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

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Abstract

Holothurians are the dominant megabenthic deposit feeders in the Peru Basin (South-East Pacific) and feed to various degrees of selectively on the heterogenous pool of sedimentary detritus, but diet preferences for most holothurian species are unknown. This study reconstructs the diets of 13 holothurian species of the orders Elasipodida, Holothuriida, and Synallactida, from bulk stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$) of holothurian body walls and guts, gut contents, and feces that were combined with compound-specific stable isotope analyses of amino acids, phospholipid-derived fatty acids, and neutral lipid-derived fatty acids in the body wall. Fatty acid concentrations showed high levels of storage lipids, an likely adaption to limited food supply to abyssal plains. Amino acid $\delta^{15}N$ isotope values allowed estimating trophic levels of holothurian species and calculating heterotrophic re-synthesis of amino acids. Fatty acids served as trophic markers for feeding on diatom- and dinoflagellate derived phytodetritus, bacteria, Foraminifera, and detritus containing the PUFA C22:1ω9-*cis*. Several holothurian species seemed to be secondary consumers of detritus, while bacteria in their guts were primary consumers of this detritus. A Sørensen–Dice coefficient based cluster analysis using data of trophic levels, levels of heterotrophic re-synthesis of amino acids, feeding selectivity, and food sources/ diet suggested three trophic groups, characterized by different trophic levels. We show that this multi-biomarker driven approach allows to deconvolve trophic niches and feeding selectivity in one of the most challenging environments on earth and to identify dependence of deep-sea species to organic matter inputs that vary with season and/or climate.

Keywords: Echinodermata, NLFA, PLFA, sea cucumber, diet, deep sea

Statements and Declarations:

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Data availability: The datasets generated during and/or analysed during the current study are available in the PANGAEA repository, **XXX**.

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Introduction

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

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

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

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

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

Material and methods

Sampling of holothurians

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

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

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

17.09.2015	-7.082	-88.469	4136.3	*Benthodytes* sp. (n = 2), *B. typica* (n = 1), *Psychronaetes hanseni* (n = 1)
18.09.2015	-7.083	-88.470	4429.4	*Amperima* sp. (n = 1), Elpidiidae gen sp. (n = 1), *Psychropotes longicauda* (n = 1), *Synallactes* 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	*B. typica* (n = 2), *Peniagone* sp. (n = 1), *Psychropotes semperiana* (n = 1), *Galatheathuria* sp. (n = 1), Synallactidae gen sp. (n = 1), *Peniagone* sp. (n = 1)

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

Analysis of amino acids

Total hydrolysable amino acids (THAA) from holothurian tissue were extracted following a modified protocol of Veuger et al. (2005): Briefly, THAAs in holothurian tissue were hydrolyzed 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 vials. A N_2-headspace was created in the vials by flushing with N_2-gas for 10 sec before the vials were closed and heated for 20 h at 110°C. After cooling, 10 µL internal L-Norleucine standard per mg dry faunal tissue (stock solution: 2.5 mg mL^{-1} L-Norleucin acidified with 100 µL 12 M HCl) was added and the solution was evaporated under N_2-flow at 60°C. THAAs from holothurian tissue were derivatized by adding 0.5 ml acidified propan-2-ol to the sample and by heating the closed vials at 110°C for 90 min. Afterwards, the vials were cooled down and the

solution was evaporated under N_2-flow at 50°C. After evaporating all solution, 200 µL dichloromethane (DCM) was added and the solution was evaporated again. When the samples were dry, 150 µL DCM and 50 µL pentafluoropropionic anhydride were added, the vials were closed and heated for 10 min at 110°C. The solvent was extracted by adding 0.5 mL chlorophorm and 1 ml phosporus-buffer to the sample, shaking it until the lower chloroform fraction was clear and centrifuging the vials with 2,000 rpm for 10 min. The chloroform fraction was transferred to GC vials and evaporated again. When the sample was completely dry, it was dissolved in ethyl acetate. Concentrations (µg C g^{-1} dry mass DM holothurian tissue) and $\delta^{13}C$ (‰), and $\delta^{15}N$ (‰) of THAAs were measured with a HP 6890 gas chromatograph (Hewlet Packard/ Agilent, USA) coupled with a DELTA-Plus Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA) on a polar analytical column (ZB5-5MS; 60m length, 0.32mm diameter, 0.25µm film thickness; Phenomenex, USA).
A list with common abbreviation of amino acids and their full name is presented in Table 2.

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

Abbreviation	Full name
Amino acids	
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
Fatty acids	
ARA	Arachidonic acid (C20:4ω6)
DHA	Docosahexaenoic acid (C22:6ω3)
EPA	Eicosapentaenoic acid (C20:5ω3)

Analysis of fatty acids

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

120ºC for 2 h; Merck Kieselgel 60) via eluting with 7 ml chloroform, 7 ml acetone, and 15 ml methanol. The aceton fraction was discarded, whereas the chloroform fraction containing the NLFAs and the methanol fraction with the PLFAs were collected in separate test tubes and evaporated to dryness.

PLFAs and NLFAs were derivatized to fatty acid methyl esters (FAMEs) by adding 1 ml methanol-toluene mix (1:1 volume/ volume), 20 µl of an internal standard (1 mg 19:0 FAME mL^{-1}), and 1 ml 0.2 M metanolic NaOH to the test tubes with the PLFAs and NLFAs extracts. After an incubation at 37ºC for 15 min, 2 ml *n*-hexane, 0.3 ml 1 M acetic acid, and 2 ml MilliQ-water were added. The solution was mixed very well and when the layers had separated, the (top) *n*-hexane layer was transferred to new test tubes. Additional 2 ml *n*-hexane were added to the previously used test tubes that contained the acetic acid-MilliQ-water solution, and the step was repeated. The *n*-hexane layer was transferred again to the new test tubes and 20 µl of a second internal standard (1 mg 12:0 FAME mL^{-1}) were added. *n*-hexane was evaporated completely and the FAMEs dissolved in 200 µl *n*-hexane were transferred to measuring vials.

The FAMEs from holothurian tissues were separated on a BPX70 column (50 m length, 0.32 mm inner diameter, 0.25 µm film thickness; SGE Analytical Science) with a HP 6890 gas chromatograph (GC; Hewlet Packard/ Agilent, USA). The FAMEs from feces and gut content were separated on a ZB5-5MS column (60 m length, 0.32 mm diameter, 0.25 µm film thickness; Phenomenex, USA) on the same GC. Concentrations (µg C g^{-1} DM holothurian tissue) and $\delta^{13}C$ values (‰) of FAMEs in holothurian tissue, feces, and gut content were measured on a Finnigan Delta Plus isotope ratio mass spectrometer (IRMS; Thermo Fisher Scientific, USA) coupled to the GC via a combustion GC-c-III interface (Thermo Fisher Scientific, USA). Identification of peaks of the FAME chromatogram were based on equivalent chain length (ECL) and peak areas were calculated using the two internal standards (12:0 and 19:0) for area correction.

A list with abbreviations and full names of several important fatty acids is presented in Table 2 and Table 3 contains dominant biomarkers.

