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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2 **Sea (Antarctica): the importance of fine-mesh towed gears and implications for**
3 **future sampling**
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17

18 **Abstract**

19 In 2008, a large sampling campaign took place in the Ross Sea between ~66°S and ~77°S during the
20 NIWA IPY-CAML voyage of R/V *Tangaroa*, as part of the Census of Antarctic Marine Life (CAML).
21 Samples of benthos were obtained by using a variety of sampling methods from 64 stations at
22 depths of between 283 m to 3490 m. Mollusca accounted for 173 living species and 1034
23 specimens, which were analysed in terms of variation in richness and composition with latitude
24 and depth, and to assess which macrofaunal size fraction contained the highest biodiversity.
25 Differences were detected in species composition with latitude (averaged across depth groups)
26 but not for depth (averaged across latitudinal groups). Richness varied locally and showed a
27 variety of patterns according to the areas and depths considered. New species accounted for ~7%
28 of the total number of species and new regional records for ~12%. Rarity was high, with a ~41% of
29 species represented by single individuals and ~63% occurring at one station only. The greatest
30 diversity was found in the fine fraction (i.e. <4.1 mm) suggesting that the systematic use of fine-
31 mesh trawling in future sampling activities can be of help in accelerating the census of Antarctic
32 mollusc fauna.
33

34 **Keywords**
35 Antarctica, Ross Sea, Victoria Land Coast, Seamounts, Benthos, Mollusca, Species Richness,
36 Abundance, Fine-mesh, Sampling efficiency, Full census
37

1

Introduction

The Ross Sea continental shelf, with an area of roughly 473 km^2, is the second largest in Antarctica after the Weddell Sea. The average depth of the shelf is 500 m, and the shelf break occurs at about 800-1000 m, from where the continental slope extends steeply down to 3000 m (Clarke et al, 2007; Scambos et al. 2007). Beyond the continental slope to the north, there are a number of seamounts and islands, including the Scott Seamount chain, the Admiralty Seamount, and the Balleny Islands.

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

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

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

The gear types used during the TAN0802 voyage, included a rough-bottom trawl, beam trawl, epibenthic sledge, and a fine-mesh epibenthic or "Brenke" sledge. The Brenke sled (Brandt et al. 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

Materials and methods

Study area and sample processing

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

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

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

Species classification

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

137   Monoplacophora, Scaphopoda, Polyplacophora and Solenogatres 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   <u>Statistical analyses</u>

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   <u>Effects of latitude and depth on species richness</u>

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   <u>Rarity</u>

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   <u>Effects of latitude and depth on species richness</u>

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   <u>Effects of latitude and depth on species composition</u>
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   <u>Comparison of species richness and completeness across body-size fractions</u>
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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405

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566

567

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

584

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.
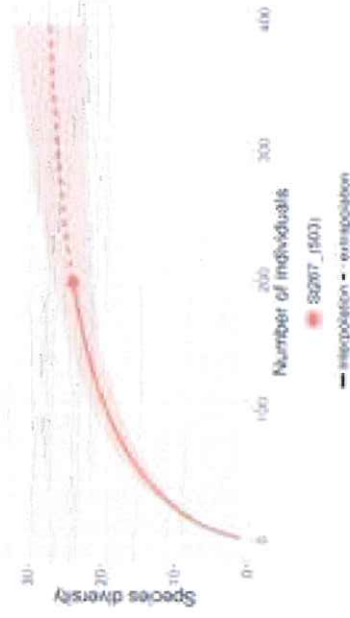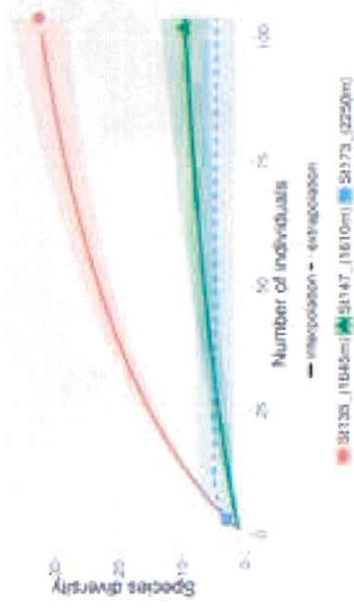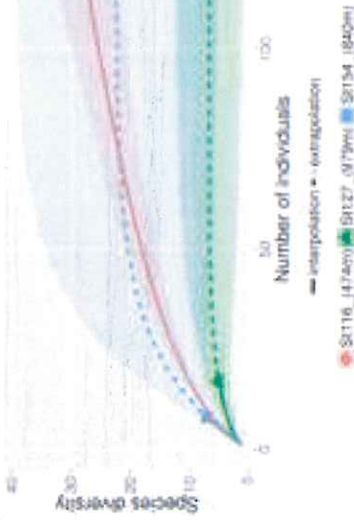
591

