



HAL
open science

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

Pierre-Yves Pascal, Yann Reynaud, Elie Poulin, Chantal de Ridder, Thomas Saucède

► **To cite this version:**

Pierre-Yves Pascal, Yann Reynaud, Elie Poulin, Chantal de Ridder, Thomas Saucède. Feeding in spatangoids: the case of *Abatus cordatus* in the Kerguelen Islands (Southern Ocean). *Polar Biology*, 2021, 44 (4), pp.795-808. 10.1007/s00300-021-02841-4 . hal-03235875

HAL Id: hal-03235875

<https://hal.science/hal-03235875>

Submitted on 7 Feb 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Polar Biology

Feeding in spatangoids: the case of *Abatus cordatus* in the Kerguelen Islands (Southern Ocean) --Manuscript Draft--

Manuscript Number:	
Full Title:	Feeding in spatangoids: the case of <i>Abatus cordatus</i> in the Kerguelen Islands (Southern Ocean)
Article Type:	Original Paper
Corresponding Author:	Pierre-Yves Pascal Institut de Systematique Evolution Biodiversite GUADELOUPE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut de Systematique Evolution Biodiversite
First Author:	Pierre-Yves Pascal
First Author Secondary Information:	
Order of Authors:	Pierre-Yves Pascal Yann REYNAUD Elie Albert POULIN Chantal DE RIDDER Thomas SAUCEDE
Funding Information:	Institut Polaire Français Paul Emile Victor (Program PROTEKÉR (N° 1044)) Dr Thomas SAUCEDE
Abstract:	<p>Irregular sea 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 <i>Abatus cordatus</i> (Verrill, 1876) is a sub-Antarctic spatangoid that is endemic to the Kerguelen Islands and Kerguelen Plateau. It presents a small depth and distribution range along with limited dispersal capabilities rendering this species particularly threatened by global warming. The feeding behaviour of <i>A. cordatus</i> was investigated using simultaneously metabarcoding and stable isotope approaches. Comparison of ingested and surrounding sediments revealed a limited selective feeding by <i>A. cordatus</i> and a limited trophic role of the meiofauna. Compared to surrounding sediments, the gut content presented i) higher carbon and nitrogen contents, potentially due to the sea urchin mucus secretion, and ii) $\delta^{15}\text{N}$ 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 <i>A. cordatus</i> revealed that all particles are not similarly transported. Fast transportation could be associated with the functioning of the siphon, allowing direct transportation of water and its associated particulate organic matter from the esophagus to the intestine. <i>A. cordatus</i> presented a gut resident time of 89 hours 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.</p>
Suggested Reviewers:	
Opposed Reviewers:	
Order of Authors Secondary Information:	

1 **FEEDING IN SPATANGOIDS: THE CASE OF *ABATUS CORDATUS* IN THE**
2 **KERGUELEN ISLANDS (SOUTHERN OCEAN)**

3
4 Pierre-Yves PASCAL¹, Yann REYNAUD², Elie POULIN³,
5 Chantal DE RIDDER⁴, Thomas SAUCEDE⁵
6

7 ¹ Institut de Systématique, Évolution, Biodiversité (ISYEB) UMR 7205, Équipe Biologie de la
8 Mangrove -Université des Antilles, 97159 Pointe-à-Pitre, Guadeloupe, France

9 ² Institut Pasteur de Guadeloupe, Morne Jolivière, 97139 les Abymes, Guadeloupe, France

10 ³ Laboratorio de Ecología Molecular, Instituto de Ecología y Biodiversidad, Facultad de Ciencias,
11 Universidad de Chile, Santiago, Chile

12 ⁴ Laboratoire de Biologie Marine (C.P. 160/15), Université Libre de Bruxelles, Brussels, Belgium

13 ⁵ Biogéosciences, UMR 6282, CNRS, EPHE, Université Bourgogne Franche-Comté, Dijon, France
14

15 *Corresponding author: pypascal@univ-ag.fr

16 Key Words: irregular sea urchin, meiofauna, nematode, selectivity, deposit feeding,
17 ingestion rate
18

19

Abstract

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

Irregular sea 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 Islands and Kerguelen Plateau. It presents a small depth and distribution range along with limited dispersal capabilities rendering this species particularly threatened by global warming. The feeding behaviour of *A. cordatus* was investigated using simultaneously metabarcoding and stable isotope approaches. Comparison of ingested and surrounding sediments revealed a limited selective feeding by *A. cordatus* and a limited trophic role of the meiofauna. Compared to surrounding sediments, the gut content presented *i*) higher carbon and nitrogen contents, potentially due to the sea urchin mucus secretion, and *ii*) $\delta^{15}\text{N}$ 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. Fast transportation could be associated with the functioning of the siphon, allowing direct transportation of water and its associated particulate organic matter from the esophagus to the intestine. *A. cordatus* presented a gut resident time of 89 hours 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.

42

Introduction

43

44

45

46

47

48

49

50

51

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 & Levinton 1987, Jumars 1993). In comparison with fermenters, deposit feeders present 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).

52

53

54

55

56

57

58

59

60

61

62

63

64

65

Among sea urchins, irregular echinoids dwell upon or inside 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. 1984). Due to their wide distribution range, large populations, burrowing activities and feeding behaviours (Ghiold 1989), heart sea urchins (Spatangoida) have long attracted the attention of paleontologists (Bromley & Asgaard 1975, Gilbert & Goldring 2008), sedimentologists (Radwanski & Wysocka 2001), physiologists (De Ridder & Jangoux 1993) and marine ecologists (Hammond 1981, De Ridder & 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 & 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

66 sediment layers increasing the depth of oxygen penetration inside the sediment (Vopel et al.
67 2007), influence the benthic carbon cycle (Osinga et al. 1997, Boon & Duineveld 2012) and
68 maintain infaunal and microbial diversity (Widdicombe et al. 2000). The volume of sediment
69 reworked by spatangoids reaches more than 60 times the volume of sediment ingested
70 (Hollertz & Duchêne 2001, Thompson & Riddle 2005). Despite this limited fraction, the
71 ingested sediment plays a structuring role due to biochemical modifications of sediment
72 within the gut (Thompson & Riddle 2005). The exact source of nutrition and dietary
73 requirements of spatangoids remain however incompletely understood (Jangoux & Lawrence
74 1982), as direct examination of marine invertebrates gut content remains problematic and
75 many consumed organisms are unrecognizable. In the last decade, new metabarcoding-based
76 techniques has been implemented (Blankenship & Yayanos 2005) but still not used for
77 spatangoids burrowers.

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

90 coasts of Kerguelen are predicted to present more acidic, fresher and warmer waters in the
91 future (Allan et al. 2013, Gutt et al. 2015). In order to monitor these modifications and their
92 impact on marine life, an integrative long-term observing system, the program PROTEKER,
93 was initiated in 2011 (Féral et al. 2016). Ocean acidification presents disproportionate
94 negative effects for echinoderms (Kurihara & Shiarayama 2004, Kurihara 2008, Collard et al.
95 2014) and preliminary results of program PROTEKER suggest that predicted changes in
96 seafloor salinity and temperature amplitudes might shift beyond the limits of *A. cordatus*
97 tolerance (Saucède et al. 2019). Due to the absence of larval stage, this endemic and narrow-
98 niche species will not be able to disperse southwards and find climatic refuge areas, which
99 raises the issue of the species potential extinction in a near future (Ledoux et al. 2012,
100 Guillaumot et al. 2018, Saucède et al. 2019). In turn, the loss of this key species may
101 significantly alter ecosystem functioning in nearshore habitats of the Kerguelen Islands and to
102 better understand this threat, it is essential to obtain a picture as complete as possible of the
103 species ecology. The aim of this study is to improve our understanding of the trophic ecology
104 of *A. cordatus*, simultaneously using metabarcoding and stable isotope approaches to evaluate
105 its selective feeding and feeding processes.

106

107 **Material and method**

108 *1) Sampling*

109 The investigated stations are located in the Morbihan Bay, in the east of the Kerguelen
110 Islands located in the southern part of the Indian Ocean, at the northern edge of the Polar
111 Front (Fig. 1). Two sampling stations were selected 500 m apart near Port-aux-Français. They
112 harbor sandy sediments with similar granulometry (median grain size of 100 μm) and are
113 localized at two distinct depths: 5 m depth for Biomar (49°21'12 S - 70°13'04 E) and 10 m
114 depth for Port Pétrolier (49°21'17 S – 70°12'44 E). Sampling was done in November 2017 by
115 scuba diving. A total of 60 specimens of *A. cordatus* were collected (mean test length of 38.0
116 \pm 3.7 mm) at Biomar ($n = 45$) and at Port Pétrolier ($n = 15$). For each station, 3 specimens
117 were analyzed for metabarcoding, 6 for natural isotopic composition whereas the remaining
118 ones were used for feeding experiments in aquaria. Surficial 5 cm of sediment (“surrounding
119 sediment samples”) were collected in each station for granulometric analysis, metabarcoding,
120 natural isotopic composition and feeding experiments.

121 *2) Metabarcoding*

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

131 PRJNA625686.

132 Denoising was performed using DADA2 software package (Callahan et al. 2016)
133 implemented in QIIME 2 (Bolyen et al. 2019) *via* q2-dada2 plugin. DADA2 allows fine-scale
134 variation identification through the characterization of amplicon sequence variants (ASV).
135 Singletons and rare ASV (below 0.001%) were removed thanks to q2-feature-table plugin
136 leading to 97 features for a total of 3046891 reads, a mean frequency of 253907 reads per
137 samples. Taxonomy was assigned to ASV using the q2-feature-classifier (Bokulich et al.
138 2018), a classify-sklearn naïve Bayes taxonomy classifier using machine learning (Pedregosa
139 et al. 2011) against the Silva 132 99_18S database (Quast et al. 2013). Normalization of ASV
140 table was done by DESeq2 (Love et al. 2014) implemented in the SHAMAN pipeline
141 (Quereda et al. 2016). Alpha diversities were calculated using the Shannon indexes. Beta-
142 diversity was calculated from the DESeq2 normalized data by a Bray-Curtis dissimilarity
143 measure. Differences in community structure observed in the surrounding sediments and in
144 the esophagus sediments were visualized using principal coordinates analysis (PCoA). Effects
145 of these variables on beta diversity were tested with permutational multivariate ANOVA
146 methods (PERMANOVA) with 999 permutations of the Bray-Curtis distance matrix. The
147 generalized linear model (GLM) was then applied to detect differences in abundance of
148 genera between variables tested with Benjamini-Hochberg false discovery rate (FDR)
149 correction. In parallel to 18S analyses, we also performed 16S metabarcoding to explore
150 differences in prokaryotic content between samples. However, as no particular bacterial
151 genera were found in the esophagus of *A. cordatus*, only the 18S results are considered in this
152 study. Workflow used for this metabarcoding is presented as supplemental (Online Resource
153 1).

154 3) *Natural isotopic composition*

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

161 All samples were frozen dried and analyzed at the Isotope Facility at the University of
162 California, Davis, using an elemental-analyzer isotope ratio mass spectrometer. The nitrogen
163 and carbon isotope ratios are expressed in the delta notation $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, as follows: $\delta X =$
164 $[(R_{\text{Reference}}/R_{\text{Sample}}) - 1] \times 1000$, where $X = \delta^{15}\text{N}$ or $\delta^{13}\text{C}$ and R is the ratio $^{15}\text{N}:^{14}\text{N}$ or $^{13}\text{C}:^{12}\text{C}$
165 in the sample and in the reference material. Results are expressed relative to atmospheric
166 nitrogen for N and to Vienna Pee Dee Belemnite (VPDB) for C, and are expressed in units of
167 ‰ \pm standard deviation (SD).

