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

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Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

## 1. Abstract

Various research projects in Europe and North Africa have recently intended to breed temperate holothurians to alleviate fishing pressure on natural populations. However, to date little is known about the nutritional requirements of East Atlantic and Mediterranean species. In this study, we propose a "natural populations" oriented approach to characterise food sources, digestive efficiency and resources allocation based on the composition of pigments and fatty acids (FA) in gut contents and tissues (muscles, gonads and digestive tract walls) of wild individuals of the species *Holothuria (Panningothuria) forskali* (Delle Chiaje, 1823) sampled in Brittany (France). Our study reveals that neither green nor red algae enter the diet of *H. forskali* in spring, and that the only fresh vegetal material found in gut contents is brown algae (very likely diatoms). The high nutritional quality of gut contents however contrasts with the detrital nature of the ingested food sources, suggesting that a trophic upgrading of organic matter occurs before digestion. In addition, unusual FA (i.e. only present in a few groups of living species) such as long-chain monounsaturated FA (especially the FA 23:1ω9) were found in large proportions in muscles and gonads and their effect on sea cucumber fitness needs further investigation.

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

## 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

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

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

station of Agrocampus-Ouest maintains 2-4 cm juveniles in 25 L plastic tanks cleaned daily, and feeds them with a mixture of microalgae produced on site (the two Bacillariophyceae *Chatoceros sp.* and *Skeletonema sp.* and the Prymnesiophyceae *Isochrysis sp.*). Finally, the AquaB Company in Plobannalec-Lesconil maintains 2-4 cm juveniles in 50 cm diameter polyamide sieves (20 µm mesh size) placed in cylindroconical tanks with a circulating filtered seawater system, and feeds them with decomposed green macroalgae (*Ulva sp.* or *Enteromorpha sp.*). Green seaweeds are gathered on nearby beaches, left to decompose during a few weeks in aerated cylindroconical tanks and the mixture is filtered before feeding events to collect particles in the size 50-300 µm. All experimental stations constantly renew the water in culture tanks with filtered seawater pumped nearby, maintaining water parameters similar to that of the ocean. The list of collected samples and major associated weaknesses is presented in Table 1. All samples were kept at -25°C and analysed within 2 months after recollection.

### 3. 2. Sample processing

Organic matter of wild *H. forskali* gut contents and nearby sediment was quantified gravimetrically after combustion of freeze-dried material in a muffle furnace (4 h at 450°C). Pigments were analysed by high performance liquid chromatography (HPLC) according to Brotas and Plante-Cuny (2003). Sub-samples of freeze-dried material (10-15 mg for sediment, gut contents and faeces and 5-10 mg for potential food sources) were incubated with 2 mL of methanol (buffered with 2% ammonium acetate) during 15 min, at -25°C in the dark. Extracts were then filtered with 0.2 µm PTFE syringe filters and analysed within 16 h using an Agilent 1260 Infinity HPLC composed of a quaternary pump (VL 400 bar), a UV–VIS photodiode array detector (DAD 1260 VL, 250–900 nm) and a 100 µl automatic sample injector refrigerated at 4°C in the dark. Chromatographic separation was carried out using a C18 column for reverse phase chromatography (Supelcosil, 25 cm long, 4.6 mm inner diameter). The solvents used were A: 0.5 M ammonium acetate in methanol and water (85:15, v:v), B: acetonitrile and water (90:10, v:v), and C: 100% ethyl acetate. The solvent gradient was set according to Brotas and Plante-Cuny (2003), with a 0.5 mL $min^{-1}$ flow rate. Identification and calibration of the HPLC peaks were performed with antheraxanthin, ββ-carotene, canthaxanthin, chlorophyll *a*, chlorophyll *b*, chlorophyll *c2*, diatoxanthin, diadinoxanthin, fucoxanthin and pheophytin *a* standards. We identified all detected peaks by their absorption spectra and relative retention times using the Agilent OpenLab software. Quantification was

performed using standard calibration curves built with repeated injections of standards over a range of dilutions. Carotenoids and chlorophyll *b* and *c* were quantified at 470 nm, chlorophyll *a* and their derivatives as well as pheopigments were quantified at 665 nm. The relative abundance of each pigment (%) was calculated from its respective concentration in the sample (µg mg $^{-1}$).