Figure 1
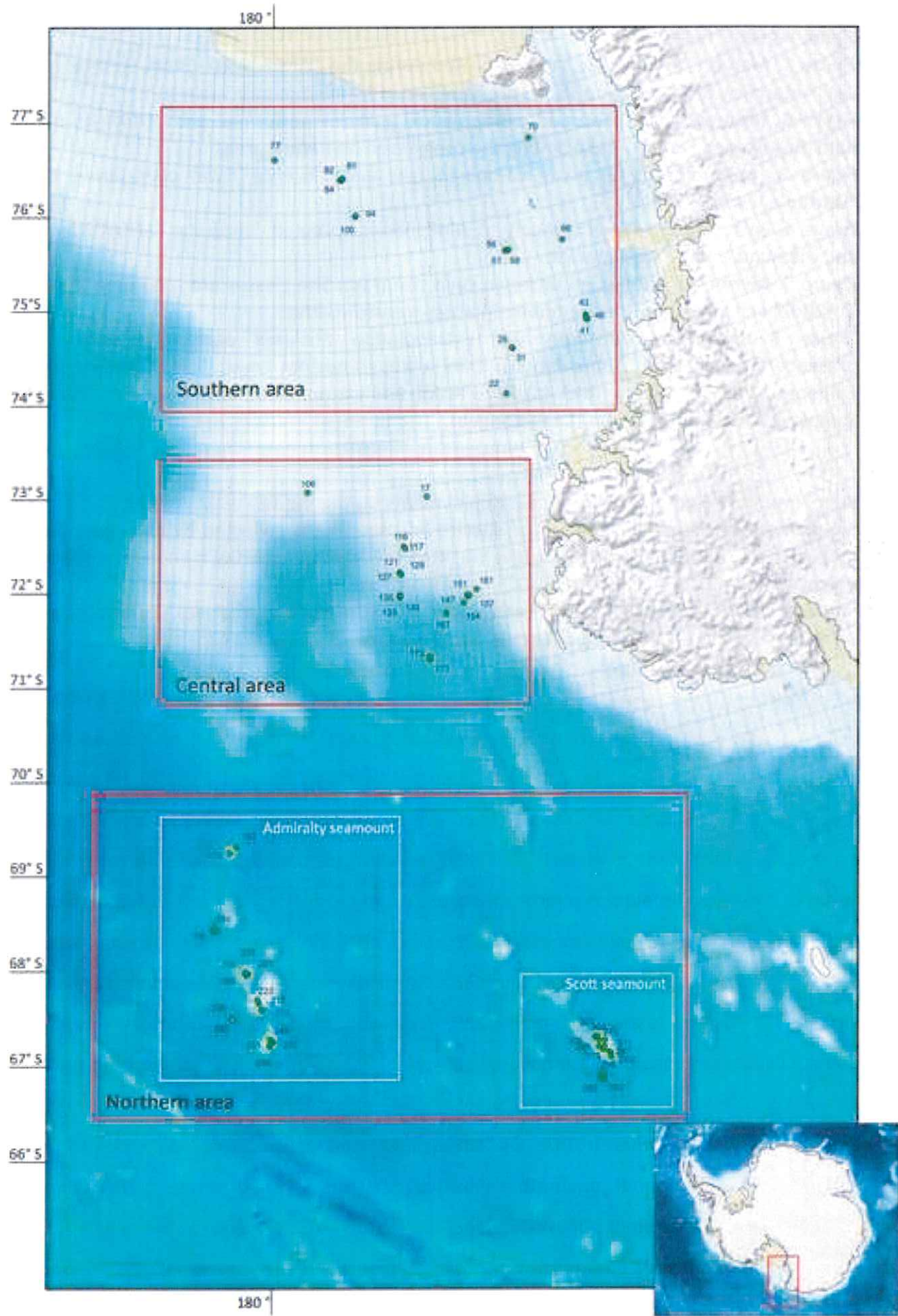
(a) Northern area abyssal
