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Mollusc species richness and abundance from shelf to abyssal depths in the Ross Sea (Antarctica): the importance of fine-mesh-towed gears and implications for future sampling

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(Article begins on next page)

38 **Introduction**

39 The Ross Sea continental shelf, with an area of roughly 473 km², is the second largest in
40 Antarctica after the Weddell Sea. The average depth of the shelf is 500 m, and the shelf break
41 occurs at about 800-1000 m, from where the continental slope extends steeply down to 3000 m
42 (Clarke et al, 2007; Scambos et al. 2007). Beyond the continental slope to the north, there are a
43 number of seamounts and islands, including the Scott Seamount chain, the Admiralty Seamount,
44 and the Balleny Islands.

45 To date, around 28 historical and recent expeditions have collected benthic material in the
46 region (Clarke et al, 2007; Griffiths et al. 2011; Schiaparelli et al. 2014 for the list of historical
47 expeditions) making the Ross Sea continental shelf one of the best-studied Antarctic seabed areas
48 (Clarke et al. 2007; Griffiths et al. 2011).

49 However, despite this significant historical sampling effort, our knowledge of the benthic
50 diversity of the Ross Sea is still incomplete, as demonstrated by recent additions of both small
51 (Rehm et al. 2007; Ghiglione et al. 2013; Lörz et al. 2013; Schiaparelli et al. 2014; Błażewicz-
52 Paszkowycz et al. 2014; Piazza et al. 2014) and large taxa, including the 3 metres tall hydroid
53 *Branchiocerianthus* sp. (Schiaparelli et al. in prep.), the stalked crinoids communities found on the
54 Admiralty seamount (Bowden et al. 2011; Eléaume et al. 2011) and the large bivalve of the genus
55 *Acesta* found on the Scott Seamount (Piazza et al. 2015). During the International Polar Year (IPY,
56 2007/2008), under the coordination of Census of Antarctic Marine Life (CAML) (Schiaparelli et al.
57 2013), substantial new sampling campaigns were undertaken in several Antarctic areas including
58 the Ross Sea, which was the focus of a research voyage, the IPY-CAML voyage (TAN0802) of the
59 R/V *Tangaroa*.

60 In this paper we focus on Mollusca (all classes but Caudofoveata and Cephalopoda which were
61 not present in the samples) one of the most extensively studied Phyla in Antarctica (Clarke and
62 Johnston 2003; Griffiths et al. 2003), collected during the TAN0802. As with the earlier *BioRoss*
63 voyage of the R/V *Tangaroa* in 2004 (Mitchell and Clark 2004; Schiaparelli et al. 2006), during the
64 TAN0802 several benthic gears were deployed at each survey site in order to document the
65 diversity of benthos. Deployment of multiple sampling gears with different mesh sizes at the same
66 location is a well-known method in biodiversity assessment to compensate for the different
67 catchability of the species (Bouchet et al. 2002; Longino et al. 2002; Clark et al. 2016).

68 The gear types used during the TAN0802 voyage, included a rough-bottom trawl, beam trawl,
69 epibenthic sledge, and a fine-mesh epibenthic or “Brenke” sledge. The Brenke sled (Brandt et al.
70 2004; Brenke 2005; Lörz et al. 2013) is specifically designed to collect organisms from the benthic

71 boundary layer and has previously been utilized in the Weddell Sea and the Atlantic sector of the
72 Southern Ocean, especially at abyssal depths (Schwabe et al. 2007; Brandt et al. 2014; Jörger et al.
73 2014). The deployment of a Brenke sled during the TAN0802 expedition was its first use in the
74 Ross Sea (Lörz et al. 2013). It also represents the second fine-mesh sampling event in the area,
75 after the deployment in 2004 of a Rauschert dredge, which provided an unexpectedly large
76 number of new records and species of molluscs for the Ross Sea (Ghiglione et al. 2013; Schiaparelli
77 et al. 2014). The use of a Brenke sled during TAN0802 thus gave an opportunity to assess the
78 distribution of mollusc biodiversity across size classes in Antarctica.

79 Answering to this question is becoming an important issue for research in Antarctica, which
80 also goes outside the geographical scope of the study, here limited to Ross Sea. In fact, by
81 considering the need of robust baseline data to measure future changes, it will be of key
82 importance to know if specific sampling gears having a small mesh size (i.e. 500 μm) have to be
83 routinely deployed in future sampling activities in order to retain the smaller fraction, i.e. the one
84 showing the highest diversity.

85 In the deep sea, the observation that faunal diversity may be higher in smaller body-size
86 fractions of the macrofauna has been clear since the 1960s, when mesh sizes smaller than 1 mm
87 began to be routinely used, revolutionizing our knowledge of deep-sea diversity (Hessler and
88 Sanders 1967). For molluscs in general, several studies highlighted that this could be a common
89 pattern with peaks in numbers of individuals and species having been found for body size between
90 0.5 and 4 mm in deep-sea gastropods in the western North Atlantic (McClain 2004), between 1.9
91 and 4.1 mm in New Caledonian reef assemblages (Bouchet et al. 2002) and in the <5 mm fraction
92 in Vanuatu reef assemblages (Albano et al. 2011). However, exceptions are also known (see
93 McClain 2004 and references therein).

94 An apparent lack of 'microfauna' in the Antarctic benthos was highlighted by Dell (1990: 264),
95 who noted that this was likely to be a consequence of sorting methods that did not retain the
96 smallest fraction of the fauna. However, fine-mesh trawling performed in 2004 with a Rauschert
97 dredge (Schiaparelli et al. 2014) provided the first evidence that the smallest mollusc fraction in
98 the Ross Sea is rich both in terms of species and specimens. If present data will confirmed this fact,
99 it is clear that new ecological questions will probably have to be asked about the evolutionary,
100 biogeographic and ecological mechanisms that may have led to a general 'miniaturization' of
101 Antarctic mollusc fauna. The present data from TAN0802 provide a more extensive dataset with
102 which to assess the diversity of Southern Ocean Mollusca in relation to body size.

103

104 **Materials and methods**

105

106 Study area and sample processing

107

108 The study area of TAN0802 covered a latitudinal range from ~66°S to ~77°S, spanning the whole
109 Ross Sea region from the Ross Ice Shelf up to the northern seamounts systems (Admiralty and
110 Scott) (Fig. 1). Three broad areas were considered, namely the “*Northern area*” (from ~66°S to
111 ~70°S), the “*Central area*” (from ~70°S to ~74°S) and the “*Southern area*” (from ~74°S to ~77°S)
112 (Hanchet et al. 2008), following a natural latitudinal gradient.

