

1 **Deconvolving feeding niches and strategies of abyssal holothurians from their stable**  
2 **isotope, amino acid, and fatty acid composition**

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15  
16 **Abstract**

17 Holothurians are the dominant megabenthic deposit feeders in the Peru Basin (South-East  
18 Pacific) and feed to various degrees of selectively on the heterogenous pool of sedimentary  
19 detritus, but diet preferences for most holothurian species are unknown. This study reconstructs  
20 the diets of 13 holothurian species of the orders Elaspodida, Holothuriida, and Synallactida,  
21 from bulk stable isotope analyses ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of holothurian body walls and guts, gut contents,  
22 and feces that were combined with compound-specific stable isotope analyses of amino acids,  
23 phospholipid-derived fatty acids, and neutral lipid-derived fatty acids in the body wall. Fatty acid  
24 concentrations showed high levels of storage lipids, an likely adaption to limited food supply to  
25 abyssal plains. Amino acid  $\delta^{15}\text{N}$  isotope values allowed estimating trophic levels of holothurian  
26 species and calculating heterotrophic re-synthesis of amino acids. Fatty acids served as trophic  
27 markers for feeding on diatom- and dinoflagellate derived phytodetritus, bacteria, Foraminifera,  
28 and detritus containing the PUFA C22:1 $\omega$ 9-*cis*. Several holothurian species seemed to be  
29 secondary consumers of detritus, while bacteria in their guts were primary consumers of this  
30 detritus. A Sørensen–Dice coefficient based cluster analysis using data of trophic levels, levels of  
31 heterotrophic re-synthesis of amino acids, feeding selectivity, and food sources/ diet suggested  
32 three trophic groups, characterized by different trophic levels. We show that this multi-biomarker  
33 driven approach allows to deconvolve trophic niches and feeding selectivity in one of the most  
34 challenging environments on earth and to identify dependence of deep-sea species to organic  
35 matter inputs that vary with season and/or climate.

36  
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38  
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47

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54

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57

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## 61 Introduction

62 Holothurians are one of the most abundant epifauna in the deep sea (Billett et al. 2001; Ruhl  
63 2007; Alt et al. 2013; Stratmann et al. 2018) and they can be suspension and deposit feeders  
64 (Massin 1982). On soft sediment, deposit feeding holothurians either dig into the sediment as  
65 funnel-feeder or conveyor belt-feeder or scavenge the surface sediment as rake feeders (Massin  
66 1982). In this way, they take up particulate organic matter that is deposited on or buried in the  
67 sediment (Roberts et al. 2000). Holothurians selectively feed on the organic sources in the  
68 sediment. The analysis of gut contents from holothurians collected at the Porcupine Abyssal  
69 Plain (PAP, NE Atlantic) showed that e.g. *Amperima rosea* Perrier, 1886, *Peniagone diaphana*  
70 Théel, 1882, and *Oneirophanta mutabilis mutabilis* Théel, 1879, feed selectively on fresh  
71 phytodetritus (FitzGeorge-Balfour et al. 2010). However, when fresh phytodetritus is scarce, *O.*  
72 *mutabilis mutabilis* feeds on more refractory detritus material (FitzGeorge-Balfour et al. 2010)  
73 which is primarily consumed by the microbial community in its gut (Romero-Romero et al.  
74 2021). Other species have a less selective feeding behavior, e.g. *Psychropotes longicauda* Théel,  
75 1882, *Molpadiodemas villosus* Théel, 1886, and *Molpadia blakei* Théel, 1886, (FitzGeorge-  
76 Balfour et al. 2010). Though it seems that feeding selectivity and diet preferences of holothurians  
77 are well known, this is actually true for very few species. For most abyssal holothurians, in fact,  
78 these information are very rudimentary (e.g. Billett 1991; Roberts et al. 2000)).

79  
80 Whereas holothurians alter the chemical composition of detritus in the sediment, this detritus  
81 composition also affects the species composition of holothurians (Wigham et al. 2003;  
82 FitzGeorge-Balfour et al. 2010). At PAP, especially *A. rosea*, *P. diaphana* and *O. mutabilis*  
83 *mutabilis* had a high concentration of carotenoids in their ovaries which are important for the  
84 reproductive success of the species (Tsushima 2007; Svensson and Wong 2011). Therefore,  
85 (Wigham et al. 2003) suggested that higher concentrations of carotenoids in the gonads of *A.*  
86 *rosea* as compared to other holothurians might give this species a reproductive advantage which  
87 could explain the so-called ‘Amperima’ event. During this event, the density of *A. rosea*  
88 increased by three orders of magnitude due to large-scale recruitment events that followed  
89 changes in the organic carbon flux to the abyssal plain, even though the total megafauna biomass  
90 did not change significantly (Billett et al. 2010).

91  
92 Amino acids, the building stones of proteins, are required to produce enzymes, structural tissue  
93 of fauna, and cell walls of bacteria (Phillips 1984; Libes 2009). Half of the 20 most common  
94 amino acids in faunal proteins can be synthesized by the organism itself (Phillips 1984), whereas  
95 the other half has to be taken up with the diet and are therefore called ‘essential’ amino acids  
96 (EAA) (Phillips 1984). Amino acids include ‘source amino acids’ (i.e., glycine, serine,  
97 phenylalanine, tyrosine, lysine), which preserve their  $\delta^{15}\text{N}$  values along the trophic chain because  
98 no new bonds are formed to the N atom nor are bonds cleaved (Chikaraishi et al. 2009). Other  
99 amino acids are ‘metabolic amino acids’ (i.e., threonine) and ‘trophic amino acids’ (i.e.,  
100 asparagine, glutamine, alanine, isoleucine, leucine, valine, proline). The  $\delta^{15}\text{N}$  values of ‘trophic  
101 amino acids’ become enriched during metabolic transamination when nitrogen bonds are cleaved  
102 (Chikaraishi et al. 2009). The larger the difference between the ‘source amino acids’ and the  
103 ‘trophic amino acids’, the higher is the trophic level of an organism, so the ratio of the  $\delta^{15}\text{N}$   
104 values of glutamic acid and phenylalanine has been used to estimate the trophic level of an  
105 organism following (Chikaraishi et al. 2009).

106

107 Fatty acids, the main components of lipids, serve as energy source, are involved in the  
108 transduction of signals, in gene expression, and are components of membranes (Burdge and  
109 Calder 2014). They contain neutral lipid-derived fatty acids (NLFAs) and phospholipid-derived  
110 fatty acids (PLFAs) (Dalsgaard et al. 2003). NLFAs are required to build wax esters and the  
111 storage lipids triacylglycerols, whereas PLFAs are necessary to build structural phospholipids of  
112 cell membranes (Dalsgaard et al. 2003). Fatty acids may be unsaturated or saturated and  
113 generally a higher number of unsaturated bonds implies that the fatty acid is more labile than a  
114 fatty acid with fewer unsaturated bonds (Pond et al. 1997). ‘Essential’ fatty acids have to be  
115 taken up with the diet because they can generally only be synthesized *de novo* by primary  
116 producers (Dalsgaard et al. 2003), except for a few hydrothermal vent shrimp species and worms  
117 that are also able to synthesize them (Pond et al. 1997, 2002). Since several fatty acids are  
118 transferred conservatively (i.e., untransformed) from primary producers and primary consumers  
119 to higher trophic levels, they may serve as trophic markers and inform about diets (Dalsgaard et  
120 al. 2003).

121  
122 To decipher feeding types and diet preferences of holothurians from the Peru Basin, compound-  
123 specific stable isotope analyses of bulk tissue, gut content, and feces were combined with  
124 compound-specific stable isotope analysis of amino acids and fatty acids. We addressed the  
125 following research questions: (1) Do the holothurian species have different trophic levels? (2)  
126 Can specific feeding strategies and diet preferences identified for the different species?

127

## 128 **Material and methods**

### 129 **Sampling of holothurians**

130 Holothurians of the putative species Elpidiidae gen sp. Théel, 1882 (n = 1), *Amperima* sp.  
131 Pawson, 1965 (n = 4), *Benthodytes* sp. Théel, 1882 (n = 2), *Benthodytes typica* Théel, 1882  
132 (n = 1), *Galatheathuria* sp. Hansen & Madsen, 1956 (n = 1), *Oneirophantha* sp. Théel, 1879  
133 (n = 1), *Psychronaetes hanseni* Pawson, 1983 (n = 1), *P. longicauda* (n = 1), *Psychropotes*  
134 *semperiana* Théel, 1882 (n = 1), *Synallactes* sp. Ludwig, 1894 (morphotype “pink”; n = 1), and  
135 Synallactidae gen sp. Ludwig, 1894 (n = 2) were collected opportunistically with the ROV with  
136 the ROV suction sampler in the Peru Basin (Table 1). As a result, sampling of several species  
137 was not balanced, but due to logistical constraints it was often limited to n = 1 or n = 2. Aboard  
138 RV *Sonne*, the length, height, and width of each holothurian specimen was measured and the  
139 specimens were dissected to separate the gut and its content from the remaining tissue. All  
140 samples were shock-frozen in liquid nitrogen and stored frozen at -20°C.

141

142 **Table 1.** Details of sampling location and collected holothurian specimens from RV *Sonne*  
143 research cruise SO242-2.

Date	Latitude (N)	Longitude (E)	Depth (m)	Putative species
05.09.2015	-7.074	-88.451	4137.0	<i>Amperima</i> sp. (n = 1), <i>Benthodytes</i> sp. (n = 1)
05.09.2015	-7.074	-88.451	4137.5	<i>Mesothuria</i> sp. (n = 1), <i>Amperima</i> sp. (n = 2),
05.09.2015	-7.074	-88.451	4136.4	<i>Oneirophanta</i> sp. (n = 1)
12.09.2015	-7.125	-88.451	4151.0	<i>Amperima</i> sp. (n = 1), <i>Benthodytes</i> sp. (n = 1), <i>B.</i> <i>typica</i> (n = 1)

17.09.2015	-7.082	-88.469	4136.3	<i>Benthodytes</i> sp. (n = 2), <i>B. typica</i> (n = 1), <i>Psychronaetes hanseni</i> (n = 1)
18.09.2015	-7.083	-88.470	4429.4	<i>Amperima</i> sp. (n = 1), Elpidiidae gen sp. (n = 1), <i>Psychropotes longicauda</i> (n = 1), <i>Synallactes</i> sp. (morphotype "pink") (n = 1)
22.09.2015	-7.126	-88.451	4150.0	Synallactidae gen sp. (n = 1)
27.09.2015	-7.078	-88.458	4141.9	<i>B. typica</i> (n = 2), <i>Peniagone</i> sp. (n = 1), <i>Psychropotes semperiana</i> (n = 1), <i>Galatheathuria</i> sp. (n = 1), Synallactidae gen sp. (n = 1), <i>Peniagone</i> sp. (n = 1)

144  
 145 Additionally, the putative holothurians species *Amperima* sp. (n = 3), *Benthodytes* sp. (n = 3), *B.*  
 146 *typica* (n = 4), *Mesothuria* sp. Ludwig, 1894 (n = 1), *Peniagone* sp. Théel, 1882 (n = 1), and  
 147 Synallactidae gen sp. Ludwig, 1894 (n = 1) from the study of (Brown et al. 2018) were used.  
 148 These specimens were collected with the ROV suction sampler in the Peru Basin and transported  
 149 to respiratory chambers to measure oxygen consumption of individual holothurian specimen over  
 150 a period of 72 hours. Aboard RV *Sonne*, the holothurians specimens were measured (length,  
 151 height, width), shock-frozen intact in liquid nitrogen, and stored at. Feces of holothurians that  
 152 defecated inside the respiratory chambers were sampled and frozen at -21°C.  
 153 In the shore-based laboratory at NIOZ-EDS (Yerseke, Netherlands), the samples were freeze-  
 154 dried and finely-ground with mortar and pestle. The organic (org.) C/  $\delta^{13}\text{C}$  and N/  $\delta^{15}\text{N}$  content  
 155 of the holothurian tissue and of the acidified holothurian gut content were measured with a  
 156 Thermo Flash EA 1112 elemental analyzer (EA; Thermo Fisher Scientific, USA) which was  
 157 coupled to a DELTA V Advantage Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher  
 158 Scientific, USA). Stable isotope values are presented in  $\delta$  notation relative to Vienna Pee Dee  
 159 Belemnite for  $\delta^{13}\text{C}$  and relative to air for  $\delta^{15}\text{N}$ .  
 160 Sediment grain size of holothurian gut content was determined by laser diffraction on freeze-  
 161 dried and sieved (<1 mm) sediment samples in a Malvern Mastersizer 2000.