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

Fatty acid	Main sources of fatty acid	Reference
Organic matter		
C18:1ω9	Highly degraded carrion-derived organic matter	(Graeve et al. 2001)
Bacteria		
i-C14:0, *i*-C15:0, *ai*-C15:0, *i*-C16:0, and C18:1ω7*cis*; C18:2ω6	Marine bacteria; gram-positive bactéria; piezotolerant bacteria	(Findlay et al. 1990; Middelburg et al. 2000; Wang et al. 2014)
10-Me-C16:0, *ai*-C17:0, *i*-C17:0, and cy-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)
Primary producers		
C16:4ω1, C16:1ω7, and EPA; $\frac{C16:1\omega7}{C16:0} > 1$ or $\frac{DHA}{EPA} < 1$	Diatoms	(Kelly and Scheibling 2012)

C18:4ω3 and DHA; $\frac{C16:1\omega7}{C16:0}$ <1 or $\frac{DHA}{EPA}$ > 1	Dinoflagellates	(Kelly and Scheibling 2012)
C18:1ω9, C18:4ω3, and ARA	Kelp	(Kelly and Scheibling 2012)
C16:0, EPA; ARA	Red macroalgae	(Bühring et al. 2002; Kelly and Scheibling 2012)
C18:1ω9, C18:4ω3, and ARA; C16:1ω5	Brown macroalgae	(Khotimchenko 1995; Kelly and Scheibling 2012)
C18:2ω6 and C18:3ω3	Seagrass	(Kelly and Scheibling 2012)
Consumers		
C20:1ω9, C22:1ω11	Calanoid copepods	(Falk-Petersen et al. 1987; Dalsgaard et al. 2003)
EPA, DHA	Hydrothermal vent bivalves	(Ben-Mlih et al. 1992)
ARA; C22:5ω5; $\frac{EPA}{ARA}$-ratio	Agglutinated foraminifera	(Larkin et al. 2014; Kharlamenko 2018)

Calculations

Concentration factors

To examine the degree to which PLFAs were concentrated between surface sediment (0 – 2cm layer; 2.32±0.51 µg C-PLFA g^{-1} DM sediment; Stratmann, unpublished) and gut content and feces, a concentration factor *CF* was calculated:

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

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

where [gut content$_{PLFA}$] corresponds to the total PLFA concentration in gut content, [feces$_{PLFA}$] to the total PLFA concentration in feces, and $[\overline{sediment_{PLFA}}]$ to the average total PLFA concentration in surface sediment.

Trophic levels

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

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

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

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

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

where σ_β is 0.9‰ and σ_Δ is 1.1‰ (Jarman et al. 2017).

Heterotrophic re-synthesis of amino acids

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

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

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

Statistics

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

Table 4. Parameters used to calculate the Sørensen–Dice coefficient β_{sor} presented as quantitative data (ranges) and categorical data. 'Food sources/ diet' includes a list of the main food sources of the investigated holothurian species which were identified by amino acid and fatty acid analysis.

Parameter	Quantitative data	Categorical data
Trophic level (*TL*)	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 *CF*	*CF* = >50–150	very selective
	CF = >150	extremely selective
Food sources/ diet		– diatom-derived phytodetritus – dinoflagellate-derived phytodetritus – detritus containing C22:1ω9-*cis* – secondary consumer of detritus – mix diet (phytodetritus primary consumer, detritus secondary consumer) – mixed diet (phytodetritus, bacteria) – mixed diet (phytodetritus, bacteria, Foraminifera)

Results

Gut content and feces of holothurians

Gut contents of holothurians in the Peru Basin weighed 1.93±3.56 g dry sediment (n = 17) and ranged from 0.11 g dry sediment for *Peniagone* sp. (n = 1) to 12.5 g dry sediment for *P. hanseni* (n = 1; Table 5). Org. C and TN content of the gut content was 5.34±4.13% (n = 17) and 1.04±0.87% (n = 17), respectively, and it contained 244±304 µg C-PLFA g^{-1} DM gut content (n = 8) and 83.3±124 µg C-NLFA g^{-1} DM gut content (n = 10) (Fig. 1a). The concentration factor *CF* for PLFAs in holothurian gut content was on average 105±131 (n = 8) and ranged from 1.17 to 335 for *Benthodytes* sp. (n = 1) and Synallactidae gen sp. (n = 1), respectively (Table 5). The average EPA/ARA-ratio for gut content was 3.20±5.58 (n = 9), the average DHA/EPA-ratio was 0.62±0.68 (n = 9), and the average C16:1ω7/C16:0-ratio was 0.81±0.85 (n = 9) (Fig. 2a).

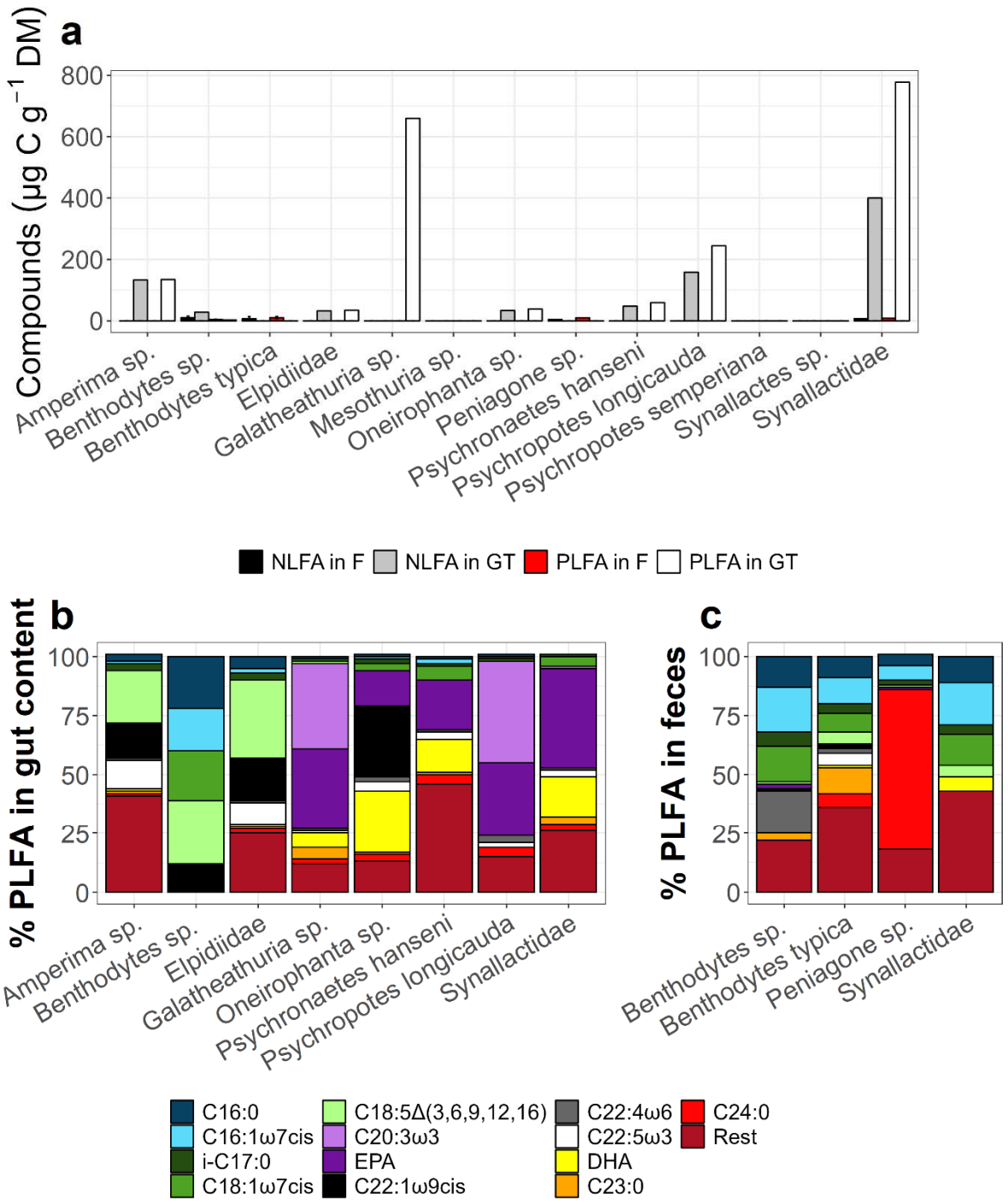


Figure 1. (a) Concentrations (µg C g^{-1} DM. C) of the compounds PLFA and NLFA in holothurian gut content (GT) and feces (F) and the contribution (%) of individual (b, c) PLFAs and (d, e) NLFAs to the total concentrations. The PLFA and NLFA pools 'Rest' include all PLFAs and NLFAs, respectively, that contribute <2.5% to total % PLFA and NLFA of the average holothurian gut content/ feces.
Error bars in (a) indicate SD.

