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Nolwenn Béhagle, Cédric Cotté, Anne Lebourges-Dhaussy, Gildas Roudaut, Guy Duhamel, et al.. Acoustic distribution of discriminated micronektonic organisms from a bi-frequency processing: the case study of eastern Kerguelen oceanic waters. *Progress in Oceanography*, 2017, 156, pp.276-289. 10.1016/j.pocean.2017.06.004 . hal-01552638

HAL Id: hal-01552638

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1 Acoustic distribution of discriminated micronektonic organisms from a bi-
2 frequency processing: the case study of eastern Kerguelen oceanic waters

3

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18

19 **Abstract**

20 Despite its ecological importance, micronekton remains one of the least investigated
21 components of the open-ocean ecosystems. Our main goal was to characterize micronektonic
22 organisms using bi-frequency acoustic data (38 and 120 kHz) by calibrating an algorithm tool
23 that discriminates groups of scatterers in the top 300 m of the productive oceanic zone east of
24 Kerguelen Islands (Indian sector of the Southern Ocean). The bi-frequency algorithm was
25 calibrated from acoustic properties of mono-specific biological samples collected with trawls,
26 thus allowing to discriminate three acoustic groups of micronekton: (i) “gas-bearing” ($\Delta S_{v,120-38} < -1$ dB), (ii) “fluid-like” ($\Delta S_{v,120-38} > 2$ dB), and (iii) “undetermined” scatterers ($-1 < \Delta S_{v,120-38} < 2$ dB). The three groups likely correspond biologically to gas-filled swimbladder fish
29 (myctophids), crustaceans (euphausiids and hyperiid amphipods), and other marine organisms
30 potentially present in these waters and containing either lipid-filled or no inclusion (*e.g.* other
31 myctophids), respectively. The Nautical Area Scattering Coefficient (NASC) was used (echo-
32 integration cells of 10m long and 1m deep) between 30 and 300m depth as a proxy of relative
33 biomass of acoustic targets. The distribution of NASC values showed a complex pattern
34 according to: (i) the three acoustically-defined groups, (ii) the type of structures (patch *vs.*
35 layers) and (iii) the timing of the day (day/night cycle). NASC values were higher at night
36 than during the day. A large proportion of scatterers occurred in layers while patches, that
37 mainly encompass gas-bearing organisms, are especially observed during daytime. This
38 method provided an essential descriptive baseline of the spatial distribution of micronekton
39 and a relevant approach to (i) link micronektonic group to physical parameters to define their
40 habitats, (ii) investigate trophic interactions by combining active acoustic and top predator
41 satellite tracking, and (iii) study the functioning of the pelagic ecosystems at various spatio-
42 temporal scales.

43

44 *Keywords:* Euphausiid, Kerguelen, Myctophid, Southern Ocean, Acoustics.

45 **1. Introduction**

46

47 Micronektonic organisms (~1-20 cm in length; [Kloser et al., 2009](#)) constitute one of
48 the most noticeable and ecologically important components of the open ocean. They amount
49 to a substantial biomass (*e.g.* estimated at > 10 000 million metric tons of mesopelagic fish in
50 oceanic waters worldwide and ~380 million metric tons of Antarctic krill in the Southern
51 Ocean; [Atkinson et al., 2009](#); [Irigoiien et al., 2014](#)) with high nutritional value ([Shaviklo and](#)
52 [Rafipour, 2013](#); [Koizumi et al., 2014](#)) leading to increasing commercial interest ([Pauly et al.,](#)
53 [1998](#)). In oceanic waters, micronekton contribute to the export of carbon from the surface to
54 deeper layers (the biological pump) through extensive daily vertical mesopelagic migrations
55 to feed on near-surface organisms at night ([Bianchi et al., 2013](#)). They play a prominent role
56 in oceanic food webs by linking primary consumers to higher predators, including
57 commercially targeted fish species and oceanic squids, together with charismatic species, such
58 as marine mammals and seabirds ([Rodhouse and Nigmatullin, 1996](#); [Robertson and Chivers,](#)
59 [1997](#); [Potier et al. 2007](#); [Spear et al. 2007](#)). Despite their ecological importance, micronekton
60 remain one of the least investigated components of the marine ecosystems, with major gaps in
61 our knowledge of their biology, ecology, and major uncertainties about their global biomass
62 ([Handegard et al., 2013](#); [Irigoiien et al., 2014](#)).