### 162 163 **Analysis of amino acids**

164 Total hydrolysable amino acids (THAA) from holothurian tissue were extracted following a  
 165 modified protocol of Veuger et al. (2005): Briefly, THAAs in holothurian tissue were hydrolyzed  
 166 by adding 0.01 to 0.02 g freeze-dried finely ground tissue to 1.5 ml 6 M HCl in 10 ml screw-cap  
 167 vials. A N<sub>2</sub>-headspace was created in the vials by flushing with N<sub>2</sub>-gas for 10 sec before the vials  
 168 were closed and heated for 20 h at 110°C. After cooling, 10  $\mu\text{L}$  internal L-Norleucine standard  
 169 per mg dry faunal tissue (stock solution: 2.5 mg mL<sup>-1</sup> L-Norleucin acidified with 100  $\mu\text{L}$  12 M  
 170 HCl) was added and the solution was evaporated under N<sub>2</sub>-flow at 60°C. THAAs from  
 171 holothurian tissue were derivatized by adding 0.5 ml acidified propan-2-ol to the sample and by  
 172 heating the closed vials at 110°C for 90 min. Afterwards, the vials were cooled down and the

173 solution was evaporated under N<sub>2</sub>-flow at 50°C. After evaporating all solution, 200 µL  
174 dichloromethane (DCM) was added and the solution was evaporated again. When the samples  
175 were dry, 150 µL DCM and 50 µL pentafluoropropionic anhydride were added, the vials were  
176 closed and heated for 10 min at 110°C. The solvent was extracted by adding 0.5 mL  
177 chlorophorm and 1 ml phosphorus-buffer to the sample, shaking it until the lower chloroform  
178 fraction was clear and centrifuging the vials with 2,000 rpm for 10 min. The chloroform fraction  
179 was transferred to GC vials and evaporated again. When the sample was completely dry, it was  
180 dissolved in ethyl acetate. Concentrations (µg C g<sup>-1</sup> dry mass DM holothurian tissue) and δ<sup>13</sup>C  
181 (‰), and δ<sup>15</sup>N (‰) of THAAs were measured with a HP 6890 gas chromatograph (Hewlet  
182 Packard/ Agilent, USA) coupled with a DELTA-Plus Isotope Ratio Mass Spectrometer (Thermo  
183 Fisher Scientific, USA) on a polar analytical column (ZB5-5MS; 60m length, 0.32mm diameter,  
184 0.25µm film thickness; Phenomenex, USA).  
185 A list with common abbreviation of amino acids and their full name is presented in Table 2.  
186

187 **Table 2.** Names and abbreviations of amino acids and fatty acids (PLFAs, NLFAs).

Abbreviation	Full name
<b>Amino acids</b>	
Ala	Alanine
Asp	Aspartic acid
Glu	Glutamic acid
Gly	Glycine
Ile	Isoleucine
Leu	Leucine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Tyr-Lys	Tyrosine and lysine combined
Val	Valine
<b>Fatty acids</b>	
ARA	Arachidonic acid (C20:4ω6)
DHA	Docosahexaenoic acid (C22:6ω3)
EPA	Eicosapentaenoic acid (C20:5ω3)

188  
189 **Analysis of fatty acids**  
190 Fatty acids (i.e., PLFAs, NLFAs) were extracted from holothurian tissue, feces and gut content  
191 following a modified Bligh and Dyer extraction method (Bligh and Dyer 1959; Boschker 2008).  
192 Freeze-dried, homogenized powder of holothurian tissue (~50 – 150 mg), feces and gut content  
193 (~150 mg – 2.0 g) were mixed with 6 ml MilliQ-water 15 ml methanol (HPLC grade, 99.8%),  
194 and 7.5% chloroform (HPLC grade, 99.5%) in pre-cleaned test tubes. The tubes were shaken for  
195 2 h, before 7.5 ml chloroform were added and the tubes were shaken again. 7.5 ml MilliQ-water  
196 were added and the tubes were stored at -21°C for 12 h for separation of the solvent layers. The  
197 lower solvent layer contained the fatty acids extract dissolved in chloroform and was transferred  
198 to pre-weighted test tubes. After determining the weight of the chloroform extract, it was  
199 fractionated into the different fatty acid classes over an activated silicic acid column (heated at

200 120°C for 2 h; Merck Kieselgel 60) via eluting with 7 ml chloroform, 7 ml acetone, and 15 ml  
 201 methanol. The acetone fraction was discarded, whereas the chloroform fraction containing the  
 202 NLFAs and the methanol fraction with the PLFAs were collected in separate test tubes and  
 203 evaporated to dryness.  
 204 PLFAs and NLFAs were derivatized to fatty acid methyl esters (FAMES) by adding 1 ml  
 205 methanol-toluene mix (1:1 volume/ volume), 20 µl of an internal standard (1 mg 19:0  
 206 FAME mL<sup>-1</sup>), and 1 ml 0.2 M methanolic NaOH to the test tubes with the PLFAs and NLFAs  
 207 extracts. After an incubation at 37°C for 15 min, 2 ml *n*-hexane, 0.3 ml 1 M acetic acid, and 2 ml  
 208 MilliQ-water were added. The solution was mixed very well and when the layers had separated,  
 209 the (top) *n*-hexane layer was transferred to new test tubes. Additional 2 ml *n*-hexane were added  
 210 to the previously used test tubes that contained the acetic acid-MilliQ-water solution, and the  
 211 step was repeated. The *n*-hexane layer was transferred again to the new test tubes and 20 µl of a  
 212 second internal standard (1 mg 12:0 FAME mL<sup>-1</sup>) were added. *n*-hexane was evaporated  
 213 completely and the FAMES dissolved in 200 µl *n*-hexane were transferred to measuring vials.  
 214 The FAMES from holothurian tissues were separated on a BPX70 column (50 m length, 0.32 mm  
 215 inner diameter, 0.25 µm film thickness; SGE Analytical Science) with a HP 6890 gas  
 216 chromatograph (GC; Hewlett Packard/ Agilent, USA). The FAMES from feces and gut content  
 217 were separated on a ZB5-5MS column (60 m length, 0.32 mm diameter, 0.25 µm film thickness;  
 218 Phenomenex, USA) on the same GC. Concentrations (µg C g<sup>-1</sup> DM holothurian tissue) and δ<sup>13</sup>C  
 219 values (‰) of FAMES in holothurian tissue, feces, and gut content were measured on a Finnigan  
 220 Delta Plus isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific, USA) coupled to  
 221 the GC via a combustion GC-c-III interface (Thermo Fisher Scientific, USA). Identification of  
 222 peaks of the FAME chromatogram were based on equivalent chain length (ECL) and peak areas  
 223 were calculated using the two internal standards (12:0 and 19:0) for area correction.  
 224 A list with abbreviations and full names of several important fatty acids is presented in Table 2  
 225 and Table 3 contains dominant biomarkers.

226  
 227 **Table 3.** Fatty acids used as biomarkers of potential food sources of holothurians from the Peru  
 228 Basin.

Fatty acid	Main sources of fatty acid	Reference
<b>Organic matter</b>		
C18:1ω9	Highly degraded carrion-derived organic matter	(Graeve et al. 2001)
<b>Bacteria</b>		
<i>i</i> -C14:0, <i>i</i> -C15:0, <i>ai</i> -C15:0, <i>i</i> -C16:0, and C18:1ω7 <i>cis</i> ;	Marine bacteria; gram-positive bacteria; piezotolerant bacteria	(Findlay et al. 1990; Middelburg et al. 2000; Wang et al. 2014)
C18:2ω6		
10-Me-C16:0, <i>ai</i> -C17:0, <i>i</i> -C17:0, and <i>cy</i> -C17:0	Sulfate-reducing and other anaerobic bacteria	(Findlay et al. 1990)
C16:1ω5	Desulfobacteraceae bacteria	(Elvert et al. 2003)
C16:1ω7	Bacteria in fish intestines	(Yano et al. 1997)
C16:1ω9	Deep-water/ benthic bacteria	(Zhao et al. 2015; Choi et al. 2015)
<b>Primary producers</b>		
C16:4ω1, C16:1ω7, and EPA; $\frac{C16:1\omega7}{C16:0} > 1$ or $\frac{DHA}{EPA} < 1$	Diatoms	(Kelly and Scheibling 2012)

C18:4 $\omega$ 3 and DHA; $\frac{C16:1\omega7}{C16:0} < 1$ or $\frac{DHA}{EPA} > 1$	Dinoflagellates	(Kelly and Scheibling 2012)
C18:1 $\omega$ 9, C18:4 $\omega$ 3, and ARA	Kelp	(Kelly and Scheibling 2012)
C16:0, EPA; ARA	Red macroalgae	(Bühning et al. 2002; Kelly and Scheibling 2012)
C18:1 $\omega$ 9, C18:4 $\omega$ 3, and ARA; C16:1 $\omega$ 5	Brown macroalgae	(Khotimchenko 1995; Kelly and Scheibling 2012)
C18:2 $\omega$ 6 and C18:3 $\omega$ 3	Seagrass	(Kelly and Scheibling 2012)
<b>Consumers</b>		
C20:1 $\omega$ 9, C22:1 $\omega$ 11	Calanoid copepods	(Falk-Petersen et al. 1987; Dalsgaard et al. 2003)
EPA, DHA ARA; C22:5 $\omega$ 5; $\frac{EPA}{ARA}$ -ratio	Hydrothermal vent bivalves Agglutinated foraminifera	(Ben-Mlih et al. 1992) (Larkin et al. 2014; Kharlamenko 2018)

229

## 230 **Calculations**

### 231 **Concentration factors**

232 To examine the degree to which PLFAs were concentrated between surface sediment (0 – 2cm  
233 layer; 2.32 $\pm$ 0.51  $\mu$ g C-PLFA g<sup>-1</sup> DM sediment; Stratmann, unpublished) and gut content and  
234 feces, a concentration factor  $CF$  was calculated:

$$235 \quad CF_{gut\ content} = \frac{[gut\ content_{PLFA}]}{[sediment_{PLFA}]}, \quad (1)$$

$$236 \quad CF_{feces} = \frac{[feces_{PLFA}]}{[sediment_{PLFA}]}, \quad (2)$$

237 where [gut content<sub>PLFA</sub>] corresponds to the total PLFA concentration in gut content, [feces<sub>PLFA</sub>]  
238 to the total PLFA concentration in feces, and [sediment<sub>PLFA</sub>] to the average total PLFA  
239 concentration in surface sediment.

240

### 241 **Trophic levels**

242 Trophic levels ( $TL$ ) of holothurian species were calculated following (Chikaraishi et al. 2009) as

$$243 \quad TL = \frac{(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4)}{7.6} + 1. \quad (3)$$

244  $\delta^{15}N_{Glu}$  is the  $\delta^{15}N$  of the amino acid glutamic acid (‰) and  $\delta^{15}N_{Phe}$  corresponds to the  $\delta^{15}N$  of  
245 the amino acid phenylalanine (‰).