Lipids were extracted following a slightly modified protocol of Bligh and Dyer (1959), as described in Meziane et al. (2007). Sub-samples of freeze-dried material (100-300 mg for sediment, gut contents and wild population faeces, 30-100 mg for potential food sources, muscles and aquacultured sea cucumber faeces and 10-30 mg for digestive tracts and gonads) were incubated with 4 mL of a water:methanol:chloroform mixture (1:2:1, v:v:v) and sonicated during 20 min at room temperature. Chloroform and water were added to the mixture to reach equal proportions of the three solvents and allow the formation of an aqueous-organic bilayer system that was separated by centrifugation (3000 rpm, 5 min). The heavy phase was retrieved and the operation was repeated a second time after completion with 2 mL of chloroform to retrieve as much lipids as possible. In addition, 15-30 µg of tricosanoic acid (23:0) provided by Sigma-Aldrich was added to every sample prior to extraction and used as internal standard. The lipid fraction, contained in the chloroform, was evaporated under nitrogen ($N_2$) flux and dried lipid extracts were saponified using a methanol:sodium hydroxide (2 N) mixture (2:1, v:v) during 1 h 30 min, at 90 °C. Finally, fatty acid esters were methylated into fatty acid methyl esters (FAME) using boron trifluoride-methanol ($BF_3$-$CH_3$-OH) and stored at −25 °C. We analysed FAME by gas chromatography (Agilent, 7890B GC) associated with triple quadrupole mass spectrometry (Agilent, 5977B MSD) equipped with an Agilent HP-5ms UI non-polar capillary column (30 m length × 0.25 mm inner diameter × 0.25 µm inner diameter) and using helium as gas carrier (1.5 mL min$^{-1}$). The oven temperature was set at 60 °C and held for 1 min, raised at 40 °C min$^{-1}$ to 170 °C and held for 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 and held for 20 min and finally increased to 240 °C at 3 °C min$^{-1}$ and held for 12 min. The two plateaux at 195 and 220 °C corresponded to the elution zones of C16 and C18 fatty acids, respectively, and temperature was held constant to improve peak delineation. After column separation, the gas carrier containing FA was split into two fractions. The largest fraction (~80%) was sent to a flame ionisation detector (FID) set at 250 °C for peaks quantification and the remaining part was send to the MSD for identification. Mass spectra were acquired in electron ionisation (EI) mode at 70 eV between 35-600 mz at a scan rate of 1.3 scan s$^{-1}$. All

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

### 3. 3. Statistical analysis

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

## 4. Results

### 4. 1. Organic matter (OM)

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

### 4. 2. Pigments

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

was not available. Most minor pigments were derivatives of major ones, and so they were combined to their closest relative compound. Major compounds and all their derivatives were summed and a selective list of pigments (plus derivatives) useful to assess the food sources of *H. forskali* was constructed (Table 2). Globally, out of chlorophyll *a*, pheophorbide *a* and pheophytin *a*, that are found in all decomposing vegetal sources, gut contents and faeces of wild sea cucumbers were dominated by chlorophyll *c* (14-20 %), fucoxanthin (3-5 %) and neoxanthin (2-3 %) with proportions relatively similar to that of sediment (Table 2). Chlorophyll *c* and fucoxanthin were major pigments of suspended particulate matter (SPM) and brown algae (diatoms and the macroalgae *Laminaria sp.*) and were also detected in faeces of aquacultured sea cucumbers fed with diatoms. Neoxanthin was found in SPM and green macroalgae (*Ulva sp.* and *Enteromorpha sp.*), but it was absent from diatoms and faeces of aquacultured individuals fed diatoms. Finally, lutein was a major pigment in red macroalgae (*Cryptopleura ramosa*) and in both fresh and decomposed green macroalgae (20-54 %) but it was almost absent from any other kind of sample (0-1.2 %), except faeces of aquacultured individuals fed with decomposed green algae where it was a major compound (11-14 %). Total concentration of pigments was 4-5 times higher in gut contents and faeces of wild animals compared to nearby sediment but no significant difference (Van der Waerden test; $p = 0.662$) was revealed between the three gut contents and the faeces (Fig. 3).