113 Benthic communities were sampled at sixty-four sampling events at depths ranging from 283
114 m to 3490 m (Fig. 1; Supplementary Table 1) by deploying four types of towed gears with different
115 mesh-sizes: Rough-bottom trawl (ORH); Beam trawl (TB); Brenke sled (SEH) (note that this
116 acronym is reported as such in agreement with the original report of Hanchet et al. 2008 but it is
117 often reported as EBS in literature), and Epibenthic sled (SEL) (Hanchet et al. 2008). Rough-bottom
118 trawl is a commercial-style fish trawl with 300 mm mesh in the forepart of the net, tapering
119 through 100 mm and 60 mm mesh sections to a 40 mm mesh cod-end. The Beam trawl is a 4 m
120 wide bottom trawl designed to sample mega-faunal benthic invertebrates and small benthic fish,
121 having a 25 mm mesh size for the whole net length. The Brenke sled has two fine-mesh with 500
122 µm nets with rigid cod-end containers arranged one above the other (Brenke 2005, here, we
123 report results from the upper and bottom net combined). The Epibenthic sled is a small sled with 1
124 m wide mouth developed for sampling mega-epifauna on rough terrain; it has a short net of 25
125 mm mesh inside a chafing cover of 100 mm mesh (Clarke and Stewart 2016). All gears were towed
126 at approximately 1 knot, except for the ORH at 3 knots.

127 Macroinvertebrates were sorted on board, preserved in 90% ethanol (or, in some cases, kept at
128 -25°C for later DNA extraction). Fine fractions from Brenke sled catches were separated from the
129 sediment through elutriation and preserved as bulk in 90% ethanol.

130

131 Species classification

132

133 In the laboratory, living specimens were sorted under a stereomicroscope, divided into
134 morphospecies and classified to the lowest possible taxonomical level. Minute species, whenever
135 necessary, were photographed using an Environmental Scanning Electron Microscopy (ESEM,
136 model Leo Stereoscan 440). In this contribution all the living fractions of Gastropoda, Bivalvia,

137 Monoplacophora, Scaphopoda, Polyplacophora and Solenogastres were considered. Cephalopoda
138 and Caudofoveata were not present in the samples.

139 Nomenclature of species was crosschecked and matched with WoRMS
140 (<http://www.marinespecies.org>; last check made on May 10, 2016). When available, molecular
141 data (COI barcodes obtained in the framework of the Italian “BAMBi” project, Barcoding of
142 Antarctic Marine Biodiversity, PNRA 2010/A1.10) were used in some cases used to split
143 morphospecies lacking sound morphological characters (e.g. for the family Velutinidae).
144 Specimens not classified to the specific level were included in the multivariate analyses and
145 reported at the level of genus or family.

146 The resulting dataset of geographic occurrences of the species will be made available through
147 ANTABIF (the Antarctic node of the Global Biodiversity Information System;
148 <http://www.biodiversity.aq>) in the collection of distributional data provided by the Italian National
149 Antarctic Museum (MNA, Section of Genoa) (<http://www.gbif.org/dataset/search?q=mna>)
150 (Ghiglione et al. in prep.).

151

152 Statistical analyses

153

154 Statistical analyses were performed to evaluate the effects of latitude and depth on species
155 richness and composition and to compare species richness across body-size fractions.

156 Since the deployment of sampling gears was not even (e.g. SEL was only used on the rough
157 bottoms of the seamounts) and the majority of specimens were collected by the Brenke sled
158 (Supplementary Fig. 1 and 2), statistical analyses were performed separately on datasets from the
159 different gears. In the specific, species richness was studied only on Brenke sled data while
160 composition was studied on presence/absence data considering all gears.

161 Rarity was evaluated in terms of number of species collected as singletons (i.e. species found
162 with a single specimen) and doubletons (i.e. species found with two specimens only), or uniques
163 (i.e. species occurring at a single station only) and duplicates (i.e. species occurring at two stations
164 only).

165

166 Effects of latitude and depth on species richness

167

168 To understand richness patterns in relation to depth and latitude, Brenke sled samples were
169 analysed through a combined analysis of rarefaction and extrapolation techniques. This analysis is

170 based on diversity accumulation curves produced on empirical estimates of the principal Hill
171 numbers (Chao et al. 2012, 2014). Individual-based (for geographic areas) and sample-based (for
172 depth) interpolation (rarefaction) and extrapolation curves (Colwell et al. 2012) were computed
173 using the online iNEXT package (Chao et al. 2016; <https://chao.shinyapps.io/iNEXTOnline/>), which
174 allows the comparison of samples taking into account sample coverage and completeness (Chao
175 and Jost 2012, Chao et al. 2014) in the R-statistical environment (<http://www.r-project.org>).
176 Uncertainty of estimations was reported in terms of 95% confidence intervals under the
177 multinomial model for the observed species sample frequencies (in the case of the individual-
178 based interpolation/extrapolation curves) or under the Bernoulli product model for the incidence
179 matrix (in the case of the sample-based interpolation/extrapolation curves) (Colwell et al. 2012).
180 The non-overlap of 95% confidence interval was used as an indicator of statistical difference
181 (Colwell et al. 2012).

182

183 Effects of latitude and depth on species composition

184

185 Species composition was evaluated through multivariate techniques to test the possible effects
186 of latitude and depth in the structure of benthic communities using presence/absence data from
187 all gears combined. In these analyses the factors “depth” (with levels: 1=0-500 m, 2=501-1000 m,
188 3≥1001 m) and “latitude” (with levels: Northern area, Central area and Southern area, in accord
189 with Schiaparelli et al. 2006) were used. Bray-Curtis similarity index was then calculated and non-
190 metric multidimensional scaling (nmMDS) performed. Two-ways ANOSIM (Clarke 1993) was used
191 to test the differences among the factors latitude and depth and decouple the covariation of
192 depth and latitude. All multivariate analyses were performed with the software PRIMER 6 of
193 Plymouth Marine Laboratory (Clarke and Gorley 2005).

194

195 Comparison of species richness across body-size fractions

196

197 The numbers of species shared among gears were visualized through Venn diagrams, prepared
198 by using *jvenny* (Bardou et al. 2014) and multivariate analyses on the factor “gear” (with levels:
199 ORH, TB, SEH and SEL) were performed on presence/absence data to statistically explore possible
200 different sampling performances of the deployed gears.

201 Extrapolation and rarefaction analyses with iNEXT were also performed to highlight the
202 completeness of the sampling (i.e. observed numbers of species compared to expected ones) on

203 incidence data (i.e. presence/absence data). Finally, in order to compare the size-spectrum of
204 species collected by each sampling gear, we counted the number of species present in different
205 size-class bins having equivalent intervals (in a logarithm transformation with base 2, following
206 Bouchet et al. 2002). The range size of the mollusc species considered was taken from the
207 literature (when available) or directly measured on the collected specimens in the case of new
208 species.

209

210 **Results**

211

212 From the 64 samples a total of 1034 living mollusc specimens belonging to 173 different species
213 were collected. The full data set consisted of 509 specimens of Gastropoda (98 species), 446
214 specimens of Bivalvia (62 species), 29 specimens of Scaphopoda (8 species), 31 specimens of
215 Polyplacophora (2 species), 8 specimens of Monoplacophora (2 species) and 11 specimens of
216 Solenogastres (not divided into morphospecies and treated at the class level). The complete list of
217 species and their occurrence in the different areas is reported in the supplementary Table 2.

