

208 respectively, and temperature was held constant to improve peak delineation. After column

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 send to the MSD for identification. Mass spectra were acquired in

212 electron ionisation (EI) mode at 70 eV between 35-600 mz at a scan rate of 1.3 scan s^{-1}. All

213 peaks in the FID chromatograms were integrated with a workflow on Agilent masshunter
214 quantitative analysis software and a routine was developed with R version 3.5.3 (R Core
215 Team 2019) to attribute to each compound the same unique identifier in every chromatogram
216 (if present). Peaks corresponding to FA were then identified by comparison of MS spectrum
217 with the NIST mass spectral library (version 2.3) and comparison of GC retention time with a
218 commercial standard (Supelco 37 component FAME mix; Sigma-Aldrich). The FA 18:1ω9
219 co-eluted with the FA 18:3ω3 and the FA 16:0iso co-eluted with the FA 16:4ω3, which was
220 taken into account for data interpretation. Results were reported in % of total FA and/or in mg
221 g^{-1} of sample dry weight.

222

223   3. 3. Statistical analysis

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

230

231 4. Results

232

233   4. 1. Organic matter (OM)

234   The highest proportion of OM was measured in sea cucumber foreguts (14.1 ± 4.5 %)
235 and the lowest proportion was found in nearby sediment (6.7 ± 2.7 %; Fig. 2). The Van der
236 Waerden test revealed a significant decrease of OM during gut transit with, on average, a one-
237 third reduction in OM content between foregut and faeces (14.1 ± 4.5 % in foregut vs. 9.1 ±
238 2.5 % in faeces; Fig. 2).

239

240   4. 2. Pigments

241   A total of 62 pigments have been separated by the HPLC system in all samples. Yet,
242 most of compounds could not be precisely identified as the corresponding purified standard

243   was not available. Most minor pigments were derivatives of major ones, and so they were

244   combined to their closest relative compound. Major compounds and all their derivatives were

245   summed and a selective list of pigments (plus derivatives) useful to assess the food sources of

246   *H. forskali* was constructed (Table 2). Globally, out of chlorophyll *a*, pheophorbide *a* and

247   pheophytin *a*, that are found in all decomposing vegetal sources, gut contents and faeces of

248   wild sea cucumbers were dominated by chlorophyll *c* (14-20 %), fucoxanthin (3-5 %) and

249   neoxanthin (2-3 %) with proportions relatively similar to that of sediment (Table 2).

250   Chlorophyll *c* and fucoxanthin were major pigments of suspended particulate matter (SPM)

251   and brown algae (diatoms and the macroalgae *Laminaria sp.*) and were also detected in faeces

252   of aquacultured sea cucumbers fed with diatoms. Neoxanthin was found in SPM and green

253   macroalgae (*Ulva sp.* and *Enteromorpha sp.*), but it was absent from diatoms and faeces of

254   aquacultured individuals fed diatoms. Finally, lutein was a major pigment in red macroalgae

255   (*Cryptopleura ramosa*) and in both fresh and decomposed green macroalgae (20-54 %) but it

256   was almost absent from any other kind of sample (0-1.2 %), except faeces of aquacultured

257   individuals fed with decomposed green algae where it was a major compound (11-14 %).

258   Total concentration of pigments was 4-5 times higher in gut contents and faeces of wild

259   animals compared to nearby sediment but no significant difference (Van der Waerden test; p

260   = 0.662) was revealed between the three gut contents and the faeces (Fig. 3).

261

262   4. 3. Fatty acids (FA)

263   We identified 37 fatty acids with the GC-FID/MSD system in all samples. As for

264   pigments, only a selective list of FA that we considered as meaningful to assess the food

265   sources of *H. forskali* has been provided (Table 3). Yet, we reported in Table 3 the sum of

266   major classes of FA that may largely inform on the general composition of samples. The

267   complete list of FA was provided for wild individual tissues (Table 4). Sexual comparisons

268   revealed no difference between male and female gut contents FA composition

269   (PERMANOVA; F = 0.375, p = 0.848 for foregut; F = 0.834, p = 0.458 for midgut; and F =

270   0.687, p = 0.632 for hindgut), no difference between digestive tracts (PERMANOVA; F =

271   0.238, p = 0.840), no difference between muscles (PERMANOVA; F = 1.045; p = 0.366) and

272   a significant difference between gonads (PERMANOVA; F = 8.667; p = 0.007).

273   Consequently, FA compositions of both gender gut contents, digestive tracts and muscles

274   were pooled, and those of gonads were displayed according to sex (Table 3 and 4).

275   Unlike for pigments composition, sediment and foregut contents differed in their FA

276 composition with higher proportions of FA 20:4ω6 (arachidonic acid; ARA), 20:5ω3

277 (eicosapentaenoic acid; EPA) and presence of the FA 23:1ω9 in the foregut and lower

278 proportions of FA 16:0 and saturated FA (SFA) in the sediment (Table 3). In contrast, freshly

279 released faeces poorly differed to nearby sediments, with only FA 23:1ω9 and 24:1ω9

280 significantly higher and the sum of branched FA (BrFA) significantly lower in faeces (Table

281 3). Highly unsaturated FA (HUFA) and long-chain monounsaturated FA (≥ 20-carbon chain

282 length; LC-MUFA) were present in low proportions in most potential food sources, while

283 they were relatively abundant in *H. forskali* tissues (Table 3 and 4). Total concentration of FA

284 strongly differed among tissues with higher concentrations in female gonads (Table 4).

285 Muscles were characterised by especially high levels of ARA and FA 23:1ω9 compared to

286 other tissues (Table 4) and different ω3/ω6 and eicosanoid ratios (Fig. 4). Male gonads were

287 significantly richer in ARA, EPA and FA 20:1ω9 compared to female gonads and poorer in

288 FA 16:0 and BrFA (Table 4).

289

290 5. Discussion

291

292   5. 1. Food sources

293   The similarity between the pigment composition of sediment and foregut content of

294 wild *H. forskali* suggests that the species gathers its vegetal food sources in the sediment.

295 However, higher organic content, total pigment concentration and total FA concentration in

296 gut contents and faeces vs. the sediment shows that the species has the ability to select its

297 food particles. Many authors already suggested that deposit-feeding holothurians are selective

298 feeders, although it is not evident whether this selection is due to chemosensory apical buds

299 (Bouland et al. 1982) or to the physical properties of ingested particles (Roberts et al. 2000).