246 Trophic levels of two different holothurian species are considered robust, when the difference in  
247 trophic levels between two species is  $\geq$ 0.44. This value corresponds to the average standard  
248 deviation of the calculated trophic level ( $\sigma_{TL}$ ) across all holothurians that was determined  
249 following equation S4 in (Jarman et al. 2017) as:



$$\sigma_{TP} = \sqrt{\left(\frac{1}{\Delta_{Glu-Phe}}\right)^2 \sigma_{\delta^{15}N_{Glu}}^2 + \left(\frac{-1}{\Delta_{Glu-Phe}}\right)^2 \sigma_{\delta^{15}N_{Phe}}^2 + \left(\frac{1}{\Delta_{Glu-Phe}}\right)^2 \sigma_{\beta}^2 + \left(\frac{-1}{\Delta^2_{Glu-Phe}} (\delta^{15}N_{Glu} - \delta^{15}N_{Phe} + \beta)\right)^2 \sigma_{\Delta_{Glu-Phe}}^2}, \quad (4)$$

251 where  $\sigma_{\beta}$  is 0.9‰ and  $\sigma_{\Delta}$  is 1.1‰ (Jarman et al. 2017).

252

### 253 Heterotrophic re-synthesis of amino acids

254 Total heterotrophic re-synthesis of amino acids ( $\sum V$ ) was approximated as the sum of variance  
 255 of individual  $\delta^{15}N$  values of the trophic amino acids alanine, aspartic acid, glutamic acid, leucine,  
 256 and proline (McCarthy et al. 2007):

$$\sum V = \sum_1^n |x_{amino\ acid} - \bar{x}_{amino\ acid}| \quad (5)$$

259  $x$  symbolized each trophic amino acid's  $\delta^{15}N$  value,  $\bar{x}$  is the average trophic amino acid's  $\delta^{15}N$   
 260 value and  $n$  is the total number of trophic amino acids used in this calculation (McCarthy et al.  
 261 2007).

262

### 263 Statistics

264 The Sørensen–Dice coefficient  $\beta_{sor}$  (Dice 1945; Sørensen 1948; Koleff et al. 2003) was  
 265 calculated using the ‘betadiver’ function in the *R* (version 4.1.2; R-Core Team 2017) package  
 266 *vegan* (version 2.6-2; (Oksanen et al. 2017)) to compare holothurian species based on their  
 267 trophic levels (*TL*), levels of heterotrophic re-synthesis of amino acids ( $\sum V$ ), feeding selectivity  
 268 based on concentration factor (*CF*), and food sources/ diet. For this purpose, the quantitative data  
 269 *TL* and  $\sum V$  were first converted into categories (Table 4) and then converted into binary  
 270 (presence/ absence) data; the categorical data ‘feeding selectivity’ (Table 4) and ‘food/sources  
 271 diet’ were also converted into binary data. Subsequently,  $\beta_{sor}$  was clustered by average linkage  
 272 clustering (unweighted pair-group method using arithmetic averages, UPGMA; Romesburg  
 273 1984) using the ‘hclust’ function in *R*. The dendrogram was prepared with *R* package *factoextra*  
 274 (version 1.0.7; Kassambara and Mundt 2020).

275

276 **Table 4.** Parameters used to calculate the Sørensen–Dice coefficient  $\beta_{sor}$  presented as  
 277 quantitative data (ranges) and categorical data. ‘Food sources/ diet’ includes a list of the main  
 278 food sources of the investigated holothurian species which were identified by amino acid and  
 279 fatty acid analysis.

Parameter	Quantitative data	Categorical data
Trophic level ( <i>TL</i> )	$TL = 2.0-2.5$	$TL_{group\ 1}$
	$TL = >2.5-3.0$	$TL_{group\ 2}$
	$TL = >3.0-3.5$	$TL_{group\ 3}$
Levels of heterotrophic re-synthesis of amino acids ( $\sum V$ )	$\sum V = 0-1.5$	$\sum V_{group\ 1}$
	$\sum V = >1.5-3.0$	$\sum V_{group\ 2}$
	$\sum V = >3.0-4.5$	$\sum V_{group\ 3}$
	$\sum V = >4.5-6.0$	$\sum V_{group\ 4}$
	$\sum V = >6.0-7.5$	$\sum V_{group\ 5}$
	$\sum V = >7.5-9.0$	$\sum V_{group\ 6}$
Feeding selectivity based on concentration	$CF = 0-10$	no selectivity
	$CF = >10-50$	selective

factor <i>CF</i>	<i>CF</i> = >50–150 <i>CF</i> = >150	very selective extremely selective
Food sources/ diet		<ul style="list-style-type: none"><li>– diatom-derived phytodetritus</li><li>– dinoflagellate-derived phytodetritus</li><li>– detritus containing C22:1<math>\omega</math>9-<i>cis</i></li><li>– secondary consumer of detritus</li><li>– mix diet (phytodetritus primary consumer, detritus secondary consumer)</li><li>– mixed diet (phytodetritus, bacteria)</li><li>– mixed diet (phytodetritus, bacteria, Foraminifera)</li></ul>

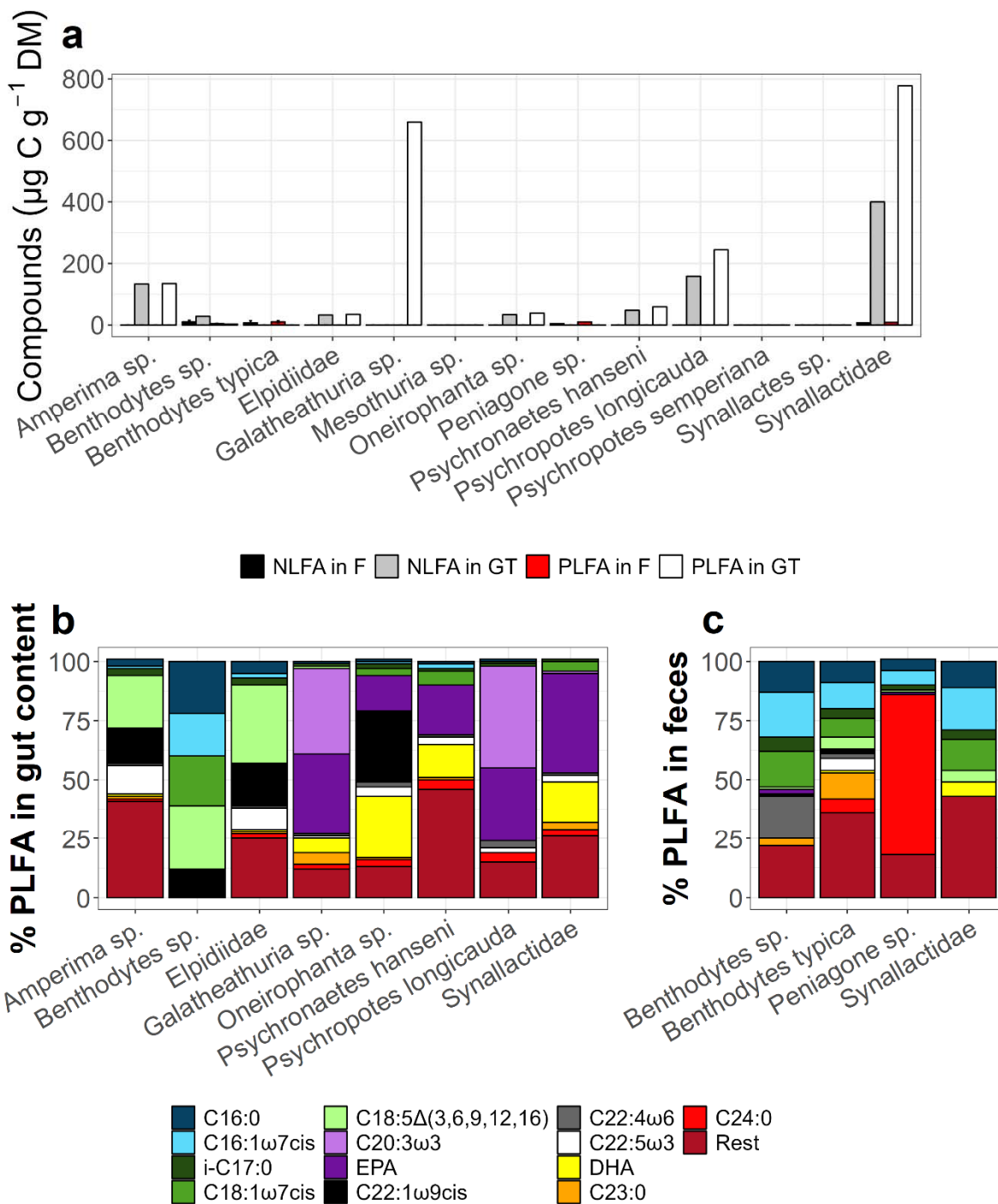
280

## 281 **Results**

### 282 **Gut content and feces of holothurians**

283 Gut contents of holothurians in the Peru Basin weighed  $1.93 \pm 3.56$  g dry sediment ( $n = 17$ ) and  
284 ranged from 0.11 g dry sediment for *Peniagone* sp. ( $n = 1$ ) to 12.5 g dry sediment for *P. hanseni*  
285 ( $n = 1$ ; Table 5). Org. C and TN content of the gut content was  $5.34 \pm 4.13\%$  ( $n = 17$ ) and  
286  $1.04 \pm 0.87\%$  ( $n = 17$ ), respectively, and it contained  $244 \pm 304$   $\mu\text{g C-PLFA g}^{-1}$  DM gut content  
287 ( $n = 8$ ) and  $83.3 \pm 124$   $\mu\text{g C-NLFA g}^{-1}$  DM gut content ( $n = 10$ ) (Fig. 1a). The concentration  
288 factor *CF* for PLFAs in holothurian gut content was on average  $105 \pm 131$  ( $n = 8$ ) and ranged  
289 from 1.17 to 335 for *Benthodytes* sp. ( $n = 1$ ) and Synallactidae gen sp. ( $n = 1$ ), respectively  
290 (Table 5). The average EPA/ARA-ratio for gut content was  $3.20 \pm 5.58$  ( $n = 9$ ), the average  
291 DHA/EPA-ratio was  $0.62 \pm 0.68$  ( $n = 9$ ), and the average C16:1 $\omega$ 7/C16:0-ratio was  $0.81 \pm 0.85$   
292 ( $n = 9$ ) (Fig. 2a).

