ORIGINAL PAPER

Feeding in spatangoids: the case of *Abatus Cordatus* in the Kerguelen Islands (Southern Ocean)

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Abstract

Irregular urchins exclusively live in marine soft bottom habitats, dwelling either upon or inside sediments and selectively picking up sediment grains and organic particles, or swallowing bulk sediment to feed on the associated organic matter. The exact food source and dietary requirements of most irregular echinoids, however, remain incompletely understood. The schizasterid species *Abatus cordatus* (Verrill, 1876) is a sub-Antarctic spatangoid that is endemic to the Kerguelen. The feeding behaviour of *A. cordatus* was investigated using simultaneously metabarcoding and stable isotope approaches. Comparison of ingested and surrounding sediments by metabarcoding revealed a limited selective ingestion of prokaryotes and eukaryotes by the urchin. Compared to surrounding sediments, the gut content had (i) higher carbon and nitrogen concentrations potentially due to selective ingestion of organic matter and/or the sea urchin mucus secretion and (ii) $\delta^{15}N$ enrichment due to the selective assimilation of lighter isotope in the gut. Feeding experiments were performed using ^{13}C and ^{15}N-enriched sediments in aquariums. The progression of stable isotope enrichment in proximal and distal parts of the digestive track of *A. cordatus* revealed that all particles are not similarly transported likely due to siphon functioning. Ingestion of water with associated dissolved and particulate organic matter should play an important role in urchin nutrition. *A. cordatus* had a gut resident time fluctuating between 76 and 101 h and an ingestion rate of 36 mg dry sediment h^{-1} suggesting that dense populations of the species may play a key ecological role through bioturbation in soft bottom shallow-water habitats of the Kerguelen Islands.

Keywords Irregular sea urchin · Selectivity · Deposit feeding · Ingestion rate · Meiofauna · Nematode

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Introduction

Aquatic sediments cover over 70% of the Earth's surface and remains poorly understood (Dorgan et al. 2006). They constitute niche habitats for deposit feeding organisms that ingest sedimented material of low nutritional value (Lopez and Levinton 1987; Jumars 1993). In comparison with fermenters, deposit feeders have short residence times of material in their guts as they principally rely on digestion and absorption of labile components diluted in large volumes of sediment (Plante et al. 1990). Due to dilution and to the uncompleted digestion of refractory material, deposit feeders need to process their food at prodigious rates reaching daily sediment ingestion of several times their own weight (Jumars 1993).

Among sea urchins, irregular echinoids dwell upon or within soft sediments and commonly exhibit highly modified modes of food intake (Mooi 1990). Most of them lack teeth and jaw (the Aristotle's lantern), they either pick up

sediment grains and organic particles using specialized tube feet, or swallow huge bulk sediment volumes to feed on the associated organic matter (De Ridder et al. 1985). Due to their wide distribution range, large populations, burrowing activities and feeding behaviours (Ghiold 1989), heart urchins (Spatangoida) have long attracted the attention of paleontologists (Bromley and Asgaard 1975; Gilbert and Goldring 2008), sedimentologists (Radwánski and Wysocka 2001), physiologists (De Ridder and Jangoux 1993) and marine ecologists (Hammond 1981; De Ridder and Saucède 2020). As they strongly modify physical and biochemical properties of marine sediments, and have disproportionally large effects on ecosystem functioning, spatangoids can be considered as keystone species of marine soft bottom ecosystems (Austen and Widdicombe 1998; Lohrer et al. 2004; Steneck 2013). The spatangoid burrowing activities in the sediment increase solutes exchange at the sediment–water interface (Bird et al. 1999), mix surface and deeper sediment layers increasing the depth of oxygen penetration inside the sediment (Vopel et al. 2007), influence the benthic carbon cycle (Osinga et al. 1997; Boon and Duineveld 2012) and maintain infaunal and microbial diversity (Widdicombe et al. 2000). The volume of sediment reworked by spatangoids reaches more than 60 times the volume of sediment ingested (Hollertz and Duchêne 2001; Thompson and Riddle 2005). Despite this limited fraction, the ingested sediment plays a structuring role due to biochemical modifications of sediment within the gut (Thompson and Riddle 2005). The exact source of nutrition and dietary requirements of spatangoids remain, however, incompletely understood (Jangoux and Lawrence 1982), as direct examination of gut content remains problematic and many consumed organisms are unrecognizable. In the last decade, new metabarcoding-based techniques has been implemented (Blankenship and Yayanos 2005) but still not used for spatangoids burrowers.

In the Southern Ocean, echinoids are common components of marine benthic communities (Fabri-Ruiz et al. 2017) and populations of spatangoids can constitute major bioturbers of surface sediments (Thompson and Riddle 2005). *Abatus cordatus* is a sub-Antarctic schizasterid spatangoid endemic to the subantarctic Kerguelen Islands and northern Kerguelen oceanic plateau. In shallow waters (0 to 3 m), it is represented by numerous, dense, and isolated populations but scattered individuals have been recorded down to 560 m depth (Poulin and Féral 1995). Like many other invertebrates in the Southern Ocean, *A. cordatus* is a brooding species with no larval dispersal stage in its development (Schatt and Féral 1991), which is a limit to its dispersal capabilities and restrains its potential distribution range (Poulin and Féral 1997; Ledoux et al. 2012). The Kerguelen archipelago is located at the confluence of Antarctic and sub-tropical water masses, near the polar front that currently shifts southwards (Weimerskirch et al. 2003). In the

context of global climate changes, the coasts of Kerguelen are predicted to present more acidic, fresher and warmer waters in the future (Allan et al. 2013; Gutt et al. 2015). In order to monitor these modifications and their impact on marine life, an integrative long-term observing system, the program PROTEKER was initiated in 2011 (Féral et al. 2016). Ocean acidification has disproportionate negative effects for echinoderms (Kurihara and Shiarayama 2004; Kurihara 2008; Collard et al. 2014) and preliminary results of program PROTEKER suggest that predicted changes in seafloor salinity and temperature amplitudes might shift beyond the limits of *A. cordatus* tolerance (Saucède et al. 2019). Due to the absence of larval stage, this endemic and narrow-niche species will not be able to disperse southwards and find climatic refuge areas, which raises the issue of the species potential extinction in a near future (Ledoux et al. 2012; Guillaumot et al. 2018, Saucède et al. 2019). In turn, the loss of this key species may significantly alter ecosystem functioning in nearshore habitats of the Kerguelen Islands and to better understand this threat, it is essential to obtain a picture as complete as possible of the species ecology. The aim of this study is to improve our understanding of the trophic ecology of *A. cordatus*, simultaneously using metabarcoding and stable isotope approaches to evaluate its selective feeding and feeding processes.