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		
		% org. C;	% TN	Concentration factor $CF_{gut\ content}$, CF_{feces}
Family: Elpidiidae				
Amperima 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
Peniagone 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
Family: Deimatidae				
Oneirophanta sp. (GT: n = 1)	3.90	2.84	0.51	16.6
Family: Laetmogonidae				
Psychronaetes hanseni (GT: n = 1)	12.5	1.94	0.38	25.6
Family: Psychropotidae				
Benthodytes 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
Benthodytes typica (F: n = 4; sediment grain size: n = 1)	0.85±0.62	1.05±0.47	0.16±0.01	4.44±1.52
Psychropotes longicauda (GT: n = 1)	1.08	1.38	0.29	105
Psychropotes semperiana (GT: n = 1)	1.78	5.25	0.80	
Family: Synallactidae				
Synallactes sp. (morphotype "pink") (GT: n = 1)	6.75	2.22	0.34	
Galatheathuria sp. (GT: n = 1)	0.51	11.4	2.10	285
Synallactidae	GT: 0.14	GT: 11.3	GT: 2.44	GT: 335

gen sp. (n = 1)	F: 0.12	F: 0.84	F: 0.17

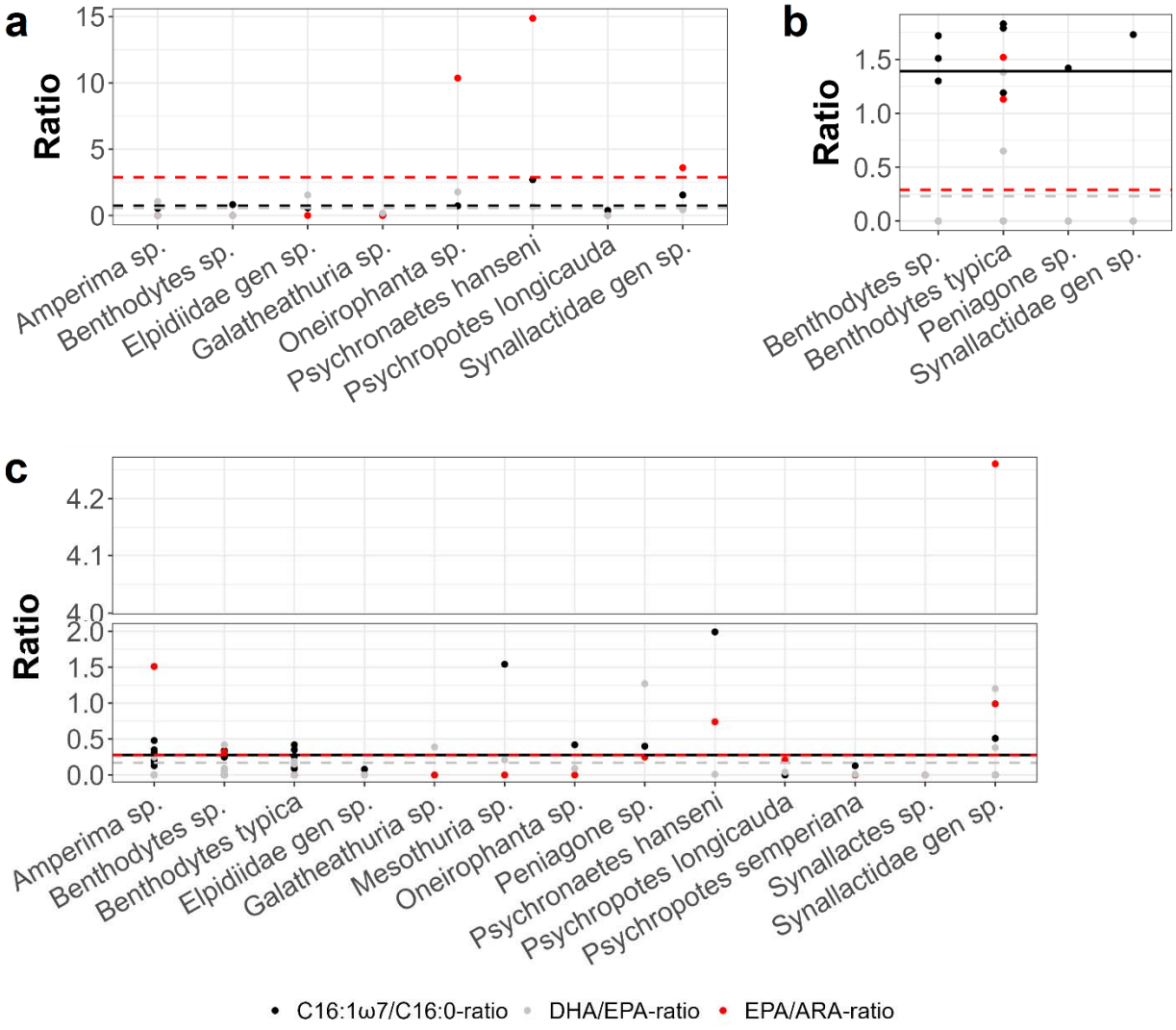


Figure 2. Ratios of C16:1ω7/C16:0, DHA/EPA, and EPA/ARA in (a) holothurian gut content, (b) holothurian feces, and (c) dried holothurian body walls. Horizontal lines show the average value of a ratio based on all samples.

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

Gut content and feces consisted to 81.2±%3.73% of silt (grain size: <63 µm; n = 6) and to 10.7±1.10% of very fine sand (grain size: 62.5 – 125 µm) (Table 6). The median grain was 15.5±2.27 µm (n = 6).

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

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

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

Chemical composition of holothurians

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

Table 7. Chemical composition of body wall (BW) and gut tissue (GT) of different holothurian species collected in the Peru Basin. Data are presented as mean±SD.