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

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

Unlike for pigments composition, sediment and foregut contents differed in their FA composition with higher proportions of FA 20:4ω6 (arachidonic acid; ARA), 20:5ω3 (eicosapentaenoic acid; EPA) and presence of the FA 23:1ω9 in the foregut and lower proportions of FA 16:0 and saturated FA (SFA) in the sediment (Table 3). In contrast, freshly released faeces poorly differed to nearby sediments, with only FA 23:1ω9 and 24:1ω9 significantly higher and the sum of branched FA (BrFA) significantly lower in faeces (Table 3). Highly unsaturated FA (HUFA) and long-chain monounsaturated FA (≥ 20-carbon chain length; LC-MUFA) were present in low proportions in most potential food sources, while they were relatively abundant in *H. forskali* tissues (Table 3 and 4). Total concentration of FA strongly differed among tissues with higher concentrations in female gonads (Table 4). Muscles were characterised by especially high levels of ARA and FA 23:1ω9 compared to other tissues (Table 4) and different ω3/ω6 and eicosanoid ratios (Fig. 4). Male gonads were significantly richer in ARA, EPA and FA 20:1ω9 compared to female gonads and poorer in FA 16:0 and BrFA (Table 4).

## 5. Discussion

### 5. 1. Food sources

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

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

Conversely, low proportions of lutein in gut contents of wild *H. forskali* indicate that the species does not feed on the red algae *Cryptopleura ramosa* found in close proximity and exhibiting high levels of this carotenoid (37.0 ± 0.4 %). More largely, all groups of red algae exhibit high contents of antheraxanthin, lutein or zeaxanthin (Schubert et al. 2006). These carotenoids were not detected or below 1 % in *H. forskali* gut contents. Lutein is also an important component of green macroalgae such as *Ulva sp.* that may reach the sea floor in this area once in decomposition but that is not ingested by *H. forskali*. High levels of lutein in the faeces of sea cucumber juveniles fed with decomposed green algae confirm that lutein can be used as a reliable tracer of green algae in the diet of *H. forskali*, and that pigment composition of farmed animal faeces roughly resembled that of their diet. Finally, low proportions of violaxanthin and FA 18:4ω3 in gut contents reveal that brown macroalgae are barely ingested, as they usually exhibit high levels of these compounds (Fleurence et al. 1994, Schmid et al. 2005). Thus, the vegetal food sources ingested by *H. forskali* in our study are mainly diatoms.

In contrast to pigments, fatty acids were not only more concentrated in foregut contents compared to the sediment but also differed in composition, suggesting that other food sources not containing pigments or exhibiting similar pigment compositions than unsorted sediment enter the diet of *H. forskali*. Higher proportions of the FA 20:4ω6, the sum of highly unsaturated FA (HUFA ≥ 20 carbon chain-length and 2 double bounds), and lower proportions of the FA 16:0 in the foregut content compared to the sediment indicate that the sea cucumbers ingest particles with especially high nutritional value. Actually, HUFA are precious compounds with roles in membrane properties and immune response (Arts and Kohler 2008, Twining et al. 2016). They are usually rapidly degraded in decaying organic matter (Wakeham 1995, Vivier et al. 2019). Such elevated proportions of HUFA in the foregut content of *H. forskali* suggest that ingested particles are upgraded regarding their nutritional value before transiting in the gut (i.e. in the nearby sediment or in the oesophagus). Trophic upgrading and enhancement of PUFA relative proportions throughout the first levels of the food web is usual in the marine realm (Desvilettes and Bec 2009), and can be mediated