300 According to Roberts et al. (2000), ingestion of coarse fragments of macrophytes is explained

301 by the low feeding selectivity of holothurians and cannot be considered as herbivory. Yet,

302 Belbachir and Mezali (2018) reported the highest Ivlev selectivity index in *H. forskali* for

303 fresh *Posidonia sp.* fragments. The *Laminaria sp.* forests in which our samples were taken are

304 very different habitats from the Posidonia seagrasses where Belbachir and Mezali (2018)

305 conducted their study, suggesting that *Posidonia sp.* fragments are not essentials in the diet of

306 *H. forskali*. Instead, high proportions of fucoxanthin and chlorophyll *c* in gut contents of wild

307   individuals indicate that diatoms and/or brown macroalgae are important food sources for *H.*

308   *forskali* in our study (Schmid et al. 2005), while neoxanthin may originate from decomposing

309   pelagic green microalgae as this carotenoid is present in high amounts in suspended

310   particulate matter (16.7 ± 0.8 %) and found almost exclusively in Prasinophyceae and

311   Chlorophyceae (Mackey et al. 1996, Brotas and Plante-Cuny 2003).

312   Conversely, low proportions of lutein in gut contents of wild *H. forskali* indicate that

313   the species does not feed on the red algae *Cryptopleura ramosa* found in close proximity and

314   exhibiting high levels of this carotenoid (37.0 ± 0.4 %). More largely, all groups of red algae

315   exhibit high contents of antheraxanthin, lutein or zeaxanthin (Schubert et al. 2006). These

316   carotenoids were not detected or below 1 % in *H. forskali* gut contents. Lutein is also an

317   important component of green macroalgae such as *Ulva sp.* that may reach the sea floor in

318   this area once in decomposition but that is not ingested by *H. forskali*. High levels of lutein in

319   the faeces of sea cucumber juveniles fed with decomposed green algae confirm that lutein can

320   be used as a reliable tracer of green algae in the diet of *H. forskali*, and that pigment

321   composition of farmed animal faeces roughly resembled that of their diet. Finally, low

322   proportions of violaxanthin and FA 18:4ω3 in gut contents reveal that brown macroalgae are

323   barely ingested, as they usually exhibit high levels of these compounds (Fleurence et al. 1994,

324   Schmid et al. 2005). Thus, the vegetal food sources ingested by *H. forskali* in our study are

325   mainly diatoms.

326   In contrast to pigments, fatty acids were not only more concentrated in foregut

327   contents compared to the sediment but also differed in composition, suggesting that other

328   food sources not containing pigments or exhibiting similar pigment compositions than

329   unsorted sediment enter the diet of *H. forskali*. Higher proportions of the FA 20:4ω6, the sum

330   of highly unsaturated FA (HUFA ≥ 20 carbon chain-length and 2 double bounds), and lower

331   proportions of the FA 16:0 in the foregut content compared to the sediment indicate that the

332   sea cucumbers ingest particles with especially high nutritional value. Actually, HUFA are

333   precious compounds with roles in membrane properties and immune response (Arts and

334   Kohler 2008, Twining et al. 2016). They are usually rapidly degraded in decaying organic

335   matter (Wakeham 1995, Vivier et al. 2019). Such elevated proportions of HUFA in the

336   foregut content of *H. forskali* suggest that ingested particles are upgraded regarding their

337   nutritional value before transiting in the gut (i.e. in the nearby sediment or in the oesophagus).

338   Trophic upgrading and enhancement of PUFA relative proportions throughout the first levels

339   of the food web is usual in the marine realm (Desvilettes and Bec 2009), and can be mediated

340 by heterotrophic protists, also named "non-pigmented" protists in the old scientific literature.

341 Other authors mentioned elevated levels of HUFA in the gut content of holothurians. Mfilinge

342 and Tsuchiya (2016) reported 21.8 % of HUFA in the foregut content of *H. leucospilota* and

343 31.4 % in *H. atra*. Both species where assumed to feed on the nearby sediment that exhibited

344 only 9.5 and 16.8 % of HUFA, respectively. High levels of HUFA in the foregut were

345 attributed to the specific selection of algae and bacteria rich detrital particles (Mfilinge and

346 Tsuchiya 2016). Although microalgae have long been considered the major *de novo* producer

347 of PUFA in marine food webs (Nichols 2003), diatoms, that are the dominant microalgae

348 consumed by *H. forskali* in our study, are usually poor in FA 20:4ω6 (Dalsgaard et al. 2003,

349 Lang et al. 2011), suggesting that the HUFA measured in the foregut content of *H. forskali*

350 may rather originate from bacteria (Nichols 2003), heterotrophic protists (Desvilettes and Bec

351 2009) and/or their symbiotic relationship (Gast et al. 2009). Ginger et al. (2000) also

352 measured high amounts of HUFA in the gut contents of holothurians, but they attributed these

353 elevated levels to the lysis of gut walls, caused by stress during animals recovery. Yet, Ginger

354 et al. (2000) worked on abyssal holothurians and change in barometric pressure during the

355 ascent phase may have caused a sufficient stress to cause cell wall lysis, which may not have

356 occurred in our study as sea cucumbers were gathered only at a few meters deep. In addition,

357 Féral (1985) measured higher lipid levels in the anterior intestine wall compared to the

358 oesophagus of *Leptosynapta galliennei* (51.9 $\pm$ 3.4 µg mg^{-1} vs. 7.6 $\pm$ 3.5 µg mg^{-1},

359 respectively). Assuming similar differences in *H. forskali*, cell wall lysis would be expected to

360 cause higher lipid contents in the midgut compared to the foregut content. The opposite was

361 measured in our study, confirming that cell wall lysis caused by the sampling stress was

362 minor. In an aquaculture perspective, further investigations would be required to determine

363 whether the nutritional upgrading of detrital organic matter takes place in the sediment and/or

364 in the foregut and which organisms mediate the biosynthesis pathways of HUFA. Actually, if

365 possible, feeding the microbiote of *H. forskali* instead of providing feeds of high nutritional

366 value may be of interest in a sustainable aquaculture perspective.

367

5. 2. Digestive efficiency

369 The significant but weak differences in OM between foregut contents and faeces

370 suggest that *H. forskali* have to process large quantities of sediment to cover its nutritional

371 needs. In the midgut (the largest compartment) of *H. forskali* found on rocky substrate in the

372 Glenan Islands, the OM content was slightly above that of the complete gut measured in

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

The absence of significant changes in relative proportions and total concentrations of pigments during gut transit indicates that *H. forskali* poorly utilises such resources, and confirms that pigments are good biomarkers of diet. In addition, these results suggest that living diatoms, that are the main pigmented food sources consumed by *H. forskali* in our study, are not digested during gut transit. Fresh pelagic microalgae were administrated to farmed juveniles. Given that *H. forskali* is a deposit feeding species, most of ingested cells were probably dead algae, which induced lower relative proportions of chlorophyll *a*, chlorophyll *c* and fucoxanthin in juveniles faeces compared to their living food sources, along with the almost exclusive presence of pheophorbide *a* in faeces, a degradation product of chlorophylls (Roy et al. 2011), that was absent from the fresh cultured microalgae. Inefficient digestion of living diatoms was also confirmed by higher relative proportions of C$_{16}$ PUFA and the FA 16:1ω7, both biomarkers of diatoms (Dalsgaard et al. 2003, Brett et al. 2009, Kelly and Schiebling 2012) in the hindgut compared to the foregut contents of wild *H. forskali*. Yet, inefficient digestion of living diatoms raises the question of reason for their ingestion, that may be due to inefficient feeding selectivity, or to the feeding on compounds that do not require cell lysis. The most abundant compounds that are released by microalgae in the adjacent environment are exopolymer secretions, mostly on the form of polysaccharides (Decho 1990, Mühlenbruch et al. 2018). Such compounds may provide a source of carbon for deposit feeders (Roberts et al. 2000), as previously evidenced in various echinoderms (e.g. in the holothurian *Isostichopus badionotus* studied by Baird and Thistle 1986 and in the brittlestar *Amphipholis gracillima* studied by Hoskins et al. 2003). In stressing conditions (as is most probably the digestive tract of holothurians for diatoms), algal cells are known to

406 increase their exudation rate (Mühlenbruchet al. 2018) especially in terms of carbohydrates

407 when they are exposed for instance to changing environmental conditions such as pH and

408 surface roughness (Riera et al. 2018). Ingest and force diatoms to release nutritive compounds

409 may thus be a very efficient way to obtain energy for a deposit-feeding organism such as *H.*

410 *forskali*.