218

219 Rarity

220

221 Out of the 173 species found, 71 were singletons, corresponding to 41.04% of the total number
222 of species, and 34 species were doubletons (representing the 19.65% of the total). In terms of
223 presence/absence data (i.e. incidence), 109 species were uniques (63.01% of the total), and 39
224 species were duplicates (22.54% of the total). Overall, ~54% of species were already reported in
225 the literature for the Ross Sea, ~12% represent new records (marked with '*' in the supplementary
226 Table 2), ~7% new species (marked with '**' in the supplementary Table 2), and ~28% have
227 uncertain status. This latter group is composed of new species or new records that are not easily
228 classifiable at present due to the unavailability of detailed iconography for some species and the
229 general need of direct comparisons with type materials, which is beyond the scope of the present
230 contribution. The Brenke sled samples contained the highest numbers of new records and new
231 species (Table 1).

232

233 Effects of latitude and depth on species richness

234

235 No differences in richness patterns were highlighted for Brenke data from the considered
236 bathymetric ranges of 0-500 m, 501-1000 m and >1000 m (Supplementary Fig. 3) on incidence
237 data. However, because latitude and depth are partially confounded, this analysis has to be
238 treated with caution. In fact, if this analysis is done on abundance data for homogeneous groups
239 of areas and depths, a variety of situations can be highlighted, indicating a high degree of
240 heterogeneity (Fig. 2). Abyssal areas, for example, can be indistinguishable in numbers of expected
241 species at depth >3000 m in the northern area (Fig. 2a) but can remarkably differ in the central
242 area in stations at almost identical depths (e.g. station 147 at 1610 m vs station 135 at 1645 m,
243 Fig. 2c). The same occurs in shelf stations (Fig. 2e) and in shelf to slope stations (Fig. 2d) but only at
244 very low numbers of individuals (i.e. <20 individuals), while at higher numbers of individuals the
245 confidence intervals are too large and do not show any difference between the stations.

246

247 Effects of latitude and depth on species composition

248

249 At the significance threshold of 0.05, composition varied (presence/absence data, all gears
250 combined), among latitudinal areas across depth groups (2-ways ANOSIM global $R=0.111$;
251 $p=0.001$), with the Northern Area being statistically distinct from the Central and Southern ones
252 (Fig. 3; Table 2). The same test performed for depths groups across latitudinal areas did not show
253 any appreciable difference due to depth (2-ways ANOSIM global $R=0.021$; $p=0.197$) (Table 3).

254

255 Comparison of species richness and completeness across body-size fractions

256

257 Due to the intrinsic sampling properties of each sampling gear, few species were common
258 between sampling gears (Fig. 4). Accordingly, the multivariate analysis performed considering the
259 factor gear (all gears combined, presence/absence data) showed that all gears differ in terms of
260 collected species (ANOSIM global $R=0.17$; $p=0.001$) (Table 4). Only the rough-bottom trawl and the
261 Beam trawl showed a higher similarity with 8 species in common (i.e. eight: *Dentalium majorinum*,
262 *Doris* sp., *Falsimargarita gemma*, *Marseniopsis mollis*, *Marseniopsis* sp., *Philobrya sublaevis*,
263 *Prodoris clavigera* and *Tritoniella* sp.) with an R value of 0.088 (Tab. 4). The highest number of
264 shared species is between the Brenke sled and the Beam trawl (i.e. ten: *Adacnarca nitens*,
265 *Dentalium majorinum*, *Limatula simillima*, *Lissarca notorcadensis*, *Philobrya sublaevis*,
266 *Propeamussium meridionale*, *Silicula rouchi*, *Thracia meridionalis*, *Tindaria antarctica*, *Yoldiella*
267 *sabrina*) despite the latter having a mesh 50 times larger than the former. Here, however, the

268 multivariate analysis indicates large differences between the two sampling gears ($R=0.222$;
269 $p=0.001$) due to the higher number of species collected by the Brenke.

270 The largest fraction of species was found in the body-size range 0.9-4.1 mm that was present
271 only in the Brenke sled samples (Fig. 5). The Brenke sled samples also provided the broadest
272 spectrum of size classes (from 0.4 to 88 mm), including some larger species that were retained by
273 other gears with larger meshes (e.g. the Beam trawl) resulting in high cumulative richness (Fig. 6a)
274 and sample completeness (Fig. 6b) curves.

275

276 Discussion

277

278 In biodiversity studies the achievement of an exhaustive list of species, i.e. a full census, for a
279 given area is an ambitious task. Generally this process is also very expensive both in terms of time
280 and costs. For these reasons, cost-effective compromises that might combine maximum sampling
281 efficiency with a minimal sampling effort are usually desirable, as long as they guarantee
282 meaningful statistical analyses.

283 In this context, several alternatives to a full census have been developed to speed the inventory
284 process. Rapid assessment techniques, for example, have been designed to rapidly evaluate the
285 biodiversity of critically important field sites around the world (more details at:
286 <http://www.conservation.org/projects/Pages/Rapid-Assessment-Program.aspx>). These surveys,
287 however, are principally meant for conservation purposes and used in prioritisation activities,
288 rather than to exhaustively inventory all species present in an area.

289 When sampling activities are accomplished, a possible shortcut to speed the 'processing time'
290 of collected species is the use of higher-taxon data as a surrogate for species richness (Gaston and
291 Williams 1993). This choice of course greatly reduces the time required for sorting into
292 Operational Taxonomic Units (OTUs) by adopting a coarser division. This method, however, needs
293 to be initially tested for the group being studied and it usually works for genus-level data only (e.g.
294 Souza et al. 2016), generally failing to give meaningful results at higher taxonomic levels.

295 However, when the target of the study is the real number of species and not a proxy for it, no
296 similar shortcuts are possible and techniques maximizing the opportunity to record the highest
297 possible number of species in time available for sampling have to be found.

298 To this aim, a well-known and effective approach that enables the collection of the number of
299 species potentially close to the real one is the simultaneous use, in the same study area, of
300 different sampling techniques. In this way, the different gears' designs partially compensate for

301 differences in species catchability, maximising sampling efficiency (Bouchet et al. 2002; Longino et
302 al. 2002). The statistical drawback of this method is that species abundances cannot be used, as
303 these come from different sampling methods, each one with its own sampling bias, for example
304 towards a given size range.

305 In Antarctica, an area of our planet where climate change impacts are expected to increase by
306 2100 (IPCC 2013, Bracegirdle et al. 2013) potentially leading to detrimental effects on the native
307 fauna, the gathering of biodiversity data is of key importance. The assessment of a reference
308 baseline for the diversity of the Antarctic marine fauna was one of the top five primary targets of
309 the Census of Antarctic Marine Life (CAML) (Schiaparelli et al. 2013) and similar research priorities
310 have also been highlighted by the recently accomplished SCAR horizon scan (Kennicutt et al. 2015)
311 where a special focus was placed on the relationships between biodiversity and ecological
312 processes.