168 4) Feeding experiments

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

173 To prepare enriched sediment, local seawater was mixed with ^{13}C glucose (99% ^{13}C -
174 enriched glucose; Euriso-top) and ^{15}N ammonium (99% ^{15}N -enriched NH_4Cl ; Euriso-top)
175 with respective concentrations of 237 mg l⁻¹ and 94 mg l⁻¹. Sediments from each station were
176 poured in distinct aquaria to form a 4 cm layer over the bottom. Sediments were then covered
177 with ^{13}C - ^{15}N enriched seawater (4 cm layer) and daily homogenized during 200 h incubations.
178 The aquaria were air bubbled aquariums and maintained at temperature of 4-6 °C under a

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

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

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

199 5) Data analyses

200 The nonparametric Kruskal-Wallis test was used to test differences in isotopic
201 composition, C and N contents and contribution of enriched sediment in diets. All statistical
202 analyses were performed using R. Values are presented as means \pm standard deviations (SD),

203 except when specified otherwise.

204

205 **Results**

206 1) *18S Metabarcoding*

207 Alpha diversity (within samples) was calculated using the Alpha and Shannon indexes
208 and revealed higher diversity in the surrounding sediments compared to the sediments filling
209 the esophagus of *A. cordatus* (Online Resource 2). Sequencing did not permit to reach
210 stabilization of rarefaction curves for the surrounding sediments samples, meaning that more
211 ASV should be characterized with deeper sequencing (Online Resource 3). Figure 2 shows a
212 PCoA graph based on beta diversities (between samples) calculated using the Bray-Curtis
213 dissimilarity distance from the taxonomic profiles, showing discrimination between
214 surrounding sediments and esophagus contents (permanova p-value < 0.029). A total of 97
215 ASV were assigned when compared to SILVA database and only 14 ASV were characterized
216 at genus taxonomic level. The genus *Protomonostroma* (green algae) and *Kalyptorhynchia*
217 (Platyhelminthes) were significantly associated with the surrounding sediments (log₂ fold
218 change >4, adjusted p-value < 0.05) and only *Acoela* genus was significantly associated with
219 the esophagus contents (log₂ fold change >4, adjusted p-value < 0.05) (Fig. 3).

220 2) *Natural isotopic composition*

221 Natural isotopic composition of the sea urchin tissues (digestive tube wall) and of
222 potential food sources is presented in figure 4. In both sampling stations, nematodes presented
223 higher $\delta^{15}\text{N}$ than *A. cordatus*. Theoretical isotopic composition of food sources of *A. cordatus*
224 were calculated according to trophic enrichment factor of $\delta^{13}\text{C}$ (1.1 ‰, (McCutchan et al.
225 2003)) and $\delta^{15}\text{N}$ (3.4 ‰, (Minagawa & Wada 1984)). In both sampling stations, this
226 theoretical food source presented an isotopic composition closer to the composition of the
227 surrounding sediment than the composition of nematodes (Fig. 4).

228 Surrounding sediment presented a C/N ratio of 5.65 ± 0.54 that is not significantly

229 different from C/N ratios observed in the digestive sediments (esophagus and rectum
230 contents) (Kruskal-Wallis, $p > 0.05$). C/N ratio, carbon and nitrogen contents (%) are similar
231 all along the digestive tube whatever the digestive stage of its content (Kruskal-Wallis, $p >$
232 0.05). In both study stations, esophagus and rectum sediments have a significantly higher
233 carbon (1.6-fold) and nitrogen (1.7-fold) contents than surrounding sediment (Kruskal-Wallis,
234 $p < 0.05$) (Fig. 5).

235 In both study stations, $\delta^{13}\text{C}$ of surrounding and of digestive sediments (esophagus and
236 rectum contents) were not significantly different (Kruskal-Wallis, $p > 0.05$). However, $\delta^{15}\text{N}$
237 sediments of the esophagus reached values of $22.4 \pm 3.3 \text{ ‰}$ (Fig. 6). In both study stations,
238 $\delta^{15}\text{N}$ values were significantly higher in the digestive tube (esophagus and rectum contents)
239 than in the surrounding sediment (Kruskal-Wallis, $p < 0.05$).

240 3) Feeding experiments

241 After incubation with ^{13}C -glucose and ^{15}N -ammonium, the surrounding sediment of
242 Biomar station presented a $\delta^{13}\text{C}$ increasing from -19.4 ± 0.1 to $564.9 \pm 3.9 \text{ ‰}$ and a $\delta^{15}\text{N}$
243 rising from 7.6 ± 0.3 to $3543.8 \pm 54.2 \text{ ‰}$. The surrounding sediment of Port Pétrolier showed
244 a $\delta^{13}\text{C}$ increasing from -18.5 ± 0.2 to $626.6 \pm 142.5 \text{ ‰}$ and a $\delta^{15}\text{N}$ rising from 9.1 ± 0.1 to
245 $2960.4 \pm 263.6 \text{ ‰}$.

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

249 For each sea urchin, the contribution of the enriched surrounding sediment to the
250 digestive content was independently evaluated using ^{13}C and ^{15}N and average difference
251 between each evaluation was $7.3 \pm 8.0 \text{ ‰}$. For Biomar station, the contribution of enriched
252 sediment to the esophagus content regularly increased forming the totality ($100 \pm 7\%$) of the

253 esophagus content after 80 hours experiment (Fig. 8). At Port Pétrolier, this contribution was
254 $81 \pm 17\%$ after 80 hours and not significantly different from values obtained for Biomar
255 (Kruskal-Wallis, $p > 0.05$). The contribution of enriched sediment to the rectum content
256 started to increase after 5 hours ($1.0 \pm 0.3\%$) and regularly increased during the experiment
257 without reaching a plateau. After 80 hours, this contribution was $82.4 \pm 23.8 \%$ for Biomar
258 and was not significantly different from the contribution of $71.5 \pm 16.4 \%$ observed for Port
259 Pétrolier (Kruskal-Wallis, $p > 0.05$). For Biomar, data on the contribution of enriched
260 sediment to the rectum content were used to fit a linear regression ($r^2 = 0.89$) revealing an
261 incubation time of 89 hours, necessary to reach a contribution of 100 %. The dissection of the
262 digestive tube of *A. cordatus* allowed measuring a dry weight sediment content of 3.23 ± 1.0
263 g. Considering that 89 hours are required to fill in its the digestive tube, *A. cordatus* would
264 present an ingestion rate of $36 \text{ mg dry sediment h}^{-1}$.

265

266

Discussion

267

Selective feeding

268

269

270

271

272

273

274

275

276

277

278

279

280

281

282

283

284

285

286

287

288

289

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 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. The simultaneous use of both methods increases the reliability of results and both leads to similar conclusions suggesting a limited selective feeding of *A. cordatus*.

When non selective, deposit feeders may ingest large amounts of sedimented material consisting of sharp-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 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 et al. 1984, De Ridder & Jangoux 1985, Hollertz & Duchêne 2001, Hollertz 2002, Boon & Duineveld 2012). However, sea urchins of the genus *Abatus* do not present 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

290 trap fine and organic-rich particles carried by ventilatory currents. This mucus is then
291 transported to the mouth by aboral floor spines along the ambulacral groove (De Ridder et al.
292 1987). This selective feeding mode using mucus was suggested for *B. lyrifera* (Hollertz 2002)
293 but not for the genus *Abatus*.

294 In the present study, the metabarcoding targeting the ADNr16S gene suggests a
295 similar microbiota composition between the sea urchin esophagus content and the
296 surrounding sediment. Despite the ecological importance and the diversity of the genus
297 *Abatus* (11 species are described in the Southern Ocean, Fabri-Ruiz et al. 2017), the gut
298 microbiota has only been recently described in the Antarctic species *Abatus agassizii*
299 (Schwob et al. 2020). Comparing the bacterial communities of the gut content and the
300 surrounding sediment, the authors observed that the same bacterial classes were present in
301 both micro-environments, although their compositions at finer taxonomic scale (OTUs) were
302 significantly different. Such a discrepancy with results from Schwob *et al.* (2020) could
303 principally be due to differences in sampling protocols as the present study has focused on the
304 esophagus bacterial community whereas the whole gut content of *A. agassizii* was analyzed
305 by Schwob *et al.* (2020). Physicochemical conditions are variable according to the digestive
306 tube regions (Thorsen 1998) and each regions could consequently present different gut
307 microbiota. The physicochemical conditions inside the esophagus are likely more similar to
308 surrounding sediment ones in comparison with the rest of the digestive tract thus explaining
309 that we did not observe any difference in the bacterial communities. The differentiation of a
310 specific microbiota would occur in the more posterior part of the digestive tract. This study
311 suggests that the feeding mechanism in *A. cordatus* would not allow the selective ingestion of
312 bacteria according to strain.

313 Meiofauna is a discrete group of small (passing through a 0.5 mm-mesh sieve), highly

314 diverse, abundant and productive organisms dwelling in sediment (Giere 2009, Schratzberger
315 & Ingels 2018). Meiofauna is a high-quality food source containing unsaturated fatty acids
316 that most metazoans are unable to produce (Leduc et al. 2009, De Troch et al. 2012) and
317 consequently, play an important role in the diet of epibenthic consumers from crustaceans
318 (Bell & Coull 1978, Nilsson et al. 1993) to vertebrates such as fish (Fitzhugh & Fleeger 1985,
319 Henry & Jenkins 1995). Heart sea urchins can affect meiofauna through predation and/or
320 alteration of chemical and physical characteristics of environment sediments (Austen &
321 Widdicombe 1998). Fatty acid composition in the foregut of *E. cordatum* suggests selective
322 ingestion of meiofauna (Boon & Duineveld 2012). In the present study, metabarcoding
323 targeting the ADNr18S gene reveals that only one organism from meiofauna was more
324 abundant in the esophagus than in surrounding sediments indicating a limited selective
325 ingestion of meiofauna by *A. cordatus*. Stable isotope composition in natural conditions or
326 after enrichment both suggest that the nematode community, the most abundant meiofauna
327 member, does not constitute a significant part of *Abatus*' diet. Such a limited trophic role of
328 meiofauna could be due to the large size of *A. cordatus* as selective ingestion of meiofauna
329 decreases with the size of consumers (Pascal et al. 2019).

330 *Acoela* is the only organism that is significantly more abundant in the esophagus of *A.*
331 *cordatus* than in surrounding sediment. Acoels are bilaterally symmetric worms in the
332 millimeter-size range (Achatz et al. 2013). Of the nearly 400 described species, the majority
333 are free-living organisms but seven species are parasites or endosymbionts associated to the
334 digestive tube of echinoderms (Jennings 1971), which could explain their higher abundance in
335 the esophagus of *A. cordatus*. The macro algae *Protomonastroma undulatum* and the
336 platyhelminth *Kalyptorhynchia* are less abundant in the sea urchin esophagus than in the
337 environment, suggesting that the sea urchin could avoid ingesting these two food items. The

338 platyhelminth can also escape predation by the sea urchin but to our knowledge, such a
339 behaviour has never been described.

340 Carbon and nitrogen contents in the esophagus sediments were respectively 1.6 and
341 1.7 times higher than in the surrounding sediments. Higher values were measured in the gut
342 content of the spatangoid *B. lyrifera* with ratios of 2 for C and 2.5 for N (Hollertz 2002). In
343 the gut content of *E. cordatum*, the organic matter is even fourfold higher than in the
344 surrounding sediment (De Ridder et al. 1984). Such high values can be explained by a
345 selective ingestion of carbon- and nitrogen- rich particles in the two last species that use
346 specialized tube feet to pick up and select these particles from the sediment surface and
347 convey them to the mouth through the vertical funnel of their burrows. Comparatively, the
348 feeding mechanism in *A. cordatus* must be less selective. The measured differences could also
349 be due to the role of mucus increasing C and N content in the ingested sediment. The mucus
350 can be produced *i*) externally by clavulae of the fascioles, transported to the mouth and
351 ingested (De Ridder et al. 1987, Hollertz 2002) or *ii*) internally in the posterior part of the
352 esophagus that produce mucus to allow sediment compaction (Holland & Ghiselin 1970, De
353 Ridder 1987).