by heterotrophic protists, also named "non-pigmented" protists in the old scientific literature. Other authors mentioned elevated levels of HUFA in the gut content of holothurians. Mfilinge and Tsuchiya (2016) reported 21.8 % of HUFA in the foregut content of *H. leucospilota* and 31.4 % in *H. atra*. Both species where assumed to feed on the nearby sediment that exhibited only 9.5 and 16.8 % of HUFA, respectively. High levels of HUFA in the foregut were attributed to the specific selection of algae and bacteria rich detrital particles (Mfilinge and Tsuchiya 2016). Although microalgae have long been considered the major *de novo* producer of PUFA in marine food webs (Nichols 2003), diatoms, that are the dominant microalgae consumed by *H. forskali* in our study, are usually poor in FA 20:4ω6 (Dalsgaard et al. 2003, Lang et al. 2011), suggesting that the HUFA measured in the foregut content of *H. forskali* may rather originate from bacteria (Nichols 2003), heterotrophic protists (Desvilettes and Bec 2009) and/or their symbiotic relationship (Gast et al. 2009). Ginger et al. (2000) also measured high amounts of HUFA in the gut contents of holothurians, but they attributed these elevated levels to the lysis of gut walls, caused by stress during animals recovery. Yet, Ginger et al. (2000) worked on abyssal holothurians and change in barometric pressure during the ascent phase may have caused a sufficient stress to cause cell wall lysis, which may not have occurred in our study as sea cucumbers were gathered only at a few meters deep. In addition, Féral (1985) measured higher lipid levels in the anterior intestine wall compared to the oesophagus of *Leptosynapta galliennei* (51.9 ± 3.4 µg $mg^{-1}$ vs. 7.6 ± 3.5 µg $mg^{-1}$, respectively). Assuming similar differences in *H. forskali*, cell wall lysis would be expected to cause higher lipid contents in the midgut compared to the foregut content. The opposite was measured in our study, confirming that cell wall lysis caused by the sampling stress was minor. In an aquaculture perspective, further investigations would be required to determine whether the nutritional upgrading of detrital organic matter takes place in the sediment and/or in the foregut and which organisms mediate the biosynthesis pathways of HUFA. Actually, if possible, feeding the microbiote of *H. forskali* instead of providing feeds of high nutritional value may be of interest in a sustainable aquaculture perspective.

### 5. 2. Digestive efficiency

The significant but weak differences in OM between foregut contents and faeces suggest that *H. forskali* have to process large quantities of sediment to cover its nutritional needs. In the midgut (the largest compartment) of *H. forskali* found on rocky substrate in the 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 ~ 8.3 ± 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, Mühlenbruchet 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

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

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

### 5. 3. Resources allocation

High contents of FA in the digestive tract of *H. forskali* are in accordance with histological observations of Féral (1985) where the author measured especially high levels of lipids in the anterior intestine walls of *Leptosynapta galliennii* (i.e. lipid droplets). Féral and Massin (1982) suggested that the digestive tract may serve as a storage organ and Allen (1968) measured highest incorporation rates of radioacetate in lipids of the gut isolated from the *rete mirabile* of *H. forskali*. All these results suggest that the fatty acid composition of holothuroids digestive tract deserves particular attention. The energy storage role of this organ has however been dismissed by Féral (1985) who did not measure any decay in the anterior intestine lipid content of adult *L. galliennii* before and after two weeks of starvation. Similarly, no seasonal difference was measured in the digestive tract lipid content of *Molpadia violacea* by Féral and Magniez (1985) whereas a storage role of the digestive tract

would probably provoke a notable depletion of stocks during winter. Yet, at the larval stage Peters-Didier and Sewell (2019) evidenced an accumulation of neutral lipids in the stomach epithelium of 14 days post-fertilisation larvae of *Australostichopus mollis*. Lipid droplets ~ 10 µm in diameter suspended in the larval blastocoel were further transported to the hyaline spheres (an enigmatic structure present in the late auricularia stage of sea cucumbers) to fuel the process of metamorphosis. Whether this accumulation of lipids in the stomach epithelium has also a role at the adult stage is still to be investigated but high incorporation rates of radioacetate in the gut of *H. forskali* measured by Allen (1968) suggest that this organ plays a major role in lipid biosynthesis. Finally, the closest similarity between ω3/ω6 FA and eicosanoid ratios between the digestive tract and the gonads compared to that of muscles suggests that the anterior intestine could exchange functional lipids with reproductive organs.

