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

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Abstract In polar areas, where benthic sampling is constrained by a series of limitations imposed by climate and logistic challenges, knowledge about the key elements required to plan a successful survey is fundamental. During the International Polar Year (IPY, 2007/2008), under the Census of Antarctic Marine Life (CAML), new sampling campaigns were undertaken in several Antarctic areas comprising the Ross Sea. In this region, the 2008 NIWA IPY-CAML voyage obtained benthos samples from shelf to abyssal depths. In the present study, we focus on the Mollusca from this expedition and on the possible variations in their richness and composition with latitude and depth. Given the use of sampling gears selective for different size fractions of the macrofauna, we also assess which size fraction contained the highest biodiversity. Differences were detected in species composition with latitude (averaged across depth groups) but not for depth (averaged across

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latitudinal groups). Richness varied locally and showed a variety of patterns depending on the areas and depths considered. The greatest diversity of molluscs was found in the fine fraction (i.e., <4.1 mm) where a considerable number of species corresponded to new species or new regional records. Rarity was high with up to ~41% of species represented by single individuals and ~63% occurring at one station only. Fine-mesh trawling appears to be of fundamental importance in accelerating the census of the fine fraction, which is the one containing the highest diversity, and is recommended for future sampling in Antarctica and in polar areas in general.

Keywords Antarctica · Ross Sea · Victoria Land Coast · Seamounts · Benthos · Mollusca · Species richness · Abundance · Fine mesh · Sampling efficiency · Full census

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 and Siciński 2014; Piazza et al. 2014) and large taxa, including the 3 m 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 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 and Stewart 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 boundary layer and has previously been utilized in the Weddell Sea and the Atlantic sector of the Southern Ocean, especially at abyssal depths (Schwabe et al. 2007; Brandt et al. 2014; Jörger et al. 2014). The deployment of a Brenke sled during the TAN0802 expedition was its first use in the Ross Sea (Lörz et al. 2013). It also represents the second fine-mesh sampling event in the area, after the deployment in 2004 of a Rauschert dredge, which provided an unexpectedly large number of new records and species of molluscs for the Ross Sea (Ghiglione et al. 2013; Schiaparelli et al. 2014). The use of a Brenke sled during TAN0802 thus gave an opportunity to assess the distribution of mollusc biodiversity across size classes in Antarctica.

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

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

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

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 64 sampling events at depths ranging from 283 to 3490 m (Fig. 1; Online Resource 1) by deploying four types of towed gears **Fig. 1** Map of sampling stations performed during the TAN0802 IPY-CAML voyage in the Ross Sea, Antarctica. Stations' coordinates are reported in the Online Resource 1



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 the 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 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 μ m 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 one knot, except for the ORH at three 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, Monoplacophora, Scaphopoda, Polyplacophora, and Solenogatres were considered. Cephalopoda and Caudofoveata were not present in the samples.

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

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

Statistical analyses

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

Since the deployment of sampling gears was not even (e.g., SEL was only used on the rough bottoms of the seamounts) and the majority of specimens were collected by the Brenke sled (Online Resource 2 and 3), statistical analyses were performed separately on data sets from the different gears. In the specific, species richness was studied only on Brenke sled data, while composition was studied on presence/absence data considering all gears. Rarity was evaluated in terms of number of species collected as singletons (i.e., species found with a single specimen) and doubletons (i.e., species found with two specimens only), or uniques (i.e., species occurring at a single station only) and duplicates (i.e., species occurring at two stations only).

Effects of latitude and depth on species richness

To understand richness patterns in relation to depth and latitude, Brenke sled samples were analysed through a combined analysis of rarefaction and extrapolation techniques. This analysis is based on diversity accumulation curves produced on empirical estimates of the principal Hill numbers (Chao and Jost 2012; Chao et al. 2014). Individual-based (for geographic areas) and samplebased (for depth) interpolation (rarefaction) and extrapolation curves (Colwell et al. 2012) were computed using the online iNEXT package (Chao et al. 2016; https:// chao.shinyapps.io/iNEXTOnline/), which allows the comparison of samples taking into account sample coverage and completeness (Chao and Jost 2012; Chao et al. 2014) in the R-statistical environment (http://www.rproject.org). Uncertainty of estimations was reported in terms of 95% confidence intervals under the multinomial model for the observed species sample frequencies (in the case of the individual-based interpolation/extrapolation curves) or under the Bernoulli product model for the incidence matrix (in the case of the sample-based interpolation/extrapolation curves) (Colwell et al. 2012). The non-overlap of 95% confidence interval was used as an indicator of statistical difference (Colwell et al. 2012).