354 In both sampling locations, $\delta^{15}\text{N}$ in the esophagus contents reached significantly
355 higher values (17 ‰) than in the surrounding sediment. $\delta^{15}\text{N}$ of an organism tissue reflects its
356 trophic position in the trophic network (Fry 2006). In Kerguelen, $\delta^{15}\text{N}$ values exceeding 17 ‰
357 are rarely reached even in organisms of higher trophic levels such as marine mammals
358 (Cherel et al. 2008) and marine birds (Camprasse et al. 2017a, Camprasse et al. 2017b). The
359 present study reveals *i*) a nematode community with $\delta^{15}\text{N}$ below 13 ‰ and *ii*) a limited
360 selective feeding behaviour of *A. cordatus*. As a result, high $\delta^{15}\text{N}$ values measured in the
361 esophagus content are unlikely due to a selective ingestion of ^{15}N enriched food items.

362 Metabarcoding indicated that eukaryotic symbionts of the genus *Acoela* are present in the
363 esophagus of *A. cordatus*. However, considering the small quantity of *Acoela*, it can be
364 reasonably assumed that this symbiont is not at the origin of the high $\delta^{15}\text{N}$ signature of the *A.*
365 *cordatus* esophagus content. As suggested earlier, a significant fraction of organic matter of
366 the esophagus could be constituted by the mucus produced externally and internally by *A.*
367 *cordatus*. However the mucus should present an isotopic composition similar to the sea urchin
368 tissues, lower than 11 ‰, and should consequently not contribute to high $\delta^{15}\text{N}$ of ingested
369 sediment. The ^{15}N enrichment in esophagus content is more probably linked with a
370 preferential assimilation of the lighter isotope due to its weaker bonds during the digestion
371 process (Fry et al. 1984). Consequently, the unabsorbed food in the gut becomes more
372 enriched in the heavier isotope (Olive et al. 2003). As catabolic reactions also favour lighter
373 isotope through production of excretory products (Minagawa & Wada 1984), the consumer
374 tissue finally become enriched in heavier isotope (Olive et al. 2003). Thanks to this
375 relationship between isotopic compositions of sources and consumers, stable isotopes are
376 routinely used to evaluate diet composition of consumers (Boecklen et al. 2011). Due to
377 limitations in sampling efforts and/or limitations in *a priori* knowledge of potential food
378 items, exhaustive sampling of food sources of a consumer is difficult and several studies
379 examined signatures of diet based on materials removed from the gastrointestinal tract (e.g.
380 Fry 1988, Peterson et al. 1993, Yatsuya & Nakahara 2004). The use of this proxy implies a
381 limited effect of ingestion and digestion on the isotopic composition of diet. A limited number
382 of studies evaluated this effect and they focused on carnivorous (Grey et al. 2002, Guelinckx
383 et al. 2008) and herbivorous (Johnson et al. 2012) fish. According to these studies, the effect
384 of ingestion on ^{13}C and ^{15}N of food can be either limited (Grey et al. 2002) or significant
385 (Guelinckx et al. 2008, Johnson et al. 2012). However, the range of ^{15}N enrichment in the

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

393 One means of diet selection is selective assimilation in the gut (Self & Jumars 1978).
394 Absorption efficiency can be estimated by comparing gut content between the proximal and
395 the distal parts of the gut (De Ridder et al. 1984). In both sampling stations, the proximal
396 (esophagus) and distal (rectum) parts of the digestive tube presented similar C and N contents,
397 and higher values than in the surrounding sediment. Similar results were observed for *B.*
398 *lyrifera* with higher C and N in defecated sediments than in the environment suggesting an
399 increase due to the presence of mucus and bacteria (Hollertz 2002). The sea urchin faeces
400 would consequently constitute a food source favourable for meio and macrofauna (Austen &
401 Widdicombe 1998). $\delta^{15}\text{N}$ of gut content is similar between first and last part of the digestive
402 tube of *A. cordatus*. Preferential assimilation of lighter isotope occurs during all the digestion
403 process and should lead to a regular increase of $\delta^{15}\text{N}$ along the digestive tract. Inversely,
404 catabolic reactions lead to a decrease of $\delta^{15}\text{N}$ in the terminal part of gut content as they favor
405 the lighter isotope eliminated through waste products (Minagawa & Wada 1984, Olive et al.
406 2003). Both reactions should be of similar intensity and would explain the reduced difference
407 of $\delta^{15}\text{N}$ between the esophagus and rectum sediments.

408 This study revealed a limited selective behaviour of *A. cordatus* as previously reported
409 for Antarctic spatangoids that present a diet relying on sediment-associated organic matter

410 with a low trophic plasticity (Michel et al. 2016).

411 *Feeding kinetic*

412 In order to measure sediment ingestion rates in *A. cordatus*, feeding experiments were
413 run in aquariums whose artificial conditions can affect the behaviour of spatangoids
414 (Thompson & Riddle 2005). Aquarium conditions (light, temperature and sediment
415 composition) were kept as close as possible to environmental ones to limit those biases.
416 According to species, spatangoids can present different activities between day and night
417 (Hammond 1982a, Thompson & Riddle 2005). To reduce this nycthemeral variability,
418 feeding experiments were run during several days (80 hours). Ingestion rates of spatangoids
419 increased with rising temperature (Hollertz & Duchêne 2001) and measured ingestion rates
420 would likely be lower during colder austral winter. For deposit feeders like spatangoids, a
421 widespread method to select food is to increase ingestion rates at sediment depth or location
422 with patch of high food quality (Hollertz 2002) and to move faster in areas of low food
423 availability (Hammond 1983). In aquarium, the sediment was homogenized and the sea
424 urchins did not have the opportunity to select patch of sediment. However, both sediments
425 used during feeding experiments were *i*) collected in areas with abundant *A. cordatus* and *ii*)
426 ingested by sea urchin in the field as revealed by metabarcoding. Moreover feeding rates were
427 similar in both sediments suggesting that underestimation due to unrealistic unpalatable
428 sediment should be limited.

429 During feeding experiments, the progression of stable isotope enrichment in proximal
430 and distal parts of *A. cordatus* digestive tube informs on digestion processes. It takes more
431 than 20 hours to completely fill the esophagus with enriched sediment whereas the rectum
432 content started to be enriched after 10 hours. Regular gut content transportation would have
433 implied a complete filling of the esophagus before apparition in distal part. Observed shift

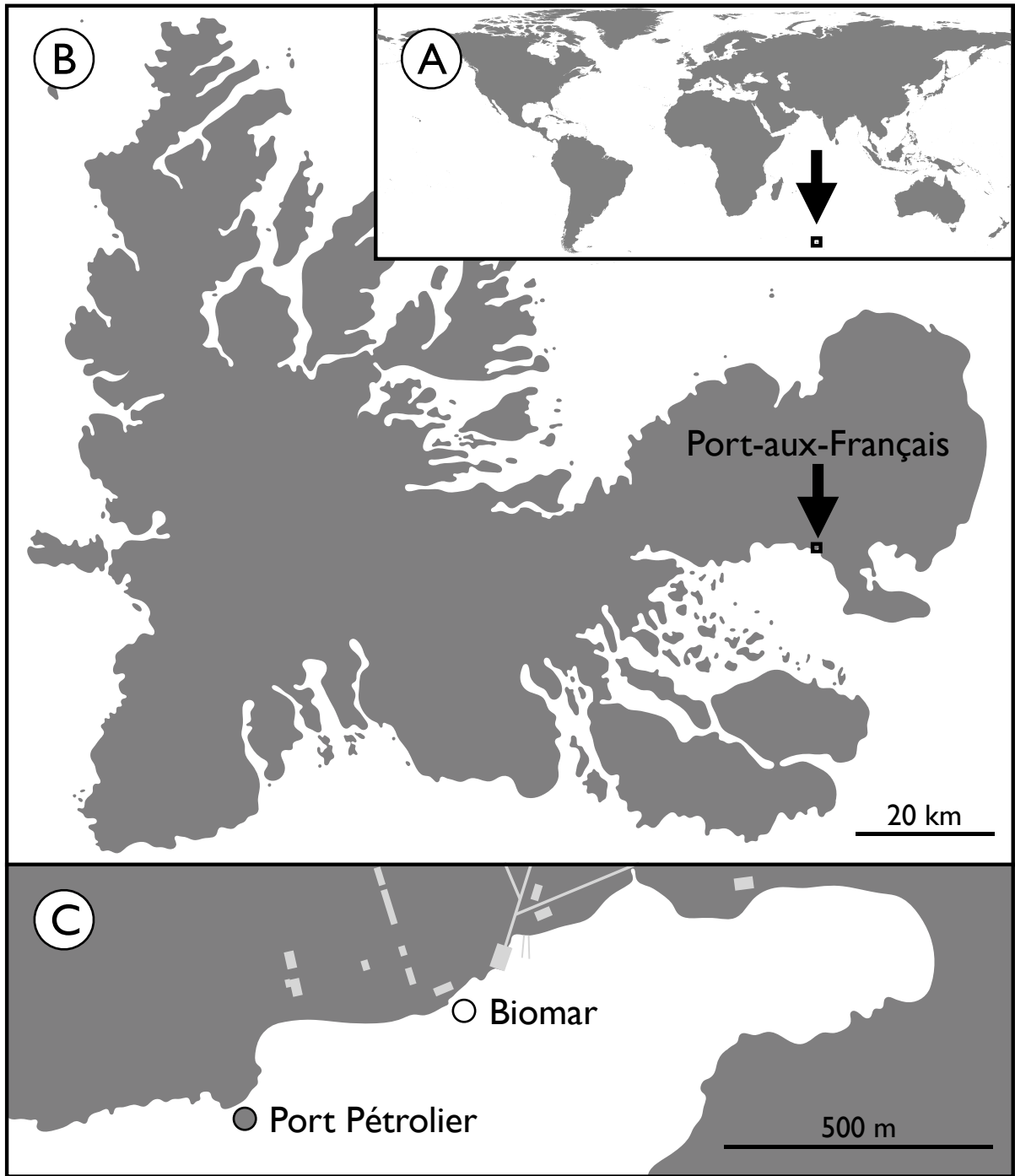
434 implies that food particles are not all transported at similar rapidity. Fast transportation could
435 be associated with the functioning of the siphon allowing rapid water circulation. Spatangoids
436 maintain ciliary currents around their test to create a water current entering in the esophagus
437 and this water is then actively pumped from the esophagus to the intestine through the siphon
438 in order to *i*) provide oxygenated water to the intestine for respiration purposes, *ii*) avoid
439 dilution of enzyme in the stomach, *iii*) facilitate advancement of content of digestive tract and
440 *iv*) carry dissolved organic matter that is assimilated in the intestine (De Ridder et al. 1984).
441 In order to produce isotopically enriched sediment used for feeding experiments of the present
442 study, sediment was incubated with ^{13}C glucose and ^{15}N ammonium and then rinsed using 4
443 cycles of dilution and sedimentation. This rinsing step removed most of non-assimilated
444 enriched isotope and enriched dissolved organic matter. During feeding experiment, high
445 amount of enrichment observed in the terminal part of the digestive tract is consequently more
446 likely due to particulate than dissolved organic matter. Through water circulation in its
447 digestive tube, the spatangoid *E. cordatum* presents a suspension feeding mode in addition to
448 deposit feeding increasing the range of exploitable food items (Rolet et al. 2012). *A. cordatus*
449 would similarly use suspended organic matter as food source but the importance of this
450 feeding mechanism remains to be investigated.