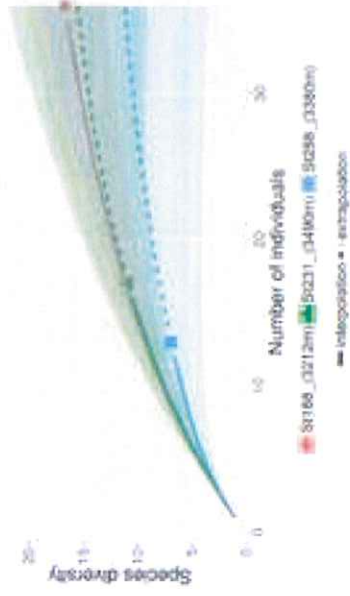
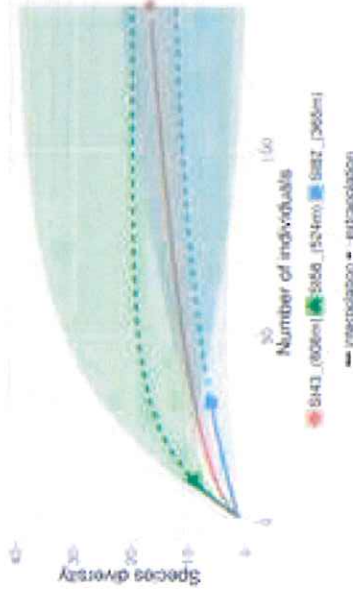**(b)** Northern