293



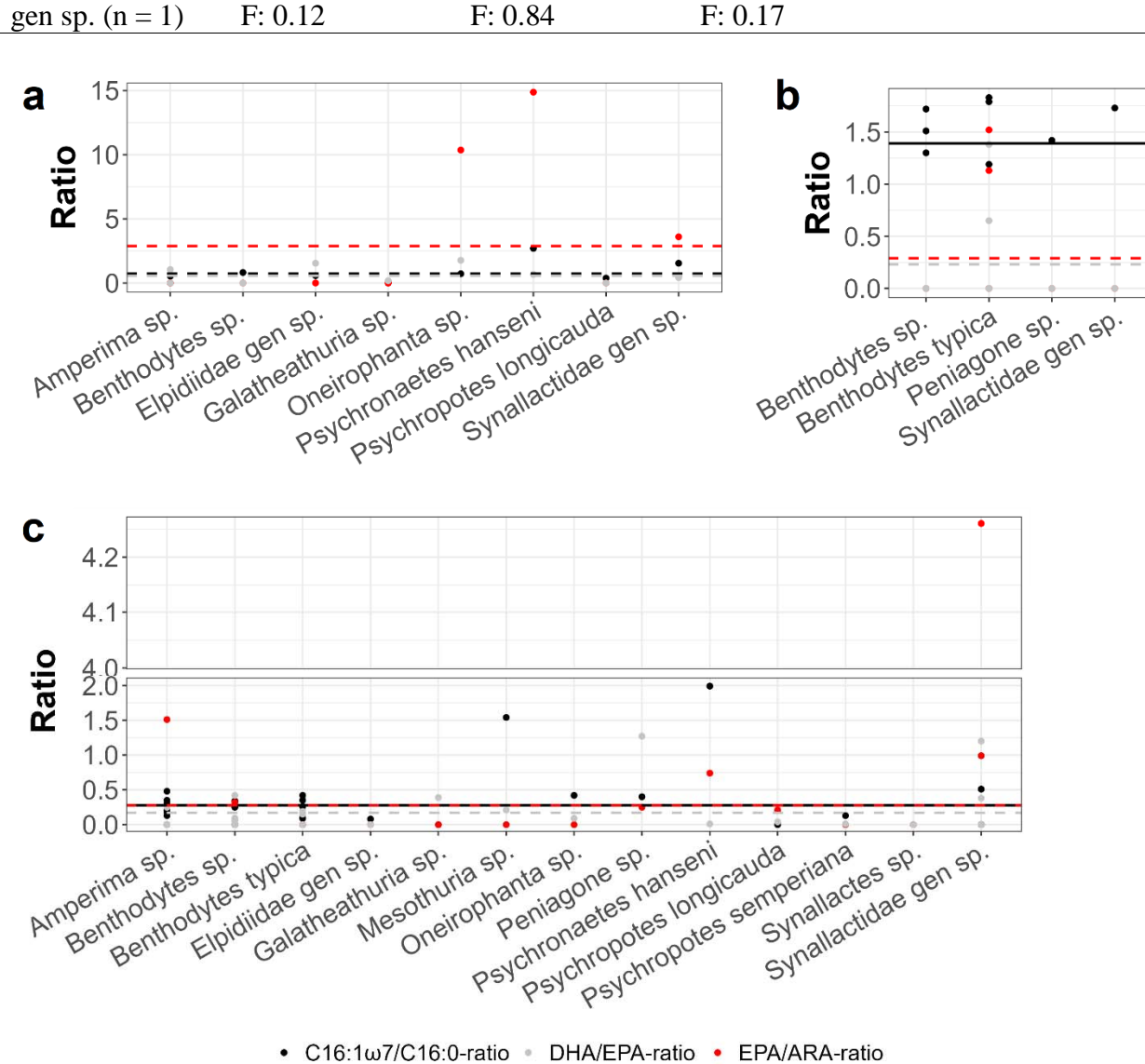
294  
295 **Figure 1.** (a) Concentrations ( $\mu\text{g C g}^{-1} \text{ DM. C}$ ) of the compounds PLFA and NLFA in  
296 holothurian gut content (GT) and feces (F) and the contribution (%) of individual (b, c) PLFAs  
297 and (d, e) NLFAs to the total concentrations. The PLFA and NLFA pools ‘Rest’ include all  
298 PLFAs and NLFAs, respectively, that contribute  $<2.5\%$  to total % PLFA and NLFA of the  
299 average holothurian gut content/ feces.  
300 Error bars in (a) indicate SD.  
301

302  
303  
304

**Table 5.** Sedimentological characteristics of holothurian gut content (GT) and feces (F). Data are presented as mean±SD.

Species	GT/ F (g dry sediment)	GT/ F composition		Concentration factor $CF_{gut}$ content, $CF_{feces}$
		% org. C;	% TN	
<b>Family: Elpidiidae</b>				
<i>Amperima</i> sp. (GT: n = 5; F: n = 1)	GT: 0.16±0.1; F: 0.54	GT: 7.76±3.85 F: 0.32	GT: 1.60±0.71 F: 0.05	GT: 58.0
Elpidiidae gen sp. (GT: n = 1)	0.88	1.57	0.35	14.9
<i>Peniagone</i> sp. (GT: n = 1; F: n = 1)	GT: 0.11; F: 0.45	GT: 3.27; F: 0.84	GT: 0.66; F: 0.13	F: 4.36
<b>Family: Deimatidae</b>				
<i>Oneirophanta</i> sp. (GT: n = 1)	3.90	2.84	0.51	16.6
<b>Family: Laetmogonidae</b>				
<i>Psychronaetes hanseni</i> (GT: n = 1)	12.5	1.94	0.38	25.6
<b>Family: Psychropotidae</b>				
<i>Benthodytes</i> sp. (GT: n = 2; F: n = 3; sediment grain size: n = 1)	GT: 2.07±2.1 F: 3.01±3.61	GT: 0.95±0.02 F: 0.70±0.16	GT: 0.16±0.01 F: 0.14±0.05	GT: 1.17 F: 1.81±0.53
<i>Benthodytes typica</i> (F: n = 4; sediment grain size: n = 1)	0.85±0.62	1.05±0.47	0.16±0.01	4.44±1.52
<i>Psychropotes longicauda</i> (GT: n = 1)	1.08	1.38	0.29	105
<i>Psychropotes semperiana</i> (GT: n = 1)	1.78	5.25	0.80	
<b>Family: Synallactidae</b>				
<i>Synallactes</i> sp. (morphotype "pink") (GT: n = 1)	6.75	2.22	0.34	
<i>Galatheathuria</i> sp. (GT: n = 1)	0.51	11.4	2.10	285
Synallactidae	GT: 0.14	GT: 11.3	GT: 2.44	GT: 335

305



306

307 **Figure 2.** Ratios of C16:1ω7/C16:0, DHA/EPA, and EPA/ARA in (a) holothurian gut content,  
 308 (b) holothurian feces, and (c) dried holothurian body walls. Horizontal lines show the average  
 309 value of a ratio based on all samples.

310

311 Feces of holothurians weighed  $1.36 \pm 2.09$  g dry sediment (n = 10) and ranged from 0.12 g dry  
 312 sediment for *Synallactidae* gen sp. (n = 1) to  $3.01 \pm 3.61$  g dry sediment for *Benthodytes* sp.  
 313 (n = 3; Table 5). Org. C and TN content of the feces was  $0.83 \pm 0.37\%$  (n = 10) and  $0.14 \pm 0.04\%$   
 314 (n = 10), respectively, and it contained  $7.73 \pm 3.60$  μg C-PLFA g<sup>-1</sup> DM sediment (n = 8) and  
 315  $7.63 \pm 5.28$  μg C-NLFA g<sup>-1</sup> DM sediment (n = 15) (Fig. 1a). In holothurian feces (n = 8), PLFAs  
 316 were on average still  $3.33 \pm 1.55$  times more concentrated compared to the upper 2 cm of  
 317 sediment ( $CF_{PLFA}$  range:  $1.81 \pm 0.53$  (n = 3) for *Benthodytes* sp. to  $4.44 \pm 1.52$  for *B. typica* (n = 3))  
 318 (Table 5). The average EPA/ARA-ratio for feces was  $0.29 \pm 0.59$  (n = 9), the average DHA/EPA-  
 319 ratio was  $0.23 \pm 0.48$  (n = 9), and the average C16:1ω7/C16:0-ratio was  $1.39 \pm 0.57$  (n = 9)  
 320 (Fig. 2b).

321 Gut content and feces consisted to  $81.2 \pm 3.73\%$  of silt (grain size:  $<63 \mu\text{m}$ ;  $n = 6$ ) and to  
 322  $10.7 \pm 1.10\%$  of very fine sand (grain size:  $62.5 - 125 \mu\text{m}$ ) (Table 6). The median grain was  
 323  $15.5 \pm 2.27 \mu\text{m}$  ( $n = 6$ ).

324

325 **Table 6.** Grain size characteristics of gut content (GT) and feces (FC).

Species	% silt fraction ( $<63 \mu\text{m}$ )	% very fine sand fraction ( $62.5 - 125 \mu\text{m}$ )	% fine sand fraction ( $125 - 250 \mu\text{m}$ )	% medium sand fraction ( $250 - 500 \mu\text{m}$ )	% coarse sand fraction ( $500 - 1000 \mu\text{m}$ )	Median grain size ( $\mu\text{m}$ )
<b>Family: Elpidiidae</b>						
Elpidiidae gen sp. (GT; $n = 1$ )	82.3	10.0	4.91	2.05	0.92	14.7
<b>Family: Deimatidae</b>						
<i>Oneirophanta</i> sp. (GT; $n = 1$ )	75.5	12.2	7.86	4.01	0.65	19.0
<b>Family: Laetmogonidae</b>						
<i>Psychronaetes</i> <i>hanseni</i> (GT; $n = 1$ )	79.0	11.7	6.67	2.48	0.33	17.5
<b>Family: Psychropotidae</b>						
<i>Benthodytes</i> sp. (F; $n = 1$ )	86.4	9.20	4.05	0.44	0.00	13.0
<i>Benthodytes</i> <i>typica</i> (F; $n = 1$ )	81.0	10.8	6.08	1.77	0.55	14.2
<b>Family: Synallactidae</b>						
Synallactidae gen sp. (F; $n = 1$ )	83.0	10.4	4.47	1.58	0.76	14.5

326

327 About  $22.3 \pm 15.5\%$  ( $n = 8$ ) of the PLFAs (Fig. 4b) and  $23.1 \pm 18.4\%$  ( $n = 7$ ) of the NLFAs  
 328 (Fig. 4d) found in holothurian gut content consisted of 'Rest', i.e., the sum of PLFAs and NLFAs  
 329 that each contributed  $<2.5\%$  to total PLFA and NLFA concentrations. The remaining PLFAs  
 330 consisted to  $7.00 \pm 6.55\%$  of saturated fatty acids (SFA),  $17.0 \pm 17.7\%$  monosaturated fatty acids  
 331 (MUFAs, i.e., fatty acids with one double bond),  $10.0 \pm 18.4\%$  polyunsaturated fatty acids  
 332 (PUFAs, i.e., fatty acids with  $\geq 2$  double bonds),  $41.5 \pm 10.6\%$  highly unsaturated fatty acids  
 333 (HUFAs, i.e., fatty acids with  $\geq 4$  double bonds), and  $2.25 \pm 1.36\%$  long-chain fatty acids  
 334 (LCFAs, i.e., fatty acids with  $\geq 24$  C atoms). NLFAs included furthermore  $36.9 \pm 11.7\%$  SFAs,  
 335  $13.0 \pm 11.0\%$  MUFAs, and  $27.0 \pm 17.3\%$  HUFAs. Feces of holothurian consisted to  $29.6 \pm 13.3\%$   
 336 ( $n = 8$ ) of the PLFAs category 'Rest' (Fig. 4c) and to  $31.4 \pm 15.6\%$  ( $n = 8$ ) of the NLFAs category  
 337 'Rest' (Fig. 4e). The other PLFAs consisted to  $20.1 \pm 9.58\%$  of SFAs,  $25.6 \pm 11.7\%$  MUFAs,  
 338  $14.1 \pm 12.4\%$  HUFAs, and  $10.6 \pm 23.7\%$  LCFAs. The NLFAs included additionally  $39.5 \pm 11.1\%$   
 339 SFAs,  $10.3 \pm 10.5\%$  MUFAs, and  $18.8 \pm 24.3\%$  HUFAs.