451 The present study suggested a gut resident time of 89 h for *A. cordatus*. This duration
452 is in the range of values (72 to 97 h) observed for the high Antarctic species *Abatus ingens*
453 (Thompson & Riddle 2005). For the temperate species *B. lyrifera*, the gut resident time is
454 linked with temperature with duration of 75 h at 7°C and 19 h at 13°C (Hollertz & Duchêne
455 2001). Another temperate species *E. cordatum* presents a digestive transit of 23-24 h (De
456 Ridder & Jangoux 1985), which is considerably higher than the 4-5 h observed for the
457 tropical species *Meoma ventricosa* (Hammond 1982b). The observed ingestion rate of 0.04 g

458 of dry sediment h^{-1} per specimen of *A. cordatus* is in the range of values obtained for the
459 Antarctic Schizasteridae species *A. ingens* (0.02 to 0.06 g h^{-1}) (Thompson & Riddle 2005) and
460 the temperate species *B. lyrifera* (0.02 to 0.08 g h^{-1}) (Hollertz & Duchène 2001). The
461 temperate spatangoid *E. cordatum* presents higher ingestion rates (0.38 g h^{-1}) but it lives in
462 nutritionally poor sandy habitats suggesting a link between sediment organic matter content
463 and ingestion rates to obtain the required nutrients (De Ridder & Jangoux 1985). The volume
464 of sediment reworked by *A. ingens* moving through sediment is 75 times greater than the
465 volume ingested implying a significant bioturbating role in the Antarctic environment
466 (Thompson & Riddle 2005). The present study reveals that *A. ingens* and *A. cordatus* present
467 similar ingestion rates (Thompson & Riddle 2005) and because local populations of *A.*
468 *cordatus* can reach high densities (Poulin & Féral 1995), it can be reasonably assumed that *A.*
469 *cordatus* can also play a key ecological role through bioturbation in nearshore benthic
470 communities of the Kerguelen islands.

471 Metabarcoding and stable isotope both suggest a limited selective feeding by *A.*
472 *cordatus*. The strong $\delta^{15}\text{N}$ enrichment of sediment in first and last part of the digestive tube
473 could be due to the selective assimilation of lighter isotope in the gut. Additional studies
474 would be necessary to determine the mechanism of this enrichment and its specificity to
475 deposit feeding mode. Feeding experiment using stable isotope enriched sediment revealed
476 different pathways of food particles in the gut of the sea urchin and allowed measurement of
477 feeding rates. The feeding activity of *A. cordatus* suggests that dense populations of the
478 species can play an important ecological role through bioturbation. The loss of this key
479 species due to global changes may consequently affect the ecosystem functioning in nearshore
480 habitats of the Kerguelen Islands.

481



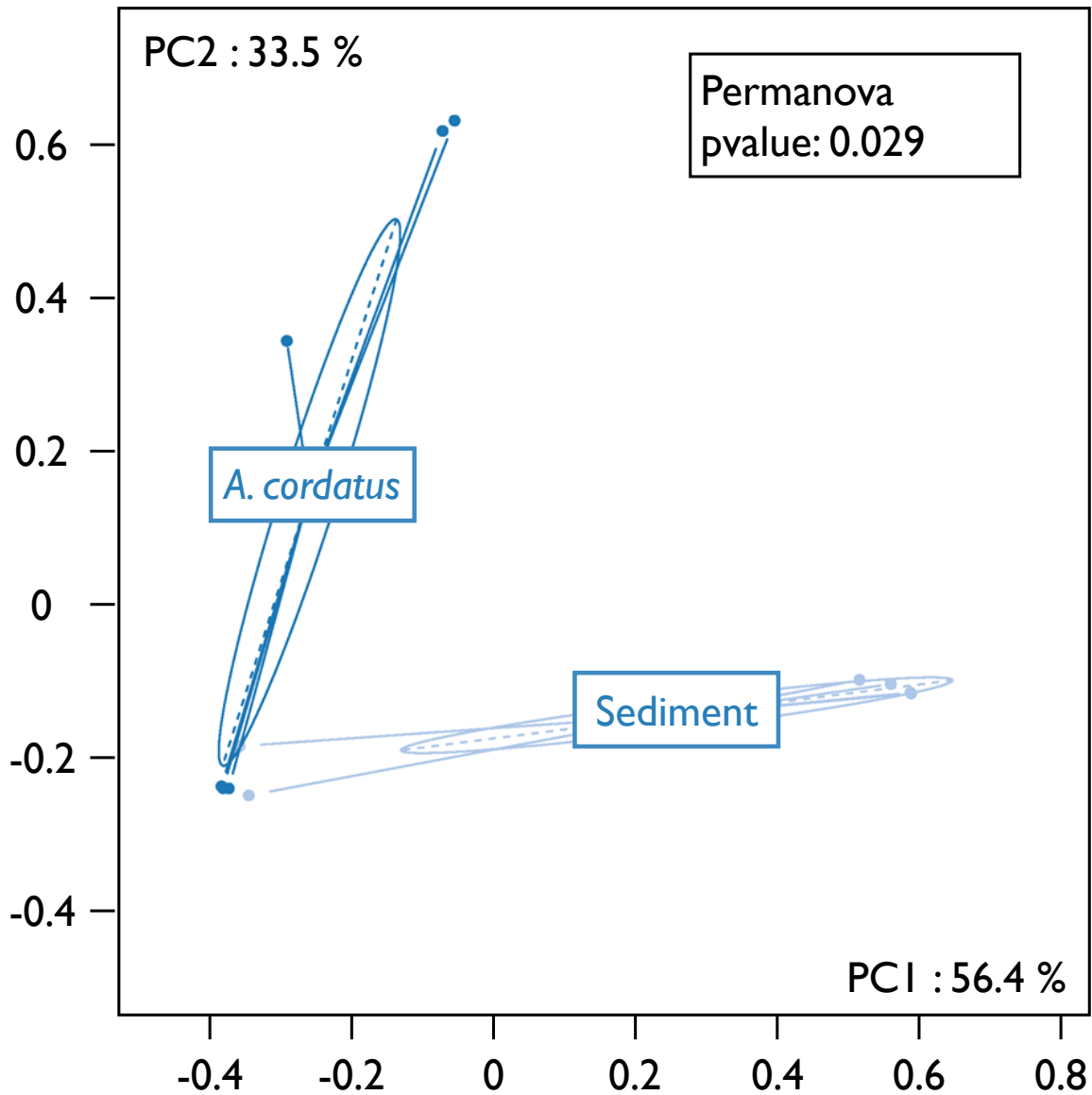
483

484 Figure 1. Positions of A) the Kerguelen archipelago in the Indian Ocean, B) Port-aux-Français

485 in Kerguelen and C) two sampling stations: Biomar (white) and Port Pétrolier (grey) in Port-

486 aux-Français

487



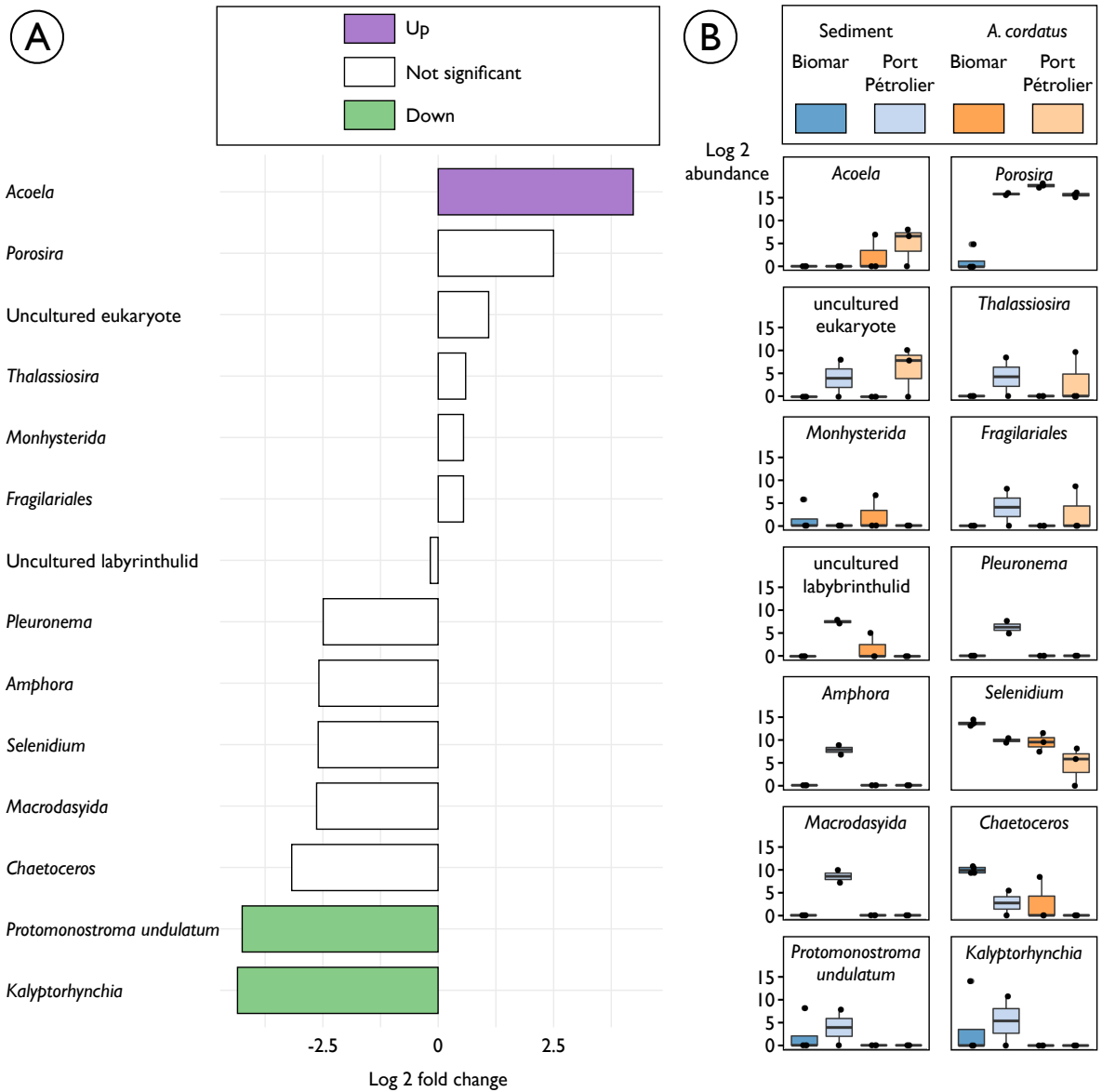
488

489 Figure 2. PCoA plots according to sample types (*A. cordatus* vs sediment) based on Bray-

490 Curtis dissimilarity matrix. PERMANOVA test based on the sample type yielded a significant

491 p-value of 0.029; 89.9% of variations were explained by the first two PC1 and PC2 axis.