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

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

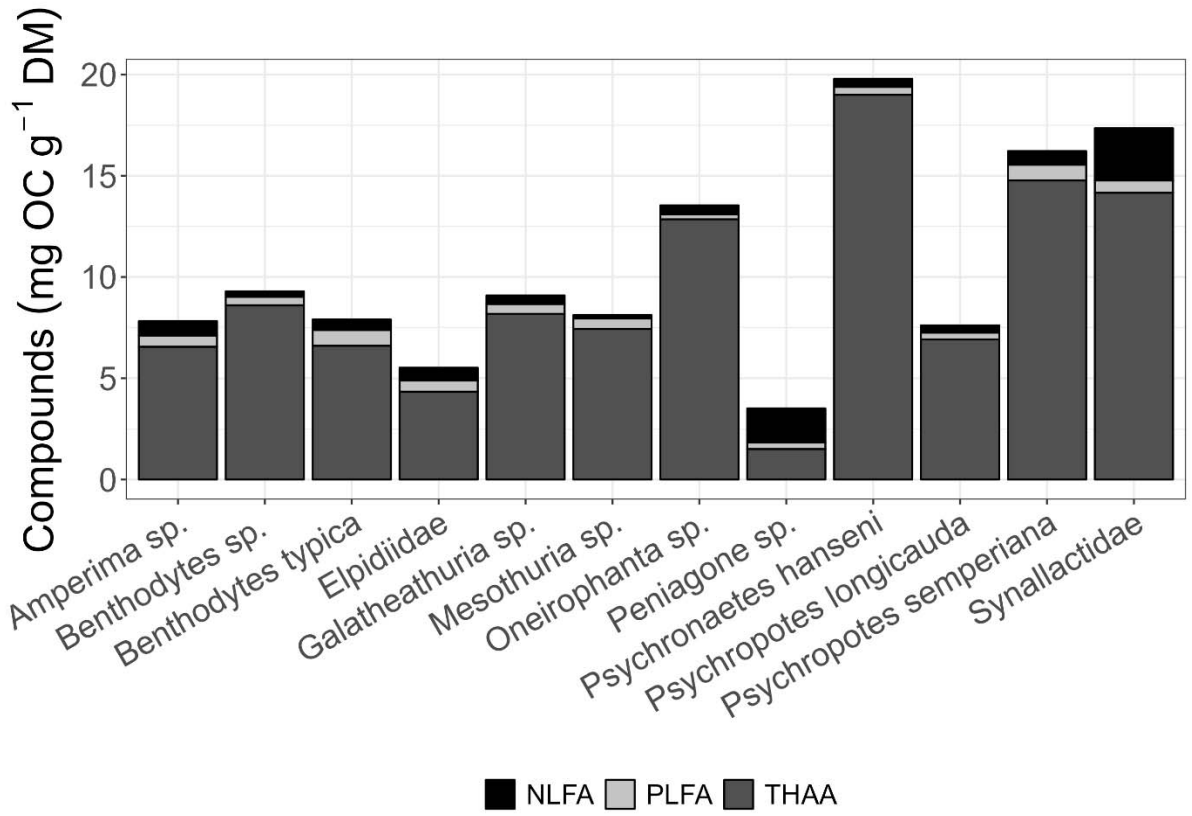


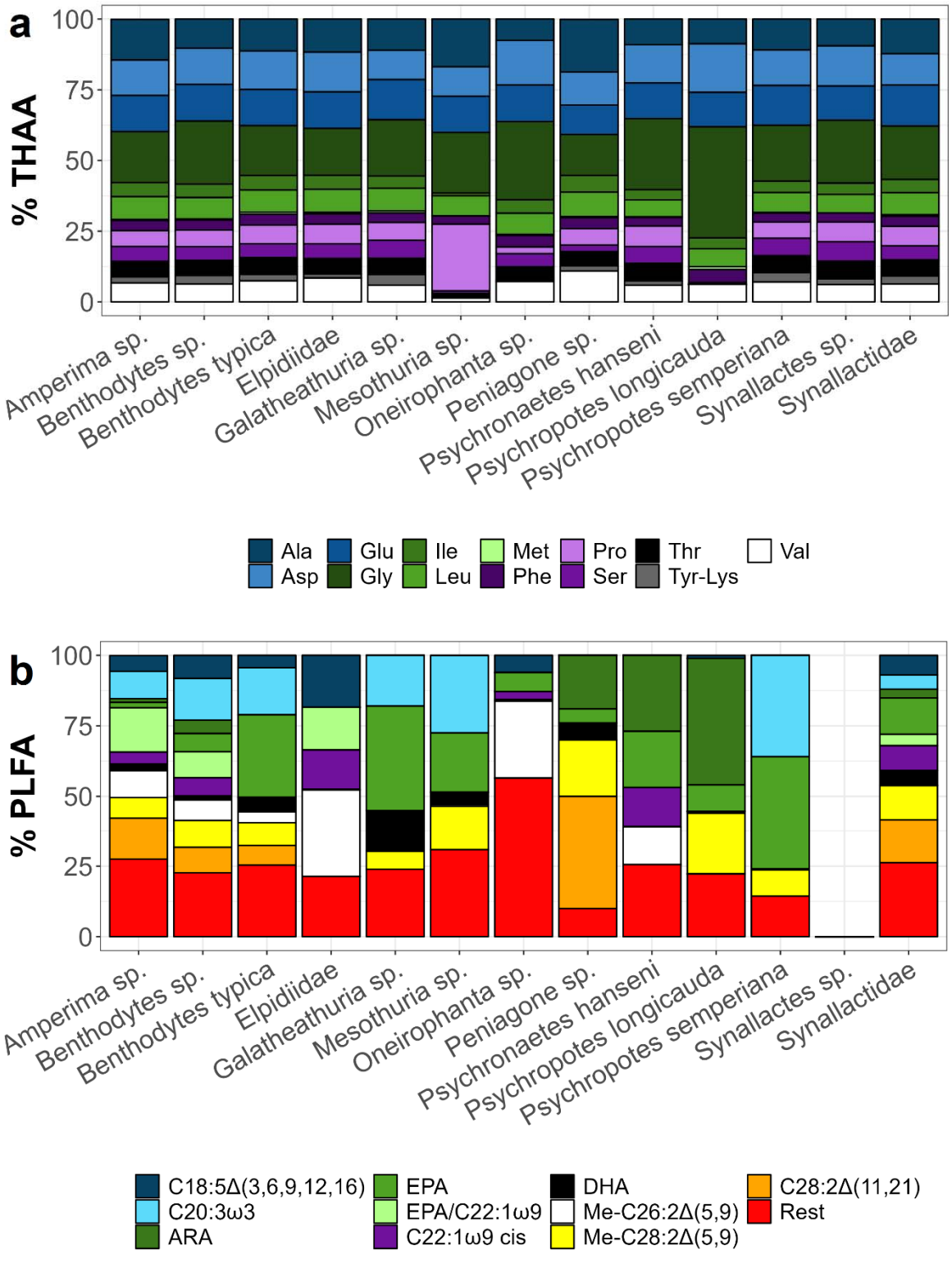
Figure 3. The compounds neutral lipid-derived fatty acids (NLFA), phospholipid-derived fatty acids (PLFA), and total hydrolysable amino acids (THAA) in dried holothurian body walls.

The THAA composition did not differ greatly among species, with the main THAAs in holothurian body walls being alanine, glycine, aspartic acid, and glutamic acid, that contributed between 55.2% (Elpidiidae; n = 1) and 77.4% (*P. longicauda*; n = 1) to total THAAs of body walls (Fig. 4a).

In contrast, the PLFA (Fig. 4b) and NLFA (Fig. 4c) composition differed strongly between species. Between 10.2% (*Peniagone* sp.; n = 1) and 56.5% (*Oneirophanta* sp.; n = 1) of the PLFAs found in holothurian body walls contributed <2.5% to the total PLFA concentration and were combined as 'Rest' (Fig. 4b). The remaining PLFAs consisted to 4.07±7.57% (n = 32) of MUFAs, 10.8±12.6% PUFAs, 32.2±14.1% HUFAs, 9.17±14.0% LCFAs, and 15.5±10.1% methyl-fatty acids. Compared to the average PLFA composition across all holothurian taxa analyzed, Elpidiidae gen sp. (n = 1) had an above average percentage of MUFAs (12.8% of total PLFAs) and methyl-fatty acids (48.1% of total PLFAs). *Oneirophanta* sp. (n = 1) had an above average percentage of SFAs (41.1% of total PLFAs) and *P. longicauda* (n = 1) had an above average percentage of HUFAs (10.0% of total PLFAs).

The NLFAs consisted for 21.6±10.4% (n = 30) of SFA, 10.1±5.80% MUFAs, 5.36±5.03% HUFAs, and 28.5±18.4% methyl-fatty acids (Fig. 4c). Between 13.8% Elpidiidae gen sp. (n = 1) and 69.6% (*Peniagone* sp.; n = 1) of the total NLFAs in holothurian body walls consisted of NLFAs that individually contributed <2.5% to the total NLFA concentration and were therefore combined as 'Rest'. In comparison to the average NLFA composition across all studied