340

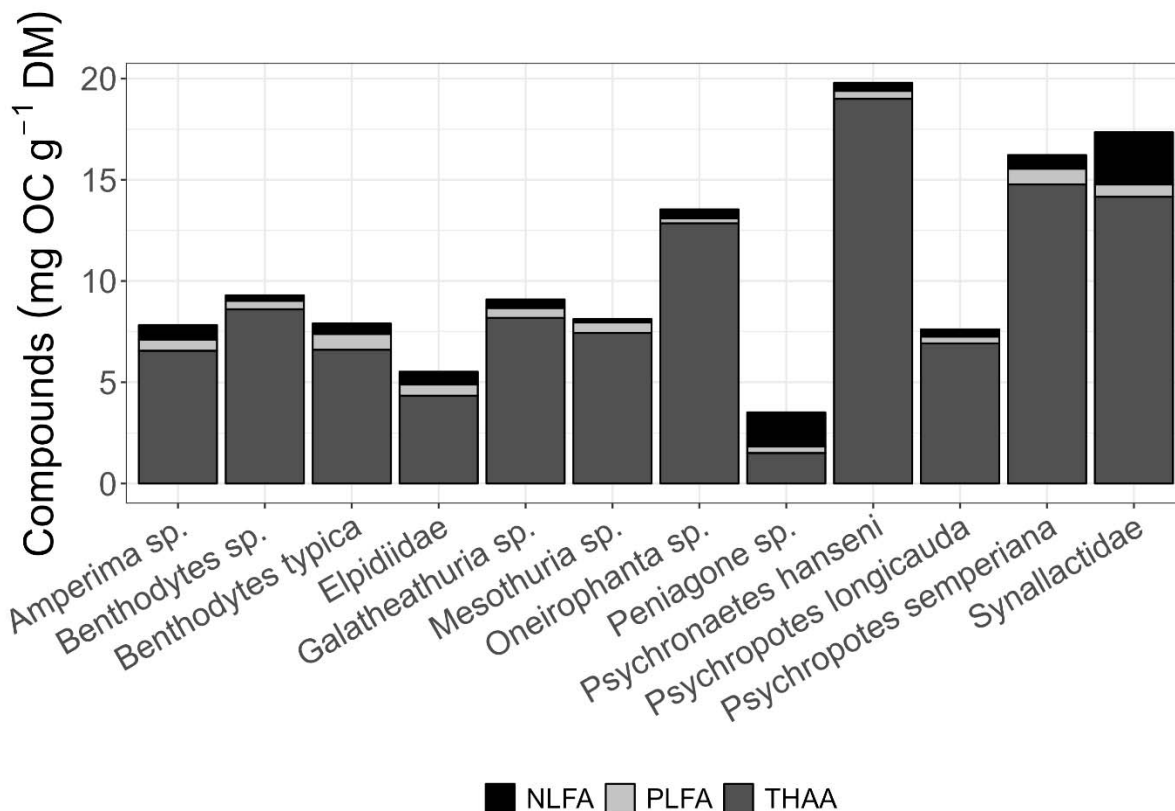
341 **Chemical composition of holothurians**

342 Holothurians in the Peru Basin consisted for  $93.0 \pm 10.2\%$  of water ( $n = 13$ ) and their dried body  
 343 walls contained  $5.87 \pm 3.50\%$  org. C and  $1.35 \pm 0.80\%$  total N ( $n = 31$ ), whereas their dried gut  
 344 tissues consisted of  $16.7 \pm 8.60\%$  org. C and  $3.76 \pm 2.18\%$  total N ( $n = 15$ ). The body wall and gut  
 345 tissue of the holothurian families Deimatidae and Laetmogonidae had the highest org. C and TN  
 346 contents, whereas the families Elpidiidae and Psychropotidae had the lowest org. C and TN  
 347 content in body wall tissue (Table 7).

348  
 349 **Table 7.** Chemical composition of body wall (BW) and gut tissue (GT) of different holothurian  
 350 species collected in the Peru Basin. Data are presented as mean  $\pm$  SD.

Species	Body wall (BW)		Gut (G)	
	% org. C	% TN	% org. C	% TN
Family: Elpidiidae (BW: $n = 10$ ; GT: $n = 4$ )	$4.48 \pm 3.25$	$1.07 \pm 0.86$	$8.02 \pm 2.15$	$1.63 \pm 0.48$
<i>Amperima</i> sp. (BW: $n = 9$ ; GT: $n = 4$ )	$4.62 \pm 3.30$	$1.10 \pm 0.88$	$8.02 \pm 2.15$	$1.63 \pm 0.48$
Elpidiidae gen sp. ( $n = 1$ )	6.94	1.73		
<i>Peniagone</i> sp. ( $n = 1$ )	0.93	0.17		
Family: Deimatidae ( $n = 1$ )	16.0	3.25	26.4	6.27
<i>Oneirophanta</i> sp. ( $n = 1$ )	16.0	3.25	26.4	6.27
Family: Laetmogonidae ( $n = 1$ )	11.7	2.46	20.6	4.42
<i>Psychronaetes hansenii</i> ( $n = 1$ )	11.7	2.46	20.6	4.42
Family: Mesothuriidae ( $n = 1$ )	5.42	1.06		
<i>Mesothuria</i> sp. ( $n = 1$ )	5.42	1.06		
Family: Psychropotidae (BW: $n = 14$ ; GT: $n = 4$ )	$5.19 \pm 2.53$	$1.21 \pm 0.60$	$19.3 \pm 7.43$	$4.15 \pm 1.78$
<i>Benthodytes</i> sp. ( $n = 6$ )	$4.53 \pm 1.54$	$1.08 \pm 0.42$		
<i>Benthodytes typica</i> (BW: $n = 5$ ; GT: $n = 1$ )	$4.19 \pm 1.64$	$0.95 \pm 0.51$	28.9 ( $n = 1$ )	6.55
<i>Psychropotes longicauda</i> ( $n = 1$ )	4.39	1.15	16.7	3.11
<i>Psychropotes semperiana</i> ( $n = 1$ )	8.41	2.09	11.2	2.53
Family: Synallactidae (BW: $n = 5$ ; GT: $n = 4$ )	$8.60 \pm 2.37$	$1.97 \pm 0.53$	$15.6 \pm 8.23$	$3.61 \pm 2.15$
<i>Synallactes</i> sp. (morphotype "pink") ( $n = 1$ )	6.63	1.42	10.5	2.07
<i>Galatheathuria</i> sp. ( $n = 1$ )	6.98	2.01	8.98	2.23
Synallactidae gen sp. (BW: $n = 3$ ; GT: $n = 2$ )	$9.80 \pm 2.41$	$2.14 \pm 0.60$	$21.4 \pm 8.15$	$5.08 \pm 2.29$

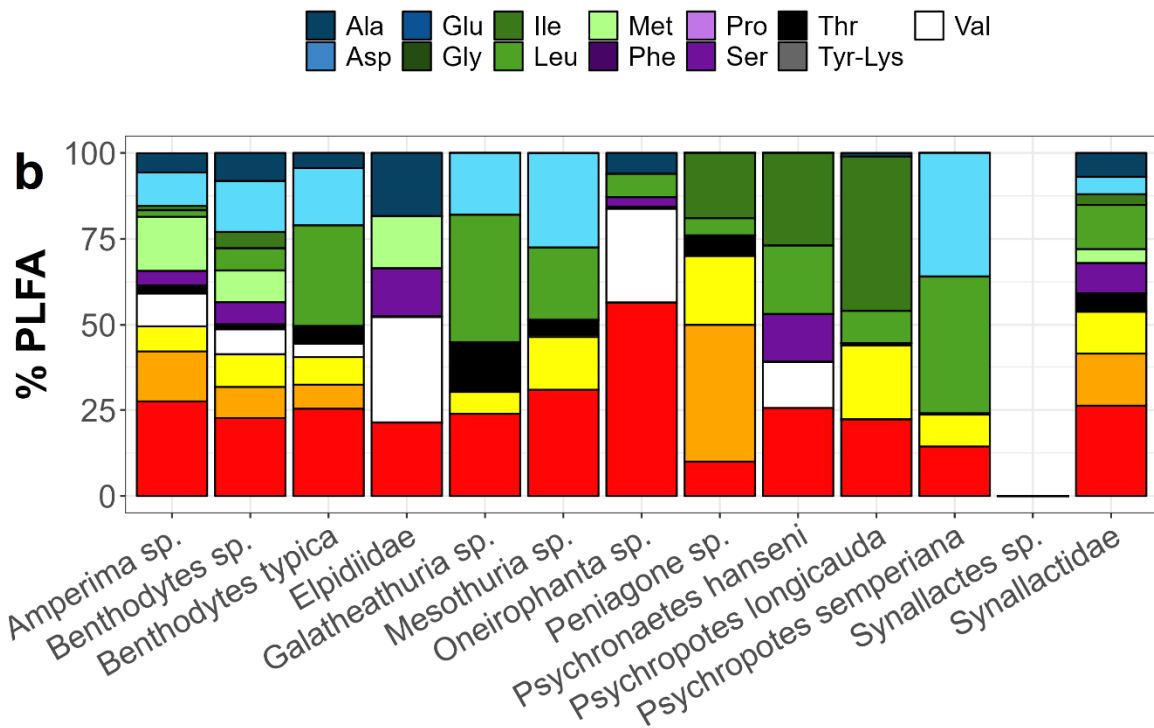
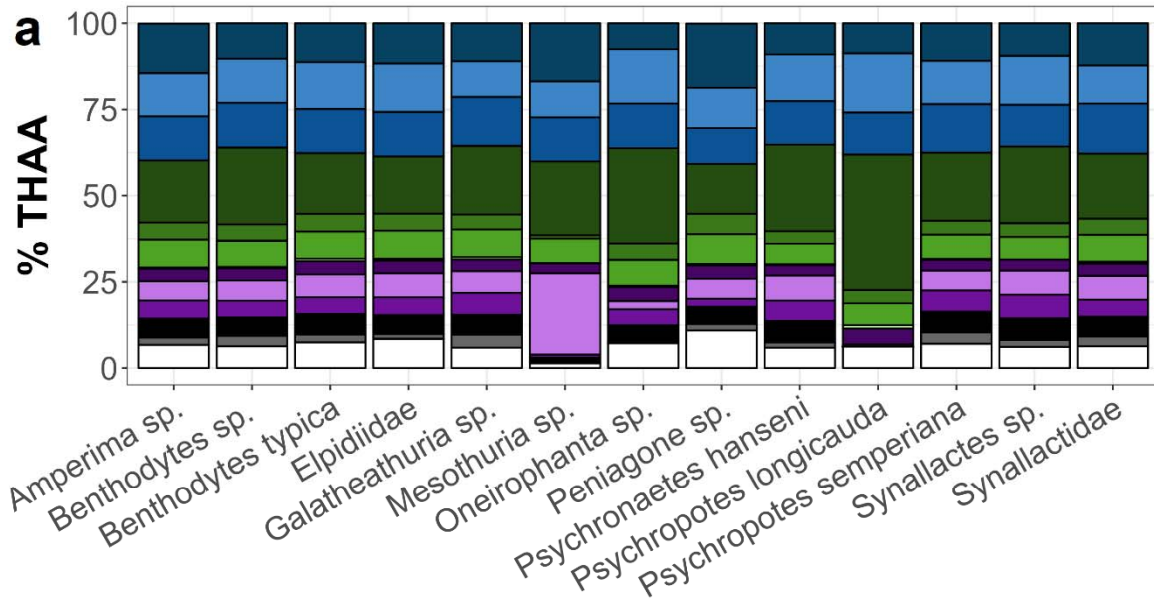
351  
 352 THAAs, PLFAs, and NLFAs contributed a total  $17.4 \pm 6.11\%$  to the org. C of all specimens  
 353 combined ( $n = 27$ ) and ranged from  $1.50 \text{ mg C g}^{-1} \text{ DM THAAs}$  (*Peniagone* sp.;  $n = 1$ ) to  
 354  $19.0 \text{ mg C g}^{-1} \text{ DM THAA}$  (*P. hansenii*;  $n = 1$ ),  $0.24 \text{ mg C g}^{-1} \text{ DM PLFAs}$  (*Oneirophanta* sp.;  
 355  $n = 1$ ) to  $0.78 \pm 0.29 \text{ mg C g}^{-1} \text{ DM PLFAs}$  (*B. typica*;  $n = 5$ ), and  $0.17 \text{ mg C g}^{-1} \text{ DM NLFAs}$   
 356 (*Mesothuria* sp.;  $n = 1$ ) to  $2.58 \pm 3.32 \text{ mg C g}^{-1} \text{ DM NLFAs}$  (Synallactidae gen sp.;  $n = 3$ ) (Fig. 3).  
 357