63 Acoustic methods have been used in fishery operations and research since 1935 ([Sund,](#)
64 [1935](#)). Stock assessment drove a continuous improvement of the methods in order to better
65 investigate the distribution and abundance of targeted marine organisms ([Simmonds and](#)
66 [MacLennan, 2005](#)). Beyond stock assessment, acoustics now extends to whole marine
67 ecosystems, being the best available tool allowing simultaneous collection of qualitative and
68 quantitative data on their biotic and even abiotic components ([Bertrand et al., 2013](#)). A major
69 limitation of acoustics is the lack of accurate taxonomic information about the ensonified

70 organisms. Hence, acoustic analytical tools to determine characteristics of biological
71 backscatters were developed by comparing and quantifying the difference of mean volume
72 backscattering strength between different frequencies. The rationale is that the acoustic
73 properties of individual species are known to vary with the operating frequencies of the echo
74 sounder. For example, both experimental and theoretical studies showed large variations in
75 the average echo energy per unit biomass due to animals from “fluid-like” to “elastic shelled”
76 organisms (Stanton et al., 1994, 1998a, 1998b). This approach has been used since the late
77 1970s to identify and quantify zooplanktonic scatterers (Greenlaw, 1977; Holliday and Pieper,
78 1980; Madureira et al., 1993a,b). Less has been done to characterize micronektonic organisms
79 from the open sea, where micronekton are diverse and include small pelagic fishes,
80 cephalopods, large crustaceans and gelatinous animals. A recent biomass estimate of mid-
81 water fish was based on the 38 kHz frequency alone (Irigoien et al., 2014). Furthermore, the
82 difference of mean volume backscattering strength between two frequencies (38 and 120 kHz)
83 was used to differentiate “fish”, “macrozooplankton” and “zooplankton” scatterers (Fielding
84 et al., 2012; Bedford et al., 2015).

85 In the Southern Ocean (water masses south of the Subtropical Front), the importance
86 of micronekton is illustrated by the considerable populations of subantarctic seabirds and
87 pinnipeds that primarily prey on schooling myctophids, swarming euphausiids and hyperiid
88 amphipods (Cooper and Brown, 1990; Woehler and Green, 1992; Guinet et al., 1996; Bocher
89 et al., 2001). However, to our knowledge, acoustic investigation of mid-water organisms in
90 lower latitudes of the Southern Ocean is limited to a few surveys (Miller, 1982; Perissinotto
91 and McQuaid 1992; Pakhomov and Froneman, 1999), and only three recent studies
92 discriminated acoustic groups by their bi-frequency characteristics (Fielding et al., 2012;
93 Saunders et al., 2013; Bedford et al., 2015).

94 The main goal of the present work was to use bi-frequency acoustic data (38 and 120
95 kHz) combined with net sampling to calibrate an algorithm tool that discriminates groups of
96 scatterers in an acoustically poorly-explored area. The targeted region was the productive
97 oceanic zone off south-eastern Kerguelen Islands, because: (i) several significant populations
98 of predators are known to feed on micronektonic organisms in the area during the summer
99 months, namely Antarctic fur seals and king penguins on mesopelagic fishes (mainly
100 myctophids) and macaroni penguins on euphausiids and hyperiids (Bost et al., 2002;
101 Charrassin et al., 2004; Lea et al., 2002; C.A. Bost and Y. Cherel, unpublished data); and (ii)
102 myctophid fishes and euphausiids were already successfully collected in the area (Duhamel et
103 al., 2000, 2005; authors' unpublished data). The rationale was that different groups of
104 micronektonic organisms (large crustaceans and mid-water fish) should be abundant in the
105 targeted area and that the bi-frequency acoustic data should allow investigating their
106 horizontal and vertical abundance patterns according to the type of structures (patches and
107 layers).

