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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 (SE Pacific) and feed to various degrees of selectivity on a heterogenous pool of sedimentary detritus, but drivers of feeding selectivity and diet preferences for most holothurian species are unknown. This study reconstructs the diets of 13 holothurian species of the orders Elasipodida, Holothuriida, and Synallactida. Bulk stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$) of holothurian body wall and gut wall tissues, gut contents, and feces 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. We further assessed how holothurians in the Peru Basin partition their resources and calculated how much of the daily particulate organic carbon (POC) flux to the area is ingested by them using information about gut contents of nine species. To assess the dependence of holothurians on fresh phytodetritus, we performed in situ pulse-chase experiments using ^{13}C- and ^{15}N-enriched phytodetritus. By measuring the uptake of this phytodetritus in fatty acids and amino acids and by comparing it with the presence of these compounds in the sediment, we calculated net accumulation and net deficiency for specific fatty acids and amino acids and discussed how climate change might affect the dependence on specific compounds. A Sørensen–Dice coefficient-based cluster analysis using data from trophic levels, levels of heterotrophic re-synthesis of amino acids, feeding selectivity, and food sources/diet suggested two major trophic groups with two optional subgroups each. Species-specific traits of locomotion, tentacle morphology, and gut structure likely allow resource partitioning and differences in selectivity among the holothurians, of which a subpopulation of 65% of all specimens can ingest 4 to 27% of the daily POC flux to the Peru Basin. Holothurians are specifically dependent on the uptake of arachidonic acid from phytodetritus, while most essential amino acids are available in the Peru Basin in sufficient concentrations.

Keywords Echinodermata · NLFA · PLFA · Sea cucumber · Diet · Deep sea · Biosynthetic pathway

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Introduction

Holothurians are abundant epifauna in the deep sea (Billett et al. 2001; Ruhl 2007; Alt et al. 2013; Stratmann et al. 2018b) and they can be suspension or deposit feeders (Massin 1982). On soft sediment, deposit-feeding holothurians either dig into the sediment as funnel-feeders or conveyor belt-feeders 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), and some species have been shown to feed selectively for specific organic compounds (Ginger et al. 2001; Witbaard et al. 2001; Wigham et al. 2003a; Hudson et al. 2003): For example, the analysis of gut contents from holothurians collected at the Porcupine Abyssal Plain (PAP, NE Atlantic) showed that *Amperima rosea* Perrier, 1886, *Peniagone diaphana* Théel,

1882, and *Oneirophanta mutabilis mutabilis* Théel, 1879, feed selectively on fresh phytodetritus (Ginger et al. 2001; Witbaard et al. 2001; 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 these examples suggest that that feeding selectivity and diet preferences of deep-sea holothurians are well known, the information is very rudimentary for most abyssal holothurian species (Billett 1991; Roberts et al. 2000).

Feeding selectivity of holothurians also affects the food availability for other benthic fauna. In fact, a non-linear regression analysis of individual biomass vs. biomass-specific phytodetritus carbon incorporation for nematodes, macrofauna, and holothurians was highly significant (Stratmann et al. 2018a). This implies that larger organisms in the deep sea might be more important for or more efficient in exploiting labile phytodetritus than smaller organisms (Stratmann et al. 2018a). For example, in the Nazaré canyon (NE Atlantic), the whole population of *Molpadia musculus* Risso, 1826, removed approximately 0.43 ± 0.13 g biopolymeric carbon (C) and 0.13 ± 0.03 g N m^{-2} d^{-1} from the sediment (Amaro et al. 2010) and at PAP, *A. rosea* and *Ellipinion molle* Théel, 1879, together with other fauna removed the phytosterols from freshly deposited phytodetritus in less than four month (Ginger et al. 2001).

Whereas holothurians alter the chemical composition of detritus in the sediment by depleting it for specific labile compounds, like sedimentary proteins (Amaro et al. 2010), this detritus composition also affects the species composition of holothurians (Wigham et al. 2003a; 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 (Wigham et al. 2003b; Tsushima 2007; Svensson and Wong 2011). Therefore, Wigham et al. (2003a) 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 C flux to the abyssal plain, even though the total megafauna biomass did not change significantly (Bett et al. 2001; Billett et al. 2001, 2010). However, the input of fresh phytodetritus will change under climate change scenarios in the future and predictions say that it will rather decrease than increase (Smith et al. 2008; Wohlers et al. 2009; Yool et al. 2017). Furthermore, warming temperatures and higher CO_2 partial pressures compared to current conditions likely lead to increasing degradation processes in the mid-water resulting in more degraded detritus reaching the deep-sea (Riebesell et al. 2007; Marsay et al. 2015). Hence, it is important to know how dependent holothurians are on fresh phytodetritus compared to the detritus present in the sediment, especially for their amino acid and fatty acid demands.

Amino acids, the building blocks 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 nitrogen (N) atom nor are bonds cleaved (McClelland and Montoya 2002; Chikaraishi et al. 2009). Other amino acids are "metabolic amino acids" (i.e., threonine) (Chikaraishi et al. 2009) and "trophic amino acids" (i.e., asparagine, glutamine, alanine, isoleucine, leucine, valine, proline) (Chikaraishi et al. 2009). The $\delta^{15}N$ values of "trophic amino acids" become enriched during metabolic transamination when N bonds are cleaved (McClelland and Montoya 2002; 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 been used to estimate the trophic level of an organism following Chikaraishi et al. (2009).

Fatty acids, the main components of lipids, have several functions, such as serving as energy source (Lindsay 1975), being involved in the transduction of signals (Graber et al. 1994; Faergeman and Knudsen 1997), and gene expression (Sampath and Ntambi 2004), and being components of membranes (van Deenen 1966; Spector and Yorek 1985). They contain neutral-lipid-derived fatty acids (NLFAs; e.g., C16:1ω7, C16:3ω3, C20:1, C20:5ω3; Maier et al. 2019; Engel et al. 2023) and phospholipid-derived fatty acids (PLFAs; e.g., C16:0, C18:1ω9 *cis*, 20:1ω9, 22:2ω6; Stratmann et al. 2022; Hoving et al. 2023) (Spector and Yorek 1985; Dalsgaard et al. 2003). NLFAs are required to build wax esters and the storage lipids triacylglycerols (Sargent and Falk-Petersen 1988; Ackman 1989), whereas PLFAs are necessary to build structural phospholipids of cell membranes (Spector and Yorek 1985). Fatty acids may be unsaturated or saturated, and generally a higher number of unsaturated bonds imply that the fatty acid is more labile than a fatty acid with fewer unsaturated bonds (Sargent 1995). "Essential" fatty acids have to be taken up with the diet (Stubbs and Smith 1990) because they can generally only be synthesized de novo by primary producers (Sargent 1995). Since several fatty acids are transferred

conservatively (i.e., untransformed) from primary producers and primary consumers to higher trophic levels (Lovern 1935), they may serve as trophic markers and inform about diets (Kharlamenko et al. 1995).

To decipher feeding types and diet preferences of holothurians and their ecological role in the Peru Basin, we combined stable isotope analyses of bulk tissue, gut content, and feces with compound-specific stable isotope analysis (CSIA) of amino acids and fatty acids. Furthermore, we performed a pulse-chase experiment with deep-sea holothurians that were fed in situ with ^{15}N and ^{13}C-enriched phytodetritus (*Skeletonema costatum* (Greville) Cleve, 1873) to compare how much of their daily amino acid and fatty acid demands holothurians can meet from ingesting sediment and which amino acids and fatty acids they have to extract from fresh phytodetritus. These two approaches allowed us to address the following research hypotheses (1) resource partitioning facilitates the high biodiversity of deposit-feeding holothurians in the Peru Basin, (2) holothurians reduce organic C availability in sediments of the Peru Basin, and (3) selective-feeding holothurians depend on amino acids and fatty acids from fresh phytodetritus.

Material and methods

Study site

The Peru Basin (Fig. 1) in the SE Pacific Ocean is a 4000 to 4400 m (Wiedicke and Weber 1996) deep polymetallic nodule-rich abyssal plain covered with a 10 to 20 cm thick layer of brown fluffy sediment (Mevenkamp et al. 2019) of silty clay and clayey silt (Grupe et al. 2001) on which nodules lay with densities of 18.1 ± 11.3 kg m^{-2} (Marchig et al. 2001). The sediment porosity is 0.93 (Vonnahme et al. 2020) and the top 1 cm of surface sediment contains 0.09 µg ml^{-1} chlorophyll-a and 0.23 µg ml^{-1} phaeopigments (Vonnahme et al. 2020). The upper 10 cm of sediment is fully oxygenated, and the oxygen penetrates the sediment down to 12 to 20 cm sediment depth (Paul et al. 2018).

Sampling of holothurians

During "JPI Healthy and Productive Seas and Ocean" RV *Sonne* cruise SO242-2 to the Peru Basin in August and September 2015 (Boetius 2015), 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 suction sampler of the remotely operated vehicle (ROV) Kiel 6000 (Fig. 1, Table S1). 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 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.

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

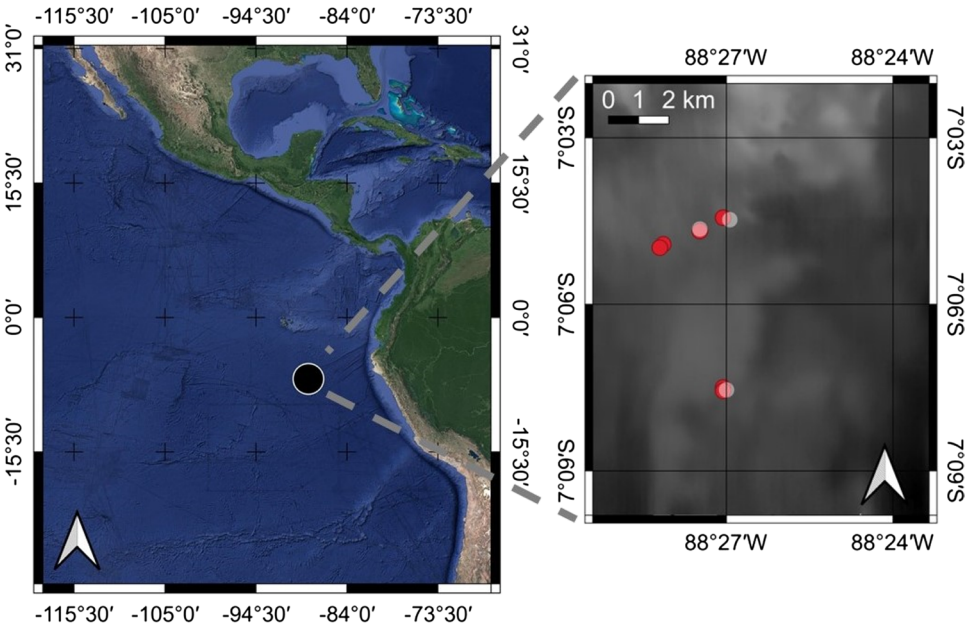

Fig. 1 Location of the Peru Basin in the Pacific Ocean (left panel) with detailed positions of holothurian sampling sites (right panel; red symbols) and in-situ experiments (white transparent symbols) during RV *Sonne* cruise SO242-2 in September–October 2015. Bathymetry data of the right panel from is Gausepohl et al. (2019, 2020) and data from of the left panel is from Google Earth. The water depth at the sampling stations ranged from 4136.3 to 4429.4 m

($n = 1$), and Synallactidae gen sp. ($n = 1$) from the study of Brown et al. (2018) were included in the analysis. These specimens were collected with the ROV suction sampler and transported to respiratory chambers to measure oxygen consumption of individual holothurian specimens over a period of 72 h. Aboard RV *Sonne*, the holothurians specimens were shock-frozen intact in liquid nitrogen and stored at − 21 °C. Feces of holothurians that defecated inside the respiratory chambers were sampled and frozen at − 21 °C. Due to the very low in-situ temperatures (1.84–1.85 °C; Brown et al. 2018), no changes in bulk and/or compound-specific composition of the feces was expected.

Sampling of sediment

Sediment samples were taken with ROV-deployed push cores and a benthic chamber lander system. After retrieval of push cores and the lander on board, the cores, and the lander chambers (484 cm^2) were sliced in two sediment intervals (0–2 cm and 2–5 cm sediment layers). Subsamples (35 ml) were taken from each sediment interval and stored frozen at − 20 °C.