The absence of sexual difference in the FA composition of gut contents, muscles and digestive tract of wild *H. forskali* suggests that food resources and digestive efficiency are not sex-dependant in this species. Sexual difference in the FA composition of gonads may thus originate from different incorporation strategies and/or conversion of FA rather than feeding. In an aquaculture perspective, a lot of studies on FA composition of gonads have been conducted on fishes, where docosahexaenoic acid (22:6ω3; DHA) is among the most abundant constituent of testes (Bell et al. 1997, Jeong et al. 2002, Beirão et al. 2015). This FA is expected to confer spermatozoids a high membrane fluidity (Stillwell and Wassal 1997) that is necessary for fecundation. It has been demonstrated experimentally that the inclusion of DHA in *Solea senegalensis* diet may improve sperm quality (Beirão et al. 2015). Yet, in our study DHA was a minor component of gonads, both in males and females. This result is consistent with previous works on other sea cucumber species, such as *Apostichopus japonicus* (< 5 % DHA in gonads; Kasai 2003), *Cucumaria frondosa* (< 2 % DHA in gonads of wild individuals and individuals fed diatoms; Gianasi et al. 2017), *Holothuria tubulosa* or *H. polii* (< 2 % DHA in gonads; Biandolino et al. 2019) and in sea urchins (< 3 % DHA in gonads; Martínez-Pita et al. 2010, Díaz de Vivar et al. 2019), suggesting that DHA is not as crucial for echinoderm reproduction as it is for fishes. Instead, membrane fluidity in echinoderms may be provided by non-methylene interrupted FA as suggested for the sea urchin *Paracentrotus lividus* (Carboni et al. 2013). Such FA could not be properly identified in our study but they may happen within the unidentified HUFA and/or co-elute with others on the non-polar HP-5ms column. In *H. forskali*, male and female gonads differ by higher proportions of eicosapentaenoic acid (20:5ω3; EPA) and arachidonic acid (20:4ω6; ARA) in

males. However, the twice as high total concentration of fatty acids in female compared to 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 to the storage of lipids for further use as an energy source by eggs, rather than for functional purpose. Maternal provisioning is actually a strong determinant of larval development in echinoderms as embryos and larvae initiate their development using nutrients provided in the eggs (Prowse et al. 2007). The lipid requirement of eggs being higher than for spermatozoids (that require energy only until fecundation), FA may not be as strictly selected, thus explaining lower HUFA proportions in female gonads compared to that of males (23.2 ± 3.2 % in female vs. 36.4 ± 7.0 in male gonads). Exploring the provisioning of essential compounds (i.e. lipids) during gametogenesis will be necessary for aquaculture purpose as broodstock conditioning may affect larval survival and development.

Finally, muscle FA composition strongly differed with that of gut contents, digestive tract and gonads, confirming that specific elements are preferentially retained and/or converted to be allocated to tissues, as hypothesised in the introduction. The preferential accumulation of PUFA in consumers relative to their diet is common in marine food webs, but the retention of long-chain MUFA is more unusual (Budge et al. 2006). The FA 23:1ω9 is found in significant proportions only in sea cucumbers, although sometimes detected as traces in other echinoderms, crustaceans and bivalves (Kaneniwa et al. 1986, Rezanka and Sigler 2008, Drazen et al. 2008). Kaneniwa et al. (1986) found higher proportions of the FA 23:1ω9 in phospholipids compared to neutral lipids of two species of sea cucumbers, suggesting that this FA has a physiological role in holothurians. It most probably does not originate from diet, as none of the potential food sources exhibited even trace amounts of this compound, and as previously proposed by Kaneniwa et al. (1986). Yet, it was detected in gut contents since the foregut, showing that the mechanism leading to its synthesis (most probably α-oxidation of the FA 24:1ω9; Kaneniwa et al. 1986) is activated at the earliest stage of the digestive process and/or that it is synthesised by endosymbionts. The absence of FA 23:1ω9 in farmed juvenile faeces indicates that the mechanism appears later in the development, that endosymbionts could not colonise individuals breed in captivity and/or that precursors of this FA have to be found in sediment microbial communities, that was absent in juvenile tanks. In an aquaculture perspective, the role and origin of this FA will require further investigation.