411   In contrast to diatom biomarkers, HUFA proportions significantly decreased during

412 gut transit (31.8 ± 9.2 % in the foregut vs. 19.8 ± 4.2 % in the hindgut), confirming that these

413 compounds (with a high nutritional value) are those that are preferentially assimilated by the

414 sea cucumbers, while increase in proportions of the FA 16:0 and the sum of SFA indicates

415 that remaining compounds are in a more advanced state of degradation with lower nutritional

416 value. The almost absence of significant differences between the FA profiles of nearby

417 sediment and sea cucumber faeces indicates that *H. forskali* have little influence on sediment

418 composition, except concerning the proportion of branched FA, indicative of bacteria

419 (Kaneda 1991, Kelly and Schiebling 2012). Actually, lower BrFA proportions in faeces ($2.0 \pm$

420 0.3 %) compared to nearby sediment (4.6 ± 1.2 %), along with similar total FA

421 concentrations, suggest that *H. forskali* has the ability to reduce bacterial loads on the sea

422 floor. Thus, as other species of sea cucumbers, *H. forskali* may be an interesting candidate for

423 integrated multitrophic aquaculture (Zamora et al. 2016), as already proposed by MacDonald

424 et al. (2003).

425

5. 3. Resources allocation

427   High contents of FA in the digestive tract of *H. forskali* are in accordance with

428 histological observations of Féral (1985) where the author measured especially high levels of

429 lipids in the anterior intestine walls of *Leptosynapta galliennii* (i.e. lipid droplets). Féral and

430 Massin (1982) suggested that the digestive tract may serve as a storage organ and Allen

431 (1968) measured highest incorporation rates of radioacetate in lipids of the gut isolated from

432 the *rete mirabile* of *H. forskali*. All these results suggest that the fatty acid composition of

433 holothuroids digestive tract deserves particular attention. The energy storage role of this organ

434 has however been dismissed by Féral (1985) who did not measure any decay in the anterior

435 intestine lipid content of adult *L. galliennii* before and after two weeks of starvation.

436 Similarly, no seasonal difference was measured in the digestive tract lipid content of

437 *Molpadia violacea* by Féral and Magniez (1985) whereas a storage role of the digestive tract

438 would probably provoke a notable depletion of stocks during winter. Yet, at the larval stage

439 Peters-Didier and Sewell (2019) evidenced an accumulation of neutral lipids in the stomach

440 epithelium of 14 days post-fertilisation larvae of *Australostichopus mollis*. Lipid droplets ~ 10

441 µm in diameter suspended in the larval blastocoel were further transported to the hyaline

442 spheres (an enigmatic structure present in the late auricularia stage of sea cucumbers) to fuel

443 the process of metamorphosis. Whether this accumulation of lipids in the stomach epithelium

444 has also a role at the adult stage is still to be investigated but high incorporation rates of

445 radioacetate in the gut of *H. forskali* measured by Allen (1968) suggest that this organ plays a

446 major role in lipid biosynthesis. Finally, the closest similarity between ω3/ω6 FA and

447 eicosanoid ratios between the digestive tract and the gonads compared to that of muscles

448 suggests that the anterior intestine could exchange functional lipids with reproductive organs.

449 The absence of sexual difference in the FA composition of gut contents, muscles and

450 digestive tract of wild *H. forskali* suggests that food resources and digestive efficiency are not

451 sex-dependant in this species. Sexual difference in the FA composition of gonads may thus

452 originate from different incorporation strategies and/or conversion of FA rather than feeding.

453 In an aquaculture perspective, a lot of studies on FA composition of gonads have been

454 conducted on fishes, where docosahexaenoic acid (22:6ω3; DHA) is among the most

455 abundant constituent of testes (Bell et al. 1997, Jeong et al. 2002, Beirão et al. 2015). This FA

456 is expected to confer spermatozoids a high membrane fluidity (Stillwell and Wassal 1997)

457 that is necessary for fecundation. It has been demonstrated experimentally that the inclusion

458 of DHA in *Solea senegalensis* diet may improve sperm quality (Beirão et al. 2015). Yet, in

459 our study DHA was a minor component of gonads, both in males and females. This result is

460 consistent with previous works on other sea cucumber species, such as *Apostichopus*

461 *japonicus* (< 5 % DHA in gonads; Kasai 2003), *Cucumaria frondosa* (< 2 % DHA in gonads

462 of wild individuals and individuals fed diatoms; Gianasi et al. 2017), *Holothuria tubulosa* or

463 *H. polii* (< 2 % DHA in gonads; Biandolino et al. 2019) and in sea urchins (< 3 % DHA in

464 gonads; Martínez-Pita et al. 2010, Díaz de Vivar et al. 2019), suggesting that DHA is not as

465 crucial for echinoderm reproduction as it is for fishes. Instead, membrane fluidity in

466 echinoderms may be provided by non-methylene interrupted FA as suggested for the sea

467 urchin *Paracentrotus lividus* (Carboni et al. 2013). Such FA could not be properly identified

468 in our study but they may happen within the unidentified HUFA and/or co-elute with others

469 on the non-polar HP-5ms column. In *H. forskali*, male and female gonads differ by higher

470 proportions of eicosapentaenoic acid (20:5ω3; EPA) and arachidonic acid (20:4ω6; ARA) in

471   males. However, the twice as high total concentration of fatty acids in female compared to

472   male gonads (78.0 ± 24.9 mg g^{-1} in female vs. 36.8 ± 3.7 mg g^{-1} in male gonads) may be due

473   to the storage of lipids for further use as an energy source by eggs, rather than for functional

474   purpose. Maternal provisioning is actually a strong determinant of larval development in

475   echinoderms as embryos and larvae initiate their development using nutrients provided in the

476   eggs (Prowse et al. 2007). The lipid requirement of eggs being higher than for spermatozoids

477   (that require energy only until fecundation), FA may not be as strictly selected, thus

478   explaining lower HUFA proportions in female gonads compared to that of males (23.2 ± 3.2

479   % in female vs. 36.4 ± 7.0 in male gonads). Exploring the provisioning of essential

480   compounds (i.e. lipids) during gametogenesis will be necessary for aquaculture purpose as

481   broodstock conditioning may affect larval survival and development.