In-situ experiment

Pulse-chase in-situ experiments were performed during RV *Sonne* cruise SO242-2 by placing large (50 × 50 cm footprint) benthic incubation chambers (Stratmann et al. 2018a) over a single holothurian specimen at the seafloor ($n = 6$, putative species: *Amperima* sp., *Benthodytes* sp., *Peniagone* sp.) (Fig. 2) with ROV Kiel 6000. The holothurians were fed by injection of 0.5 g dry mass (DM) freeze-dried phytodetritus (i.e., *S. costatum*; equivalent to 40 mmol C m^{-2} and 5 mmol N m^{-2}) that was enriched in $\delta^{13}C$ and $\delta^{15}N$ (29 at% ^{13}C and 37 at% ^{15}N) into the chamber. After 3 days of incubation, the experiment was terminated, and the holothurians were collected via the suction pump of the ROV Kiel 6000. Aboard the vessel, the holothurians were dissected to separate the gut and its content from the remaining tissue. All samples were flash-frozen in liquid nitrogen and stored frozen at − 20 °C.

Laboratory analyses

Bulk analyses

In the shore-based laboratory at NIOZ-EDS (Yerseke, Netherlands), the samples were freeze-dried and finely-ground with mortar and pestle. Total C (TC)/$\delta^{13}C$ and total N (TN)/$\delta^{15}N$ content of the holothurian body wall tissue was measured in not acidified samples and organic C (org. C)/$\delta^{13}C$ was measured in acidified tissue samples 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). Org. C/$\delta^{13}C$ and TN/$\delta^{15}N$ contents of acidified holothurian gut contents, feces, and sediment were measured with the same EA-IRMS and the $\delta^{13}C$ and $\delta^{15}N$ data were normalized against L-glutamic acid isotope reference material USGS40 (Qui et al. 2004) and USGS41a (Qi et al. 2016) following Sharp (2017). Stable isotope values are presented in δ notation relative to Vienna Pee Dee Belemnite for $\delta^{13}C$ and relative to air for $\delta^{15}N$.

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

Hydrolysable amino acids (HAA) from holothurian body wall tissue and phytodetritus were extracted following a modified protocol of Veuger et al. (2005). Briefly, HAAs in holothurian tissue and HAAs in phytodetritus were hydrolyzed by adding 0.01 to 0.02 g freeze-dried finely ground holothurian tissue and 0.01 to 0.02 g phytodetritus, respectively, to 1.5 ml 6 M HCl in

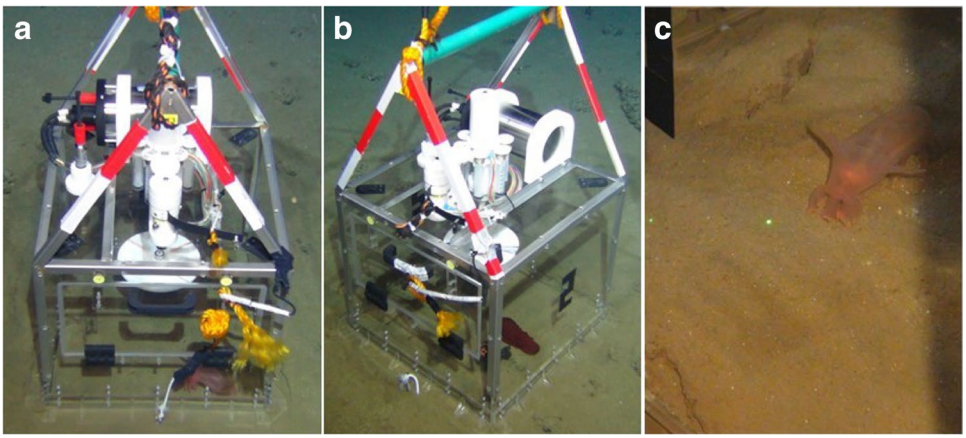

Fig. 2 Incubation of **a** *Amperima* sp.; **b** *Benthodytes* sp.; and **c** *Peniagone* sp. inside the benthic incubation chamber during the in situ pulse-chase experiments at the Peru Basin at ~ 4100 m water depth. Photos by ROV Kiel 6000 (GEOMAR, Germany)

10 ml screw-cap vials. A N_2-headspace was created in the vials by flushing with N_2-gas for 10 s 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-Norleucine acidified with 100 µl 12 M HCl) was added and the solution was evaporated under N_2-flow at 60 °C. HAAs 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 chloroform and 1 ml phosphorus-buffer to the sample, shaking it until the lower chloroform fraction was clear and centrifuging the vials with 2000 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 body wall tissue), and $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of HAAs were measured with a HP 6890 gas chromatograph (Hewlett Packard/ Agilent, USA) coupled with a DELTA-Plus Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA) on a polar analytical column (ZB5-5MS; 60 m length, 0.32 mm diameter, 0.25 µm film thickness; Phenomenex, USA). The concentrations of amino acids were determined using L-Norleucine as internal standard and $\delta^{13}C$ and $\delta^{15}N$ data of amino acids were standardized against L-Norleucine which had $\delta^{13}C$ and $\delta^{15}N$ values of -31.31 ± 0.14 ‰ and -4.45 ± 0.05 ‰, respectively, as determined by EA-IRMS prior to its application as internal standard.

Following Veuger et al. (2005), individual HAAs were identified based on their retention time in relation to the L-Norleucine standard. Asparagine *and* aspartic acids, and glutamine *and* glutamic acid, however, were reported as sums, because during the hydrolysis asparagine is converted to aspartic acid and glutamine to glutamic acid (Uhle et al. 1997; Erbe 1999). Except for histidine, cysteine, tryptophan, and arginine, all other 20 "common" HHA can be detected with this method (Erbe 1999), but the recovery of methionine can be ~ 30% (Maier et al. 2019). Therefore, methionine was excluded from the analysis. C concentrations were also corrected for the C-atoms added during the derivatization step.

Analysis of fatty acids

Lipids were extracted from holothurian body wall tissue, phytodetritus, 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), phytodetritus (~ 1 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 lipid 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 120 °C for 2 h; Merck Kieselgel 60) via eluting with 7 ml chloroform, 7 ml acetone, and 15 ml methanol. The acetone 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 C19:0 FAME mL^{-1}), and 1 ml 0.2 M methanolic NaOH to the test tubes with the PLFAs and NLFAs extracts. After an incubation at 37 °C 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 was 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 C12:0 FAME mL^{-1}) was 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 and phytodetritus were separated on a very polar BPX70 column (50 m length, 0.32 mm inner diameter, 0.25 µm film thickness; SGE Analytical Science) with a HP 6890 GC (Hewlett Packard/ Agilent, USA). The FAMEs from sediment, feces, and gut content were separated on a polar 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 body wall tissue) and $\delta^{13}C$ values (‰) of FAMEs in holothurian body wall tissue, feces, and gut content were measured on a Finnigan Delta Plus IRMS (Thermo Fisher Scientific, USA) coupled to the GC via a combustion GC-c-III interface (Thermo Fisher Scientific, USA). Five reference material FAMEs (C14:0 FAME, C16:0 FAME, C18:0 FAME, C20:0 FAME, C24:0 FAME) from Schimmelmann Research (Indiana University Bloomington, USA) were combined in a mix and served as standard against which the $\delta^{13}C$ and $\delta^{15}N$ data were normalized following (Sharp 2017).

The peaks of FAMEs in the chromatograms were identified as specific fatty acids based on their retention time in relation

to the internal standards C12:0 FAME and C19:0 FAME, and the omnipresent peak of C16:0 following Boschker et al. (1999). The retention times of certified reference FAME standards from Sigma-Aldrich (USA; product numbers: L-7272, T-0627, M-3378, P-6250, P-5177, H-4515, S-5376, N-5377, A-3881, P-9667, P-0203, E-4762, B-3271, T-9900, L-6766, -4754), from Larodan (Sweden; product numbers: 20–1603-4, 20–1812-9, 20–1802-13, 20–1840-4, 20–2004-7, 20–2003-9, 20–2205-9, 90–1100, 90–1051, 90–1054, 21–1610-7, 21–1710-7, 21–1810-7, 21–1413-7, 21–1615-7, 11–1412-7), from Supelco® Analytical (USA; product numbers: Supelco 47,033, Supelco-47033, Supelco-47015-U, Supelco-47085-U, Supelco-47080-U), and from Schimmelmann Research (fatty acid mixture F8-4) measured on the same GC columns served as references. Further peaks in the chromatograms were confirmed by gas chromatography/mass spectrometry (GC/MS) following Kurkiewicz et al. (2003). Whenever, the identification of chromatogram peaks was not unique, because fatty acids co-eluted, both fatty acids were reported as "fatty acid 1/ fatty acid 2."

C concentrations were calculated based on peak area of the chromatogram peaks using the two internal standards (C12:0 FAME and C19:0 FAME) for area correction and corrected for the C-atoms added during the derivatization step.

A list with of dominant fatty biomarkers is presented in Table 1.

Data analyses

Concentration factors

To examine the degree to which PLFAs were concentrated between surface sediment (0–2 cm layer) 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 mean total PLFA concentration in surface sediment.

Trophic levels

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

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

Table 1 Fatty acids used as biomarkers of potential food sources of holothurians from the Peru Basin. Abbreviations: *ARA* arachidonic acid (C20:4ω6), *DHA* docosahexaenoic acid (C22:6ω3), *EPA* eicosapentaenoic acid (C20:5ω3)

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 bacteria; 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	(Kharlamenko et al. 1995; Budge and Parrish 1998; Dalsgaard et al. 2003; Parrish et al. 2005)
C18:4ω3 and DHA; $\frac{C16:1\omega7}{C16:0} < 1$ or $\frac{DHA}{EPA} > 1$	Dinoflagellates	(Budge and Parrish 1998; Dalsgaard et al. 2003; Parrish et al. 2005)
Consumers		
C20:1ω9, C22:1ω11	Calanoid copepods	(Falk-Petersen et al. 1987; Dalsgaard et al. 2003)
ARA; C22:5ω5; $\frac{EPA}{ARA}$–ratio	Agglutinated foraminifera	(Larkin et al. 2014; Kharlamenko 2018)

$\delta^{15}N_{Glu}$ is the $\delta^{15}N$ of the amino acid glutamic acid (‰) and $\delta^{15}N_{Phe}$ corresponds to the $\delta^{15}N$ of the amino acid phenylalanine (‰). β is the fractionation between glutamic acid and phenylalanine at trophic level 1 and $\Delta_{Glu\text{-}Phe}$ is the ^{15}N enrichment in glutamic acid relative to phenylalanine.

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

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

where σ_{β} is 0.9‰, σ_{Δ} is 1.1‰, and β is the fractionation between glutamic acid and phenylalanine (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 $\delta^{15}N$ values of the trophic amino acid alanine, aspartic acid, glutamic acid, leucine, and proline (McCarthy et al. 2007):

$$\sum V = \sum_{1}^{n} \left| x_{amino\ acid} - \overline{x_{amino\ acid}} \right| \quad (5)$$

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

Bayesian mixing model

The relative contribution of potential food sources (Table 2) to the diets of the different holothurian species (i.e., consumers) was estimated using Bayesian mixing models based on the natural abundance stable isotopic composition ($\delta^{13}C$, $\delta^{15}N$) of food sources and holothurians.

The Bayesian analysis was conducted using the *R* (version 4.3.0; R-Core Team 2022) package *rjags* (version 4.3.1; Stock et al. 2020) and the mixing models were performed using the *R* package *MixSIAR* (version 3.1.12; Stock et al. 2018, 2020). A general trophic enrichment factor of 0.5‰ for C and 3.4‰ for N (Fry 2006) was applied for all food sources. The mixing models for the fixed factor "species" were fitted using the Markov Chain Monte Carlo (MCMC) method on 300,000 iterations for informative priors (i.e., $\alpha = (1, 1, 1, 0.5, 0.5, 0.5)$ for sedimentary detritus, phytodetritus, heterotrophic prokaryotes, Foraminifera, Fungi, and Nematoda, respectively). Model convergence was diagnosed with Gelman-Rubin diagnostics $\widehat{R}$ ($\widehat{R} < 1.05$) (Gelman and Rubin 1992; Gelman et al. 2014; Roy 2020) and Geweke diagnostics Z_n (Geweke 1991; Roy 2020) being similar in all three chains. The model solutions are presented as mean (%) with 95% and 75% Bayesian credibility intervals (BCI).