area shallow
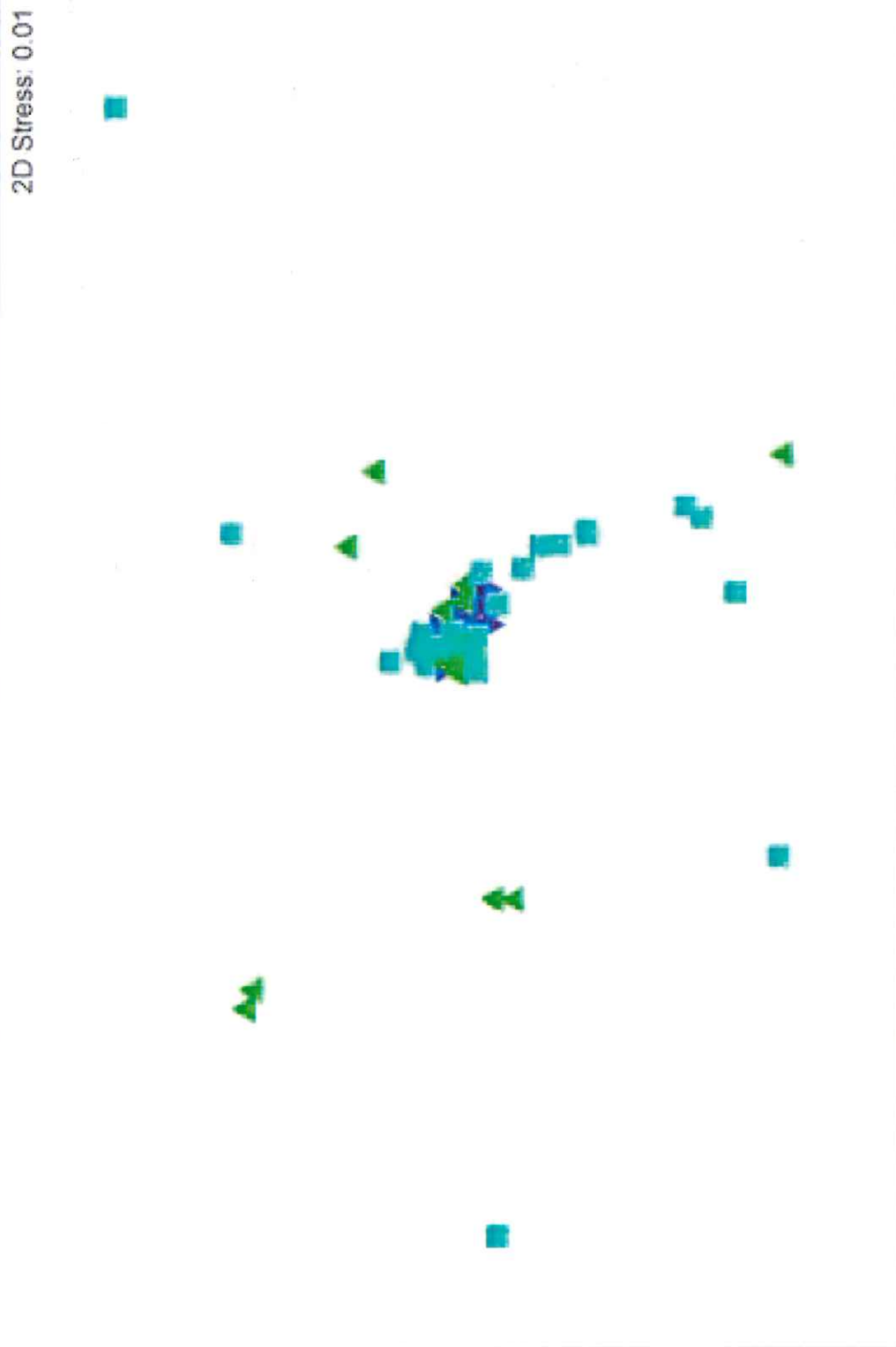
(c) Central area abyssal