108 2. Materials and methods

109

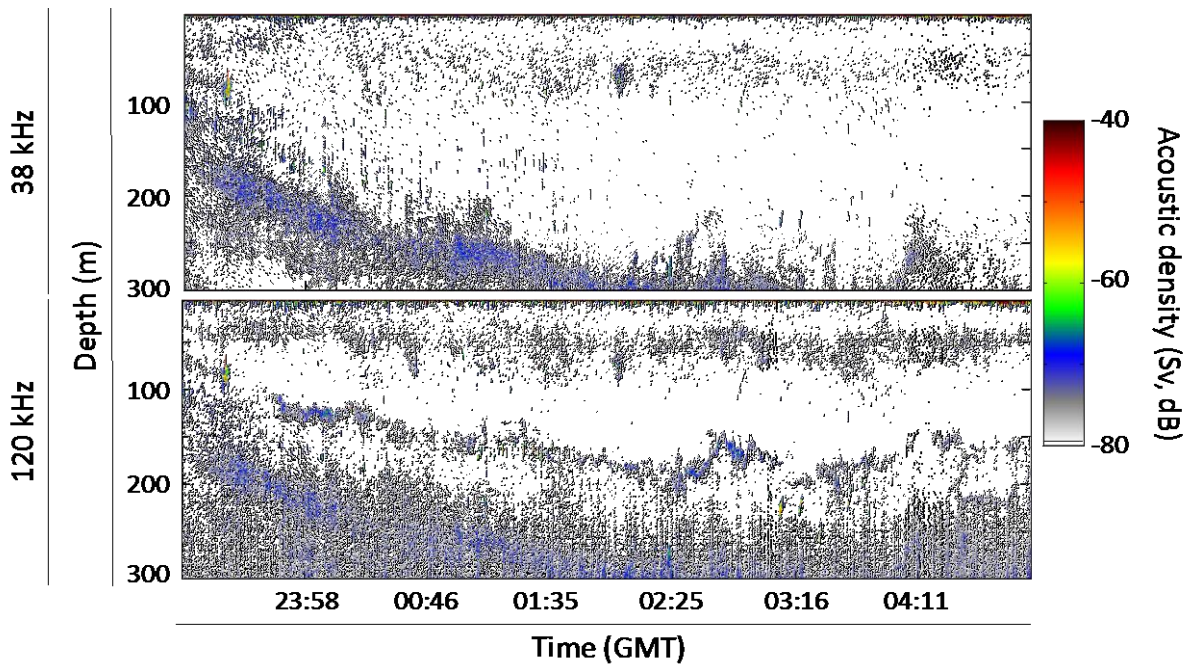
110 The oceanographic cruise (MD197/MYCTO) was carried out during the austral
111 summer 2013-2014 on board the R/V *Marion Dufresne II*. The overall dataset was based on
112 1,320 km of acoustic data in oceanic waters off Kerguelen Islands during 14 consecutive days
113 of recording.

114

115 2.1. Acoustic sampling

116 *In situ* acoustic data were recorded day and night during the period 23 January-5
117 February 2014. Measurements were made when cruising at a speed of 8 knots, using a Simrad
118 EK60 split-beam echo sounder operating simultaneously at 38 and 120 kHz. The transducers
119 were hull-mounted at a depth of 6 m below the water surface. An offset of 30 m below the
120 surface was applied to account for: (i) the depth of the transducers, (ii) the acoustic Fresnel
121 zone, and (iii) the acoustic interference from surface turbulence. Acoustic data were thus
122 collected on a vertical range from 30 to 300 m according to the 120 kHz range (Fig. 1). The
123 limited depth of 300m is considered in the interpretation of mid-water organisms
124 distributions, especially for those which are known to perform vertical migration according to
125 the day/night cycle (diel vertical migration; Lebourges-Dhaussy et al., 2000; Benoit-Bird et
126 al., 2009) (see section 4.2 below). Indeed, most of these organisms were sampled at night but
127 only epipelagic and some mesopelagic organisms were observable during the day within this
128 depth range.

129



130 **Fig. 1.** 38 and 120 kHz echograms representing acoustic density (in color, S_v in dB) recorded
 131 on the 24th of January 2014 morning from 30 to 300m depth in east waters off Kerguelen.