482   Finally, muscle FA composition strongly differed with that of gut contents, digestive

483   tract and gonads, confirming that specific elements are preferentially retained and/or

484   converted to be allocated to tissues, as hypothesised in the introduction. The preferential

485   accumulation of PUFA in consumers relative to their diet is common in marine food webs,

486   but the retention of long-chain MUFA is more unusual (Budge et al. 2006). The FA 23:1ω9 is

487   found in significant proportions only in sea cucumbers, although sometimes detected as traces

488   in other echinoderms, crustaceans and bivalves (Kaneniwa et al. 1986, Rezanka and Sigler

489   2008, Drazen et al. 2008). Kaneniwa et al. (1986) found higher proportions of the FA 23:1ω9

490   in phospholipids compared to neutral lipids of two species of sea cucumbers, suggesting that

491   this FA has a physiological role in holothurians. It most probably does not originate from diet,

492   as none of the potential food sources exhibited even trace amounts of this compound, and as

493   previously proposed by Kaneniwa et al. (1986). Yet, it was detected in gut contents since the

494   foregut, showing that the mechanism leading to its synthesis (most probably α-oxidation of

495   the FA 24:1ω9; Kaneniwa et al. 1986) is activated at the earliest stage of the digestive process

496   and/or that it is synthesised by endosymbionts. The absence of FA 23:1ω9 in farmed juvenile

497   faeces indicates that the mechanism appears later in the development, that endosymbionts

498   could not colonise individuals breed in captivity and/or that precursors of this FA have to be

499   found in sediment microbial communities, that was absent in juvenile tanks. In an aquaculture

500   perspective, the role and origin of this FA will require further investigation.

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3   503   6. Conclusion
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6   504   Our study reveals that neither green nor red algae enter the diet of *H. forskali* in the
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8   505   Glenan Islands in spring, and that the only fresh vegetal material found in gut contents is
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10   506   diatoms. These diatoms are however poorly digested during gut transit and the utility of their
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12   507   ingestion remains unclear. In addition, gut contents exhibit a high nutritional quality that
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14   508   contrasts with the detrital nature of ingested diet, and suggesting that a trophic upgrading of
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16   509   OM occurs before digestion. To date, the few attempts that were made to feed *H. forskali*
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18   510   have been focusing on macroalgae that are actually cheap, easy to obtain and achieving
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20   511   relatively good results. Yet, as they may not constitute the natural food resources of *H.*
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22   512   *forskali* their use could lead to unexpected difficulties (e.g. health issues, poor reproduction
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24   513   efficiency) and/or sub-optimal growth rate. Our study aims to open up a wider range of
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26   514   nutritional options for the rearing of *H. forskali* (e.g. microbial cultures, food industry
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28   515   discards, dejections of other animals in multitrophic systems). Finally, unusual fatty acids
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30   516   such as non-methylene interrupted FA or the FA 23:1ω9 may play a non-negligible
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32   517   physiological role in *H. forskali*. How these compounds are affected by nutrition in
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34   518   aquaculture and their effect on sea cucumber fitness needs further investigation for a
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36   519   sustainable production of holothurians.
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41   521   7. References
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753   Table 1: List of collected samples and major weaknesses associated

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 (*Chatoceros sp.*, *Skeletonema sp.* and *Isochrysis sp.*)	Faeces and feed	2-4 cm juveniles
AquaB company	Decomposed *Ulva sp.* or *Enteromorpha sp.*	Faeces, feed and fresh macroalgae	2-4 cm juveniles

754

755 Table 2: Selected proportions (%) of pigments in samples (mean ± SD). Anthera = Antheraxanthin; Chla (b) (c) = Chlorophyll a (b) (c); Fucox =

756 Fucoxanthin; Neox = Neoxanthin; Viola = Violanxanthin; Pheopd = Pheophorbide a; Pheopt = Pheophytin a.

	n	Anthera	Chla	Chlb	Chlc	Fucox	Lutein	Neox	Viola	Pheopd	Pheopt	Others	Total concentration
Food sources													
Laminaria sp. (brown macroalgae)	2	0.0 ± 0.0	6.6 ± 0.6	0.0 ± 0.0	8.4 ± 0.1	38.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	15.1 ± 0.2	0.0 ± 0.0	26.9 ± 0.2	4.5 ± 0.1	2.3 ± 0.2
Cryptopleura ramosa (red macroalgae)	2	0.0 ± 0.0	15.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.0	37.0 ± 0.4	0.0 ± 0.0	1.5 ± 0.0	0.0 ± 0.0	39.4 ± 0.7	6.4 ± 1.0	7.0 ± 0.4
Ulva sp. (green macroalgae)	2	1.7 ± 0.1	10.5 ± 0.1	5.9 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	53.4 ± 0.3	6.8 ± 0.5	18.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	3.6 ± 0.1	8.3 ± 0.1
Decomposed *Ulva* sp.	2	1.4 ± 0.0	3.2 ± 0.0	3.8 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	32.4 ± 2.0	3.9 ± 0.3	11.1 ± 2.6	25.4 ± 1.9	17.2 ± 3.0	1.5 ± 0.1	22.5 ± 5.0
Enteromorpha sp. (green macroalgae)	2	5.7 ± 0.5	8.2 ± 0.4	3.7 ± 0.2	0.0 ± 0.0	2.4 ± 0.1	39.7 ± 0.6	12.4 ± 0.8	23.0 ± 0.3	0.0 ± 0.0	1.7 ± 0.5	3.3 ± 0.3	15.2 ± 2.4
Decomposed *Enteromorpha* sp.	2	5.6 ± 1.0	1.7 ± 0.2	1.2 ± 0.1	0.0 ± 0.0	3.5 ± 0.4	20.7 ± 1.3	2.4 ± 0.1	7.4 ± 0.2	20.5 ± 1.9	34.5 ± 3.2	2.4 ± 0.4	5.9 ± 2.2
Cultured microalgae	2	0.0 ± 0.0	10.7 ± 0.4	0.0 ± 0.0	29.7 ± 4.0	52.6 ± 5.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.9 ± 2.2	n.d.
Shelfish Diet 1800	2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	13.1 ± 0.0	7.8 ± 0.3	0.9 ± 0.1	4.7 ± 0.3	0.4 ± 0.0	71.6 ± 0.7	0.2 ± 0.0	1.3 ± 0.0	256.8 ± 4.0
Grower Fertil + fresh mussels	2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	29.0 ± 1.7	0.0 ± 0.0	2.2 ± 0.6	0.0 ± 0.0	64.5 ± 2.0	3.7 ± 0.7	0.6 ± 0.1	4.9 ± 1.7
Suspended particulate matter	2	0.0 ± 0.0	7.5 ± 0.3	0.2 ± 0.0	44.0 ± 0.3	23.0 ± 0.1	0.0 ± 0.0	16.7 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	2.9 ± 0.0	5.8 ± 0.2	3.6 ± 1.1
Faeces of aquacultured individuals													
Juveniles fed decomposed *Ulva* sp.	3	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11.4 ± 1.2	1.2 ± 0.2	3.7 ± 0.4	74.0 ± 2.8	7.8 ± 0.9	0.8 ± 0.2	24.8 ± 3.2
Juveniles fed decomposed *Enteromorpha* sp.	3	5.1 ± 1.7	0.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 1.0	14.4 ± 2.5	0.0 ± 0.0	3.7 ± 0.2	36.4 ± 3.5	26.6 ± 3.5	8.4 ± 1.3	3.1 ± 1.7
Juveniles fed fresh microalgae	3	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	4.4 ± 0.6	1.4 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	91.3 ± 1.5	1.8 ± 1.0	1.0 ± 0.6	74.2 ± 27.1
Adults fed Shellfish Diet 1800 + Grower Fertil + fresh mussels	3	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	9.1 ± 1.3	6.9 ± 2.5	0.4 ± 0.1	3.1 ± 0.4	0.5 ± 0.3	77.7 ± 5.3	1.6 ± 0.6	0.5 ± 0.3	25.5 ± 21.2
Wild *H. forskali* samples													
Nearby sediment	4	0.0 ± 0.0	0.7 ± 0.4	0.2 ± 0.1	12.2 ± 3.1	5.9 ± 2.6	1.0 ± 0.2	2.5 ± 0.8	0.5 ± 0.4	71.4 ± 4.7	3.9 ± 1.1	1.6 ± 0.2	1.4 ± 0.9
Foregut content	10	0.0 ± 0.0	0.4 ± 0.4	0.2 ± 0.1	14.9 ± 5.2	4.9 ± 2.3	0.7 ± 0.3	2.8 ± 0.6	0.7 ± 0.1	66.3 ± 9.0	6.9 ± 4.5	2.2 ± 0.7	5.9 ± 2.2
Midgut content	10	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	16.5 ± 4.0	3.1 ± 0.6	0.7 ± 0.2	2.5 ± 0.5	0.7 ± 0.2	69.6 ± 4.9	4.4 ± 2.4	2.3 ± 1.0	7.0 ± 3.0
Hindgut content	10	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	16.4 ± 3.1	3.4 ± 0.5	0.7 ± 0.1	2.4 ± 0.5	0.7 ± 0.1	71.4 ± 3.4	2.7 ± 1.3	1.9 ± 0.4	7.3 ± 3.2
Freshly released faeces	4	0.0 ± 0.0	0.3 ± 0.1	0.1 ± 0.0	19.6 ± 2.9	3.2 ± 0.7	1.2 ± 0.4	2.2 ± 0.1	0.7 ± 0.1	67.1 ± 5.5	3.6 ± 1.7	2.0 ± 0.3	6.5 ± 3.0