Cluster analysis of Sørensen–Dice coefficient β_{sor}

The Sørensen–Dice coefficient β_{sor} (Dice 1945; Sørensen 1948; Koleff et al. 2003) was calculated using the "betadiver" function in the *R* 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 3) and then converted into binary (presence/absence) data. The categorical data "feeding selectivity" (Table 3) 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 optimal number of clusters was identified by finding a consensus among the results of 20 different clustering algorithms using the "n_clusters" function in the R package *parameter* (version 0.21.1; Lüdecke et al. 2020). The dendrogram was prepared with *R* package *factoextra* (version 1.0.7; Kassambara and Mundt 2020).

Table 2 Stable isotopic composition of potential food sources of holothurians used in the Bayesian mixing model

Food source	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	*n*	Reference
Bulk sediment	− 20.7 ± 0.6	10.3 ± 2.0	4	This study
Diatom-derived phytodetritus	− 12.8 ± 0.2	1.9 ± 0.3	3	Unpublished data
Heterotrophic prokaryotes	− 15.6 ± 1.5	2.4 ± 1.4	4	(Pagès et al. 2014)
Foraminifera	− 19.2 ± 0.5	8.7 ± 1.3	33	(Nomaki et al. 2008)
Fungi	− 25.8 ± 0.6	0.9 ± 1.6	12	(Gutiérrez et al. 2020)
Nematoda	− 19.3 ± 0.4	6.5 ± 5.8	3	(Stratmann et al. 2018a)

^{13}C and ^{15}N incorporation

The bulk incorporations of phytodetritus C and N into holothurian body wall tissue and the incorporation of ^{13}C and ^{15}N into holothurian HAAs, PLFAs, and NLFAs were calculated as follows:

$$R_{bulk\ tissue,\ HAA,\ PLFA,\ NLFA} = \left(\frac{\delta^{13}C}{1000} + 1\right) \times R_{standard}, \quad (6)$$

or

$$R_{bulk\ tissue,\ HAA,\ PLFA,\ NLFA} = \left(\frac{\delta^{15}N}{1000} + 1\right) \times R_{standard} \quad (7)$$

where $R_{standard}$ is 0.0111802 in case of C and $R_{standard}$ is 0.0036782 in case of N. The fractions (F) of the ^{13}C and ^{15}N isotopes in the enriched tissue and HAAs, PLFAs, NLFAs (F_{sample}), and background tissue (i.e., *Amperima* sp., *Benthodytes* sp., *Peniagone* sp. specimens collected in "Sampling of holothurians") and HAAs, PLFAs, and NLFAs ($F_{background}$) were calculated as follows:

$$F = \frac{^{13}C}{\left(^{13}C + {}^{12}C\right)} = \frac{R}{(R+1)} \quad (8)$$

or

$$F = \frac{^{15}N}{\left(^{15}N + {}^{14}N\right)} = \frac{R}{(R+1)} \quad (9)$$

Subsequently, the incorporations of phytodetritus C and N (I) in the tissue were calculated as follows:

$$I = \frac{\left(F_{sample} - F_{background}\right) \times total\ C}{phytodetritus\ enrichment} \quad (10)$$

or

$$I = \frac{\left(F_{sample} - F_{background}\right) \times total\ N}{phytodetritus\ enrichment} \quad (11)$$

The incorporations of ^{13}C and ^{15}C in HAAs, PLFAs, and NLFAs were calculated as follows:

$$\mathrm{I} = \left(\mathrm{F_{sample}} - \mathrm{F_{background}}\right) \times \mathrm{total}\ C \quad (12)$$

or

$$\mathrm{I} = \left(\mathrm{F_{sample}} - \mathrm{F_{background}}\right) \times \mathrm{total\ N} \quad (13)$$

For the calculation of ^{13}C incorporation into PLFAs and NLFAs, only PLFAs and NLFAs were considered that were present in at least four out of five samples.

Statistical analysis

To test whether the proportions of individual amino acids and fatty acids to total amino acids and fatty acids in (i) sediments compared to holothurians with natural abundance stable isotope composition and in (ii) phytodetritus compared to holothurians in the pulse-chase experiment were

Table 3 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 factor *CF*	*CF* = 0–10	No selectivity
	CF = > 10–50	Selective
	CF = > 50–150	Very selective
	CF = > 150	Extremely selective
Food sources/diet		– Diatom-derived phytodetritus
		– Dinoflagellate-derived phytodetritus
		– Secondary consumer of detritus
		– Sedimentary detritus
		– Foraminifera
		– Bacteria

statistically significantly different (level of significance: $\alpha = 0.05$), Wilcoxon Rank Sum Test W (also known as Mann Whitney U Test) was performed. This test allows to compare means of two different samples that are not normally distributed.

All data are presented as mean ± 1 standard deviation (SD). A list with the number of replicates per specific parameter and holothurian species is presented in Table S2.

Results

Chemical composition of sediment

Sediment in the Peru Basin consisted of 0.63 ± 0.01% org. C in the upper 2 cm of sediment ($n = 4$) and 0.62 ± 0.03% org. C in the 2 to 5 cm sediment depth layer ($n = 4$). TN content in each sediment layer was $0.13 \pm 1.98 \times 10^{-3}$% N and the C/N ratios were 5.44 ± 0.02 (0–2 cm) and 5.45 ± 0.19 (2–5 cm). The sediment had $\delta^{13}C$ values of − 20.7 ± 0.6‰ (0–2 cm; $n = 4$) and − 21.8 ± 1.2‰ (2–5 cm; $n = 4$), and $\delta^{15}N$ values of 10.3 ± 2.0‰ (0–2 cm; $n = 4$) and 8.20 ± 0.8‰ (2–5 cm; $n = 4$).

The surface sediment layer (0–2 cm; $n = 4$) contained 18.5 ± 7.16 µmol HAA g^{-1} dry mass (DM) sediment, whereas the 2 to 5 cm layer ($n = 4$) had 18.6 ± 5.73 µmol HAA g^{-1} DM sediment. Alanine, glycine, and isoleucine contributed between 51.0 ± 2.28% (0–2 cm; $n = 4$) and 52.8 ± 3.82% (2–5 cm; $n = 4$) to the HAA concentration.

The surface sediment layer (0–2 cm; $n = 3$) contained 0.19 ± 0.04 µmol C-PLFA g^{-1} DM sediment, whereas the 2 to 5 cm layer ($n = 3$) had 0.15 ± 0.08 µmol C-PLFA g^{-1} DM sediment. The PLFAs C16:0, C16:1ω7 *cis*, and 18:1ω7 *cis*/18:1ω9 *trans* contributed 34.9 ± 11.1% (2–5 cm; $n = 3$) to 44.4 ± 3.16% (0–2 cm; $n = 3$) to the total PLFA concentration in the respective sediment layers.

Gut content and feces of holothurians

Gut contents of holothurians in the Peru Basin weighed 1.93 ± 3.56 g DM gut content and ranged from 0.11 g DM gut content for *Peniagone* sp. to 12.5 g DM gut content for *P. hanseni* (Table 4). Org. C and TN contents of the gut content of all investigated holothurian specimens were 5.34 ± 4.13% and 1.04 ± 0.87%, respectively, and it contained 244 ± 304 µg C-PLFA g^{-1} DM gut content and 83.3 ± 124 µg C-NLFA g^{-1} DM gut content (Fig. 3a). The concentration factor $CF_{gut\ content}$ for PLFAs in holothurian gut content was on average 105 ± 131 and ranged from 1.17 to 335 for *Benthodytes* sp. and Synallactidae gen sp., respectively (Table 4). The mean eicosapentaenoic acid (EPA, C20:5ω3)/arachidonic acid (ARA, C20:4ω6)-ratio for gut content across all holothurians was 3.20 ± 5.58, the mean docosahexaenoic acid (DHA, C22:6ω3)/EPA-ratio was 0.62 ± 0.68, and the mean C16:1ω7/C16:0-ratio was 0.81 ± 0.85 (Fig. 4a).

Feces of holothurians weighed 1.36 ± 2.09 g DM feces and ranged from 0.12 g DM feces for Synallactidae gen sp. to 3.01 ± 3.61 g DM feces for *Benthodytes* sp. (Table 4). Org. C and TN content of the feces was 0.83 ± 0.37% and 0.14 ± 0.04%, respectively, and it contained 7.73 ± 3.60 µg C-PLFA g^{-1} DM feces and 7.63 ± 5.28 µg C-NLFA g^{-1} DM feces (Fig. 3a). In holothurian feces, PLFAs were on average still 3.33 ± 1.55 times more concentrated compared to the upper 2 cm of sediment (CF_{feces} range: 1.81 ± 0.53 for *Benthodytes* sp. to 4.44 ± 1.52 for *B. typica*) (Table 4). The mean EPA/ARA-ratio for feces was 0.29 ± 0.59, the mean DHA/EPA-ratio was 0.23 ± 0.48, and the mean C16:1ω7/C16:0-ratio was 1.39 ± 0.57 (Fig. 4b).

Gut content and feces consisted of 81.2 ± 3.73% silt (grain size: < 63 µm) and of 10.7 ± 1.10% very fine sand (grain size: 62.5–125 µm) (Table 5). The median grain was 15.5 ± 2.27 µm.

Most of the PLFAs (Fig. 3b; Fig. S1a) and the NLFAs (Fig. 3d; Fig. S1c) found in holothurian gut content consisted of highly unsaturated fatty acids (HUFA, i.e., fatty acids with ≥ 4 double bonds; 47.7 ± 14.6%) and saturated fatty acids (SFA; 40.5 ± 11.3%), respectively, followed by monosaturated fatty acids (MUFA), SFA, and polyunsaturated fatty acids (PUFAs, i.e., fatty acids with ≥ 2 double bonds) in case of PLFAs, and followed by HUFA and MUFA in case of NLFA.

Feces of holothurians consisted of 33.0 ± 10.7% PLFA-SFA (Fig. 3c; Fig. S1b) and of 50.4 ± 12.8% NLFA-SFA (Fig. 3e; Fig. S1d). The other PLFAs consisted of 31.7 ± 10.5% MUFA, 16.1 ± 11.4% HUFAs, and 10.6 ± 23.7% long-chain fatty acids (LCFA, i.e., fatty acid with ≥ 24 C atoms). The NLFAs included additionally 20.2 ± 23.5% HUFA and 14.8 ± 11.7% MUFA.

Chemical composition of holothurians

Holothurians in the Peru Basin consisted of 93.0 ± 10.2% water and their dried body walls contained 5.08 ± 2.89% org. C and 1.33 ± 0.80% TN, whereas their dried gut tissues consisted of 17.1 ± 8.43% org. C and 3.76 ± 2.18% TN. The body wall and gut wall 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 S3).

The HAA composition did not differ greatly among species, with the main HAAs in holothurian body walls being alanine, glycine, aspartic acid, and glutamic acid that contributed between 55.6% (Elpidiidae) and 78.2% (*P. longicauda*) to total HAAs of body walls (Fig. 5a).

Table 4 Sedimentological characteristics of holothurian gut content (GT) and feces (*F*). Data are presented as mean ± 1 SD and number of replicates (*n*) is presented in Table S2

Species	g dry sediment		Composition				Concentration factor	
			% org. C		% TN			
	GT	*F*	GT	*F*	GT	*F*	$CF_{gut\ content}$	CF_{feces}
Family: Elpidiidae								
Amperima sp.	0.16 ± 0.1	0.54	7.76 ± 3.85	0.32	1.60 ± 0.71	0.05	58.0	
Elpidiidae gen sp.	0.88		1.57		0.35		14.9	
Peniagone sp.	0.11	0.45	3.27	0.84	0.66	0.13		4.36
Family: Deimatidae								
Oneirophanta sp.	3.90		2.84		0.51		16.6	
Family: Laetmogonidae								
Psychronaetes hanseni	12.5		1.94		0.38		25.6	
Family: Psychropotidae								
Benthodytes sp.	2.07 ± 2.1	3.01 ± 3.61	0.95 ± 0.02	0.70 ± 0.16	0.16 ± 0.01	0.14 ± 0.05	1.17	1.81 ± 0.53
Benthodytes typica	0.85 ± 0.62		1.05 ± 0.47		0.16 ± 0.01		4.44 ± 1.52	
Psychropotes longicauda	1.08		1.38		0.29		105	
Psychropotes semperiana	1.78		5.25		0.80			
Family: Synallactidae								
Synallactes sp. (morphotype "pink")	6.75		2.22		0.34			
Galatheathuria sp.	0.51		11.4		2.10		285	
Synallactidae gen sp.	0.14	0.12	11.3	0.84	2.44	0.17	335	

Across all holothurians, the metabolic amino acid threonine had a mean $\delta^{15}N$ value of − 9.84 ± 8.20‰ and source amino acids had a total mean $\delta^{15}N$ value of 7.49 ± 7.81‰ (Fig. S3). The $\delta^{15}N$ values of all trophic amino acids asparagine, glutamine, alanine, isoleucine, leucine, valine, and proline averaged 20.4 ± 4.95‰. The highest $\delta^{15}N$ values for the metabolic amino acid were measured in body wall tissue of *Amperima sp.* (− 3.59 ± 2.19‰) and the lowest value was measured in body wall tissue of *P. longicauda* (− 34.4‰). The highest $\delta^{15}N$ values of source amino acids were measured in *P. longicauda* (13.3 ± 30.3‰), whereas the lowest value was found in *Oneirophanta* sp. (− 3.75 ± 5.05‰). *P. longicauda* had the lowest $\delta^{15}N$ values of trophic amino acids (16.6 ± 3.76‰), whereas *P. semperiana* had the highest $\delta^{15}N$ value (24.7 ± 3.39‰).