132

133 Transducers were calibrated following the procedures recommended in Foote et al.
 134 (1987). Settings that were used during data acquisition are summarized in Table 1. Movies+
 135 software (Ifremer development) was used for assessing visually the quality of the data prior
 136 further analyses. Depending on this quality assessment, data were filtered using an in-house
 137 tool (Béhagle et al., 2015) computed with Matlab (MATLAB 7.11.0.584, Release 2010b) and
 138 Movies3D software (Ifremer development) to remove ADCP (Acoustic Doppler Current
 139 Profiler) interference, background noise, and both attenuated- and elevated-signals. Then, an
 140 echo-integration by layer, with a threshold set at -80 dB to exclude scatterers which are not
 141 representative of micronektonic organisms, was applied on filtered acoustic data with an
 142 echo-integration cell size fixed at 3 pings per 1 m depth in order to smooth variability while
 143 keeping as much information as possible.

144 From echo-integration, volume backscattering strength (S_v , dB re 1 m^{-1}) was used to
 145 assess the mean echo level on both 38 and 120 kHz and thus to evaluate differences of relative
 146 frequency response of the organisms considered (*see section 2.3* below). Also, the acoustic

147 density of scatterers was estimated by calculating the Nautical Area Scattering Coefficient
 148 (NASC, s_A , $m^2 \cdot nmi^{-2}$; [MacLennan et al., 2002](#)). NASC was used as a proxy of relative
 149 biomass of acoustic targets, assuming that the composition of the scattering layers and the
 150 resulting scattering properties of biological organisms are homogeneous (*e.g.* [Simmonds and](#)
 151 [MacLennan, 2005](#); [Lawson et al., 2008](#)).

152

153 **Table 1.** Simrad EK60 echo sounder parameter settings onboard the R/V *Marion Dufresne II*
 154 during the MD197/MYCTO cruise in January-February 2014.

	38 kHz	120 kHz
Max. power (W)	1000	250
Pulse duration (ms)	1.024	1.024
Ping interval (s)	1.5	1.5
Target Strength 'TS' gain (dB)	24.65	27.03
Area backscattering coefficient (Sa) correction	-0.75	-0.30
Sample length (m)	0.189	0.189

155

156 2.2. Biological sampling

157 To determine the species and size composition of the dominant scatterers, trawling of
 158 micronektonic animals was conducted using the Mesopelagos trawl that was designed by
 159 Ifremer (fisheries biology and technology laboratory, LTBH, Lorient, France) ([Meillat, 2012](#)).
 160 The non-closing trawl vertical and horizontal openings varied between 5 and 6 m and 10 and
 161 12 m, respectively. The trawl has a mesh size of 4 cm in the wings, reducing to 5 mm at the
 162 codend during sampling. A terminal rigid collector was fixed on the codend in order to collect
 163 micronektonic organisms in good conditions. A Scanmar acoustic device ([Åsgårdstrand,](#)
 164 Norway) was attached on the net to monitor in real time the depth of trawling simultaneously
 165 to acoustic measurements ([Williams and Koslow, 1997](#)). The net was also equipped with an