Material and method

Sampling

The investigated stations are located in the Morbihan Bay, in the east of the Kerguelen Islands located in the southern part of the Indian Ocean, at the northern edge of the Polar Front (Fig. 1). Two sampling stations were selected 500 m apart near Port-aux-Français. They harbor sandy sediments with similar granulometry (median grain size of 100 μm with a fraction < 63 μm representing 0.7% of the sediment) and are localized at two distinct depths: 5 m depth for Biomar (49°21′12 S–70°13′04 E) and 10 m depth for Port Pétrolier (49°21′17 S–70°12′44 E). Sampling was done in November 2017 by scuba diving. A total of 60 specimens of *A. cordatus* were collected (mean test length of 38.0 ± 3.7 mm) at Biomar ($n = 45$) and at Port Pétrolier ($n = 15$). For each station, 3 freshly collected specimens were analyzed for metabarcoding and 6 for natural isotopic composition. The remaining ones were used for feeding experiments and they were collected less than one hour before they were placed in aquaria with sediment to avoid starvation and experiments started less than two days later. Surficial 5 cm of sediment ("surrounding sediment samples") were collected in each station for granulometric analysis, metabarcoding, natural isotopic composition and feeding experiments.

Fig. 1 Positions of **a** the Kerguelen archipelago in the Indian Ocean, **b** Port-aux-Français in Kerguelen and **c** two sampling stations: Biomar (white) and Port Pétrolier (grey) in Port-aux-Français
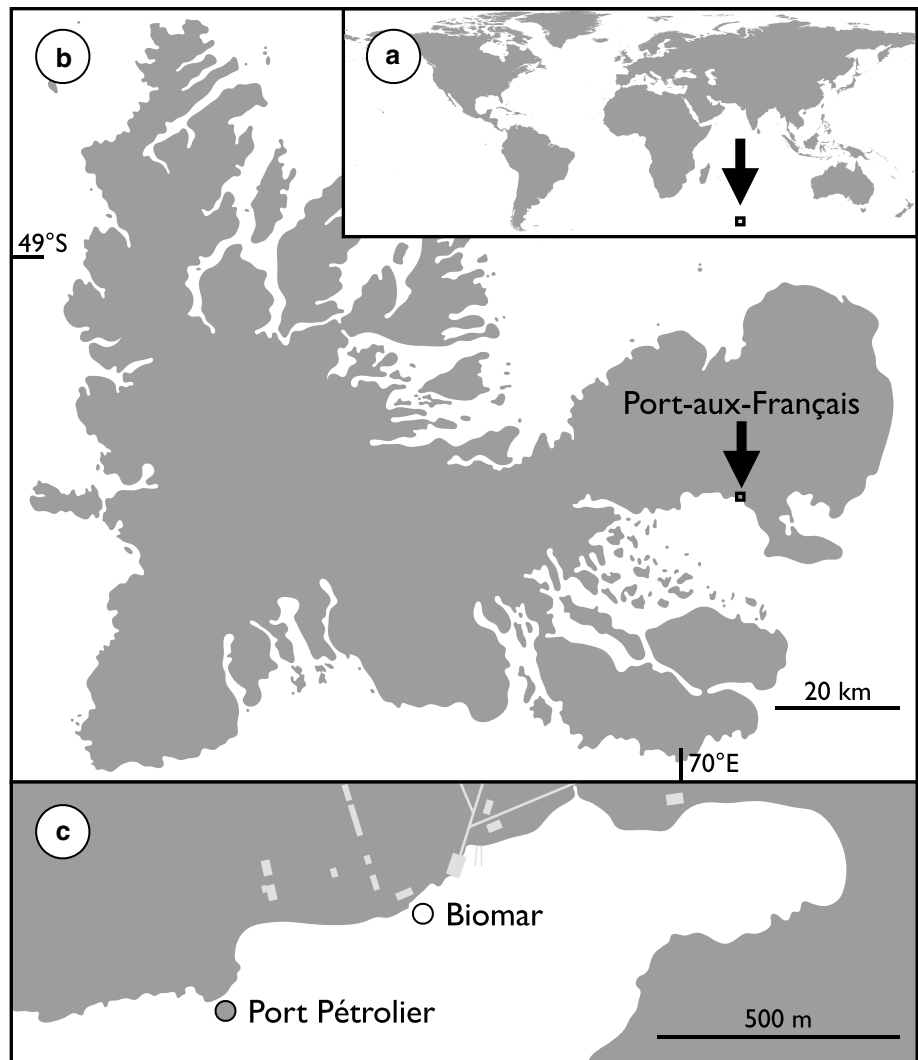
Metabarcoding

In each station, the surrounding sediment (3 samples) and the sediment filling the esophagus of *A. cordatus* (3 samples) were collected and stored in RNA-later prior to DNA extraction. DNA was extracted with the DNeasy PowerSoil Kit (Qiagen) kit following manufacturer instructions. V4 region of ADNr18S was targeted for metabarcoding analyses. The libraries were prepared with the Nextera XT kit (Illumina), and sequencing was performed with the MiSeq system (Illumina). Paired-end reads were trimmed and filtered with an AlienTrimmer (Criscuolo and Brisse 2013) at a Phred quality score threshold of 28 on a minimum length of 70 nucleotides generating 164–276 bp reads. All FASTQ data files have been deposited in NCBI-SRA public archives under the BioProject accession number PRJNA625686.

Denoising was performed using DADA2 software package (Callahan et al. 2016) implemented in QIIME 2 (Bolyen et al. 2019) via q2-dada2 plugin. DADA2 allows fine-scale variation identification through the characterization of amplicon sequence variants (ASV). Singletons and rare ASV (bellow 0.001%) were removed thanks to q2-feature-table plugin leading to 97 features for a total of 3,046,891 reads, a mean frequency of 253,907 reads per samples. Taxonomy was assigned to ASV using the q2-feature-classifier (Bokulich et al. 2018), a classify-sklearn naïve Bayes taxonomy classifier using machine learning (Pedregosa et al. 2011) against the Silva 132 99_18S database (Quast et al. 2013). Normalization of ASV table was done by DESeq2 (Love et al. 2014) implemented in the SHAMAN pipeline (Quereda et al. 2016). Alpha diversities were calculated using the Shannon indexes. Beta diversity was calculated from the DESeq2 normalized data by a Bray–Curtis dissimilarity measure. Differences in community structure observed in the surrounding sediments and in the esophagus sediments were visualized using principal coordinates analysis (PCoA). Effects of these variables on beta diversity were tested with permutational multivariate ANOVA methods

(PERMANOVA) with 999 permutations of the Bray–Curtis distance matrix. The generalized linear model (GLM) was then applied to detect differences in abundance of genera between variables tested with Benjamini-Hochberg false discovery rate (FDR) correction. In parallel to 18S analyses, we also performed 16S metabarcoding to explore differences in prokaryotic content between samples. As bacterial community from the esophagus were not significantly different from bacterial community of surrounding sediment, only the 18S results are considered in this study. Workflow used for this metabarcoding is presented as supplemental (Electronic Supplementary Material 1).

Natural isotopic composition

Isotopic compositions of the surrounding sediment were measured with samples from each station. Surrounding sediment was agitated and decanted in tap water in order to extract meiofauna. For each sample, 700 specimens of nematode were haphazardly handpicked under dissecting microscope, rinsed to remove adhering particle and transferred to tin cup. *A. cordatus* were dissected to collect (i) tissue from the digestive tract and compact content of the (ii) esophagus and (iii) rectum.