Effects of latitude and depth on species composition

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

Comparison of species richness across body-size fractions

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

Extrapolation and rarefaction analyses with iNEXT were also performed to highlight the completeness of the sampling (i.e., observed numbers of species compared to expected ones) on incidence data (i.e., presence/absence data). Finally, to compare the size-spectrum of species collected by each sampling gear, we counted the number of species present in different size-class bins having equivalent intervals (in a logarithm transformation with base 2, following Bouchet et al. 2002). The range size of the mollusc species considered was taken from the literature (when available) or directly measured on the collected specimens in the case of new species.

Results

From the 64 samples, a total of 1034 living mollusc specimens belonging to 173 different species were collected. The full data set consisted of 509 specimens of Gastropoda (98 species), 446 specimens of Bivalvia (62 species), 29 specimens of Scaphopoda (8 species), 31 specimens of Polyplacophora (2 species), 8 specimens of Monoplacophora (2 species), and 11 specimens of Solenogastres (not divided into morphospecies and treated at the class level). The complete list of species and their occurrence in the different areas is reported in the Online Resource 4.

Rarity

Out of the 173 species found, 71 were singletons, corresponding to 41.04% of the total number of species, and 34 species were doubletons (representing the 19.65% of the total). In terms of presence/absence data (i.e., incidence), 109 species were uniques (63.01% of the total), and 39 species were duplicates (22.54% of the total). Overall, ~54% of species were already reported in the literature for the Ross Sea, ~12% represent new records (marked with '*' in the Online Resource 4), ~7% new species (marked with '**' in the Online Resource 4), and ~28% have uncertain status. This latter group is composed of new species or new records that are not easily classifiable at present due to the unavailability of detailed iconography for some species and the general need of

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

Gear	New records	New species	No. of speci- mens
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

direct comparisons with type materials, which is beyond the scope of the present contribution. The Brenke sled samples contained the highest numbers of new records and new species (Table 1).

Effects of latitude and depth on species richness

No differences in richness patterns were highlighted for Brenke data from the considered bathymetric ranges of 0-500 m, 501-1000 m, and >1000 m (Online Resource 5) on incidence data. However, because latitude and depth are partially confounded, this analysis has to be treated with caution. In fact, if this analysis is done on abundance data for homogeneous groups of areas and depths, a variety of situations can be highlighted, indicating a high degree of heterogeneity (Fig. 2). Abyssal areas, for example, can be indistinguishable in numbers of expected species at depth >3000 m in the northern area (Fig. 2a) but can remarkably differ in the central area in stations at almost identical depths (e.g., station 147 at 1610 m vs station 135 at 1645 m, Fig. 2c). The same occurs in shelf stations (Fig. 2e) and in shelf to slope stations (Fig. 2d) but only at very low numbers of individuals (i.e., <20 individuals), while at higher numbers of individuals, the confidence intervals are too large and do not show any difference between the stations.

Effects of latitude and depth on species composition

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



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

Comparison of species richness and completeness across body-size fractions

Due to the intrinsic sampling properties of each sampling gear, few species were common between sampling gears (Fig. 4). Accordingly, the multivariate analysis performed considering the factor gear (all gears combined, presence/absence data) showed that all gears differ in terms of collected species (ANOSIM global R=0.17; p=0.001)

(Table 4). Only the rough-bottom trawl and the Beam trawl showed a higher similarity with eight species in common (i.e., eight: *Dentalium majorinum, Doris* sp., *Falsimargarita gemma, Marseniopsis mollis, Marseniopsis* sp., *Philobrya sublaevis, Prodoris clavigera*, and *Tritoniella* sp.) with an *R* value of 0.088 (Table 4). The highest number of shared species is between the Brenke sled and the Beam trawl (i.e., ten: *Adacnarca nitens, D. majorinum, Limatula simillima, Lissarca notorcadensis, P. sublaevis,*



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

Propeamussium meridionale, Silicula rouchi, Thracia meridionalis, Tindaria antarctica, and Yoldiella sabrina) despite the latter having a mesh 50 times larger than the former. Here, however, the multivariate analysis indicates large differences between the two sampling gears (R=0.222; p=0.001) (Table 4) due to the higher number of species collected by the Brenke.