492



493

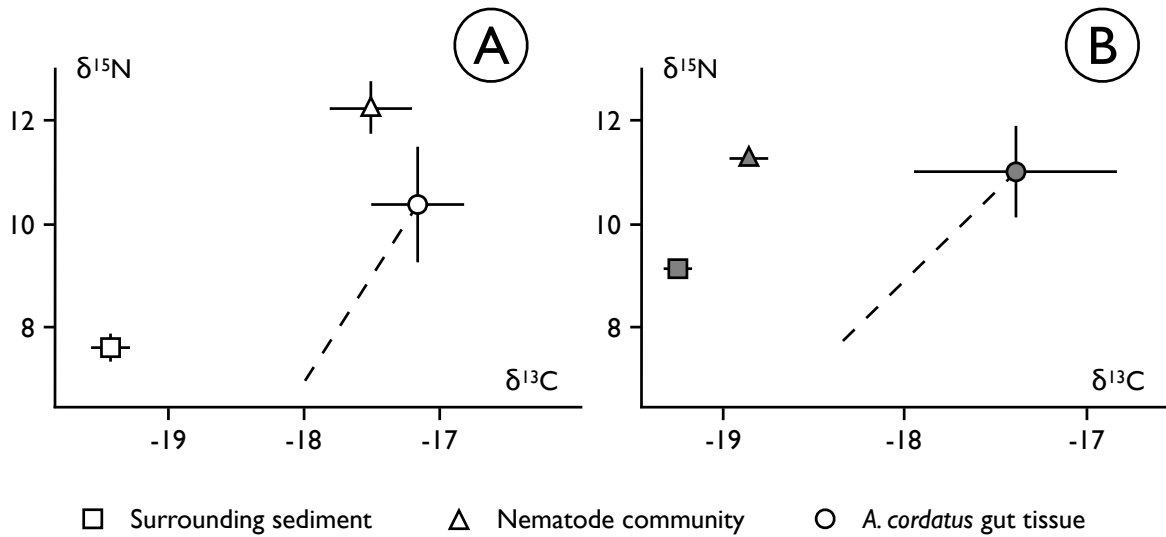
494 Figure 3. A) Barplot of eukaryotic genera significantly associated (p-value < 0,05) to *A.*

495 *cordatus* (purple) and to sediment samples (green). B) Boxplot of the log2 abundances of the

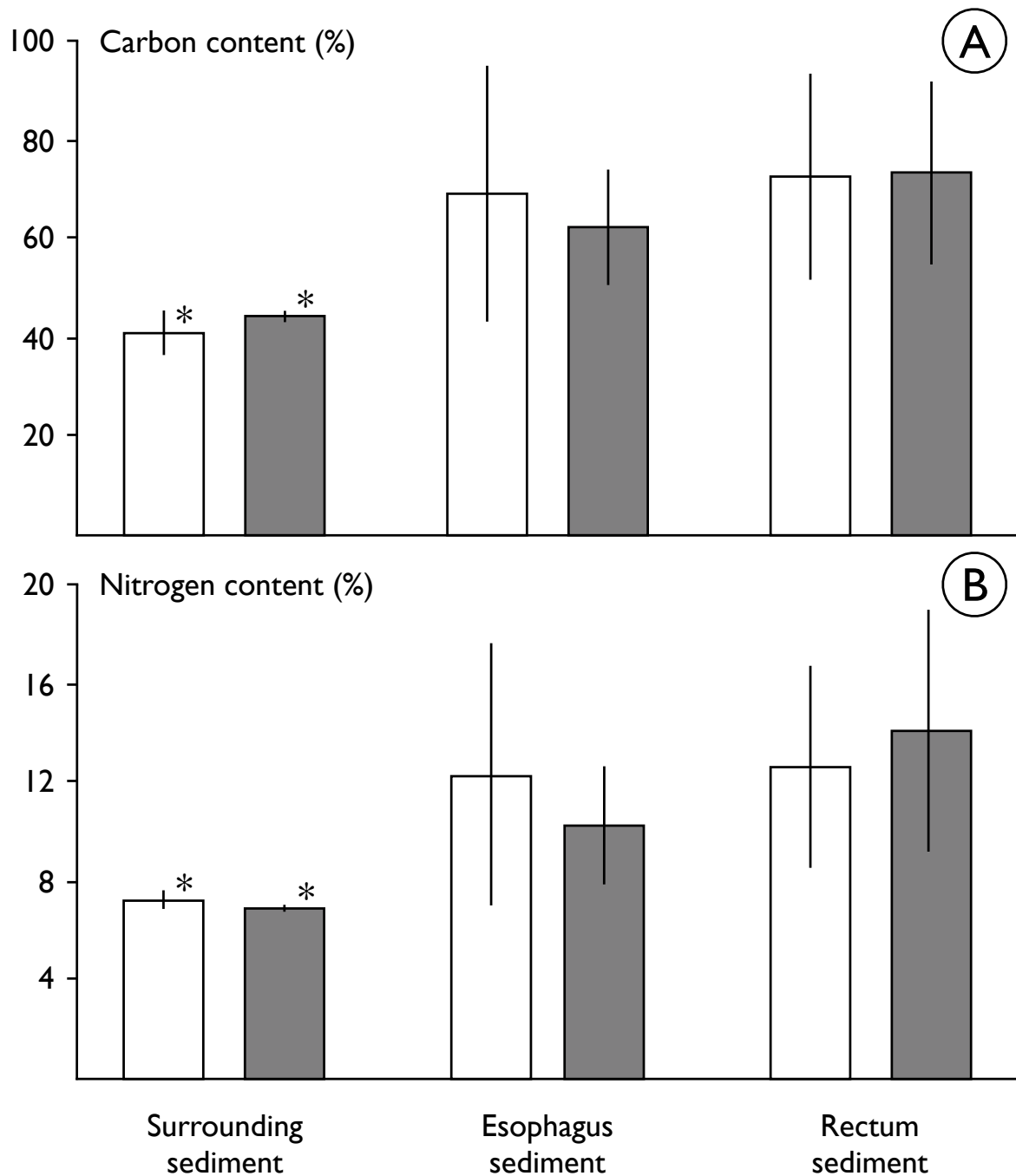
496 14 genera studied in sediments and *A. cordatus* samples on the 2 sites Biomar and Port

497 Pétrolier.

498



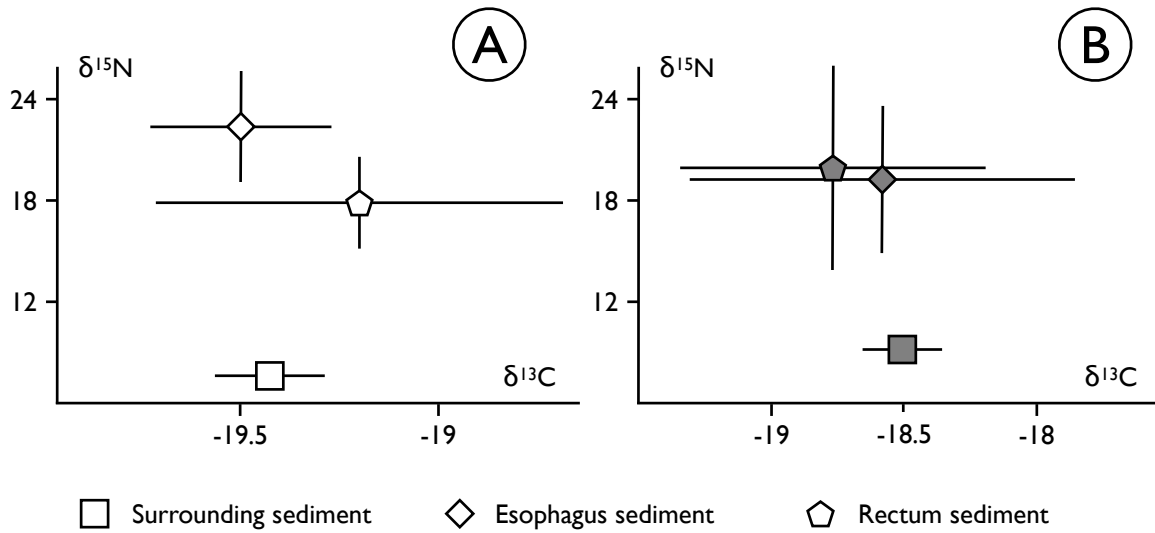
499
 500 Figure 4. Isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; \pm SD; $n = 6$) of surrounding sediment,
 501 nematode community and gut tissue of *A. cordatus* in A) Biomar (white) and B) Port Pétrolier
 502 (grey). Dotted line represents the theoretical isotopic composition of food source of *A.*
 503 *cordatus*, taking into account trophic enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of 1.1 (McCutchan et al.
 504 2003) and 3.4 ‰ (Minagawa & Wada 1984) respectively.
 505



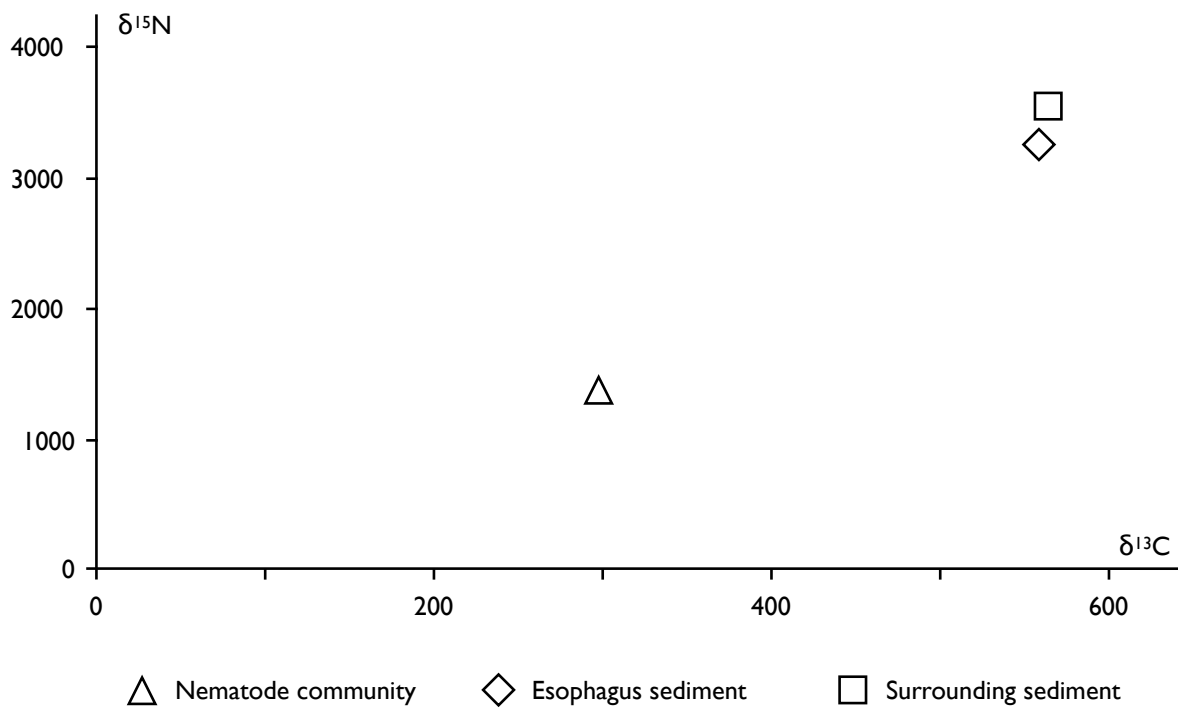
506

507 Figure 5. A) Carbon and B) nitrogen contents (\pm SD; $n = 6$) of sediment and ingested and
 508 digested sediment by *A. cordatus* in Biomar (white) and Port Pétrolier (grey). *: Significant
 509 differences between surrounding sediments and sediments from the digestive tube (Kruskal-
 510 Wallis tests, $p < 0.05$)