All samples were frozen dried and analyzed at the Isotope Facility at the University of California, Davis, using an elemental-analyzer isotope ratio mass spectrometer. This type of analyze provide nitrogen and carbon concentrations for each sample. The nitrogen and carbon isotope ratios are expressed in the delta notation $\delta^{15}N$ and $\delta^{13}C$, as follows: $\delta X = [(R_{Reference}/ R_{Sample}) - 1] \times 1000$, where $X = \delta^{15}N$ or $\delta^{13}C$ and R is the ratio $^{15}N: ^{14}N$ or $^{13}C: ^{12}C$ in the sample and in the reference material. Results are expressed relative to atmospheric nitrogen for N and to Vienna Pee Dee Belemnite (VPDB) for C, and are expressed in units of ‰ ± standard deviation (SD).

Feeding experiments

The experiments were performed in aquaria. Surrounding sediment was enriched in ^{13}C and ^{15}N and placed in contact with *A. cordatus*. Evolution of the isotopic compositions of the digestive tube contents (esophagus and rectum) were compared during successive incubation times to provide an insight into food processes along the digestive tube.

To prepare enriched sediment, local seawater was mixed with ^{13}C glucose (99% ^{13}C-enriched glucose; Euriso-top) and ^{15}N ammonium (99% ^{15}N-enriched NH_4Cl; Euriso-top) with respective concentrations of 237 mg l^{-1} and 94 mg l^{-1}. Sediments from each station were poured in several aquaria to form a 4 cm layer over the bottom. Sediments were then covered with ^{13}C-^{15}N-enriched seawater (4 cm layer) and daily homogenized during 200 h incubations. The aquaria were

air bubbled and maintained at temperature of 4–6 °C under a local light–dark cycle. At the end of the incubation, sediments were rinsed with two times their volume of seawater, overlaying water was removed after total sedimentation and this rinsing protocol was repeated four times. Before to be used in the experiments, *A. cordatus* individuals were kept less than two days in bubbled aquaria containing sediment to avoid starvation. At the beginning of the feeding experiments, ^{13}C-^{15}N-enriched sediments were sampled to determine their initial isotopic compositions and the occurring meiofauna as previously described. Each aquarium (0.1 m²) contains 3.5 L of ^{13}C-^{15}N-enriched sediment, 14.5 L of seawater and 6 *A. cordatus* individuals. During the experiments, the aquaria were maintained in conditions previously described. Six incubation times were tested for Biomar sediments (2, 5, 10, 20, 40 and 80 h) whereas only an 80 h-long experiment was tested for Port Pétrolier sediments. At the end of incubation, the sea urchins were collected, frozen (− 80 °C), thawed and dissected to isolate the contents of their esophagus and rectum. Isotope samples were treated as previously described.

The contribution of enriched sediment to the contents of esophagus and rectum of *A. cordatus* was evaluated considering (i) the enrichment of the contents of esophagus and rectum since the beginning of feeding experiments and (ii) the isotopic composition of enriched sediment. This evaluation was done for ^{13}C and ^{15}N and average values were calculated for each sea urchin from each sampling station and for ingested and digested sediments.

The weight of sediment contained in the entire digestive tube of *A. cordatus* was measured through the dissection of 20 individuals.

Data analyses

The nonparametric Kruskal–Wallis test was used to test differences in isotopic composition, C and N concentrations and contribution of enriched sediment in diets. A linear regression model was used to fit the contribution of enriched sediment to the rectum content and estimate the total incubation time for a contribution of 100%. Model uncertainty was estimated by computing minimum and maximum slope values based on observed data variation. All statistical analyses were performed using R. Values are presented as means ± standard deviations (SD), except when specified otherwise.

Results

Metabarcoding

Alpha diversity (within samples) was calculated using the Alpha and Shannon indexes and revealed higher diversity

in the surrounding sediments compared to the sediments filling the esophagus of *A. cordatus* (Electronic Supplementary Material 2). Sequencing did not attain stabilization of rarefaction curves for the surrounding sediment samples, meaning that more ASV should be characterized with deeper sequencing (Electronic Supplementary Material 3). Figure 2 shows a PCoA graph based on beta diversities (between samples) calculated using the Bray–Curtis dissimilarity distance from the taxonomic profiles, showing discrimination between surrounding sediments and esophagus contents (permanova *p*-value < 0.029). A total of 97 ASV were assigned when compared to SILVA database and only 14 ASV were characterized at genus taxonomic level. The genus *Protomonostroma* (green algae) and *Kalyptorhynchia* (Platyhelminthes) were significantly associated with the surrounding sediments (log2 fold change > 4, adjusted *p*-value < 0.05) and only *Acoela* genus was significantly associated with the esophagus contents (log2 fold change > 4, adjusted *p*-value < 0.05) (Fig. 3).

Natural isotopic composition

Natural isotopic compositions of the sea urchin tissues (digestive tube wall) and of potential food sources (surrounding sediment and nematode community) are presented in Fig. 4. In both sampling stations, nematodes had higher $\delta^{15}N$ than *A. cordatus*. Theoretical isotopic composition of food sources of *A. cordatus* were calculated according to trophic enrichment factor of $\delta^{13}C$ [1.1 ‰, (McCutchan et al. 2003)] and $\delta^{15}N$ [3.4 ‰, (Minagawa and Wada 1984)]. In both sampling stations, this theoretical food source presented

an isotopic composition closer to the composition of the surrounding sediment than the composition of nematodes (Fig. 4).

Surrounding sediment had a C/N ratio of 5.65 ± 0.54 that is not significantly different from C/N ratios observed in the digestive sediments (esophagus and rectum contents) (Kruskal–Wallis, $p > 0.05$). C/N ratio, carbon and nitrogen concentrations (%) are similar in both parts of the digestive tube whatever the digestive stage of its content (Kruskal–Wallis, $p > 0.05$). In both study stations, esophagus and rectum sediments have a significantly higher carbon (1.6-fold) and nitrogen (1.7-fold) contents than surrounding sediment (Kruskal–Wallis, $p < 0.05$) (Fig. 5).

In both study stations, $\delta^{13}C$ of surrounding and of digestive sediments (esophagus and rectum contents) were not significantly different (Kruskal–Wallis, $p > 0.05$). However, $\delta^{15}N$ sediments of the esophagus reached values of 22.4 ± 3.3 ‰ (Fig. 6). In both study stations, $\delta^{15}N$ values were significantly higher in the digestive tube (esophagus and rectum contents) than in the surrounding sediment (Kruskal–Wallis, $p < 0.05$).

Feeding experiments

After incubation with ^{13}C glucose and ^{15}N ammonium, the surrounding sediment of Biomar station had a $\delta^{13}C$ increasing from -19.4 ± 0.1 to 564.9 ± 3.9 ‰ and a $\delta^{15}N$ rising from 7.6 ± 0.3 to 3543.8 ± 54.2 ‰. The surrounding sediment of Port Pétrolier showed a $\delta^{13}C$ increasing from -18.5 ± 0.2 to 626.6 ± 142.5 ‰ and a $\delta^{15}N$ rising from 9.1 ± 0.1 to 2960.4 ± 263.6 ‰.

After 80 h of incubation in the presence of enriched sediment, the digestive content of *A. cordatus* had ^{13}C and ^{15}N isotopic compositions closer to the enriched surrounding sediment than to the enriched nematodes (Fig. 7).