757 *n.d. not determined*

758 Table 3: Selected fatty acid proportions in samples (mean ± SD). Letters in exponent indicate significant differences between nearby sediment, gut
759 contents and faeces of wild individuals (Van der Waerden test, α = 5%).
760

	n	16:0	ΣSFA	16:1ω7	20:1ω9	22:1ω9	23:1ω9	24:1ω9	Σ LC-MUFA	Σ MUFA
Food sources										
Laminaria sp. (brown macroalgae)	2	21.1 ± 1.0	30.0 ± 1.6	3.1 ± 0.0	0.3 ± 0.0				0.5 ± 0.1	28.3 ± 0.8
Cryptopleura ramosa (red macroalgae)	2	61.9 ± 1.1	77.6 ± 1.0	3.6 ± 0.5	0.6 ± 0.1				0.6 ± 0.1	14.4 ± 0.2
Ulva sp. (green macroalgae)	2	44.8 ± 0.9	51.8 ± 0.7	4.2 ± 0.2					0.2 ± 0.0	33.1 ± 0.0
Decomposed *Ulva* sp.	2	16.6 ± 1.0	29.8 ± 0.3	17.9 ± 2.6	0.4 ± 0.1	0.1 ± 0.1		0.1 ± 0.1	1.3 ± 0.3	46.9 ± 0.1
Enteromorpha sp. (green macroalgae)	2	29.7 ± 0.8	36.4 ± 1.3	3.8 ± 0.4	0.2 ± 0.0				0.2 ± 0.0	32.4 ± 0.5
Decomposed Enteromorpha sp.	2	18.7 ± 2.1	34.1 ± 3.1	10.7 ± 0.9	0.6 ± 0.1	0.1 ± 0.0		0.2 ± 0.1	2.4 ± 0.2	38.8 ± 2.9
Cultured diatoms (brown microalgae)	2	27.4 ± 3.2	64.2 ± 3.4	12.4 ± 4.9						20.2 ± 1.8
Shelfish Diet 1800	2	14.9 ± 0.0	23.7 ± 0.1	9.5 ± 0.2	0.4 ± 0.0	0.9 ± 0.0			1.5 ± 0.0	31.4 ± 0.0
Grower Fertil + fresh mussels	2	19.6 ± 1.4	29.8 ± 1.6	10.0 ± 2.0	3.2 ± 0.1	0.3 ± 0.0		0.2 ± 0.0	4.9 ± 0.0	32.3 ± 0.1
Suspended particulate matter	2	33.7 ± 1.5	66.9 ± 4.2	7.0 ± 2.2	0.4 ± 0.1	0.6 ± 0.4			1.4 ± 0.5	22.8 ± 3.2
Faeces of farmed individuals										
Juveniles fed decomposed *Ulva* sp.	3	22.7 ± 6.0	47.7 ± 9.6	13.1 ± 2.2	0.3 ± 0.3				0.9 ± 0.5	34.5 ± 6.8
Juveniles fed decomposed *Enteromorpha* sp.	3	29.4 ± 6.4	53.9 ± 11.5	5.8 ± 2.2	0.2 ± 0.4				0.5 ± 0.8	30.0 ± 5.8
Juveniles fed fresh microalgae	3	31.8 ± 17.8	60.9 ± 28.1	9.6 ± 7.6	0.3 ± 0.2	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.7 ± 0.7	22.2 ± 16.9
Adults fed Shelfish Diet 1800 + Grower Fertil + fresh mussels	3	29.1 ± 2.5	45.6 ± 1.1	9.4 ± 1.1	5.6 ± 1.7	0.3 ± 0.1	0.8 ± 1.3	0.2 ± 0.0	8.1 ± 0.6	33.4 ± 0.8
Wild *H. forskali* samples										
Nearby sediment	4	23.0 ± 2.9[a]	42.0 ± 3.1[a]	11.3 ± 4.7[bc]	3.3 ± 2.3	0.5 ± 0.4		0.1 ± 0.2[c]	5.0 ± 3.0[b]	30.9 ± 2.7
Foregut content	10	12.0 ± 6.0[b]	30.7 ± 7.4[b]	9.9 ± 4.0[c]	2.3 ± 1.1	0.4 ± 0.3	2.2 ± 0.6[a]	1.7 ± 1.1[a]	9.4 ± 3.2[a]	29.1 ± 3.2
Midgut content	10	14.1 ± 3.7[b]	33.5 ± 3.6[b]	14.0 ± 3.7[ab]	1.0 ± 0.5	0.7 ± 0.4	1.3 ± 0.6[b]	1.2 ± 0.5[a]	6.2 ± 1.6[b]	30.7 ± 2.1
Hindgut content	10	21.3 ± 5.2[a]	39.7 ± 6.0[a]	17.1 ± 3.7[a]	1.3 ± 0.5	0.3 ± 0.4	1.0 ± 0.7[b]	0.5 ± 0.4[bc]	4.2 ± 2.1[b]	30.6 ± 3.2
Freshly released faeces	4	23.7 ± 0.9[a]	40.6 ± 1.1[a]	15.0 ± 1.8[ab]	2.1 ± 0.6	0.2 ± 0.1	2.2 ± 1.0[a]	0.9 ± 0.3[ab]	6.4 ± 2.1[ab]	31.9 ± 2.8