511



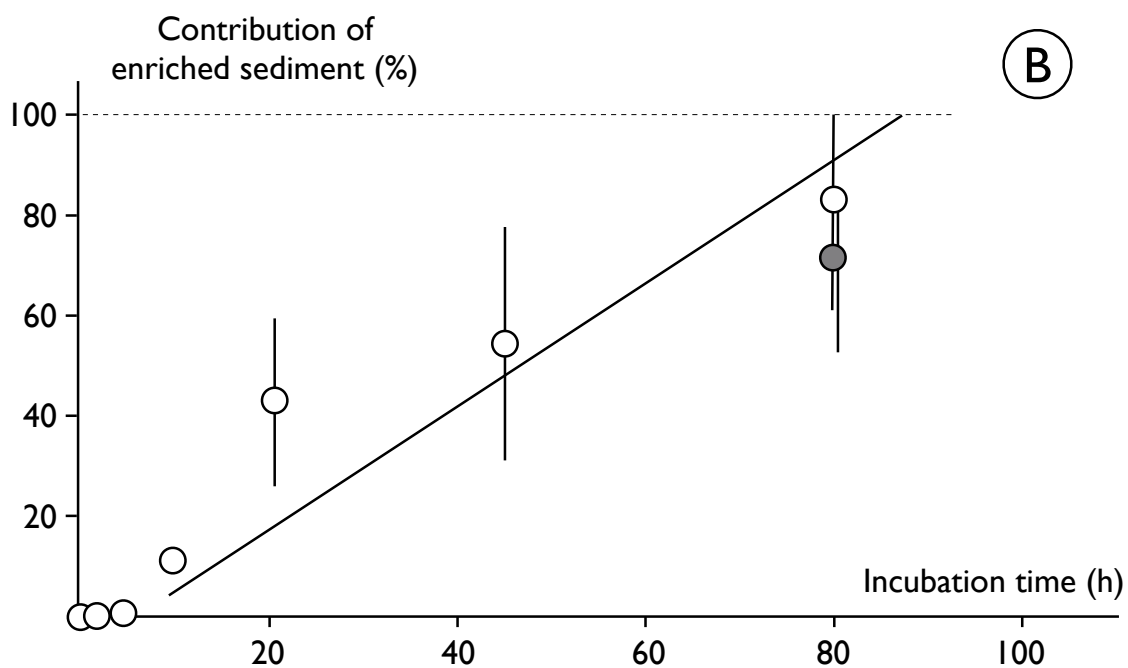
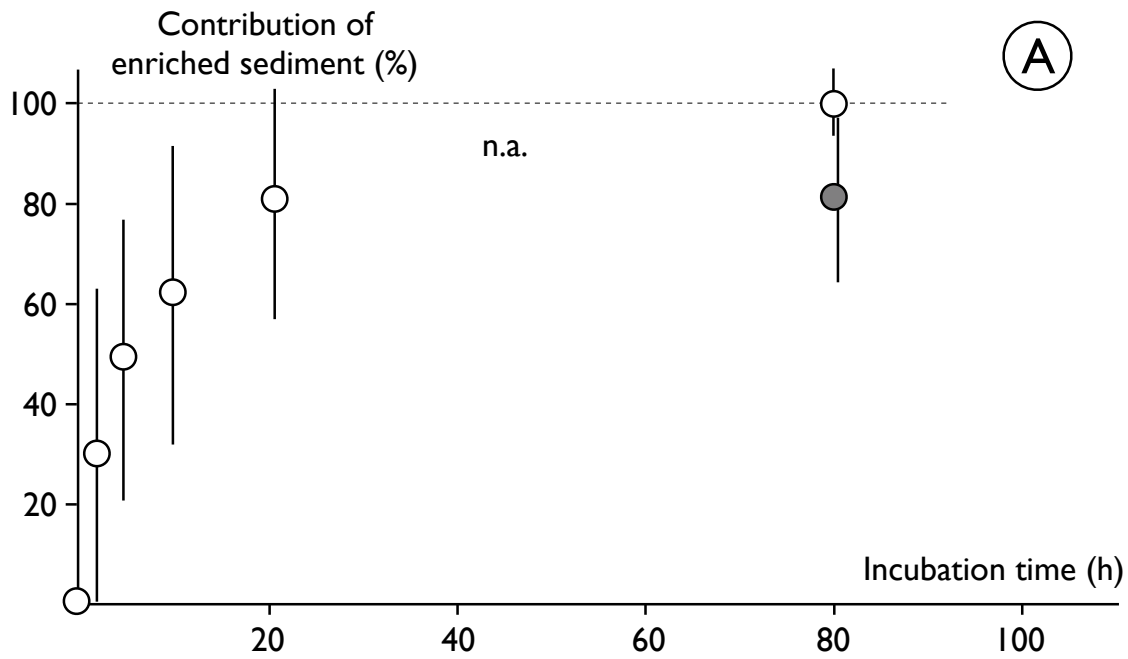
512
 513 Figure 6. Isotopic composition ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$; \pm SD; $n = 6$) of surrounding sediment and of
 514 sediment from the digestive tube of *A. cordatus* in A) Biomar (white) and B) Port Pétrolier
 515 (grey)
 516



517

518 Figure 7. Isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; \pm SD; $n = 6$) of stable isotope enriched source
 519 used in feeding experiment (Biomar nematode community and sediment) and of the sediment
 520 ingested by *A. cordatus* (esophagus content) after an 80 h incubation time.

521



522

523 Figure 8. Contribution of ^{13}C , ^{15}N enriched sediment to the total sediment (in %) in A)

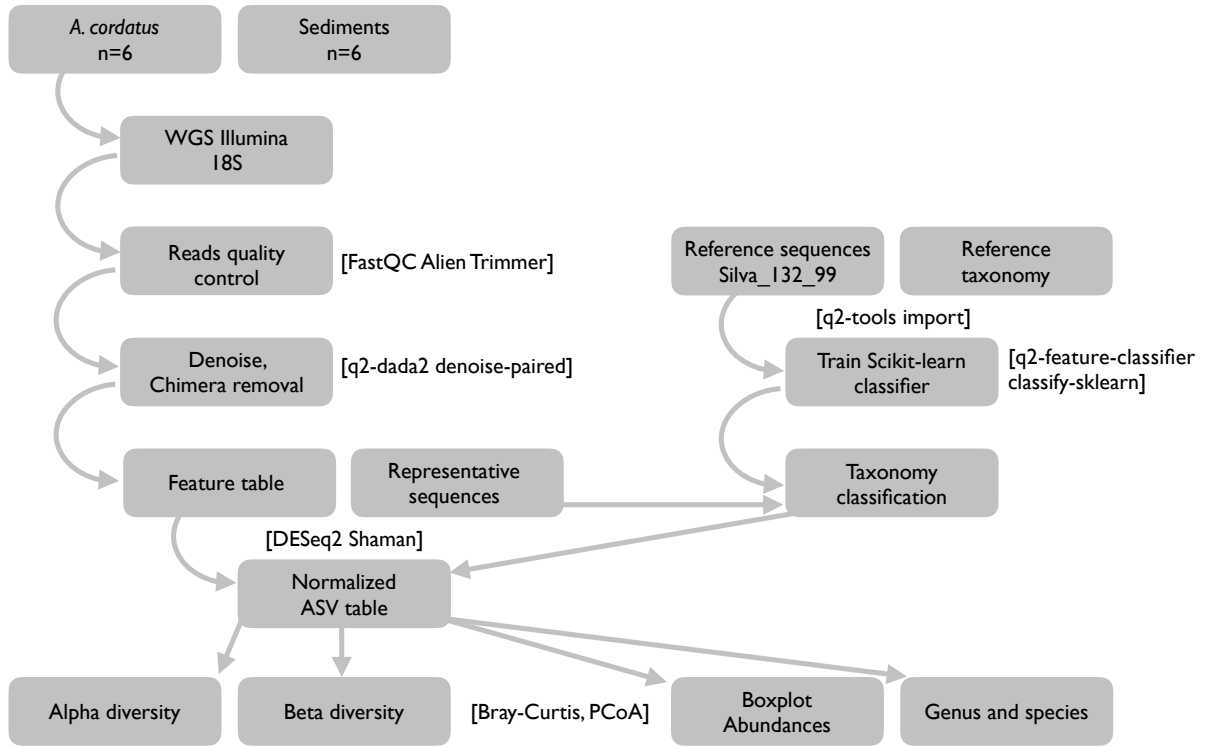
524 esophagus sediment and B) rectum sediment of *A. cordatus* according to the duration of

525 incubation (h) with enriched sediment from Biomar (white) and Port Pétrolier (grey) (means \pm

526 sd, $n = 6$). The linear regression was evaluated with data of ingested sediment from Biomar

527 ($r^2 = 0.98$).

528 30



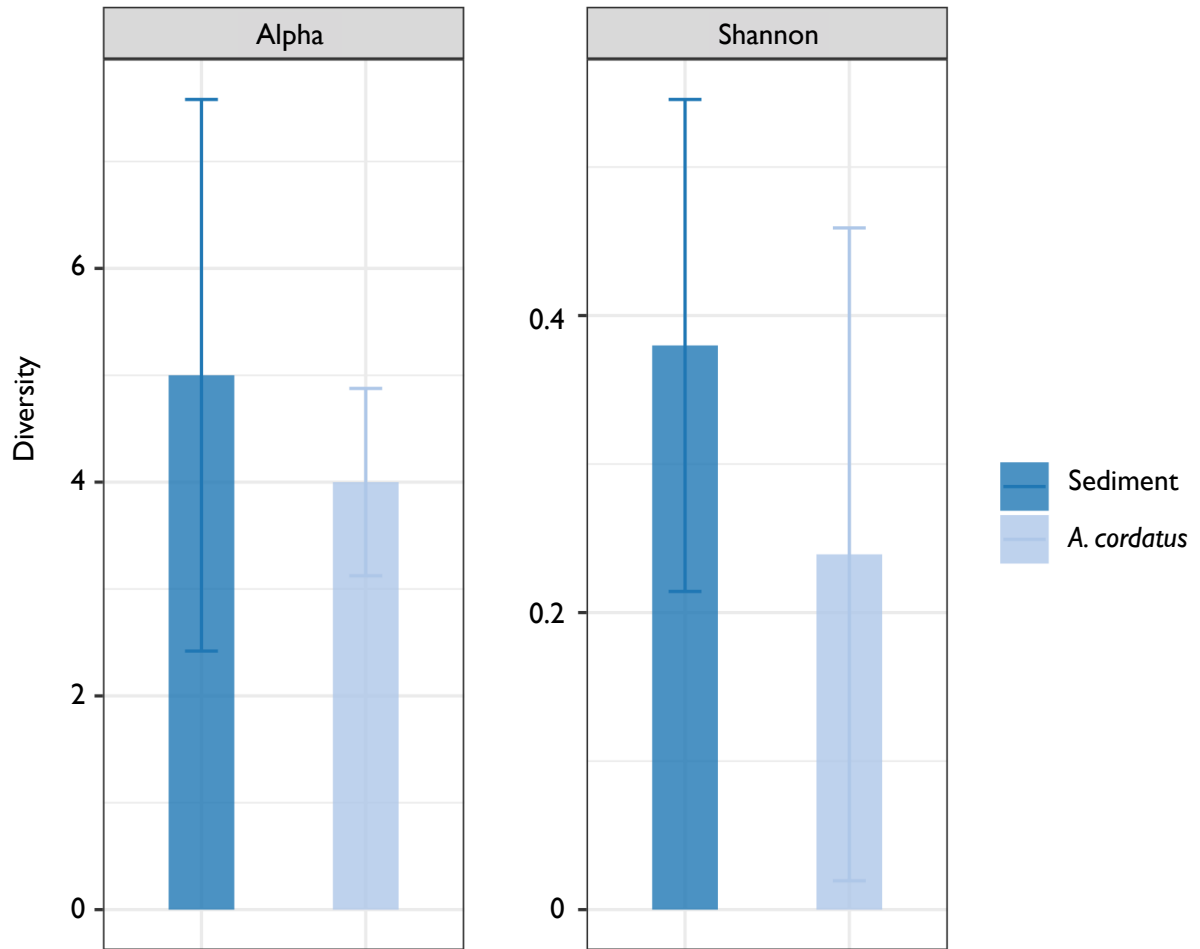
529

530 Online Resource 1. Workflow implemented for eukaryotic metataxonomic analyses; ASV

531 were extracted using QIIME2 pipeline; statistical analyses were performed using