For each sea urchin, the contribution of the enriched surrounding sediment to the digestive content was independently evaluated using ^{13}C and ^{15}N and average difference between each evaluation was $7.3 \pm 8.0\%$. For Biomar station, the contribution of enriched sediment to the esophagus content regularly increased forming the totality ($100 \pm 7\%$) of the esophagus content after 80 h experiment (Fig. 8). At Port Pétrolier, this contribution was $81 \pm 17\%$ after 80 h and not significantly different from values obtained for Biomar (Kruskal–Wallis, $p > 0.05$). The contribution of enriched sediment to the rectum content started to increase after 5 h ($1.0 \pm 0.3\%$) and regularly increased during the experiment without reaching a plateau. After 80 h, this contribution was $82.4 \pm 23.8\%$ for Biomar and was not significantly different from the contribution of $71.5 \pm 16.4\%$ observed for Port Pétrolier (Kruskal–Wallis, $p > 0.05$). For Biomar, data on the contribution of enriched sediment to the rectum content were
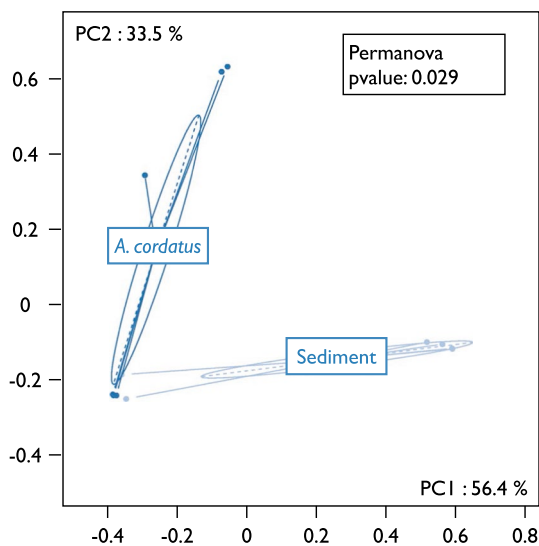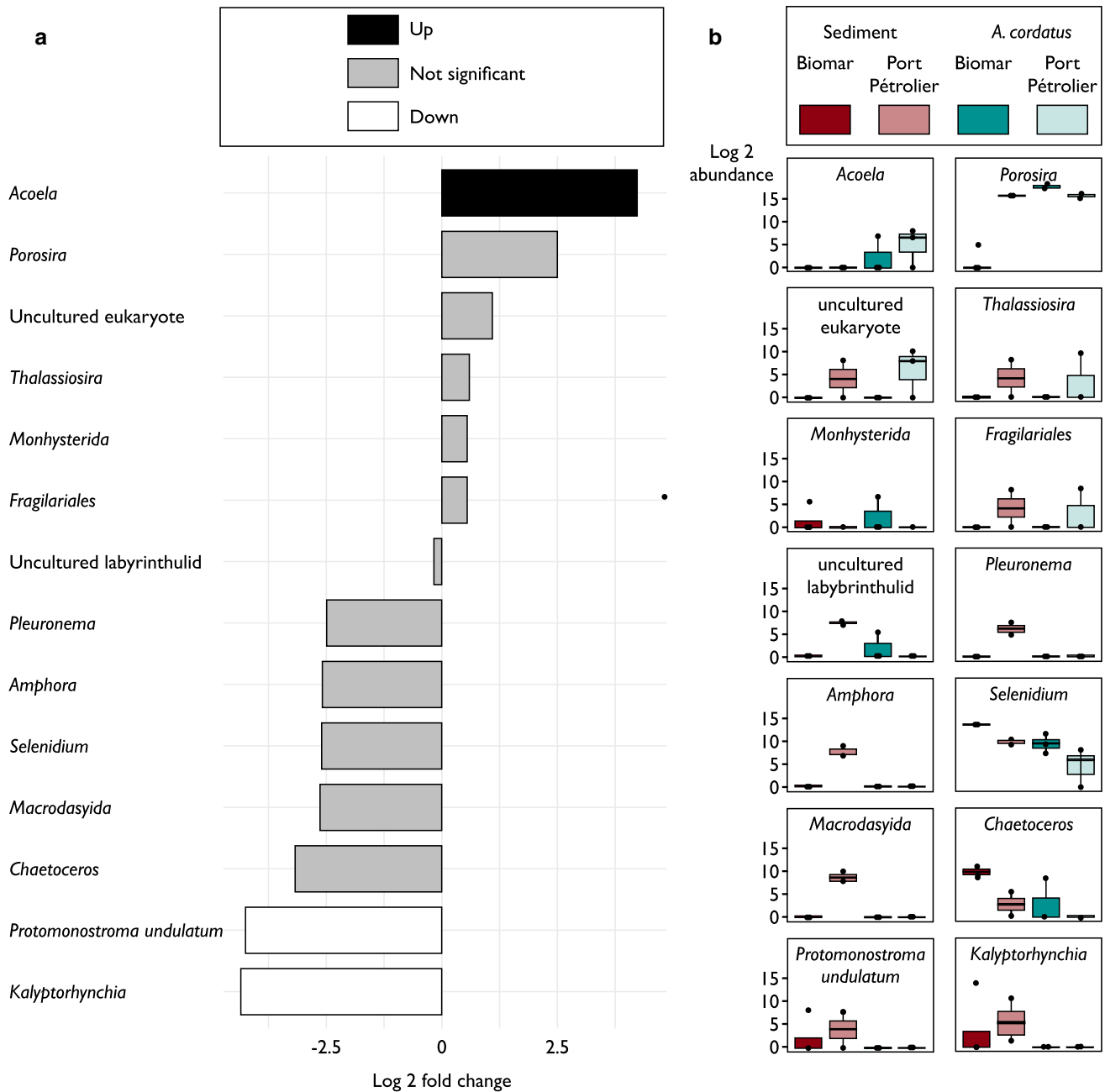

Fig. 2 PCoA plots according to sample types (*Abatus cordatus vs* sediment) based on Bray–Curtis dissimilarity matrix. PERMANOVA test based on the sample type yielded a significant *p*-value of 0.029; 89.9% of variations were explained by the first two PC1 and PC2 axis

Fig. 3 **a** Barplot of eukaryotic genera significantly associated (*p*-value < 0.05) to *A. cordatus* (green) and to sediment samples (red). **b** Boxplot of the log2 abundances of the 14 genera studied in sediments and *A. cordatus* samples on the 2 sites Biomar and Port Pétrolier

used to fit a linear regression ($r^2 = 0.89$) modeling an average incubation time of 86 h (minimum time of 76 h and maximum time of 101 h when considering model slope uncertainty), necessary to reach a contribution of 100%. The dry weight of sediment removed in its entirety from the gut was 2.23 ± 1.0 g. Considering that 86 h are required to fill in the digestive tube according to linear regression, *A. cordatus* would have an ingestion rate of 36 mg dry sediment h^{-1}.