## 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 23:1ω9 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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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 |

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

*n.d. not determined*

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

| | 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 |

*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 $g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| **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 ± 0.5 | 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 |
| **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*

Table 4: Fatty acid proportions of wild *H. forskali* tissues (mean ± SD). Letters in exponent indicate significant differences between groups (Van der Waerden test, α = 5%). Note that traces of 18:3ω3 may have co-eluted with 18:1ω9 and traces of 16:0iso may have co-eluted with 16:4ω3. LC-MUFA Long-Chain MUFA ; HUFA Highly Unsaturated FA

| 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$^{b}$** |

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

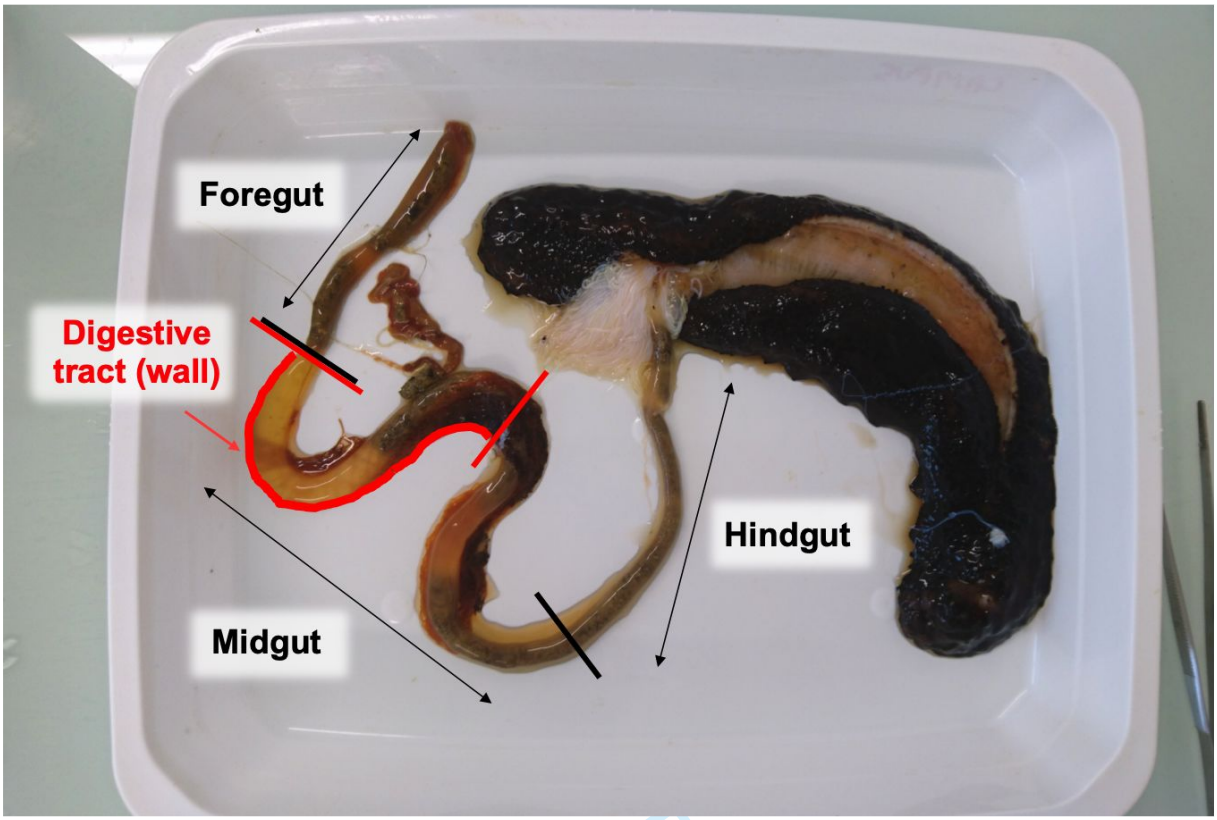


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Fig. 2: Boxplot of the organic matter content (%) in wild *H. forskali* samples and nearby sediment. Broad lines indicate median, box edges refer to 1st and 3rd quartiles and circles indicate outliers. Letters indicate significant differences between samples (Van der Waerden test, α = 5%)

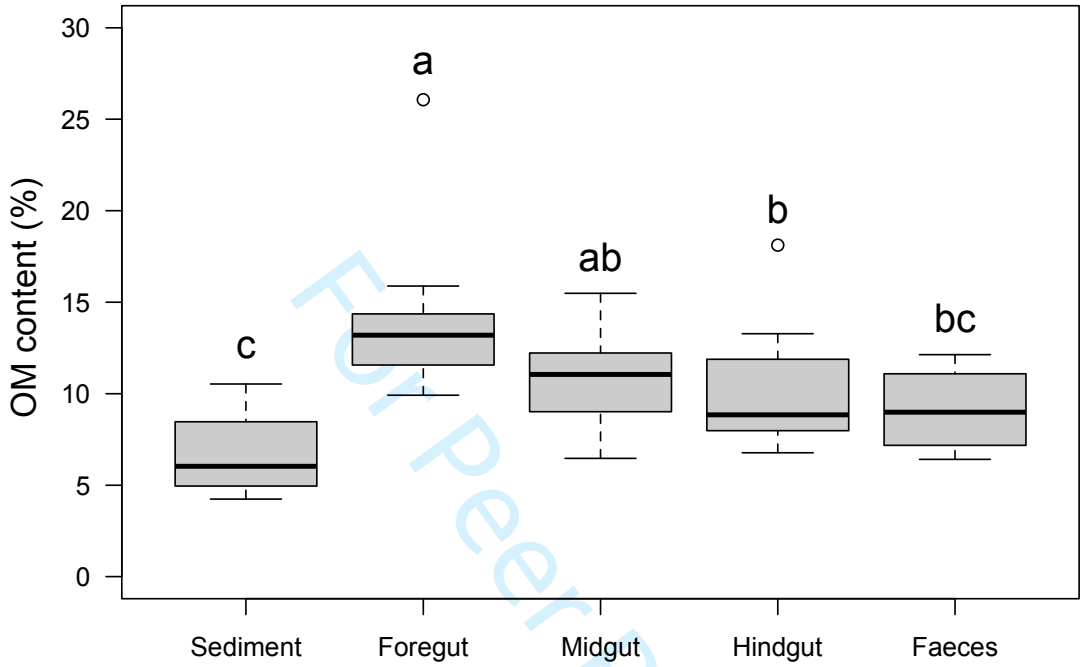
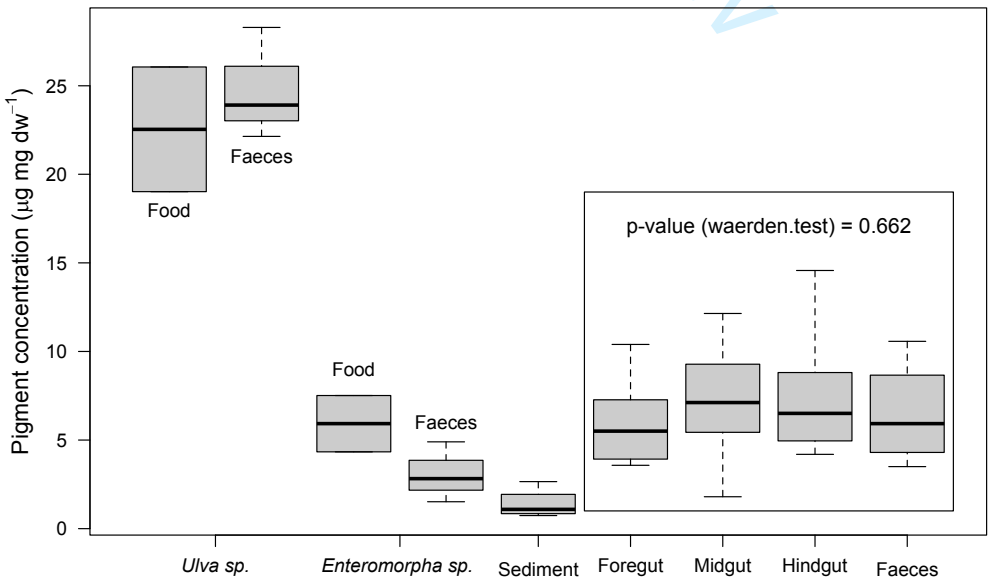