The PLFA (Fig. 5b; Fig. S2a) and NLFA (Fig. 5c; Fig. S2b) compositions differed strongly among species. Between 1.75% (Elpidiidae gen sp.) and 36.6% (*P. semperiana*) of the PLFAs found in holothurian body walls consisted of PUFAs. The other PLFA classes detected in the body walls were HUFAs (36.9 ± 13.3%), methyl-fatty acids (16.2 ± 10.1%), LCFAs (15.7 ± 15.5%), MUFAs (9.08 ± 12.3%), and SFAs (8.62 ± 6.36%). Compared to the mean PLFA composition across all holothurian taxa analyzed, *P. hanseni* had an above mean percentage of MUFAs (17.7% of total PLFAs) and a below mean percentage of PUFAs (0.80% of total PLFAs). *Oneirophanta* sp. had an above mean percentage of SFAs (39.3% of total PLFAs) and *Galatheathuria* sp. had an above mean percentage of HUFAs (36.9% of total PLFAs).

The NLFAs consisted of 32.1 ± 12.7% SFAs, of 15.4 ± 6.39% MUFAs, of 3.75 ± 2.86% PUFAs, of 13.4 ± 6.86% HUFAs, of 4.41 ± 11.0% LCFA, and of 31.0 ± 16.7% methyl-fatty acids. In comparison to the mean NLFA composition across all studied holothurian taxa, *Oneirophanta* sp. had an above mean percentage of SFA (60.4% of total NLFAs). *P. hanseni* had an above mean percentage of MUFAs (32.7% of total NLFAs), *P. semperiana* had an above mean percentage of HUFAs (23.5% of total NLFAs), and Elpidiidae gen sp. had an above mean percentage of methyl-fatty acids (48.1%).

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. to 1.75 ± 2.23 for Synallactidae gen sp. (Fig. 4c). In comparison, the ratio of DHA to EPA, i.e., the DHA/EPA-ratio, ranged from 0.01 for *P. hanseni* to 1.27 for *Peniagone* sp. (Fig. 4c). 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. 4c).

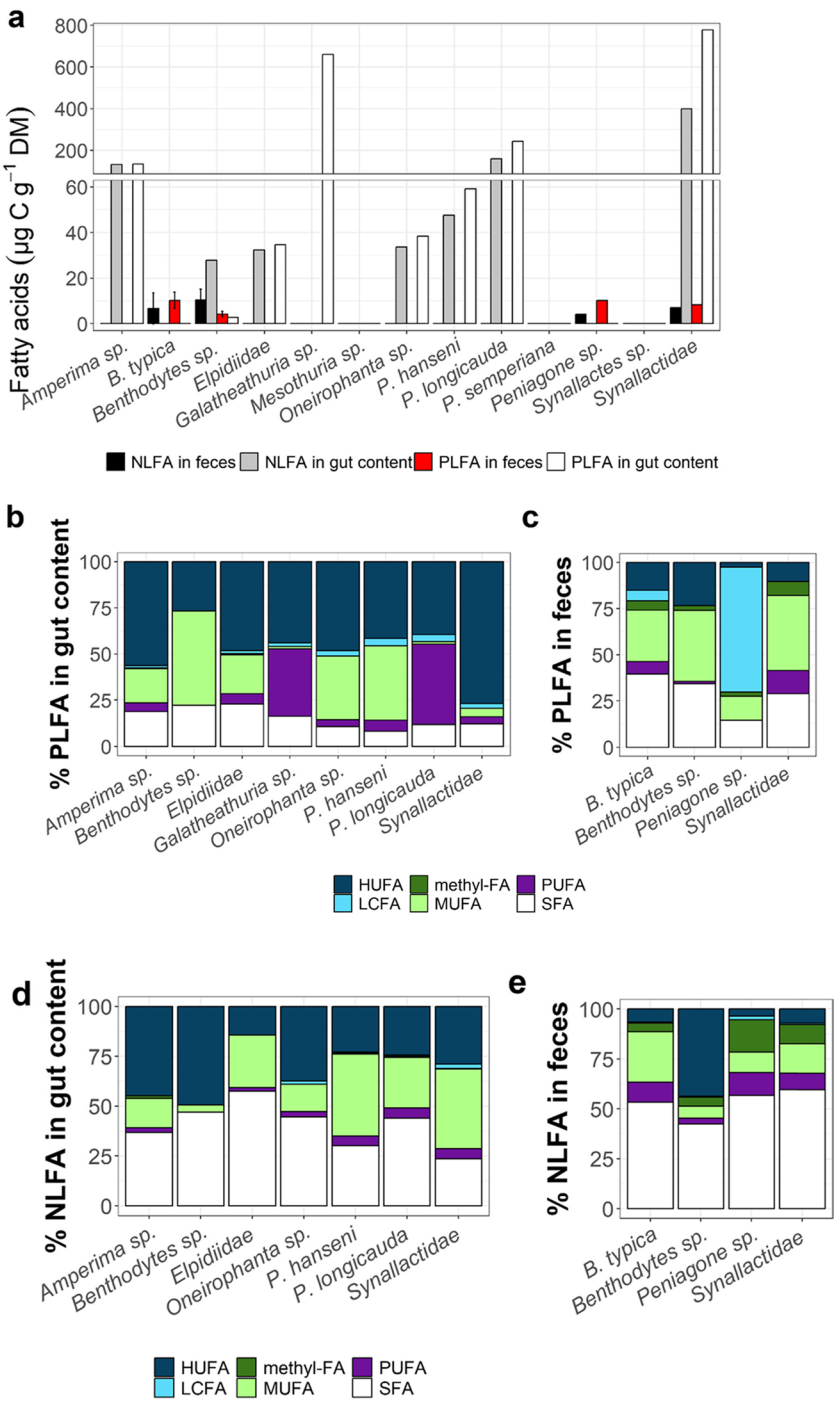

Fig. 3 **a** Concentrations (µg C g^{-1} DM) of phospholipid-derived fatty acids (PLFA) and neutral-lipid-derived fatty acid (NLFA) in holothurian gut content and feces and the contribution (%) of individual **b**, **c** PLFA classes and **d**, **e** NLFA classes to the total concentrations. Error bars in (a) indicate 1 SD. Abbreviations: HUFA, highly unsaturated fatty acid; LCFA, long-chain fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

Trophic position of holothurians and recycling of amino acids

Holothurians in the Peru Basin had a mean δ^{13}org. C value of -17.5 ± 0.89‰ with a minimum value of -16.6‰ for *P. semperiana* and a maximum value of -18.4‰ for *Galatheathuria* sp. The mean δ^{15}N value was 11.6 ± 1.47‰ with a minimum value of 9.84‰ for *Peniagone* sp. and a maximum value of 14.3‰ for *Oneirophanta* sp (Fig. 6a).

Trophic level (TL) estimates for holothurians in the Peru Basin, based on the δ^{15}N values of the HAA glutamic acid and alanine, ranged from 2.0 (*P. hanseni*; σ_{TL}: ±0.44) to 3.5 (*Oneirophanta sp.*; σ_{TL}: ±0.44) (Fig. 6b).

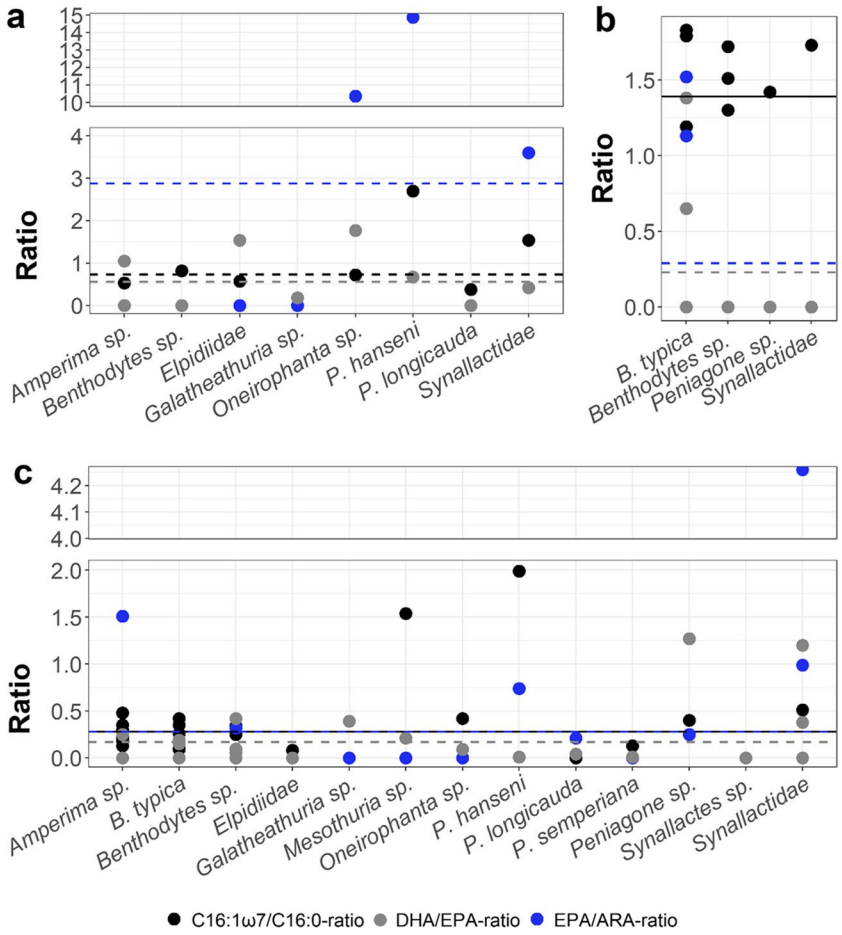

Fig. 4 Ratios of C16:1ω7/C16:0, DHA/EPA, and EPA/ARA in **a** holothurian gut content; **b** holothurian feces; and **c** holothurian body wall tissue. Horizontal lines show the mean value of a ratio based on all samples. Abbreviations: ARA, arachidonic acid (C20:4ω6); DHA, docosahexaenoic acid (C22:6ω3), EPA, eicosapentaenoic acid (C20:5ω3)

Table 5 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)	82.3	10.0	4.91	2.05	0.92	14.7
Family: Deimatidae						
Oneirophanta sp. (GT)	75.5	12.2	7.86	4.01	0.65	19.0
Family: Laetmogonidae						
Psychronaetes hanseni (GT)	79.0	11.7	6.67	2.48	0.33	17.5
Family: Psychropotidae						
Benthodytes sp. (F)	86.4	9.20	4.05	0.44	0.00	13.0
Benthodytes typica (F)	81.0	10.8	6.08	1.77	0.55	14.2
Family: Synallactidae						
Synallactidae gen sp. (F)	83.0	10.4	4.47	1.58	0.76	14.5

$\sum V$ 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. 6b).