Discussion

Selective feeding

Metabarcoding of the esophagus content of *A. cordatus* was used to evaluate its diet composition and to list its ingested food items. This approach gives a snapshot of the sea urchin's diet as it only considers the last food intake. Analyzing the natural isotopic composition of an animal

Fig. 4 Isotopic composition (δ^{13}C and δ^{15}N; $\pm$SD; $n=6$) of surrounding sediment, nematode community and gut tissue of *A. cordatus* in **a** Biomar (white) and **b** Port Pétrolier (grey). Dotted line represents the theoretical isotopic composition of food source of *A. cordatus*, taking into account trophic enrichment of δ^{13}C and δ^{15}N of 1.1 (McCutchan et al. 2003) and 3.4 ‰ (Minagawa and Wada 1984) respectively

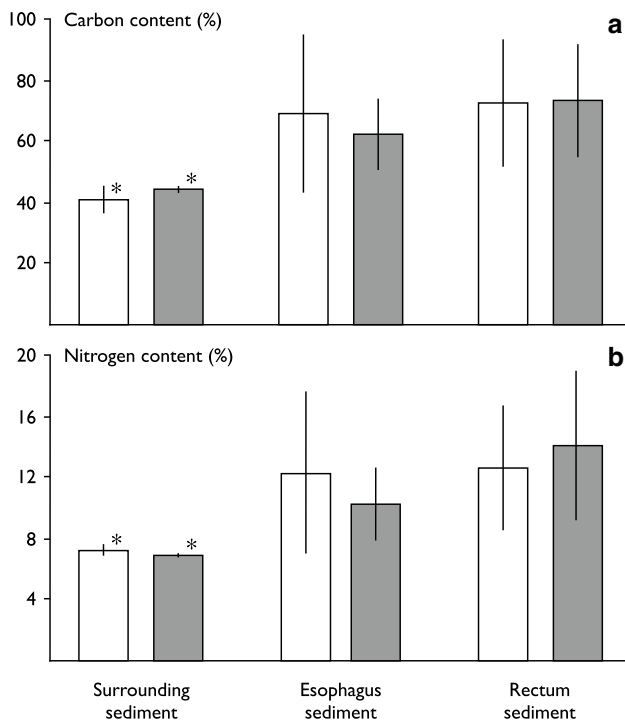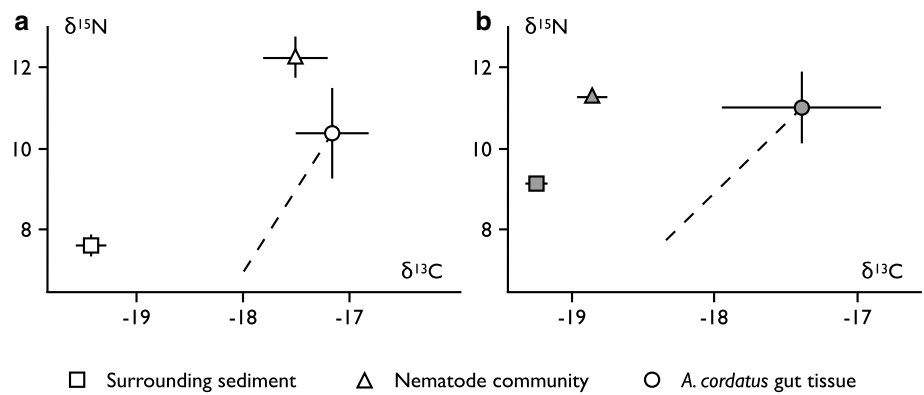**Fig. 5 a** Carbon and **b** nitrogen concentrations ($\pm$SD; $n=6$) of sediment and ingested and digested sediment by *A. cordatus* in Biomar (white) and Port Pétrolier (grey). *Significant differences between surrounding sediments and sediments from the digestive tube (Kruskal–Wallis tests, $p < 0.05$)

through selective ingestion. Compared to other echinoids, irregular sea urchins present a drastically different mode of food intake as they lack an Aristotle's lantern with the mouth directly opening into the esophagus (Holland 2013). Spatangoid species (*e.g. Echinocardium cordatum* and *Brissopsis lyrifera*) dwell in burrows connected to the sediment surface by a funnel. Particulate organic matter trapped in this funnel can be selectively transferred to the ventrally-located mouth using specialized aboral tube feet (De Ridder and Jangoux 1985; De Ridder et al. 1985; Hollertz and Duchêne 2001; Hollertz 2002; Boon and Duineveld 2012). However, sea urchins of the genus *Abatus* do not have such a sophisticated funnel feeding behaviour and consequently has no direct access to the organic particles occurring at the surface of the sediments. Spatangoids can also exudate mucus that trap fine and organic-rich particles carried by ventilatory currents. This mucus is then transported to the mouth by aboral floor spines along the ambulacral groove (De Ridder et al. 1987). This selective feeding mode using mucus was suggested for *B. lyrifera* (Hollertz 2002) but not for the genus *Abatus*.

In the present study, the metabarcoding targeting the ADNr16S gene suggests a similar microbiota composition between the sea urchin esophagus content and the surrounding sediment. Despite the ecological importance and the diversity of the genus *Abatus* (11 species are described in the Southern Ocean, Fabri-Ruiz et al. 2017), the gut microbiota has only been recently described in the Antarctic species *Abatus agassizii* (Schwob et al. 2020). Comparing the bacterial communities of the gut content and the surrounding sediment, Schwob et al. (2020) observed that the same bacterial classes were present in both micro-environments, although their compositions at finer taxonomic scale (OTUs) were significantly different. Such a discrepancy with results from Schwob et al. (2020) could principally be due to differences in sampling protocols as the present study has focused on the esophagus bacterial community whereas the whole gut content of *A. agassizii* was analyzed by Schwob et al. (2020). Physicochemical conditions are variable according to the

tissues offers the advantage of integrating diet over a longer period (Fry 2006) but it does not offer such a precision in the list of ingested food items. This limitation is partially due to difficulties in the isolation of small food particles as for instance, 700 nematode individuals per sample were needed to determine their isotopic composition in the present study.

When non selective, deposit feeders may ingest large amounts of sedimented material consisting of sharped-edged mineral grains and of particulate organic matter (Jumars 1993). Transportation of unpalatable sediment in the digestive tube is energy intensive and this cost could be reduced