313 The data reported here from the extensive sampling of the NIWA TAN0802 IPY-CAML voyage
314 provide a benchmark from which to measure future changes in the Ross Sea and also provide a
315 key test to evaluate our knowledge gaps and more specific gear-related sampling issues.

316 The results of our study demonstrate statistical differences in species composition between the
317 Northern Area and the Central and Southern ones, but no variation related to depth across
318 latitudinal areas. If only richness data are taken into account, by considering Brenke sled samples,
319 a variety of patterns according to area and depth can be appreciated, denoting an overall large
320 variability between samples even from purportedly similar areas.

321 As a whole, these results suggest the existence of complex patterns and non-linear correlations
322 between environmental determinants and the composition of benthic communities in the Ross
323 Sea. This is in substantial agreement with Cummings et al. (2010), where all available literature for
324 the Ross Sea was reviewed in search for a common pattern determined by latitude, depth, or any
325 other important explanatory variable. However, Cummings et al. (2010) found no clear trends, the
326 outcomes of the studies varying by group considered, location and gear used. It is probable that
327 macrobenthic assemblages in the Ross Sea are strongly influenced by a 'seafloor-habitat' control
328 effect, defined by depth, slope, current speed immediately above the seabed and organic content
329 of seafloor sediments as already suggested by Barry et al. (2003).

330 Besides the results focused on latitudinal or depth trends, the NIWA TAN0802 IPY-CAML data
331 also allow for the evaluation of sampling performances of different gears and their relative
332 contribution to a biodiversity studies when performed at a large geographical scale. In particular,
333 among the considered gears, the catches of the fine-mesh sampling gear (i.e. Brenke sled) allowed

334 for testing if the micromolluscs represented the larger proportion of the total molluscan fauna
335 both in terms of richness and abundance. In accordance with what was found from fine-mesh
336 samples obtained by a Rauschert dredge during the Latitudinal Gradient Program (R/V *Italica*
337 2004) expedition (Ghiglione et al. 2013; Schiaparelli et al. 2014), the Brenke sled provided the
338 highest number of species and specimens compared to the other standard sampling gears
339 (Supplementary Figure 2) and, in turn, of new species and new records for the area (Table 1;
340 Supplementary Table 2 symbols "*" and "***" respectively).

341 Previously, the Brenke sled was used in several localities out of the Antarctic area (Brandt et al.
342 1995; Linse and Brandt 1998; Linse 2004; Kaiser et al. 2008; Kaiser et al. 2009; Brandt et al. 2013;
343 Brandt et al. 2015) while, inside the Polar Front, it was deployed in the Weddell Sea and
344 periantarctic areas only, especially in the abysses (Schwabe et al. 2007; Brandt et al. 2014; Jörger
345 et al. 2014). In a few cases the benthic organisms collected with this gear were compared with the
346 other gears, e.g. vs a box-corer (Hilbing 2004) or vs an Agassiz trawl (Schwabe et al. 2007;). In all
347 these cases, however, no quantitative and statistical comparisons between the sampling gears
348 performances were performed.

349 At the beginning of our study we were not expecting many new findings from the TAN0802
350 voyage, at least for the shelf area, given the extensive sampling effort done in the past along the
351 Ross Sea shelf (e.g. Rehm et al. 2007; Ghiglione et al. 2013; Błażewicz-Paszkowycz et al. 2014;
352 Piazza et al. 2014; Schiaparelli et al. 2014) and new records were only expected from deeper
353 strata, only rarely investigated in the past.

354 However, the TAN0802 data suggest that for molluscs, even in shallow waters, we are still far
355 from a complete knowledge as more new records and new species are continuously added to the
356 general inventory of the Ross Sea molluscs. For the Ross Sea area, Dell (1990) reported a total of
357 193 species (considering the classes Gastropoda, Bivalvia, Polyplacophora and Scaphopoda). In the
358 last 25 years and, in particular, following the expeditions of the last decade, this number has
359 doubled, increasing up to 392 species (belonging to the same mollusc classes considered in Dell
360 1990).

361 The number of new records and new species added to the Ross Sea inventory has been
362 dramatic. It increased by 20% after the Latitudinal Gradient Program (R/V *Italica* 2004) and
363 TAN0402 BioRoss voyage (R/V *Tangaroa* 2004) expeditions (Schiaparelli et al. 2006), followed by
364 further 18% after the Latitudinal Gradient Program (R/V *Italica* 2004) expedition (thanks to the use
365 of a Rauschert dredge, Ghiglione et al. 2013; Schiaparelli et al. 2014) and by another 19% after the
366 TAN0802 (present data).

367 The fact that any additional sampling performed is bringing new records even in shelf areas is a
368 conundrum that can be reasonably explained by not only the increase of sampling effort, but also
369 by the fact that part of the recent sampling is based on novel sampling methods, i.e. fine-mesh
370 trawling. When this method is adopted, the proportion of new species that can be found is very
371 high. This is not true only for molluscs as similar results in the Ross Sea were also found for
372 Tanaidacea (Pabis et al. 2015) and Isopoda (Lörz et al. 2013) with 85% and 72% of new species
373 respectively.

374 Of course, species richness is just one aspect of biodiversity, and it might even not be the target
375 of a survey focused on the understanding of specific causal factors and the relationship between
376 environmental features and benthic community structure. In these cases, the use of 'standard'
377 gears, as grabs (Cummings et al. 2010) or box-corers (Barry et al. 2003), is the only feasible
378 solution for evaluating the possible influence of specific features on the distribution of benthic
379 organisms, such as the percentage of fine sand and silt and the ratio of sediment chlorophyll a to
380 phaeophytin (as in Cummings et al. 2010) or the organic percentage in seafloor sediments (as in
381 Barry et al. 2003).

382 In contrast, towed gears, provide a cross-habitat description, integrating different habitats and
383 communities but, regrettably, do not allow the explanation of species richness and abundances
384 based on specific environmental variables. However, when taxonomic richness is the targeted
385 variable and the study has the aim to evaluate diversity over large spatial scales, fine-mesh towed
386 gears ensure the best efficiency in catching highest numbers of species and specimens. In polar
387 areas, where sampling constraints may be really high, such sampling methods could accelerate our
388 knowledge increase of diversity and hence build the basis of future, more detailed sampling
389 activities, to be performed by using purely quantitative methods, such as grabs or box-corers.

390 Our results suggest that sampling with fine-mesh towed gears if routinely included in future
391 benthic sampling activities in Antarctica, could greatly extend our knowledge of biodiversity,
392 especially in areas where limited sampling has been performed in the past.