Mixing model results (MixSIAR)

Bayesian mixing model results suggested that potential food sources of holothurians in the Peru Basin were (in order)

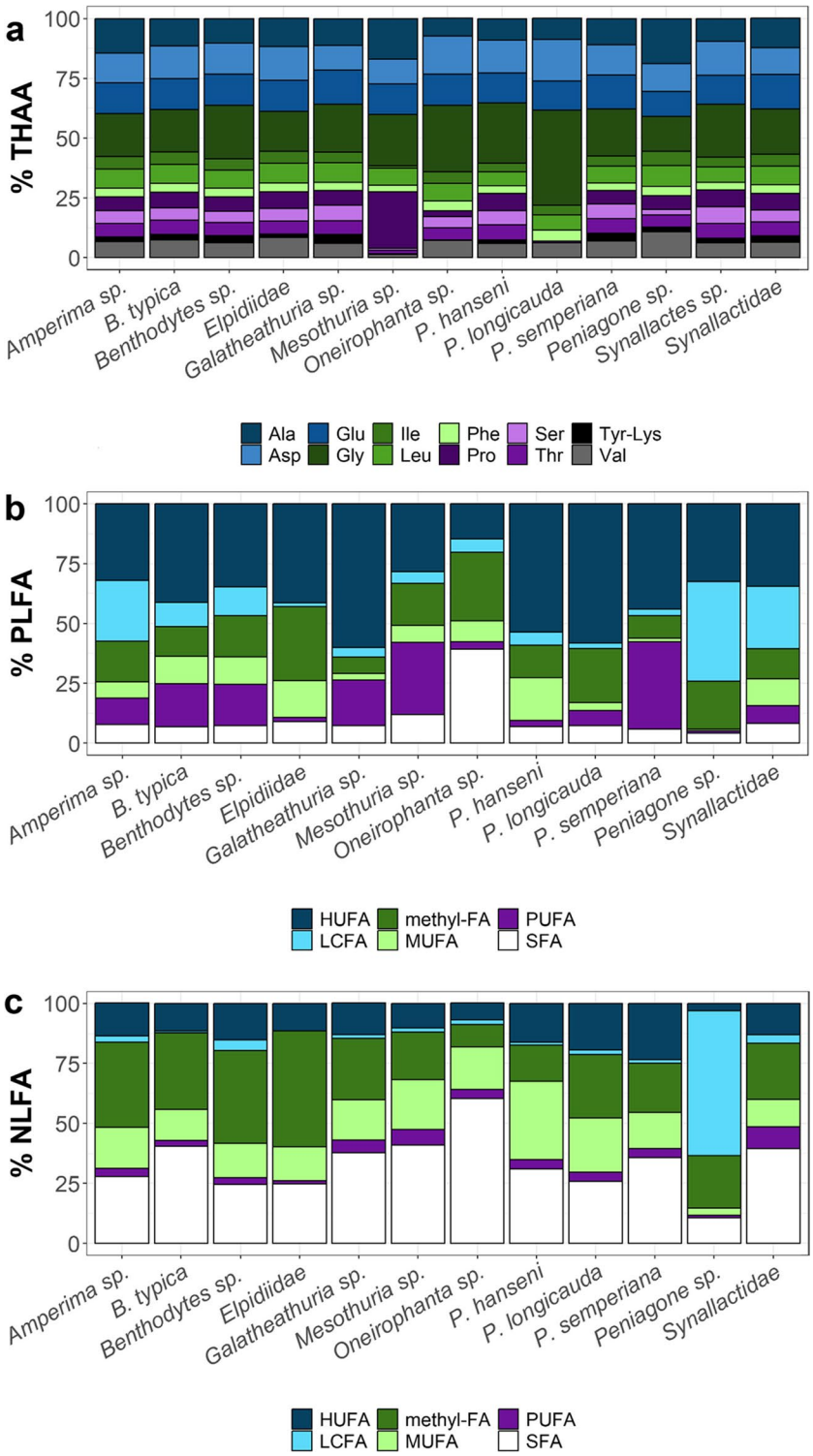

Fig. 5 Contribution (%) of individual **a** hydrolysable amino acids (HAAs); **b** phospholipid-derived fatty acid (PLFA) classes; and **c** neutral-lipid-derived fatty acid (NLFA) classes to the total concentrations in holothurian body wall tissue. Abbreviations of HAAs: Ala, alanine; Asp, aspartic 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. Abbreviations of PLFA and NLFA classes: HUFA, highly unsaturated fatty acid; LCFA, long-chain fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid

Foraminifera, sedimentary detritus, Nematoda, phytodetritus, prokaryotes, and Fungi (< 0.1%) (Fig. 7).

The contribution of Foraminifera ranged from 31.0 ± 28.2% (5–95% Bayesian credibility intervals BCI, 0.70–86.2%) to 39.1 ± 23.6% (5–95% BCI, 1.30–77.2%) and 38.6 ± 27.7% (5–95% BCI, 1.10–82.6%) to the total food sources of *P. semperiana*, *Benthodytes* sp., and *B. typica*, respectively, whereas sedimentary detritus potentially contributed between 17.5 ± 15.8% (5–95% BCI, 0.80–48.7%) and 23.8 ± 21.5% (5–95% BCI, 0.70–65.6%) to the total diet of *Peniagone* sp. and *Galatheathuria* sp. Phytodetritus was suggested to account for 8.50 ± 6.80% (5–95% BCI,

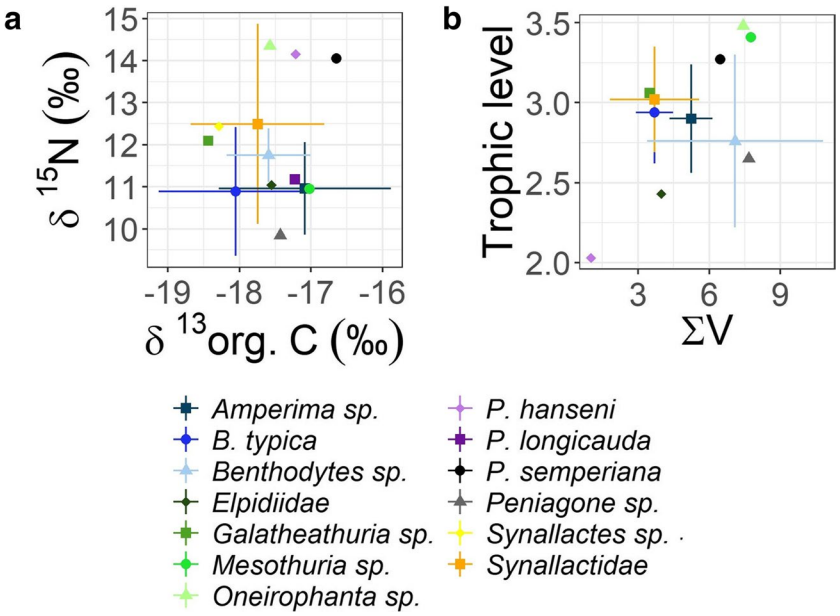

Fig. 6 **a** Isotopic composition of carbon (δ^{13}org. C, ‰) and N (δ^{15}N, ‰) of holothurian body wall tissue from the Peru Basin; **b** trophic level (TL) and heterotrophic enrichment factor $\sum V$ of holothurians. Error bars indicate 1 SD.

0.70–22.0%) to 16.9 ± 11.8% (5–95% BCI, 1.20–37.8%) to the food sources of *Oneirophanta* sp. and *Peniagone* sp., and heterotrophic prokaryotes contributed between 7.80 ± 16.1% (5–95% BCI, 0.30–24.3%) and 16.1 ± 15.5% (5–95% BCI, 0.40–47.1%) to the diets of the same species. However, the large 25%/75% and 5%/95% BCI ranges (Fig. 7) indicate that the capacity of the models to resolve the contributions of potential food sources was low, likely due to the implementation of published stable isotopic composition data that were mostly not site-specific. Hence, the model outcomes should be interpreted with care.

Fresh phytodetritus uptake by holothurians

Within 3 days, the holothurians in the feeding experiment took up 0.021 ± 0.014 mmol phytodetritus C mmol^{-1} org. C (*Amperima* sp., 0.017 ± 0.014; *Peniagone* sp., 0.039; *Benthodytes* sp., 0.018) and 0.017 ± 0.013 mmol phytodetritus N mmol^{-1} TN (*Amperima* sp., 0.013 ± 0.012; *Peniagone* sp., 0.036; *Benthodytes* sp., 0.016). Gut content of holothurians collected after 3 days still contained 0.122 ± 0.223 mmol phytodetritus C g^{-1} DM gut content and 0.083 ± 0.164 mmol phytodetritus N g^{-1} DM gut content. The feces of 1 *Amperima* sp. specimen even included 0.036 mmol phytodetritus C g^{-1} DM feces and 0.028 mmol phytodetritus N g^{-1} DM feces.

Holothurians incorporated 0.95 ± 0.78 µmol ^{13}C-HAA mmol^{-1} org. C (min, 0.51, *Benthodytes* sp.; max, 1.06 ± 1.05, *Amperima* sp.) and 5.53 ± 4.28 µmol ^{15}N-HAA mmol^{-1} TN (min, 3.05, *Benthodytes* sp.; 6.53 ± 5.66, *Amperima* sp.) (Fig. 8a).

Most of the ^{13}C in HAAs consisted of ^{13}C in glutamic acid (21.3 ± 8.93%), in aspartic acid (16.9 ± 11.1%), and in

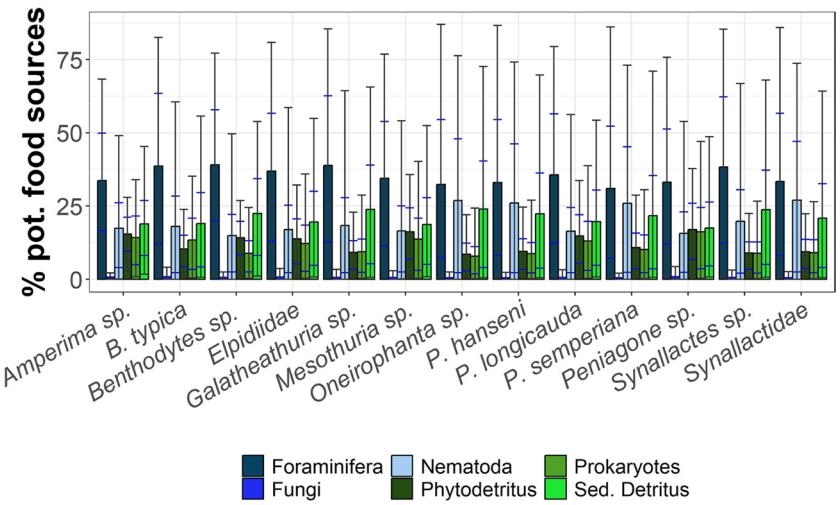

Fig. 7 Results of SIAR Bayesian mixing model showing the mean contribution of each potential food source (%) to the diet of the different holothurian species in the Peru Basin. Error bars in blue indicate 25% and 75% Bayesian credibility intervals (BCI), and error bars in gray present 5% and 95% BCI

alanine (15.3 ± 4.86%) (Fig. 9a), whereas most of the ^{15}N in HAAs was incorporated in the amino acids glutamic acid (19.5 ± 1.46%), alanine (17.5 ± 5.25%), and aspartic acid (13.7 ± 4.91%) (Fig. 9b).

Holothurians incorporated 0.007 ± 0.007 µmol ^{13}C-PLFA mmol^{-1} org. C (min, 0.002, *Benthodytes* sp.; max, 0.018, *Peniagone* sp.) and 0.064 ± 0.030 µmol ^{13}C-NLFA mmol^{-1} org. C (min, 0.059 ± 0.050, *Amperima* sp.; max, 0.075, *Peniagone* sp.) (Fig. 8b). Most of the ^{13}C in PLFAs consisted of ^{13}C in C22:1ω9 *cis* (40.3 ± 23.1%), in C16:1ω7 *cis* (13.4 ± 7.33%), and in C16:0 (11.4 ± 7.53%) (Fig. 9c), whereas most of the ^{13}C in NLFAs was incorporated in C22:1ω9 *cis* (32.1 ± 23.2%), C16:1ω7 *cis* (20.5 ± 9.16%), C20:0 (12.9 ± 8.49), and C14:0 (13.0 ± 3.30%) (Fig. 9d).

Dependence of holothurians on sediments and fresh phytodetritus

The HAAs aspartic acid, glutamic acid, leucine, phenylalanine, threonine, and valine occurred in statistically higher proportions in holothurians from the Peru Basin than in the surface sediment (0–2 cm) (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10a), implying a net accumulation of these amino acids. In comparison, the amino acids glycine, serine, and tyrosine and lysine combined showed a net deficiency, i.e., their proportions were statistically significantly higher in the surface sediment compared to the tissue of holothurians (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10a).

In the in-situ experiment, the difference in mole percentages of amino acids indicated a net deficiency of holothurians in aspartic acid, glutamic acid, leucine, and phenylalanine (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10a). In comparison, holothurians from this experiment had a net accumulation of glycine and threonine compared to the phytodetritus (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10a).