Fig. 6 Isotopic composition (δ^{15}N and δ^{13}C; ±SD; $n=6$) of surrounding sediment and of sediment from the digestive tube of *A. cordatus* in **a** Biomar (white) and **b** Port Pétrolier (grey)
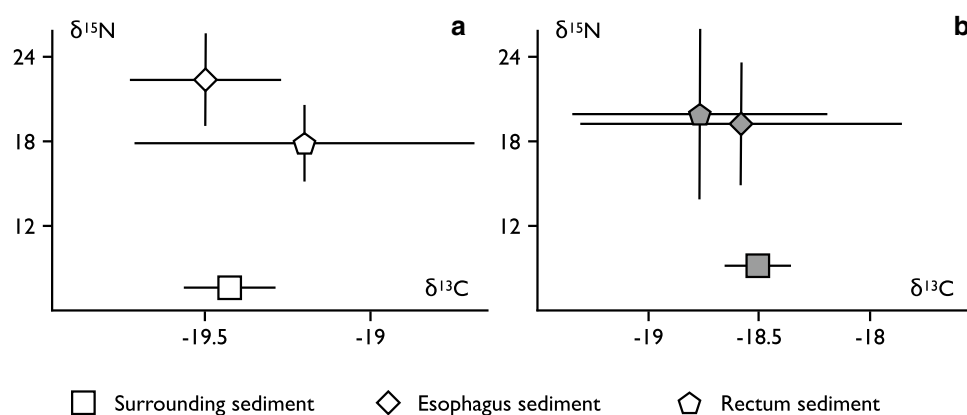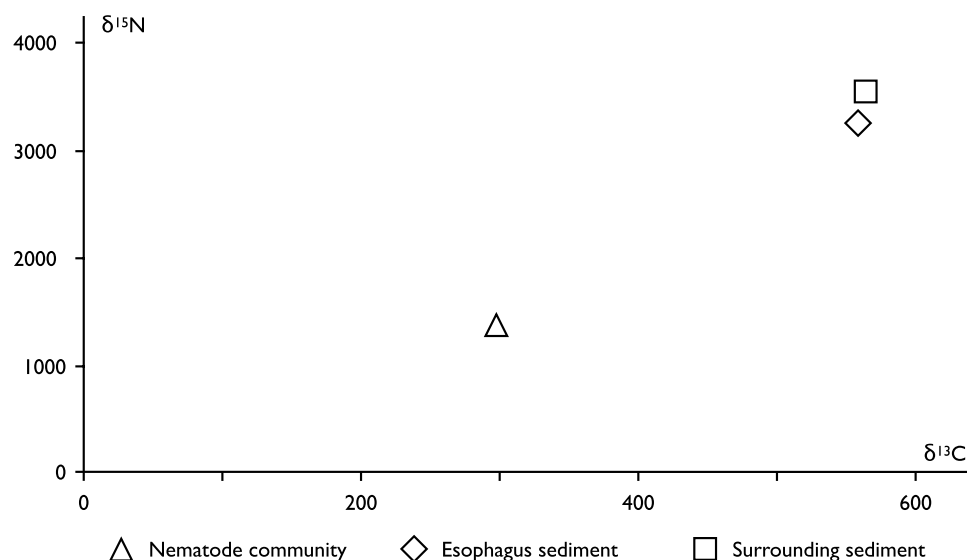

Fig. 7 Isotopic composition (δ^{13}C and δ^{15}N; ±SD; $n=6$) of stable isotope enriched source used in feeding experiment (Biomar nematode community and sediment) and of the sediment ingested by *Abatus cordatus* (esophagus content) after an 80 h incubation time


digestive tube regions (Thorsen 1998) and each regions could consequently have different gut microbiota. The physicochemical conditions inside the esophagus are likely more similar to surrounding sediment ones in comparison with the rest of the digestive tract thus explaining that we did not observe any difference in the bacterial communities. The differentiation of a specific microbiota would occur in the more posterior part of the digestive tract. This study suggests that the feeding mechanism in *A. cordatus* would not allow the selective ingestion of bacteria according to strain.

Meiofauna is a discrete group of small (passing through a 0.5 mm-mesh sieve), highly diverse, abundant and productive organisms dwelling in sediment (Giere 2009; Schratzberger and Ingels 2018). Meiofauna is a high-quality food source containing unsaturated fatty acids that most metazoans are unable to produce (Leduc et al. 2009; De Troch et al. 2012) and consequently, play an important role in the diet of epibenthic consumers from crustaceans (Bell and Coull 1978; Nilsson et al. 1993) to vertebrates such as fish (Fitzhugh and Fleeger 1985; Henry and Jenkins 1995).

Heart urchins can affect meiofauna through predation and/or alteration of chemical and physical characteristics of environment sediments (Austen and Widdicombe 1998). Using tube feet, several species of heart urchins such as *E. cordatum* presents important ability to select their food and fatty acid composition of their foregut suggests selective ingestion of meiofauna (Boon and Duineveld 2012). In the present study, metabarcoding targeting the ADNr18S gene reveals that only one organism from meiofauna was more abundant in the esophagus than in surrounding sediments indicating a limited selective ingestion of meiofauna by *A. cordatus*. Stable isotope composition in natural conditions or after enrichment both suggest that the nematode community, the most abundant meiofauna member, does not constitute a significant part of *Abatus*' diet. Such a limited trophic role of meiofauna could be due to the large size of *A. cordatus* as selective ingestion of meiofauna decreases with the size of consumers (Pascal et al. 2019).

Acoela is the only organism that is significantly more abundant in the esophagus of *A. cordatus* than in surrounding

Fig. 8 Contribution of ^{13}C, ^{15}N-enriched sediment to the total sediment (in %) in **a** esophagus sediment and **b** rectum sediment of *Abatus cordatus* according to the duration of incubation (h) with enriched sediment from Biomar (white) and Port Pétrolier (grey) (means ± sd, n = 6). The linear regression was evaluated with data of ingested sediment from Biomar ($r^2 = 0.89$) and data variation was used to produce minimum and maximum uncertainty slopes
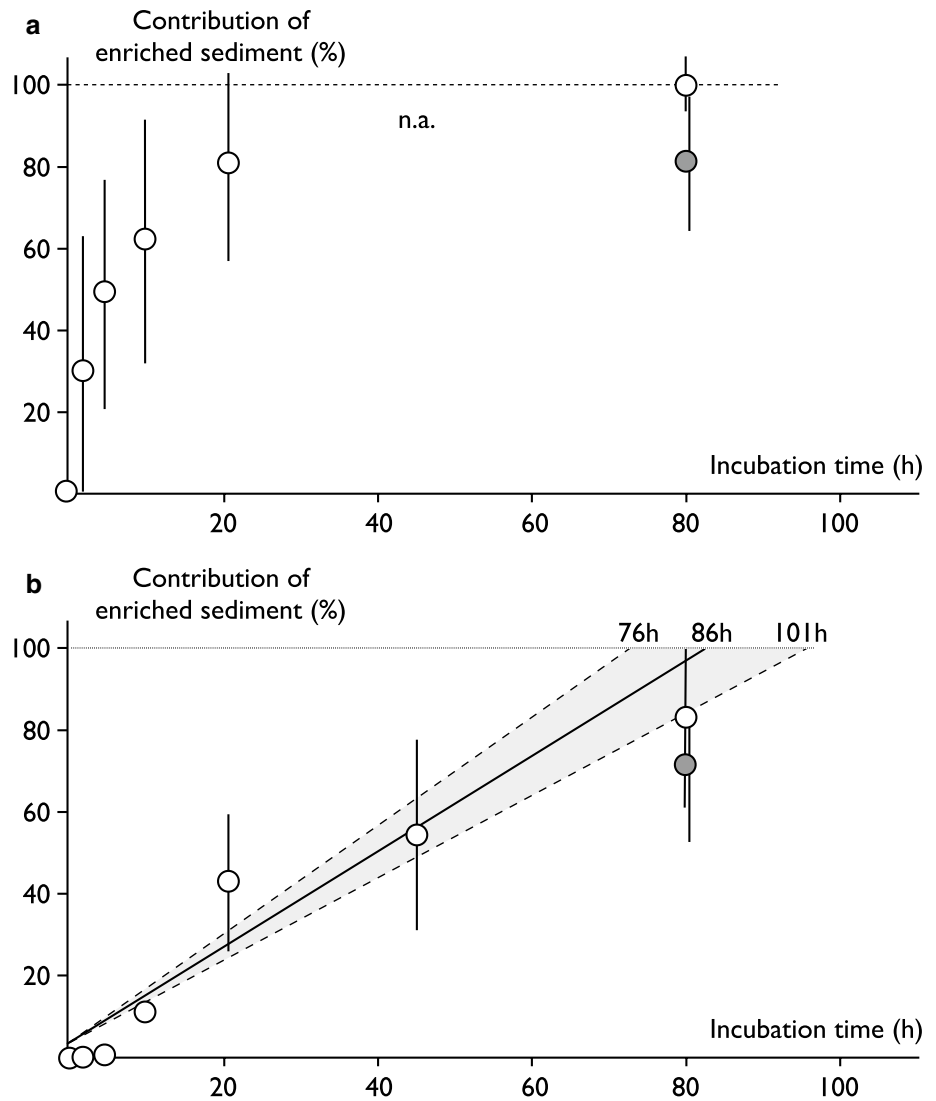
sediment. Acoels are bilaterally symmetric worms in the millimeter-size range (Achatz et al. 2013). Of the nearly 400 described species, the majority are free-living organisms but seven species are parasites or endosymbionts associated to the digestive tube of echinoderms (Jennings 1971), which could explain their higher abundance in the esophagus of *A. cordatus*. The macro algae *Protomonastroma undulatum* and the platyhelminthes *Kalyptorhynchia* are less abundant in the sea urchin esophagus than in the environment, suggesting that the sea urchin could avoid ingesting these two food items. The platyhelminthes can also escape predation by the sea urchin but to our knowledge, such a behaviour has never been described.