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

Discussion

In biodiversity studies, the achievement of an exhaustive list of species, i.e., a full census, for a given area is an ambitious task. In general, this process is also very expensive in terms of both time and costs. For these reasons, cost effective compromises that might combine maximum sampling efficiency with a minimal sampling effort are usually desirable, as long as they guarantee meaningful statistical analyses. In this context, several alternatives to a full census have been developed to speed the inventory process. Rapid assessment techniques, for example, have been designed to rapidly evaluate the biodiversity of critically important field sites around the world (more details at: http://www. conservation.org/projects/Pages/Rapid-Assessment-Program.aspx). These surveys, however, are principally meant for conservation purposes and used in prioritisation activities, rather than to exhaustively inventory all species present in an area.

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

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

To this aim, a well-known and effective approach that enables the collection of the number of species potentially close to the real one is the simultaneous use, in the same

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

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

Table 2Two-way ANOSIManalysis for all gears combined(presence/absence data).Tests for differences betweenlatitudinal area groups acrossdepth factor groups

	R	Sign. (%)	Actual permuta- tions	Observed
Global <i>R</i>	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

Fig. 4 Venn diagram showing the number of species collected during the TAN0802 by each gear and the number of shared species



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

	R	Sign. (%)	Actual permuta- tions	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

study area, of different sampling techniques. In this way, the different gears' designs partially compensate for differences in species catchability, maximizing sampling efficiency (Bouchet et al. 2002; Longino et al. 2002). The statistical drawback of this method is that species abundances cannot be used, as these come from different sampling methods, each one with its own sampling bias, for example, towards a given size range.

In Antarctica, an area of our planet where climate change impacts are expected to increase by 2100 (IPCC 2013; Bracegirdle and Stephenson 2012) potentially leading to detrimental effects on the native fauna, the gathering of biodiversity data is of key importance. The assessment

of a reference baseline for the diversity of the Antarctic marine fauna was one of the top five primary targets of the Census of Antarctic Marine Life (CAML) (Schiaparelli et al. 2013) and similar research priorities have also been highlighted by the recently accomplished SCAR horizon scan (Kennicutt et al. 2015), where a special focus was placed on the relationships between biodiversity and ecological processes.

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

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

As a whole, these results suggest the existence of complex patterns and non-linear correlations between environmental determinants and the composition of benthic communities in the Ross Sea. This is in substantial agreement with Cummings et al. (2010), where all available literatures for the Ross Sea were reviewed in search for a common pattern determined by latitude, depth, or any other important explanatory variable. However, Cummings et al. (2010) found no clear trends, the outcomes Fig. 5 Number of species occurring in each size class. Size classes are according to Bouchet et al. (2002) and have equivalent intervals in a \log_2 transformation



0

0.0

0.2



●ORH**▲**SEH**■**SEL**■**TB **Fig. 6** Richness rarefaction and extrapolation analyses performed and extrapo

and extrapolation output for the factor gear. ${\bf b}$ Sample coverage output from the factor gear

0.4

Sample coverage

- interpolation - · extrapolation

🖲 ORH 📥 SEH 🚍 SEL 금 TB

0.6

of the studies varying by group considered, location, and gear used. It is probable that macrobenthic assemblages in the Ross Sea are strongly influenced by a 'seafloorhabitat' control effect, defined by depth, slope, current speed immediately above the seabed and organic content of seafloor sediments as already suggested by Barry et al. (2003).

with iNEXT on presence/absence data. ORH rough-bottom trawl,

SEH Brenke sled, SEL Epibenthic sled, TB beam trawl. a Rarefaction

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

0.8

micromolluscs represented the larger proportion of the total molluscan fauna in terms of both richness and abundance. In accordance with what was found from fine-mesh samples obtained by a Rauschert dredge during the Latitudinal Gradient Program (R/V *Italica* 2004) expedition (Ghiglione et al. 2013; Schiaparelli et al. 2014), the Brenke sled provided the highest number of species and specimens compared to the other standard sampling gears (Online Resource 3) and, in turn, of new species and new records for the area (Table 1; Online Resource 4 symbols "*" and "**", respectively).

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

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

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

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

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

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

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

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

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