393

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395

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404

405

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407

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567

568 **Figure legend:**

569 **Figure 1.** Map of sampling stations performed during the TAN0802 IPY-CAML voyage in the Ross
570 Sea, Antarctica. Stations' coordinates are reported in Supplementary Table 1.

571 **Figure 2.** Richness rarefaction and extrapolation analyses performed with iNEXT on abundance
572 data (Brenke sled stations only) among the considered latitudinal areas.

573 **Figure 3.** MDS plot of all gears combined (presence/absence data) considering the factor
574 "Latitudinal area".

575 **Figure 4.** Venn diagram showing the number of species collected during the TAN0802 by each gear
576 and the number of shared species.

577 **Figure 5.** Number of species occurring in each size class. Size classes are according to Bouchet et
578 al. (2002) and have equivalent intervals in a \log_2 transformation.

579 **Figure 6.** Richness rarefaction and extrapolation analyses performed with iNEXT on
580 presence/absence data. Abbreviations: ORH = Rough-bottom trawl; SEH = Brenke sled; SEL =
581 Epibenthic sled; TB = Beam trawl. **(a).** Rarefaction and extrapolation output for the factor gear. **(b).**
582 Sample coverage output from the factor gear.

583

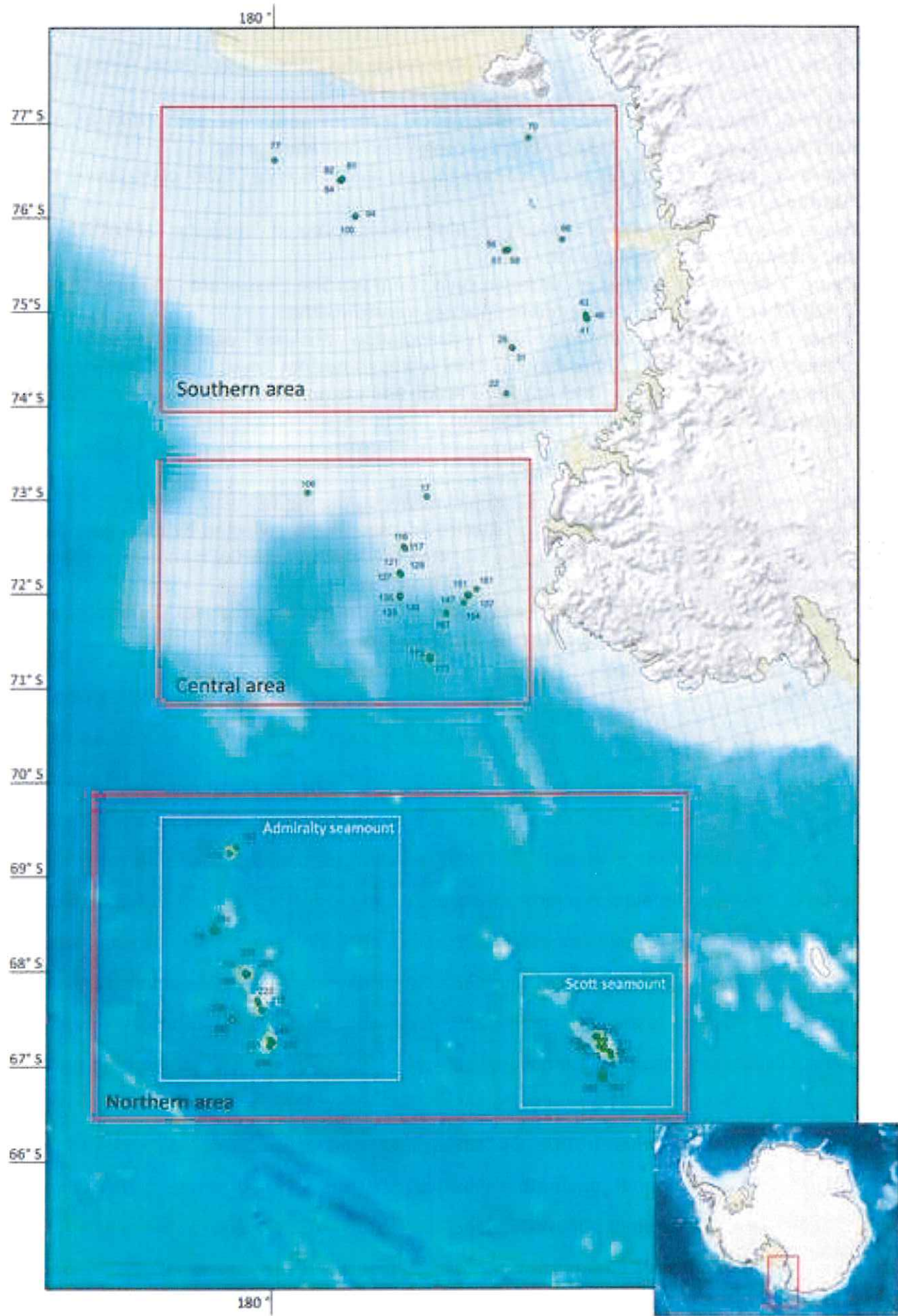
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585 **Supplementary Figure 1.** Maps of gear deployments during the TAN0802 voyage.

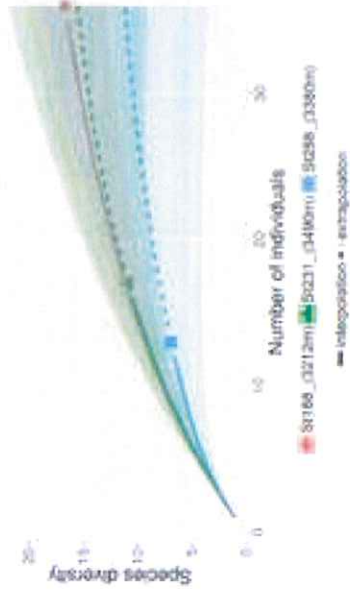
586 **Supplementary Figure 2.** Numbers of species and specimens collected by each gear divided per
587 mollusc class. Abbreviations: BIV = Bivalvia; GAS = Gastropoda; MON = Monoplacophora; POL =
588 Polyplacophora; SCA = Scaphopoda; SOL = Solenogastres

589 **Supplementary Figure 3.** Richness rarefaction and extrapolation analyses performed with iNEXT
590 on incidence data (Brenke sled data only) for the bathymetric classes considered.