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



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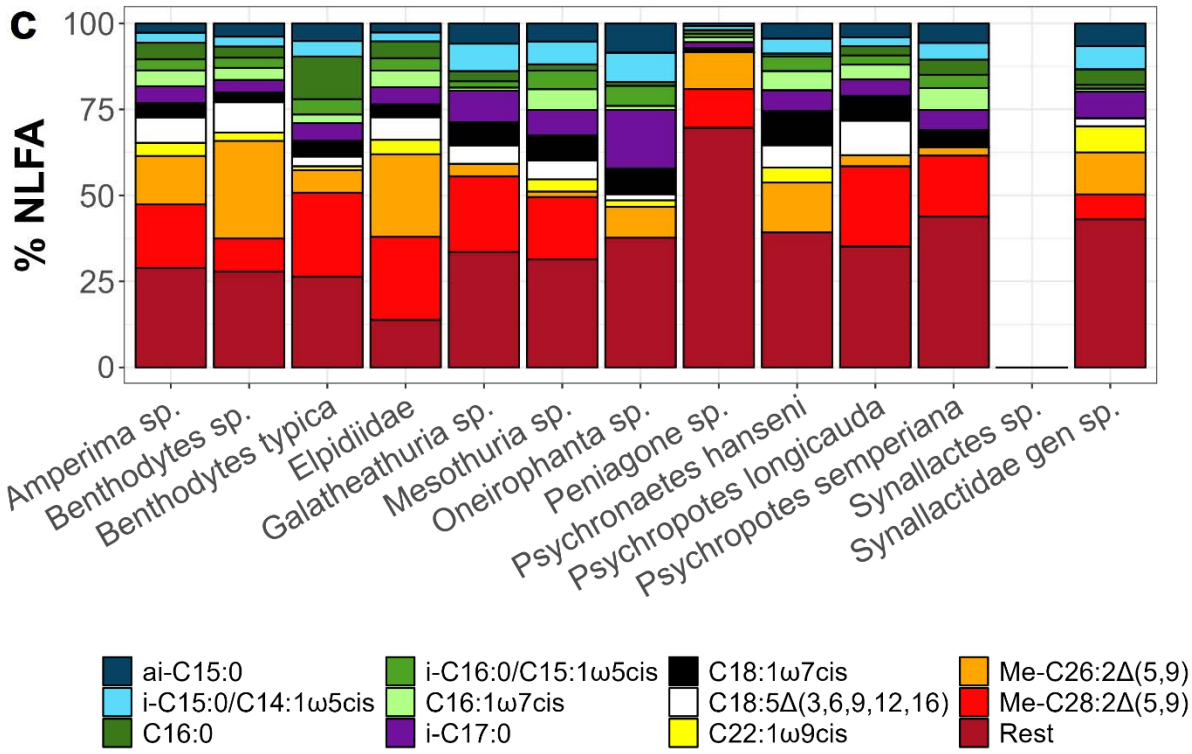

Figure 4. Contribution (%) of individual (a) THAAs, (b) PLFAs, and (c) NLFAs to the total concentrations. The PLFA and NLFA pools 'Rest' include all PLFAs and NLFAs, respectively, that contribute <2.5% to total % PLFA and NLFA of the average holothurian tissue.

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

Trophic position of holothurians and recycling of amino acids

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

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

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

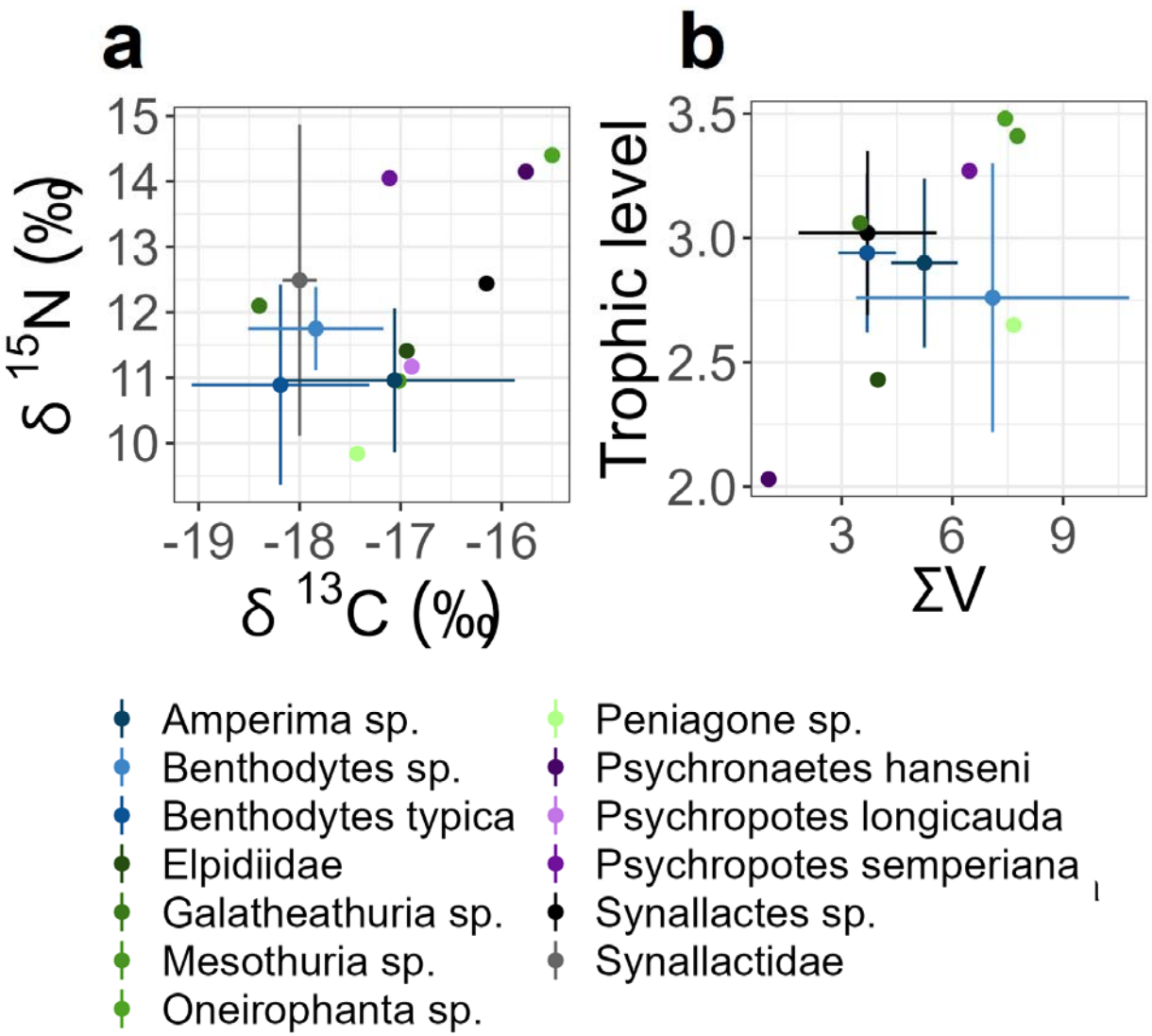

Figure 5. (a) Isotopic composition of carbon ($\delta^{13}C$, ‰) and nitrogen ($\delta^{15}N$, ‰) of holothurian body wall tissue from the Peru Basin. (b) Trophic position and heterotrophic enrichment factor of holothurians. Error bars in indicate SD.

Discussion

Fatty acid composition of holothurians

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

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

Holothurians trophic level and inferred feeding strategy

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

Order Elasipodida

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

Elpidiidae gen sp. (**family Elpidiidae**) has a trophic level of 2.4 and medium level of heterotrophic re-synthesis of amino acids ($\sum V$ = 3.98). This species is a selective deposit feeder ($CF_{gut\ content}$ = 14.9) that preferentially feeds upon the PUFA C22:1ω9*cis* which is present in high percentages in its gut content (18%) and in its body tissue (29.0%).

The bentho-pelagic *Peniagone* sp. of the **family Elpidiidae** has an estimated trophic level of 2.7 and a very high level of heterotrophic re-synthesis of amino acids ($\sum V$ = 7.66). This species has a 'sweeping' feeding style (Roberts et al. 2000) and assimilates fresh phytodetritus (Iken et al. 2001) with medium efficiency, as the PLFA concentration in its feces (CF_{feces}) is four times higher than in the surface sediment (this study). In the Peru Basin, *Peniagone* sp. seems to feed on diatom-derived phytodetritus ($\frac{C16:1\omega7}{C16:0}$ -ratio$_{\text{feces}}$ = 1.42; this study).