761

762 *n.d. not determined*

763

	n	Σ C16PUFA	18:4ω3	20:4ω6 (ARA)	20:5ω3 (EPA)	22:6ω3 (DHA)	Σ HUFA	Σ PUFA	Σ BrFA	Total concentration (mg g^{-1})
Food sources										
Laminaria 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
Cryptopleura ramosa (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
Ulva 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
Decomposed *Ulva* 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
Enteromorpha 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
Decomposed *Enteromorpha* 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
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
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
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
Faeces of aquacultured individuals										
Juveniles fed decomposed *Ulva* 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
Juveniles fed decomposed *Enteromorpha* 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
Juveniles fed fresh microalgae	3	1.9 ± 1.7	0.5 ± 0.1	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
Wild *H. forskali* samples										
Nearby sediment	4	1.8 ± 1.0[b]	1.1 ± 0.6	3.7 ± 1.2[c]	9.2 ± 3.9	2.6 ± 1.0	17.1 ± 6.9[c]	21.0 ± 6.5[c]	4.5 ± 1.2[a]	0.3 ± 0.1[d]
Foregut content	10	1.7 ± 0.8[b]	1.3 ± 0.3	8.6 ± 2.7[a]	15.0 ± 5.3	5.1 ± 4.9	31.8 ± 9.2[a]	34.9 ± 8.7[a]	3.1 ± 1.0[b]	2.6 ± 1.3[a]
Midgut content	10	2.5 ± 0.7[ab]	1.6 ± 0.2	6.3 ± 1.7[b]	13.2 ± 2.6	3.2 ± 0.9	24.7 ± 5.0[ab]	29.5 ± 4.7[ab]	4.1 ± 1.3[a]	1.1 ± 0.4[b]
Hindgut content	10	2.9 ± 0.5[a]	1.5 ± 0.2	4.6 ± 1.4[c]	11.1 ± 2.2	2.7 ± 0.9	19.8 ± 4.2[bc]	24.9 ± 4.2[bc]	2.7 ± 0.9[bc]	0.7 ± 0.5[c]
Freshly released faeces	4	2.7 ± 0.5[ab]	1.6 ± 0.4	5.0 ± 1.1[bc]	10.1 ± 1.7	2.0 ± 0.7	18.3 ± 2.4[bc]	23.5 ± 2.4[bc]	2.0 ± 0.3[c]	0.4 ± 0.2[cd]

n.d. not determined

768 Table 4: Fatty acid proportions of wild *H. forskali* tissues (mean ± SD). Letters in exponent
769 indicate significant differences between groups (Van der Waerden test, α = 5%). Note that
770 traces of 18:3ω3 may have co-eluted with 18:1ω9 and traces of 16:0iso may have co-eluted
771 with 16:4ω3. 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)		
Saturated												
12:0	0.3	±	0.1[a]	0.1	±	0.1[b]	0.1	±	0.0[b]	0.1	±	0.1[b]
14:0	2.5	±	0.4[c]	3.9	±	0.8[b]	5.3	±	0.5[a]	2.2	±	1.5[c]
15:0	0.7	±	0.2[b]	0.7	±	0.2[b]	1.3	±	0.2[a]	0.5	±	0.3[b]
16:0	5.3	±	0.9[b]	6.0	±	1.1[b]	8.9	±	0.9[a]	5.0	±	1.7[b]
17:0	0.7	±	0.1[c]	0.9	±	0.1[b]	1.1	±	0.1[a]	1.4	±	0.5[a]
18:0	4.6	±	0.4[c]	7.2	±	0.7[b]	6.5	±	0.8[b]	9.4	±	1.9[a]
19:0	1.2	±	0.1[b]	1.7	±	0.3[a]	1.2	±	0.1[b]	1.7	±	0.1[a]
20:0	2.9	±	0.2[ab]	2.8	±	0.4[b]	2.3	±	0.2[c]	3.8	±	1.2[a]
21:0	1.8	±	0.3[a]	1.4	±	0.3[b]	0.9	±	0.1[c]	1.2	±	0.1[b]
22:0	1.8	±	0.1[a]	1.5	±	0.4[a]	1.2	±	0.2[b]	1.1	±	0.1[b]
∑SFA	21.8	±	1.5	26.2	±	2.4	28.7	±	1.0	26.4	±	0.9
Monounsaturated												
16:1ω9	0.3	±	0.1[b]	0.3	±	0.1[b]	0.7	±	0.2[a]	0.1	±	0.2[c]
16:1ω7	3.9	±	0.5[b]	8.3	±	1.8[a]	9.5	±	1.1[a]	4.4	±	2.7[b]
16:1ω5	0.4	±	0.1[bc]	0.4	±	0.1[b]	0.8	±	0.2[a]	0.3	±	0.1[c]
18:1ω9	2.2	±	0.4[c]	2.6	±	0.3[b]	3.9	±	0.5[a]	2.2	±	0.7[c]
18:1ω7	3.7	±	0.4[c]	5.7	±	0.4[b]	6.4	±	0.7[ab]	7.9	±	2.9[a]
20:1ω9	7.3	±	1.0[a]	4.5	±	0.4[bc]	3.9	±	0.8[c]	5.5	±	1.0[b]
20:1ω7	1.3	±	0.1[b]	1.6	±	0.2[a]	1.4	±	0.1[b]	2.0	±	0.3[a]
22:1ω9	1.2	±	0.2[a]	0.6	±	0.1[c]	0.8	±	0.2[a]	0.6	±	0.2[bc]
22:1ω7	1.8	±	0.2[a]	1.8	±	0.3[a]	1.4	±	0.3[b]	1.5	±	0.4[ab]
23:1ω9	7.7	±	1.5[a]	3.5	±	0.6[b]	2.1	±	0.9[c]	3.6	±	1.3[bc]
24:1ω9	3.5	±	0.7[a]	1.4	±	0.2[b]	1.4	±	0.3[b]	1.8	±	0.3[b]
∑LC-MUFA	22.9	±	3.3[a]	13.3	±	1.4[b]	11.0	±	2.1[c]	15.0	±	2.8[b]
∑MUFA	33.5	±	2.1[a]	30.7	±	1.3[b]	32.4	±	1.7[ab]	29.9	±	3.8[b]
Polyunsaturated												
16:4ω3	1.1	±	0.1[c]	1.6	±	0.3[b]	2.4	±	0.4[a]	1.1	±	0.6[c]
16:3ω4	0.5	±	0.1[c]	0.7	±	0.2[b]	1.1	±	0.4[a]	0.4	±	0.2[c]
16:2ω4	0.4	±	0.1[b]	0.7	±	0.3[a]	0.8	±	0.2[a]	0.4	±	0.3[b]
18:4ω3	1.3	±	0.3	1.7	±	0.3	1.8	±	0.4	1.4	±	0.7
18:2ω6	0.8	±	0.2[c]	0.9	±	0.1[b]	1.3	±	0.2[a]	0.7	±	0.1[c]
20:4ω6	19.5	±	0.9[a]	11.0	±	1.9[b]	7.4	±	1.1[c]	12.8	±	2.5[b]
20:5ω3	11.9	±	1.5[b]	15.9	±	1.8[a]	11.5	±	1.8[b]	18.5	±	4.0[a]
20:2ω6	0.8	±	0.2[b]	1.3	±	0.3[a]	1.0	±	0.1[a]	1.4	±	0.6[a]
Unidentified HUFA	1.3	±	0.2	1.0	±	0.2	0.8	±	0.1	0.9	±	0.1
22:5ω6	1.1	±	0.1[a]	0.7	±	0.2[b]	0.5	±	0.1[c]	0.8	±	0.2[b]
22:6ω3	2.0	±	0.4[b]	2.7	±	0.5[a]	2.0	±	0.6[b]	2.1	±	0.6[ab]
∑C16PUFA	0.9	±	0.2[b]	1.4	±	0.4[a]	1.9	±	0.6[a]	0.8	±	0.5[b]
∑HUFA	36.6	±	2.0[a]	32.6	±	4.4[a]	23.2	±	3.2[b]	36.4	±	7.0[a]
∑PUFA	38.9	±	1.8[a]	36.5	±	3.8[a]	28.7	±	3.3[b]	39.2	±	5.5[a]
Branched												
14:0iso	0.5	±	0.1[bc]	0.6	±	0.1[b]	1.0	±	0.2[a]	0.3	±	0.2[c]
15:0iso	1.7	±	0.4[bc]	2.1	±	0.4[b]	3.6	±	0.6[a]	1.2	±	0.8[c]
15:0anteiso	0.7	±	0.2[bc]	0.8	±	0.2[b]	1.4	±	0.3[a]	0.5	±	0.3[c]
17:0iso	0.8	±	0.1[c]	1.1	±	0.1[b]	1.6	±	0.2[a]	0.9	±	0.3[c]
17:0anteiso	0.4	±	0.1[b]	0.4	±	0.0[b]	0.6	±	0.1[a]	0.3	±	0.1[b]
∑BrFA	4.1	±	0.8[bc]	4.9	±	0.9[b]	8.3	±	1.3[a]	3.2	±	1.8[c]
∑FA (mg g⁻¹)	5.2	±	1.8[c]	40.1	±	10.1[b]	78.0	±	24.9[a]	36.8	±	3.7[b]