Fig. 3: Total pigment concentration in aquaculture and wild samples (µg mg $dw^{-1}$). Broad lines indicate median and box edges refer to 1st and 3rd quartiles.



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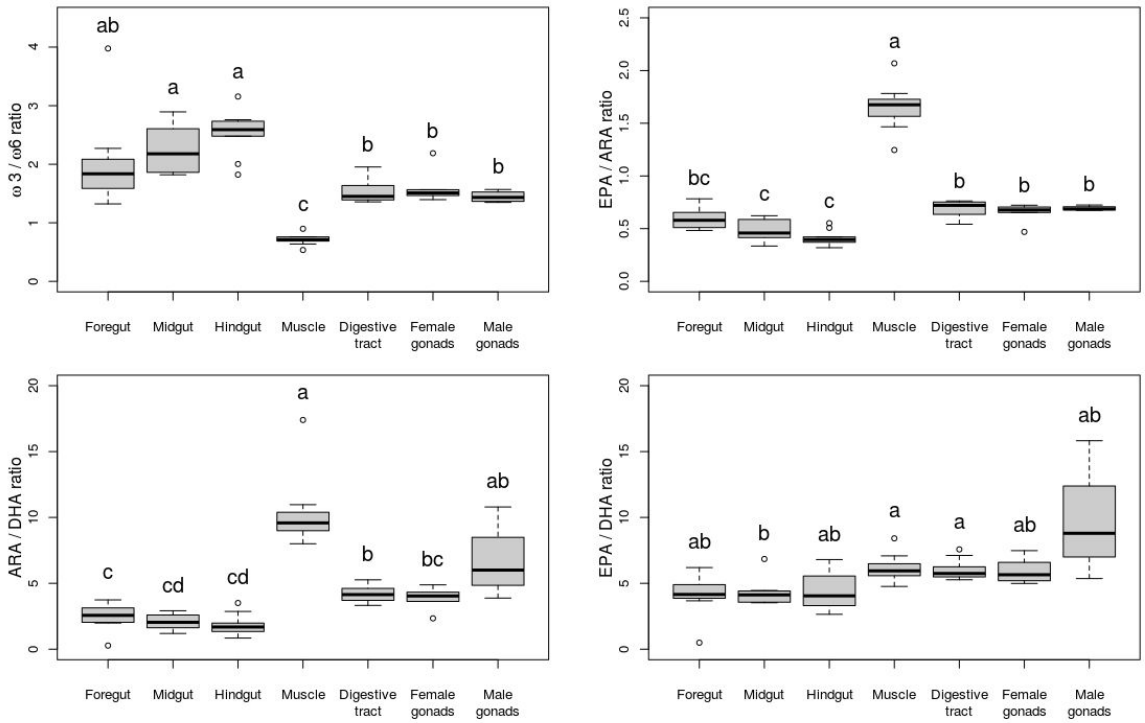
788 Fig. 4: Eicosanoid ratios in gut content and tissues of wild *H. forskali* samples. Letters
789 indicate significant differences between samples (Van der Waerden test, α = 5%). Broad lines
790 indicate median, box edges refer to 1st and 3rd quartiles and circles indicate outliers.

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