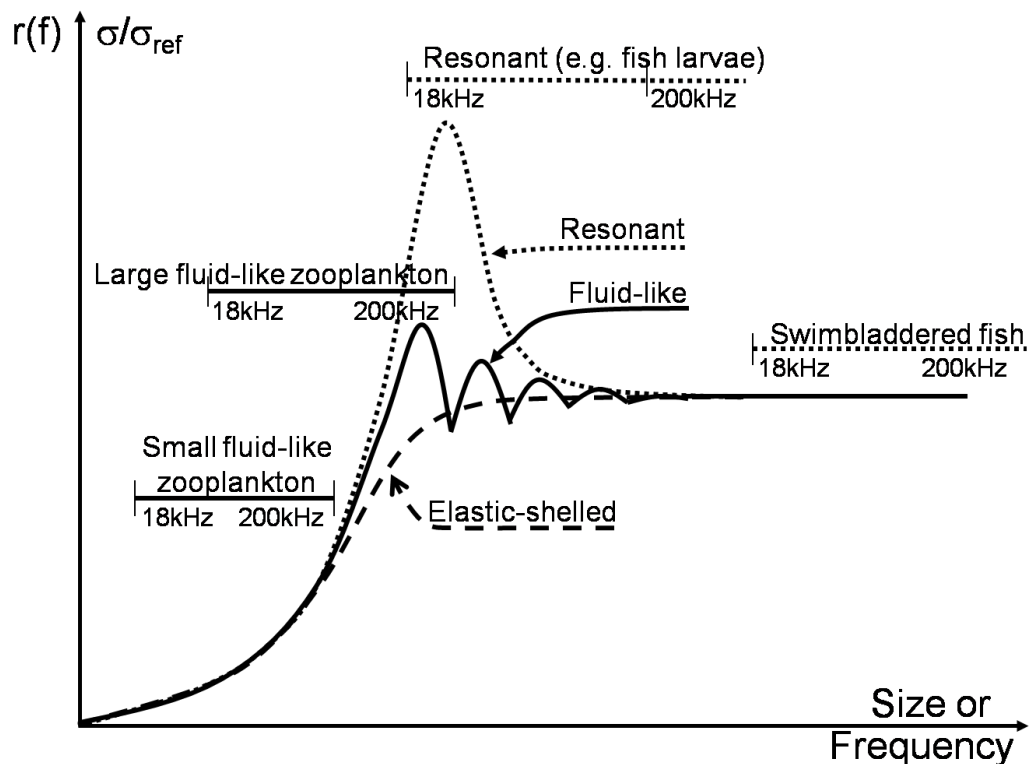
166 elephant seal tag (Sea Mammal Research Unit, UK) that was fixed on the trawl headline. The
167 tag was a multisensor data logger recording pressure (accuracy of 2 dbar) and hence depth,
168 temperature, salinity and fluorescence (Blain et al., 2013). Only depth data were analyzed in
169 the present work, thus providing an accurate time/depth profile for each tow. The trawl was
170 towed for 30 min at targeted depth at a speed of 1.5-2.5 knots. All catches were sorted by
171 species or lowest identifiable taxonomic groups, measured and weighed. While Antarctic krill
172 (*Euphausia superba*) does not occur in the area, collected taxa were representative of the
173 Polar Frontal Zone and Polar Front, including zooplankton-like organisms (*i.e.* euphausiids,
174 amphipods, large copepods and non-gaseous gelatinous organisms), fish-like organisms (*i.e.*
175 fish with a gas-filled swimbladder and gaseous gelatinous organisms), and other organisms
176 (*i.e.* fish without a gas-filled swimbladder and small squids). Most of the 39 pelagic hauls
177 conducted during this survey had mixed catches and were not further considered here. Indeed,
178 to be able to calibrate as correctly as possible a bi-frequency algorithm in this area, we chose
179 to use only mono-specific trawls. Two trawls were suitable for acoustic mark identification,
180 because almost all the catches consisted of one single species in large quantity (*see section 2.3*
181 below).

182

183 *2.3. Bi-frequency method calibration*

184 The acoustic properties of biological organisms vary with the operating frequency of
185 the echo sounder. Therefore, comparing the echo levels of individual scatterers ensonified at
186 different frequencies is likely to provide information on the types of targets that are present in
187 the water column (Madureira et al., 1993a,b; Kang et al., 2002). According to the literature,
188 zooplankton-like and non-gaseous gelatinous organisms have an increasing relative frequency
189 response between 38 and 120 kHz (Stanton and Chu, 2000; David et al., 2001; Lavery et al.,
190 2002; Korneliussen and Ona, 2003), whereas fish with a gas-filled swimbladder and gaseous

191 gelatinous organisms have a stable to decreasing relative frequency response between 38 and
 192 120 kHz, depending on the size of the gaseous inclusion (Warren et al., 2001; Kloser et al.,
 193 2002; Korneliussen and Ona, 2003) (Fig. 2). Thus, using the difference of reflectance of well-
 194 characterized biological samples collected by trawls, we determined thresholds to obtain the
 195 best compromise to separate three acoustic groups of organisms.
 196