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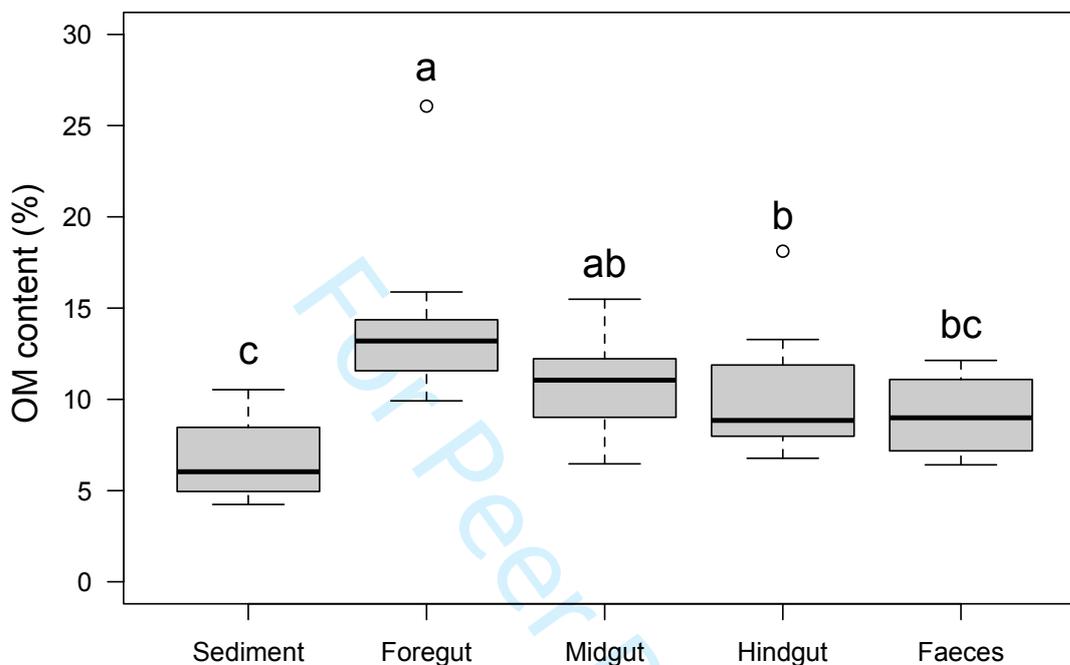
775   Delle Chiaje, 1823
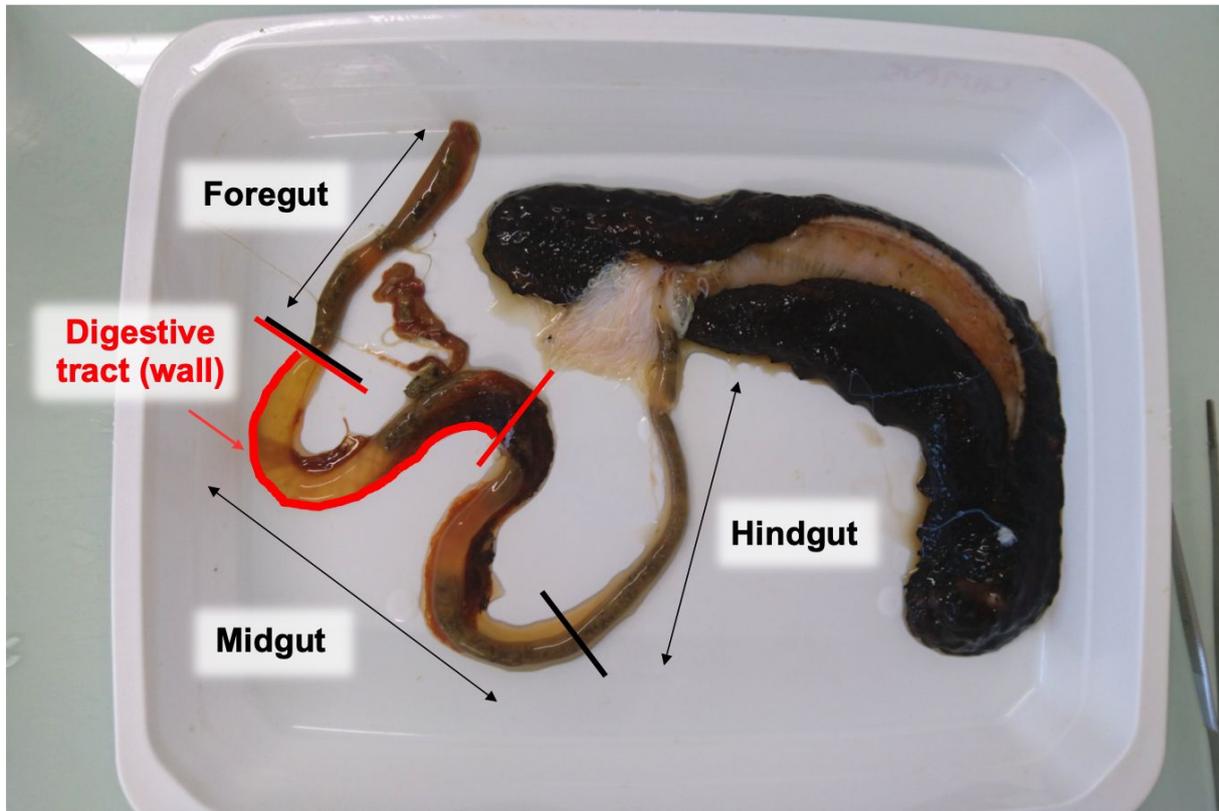