Carbon and nitrogen concentrations in the esophagus sediments were, respectively, 1.6 and 1.7 times higher than in the surrounding sediments. Higher values were measured in the gut content of the spatangoid *B. lyrifera* with ratios of 2 for C and 2.5 for N (Hollertz 2002). In the gut content

of *E. cordatum,* the organic matter is even fourfold higher than in the surrounding sediment (De Ridder et al. 1985). Such high values can be explained by a selective ingestion of carbon- and nitrogen- rich particles. The higher ratios of the two last species would be due to higher selective efficiency due to the use of specialized tube feet to pick up and select these particles from the sediment surface and convey them to the mouth through the vertical funnel of their burrows. Even if *A. cordatus* do not present tube feet and is less efficient in food selectivity, this species presents the ability to ingest specifically food items with higher carbon and nitrogen concentrations.

In both sampling locations, δ^{15}N in the esophagus contents reached significantly higher values (17 ‰) than in the surrounding sediment. δ^{15}N of an organism tissue reflects its trophic position in the trophic network (Fry 2006). In Kerguelen, δ^{15}N values exceeding 17 ‰ are rarely reached even in organisms of higher trophic levels such as marine

mammals (Cherel et al. 2008) and marine birds (Camprasse et al. 2017a, b). The present study reveals (i) a nematode community with δ^{15}N below 13 ‰ and (ii) a limited selective feeding behaviour of *A. cordatus*. As a result, high δ^{15}N values measured in the esophagus content are unlikely due to a selective ingestion of ^{15}N-enriched food items. Metabarcoding indicated that eukaryotic symbionts of the genus *Acoela* are present in the esophagus of *A. cordatus*. However, considering the small quantity of *Acoela*, it can be reasonably assumed that this symbiont is not at the origin of the high δ^{15}N signature of the *A. cordatus* esophagus content. The measured differences could also be due to the role of mucus changing isotopic composition in the ingested sediment. The mucus can be produced (i) externally by clavulae of the urchin's fascioles, transported to the mouth and ingested (De Ridder et al. 1987; Hollertz 2002) or (ii) internally in the posterior part of the esophagus that produces mucus to allow sediment compaction (Holland and Ghiselin 1970; De Ridder 1987). However, the mucus should have an isotopic composition similar to the sea urchin tissues, lower than 11 ‰, and should consequently not contribute to high δ^{15}N of ingested sediment. The ^{15}N enrichment in esophagus content is more probably linked with a preferential assimilation of the lighter isotope by the urchin due to its weaker bonds during the digestion process (Fry et al. 1984). Heart urchins ingest sediment with associated particulate and dissolved organic matter. Part of this detritus material is labile through natural transformation by sediment microorganisms. Heart urchins present the ability to assimilate this labile material all along their digestive tube and particularly in the first digestive loop (De Ridder et al. 1985; Rolet and De Ridder 2012; Rolet et al. 2012). Through preferential ingestion of lighter isotope in esophagus, the unabsorbed food remaining in the gut would become more enriched in the heavier isotope (Olive et al. 2003). As catabolic reactions also favour lighter isotope (Minagawa and Wada 1984), the consumer tissue finally become enriched in heavier isotope (Olive et al. 2003). Thanks to this relationship between isotopic compositions of sources and consumers, stable isotopes are routinely used to evaluate diet composition of consumers (Boecklen et al. 2011). Due to limitations in sampling efforts and/or limitations in a priori knowledge of potential food items, exhaustive sampling of food sources of a consumer is difficult and several studies examined signatures of diet based on materials removed from the gastrointestinal tract (e.g. Fry 1988; Peterson et al. 1993; Yatsuya and Nakahara 2004). The use of this proxy implies a limited effect of ingestion and digestion on the isotopic composition of diet. A limited number of studies evaluated this effect and they focused on carnivorous (Grey et al. 2002; Guelinckx et al. 2008) and herbivorous (Johnson et al. 2012) fish. According to these studies, the effect of ingestion on ^{13}C and ^{15}N of food can be either limited (Grey

et al. 2002) or significant (Guelinckx et al. 2008; Johnson et al. 2012). However, the range of ^{15}N enrichment in the esophagus of *A. cordatus* is higher than the values obtained in these studies. This could be due to specificities of digestion processes associated with deposit feeding. Care should be taken in determining isotopic signatures of diet based on isotopic composition of gut content (Guelinckx et al. 2008; Johnson et al. 2012) and this method would not be adapted to evaluate selective feeding for deposit feeders. As in the present study the isotopic shift *i*) appeared higher for ^{15}N than for ^{13}C (Guelinckx et al. 2008) and ii) occurred in the first part of the digestive tract (Guelinckx et al. 2008; Johnson et al. 2012).

One means of diet selection is selective assimilation in the gut (Self and Jumars 1978). Absorption efficiency can be estimated by comparing gut content between the proximal and the distal parts of the gut (De Ridder et al. 1985). In both sampling stations, the proximal (esophagus) and distal (rectum) parts of the digestive tube had similar C and N concentrations, and higher values than in the surrounding sediment. Similar results were observed for *B. lyrifera* with higher C and N in defecated sediments than in the environment suggesting an increase due to the presence of mucus and bacteria (Hollertz 2002). The sea urchin faeces would consequently constitute a food source favourable for meio and macrofauna (Austen and Widdicombe 1998). δ^{15}N of gut content is similar between first and last part of the digestive tube of *A. cordatus*. Preferential assimilation of lighter isotope occurs during all the digestion process and should lead to a regular increase of δ^{15}N along the digestive tract. Inversely, catabolic reactions lead to a decrease of δ^{15}N in the terminal part of gut content as they favor the lighter isotope eliminated through waste products (Minagawa and Wada 1984; Olive et al. 2003). Both reactions should be of similar intensity and would explain the reduced difference of δ^{15}N between the esophagus and rectum sediments.