An assessment of PLFAs that contributed on average ≥ 1% to total PLFAs in body wall tissue of holothurians from the Peru Basin showed that the PLFAs C18:5ω3, C18:5(n-3,6,9,12,16), C20:0, docosapentaenoic acid (DPA, C22:5ω3), DHA, C24:0, and Me-C26:2Δ(5,9) were present in statistically higher proportions in holothurians than in surface sediment (0–2 cm) (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10b, c) indicating a net accumulation of these PLFAs in holothurian tissue. The PLFAs C16:0, C18:0, C18:1ω7 *cis*, and ARA had net deficiencies in holothurians (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10b, c).

In the in-situ experiment, the difference in mole percentages of PLFA (≥ 1% of total PLFAs in body-wall tissue in in-situ holothurians) pointed towards a net accumulation of C18:0, C18:5ω3, C18:5(n-3,6,9,12,16), C20:0, C20:2ω9, EPA/C22:1ω9, C21:0, DPA, C24:0, C26:2Δ(9,19), and Me-C26:2Δ(5,9) in holothurian tissue (Wilcoxon rank sum tests W, $p \leq 0.05$; Table S4, Fig. 10b, c). In comparison, holothurians had a net deficiency of EPA, Me-C28:2Δ(5,9) and C28:2Δ(11,21) (Wilcoxon rank sum test W, $p < 0.04$; Table S4, Fig. 10b, c) compared to the phytodetritus.

Discussion

For most of the holothurian species present in the Peru Basin, very little to no information is available about their feeding strategies and diet preferences. Therefore, we first infer feeding strategies for holothurians based on CSIA, and subsequently, we explore whether resource

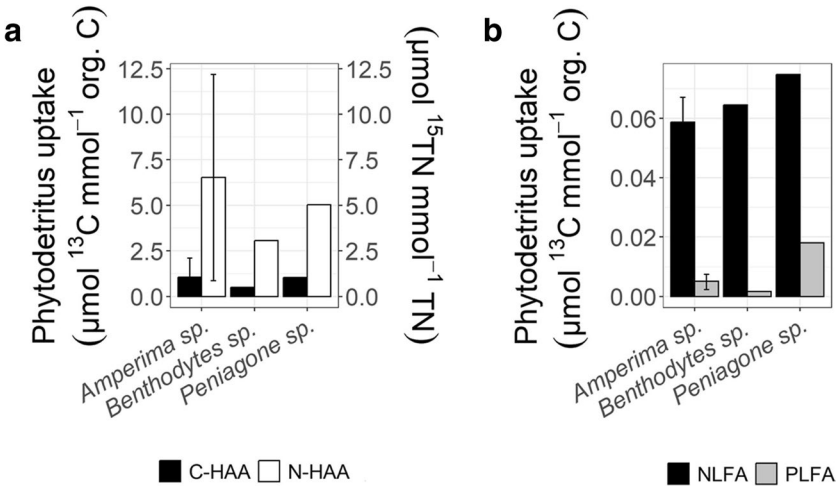

Fig. 8 Uptake of phytodetritus C and N in the compounds **a** HAAs (µmol ^{13}C mmol^{-1} org. C, µmol ^{15}N mmol^{-1} TN); **b** PLFAs (µmol ^{13}C mmol^{-1} org. C) and NLFAs (µmol ^{13}C mmol^{-1} org. C) of holothurian tissue from the *in-situ* experiment. Error bars indicate 1 SD

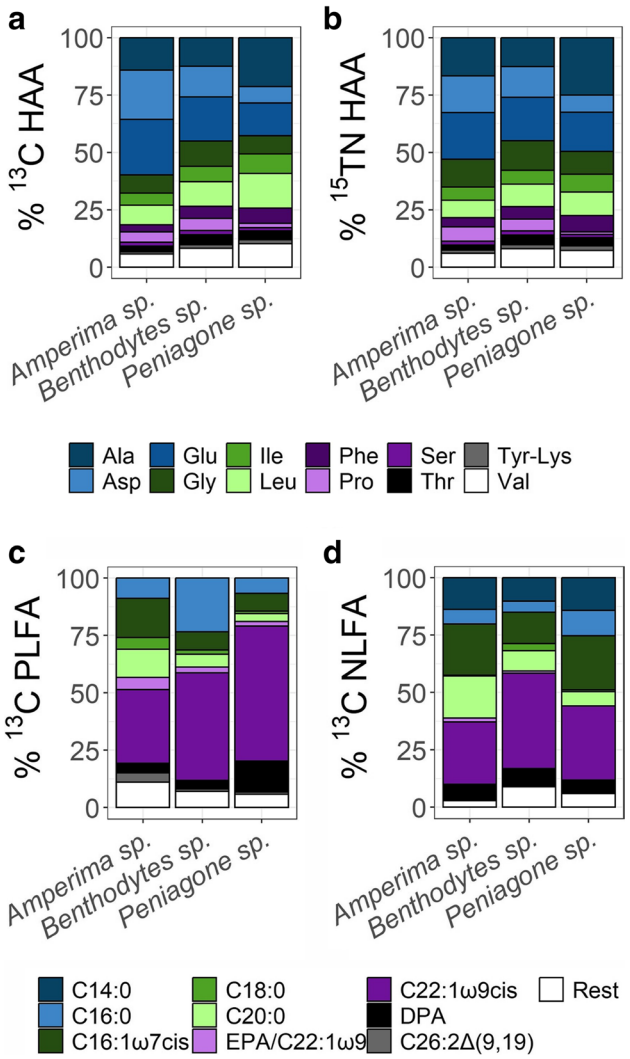

Fig. 9 Contribution (%) of individual **a** phytodetritus C and **b** N uptake in hydrolysable amino acids (HAAs) to total phytodetritus C and N uptake in HAAs and **c**, **d** contribution (%) of phytodetritus C uptake by individual phospholipid-derived fatty acids (PLFAs) and neutral-lipid-derived fatty acids (NLFAs) to total phytodetritus C uptake in PLFAs and NLFAs of holothurian body wall tissue from the in-situ experiment. The PLFA and NLFA pools "Rest" include all PLFAs and NLFAs, respectively, that contribute < 2.5% to total % phytodetritus C uptake in PLFAs or NLFAs of the mean holothurian body wall tissue from the in-situ experiment. Abbreviations of HAAs: 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. Abbreviations of PLFAs and NLFAs: DPA, docosapentaenoic acid (C22:5ω3); EPA, eicosapentaenoic acid (C20:5ω3)

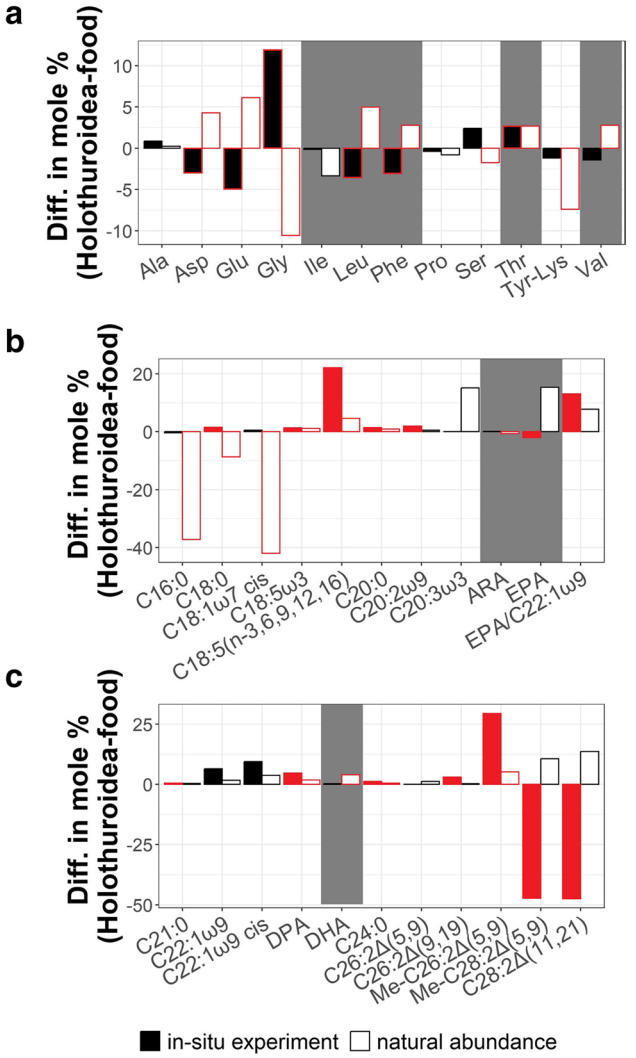

Fig. 10 Comparison of the mean difference in mole percentages **a** of hydrolysable amino acids (HAAs) and **b**, **c** phospholipid-derived fatty acids (PLFAs) between holothurian body wall tissue and the corresponding food source (natural abundance holothurians with the 0–2 cm sediment layer, white bars; holothurians from the in-situ experiment with ^{13}C- and ^{15}N-enriched phytodetritus, black bars). The gray shading highlights "essential" amino acids (Phillips 1984) and fatty acids (Glencross 2009). Statistical differences in mole percentages between were determined by Wilcoxon rank sum tests (Table S4) and indicated by the red outlines. Abbreviations of HAAs: 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. Abbreviations of PLFAs and NLFAs: ARA, arachidonic acid (C20:4ω6); DHA, docosahexaenoic acid (C22:6ω3); DPA, docosapentaenoic acid (C22:5ω3); EPA, eicosapentaenoic acid (C20:5ω3)

partitioning exists that could facilitate the high holothurian biodiversity in the Peru Basin. Finally, we discuss how holothurians affect organic C availability in sediments of the Peru Basin, and whether holothurians are dependent on amino acids and phospholipid-derived fatty acids from fresh phytodetritus.

Holothurians trophic level and inferred feeding strategy

Based on the δ^{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. to 14.4‰ for *Oneirophanta* sp. 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 CSIA (amino acids, fatty acids) with results of a Bayesian mixing model, biomarkers, grain size analysis, and concentration factors for PLFAs.

CSIA of amino acids allows to calculate the total heterotrophic re-synthesis of amino acids $\sum V$ which is a proxy for "heterotrophic reworking of proteinaceous material" (McCarthy et al. 2007). In the context of holothurians, $\sum V_{body-wall\ tissue} > \sum V_{sediment}$ indicates that the heterotrophic gut microbiome re-works detritus that was ingested by the holothurians or that holothurians selectively consume material that has undergone more re-working. Among various holothurian species, species with a low $\sum V$ value likely ingest more phytodetritus than sedimentary detritus compared to species with a high $\sum V$ value, as McCarthy et al. (2007) detected a linear increase in $\sum V$ from algae, via zooplankton, to detrital material.

When deep-sea holothurians have source amino acids in their gut contents that are enriched in ^{15}N relative to sediments, these holothurians are likely secondary consumers of detritus, whereas the microbial community in their guts are primary consumers of detritus (Romero-Romero et al. 2021).

Ratios of the fatty acids C16:1ω7 to C16:0 and DHA to EPA are biomarkers for diatoms when $\frac{C16:1\omega7}{C16:0} > 1$ or $\frac{DHA}{EPA} < 1$ (Kharlamenko et al. 1995; Budge and Parrish 1998; Dalsgaard et al. 2003; Parrish et al. 2005) and for dinoflagellates when $\frac{C16:1\omega7}{C16:0} < 1$ or $\frac{DHA}{EPA} > 1$ (Budge and Parrish 1998; Dalsgaard et al. 2003; Parrish et al. 2005).

Order Elasipodida

P. hanseni ($n = 1$) is a deposit feeder of the family Laetmogonidae, which has a trophic level of 2.0, a low level of heterotrophic re-synthesis of amino acids and feeds selectively 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 analyzed and in its gut content, (large) parts of the sedimentary detritus likely consist of diatom-derived phytodetritus.

Elpidiidae gen sp. ($n = 1$) (family Elpidiidae) has a trophic level of 2.4 and medium level of heterotrophic re-synthesis of amino acids. This species is a selective deposit feeder that might ingest sedimentary detritus (Bayesian mixing model, 19.5 ± 17.6% contribution; 5–95% BCI, 0.80–54.9%) and/or Foraminifera (Bayesian mixing model, 36.9 ± 25.9% contribution; 5–95% BCI, 1.00–80.9%). Though, this result should be interpreted with caution due to the low capability of the Bayesian mixing model to constrain the contribution of the potential food sources.