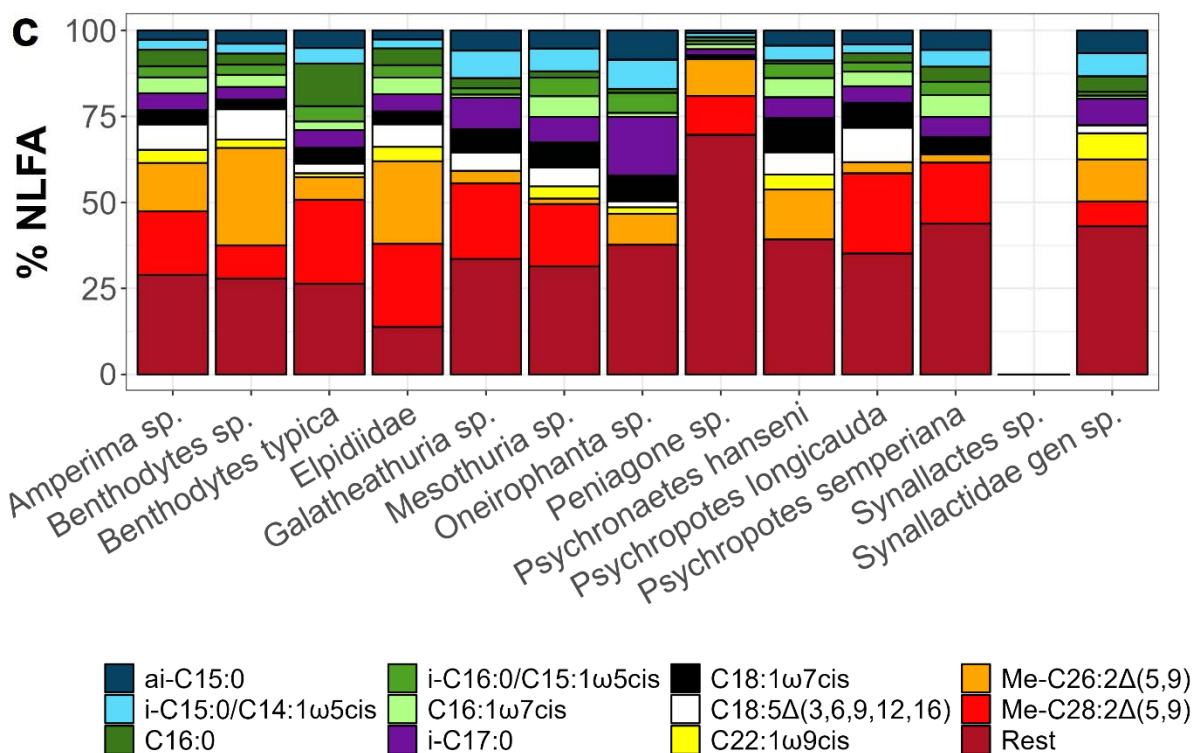
358  
 359 **Figure 3.** The compounds neutral lipid-derived fatty acids (NLFA), phospholipid-derived fatty  
 360 acids (PLFA), and total hydrolysable amino acids (THAA) in dried holothurian body walls.  
 361  
 362 The THAA composition did not differ greatly among species, with the main THAAs in  
 363 holothurian body walls being alanine, glycine, aspartic acid, and glutamic acid, that contributed  
 364 between 55.2% (Elpidiidae; n = 1) and 77.4% (*P. longicauda*; n = 1) to total THAAs of body  
 365 walls (Fig. 4a).  
 366 In contrast, the PLFA (Fig. 4b) and NLFA (Fig. 4c) composition differed strongly between  
 367 species. Between 10.2% (*Peniagone* sp.; n = 1) and 56.5% (*Oneirophanta* sp.; n = 1) of the  
 368 PLFAs found in holothurian body walls contributed <2.5% to the total PLFA concentration and  
 369 were combined as ‘Rest’ (Fig. 4b). The remaining PLFAs consisted to 4.07±7.57% (n = 32) of  
 370 MUFAs, 10.8±12.6% PUFAs, 32.2±14.1% HUFAs, 9.17±14.0% LCFAs, and 15.5±10.1%  
 371 methyl-fatty acids. Compared to the average PLFA composition across all holothurian taxa  
 372 analyzed, Elpidiidae gen sp. (n = 1) had an above average percentage of MUFAs (12.8% of total  
 373 PLFAs) and methyl-fatty acids (48.1% of total PLFAs). *Oneirophanta* sp. (n = 1) had an above  
 374 average percentage of SFAs (41.1% of total PLFAs) and *P. longicauda* (n = 1) had an above  
 375 average percentage of HUFAs (10.0% of total PLFAs).  
 376 The NLFAs consisted for 21.6±10.4% (n = 30) of SFA, 10.1±5.80% MUFAs, 5.36±5.03%  
 377 HUFAs, and 28.5±18.4% methyl-fatty acids (Fig. 4c). Between 13.8% Elpidiidae gen sp. (n = 1)  
 378 and 69.6% (*Peniagone* sp.; n = 1) of the total NLFAs in holothurian body walls consisted of  
 379 NLFAs that individually contributed <2.5% to the total NLFA concentration and were therefore  
 380 combined as ‘Rest’. In comparison to the average NLFA composition across all studied



381 holothurian taxa, *Oneirophanta* sp. (n = 1) had an above average percentage of SFA (41.1% of  
382 total NLFAs). *P. hanseni* (n = 1) had an above average percentage of MUFAs (19.9% of total  
383 NLFAs), *P. longicauda* had an above average percentage of HUFAs (10.0% of total NLFAs),  
384 and Elpidiidae gen sp. had an above average percentage of methyl-fatty acids (48.1%).  
385



386



387  
388 **Figure 4.** Contribution (%) of individual (a) THAAs, (b) PLFAs, and (c) NLFAs to the total  
389 concentrations. The PLFA and NLFA pools ‘Rest’ include all PLFAs and NLFAs, respectively,  
390 that contribute <2.5% to total % PLFA and NLFA of the average holothurian tissue.

391  
392 The ratio of the essential phospholipid-derived PUFAs EPA to ARA, i.e., the EPA/ARA-ratio,  
393 ranged from  $0.05 \pm 0.13$  for *Benthodytes* sp. (n = 6) to  $1.75 \pm 2.23$  for Synallactidae gen sp. (n = 3)  
394 (Fig. 2c). In comparison, the ratio of DHA to EPA, i.e., the DHA/EPA-ratio, ranged from 0.01  
395 for *P. hansenii* (n = 1) to 1.27 for *Peniagone* sp. (n = 1) (Fig. 2c). Due to the absence of the  
396 PUFAs ARA and/ or EPA in holothurian body wall tissue, no EPA/ARA-ratios were calculated  
397 for *B. typica*, *Elpidiidae* gen sp., *Galatheathuria* sp., *Mesothuria* sp., *Oneirophanta* sp., and *P.*  
398 *semperiana*. *Elpidiidae* gen sp. lacked both, DHA and EPA, and therefore no DHA/EPA-ratio  
399 could be calculated (Fig. 2c).

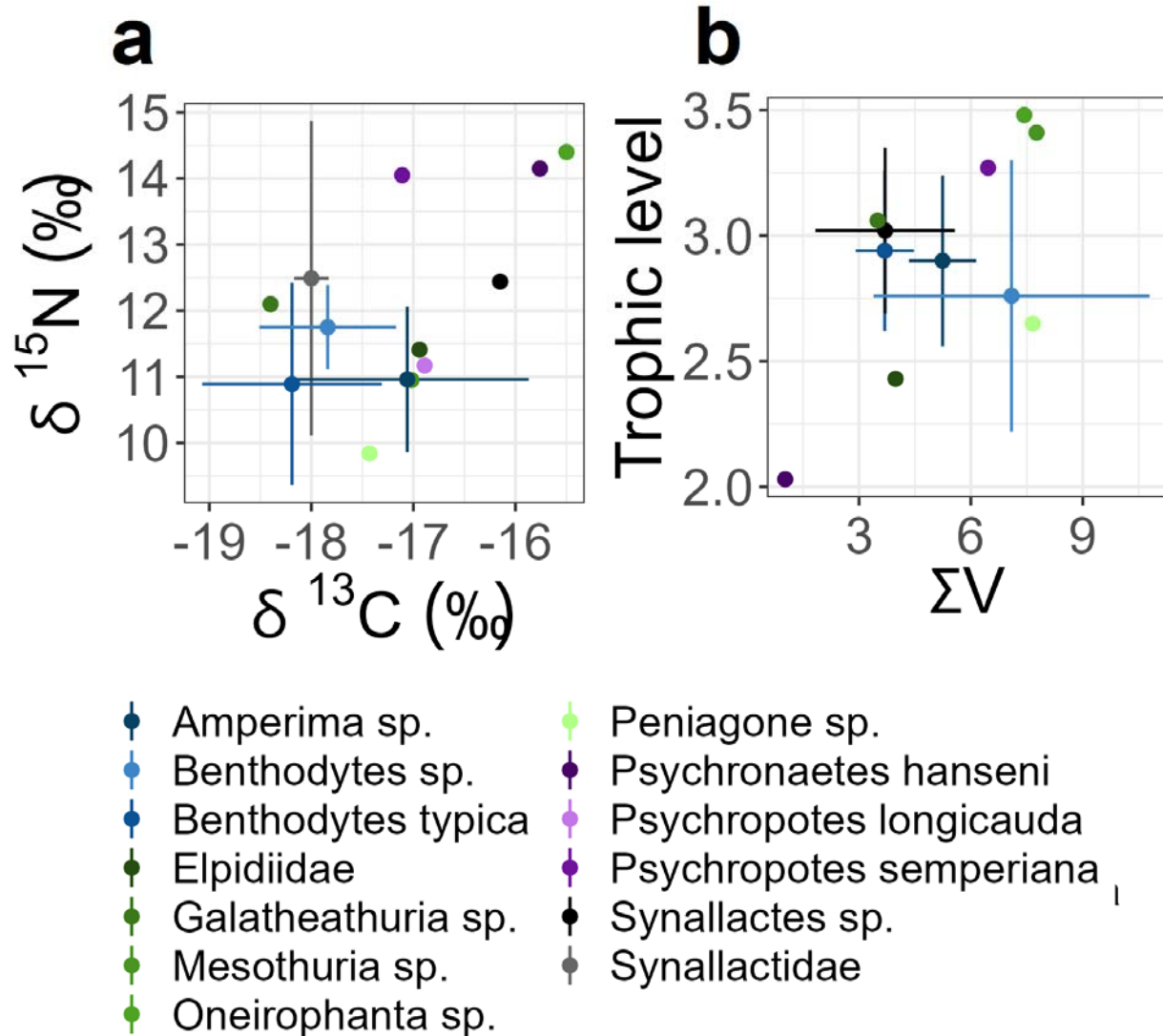
#### 400 401 **Trophic position of holothurians and recycling of amino acids**

402 Holothurians in the Peru Basin had an average  $\delta^{13}\text{C}$ -value of  $-17.4 \pm 1.02\text{‰}$  (n = 31) with a  
403 minimum value of  $-18.4\text{‰}$  for *Galatheathuria* sp. (n = 1) and a maximum value of  $-15.5\text{‰}$  for  
404 *Oneirophanta* sp. (n = 1). The average  $\delta^{15}\text{N}$ -value was  $11.6 \pm 1.47\text{‰}$  (n = 31) with a minimum  
405 value of  $9.84\text{‰}$  for *Peniagone* sp. (n = 1) and a maximum value of  $14.4\text{‰}$  for *Oneirophanta* sp.  
406 (n = 1) (Fig. 5a).