532 SHAMAN pipeline.

533

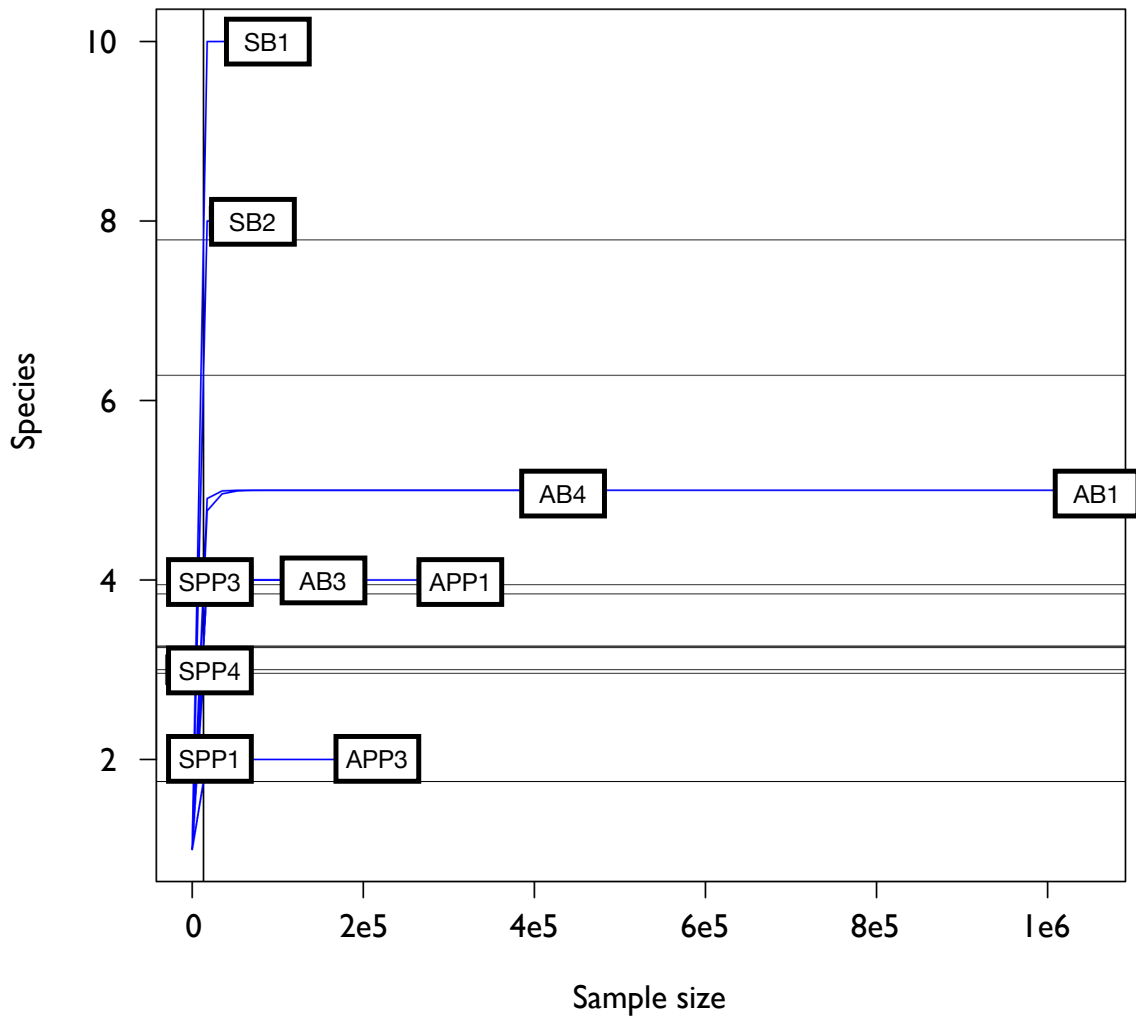


534

535 Online Resource 2. Alpha diversity analysis based on alpha and Shannon indexes for
536 sediments and *A. cordatus* samples; error bars represent 95% confidence intervals.

537

538



539

540 Online Resource 3. Rarefaction curves of ASV for each sample.

541

542

Acknowledgements

543

544

545

546

547

548

549

This study is a contribution to program PROTEKER (No. 1044) of the French Polar Institute and LTSER Zone ATelier Antarctique (ZATA, France). Work in the field also benefited from the support of the National Nature Reserve of the French Southern Territories and its staff and from Chilean PIA CONICYT ACT172065 for EP. We are particularly indebted to Gilles Marty, Sebastien Motreuil and to the crew of the boat *Le Commerson* for their invaluable help and support in the field. We would like to thank Stanislas Dubois for helpful comments about stable isotope data.

550

551

Metabarcoding sequencing performed at Biomics Platform, C2RT, Institut Pasteur, Paris, France, supported by France Génomique (ANR-10-INBS-09-09) and IBISA.

References

- 553 Achatz JG, Chiodin M, Salvenmoser W, Tyler S, Martinez P (2013) The Acoela: on their kind
554 and kinships, especially with nemertodermatids and xenoturbellids (Bilateria incertae
555 sedis). *Org Divers Evol* 13:267-286
- 556 Allan EL, Froneman PW, Durgadoo JV, McQuaid CD, Ansorge IJ, Richoux NB (2013)
557 Critical indirect effects of climate change on sub-Antarctic ecosystem functioning.
558 *Ecol Evol* 3:2994-3004
- 559 Austen MC, Widdicombe S (1998) Experimental evidence of effects of the heart urchin
560 *Brissopsis lyrifera* on associated subtidal meiobenthic nematode communities. *J Exp*
561 *Mar Biol Ecol* 222:219-238
- 562 Bell SS, Coull BC (1978) Field evidence that shrimp predation regulates meiofauna.
563 *Oecologia* 35:141-148
- 564 Bird FL, Ford PW, Hancock GJ (1999) Effect of burrowing macrobenthos on the flux of
565 dissolved substances across the water-sediment interface. *Mar Freshwater Res* 50:523-
566 532
- 567 Blankenship LE, Yayanos AA (2005) Universal primers and PCR of gut contents to study
568 marine invertebrate diets. *Mol Ecol* 14:891-899
- 569 Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotope in trophic
570 ecology. *Annu Rev Ecol Syst* 42:411-440
- 571 Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, Huttley GA, Caporaso
572 JG (2018) Optimizing taxonomic classification of marker-gene amplicon sequences
573 with QIIME 2's q2-feature-classifier plugin. *Microbiome* 17:90
- 574 Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H,
575 Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A,
576 Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK,
577 Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C,
578 Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson
579 DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C,
580 Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR,
581 Keim P, Kelley ST, Knights D, Koester I, Kosciolk T, Kreps J, Langille MGI, Lee J,
582 Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald
583 D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-
584 Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML,
585 Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M,
586 Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh
587 P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-
588 Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber
589 KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R,
590 Caporaso JG (2019) Reproducible, interactive, scalable and extensible microbiome
591 data science using QIIME 2. *Nat biotechnol* 37:852-857
- 592 Boon AR, Duineveld GCA (2012) Phytopigments and fatty acids in the gut of the deposit-
593 feeding heart urchin *Echinocardium cordatum* in the southern North Sea: Selective
594 feeding and its contribution to the benthic carbon budget. *J Sea Res* 67:77-84
- 595 Bromley RG, Asgaard U (1975) Sediment structures produced by a spatangoid echinoid: a
596 problem of preservation. *Bull Geol Soc Den* 24:261-281
- 597 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2:

598 High-resolution sample inference from illumina amplicon data. *Nat Methods* 13:581-
599 583

600 Camprasse ECM, Cherel Y, Arnould JPY, Hoskins AJ, Bustamante P, Bost CA (2017a) Mate
601 similarity in foraging Kerguelen shags: a combined bio-logging and stable isotope
602 investigation. *Mar Ecol Prog Ser* 578:183-196

603 Camprasse ECM, Cherel Y, Bustamante P, Arnould JPY, Bost CA (2017b) Intra- and inter-
604 individual variation in the foraging ecology of a generalist subantarctic seabird, the
605 gentoo penguin. *Mar Ecol Prog Ser* 578:227-242

606 Cherel Y, Ducatez S, Fontaine C, Richard P, Guinet C (2008) Stable isotopes reveal the
607 trophic position and mesopelagic fish diet of femal southern elephant seals breeding
608 on the Kerguelen Islands. *Mar Ecol Prog Ser* 370:239-247

609 Collard M, De Ridder C, David B, Dehairs F, Dubois P (2014) Could the acid-base status of
610 Antarctic sea urchins indicate a better than expected resiliens to near-future ocean
611 acidification? *Glob Change Biol* 21:605-617

612 Criscuolo A, Brisse S (2013) AlienTrimmer: a tool to quickly and accurately trim off multiple
613 short contaminant sequences from high-throughput sequencing reads. *Genomics*
614 102:500-506

615 De Ridder C (1987) Mécanique digestive chez l'échinide fouisseur *Echinocardium cordatum*
616 (echinodermata). *Bull Soc Sci Nat*:65-70

617 De Ridder C, Jangoux M (1985) Origine des sédiments ingérés et durée du transit digestif
618 chez l'oursin spatangide, *Echinocardium cordatum* (Pennant) (Echinodermata). *Ann*
619 *Inst Oceanogr* 61

620 De Ridder C, Jangoux M (1993) The digestive tract of the spatangoid echinoid
621 *Echinocardium cordatum* (Echinodermata): morphofunctional study. *Acta Zool*
622 74:337-351

623 De Ridder C, Jangoux M, de Vos L (1987) Frontal ambulacrum and peribuccal areas of the
624 spatangoid echinoid *Echinocardium cordatum* (Echinodermata): a functional entity in
625 feeding mechanism. *Mar Biol* 94:613-624

626 De Ridder C, Jangoux M, Van Impe E (1984) Food selection and absorption efficiency in the
627 spatangoid echinoid, *Echinocardium cordatum* (Echinodermata). In: Keegan B,
628 O'Connor B (eds) Fifth International Echinoderm Conference. Balkema, Galway

629 De Ridder C, Saucède T (2020) *Echinocardium cordatum*. In: Lawrence JM (ed) Sea urchins
630 : Biology and Ecology, Book 43. Elsevier, Amsterdam

631 De Troch M, Boeckx P, Cnudde C, Van Gansbeke D, Vanreusel A, Vincx M, Caramujo M-J
632 (2012) Bioconversion of fatty acids at the basis of marine food webs: insight from
633 compound-specific stable isotope analysis. *Mar Ecol Prog Ser* 465:53-67

634 Dorgan KM, Jumars PA, Johnson BD, Boudreau BP (2006) Macrofaunal burrowing: the
635 medium is the message. *Ocean Mar Biol Ann Rev* 44:85-121

636 Fabri-Ruiz S, Saucède T, Danis B, David B (2017) Southern Ocean Echinoids database. An
637 updated version of Antarctic, Sub-Antarctic and cold temperate echinoid database.
638 *ZooKeys* 697

639 Féral JP, Saucède T, Poulin E, Marschal C, Marty G, Roca JC, Motreuil S, Beurrier JP (2016)
640 PROTEKER: implementation of a submarine observatory at the Kerguelen Islands
641 (Southern Ocean). *Underw Technol* 34:3-10

642 Fitzhugh GR, Fleeger JW (1985) Goby (Pisces: Gobiidae) interactions with meiofauna and
643 small macrofauna. *Bull Mar Sci* 36:436-444

644 Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic
645 compositions. *Limnol Oceanogr* 33:1182-1190