The benthopelagic *Peniagone* sp. ($n = 1$) of the family Elpidiidae has an estimated trophic level of 2.7 and a very high level of heterotrophic re-synthesis of amino acids. This species has a "sweeping" feeding style (Roberts et al. 2000) and was found to assimilate fresh phytodetritus at PAP (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, but likely also on sedimentary detritus as indicated by the high $\sum V$ value. In fact, the poorly resolved Bayesian mixing model suggests an equal contribution of phytodetritus (16.9 ± 11.8%; 5–95% BCI, 1.20–37.8%) and sedimentary detritus (17.5 ± 15.8%; 5–95% BCI, 0.80–48.7%) to the holothurian diet.

Amperima sp. ($n = 9$) 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. This species is a very selective surface deposit feeder 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. 2003a). Based on the PLFA composition of its gut content, we found that *Amperima* sp. from the Peru Basin likely feeds on dinoflagellate-derived phytodetritus. 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 diet preferences of the well-studied *Amperima* sp. can differ substantially between ocean basins.

Benthodytes sp. ($n = 6$) 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. 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. However, it likely does not or only moderately selects for specifically detritus-enriched particles. 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. hosts a large biomass of living heterotrophic prokaryotes. Though, the mean $\delta^{15}N_{source\ AA}$-value is below the mean $\delta^{15}N_{source\ AA}$-value across all holothurians investigated in this study, we hypothesize that *Benthodytes* sp. is a secondary consumer, and its microbial gut community is the primary consumer of detritus.

B. typica ($n = 5$) belongs to the family Psychropotidae and its trophic level is comparable (2.9) to the trophic level of *Benthodytes* sp. This species has a medium level of heterotrophic re-synthesis of amino acids and feeds selectively on smaller particles (medium grain size: 14.2 µm) from the ambient sediment. These smaller particles contain a four times higher PLFA concentration than the surrounding sediment and consist partially of diatom-derived phytodetritus. Reliance on phytodetritus is confirmed by the PLFA composition of *B. typica* body walls. In addition, this species either feeds selectively on sediment-bound prokaryotes or hosts prokaryotes because bacteria-specific PLFAs (i.e., C16:0, C16:1ω7 *cis*, and C18:1ω7 *cis*) contribute almost 30% to the total PLFA composition in feces. Also, the mean $\delta^{15}N_{source\ AA}$-value is enriched compared to the mean $\delta^{15}N_{source\ AA}$-value across all holothurians. Since 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 the gut microbiome.

P. longicauda ($n = 1$) from the family Psychropotidae has a medium level of heterotrophic re-synthesis of amino acids. Feeding selectivity was the highest in our data, though, surprisingly, at PAP this species was found to feed less selectively than *P. diaphana* (FitzGeorge-Balfour et al. 2010). *P. longicauda*'s diet consists likely mainly of diatom-derived phytodetritus, 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 on it sporadically, because 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.

P. semperiana ($n = 1$) (family Psychropotidae) has an estimated trophic level of 3.3, likely related to the high level of heterotrophic re-synthesis of amino acids. 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 might also consume diatom-derived phytodetritus.

Order Holothuriida

Mesothuria sp. ($n = 1$) 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. Indeed, in a study on the Hawaiian slope, gut contents of *Mesothuria carnosa* Fisher, 1907, 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 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. ($n = 1$) 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. This species feeds selectively 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 (Mevenkamp et al. 2019). The specimen collected in the Peru Basin likely fed on diatom-derived phytodetritus 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, whereby 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 composition in the gut content of *Oneirophanta* sp. Therefore, the diet preferences of this species in the Peru Basin are less clear.

Synallactidae gen sp. ($n = 3$) (family Synallactidae) has an estimated trophic level of 3.0 ± 1.5 and a medium level of heterotrophic re-synthesis of amino acids. It feeds extremely selectively and consumes particles of a median grain size that is smaller than the median grain size of the surface sediment in the Peru Basin. The PLFA composition of the body wall and the gut content of Synallactidae gen sp. indicates that this species predates upon agglutinated foraminiferans, and it consumes diatom-derived phytodetritus. However, it

is not possible to differentiate whether Synallactidae gen sp. is a primary consumer of the phytodetritus or a secondary consumer, whereupon the foraminiferans are the primary consumer. The PLFA composition of the feces shows that this holothurian species is also a bacterivore as bacteria specific PLFAs (i.e., C16:0, C16:1ω7 *cis*, and C18:1ω7 *cis*) contribute 42% to the total PLFA composition in the feces. If Synallactes hosted a large community of living bacteria, we would expect to detect a significant amount of bacteria-specific PLFAs in the gut content and a higher level of heterotrophic re-synthesis of amino acids. Additionally, the mean $\delta^{15}N_{source\ AA}$-value of its body-wall tissue would likely be enriched compared to the mean $\delta^{15}N_{source\ AA}$-value across all species. Therefore, we assume that Synallactidae gen sp. has a mixed diet consisting of foraminiferans, bacteria, and phytodetritus.

Galatheathuria sp. the family Synallactidae has an estimated trophic level of 3.1 and a medium level of heterotrophic re-synthesis of amino acids. Like Synallactidae gen sp., it feeds extremely selectively) and *Galatheathuria* sp. seems to consume preferably diatom-derived phytodetritus.

Classification of holothurian trophic groups

Here, we propose a classification system of trophic groups for holothurians from the Peru Basin (Fig. 11). It is based on cluster analysis of trophic levels, heterotrophic re-synthesis level of amino acids, feeding selectivity, and diet preferences. Trophic group 1 has a narrow trophic level between 2.7 and 2.9, feeds either very selectively or unselectively on phytodetritus and sedimentary detritus. Though, not statistically confirmed, we suggest splitting this trophic group 1 in two subgroups: Trophic group 1a includes the very selectively feeding *Amperima* sp. with its dinoflagellate-derived detritus diet and trophic group 1b comprises the unselective feeders *Benthodytes* sp., *B. typica*, and *Peniagone* sp. that consume diatom-derived phytodetritus and sedimentary detritus.

Trophic group 2, in comparison, has a very large trophic level range between 2.0 and 3.5. This trophic group could be split again into two subgroups: Trophic group 2a (*P. longicauda*, *Galatheathuria* sp., Synallactidae gen sp., *Mesothuria* sp., *P. semperiana*) has a trophic level between 3.0 and 3.4 and feeds extremely selectively on phytodetritus or on a mixed diet with phytodetritus, sedimentary detritus, and bacteria. Trophic group 2b consists of Elpidiidae gen sp., *P. hanseni*, and *Oneirophanta* sp. that feed selectively on phytodetritus, foraminiferans, sedimentary detritus, and bacteria.

Potential resource partitioning and its importance for holothurian biodiversity in the Peru Basin

With 23 different holothurian morphotypes observed, the holothurian biodiversity in the abyssal plain of the Peru Basin is higher than in the abyssal plains around the Bullard Fracture Zone and the South Orkney Islands (Southern Ocean; Ogawa et al. 2022), the abyssal central Cantabrian Sea (NE Atlantic; Fernández-Rodríguez et al. 2019), the abyssal Aleutian Basin (Bering Sea, NE Pacific; Sigwart et al. 2023), and at PAP (Iken et al. 2001), but lower than at Station M (NE Pacific; Kuhnz et al. 2020) and in the Clarion-Clipperton Fracture Zone (CCZ, central N Pacific; Simon-Lledó et al. 2023a, b). The co-existence of so many deposit-feeding species of the same taxonomic class is astonishing, as one might expect a strong competition among the different taxa leading ultimately to taxa being outcompeted.

In the 1970s, Menge and Sutherland (1976) synthesized that high local species diversity is maintained by predation

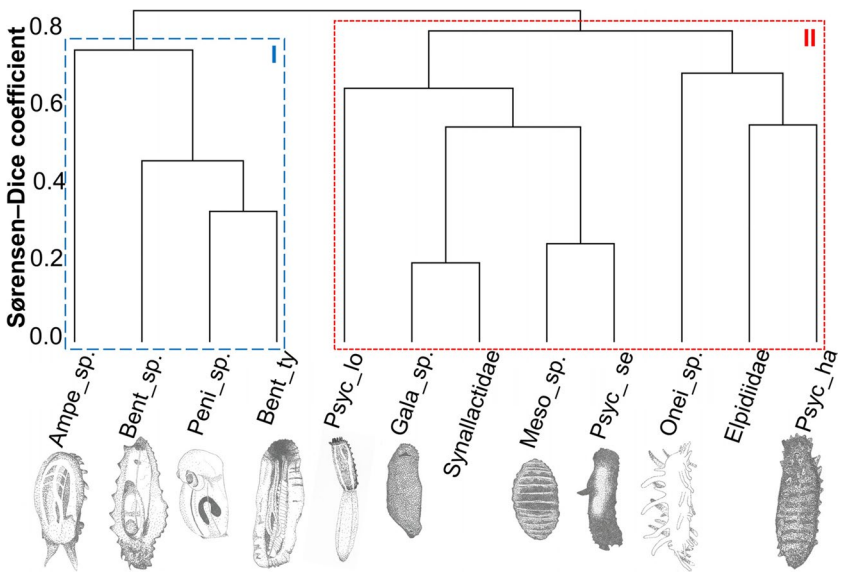

Fig. 11 Dendrogram of the Sørensen–Dice coefficient calculated for holothurian species from the Peru Basin based. Trophic group I includes *Amperima* sp. (Ampe_sp.), *Benthodytes* sp. (Bent_sp.), *Peniagone* sp. (Peni_sp.), and *Benthodytes typica* (Bent_ty). Trophic group II comprises *Psychropotes longicauda* (Psyc_lo), *Galatheathuria* sp. (Gala_sp.), Synallactidae gen sp. (Synallactidae), *Mesothuria* sp. (Meso_sp.), *Psychropotes semperiana* (Psyc_se), *Oneirophanta* sp. (Onei_sp.), Elpidiidae gen sp. (Elpidiidae), and *Psychronaetes hanseni* (Psyc_ha). Illustrations of holothurians by Tanja Stratmann

(i.e., predation hypothesis; Paine 1966, 1971) and competition (i.e., competition hypothesis; Dobzhansky 1950). To reduce this interspecific competition, similar species may subdivide their use of the available food sources (Schoener 1974; Winemiller and Pianka 1990), a process known as resource partitioning. Such resource partitioning along a decomposition gradient of food sources likely accounts for the high diversity of detritivores in soils (Schneider et al. 2004; Chahartaghi et al. 2005; Hishi et al. 2007). In an experiment with shallow-water benthic deposit-feeding echinoderms (i.e., holothurians and echinoids), however, species-specific traits and not resource partitioning explained the co-occurrence of species of the same feeding guild (Godbold et al. 2009).

The high holothurian biodiversity in the Peru Basin, in contrast, seems to be maintained by a combination of resource partitioning and particular selectivity, likely due to species-specific traits, such as different tentacle morphology (Roberts and Moore 1997; Pierrat et al. 2022), feeding rates and gut residence time (Godbold et al. 2009; Durden et al. 2020), and/or mobility (Smith et al. 1993; Kaufmann and Smith 1997; Miguez-Salas et al. 2020). For instance, *Amperima* sp. as the only member of trophic group 1a ("Classification of holothurian trophic groups") moves non-randomly (Bluhm and Gebruk 1999) in a uniform direction with a mean speed of 3.3 ± 1.8 cm h^{-1} (Wigham 2002). It moves mostly slowly and is stationary only 22% of the time (Wigham 2002). In fact, at PAP, *A. rosea* had a 20 times higher tracking rate between 1997 and 1998 than the other holothurians present (Bett et al. 2001). As *Amperima* sp. shares the same tentacle structure (peltate) with *Benthodytes* sp. and *P. longicauda* (Roberts et al. 2000), we hypothesize, that indeed, mobility is the specific trait that enables this species to create its own feeding niche and to partition its food sources from the resources of the other holothurian species.

In comparison, the member of trophic group 1b *Peniagone* sp. can swim (Stratmann, personal observation; Pawson and Foell 1986; Bluhm and Gebruk 1999; Gong et al. 2020) and it was estimated that *Peniagone leander* Pawson & Foell 1986, spends about half of its time at the seafloor and the other half swimming within 50 m above the seafloor (Pawson and Foell 1986). At the seafloor, *Peniagone vitrea* Théel, 1882, moves with 8.1 ± 7.9 cm h^{-1} (Smith et al. 1993) to 10.1 ± 12.9 cm h^{-1}(Kaufmann and Smith 1997) in a "running pattern" (frequency of records, 46%) and in large loops (39%) (Kaufmann and Smith 1997). The species is therefore faster than *Amperima* sp., but slower than *O. mutabilis mutabilis* (64.6 ± 68.4 cm h^{-1}) and *Synallactes profundi* (Koehler & Vaney, 1905) (12.7 ± 11.9 cm h^{-1}) (Kaufmann and Smith 1997). *Peniagone* sp. has simple peltate tentacles (Roberts et al. 2000) and uses selective ingestion as primary feeding strategy (Purinton et al. 2008). Hence, in the Peru Basin this species probably co-occurs with the other holothurians due to its specific mobility trait and its selectivity.