(d) Central area shallow
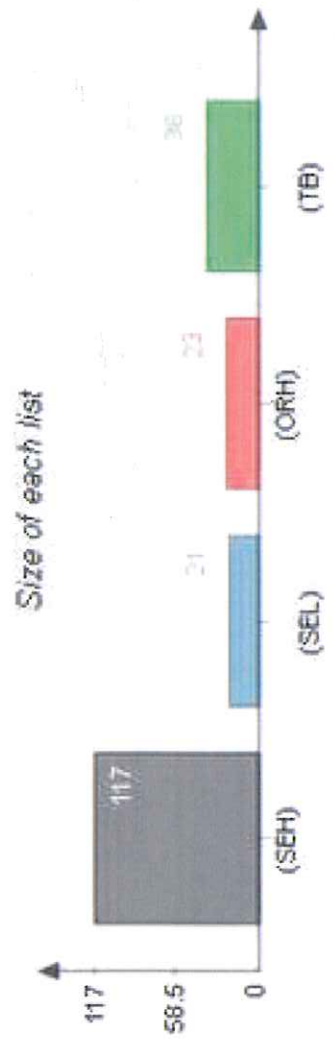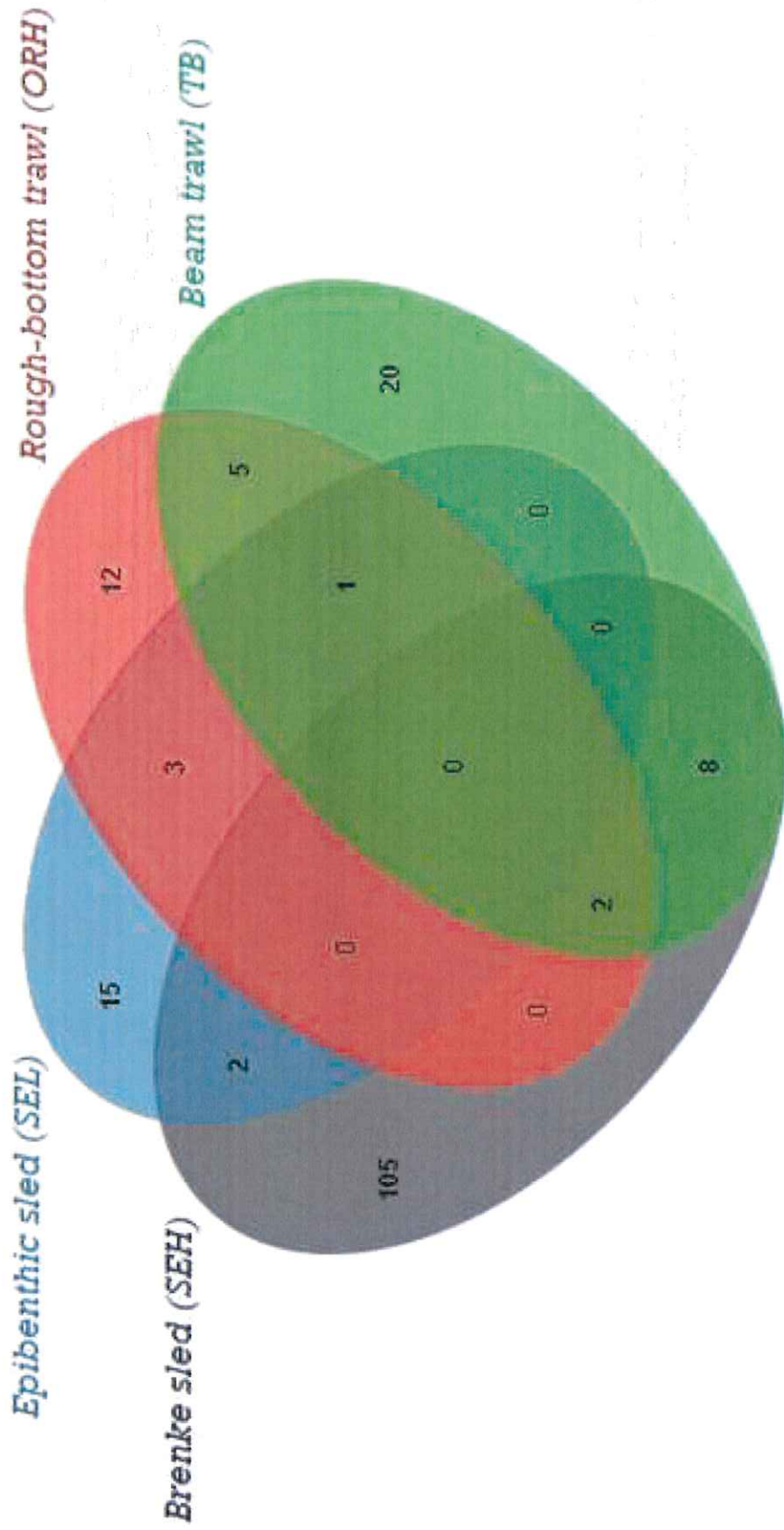
(e) Southern area