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

concentrations of chlorophyll-*a* compared to surface sediment or phytodetritus (FitzGeorge-Balfour et al. 2010). A more detailed analysis of the phytopigments in this gut content revealed that *A. rosea* at PAP feeds preferentially on cyanobacteria-derived phytodetritus (Wigham et al. 2003). Based on the PLFA composition of its gut content, we found that *Amperima* sp. from the Peru Basin likely feeds on dinoflagellate-derived phytodetritus ($\frac{C16:1\omega7}{C16:0}$ -ratio$_{\text{gut content}}$ = 0.53; this study). Also the body wall fatty acid composition in our study differs substantially from specimens from PAP, as the PLFA profile of PAP specimens is dominated by EPA, DHA, ARA, and C18:0 (Hudson et al. 2004), whereas the PLFA profile of Peru Basin specimens is characterized mostly by EPA co-eluted with C22:1ω9, Me-C26:2Δ(5,9), and C28:2Δ(11,21). Hence, it seems that the feeding niche of the well-studied *Amperima* sp. can differ substantially between ocean basins.

Benthodytes sp. from the **family Psychropotidae** has an estimated trophic level of 2.8±0.5 and a very high level of heterotrophic re-synthesis of amino acids ($\sum V$ = 7.09±3.70). It feeds with a 'sweeper' feeding style (Roberts et al. 2000) selectively on smaller sediment particles (medium grain size:13.0 µm) from the surface sediment (medium grain size: 20.8±0.3 µm; Mevenkamp et al., 2019). However, it likely does not or only moderately selects for specifically detritus-enriched particles ($CF_{gut\ content}$ = 1.17; CF_{feces} = 1.81±0.53). In fact, the high percentage of the bacteria-biomarker PLFAs C16:0, C16:1ω7*cis*, and C18:1ω7*cis* in its gut content and feces, and the very high level of heterotrophic re-synthesis of amino acids indicates *Benthodytes* sp. might host a large biomass of living heterotrophic prokaryotes. Unfortunately, in this study no amino acids from gut content or feces were extracted to assess whether this species concentrates detritus that is highly enriched in amino acids. Such an observation was interpreted by Romero-Romero et al., (2021) as a sign that deep-sea holothurians from Station M are secondary consumers of detritus, whereas the microbial community in their guts are primary consumers of detritus. Therefore, we hypothesize that also *Benthodytes* sp. is a secondary consumer, and its microbial gut community is the primary consumer of detritus.

Benthodytes typica belongs to the **family Psychropotidae** and its trophic level is estimated to be a bit higher (2.9) than the trophic level of *Benthodytes* sp. This species has a medium level of heterotrophic re-synthesis of amino acids ($\sum V$ = 3.69) and feeds selectively on smaller particles (medium grain size: 14.2 µm) from the ambient sediment (20.8±0.3 µm; Mevenkamp et al., 2019). These smaller particles contain an at least four times higher concentration of PLFAs than the surrounding sediment (CF_{feces} = 4.44±1.52) and consist partially of diatom-derived phytodetritus ($\frac{C16:1\omega7}{C16:0}$-ratio$_{\text{feces}}$ = 1.20±0.85). Reliance on phytodetritus is confirmed by the PLFA composition of *B. typica* body walls ($\frac{DHA}{EPA}$-ratio$_{\text{body wall}}$ = 0.13±0.08). In addition, this species either feeds selectively on sediment-bound prokaryotes or hosts prokaryotes as bacteria-specific PLFAs (i.e., C16:0, C16:1ω7*cis*, and C18:1ω7*cis*) contribute almost 30% to the total PLFA composition in feces, but were not detected in the body wall with >2.5% of total PLFAs. As a medium level of heterotrophic re-synthesis of amino acids was measured, *B. typica* likely has a mixed diet. In this diet, this holothurian species consumes phytodetritus as primary consumer and other types of detritus as secondary consumer following primary processing by a bacterial gut community.

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

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

Order Holothuriida

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

Order Synallactida

Oneirophanta sp. as member of the **family Deimatidae** has an estimated trophic level of 3.5 and a very high level of heterotrophic re-synthesis of amino acids ($\sum V$ = 7.43). This species feeds selectively ($CF_{gut\ content}$ = 16.6) with a 'raker' feeding style (Roberts et al. 2000) and takes up particles with a median grain size of 19.0 µm, which is slightly smaller than the median grain size of sediment particles in the Peru Basin (20.8±0.3 µm; Mevenkamp et al., 2019). The specimen collected in the Peru Basin likely fed on diatom-derived phytodetritus ($\frac{DHA}{EPA}$-ratio$_{\text{body wall}}$ = 0.09) and maybe on bacteria. The very high level of heterotrophic re-synthesis of amino acids and the high trophic level of *Oneirophanta* sp. points to the role of a secondary consumer of detritus, whereas a big biomass of microbial gut community serves as first consumers. However, bacteria-specific PLFAs C16:0, C16:1ω7*cis*, and C18:1ω7*cis*, that were detected in 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±1.5 and a
566 medium level of heterotrophic re-synthesis of amino acids ($\sum V$ = 3.70±1.87). It feeds extremely
567 selectively ($CF_{gut\ content}$ = 335) and consumes particles of a median grain size (14.5 µm) that is
568 smaller than the median grain size of the surface sediment in the Peru Basin (20.8±0.3 µm;
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 predates upon agglutinated foraminiferans ($\frac{EPA}{ARA}$-
571 ratio$_{\text{body wall}}$ = 1.75±2.23) and it consumes diatom-derived detritus ($\frac{DHA}{EPA}$-ratio$_{\text{body wall}}$ = 0.13±0.08,
572 $\frac{DHA}{EPA}$-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 C16:1ω7*cis*, and C18:1ω7*cis*) 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 ($CF_{gut\ content}$ = 285) and *Galatheathuria* sp. seems to
585 consume preferably diatom-derived detritus ($\frac{DHA}{EPA}$-ratio$_{\text{body wall}}$ = 0.39, $\frac{DHA}{EPA}$-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 δ^{15}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