407 TL estimates for holothurians in the Peru Basin, based on the  $\delta^{15}\text{N}$  values of the THAA glutamic  
408 acid and alanine, ranged from 2.0 (*P. hansenii*, n = 1) to 3.5 (*Oneirophanta* sp., n = 1) (Fig. 5b).

409 values ranged from 1.02 to 7.76, with *P. hansenii* having the lowest heterotrophic enrichment  
410 and *Mesothuria* sp. having the highest heterotrophic enrichment (Fig. 5b).

411



412  
 413 **Figure 5.** (a) Isotopic composition of carbon ( $\delta^{13}\text{C}$ , ‰) and nitrogen ( $\delta^{15}\text{N}$ , ‰) of holothurian  
 414 body wall tissue from the Peru Basin. (b) Trophic position and heterotrophic enrichment factor  
 415 of holothurians. Error bars indicate SD.

416  
 417 **Discussion**

418 **Fatty acid composition of holothurians**

419 Deep-sea megabenthic invertebrates consist to 4.5% DM (cnidarians) to 44.9% DM  
 420 (crustaceans) of lipids (Drazen et al. 2008a), whereupon holothurians have lipid contents of <1%  
 421 DM to 5.8% DM (Drazen et al. 2008b). The largest lipid fraction is phospholipids with 14.5%  
 422 total lipids (crustaceans, Drazen et al. 2008a) to 95.2% total lipids (holothurians; Drazen et al.  
 423 2008b). The neutral lipids wax esters and triacylglycerol contribute between <1% (polychaetes)  
 424 and 83% (crustaceans) to total lipids (Drazen et al. 2008a) and also holothurians consist only of  
 425 <1% to 2.6% total lipids wax esters and triacylglycerol (Drazen et al. 2008c). Holothurians from  
 426 the Peru Basin contain between 0.16% DM (*Oneirophanta* sp.) and 2.30% DM (*B. typica*)  
 427 PLFAs, components of phospholipids, and 0.31% DM (*Oneirophanta* sp.) to 4.71% DM

428 (*Peniagone* sp.) NLFAs, elements of neutral lipids. Hence, they have a relatively high neutral  
429 fatty acid content compared to holothurians from Station M (NE Pacific) (Drazen et al. 2008b).  
430 This might be related to differences in food availability at the two study sites: The abyssal  
431 seafloor at Station M receives on average  $22.3 \text{ g C m}^{-2} \text{ yr}^{-1}$  particulate organic carbon (POC)  
432 (Baldwin et al. 1998), whereas the POC flux to the Peru Basin is estimated to be  $1.49 \text{ g C m}^{-2} \text{ yr}^{-1}$   
433 (Haeckel et al. 2001). As a result, holothurians from the Peru Basin might be adapted to a more  
434 food-limited environment by building higher concentrations of storage lipids when they  
435 encounter fresh phytodetritus than holothurians at Station M.

436

### 437 **Holothurians trophic level and inferred feeding strategy**

438 Based on the  $\delta^{15}\text{N}$  value of body wall tissue, Iken et al., (2001) identified three trophic groups  
439 among holothurians from PAP: Group A had  $\delta^{15}\text{N}$  values from 10.8 to 12.3‰, group B's  $\delta^{15}\text{N}$   
440 values ranged from 13.2 to 13.9‰, and group C had  $\delta^{15}\text{N}$  values from 15.6 to 16.2‰. The  $\delta^{15}\text{N}$   
441 values of holothurian tissue from the Peru Basin investigated in this study were lower and ranged  
442 from 9.84‰ for *Peniagone* sp. (n = 1) to 14.4‰ for *Oneirophanta* sp. (n = 1). Instead of basing  
443 our classification of holothurians from the Peru Basin solely on  $\delta^{15}\text{N}$  values, we combined data  
444 of trophic level based on compound-specific stable isotope analysis with biomarkers, grain size  
445 analysis, and concentration factors for PLFAs.

446

### 447 **Order Elasipodida**

448 *Psychronaetes hanseni* is a deposit feeder of the **family Laetmogonidae**, which has a trophic  
449 level of 2.0, low level of heterotrophic re-synthesis of amino acids ( $\sum V = 1.02$ ) and feeds  
450 selectively ( $CF_{\text{gut content}} = 25.6$ ) on sedimentary detritus particles of a medium grain size of  
451  $17.5 \mu\text{m}$  which is smaller than the medium grain size of the upper 5 cm of sediment  
452 ( $20.8 \pm 0.3 \mu\text{m}$ ; Mevenkamp et al., 2019). Based on the biomarkers present in the body wall tissue  
453 of the specimen analysed and in its gut content, parts of the sedimentary detritus likely consists  
454 of diatom-derived phytodetritus ( $\frac{\text{DHA}}{\text{EPA}}\text{-ratio}_{\text{body wall}} = 0.01$ ,  $\frac{\text{DHA}}{\text{EPA}}\text{-ratio}_{\text{gut content}} = 0.67$ ).

455

456 Elpidiidae gen sp. (**family Elpidiidae**) has a trophic level of 2.4 and medium level of  
457 heterotrophic re-synthesis of amino acids ( $\sum V = 3.98$ ). This species is a selective deposit feeder  
458 ( $CF_{\text{gut content}} = 14.9$ ) that preferentially feeds upon the PUFA C22:1 $\omega$ 9cis which is present in high  
459 percentages in its gut content (18%) and in its body tissue (29.0%).

460

461 The benthopelagic *Peniagone* sp. of the **family Elpidiidae** has an estimated trophic level of 2.7  
462 and a very high level of heterotrophic re-synthesis of amino acids ( $\sum V = 7.66$ ). This species has  
463 a 'sweeping' feeding style (Roberts et al. 2000) and assimilates fresh phytodetritus (Iken et al.  
464 2001) with medium efficiency, as the PLFA concentration in its feces ( $CF_{\text{feces}}$ ) is four times  
465 higher than in the surface sediment (this study). In the Peru Basin, *Peniagone* sp. seems to feed  
466 on diatom-derived phytodetritus ( $\frac{\text{C}_{16:1\omega7}}{\text{C}_{16:0}}\text{-ratio}_{\text{feces}} = 1.42$ ; this study).

467

468 *Amperima* sp. belongs to the **family Elpidiidae** and its trophic level was estimated to be  $2.9 \pm 0.3$ ,  
469 potentially due to a medium level of heterotrophic re-synthesis of amino acids  
470 ( $\sum V = 5.24 \pm 0.90$ ). This species is a very selective surface deposit feeder ( $CF_{\text{gut content}} = 58.0$ )  
471 with a 'sweeping' feeding style (Roberts et al. 2000) that grazes on very fresh phytodetritus on  
472 the surface sediment (Iken et al. 2001). As a result, the gut content of *A. rosea* at PAP has higher

473 concentrations of chlorophyll-*a* compared to surface sediment or phytodetritus (FitzGeorge-  
474 Balfour et al. 2010). A more detailed analysis of the phytopigments in this gut content revealed  
475 that *A. rosea* at PAP feeds preferentially on cyanobacteria-derived phytodetritus (Wigham et al.  
476 2003). Based on the PLFA composition of its gut content, we found that *Amperima* sp. from the  
477 Peru Basin likely feeds on dinoflagellate-derived phytodetritus ( $\frac{C_{16:1\omega7}}{C_{16:0}}$ -ratio<sub>gut content</sub> = 0.53; this  
478 study). Also the body wall fatty acid composition in our study differs substantially from  
479 specimens from PAP, as the PLFA profile of PAP specimens is dominated by EPA, DHA, ARA,  
480 and C18:0 (Hudson et al. 2004), whereas the PLFA profile of Peru Basin specimens is  
481 characterized mostly by EPA co-eluted with C22:1 $\omega$ 9, Me-C26:2 $\Delta$ (5,9), and C28:2 $\Delta$ (11,21).  
482 Hence, it seems that the feeding niche of the well-studied *Amperima* sp. can differ substantially  
483 between ocean basins.

484  
485 *Benthodytes* sp. from the **family Psychropotidae** has an estimated trophic level of 2.8 $\pm$ 0.5 and a  
486 very high level of heterotrophic re-synthesis of amino acids ( $\sum V = 7.09\pm 3.70$ ). It feeds with a  
487 ‘sweeper’ feeding style (Roberts et al. 2000) selectively on smaller sediment particles (medium  
488 grain size: 13.0  $\mu$ m) from the surface sediment (medium grain size: 20.8 $\pm$ 0.3  $\mu$ m; Mevenkamp et  
489 al., 2019). However, it likely does not or only moderately selects for specifically detritus-  
490 enriched particles ( $CF_{gut content} = 1.17$ ;  $CF_{feces} = 1.81\pm 0.53$ ). In fact, the high percentage of the  
491 bacteria-biomarker PLFAs C16:0, C16:1 $\omega$ 7*cis*, and C18:1 $\omega$ 7*cis* in its gut content and feces, and  
492 the very high level of heterotrophic re-synthesis of amino acids indicates *Benthodytes* sp. might  
493 host a large biomass of living heterotrophic prokaryotes. Unfortunately, in this study no amino  
494 acids from gut content or feces were extracted to assess whether this species concentrates detritus  
495 that is highly enriched in amino acids. Such an observation was interpreted by Romero-Romero  
496 et al., (2021) as a sign that deep-sea holothurians from Station M are secondary consumers of  
497 detritus, whereas the microbial community in their guts are primary consumers of detritus.  
498 Therefore, we hypothesize that also *Benthodytes* sp. is a secondary consumer, and its microbial  
499 gut community is the primary consumer of detritus.

500  
501 *Benthodytes typica* belongs to the **family Psychropotidae** and its trophic level is estimated to be  
502 a bit higher (2.9) than the trophic level of *Benthodytes* sp. This species has a medium level of  
503 heterotrophic re-synthesis of amino acids ( $\sum V = 3.69$ ) and feeds selectively on smaller particles  
504 (medium grain size: 14.2  $\mu$ m) from the ambient sediment (20.8 $\pm$ 0.3  $\mu$ m; Mevenkamp et al.,  
505 2019). These smaller particles contain an at least four times higher concentration of PLFAs than  
506 the surrounding sediment ( $CF_{feces} = 4.44\pm 1.52$ ) and consist partially of diatom-derived  
507 phytodetritus ( $\frac{C_{16:1\omega7}}{C_{16:0}}$ -ratio<sub>feces</sub> = 1.20 $\pm$ 0.85). Reliance on phytodetritus is confirmed by the  
508 PLFA composition of *B. typica* body walls ( $\frac{DHA}{EPA}$ -ratio<sub>body wall</sub> = 0.13 $\pm$ 0.08). In addition, this  
509 species either feeds selectively on sediment-bound prokaryotes or hosts prokaryotes as bacteria-  
510 specific PLFAs (i.e., C16:0, C16:1 $\omega$ 7*cis*, and C18:1 $\omega$ 7*cis*) contribute almost 30% to the total  
511 PLFA composition in feces, but were not detected in the body wall with >2.5% of total PLFAs.  
512 As a medium level of heterotrophic re-synthesis of amino acids was measured, *B. typica* likely  
513 has a mixed diet. In this diet, this holothurian species consumes phytodetritus as primary  
514 consumer and other types of detritus as secondary consumer following primary processing by a  
515 bacterial gut community.