Metabarcoding revealed that *A. cordatus* would not be selective enough to ingest specifically prokaryots and eukaryots as previously reported for Antarctic spatangoids that have a diet relying on sediment-associated organic matter with a low trophic plasticity (Michel et al. 2016). However their feeding mechanism would allow this urchin to ingest food with higher carbon and nitrogen concentrations than surrounding sediment.

Feeding kinetic

In order to measure sediment ingestion rates in *A. cordatus*, feeding experiments were run in aquariums whose artificial conditions can affect the behaviour of spatangoids (Thompson and Riddle 2005). Aquarium conditions (light, temperature and sediment composition) were kept as close as possible to environmental ones to limit those

biases. According to species, spatangoids can present different activities between day and night (Hammond 1982a; Thompson and Riddle 2005). To reduce this nychthemeral variability, feeding experiments were run during several days (80 h). Ingestion rates of spatangoids increased with rising temperature (Hollertz and Duchêne 2001) and measured ingestion rates would likely be lower during colder austral winter. For deposit feeders like spatangoids, a widespread method to select food is to take advantage of heterogenous distribution of food in sediment: bulk sediment is ingested (i) at higher rates at sediment depth or location presenting patch of high food quality (Hollertz 2002) or (ii) at lower rates with faster movements in areas of low food availability (Hammond 1983). In aquarium, the sediment was initially homogenized and during the experiment the bioturbating activity of urchin likely maintain the sediment homogenization (Thompson and Riddle 2005). As a result, the sea urchins did not have the opportunity to select patch of sediment. However, both sediments used during feeding experiments were (i) collected in areas with abundant *A. cordatus* and (ii) ingested by sea urchin in the field as revealed by metabarcoding. Moreover feeding rates were similar in both sediments suggesting that underestimation due to unrealistic unpalatable sediment should be limited.

During feeding experiments, the progression of stable isotope enrichment in proximal and distal parts of *A. cordatus* digestive tube informs on digestion processes. It takes more than 20 h to completely fill the esophagus with enriched sediment whereas the rectum content started to be enriched after 10 h. Regular gut content transportation would have implied a complete filling of the esophagus before apparition in distal part. Observed shift implies that food particles are not all transported at similar rapidity. Fast transportation could be associated with the functioning of the siphon allowing rapid circulation of water and small particles associated. Spatangoids maintain ciliary currents around their test to create a water current entering in the esophagus and this water is then actively pumped from the esophagus to the intestine through the siphon in order to (i) provide oxygenated water to the intestine for respiration purposes, (ii) avoid dilution of enzyme in the stomach, (iii) facilitate advancement of content of digestive tract and (iv) carry dissolved organic matter that is assimilated in the intestine (De Ridder et al. 1985). In order to produce isotopically enriched sediment used for feeding experiments of the present study, sediment was incubated with ^{13}C glucose and ^{15}N ammonium both being rapidly assimilated by sediment bacteria (van Oevelen et al. 2006; Pascal et al. 2008). Sediment was then rinsed using 4 cycles of dilution and sedimentation allowing to remove most of non-assimilated-enriched isotope and enriched dissolved organic matter. After the labelling and rinsing steps, the emission of enriched dissolved organic matter by enriched bacteria cannot be excluded and

enrichment in *A. cordatus* gut can be due to dissolved and/or particulate organic matter. Through water circulation in its digestive tube, the spatangoid *E. cordatum* has a suspension feeding mode in addition to deposit feeding increasing the range of exploitable food items (Rolet et al. 2012). Higher carbon and nitrogen concentrations in gut content and rapid transfer of enriched isotope in the digestive tube both suggest that *A. cordatus* would similarly use suspended organic matter as food source but the importance of this feeding mechanism remains to be investigated.

The present study suggested a gut resident time fluctuating between 76 and 101 h for *A. cordatus*. This duration is in the range of values (72 to 97 h) observed for the high Antarctic species *Abatus ingens* (Thompson and Riddle 2005). For the temperate species *B. lyrifera*, the gut resident time is linked with temperature with duration of 75 h at 7 °C and 19 h at 13 °C (Hollertz and Duchêne 2001). Another temperate species *E. cordatum* has a digestive transit of 23–24 h (De Ridder and Jangoux 1985), which is considerably higher than the 4–5 h observed for the tropical species *Meoma ventricosa* (Hammond 1982b). The observed ingestion rate of 0.04 g of dry sediment h^{-1} per specimen of *A. cordatus* is in the range of values obtained for the Antarctic Schizasteridae species *A. ingens* (0.02 to 0.06 g h^{-1}) (Thompson and Riddle 2005) and the temperate species *B. lyrifera* (0.02 to 0.08 g h^{-1}) (Hollertz and Duchêne 2001). The temperate spatangoid *E. cordatum* presents higher ingestion rates (0.38 g h^{-1}) but it lives in nutritionally poor sandy habitats suggesting a link between sediment organic matter content and ingestion rates to obtain the required nutrients (De Ridder and Jangoux 1985). The volume of sediment reworked by *A. ingens* moving through sediment is 75 times greater than the volume ingested implying a significant bioturbating role in the Antarctic environment (Thompson and Riddle 2005). The present study reveals that *A. ingens* and *A. cordatus* have similar ingestion rates (Thompson and Riddle 2005) and because local populations of *A. cordatus* can reach high densities (Poulin and Féral 1995), it can be reasonably assumed that *A. cordatus* can also play a key ecological role through bioturbation in nearshore benthic communities of the Kerguelen Islands.

Comparison of ingested and surrounding sediments by metabarcoding revealed a limited selective ingestion of prokaryotes and eukaryotes by *A. cordatus*. However, higher carbon and nitrogen concentrations of their gut content suggest a selective ingestion of organic matter. The strong $\delta^{15}N$ enrichment of sediment in first and last part of the digestive tube could be due to the selective assimilation of lighter isotope in the gut. Additional studies would be necessary to determine the mechanism of this enrichment and its specificity to deposit feeding mode. Feeding experiment using stable isotope-enriched sediment allowed measurement of feeding rates and indicated that, in the gut of the sea urchin,

food particles can be processed more rapidly than sediment particles. This rapid transfer of dissolved and/or particulate organic matter is likely due to water circulation in the digestive tube allowing *A. cordatus* to collect food through suspension feeding. The feeding activity of *A. cordatus* suggests that dense populations of the species can play an important ecological role through bioturbation. The loss of this key species due to global changes may consequently affect the ecosystem functioning in nearshore habitats of the Kerguelen Islands.

Author contributions PYP and TS conceived and design research. PYP, TS and EP realized the field work. PYP and TS conducted laboratory experiments. YR realized all the metabarcoding study. PYP wrote the manuscript. All authors read and approved the manuscript.

Declarations

Conflict of interest Authors have no conflict of interest to declare.

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