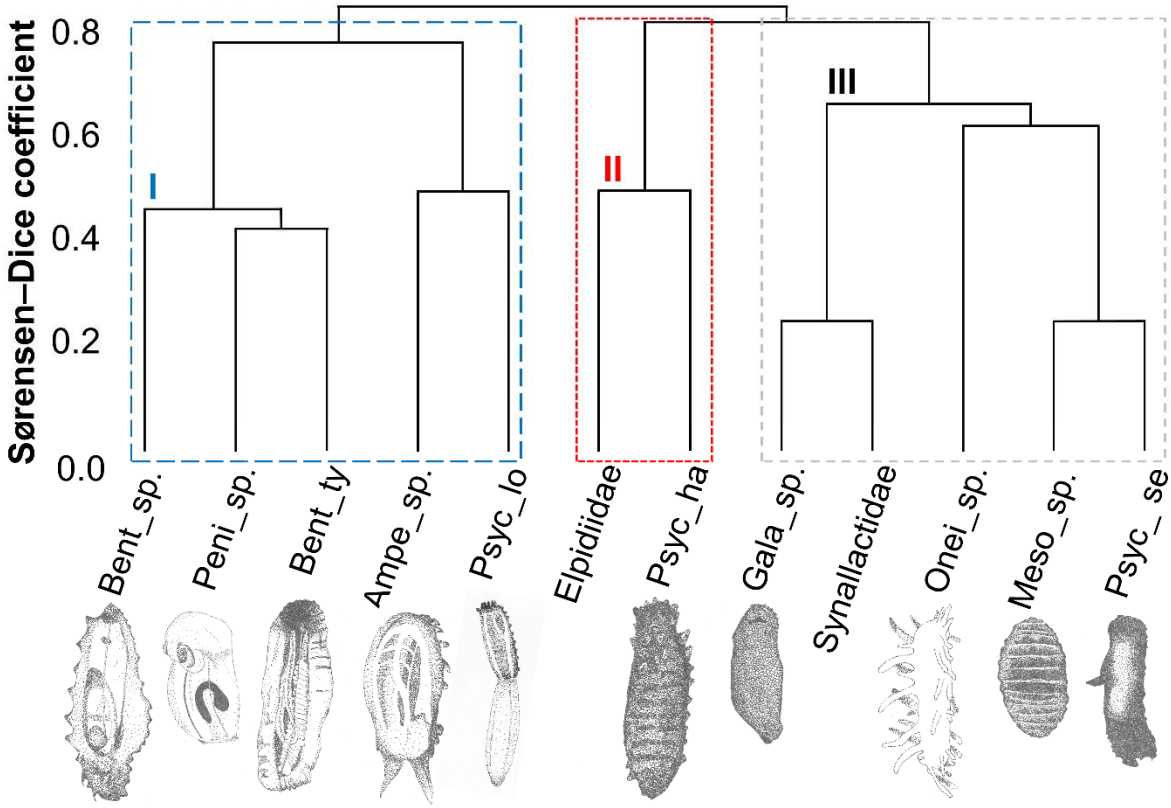

Figure 6. Dendrogram of the Sørensen–Dice coefficient calculated for holothurian species from the Peru Basin based. Trophic group I includes *Amperima* sp. (Ampe_sp.), *Benthodytes* sp. (Bent_sp.), *Benthodytes typica* (Bent_ty), *Peniagone* sp. (Peni_sp.), and *Psychropotes longicauda* (Psyc_lo). Trophic group II comprises Elpidiidae gen sp. (Elpidiidae) and *Psychronaetes hanseni* (Psyc_ha), and trophic group III contains *Galatheathuria* sp. (Gala_sp.), *Mesothuria* sp. (Meso_sp.), *Oneirophanta* sp. (Onei_sp.), *Psychropotes semperiana* (Psyc_se), and Synallactidae gen sp. (Synallactidae).
Illustrations of holothurians by Tanja Stratmann.

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References

Alt CHS, Rogacheva A, Boorman B, et al (2013) Trawled megafaunal invertebrate assemblages from bathyal depth of the Mid-Atlantic Ridge (48°-54°N). Deep-Sea Research II 98:326–340. https://doi.org/10.1016/j.dsr2.2013.02.003

Baldwin RJ, Glatts RC, Smith KL (1998) Particulate matter fluxes into the benthic boundary layer at a long time-series station in the abyssal NE Pacific: composition and fluxes. Deep-Sea Research II 45:643–665

621 Ben-Mlih F, Marty JC, Fiala-Medioni A (1992) Fatty acid composition in deep hydrothermal vent
622 symbiotic bivalves. J Lipid Res 33:1797–1806. https://doi.org/10.1016/s0022-2275(20)41337-9

623 Billett DSM (1991) Deep-sea holothurians. Oceanography and Marine Biology 29:259–317

624 Billett DSM, Bett BJ, Reid WDK, et al (2010) Long-term change in the abyssal NE Atlantic: The "Amperima
625 Event" revisited. Deep-Sea Research II 57:1406–1417. https://doi.org/10.1016/j.dsr2.2009.02.001

626 Billett DSM, Bett BJ, Rice AL, et al (2001) Long-term change in the megabenthos of the Porcupine
627 Abyssal Plain (NE Atlantic). Prog Oceanogr 50:325–348. https://doi.org/10.1016/S0079-
628 6611(01)00060-X

629 Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol
630 37:911–917. https://doi.org/10.1139/o59-099

631 Boschker H (2008) Linking microbial community structure and functioning: Stable isotope (13C) labeling
632 in combination with PLFA analysis. Molecular Microbial Ecology Manual II 1673–1688.
633 https://doi.org/10.1007/978-1-4020-2177-0_807

634 Brown A, Hauton C, Stratmann T, et al (2018) Metabolic rates are significantly lower in abyssal
635 Holothuroidea than in shallow-water Holothuroidea. R Soc Open Sci 5:172162.
636 https://doi.org/10.1098/rsos.172162

637 Bühring SI, Koppelmann R, Christiansen B, Weikert H (2002) Are Rhodophyceae a dietary component for
638 deep-sea holothurians? Journal of the Marine Biological Association of the United Kingdom
639 82:347–348. https://doi.org/10.1017/S0025315402005556

640 Burdge GC, Calder PC (2014) Introduction to fatty acids and lipids. In: World Review of Nutrition and
641 Dietetics. pp 1–16

642 Chikaraishi Y, Ogawa NO, Kashiyama Y, et al (2009) Determination of aquatic food-web structure based
643 on compound-specific nitrogen isotopic composition of amino acids. Limnol Oceanogr Methods
644 7:740–750. https://doi.org/10.4319/lom.2009.7.740

645 Choi A, Song J, Joung Y, et al (2015) Lentisphaera profundi sp. nov., Isolated from deep-sea water. Int J
646 Syst Evol Microbiol 65:4186–4190. https://doi.org/10.1099/ijsem.0.000556

647 Dalsgaard J, st. John M, Kattner G, et al (2003) Fatty acid trophic markers in the pelagic marine
648 environment. Adv Mar Biol 46:225–340. https://doi.org/10.1016/S0065-2881(03)46005-7

649 Dice LR (1945) Measures of the amount of ecological association between species. Ecology 26:297–302

650 Drazen J, Phleger C, Guest M, Nichols P (2008a) Lipid, sterols and fatty acids of abyssal polychaetes,
651 crustaceans, and a cnidarian from the northeast Pacific Ocean: food web implications. Mar Ecol
652 Prog Ser 372:157–167. https://doi.org/10.3354/meps07707

653 Drazen JC, Phleger CF, Guest MA, Nichols PD (2008b) Lipid, sterols and fatty acid composition of abyssal
654 holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications.
655 Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 151:79–87.
656 https://doi.org/10.1016/j.cbpb.2008.05.013

657 Drazen JC, Phleger CF, Guest MA, Nichols PD (2008c) Lipid, sterols and fatty acid composition of abyssal
658 holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications. Comp
659 Biochem Physiol B Biochem Mol Biol 151:79–87. https://doi.org/10.1016/j.cbpb.2008.05.013

660 Elvert M, Boetius A, Knittel K, Barker Jørgensen BO (2003) Characterization of Specific Membrane Fatty
661 Acids as Chemotaxonomic Markers for Sulfate-Reducing Bacteria Involved in Anaerobic Oxidation
662 of Methane. https://doi.org/10.1080/01490450390241071

663 Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the Sub-
664 Arctic food web. Polar Biol 8:115–120

665 Findlay RH, Trexler MB, Guckerte JB, White DC (1990) Laboratory study of disturbance in marine
666 sediments: response of a microbial community. Mar Ecol Prog Ser 62:121–133

667 FitzGeorge-Balfour T, Billett DSM, Wolff GA, et al (2010) Phytopigments as biomarkers of selectivity in
668 abyssal holothurians; interspecific differences in response to a changing food supply. Deep-Sea
669 Research II 57:1418–1428. https://doi.org/10.1016/j.dsr2.2010.01.013

670 Ginger ML, Santos VLCS, Wolff GA (2000) A preliminary investigation of the lipids of abyssal holothurians
671 from the north-east Atlantic Ocean. Journal of the Marine Biological Association of the UK 80:139–
672 146. https://doi.org/10.1017/s0025315499001654

673 Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: A
674 penetrating approach to estimate feeding modes of Antarctic amphipods. Polar Biol 24:853–862.
675 https://doi.org/10.1007/s003000100295

676 Haeckel M, König I, Riech V, et al (2001) Pore water profiles and numerical modelling of biogeochemical
677 processes in Peru Basin deep-sea sediments. Deep-Sea Research I 48:3713–3736

678 Hudson IR, Pond DW, Billett DSM, et al (2004) Temporal variations in fatty acid composition of deep-sea
679 holothurians: evidence of bentho-pelagic coupling. Mar Ecol Prog Ser 281:109–120