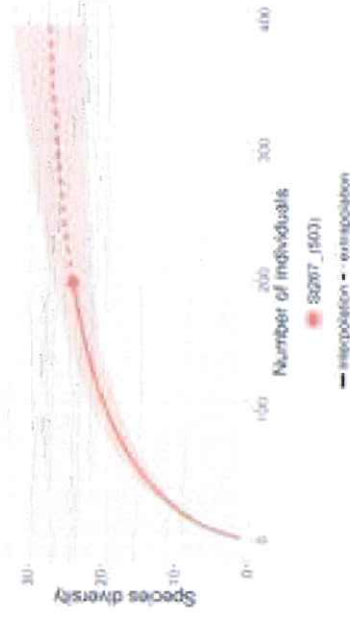
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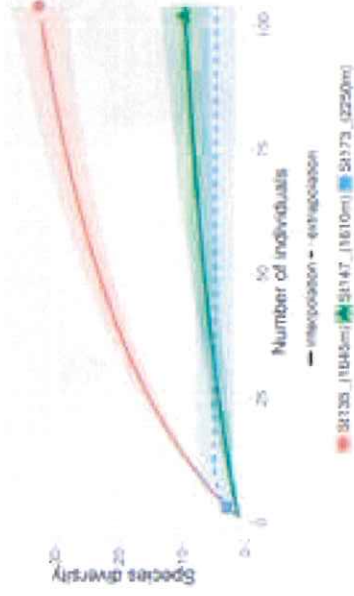
(a) Northern area abyssal