516

517 *Psychropotes longicauda* from the **family Psychropotidae** has a medium level of heterotrophic  
518 re-synthesis of amino acids ( $\sum V = 5.13$ ). Feeding selectively was the highest in our data ( $CF_{gut}$   
519  $content = 105$ ), though, surprisingly at PAP this species was found to feed less selectively than  
520 *Peniagone diaphana* (FitzGeorge-Balfour et al. 2010). *P. longicauda*'s diet consists likely of  
521 diatom-derived phytodetritus ( $\frac{DHA}{EPA}$ -ratio<sub>body wall</sub> = 0.04), but it is also possible that *P. longicauda*  
522 consumes filamentous Rhodophyceae. This algae has been found in gelatinous detritus in the  
523 deep sea of the NE Atlantic and Bühring et al., (2002) speculated that *P. longicauda* might feed  
524 it sporadically, as the body walls of *P. longicauda* from specimens collected at PAP and in the  
525 Peru Basin contain EPA, a PLFA typical for Rhodophyceae, at relatively high concentrations  
526 (31% of total PLFA, this study; ~24% of total fatty acids at PAP (Ginger et al. 2000)).  
527 Additionally, at PAP 70 to 80% of the gut content of this species contained sediment (Iken et al.  
528 2001), which might originate from foraminiferans that Roberts and Moore, (1997) found in its  
529 guts together with radiolarians, harpacticoids, nematodes, spicules, and diatoms.

530  
531 *Psychropotes semperiana* (**family Psychropotidae**) has an estimated trophic level of 3.3, likely  
532 related to the high level of heterotrophic re-synthesis of amino acids ( $\sum V = 6.46$ ). This species  
533 has been classified as surface deposit feeder (Iken et al. 2001) and based on the biomarkers in the  
534 body tissue of a specimen collected in the Peru Basin, it consumes diatom-derived phytodetritus  
535 ( $\frac{DHA}{EPA}$ -ratio<sub>body wall</sub> = 0.01, this study).

#### 536 537 **Order Holothuriida**

538 *Mesothuria* sp. belongs to the **family Mesothuriidae** and has an estimated trophic level of 3.4.  
539 This species could be a subsurface (Iken et al. 2001) or surface deposit feeder (Miller et al. 2000)  
540 with a 'raker' feeding style (Roberts et al. 2000) or feeding with a 'wiping' motion (Hudson et  
541 al. 2005). The PLFA composition of its body walls suggests that *Mesothuria* sp. likely consumes  
542 diatom-derived phytodetritus ( $\frac{DHA}{EPA}$ -ratio<sub>body wall</sub> = 0.21). Indeed, in a study on the Hawaiian slope,  
543 gut contents of *Mesothuria carnosa* had a 2.7 fold enrichment of chlorophyll a pointing towards  
544 selective feeding on phytodetritus (Miller et al. 2000). Furthermore, the very high level of  
545 heterotrophic re-synthesis of amino acids ( $\sum V = 7.76$ ) from the Peru Basin suggests that  
546 *Mesothuria* sp. might also be a secondary consumer of detritus. However, we lack information  
547 about its gut content to confirm that it hosts a big(ger) living microbial biomass in its gut that is  
548 the primary consumer of detritus.

#### 549 550 **Order Synallactida**

551 *Oneirophanta* sp. as member of the **family Deimatidae** has an estimated trophic level of 3.5 and  
552 a very high level of heterotrophic re-synthesis of amino acids ( $\sum V = 7.43$ ). This species feeds  
553 selectively ( $CF_{gut content} = 16.6$ ) with a 'raker' feeding style (Roberts et al. 2000) and takes up  
554 particles with a median grain size of 19.0  $\mu\text{m}$ , which is slightly smaller than the median grain  
555 size of sediment particles in the Peru Basin ( $20.8 \pm 0.3 \mu\text{m}$ ; Mevenkamp et al., 2019). The  
556 specimen collected in the Peru Basin likely fed on diatom-derived phytodetritus ( $\frac{DHA}{EPA}$ -ratio<sub>body</sub>  
557  $_{wall} = 0.09$ ) and maybe on bacteria. The very high level of heterotrophic re-synthesis of amino  
558 acids and the high trophic level of *Oneirophanta* sp. points to the role of a secondary consumer  
559 of detritus, whereas a big biomass of microbial gut community serves as first consumers.  
560 However, bacteria-specific PLFAs C16:0, C16:1 $\omega$ 7cis, and C18:1 $\omega$ 7cis, that were detected in  
561 high concentrations in the gut content of *Benthodytes* sp., contribute only 5% to the total PLFA

562 composition in the gut content of *Oneirophanta* sp. Therefore, the diet preferences of this species  
563 in the Peru Basin is less clear.

564

565 Synallactidae gen sp. (**family Synallactidae**) has an estimated trophic level of  $3.0 \pm 1.5$  and a  
566 medium level of heterotrophic re-synthesis of amino acids ( $\sum V = 3.70 \pm 1.87$ ). It feeds extremely  
567 selectively ( $CF_{\text{gut content}} = 335$ ) and consumes particles of a median grain size ( $14.5 \mu\text{m}$ ) that is  
568 smaller than the median grain size of the surface sediment in the Peru Basin ( $20.8 \pm 0.3 \mu\text{m}$ ;  
569 Mevenkamp et al., 2019). The PLFA composition of the body wall and the gut content of  
570 Synallactidae gen sp. indicates that this species predares upon agglutinated foraminiferans ( $\frac{EPA}{ARA}$ -  
571  $\text{ratio}_{\text{body wall}} = 1.75 \pm 2.23$ ) and it consumes diatom-derived detritus ( $\frac{DHA}{EPA}$ - $\text{ratio}_{\text{body wall}} = 0.13 \pm 0.08$ ,  
572  $\frac{DHA}{EPA}$ - $\text{ratio}_{\text{gut content}} = 0.42$ , this study). However, it is not possible to differentiate whether  
573 Synallactidae gen sp. is a primary consumer of the phytodetritus or a secondary consumer,  
574 whereupon the foraminiferans are the primary consumer. The PLFA composition of the feces  
575 shows that this holothurian species is also a bacterivore as bacteria-specific PLFAs (i.e., C16:0,  
576 C16:1 $\omega$ 7cis, and C18:1 $\omega$ 7cis) contribute 42% to the total PLFA composition in the feces. If  
577 Synallactes hosts a large community of living bacteria, we would expect to detect a significant  
578 amount of bacteria-specific PLFAs in the gut content and a higher level of heterotrophic re-  
579 synthesis of amino acids. Therefore we assume that Synallactidae gen sp. has a mixed diet  
580 consisting of foraminiferans, bacteria, and phytodetritus.

581

582 *Galatheathuria* sp. from the **family Synallactidae** has an estimated trophic level of 3.1 and a  
583 medium level of heterotrophic re-synthesis of amino acids ( $\sum V = 3.50$ ). Similar to Synallactidae  
584 gen sp. it feeds extremely selectively ( $CF_{\text{gut content}} = 285$ ) and *Galatheathuria* sp. seems to  
585 consume preferably diatom-derived detritus ( $\frac{DHA}{EPA}$ - $\text{ratio}_{\text{body wall}} = 0.39$ ,  $\frac{DHA}{EPA}$ - $\text{ratio}_{\text{gut content}} = 0.18$ ).

586

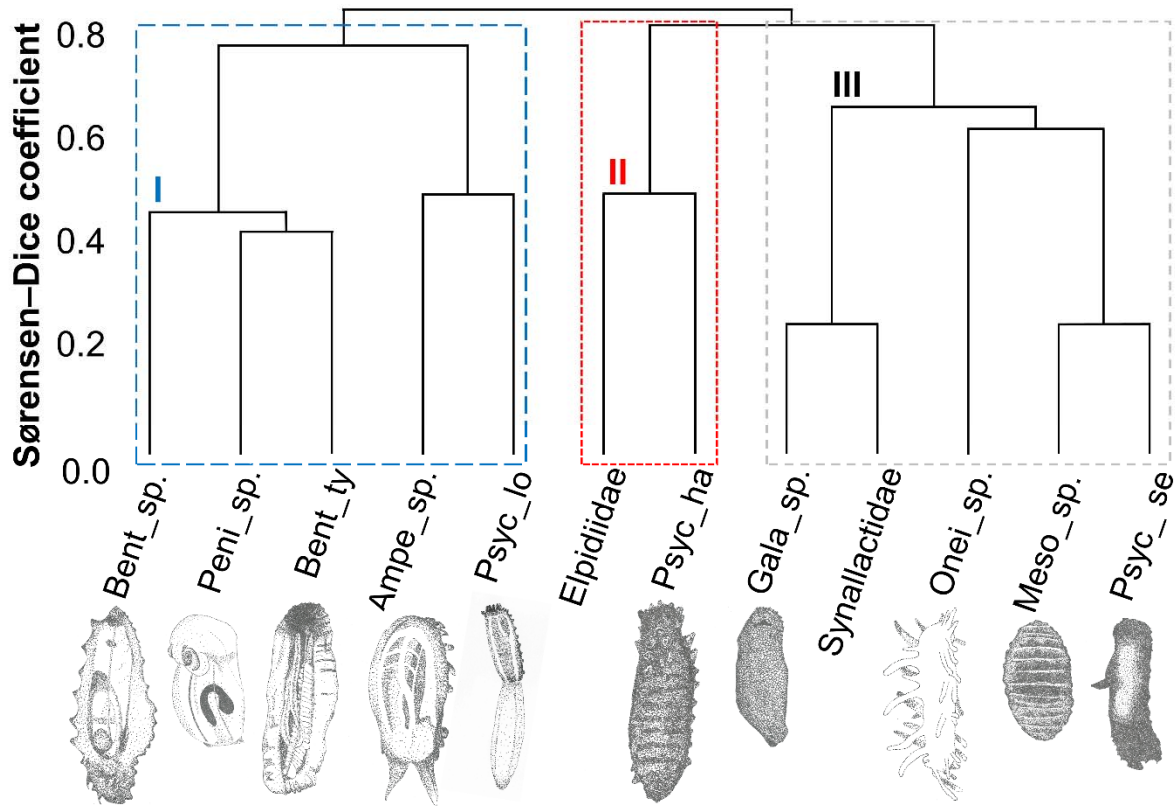
### 587 **Classification of holothurian trophic groups**

588 Here, we propose a classification system of trophic groups for holothurians from the Peru Basin  
589 (Fig. 6). It is based on cluster analysis of trophic levels, heterotrophic re-synthesis level of amino  
590 acids, feeding selectivity, and diet preferences, instead of on  $\delta^{15}\text{N}$  value of body wall tissue.

591 **Trophic group 1** has a trophic level between 2.7 and 3.0, a very diverse diet preference, and  
592 includes the species *Amperima* sp., *Benthodytes* sp., *B. typica*, *Peniagone* sp., and *Psychropotes*  
593 *longicauda*. **Trophic group 2** has a low trophic level of 2.0 to 2.4 and feeds selectively. It  
594 includes Elpidiidae gen sp. and *P. hanseni* and **trophic group 3** has a trophic level between 3.0  
595 and 3.5 with a mixed diet and diatom-derived phytodetritus-based diet. It consists of the species  
596 *Galatheathuria* sp., *Mesothuria* sp., *Oneirophanta* sp., *P. semperiana*, and Synallactidae gen sp.

597





598  
 599 **Figure 6.** Dendrogram of the Sørensen–Dice coefficient calculated for holothurian species from  
 600 the Peru Basin based. Trophic group I includes *Amperima* sp. (Ampe\_sp.), *Benthodytes* sp.  
 601 (*Bent\_sp.*), *Benthodytes typica* (*Bent\_ty*), *Peniagone* sp. (Peni\_sp.), and *Psychropotes*  
 602 *longicauda* (Psyc\_lo). Trophic group II comprises Elpidiidae gen sp. (Elpidiidae) and  
 603 *Psychronaetes hansenii* (Psyc\_ha), and trophic group III contains *Galatheathuria* sp. (Gala\_sp.),  
 604 *Mesothuria* sp. (Meso\_sp.), *Oneirophanta* sp. (Onei\_sp.), *Psychropotes semperiana* (Psyc\_se),  
 605 and Synallactidae gen sp. (Synallactidae).  
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613  
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