680 Hudson IR, Wigham BD, Solan M, Rosenberg R (2005) Feeding behaviour of deep-sea dwelling
681 holothurians: Inferences from a laboratory investigation of shallow fjordic species. Journal of
682 Marine Systems 57:201–218. https://doi.org/10.1016/j.jmarsys.2005.02.004

683 Iken K, Brey T, Wand U, et al (2001) Food web structure of the benthic community at the Porcupine
684 Abyssal Plain (NE Atlantic): a stable isotope analysis. Prog Oceanogr 50:383–405

685 Jarman CL, Larsen T, Hunt T, et al (2017) Diet of the prehistoric population of Rapa Nui (Easter Island,
686 Chile) shows environmental adaptation and resilience. Am J Phys Anthropol 164:343–361.
687 https://doi.org/10.1002/ajpa.23273

688 Kassambara A, Mundt F (2020) factoextra: Extract and Visualize the Results of Multivariate Data
689 Analyses

690 Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser
691 446:1–22. https://doi.org/10.3354/meps09559

692 Kharlamenko VI (2018) Abyssal foraminifera as the main source of rare and new polyunsaturated fatty
693 acids in deep-sea ecosystems. Deep-Sea Research II 154:358–364.
694 https://doi.org/10.1016/j.dsr2.2017.10.015

695 Khotimchenko S v (1995) Uncommon 16:1(n-5) acid from Dictyota dichotoma and fatty acids of some
696 brown algae of Dictyotaceae. Phytochemistry 38:1411–1415

697 Koleff P, Gaston KJ, Lennon JJ (2003) Measuring beta diversity for presence-absence data. Journal of
698 Animal Ecology 72:367–382. https://doi.org/10.1046/j.1365-2656.2003.00710.x

699 Larkin KE, Gooday AJ, Woulds C, et al (2014) Uptake of algal carbon and the likely synthesis of an
700 "essential" fatty acid by Uvigerina ex. gr. semiornata (Foraminifera) within the Pakistan margin
701 oxygen minimum zone: Evidence from fatty acid biomarker and 13C tracer experiments.
702 Biogeosciences 11:3729–3738. https://doi.org/10.5194/bg-11-3729-2014

703 Libes S (2009) Introduction to marine biogeochemistry, 2nd edn. Academic Press, Inc., Burlington

704 Massin C (1982) Food and feeding mechanisms: Holothuroidea. In: Jangoux M, Lawrence JM (eds)
705 Echinoderm Nutrition. Balkema, Rotterdam, pp 43–55

706 McCarthy MD, Benner R, Lee C, Fogel ML (2007) Amino acid nitrogen isotopic fractionation patterns as
707 indicators of heterotrophy in plankton, particulate, and dissolved organic matter. Geochim
708 Cosmochim Acta 71:4727–4744. https://doi.org/10.1016/j.gca.2007.06.061

709 Mevenkamp L, Guilini K, Boetius A, et al (2019) Responses of an abyssal meiobenthic community to
710 short-term burial with crushed nodule particles in the South-East Pacific. Biogeosciences 16:2329–
711 2341. https://doi.org/10.5194/bg-16-2329-2019

712 Middelburg JJ, Barranguet C, Boschker HTS, et al (2000) The fate of intertidal microphytobenthos
713 carbon: An *in situ* ^{13}C-labeling study. Limnol Oceanogr 45:1224–1234.
714 https://doi.org/10.4319/lo.2000.45.6.1224

715 Miller RJ, Smith CR, Demaster DJ, Fornes WL (2000) Feeding selectivity and rapid particle processing by
716 deep-sea megafaunal deposit feeders: A 234 Th tracer approach. J Mar Res 58:653–673

717 Oksanen J, Blanchet FG, Friendly M, et al (2017) vegan: Community ecology package.

718 Phillips NW (1984) Role of different microbes and substrates as potential suppliers of specific, essential
719 nutrients to marine detritivores. Bull Mar Sci 35:283–298

720 Pond D, Dixon D, Bell M, et al (1997) Occurrence of 16:2(n-4) and 18:2(n-4) fatty acids in the lipids of the
721 hydrothermal vent shrimps Rimicaris exoculata and Alvinocaris markensis: Nutritional and trophic
722 implications. Mar Ecol Prog Ser 156:. https://doi.org/10.3354/meps156167

723 Pond DW, Allen CE, Bell M v., et al (2002) Origins of long-chain polyunsaturated fatty acids in the
724 hydrothermal vent worms Ridgea piscesae and Protis hydrothermica. Mar Ecol Prog Ser 225:219–
725 226. https://doi.org/10.3354/meps225219

726 R-Core Team (2017) R: A language and environment for statistical computing

727 Roberts D, Gebruk A v., Levin V, Manship BAD (2000) Feeding and digestive strategies in deposit-feeding
728 holothurians. Oceanography and Marine Biology: An Annual Review 38:257–310

729 Roberts D, Moore HM (1997) Tentacular diversity in deep-sea deposit-feeding holothurians: implications
730 for biodiversity in the deep sea. Biodivers Conserv 6:1487–1505

731 Romero-Romero S, Miller EC, Black JA, et al (2021) Abyssal deposit feeders are secondary consumers of
732 detritus and rely on nutrition derived from microbial communities in their guts. Sci Rep 11:.
733 https://doi.org/10.1038/s41598-021-91927-4

734 Romesburg HC (1984) Cluster analysis for reseasrchers. Lifetime Learning Publications

735 Ruhl HA (2007) Abundance and size distribution dynamics of abyssal epibenthic megafauna in the
736 northeast Pacific. Ecology 88:1250–1262. https://doi.org/10.1890/06-0890

737 Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on
738 similarity of species content and its application to analysis of the vegetation on Danish commons.
739 Det Kongelige Danske Videnskabernes Selskab, Copenhaguen

740 Stratmann T, Voorsmit I, Gebruk A v., et al (2018) Recovery of Holothuroidea population density,
741 community composition and respiration activity after a deep-sea disturbance experiment. Limnol
742 Oceanogr 63:2140–2153. https://doi.org/10.1002/lno.10929

743 Svensson PA, Wong BBM (2011) Carotenoid-based signals in behavioural ecology: A review. Behaviour
744 148:131–189. https://doi.org/10.1163/000579510X548673

745 Tsushima M (2007) Carotenoids in sea urchins. In: Miller Lawrence J (ed) Edible Sea Urchins: Biology and
746 Ecology. Elsevier Science B.V., Amsterdam, pp 159–166

747 Veuger B, Middelburg JJ, Boschker HTS, Houtekamer M (2005) Analysis of ^{15}N incorporation into D-
748 alanine: A new method for tracing nitrogen uptake by bacteria. Limnol Oceanogr Methods 3:230–
749 240. https://doi.org/10.4319/lom.2005.3.230

750 Wang J, Li J, Dasgupta S, et al (2014) Alterations in membrane phospholipid fatty acids of gram-positive
751 piezotolerant bacterium sporosarcina sp. DSK25 in response to growth pressure. Lipids 49:347–
752 356. https://doi.org/10.1007/s11745-014-3878-7

753 Wigham BD, Hudson IR, Billett DSM, Wolff GA (2003) Is long-term change in the abyssal Northeast
754 Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea
755 holothurians. Prog Oceanogr 59:409–441. https://doi.org/10.1016/j.pocean.2003.11.003

756 Yano Y, Nakayama A, Yoshida K (1997) Distribution of polyunsaturated fatty acids in bacteria present in
757 intestines of deep-sea fish and shallow-sea poikilothermic animals. Appl Environ Microbiol
758 63:2572–2577

759 Zhao JX, Liu QQ, Zhou YX, et al (2015) Alkalimarinus sediminis gen. nov., sp. nov., isolated from marine
760 sediment. Int J Syst Evol Microbiol 65:3511–3516. https://doi.org/10.1099/ijsem.0.000446

761