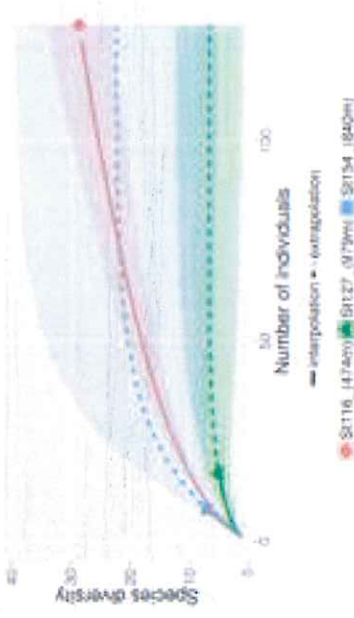
(b) Northern area shallow



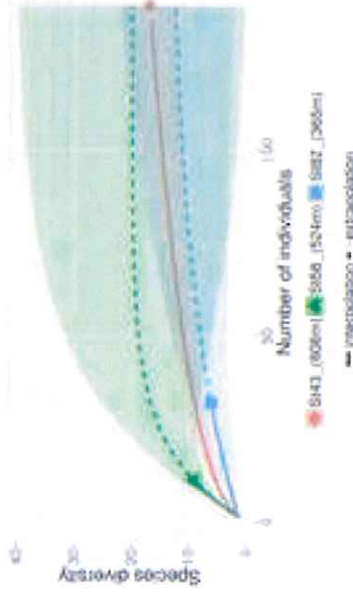
(c) Central area abyssal



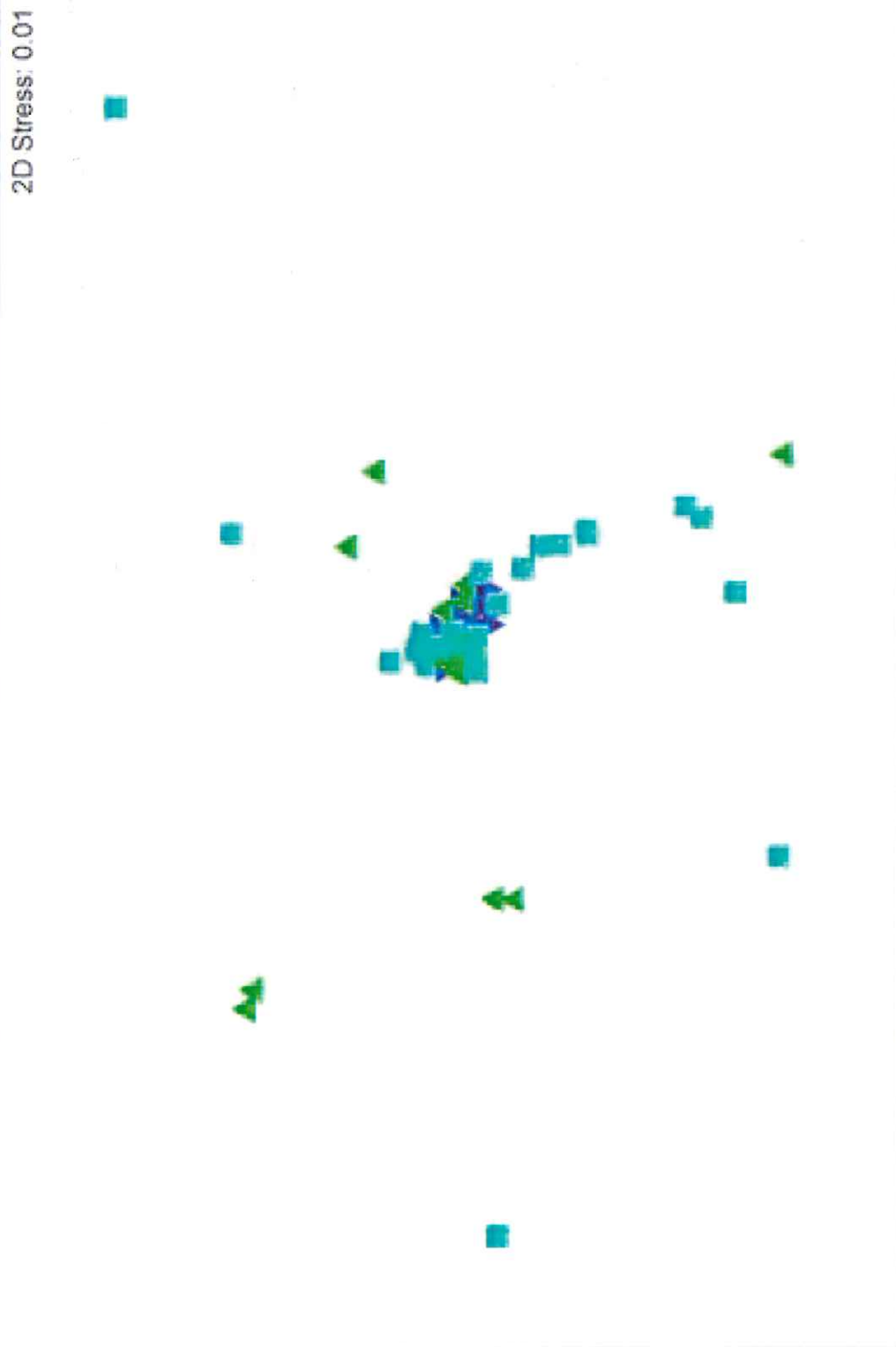
(d) Central area shallow



(e) Southern area

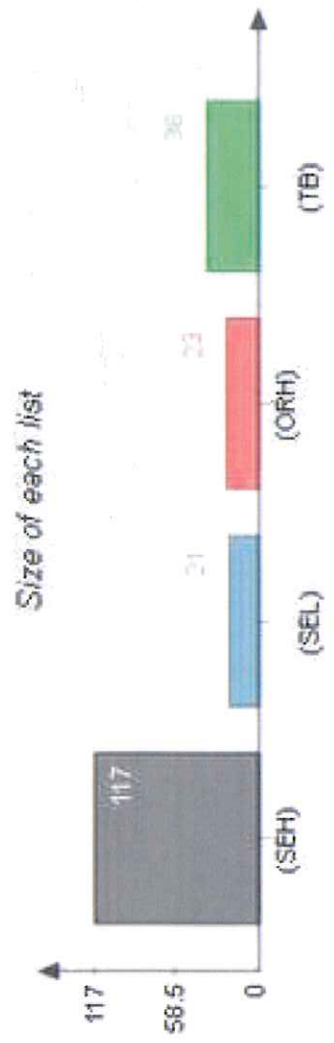
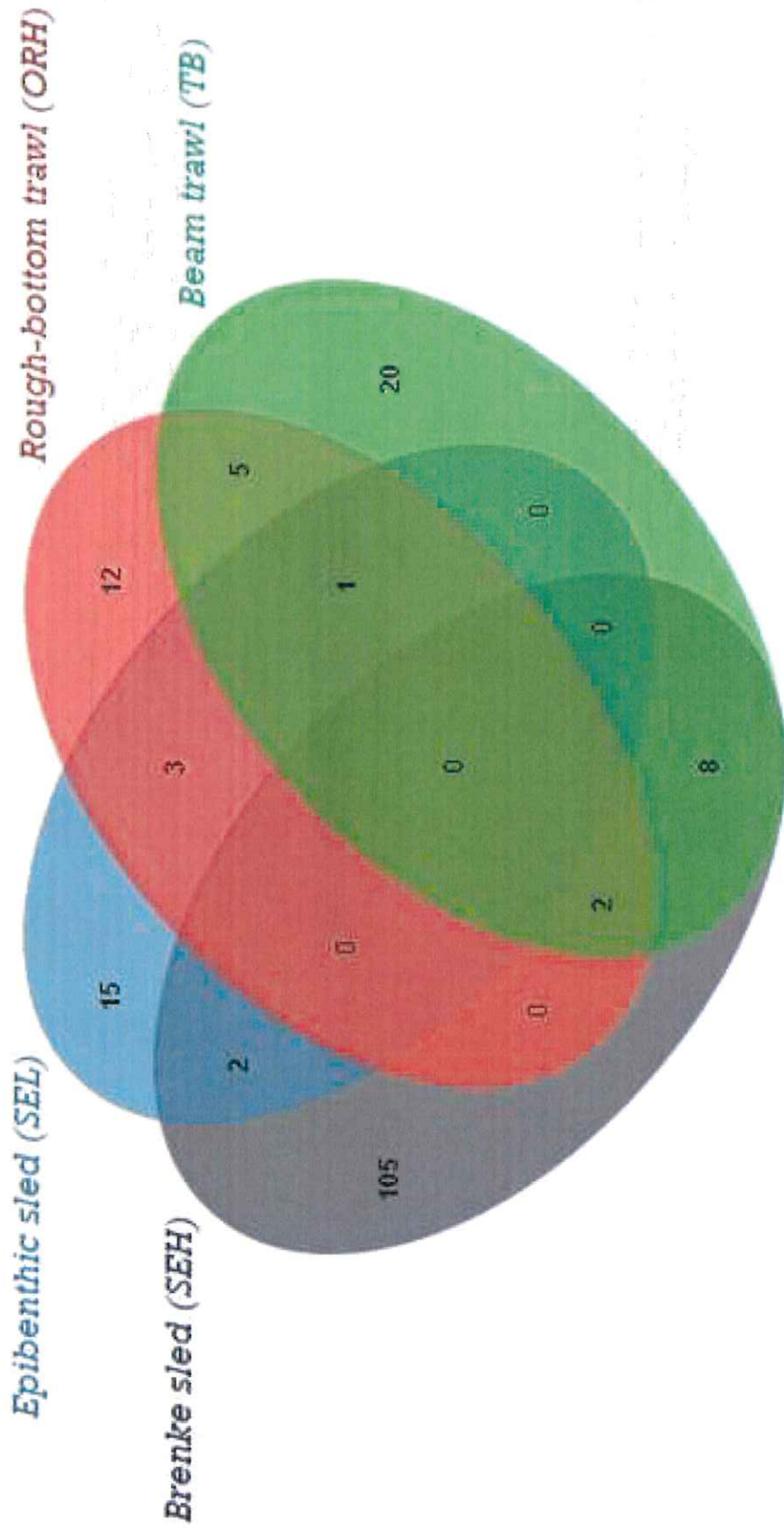