P. longicauda as an example of trophic group 2a moves at the seafloor with a speed of 2.6 cm h^{-1} (Wigham 2002) and it may be able to "sail" using its velum (Gebruk 1995). This species has peltate tentacles (Roberts and Moore 1997) and a complex gut system with a more extended pharynx and rectum compared to *Oneirophanta* sp. and *M. villosus* (Théel, 1886) (Moore et al. 1995). This type of gut system might function as a mixing chamber (Penry and Jumars 1987) in which fermentation occurs (Roberts et al. 2000). Following that line of thought, the pattern we see in our data might not show extreme selectivity of food sources, but very efficient fermentation. However, as Khripounoff and Sibuet (1980) found a four times enrichment of organic C in the particles in *P. longicauda*'s pharynx compared to the ambient sediment, we propose that in the Peru Basin this species combines selective feeding with efficient fermentation.

Oneirophanta sp. (trophic group 2b) is the fastest holothurian species in this study and moves non-randomly with a speed of 84.8 ± 80.9 cm h^{-1} (Smith et al. 1993) to 128.9 ± 68.3 cm h^{-1} (Smith et al. 1997) in a "running pattern" (frequency of records, 60%) (Kaufmann and Smith 1997). With its digitate tentacles (Roberts and Moore 1997) and its fast locomotion, *Oneirophanta* sp. is adapted to exploit fast fresh phytodetritus (Roberts et al. 2000; Witbaard et al. 2001; Wigham et al. 2003a) and replaces its gut content completely every 5 days (Witbaard et al. 2001). When *P. longicauda* and *Oneirophanta sp.* co-occur like in the Peru Basin, *Oneirophanta sp.* outcompetes *P. longicauda* when fresh phytodetritus is available, but when fresh/labile detritus is rare, both species compete for the same material (Neto et al. 2006; FitzGeorge-Balfour et al. 2010).

Hence, we suggest that the high biodiversity of holothurians in the Peru Basin is maintained by differences in species-specific traits (i.e., mobility, tentacle morphology, gut structure) which allow resource partitioning and selectivity.

The effect of holothurians on sedimentary organic carbon availability in the Peru Basin

Nine of the 13 species analyzed in this study were detected visually in an area of the Peru Basin that Stratmann et al. (2018b) called "reference site." There, they had a density of 157 ± 9 ind. ha^{-1} (Stratmann et al. 2018b) and contributed 65% to total holothurian density in the Peru Basin. For a subgroup of these species for which data on gut contents (Table 5) and densities (Stratmann et al. 2018b) were available, it was possible to calculate the gut sediment stock. Together, *Amperima* sp., *Benthodytes* sp., Elpidiidae gen sp., *Oneirophanta* sp., *Peniagone* sp., *P. hanseni*, and *Synallactes* sp. (morphotype "pink") hold a gut sediment stock of 0.02 g DM gut content m^{-2}. In comparison, in the Santa

Catalina Basin (NE Pacific) the three dominant surface deposit feeders *Pannychia moseleyi* Théel, 1882 (holothurian), *Chiridota* sp. Eschscholtz, 1829 (holothurian), and *Bathybembix bairdii* Dall, 1889 (gastropod) have a gut standing stock of 0.27 g DM gut content m^{-2} (Miller et al. 2000). Even though, it seems as if the holothurians in the Peru Basin have a very small gut sediment stock, the species used to calculate the stocks include only one (*Amperima* sp.) of the three dominant holothurian species in the Peru Basin. Therefore, a direct comparison of these two datasets is not possible, but it is likely that the gut sediment stock of holothurians in the Peru Basin is comparable to the gut sediment stock of the dominant surface deposit feeders in the Santa Catalina Basin.

Based on the sediment stocks, it is possible to estimate sediment ingestion rates and upscale how much of the sediment in the Peru Basin is bioturbated by 65% of all holothurians. Unfortunately, we lacked information about gut residence times (GRTs) for most holothurian species for which we had gut sediment stock data. Therefore, we approximated min and max ranges of sediment ingestion rates by applying the same GRTs that were used previously in the literature ($min_{GRT} = 1$ day, Miller et al. 2000; $max_{GRT} = 6$ days, Lauerman et al. 1997) and multiplying it with a gut sediment stock of 0.02 g DM gut content m^{-2}. Hence, 65% of the holothurians reworked between 1.2 and 7.3 g DM sediment m^{-2} $year^{-1}$. In comparison, the shallow-water tropical coral reef holothurians *Holothuria atra* Jaeger, 1833, and *Stichopus chloronotus* Brandt, 1835, consume 4.6 kg DM sediment m^{-2} $year^{-1}$ at Lizard Island (Great Barrier Reef, Australia, SW Pacific) which implies they bioturbate all the sediment in the upper 5 mm (Uthicke 1999). *Holothuria scabra* Jaeger, 1833, populations in Fiji (SW Pacific) even ingest 10.5 kg DM sediment m^{-2} $year^{-1}$ (Lee et al. 2018) and in W Australia *Holothuria whitmaei* Bell, 1887, ingests yearly between 2 and 14% of all the available sediment (0 – 5 mm sediment depth) in 1 ha (Shiell and Knott 2010).

It was estimated that *P. moseleyi*, *Chiridota* sp., and *B. bairdii* process 39 – 52% of the daily particulate organic carbon (POC) flux to the Santa Catalina Basin (Miller et al. 2000), whereas at Station M, mobile invertebrate megabenthos including holothurians could process 0.2 to 4% of the daily POC flux that reaches the seafloor (Smith et al. 1993; Lauerman et al. 1997). Hence, an estimated ingestion of 4 to 27% of the daily POC flux to the Peru Basin (1.3 mg C m^{-2} d^{-1}; Hoving et al. 2023) by 65% of the holothurian community seems reasonable.

Not all the organic material that holothurians ingest is assimilated. Instead, a substantial fraction is egested as feces. In fact, the fecal cast of a holothurian in the in-situ experiment still contained 0.036 mmol phytodetritus C g^{-1} DM feces and 0.028 mmol phytodetritus N g^{-1} DM feces from the *S. costatum* with which it was fed. Also, in the Nazaré canyon, the fecal casts of *M. musculus* still contained 4.04 ± 0.61 mg biopolymeric C g^{-1} DM sediment (Amaro et al. 2010). Hence, holothurians increase the patchiness of organic C distribution in abyssal marine surface sediments by producing fecal casts which, in theory, could be an important food source for detritivores/coprophagous feeders. However, a time-lapse camera study in 1978 showed that the fecal cast of a holothurian was still distinguishable after 202 days (Paul et al. 1978) pointing towards a low number of abyssal coprophagous feeders that target holothurian fecal casts.

Dependence of holothurians on fresh phytodetritus

In the in-situ experiment, the holothurians assimilated the *S. costatum*-derived PLFAs C14:0, C16:0, C16:1ω7 *cis*, and EPA (Volkman et al. 1989; Table S5), whereas the largest fraction of ^{13}C-PLFAs and ^{13}C-NLFAs (PLFAs: 32 – 59%; NLFAs: 27 – 42%; Fig. 9) was C22:1ω9 *cis*. This fatty acid was not present in *S. costatum* (Table S5), but it can be biosynthesized via carbon-chain elongation of the precursor C18:1ω9 *cis* (Ackman and Castell 1966) which existed in *S. costatum* (Table S5). However, the ecologically/physiologically most interesting ^{13}C-PLFA and ^{13}C-NLFA is DPA, which was not detected in *S. costatum* (Table S5). This fatty acid is biosynthesized from EPA via a two carbon-chain elongation (Mansour et al. 2005) and is used by the shallow-water ophiuroid *Amphiura (Amphiura) elandiformis* A.M. Clark, 1966, to synthesize DHA via Δ4 desaturation (Mansour et al. 2005). One of the *Amperima* sp. specimens in the in-situ experiment was in fact able to biosynthesize DPA (0.080 nmol ^{13}C-PLFA $mmol^{-1}$ org. C) and DHA (0.019 nmol ^{13}C-PLFA $mmol^{-1}$ org. C) indicating that this biosynthetic pathway can be performed by a broader range of taxonomic classes, including ophiuroids (Mansour et al. 2005), asteroids (Jeffreys et al. 2009), holothurians (this study), arthropods (Jeffreys et al. 2009), polychaetes (Jeffreys et al. 2009), and anthozoans (Jeffreys et al. 2009). Furthermore, it implies that holothurians, or at least *Amperima* sp., are less dependent on ingesting all "essential" fatty acids with their food as they can biosynthesize DHA themselves when EPA is available.

The ability of *Amperima* sp. to biosynthesize DHA from a precursor fatty acid might explain why holothurians in the Peru Basin showed a net accumulation in this "essential" fatty acid (Fig. 10c), while they had a net deficiency in ARA. They could not remedy this deficiency in ARA by ingesting fresh phytodetritus in the in-situ experiment because *S. costatum* did not contain this specific fatty acid. Besides these "essential" fatty acids, holothurians also had a net deficiency in C16:0, C18:0, and C18:1ω1 *cis* (Fig. 10b). C16:0 and C18:1ω1 *cis* are fatty acids that are present in bacteria (Table 1). Hence, a deficiency in these fatty acids could imply that holothurians either did not ingest enough bacteria

or bacteria-covered particles (Pierrat et al. 2022) or that the microbiome in their guts did not produce these fatty acids in sufficient quantities.

Holothurians from the Peru Basin had a net accumulation of most EAAs (leucine, phenylalanine, threonine, valine), whereas the specimens from the in-situ experiment showed a net deficiency in them. This indicates that these specific EAAs are not limited in the Peru Basin, but instead are available in sufficient concentrations so that a pulse of fresh phytodetritus will likely not be completely depleted.

Holothurians are not only dependent on specific "essential" fatty acids and EAAs that they have to take up by grazing upon phytodetritus or biosynthesize partly themselves. They also depend on phytodetritus containing the required chemical composition which changes depending on the growth conditions of phytoplankton (Grosse et al. 2017, 2019). Already from 2000 to 2015 the pelagic phytoplankton community has changed on a global scale with either a decrease in diatom abundance, and/ or a change in the physiology of phytoplankton (Lorrain et al. 2020). For the Peru Basin, no further changes in phytoplankton species richness are predicted for the period from 2081 to 2100 (Benedetti et al. 2021), but less POC flux is predicted to reach the abyssal seafloor in 2100 compared to present day conditions (Sweetman et al. 2017). This implies that holothurians in the Peru Basin will be more dependent on pulses of fresh phytodetritus—though not on *S. costatum*—in the future than at present day as the sediment will likely contain less "essential" fatty acids and EAAs that originated from surface primary producers and eventually sank to the seafloor than now.

Conclusion

This study identified feeding strategies and diet preferences of 13 putative holothurian species from the Peru Basin which can be classified in two major trophic groups based on trophic level, level of heterotrophic re-synthesis of amino acids, feeding selectivity, and diet preferences. The differences in selectivity and resource partitioning were likely related to species-specific variability in mobility, tentacle morphology, and gut structure and allowed the high biodiversity of holothurians in the abyssal plain of the Peru Basin. It was estimated that a subgroup of the holothurian community is able to process between 4 and 27% of the daily POC flux and, in doing so, increases the heterogeneity of organic matter availability in the Peru Basin. Under current conditions, holothurians are limited in the "essential" fatty acid ARA, while at least one species of the assemblage is able of biosynthesizing DHA from EPA. EAA is still available in sufficient concentrations in the environment, but this might change in the future when less POC flux reaches the abyssal plains.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for animal testing, animal care, and use of animals were followed by the authors.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities and are mentioned in the acknowledgements, if applicable. The study is compliant with CBD and Nagoya protocols.

Data availability The datasets generated during and/or analyzed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.10188555.

Author contribution TS and DvO conceived the study; TS, DvO, and AKS performed fieldwork; TS and PvB performed lab analysis; TS drafted the manuscript; and TS, DvO, PvB, and AKS contributed to revising the manuscript to its final version which was approved by all.

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