646 Fry B (2006) Stable isotope ecology. Springer, New-York

- 647 Fry B, Anderson RK, Entzeroth L, Bird JL, Parker PL (1984) ^{13}C enrichment and oceanic
648 food web structure in the Northwestern Gulf of Mexico. *Contrib Mar Sci* 27:49-63
- 649 Ghiold J (1989) Species distribution of irregular echinoids. *Biol Oceanogr* 6:79-162
- 650 Giere O (2009) *Meiobenthology: the microscopic motile fauna of aquatic sediments.*
651 Springer, Berlin
- 652 Gilbert JM, Goldring R (2008) Spatangoid-produced ichnofabrics (Bateig Limestone,
653 Miocene, Spain) and the preservation of spatangoid trace fossils. *Palaeogeogr*
654 *Palaeoclimatol Palaeoecol* 270:299-310
- 655 Grey J, Thackeray SJ, Jones RI, Shine A (2002) Ferox trout (*Salmo trutta*) as « Russian
656 dolls »: complementary gut content and stable isotope analyses of the Loch Ness
657 foodweb. *Freshw Biol* 47:1235-1243
- 658 Guelinckx J, Dehairs F, Ollevier F (2008) Effect of digestion on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish-gut
659 contents. *J Fish Biol* 72:301-309
- 660 Guillaumot C, Fabri-Ruiz S, Martin A, Eléaume M, Danis B, Féral JP, Saucède T (2018)
661 Benthic species of the Kerguelen Plateau show contrasting distribution shifts in
662 response to environmental changes. *Ecol Evol* 8:6210-6225
- 663 Gutt J, Bertler N, Bracegirdle TJ, Bushmann A, Comiso J, Hosie G, Isla E, Schloss IR, Smith
664 CR, Tournadre J, Xavier JC (2015) The Southern Ocean ecosystem under multiple
665 climate change stress: an integrated circumpolar assessment. *Glob Change Biol*
666 21:1434-1453
- 667 Hammond LS (1981) An analysis of grain size modification in biogenic carbonate sediments
668 by deposit-feeding holothurians and echinoids (Echinodermata). *Limnol Oceanogr*
669 26:898-906
- 670 Hammond LS (1982a) Analysis of grain-size selection by deposit-feeding holothurians and
671 echinoids (Echinodermata) from a shallow reef lagoon, Discovery Bay, Jamaica. *Mar*
672 *Ecol Prog Ser* 8:25-36
- 673 Hammond LS (1982b) Patterns of feeding and activity in deposit-feeding holothurians and
674 echinoids (echinodermata) from a shallow back-reef lagoon, Discovery Bay, Jamaica.
675 *Bull Mar Sci* 32:549-571
- 676 Hammond LS (1983) Nutrition of deposit-feeding holothuroids and echinoids
677 (Echinodermata) from a shallow reef lagoon, Discovery Bay, Jamaica. *Mar Ecol Prog*
678 *Ser* 10:297-305
- 679 Henry BA, Jenkins GP (1995) The impact of predation by the girded goby, *Nesogobius* sp. 1,
680 on abundances of meiofauna and small macrofauna. *J Exp Mar Biol Ecol* 191:223-238
- 681 Holland ND (2013) Digestive system. In: Lawrence JM (ed) *Sea urchins: biology and*
682 *ecology.* Academic Press, San Diego
- 683 Holland ND, Ghiselin MT (1970) A comparative study of gut mucuous cells in thirty-seven
684 species of the class Echinoidea (Echinodermata). *Biol Bull (Woods Hole)* 138:286-
685 305
- 686 Hollertz K (2002) Feeding biology and carbon budget of the sediment-burrowing heart urchin
687 *Brissopsis lyrifera* (Echinoidea: Spatangoida). *Mar Biol* 140:959-969
- 688 Hollertz K, Duchêne J-C (2001) Burrowing behaviour and sediment reworking in the heart
689 urchin *Brissopsis lyrifera* Forbes (Spatangoida). *Mar Biol* 139:951-957
- 690 Jangoux M, Lawrence JM (1982) *Echinoderm nutrition.* Balkema Press, Rotterdam
- 691 Jennings JB (1971) Parasitism and commensalism in the Turbellaria. *Adv Parasit* 9:1-32
- 692 Johnson JS, Raubenheimer D, Bury SJ, Clements KD (2012) Effect of ingestion on the stable
693 isotope signatures of marine herbivorous fish diets. *J Exp Mar Biol Ecol* 438:137-143
- 694 Jumars PA (1993) Gourmands of mud: diet selection in marine deposit feeders. In: Hughes
695 RN (ed) *Mechanisms of diets choice.* Blackwell Scientific Publishers, Oxford

- 696 Kurihara H (2008) Effects of CO₂-driven ocean acidification on the early developmental
697 stages of invertebrates. *Mar Ecol Prog Ser* 373:275-284
- 698 Kurihara H, Shiarayama Y (2004) Effects of increased atmospheric CO₂ on sea urchin early
699 development. *Mar Ecol Prog Ser* 274:161-169
- 700 Ledoux JB, Tarnowska K, Gérard K, Lhuiller E, Jacquemin B, Weydmann A, Féral JP,
701 Chenuil A (2012) Fine-scale spatial genetic structure in the brooding sea urchin
702 *Abatus cordatus* suggests vulnerability of the Southern Ocean marine invertebrates
703 facing global change. *Pol Biol* 35:611-623
- 704 Leduc D, Probert PK, Duncan A (2009) A multi-method approach for identifying meiofaunal
705 trophic connections. *Mar Ecol Prog Ser* 383:95-111
- 706 Lohrer AM, Thrush SF, Gibbs M (2004) Bioturbators enhance ecosystem function through
707 complex biogeochemical interactions. *Nature* 431:1092-1095
- 708 Lopez GR, Levinton JS (1987) Ecology of deposit-feeding animals in marine sediments. *Q*
709 *Rev Biol* 62:235-260
- 710 Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for
711 RNA-seq data with DESeq2. *Genome Biol* 15:550
- 712 McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for
713 stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378-390
- 714 Michel LN, David B, Dubois P, Lepoint G, De Ridder C (2016) Trophic plasticity of
715 Antarctic echinoids under contrasted environmental conditions. *Pol Biol* 39:913-923
- 716 Minagawa M, Wada E (1984) Stepwise enrichment of ¹⁵N along food chain: further evidence
717 and the $\delta^{15}\text{N}$ and animal age. *Geochim Cosmochim Acta* 48:1135-1140
- 718 Mooi R (1990) Paedomorphosis, Aristotle's lantern, and the origin of the sand dollar
719 (Echinodermata: Clypeasteroidea). *Paleobiology* 16:25-48
- 720 Nilsson P, Sundback K, Jonsson B (1993) Effect of the brown shrimp *Crangon crangon* on
721 endobenthic macrofauna, meiofauna and meiofaunal grazing rates. *Neth J Sea Res*
722 31:95-106
- 723 Olive PJW, Pinnegar JK, Polunin NVC, Richards G, Welch R (2003) Isotope trophic-step
724 fractionation: a dynamic equilibrium model. *J Anim Ecol* 72:608-617
- 725 Osinga R, Kop AJ, Malschaert JFP, van Duyl FC (1997) Effects of the sea urchin
726 *Echinocardium cordatum* on bacterial production and carbon flow in experimental
727 benthic systems under increasing organic loading. *J Sea Res* 37:109-121
- 728 Pascal PY, Bocher P, Lefrançois C, Nguyen TH, Chevalier J, Dupuy C (2019) Meiofauna
729 versus macrofauna as food resource in a tropical intertidal mudflat. *Mar Biol* 166:144
- 730 Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, Blondel M,
731 Prettenhofer P, Weiss R, Dubourg V, Vanderplas J, Passos A, Cournapeau D, Brucher
732 M, Perrot M, Duchesnay E (2011) Scikit-learn: Machine Learning in Python. *J Mach*
733 *Learn Res* 12:2825-28300
- 734 Peterson B, Fry B, Deegan LA, Hershey A (1993) The trophic significance of epilithic algal
735 production in a fertilized tundra river ecosystem. *Limnol Oceanogr* 38:872-878
- 736 Plante C, Jumars PA, Baross JA (1990) Digestive associations between marine detritivores
737 and bacteria. *Annu Rev Ecol Syst* 21:93-127
- 738 Poulin E, Féral JP (1995) Pattern of spatial distribution of a brood-protecting schizasterid
739 echinoid, *Abatus cordatus*, endemic to the Kerguelen Islands. *Mar Ecol Prog Ser*
740 118:179-186
- 741 Poulin E, Féral JP (1997) Genetic structure of the brooding sea urchin *Abatus cordatus*, an
742 endemic of the subantarctic Kerguelen Island. In: Mooi R, Telford M (eds) 9th
743 Echinoderm conference. Balkema, San Fransisco
- 744 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013)

745 The SILVA ribosomal RNA gene database project: improved data processing and
746 web-based tools. , 41(Database issue), D590–D596. doi:10.1093/nar/gks1219. Nucleic
747 acids research 41:D590-D596

748 Quereda JJ, Dussurget O, Nahori MA, Ghazlane A, Volant S, Dillies MA, Regnault B,
749 Kennedy S, Mondot S, Villoing B, Cossart P, Pizarro-Cerda J (2016) Bacteriocin from
750 epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. P Natl
751 Acad Sci USA 113:5706-5711

752 Radwanski A, Wysocka A (2001) Mass aggregation of Middle Miocene spine-coated
753 echinoids *Echinocardium* and their integrated eco-taphonomy. Acta Geol Pol 51:295-
754 316

755 Rolet G, Ziegler A, De Ridder C (2012) Presence of a seawater-filled caecum in
756 *Echinocardium cordatum* (Echinoidea: Spatangoida). J Mar Biol Ass U K 92:379-385

757 Saucède T, Guillaumot C, Michel L, Fabri-Ruiz S, Bazin A, Cabessut M, García-Berro A,
758 Mateos A, Mathieu O, De Ridder C, Dubois P, Danis B, David B, Díaz C, Lepoint G,
759 Motreuil S, Poulin E, Féral JP Modelling species response to climate change in sub-
760 Antarctic islands: echinoids as a case study for the Kerguelen plateau. In: Welsford D,
761 Dell J, Duhamel G (eds). Proc The Kerguelen Plateau: marine ecosystem and
762 fisheries. Australian Antarctic Division

763 Schatt P, Féral JP (1991) The brooding cycle of *Abatus cordatus* (Echinodermata:
764 Spatangoida) at Kerguelen Islands. Pol Biol 11:283-292

765 Schratzberger M, Ingels J (2018) Meiofauna matters: the role of meiofauna in benthic
766 ecosystems. J Exp Mar Biol Ecol 502:12-25

767 Schwob G, Cabrol L, Poulin E, Orlando J (2020) Characterization of the gut microbiota of the
768 antarctic heart urchin (Spatangoida) *Abatus agassizzi*. Front Microbiol 11:308

769 Self RFL, Jumars PA (1978) New resource axes for deposit feeders? J Mar Res 36:627-641

770 Steneck RS (2013) Sea urchins as drivers of shallow marine community structure. In:
771 Lawrence JM (ed) Sea urchins: Biology and ecology. Academic Press, San Diego

772 Thompson BAW, Riddle MJ (2005) Bioturbation behaviour of the spatangoid urchin *Abatus*
773 *ingens* in Antarctic marine sediments. Mar Ecol Prog Ser 290:135-143

774 Thorsen MS (1998) Microbial activity, oxygen status and fermentation in the gut of the
775 irregular sea urchin *Echinocardium cordatum* (Spatangoida: echinodermata). Mar Biol
776 132:423-433

777 Vopel K, Vopel A, Thistle D, Hancock N (2007) Effects of spatangoid heart urchins on O₂
778 supply into coastal sediment. Mar Ecol Prog Ser 333:161-171

779 Weimerskirch H, Inchausti P, Guinet C, Barbraud C (2003) Trends in birds and seals
780 populations as indicators of a system shift in the Southern Ocean. Antarct Sci 15:249-
781 256

782 Widdicombe S, Austen MC, Kendall MA, Warwick RM, Jones MB (2000) Bioturbation as a
783 mechanism for setting and maintaining levels of diversity in subtidal macrobenthic
784 communities. Hydrobiologia 440:369-377

785 Yatsuya K, Nakahara H (2004) Diet and stable isotope ratios of gut contents and gonad of the
786 sea urchin *Anthorcidaris crassispina* (A. Agassiz) in two different adjacent habitats,
787 the *Sargassum* area and *Corallina* area. Fish Sci 700:285-292

788