Transform: Presence/absence
Resemblance: S17 Bray Curtis similarity

2D Stress: 0.01

Latitudinal Area
■ Northern Area
▲ Central Area
▶ Southern Area

Epibenthic sled (SEL)

Rough-bottom trawl (ORH)

Beam trawl (TB)

Brenke sled (SEH)

15

12

3

2

5

1

0

20

0

0

0

0

0

0

8

2

105

Size of each list

117

58.5

0

117

31

23

96

(SEH)

(SEL)

(ORH)

(TB)

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

| Brenke sled (SEH) | Rough-bottom trawl (ORH) | Beam trawl (TB) | Epibenthic sled (SEL) |

Number of species

Size class

(a)

Species diversity

200·
150·
100·
50·
0·

Number of sampling units

10   20

— interpolation ═ · extrapolation

● ORH ▲ SEH ■ SEL ▦ TB

(b)

Species diversity

200·
150·
100·
50·
0·

Sample coverage

0.0   0.2   0.4   0.6   0.8

— interpolation ═ · extrapolation
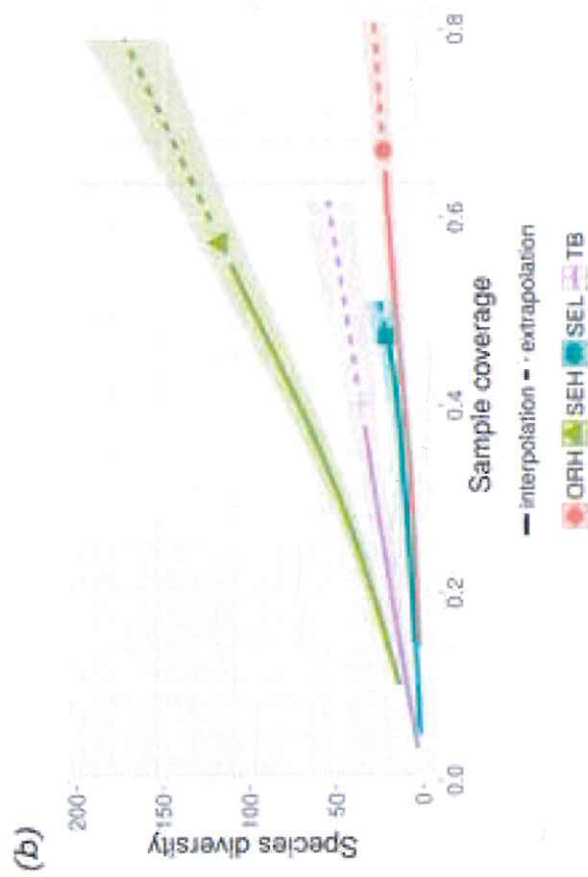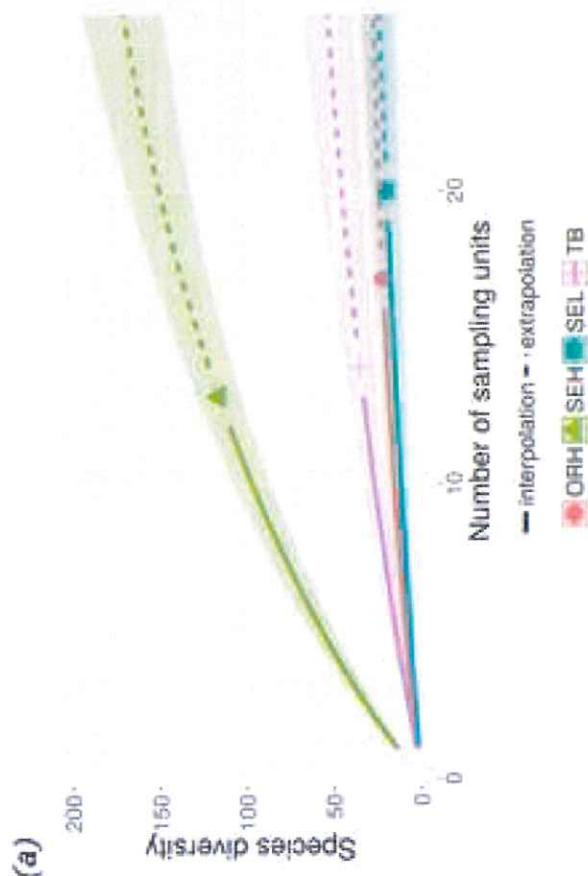
● ORH ▲ SEH ■ SEL ▦ TB
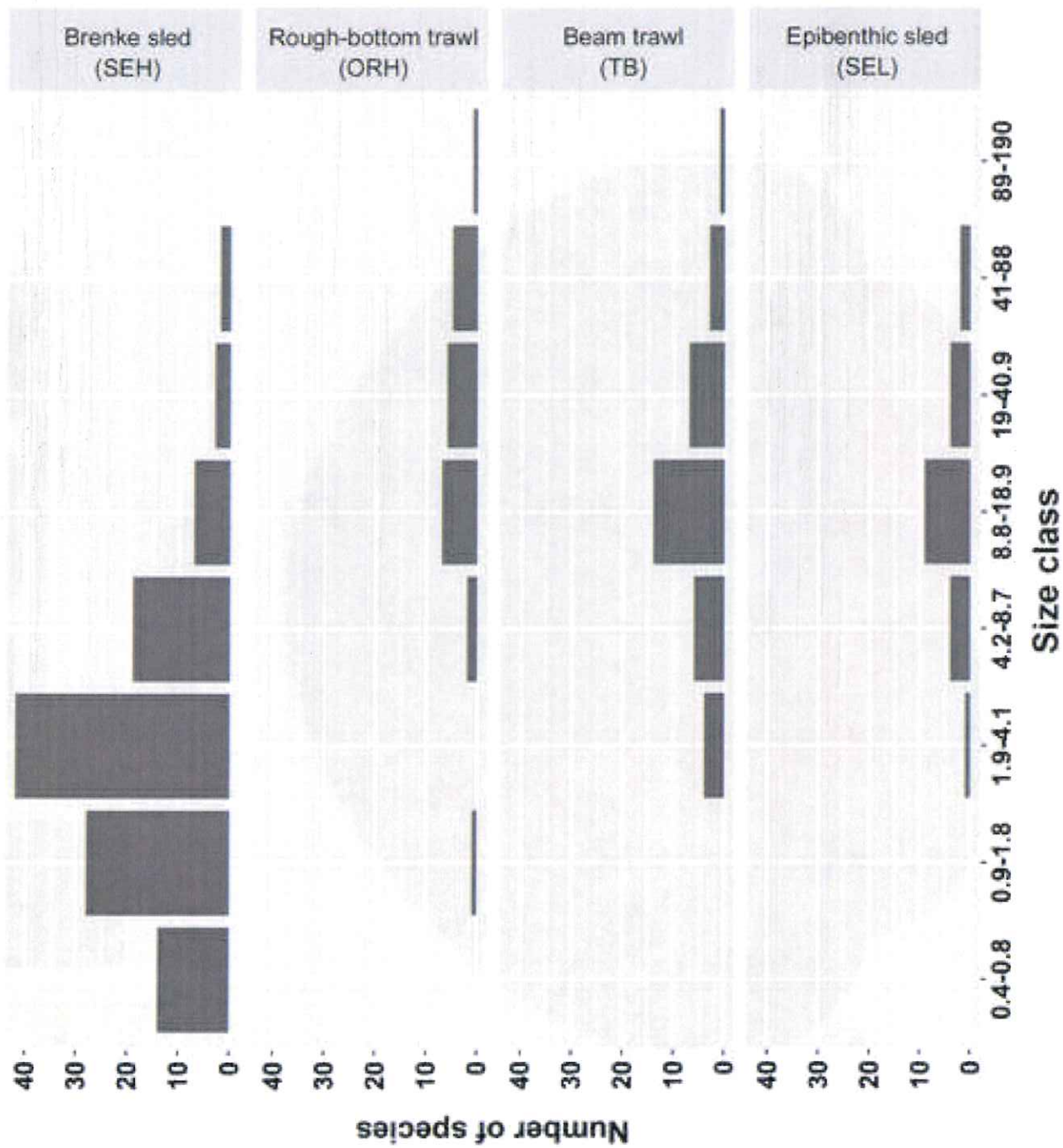
Table 1. Sampling performance with different deployed gears showing new records and new species.

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		
Pairwise test				
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		
Pairwise test				
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		
Pairwise test				
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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