Transform: Presence/absence
Resemblance: S17 Bray Curtis similarity

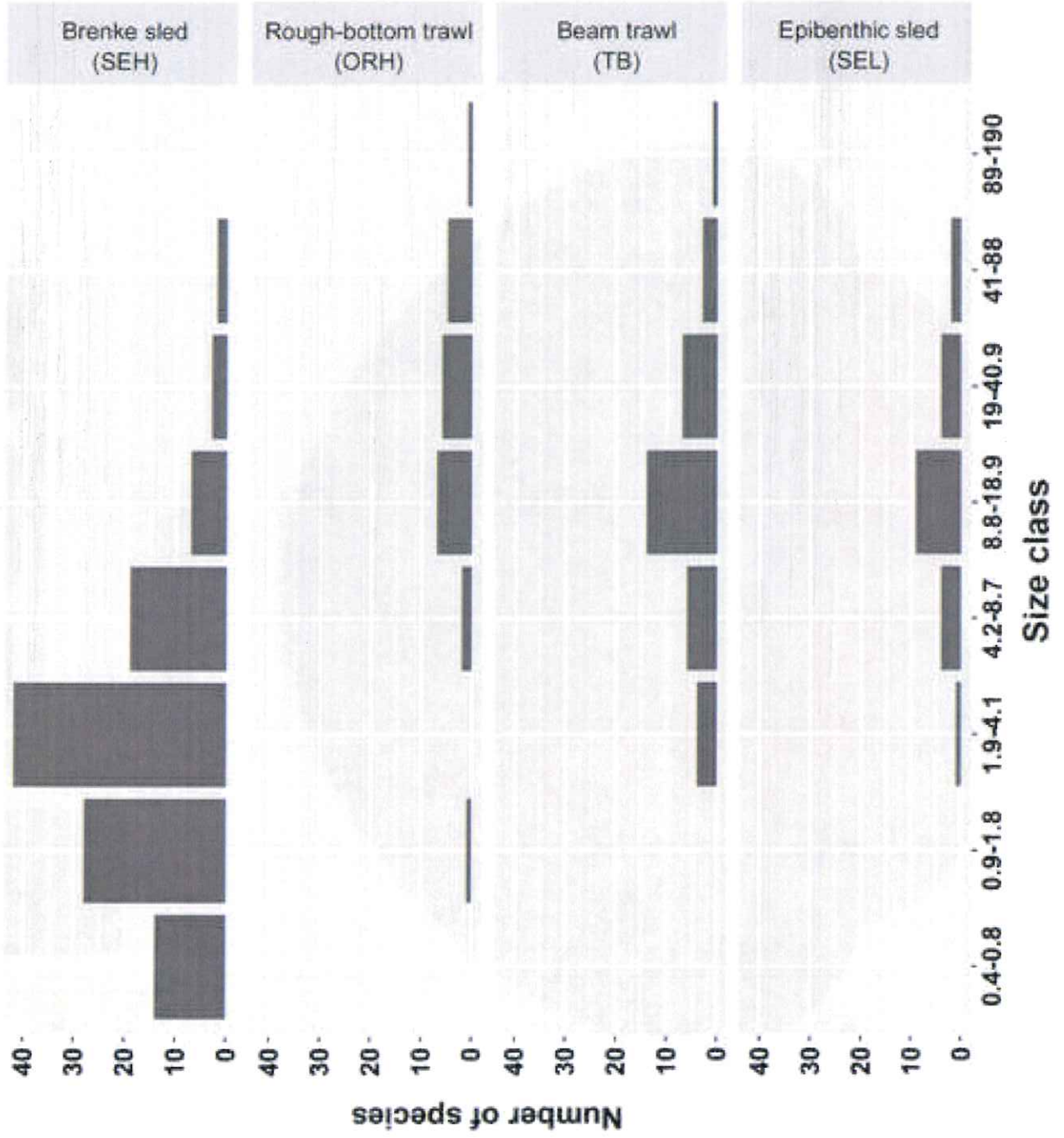


Latitudinal Area

- Northern Area
- ▲ Central Area
- ▼ Southern Area



Number of species occurring at each size class (in mm)



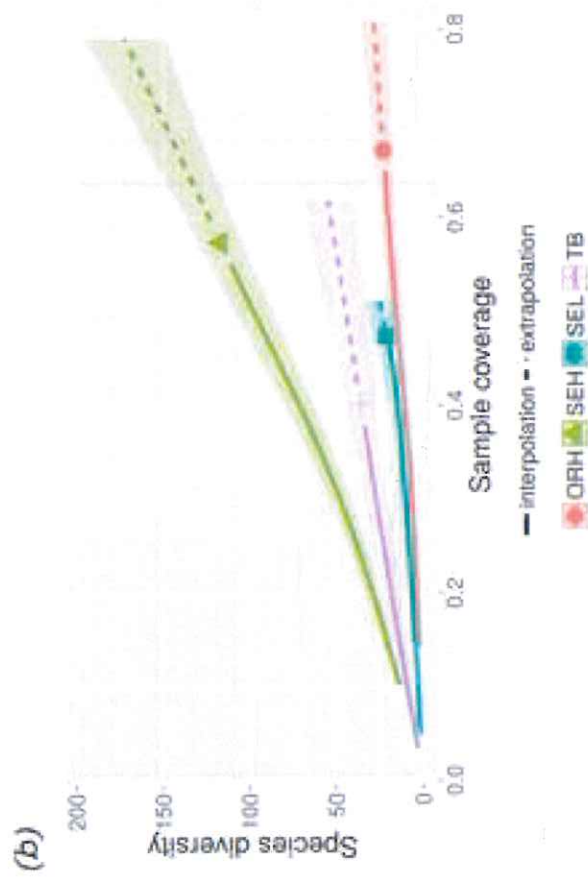
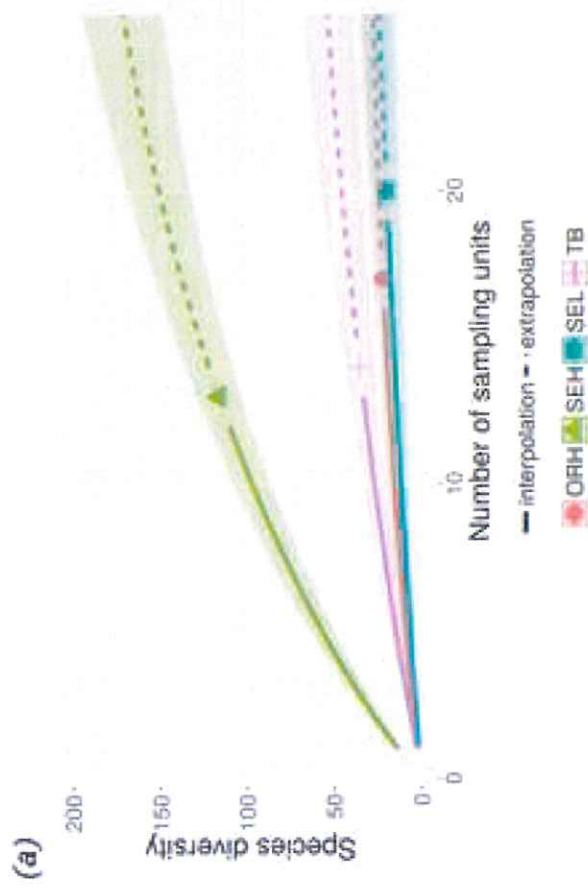


Table 1. Sampling performance with different deployed gears showing new records and new species.

Gear	New records	New species	N° of specimens
Brenke sled (SEH)	15	8	202
Beam trawl (TB)	4	2	7
Epibenthic sled (SEL)	2	1	16
Rough-bottom trawl (ORH)	0	1	3
Total			228

Table 2. Two-ways ANOSIM analysis for all gears combined (presence/absence data). Tests for differences between latitudinal area groups across depth factor groups.

	R	Sign. (%)	Actual permutations	Observed
Global R	0.111	0.1		
<u>Pairwise test</u>				
Central Area vs Southern Area	0.089	10	999	99
Central Area vs Northern Area	0.074	0.3	999	2
Southern Area vs Northern Area	0.169	0.1	999	0

Table 3. Two-ways ANOSIM analysis for all gears combined (presence/absence data). Tests for differences between depth factor groups across latitudinal area groups.

	R	Sign. (%)	Actual permutations	Observed
Global R	0.021	19.7		
<u>Pairwise test</u>				
0-500m vs 501-1000m	- 0.011	59.9	999	598
0-500m vs >1001m	0.059	9.9	999	98
501-1000m vs >1001m	0.037	12.4	999	123

Table 4. One-way ANOSIM analysis for factor gear considering all gears combined (presence/absence data).

	R	Sign. (%)	Actual permutations	Observed
Global R	0.17	0.1		
<u>Pairwise test</u>				
ORH - Beam trawl	0.088	1.5	999	14
ORH - Brenke sled	0.377	0.1	999	0
ORH - Epibenthic sled	0.138	0.1	999	0
Beam trawl - Brenke sled	0.222	0.1	999	0
Beam trawl - Epibenthic sled	0.075	0.1	999	0
Brenke sled - Epibenthic sled	0.195	0.1	999	0

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Electronic Supplementary Material
Supplementary Figure 1_ok2017.tif