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779   Fig. 2: Boxplot of the organic matter content (%) in wild *H. forskali* samples and nearby

780   sediment. Broad lines indicate median, box edges refer to 1st and 3rd quartiles and circles

781   indicate outliers. Letters indicate significant differences between samples (Van der Waerden
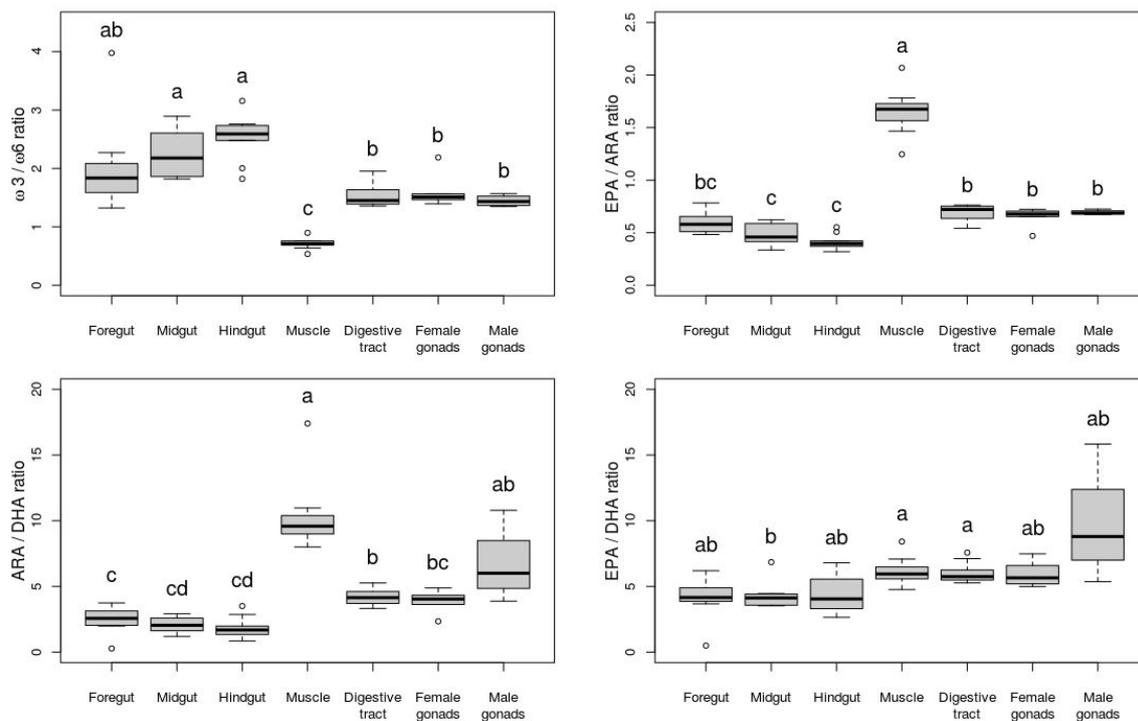
782   test, $\alpha = 5\%$)

783

784   Fig. 3: Total pigment concentration in aquaculture and wild samples ($\mu g\ mg\ dw^{-1}$). Broad

785   lines indicate median and box edges refer to 1st and 3rd quartiles.

786
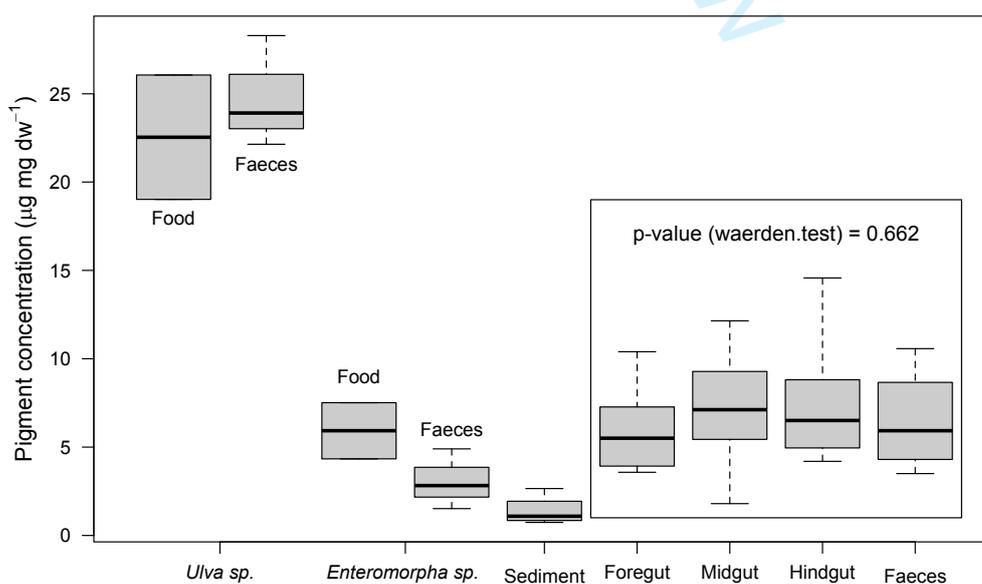
787

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, α = 5%). Broad lines

790   indicate median, box edges refer to 1st and 3rd quartiles and circles indicate outliers.

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