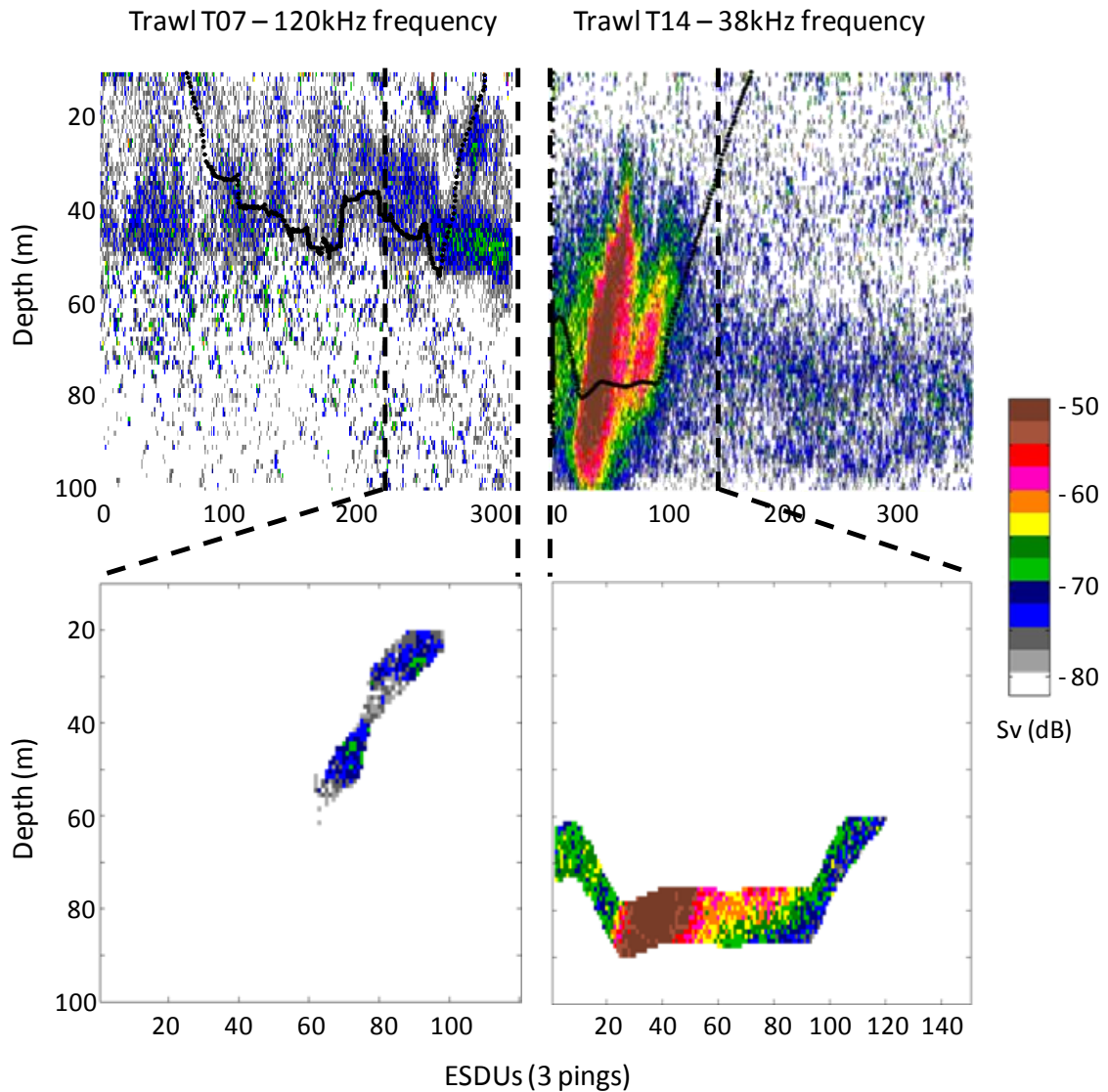


197
 198 **Fig. 2.** Schematic description of the relative frequency response, $r(f)$. Horizontal lines indicate
 199 typical range positions of selected acoustic categories when measured at frequencies 18-200
 200 kHz. Source: Korneliussen and Ona (2003).

201
 202 Firstly, “fluid-like” organisms were discriminated from “gas-bearing” organisms
 203 according to trawl sampling and to acoustic properties of scatterers at 120 and 38 kHz,
 204 respectively (Simmonds and MacLennan, 2005). Thresholds used in the bi-frequency
 205 algorithm to discriminate acoustic groups were fixed using acoustic data from two relevant

206 trawls, which were selected according to: (i) their depth (only trawls between the surface and
207 200 m depth were considered to minimize as much as possible interference from the saturated
208 outgoing signal at 120 kHz), and (ii) the quality of their acoustic data (mainly depending on
209 the weather; only trawls with more than 50% of clean pings were considered). Two night
210 trawls (T07, 50 m depth and T14, 70 m depth) were mono-specific in their composition,
211 containing almost exclusively adults of subantarctic krill *Euphausia vallentini* (15-24 mm
212 long) and juveniles of the demersal fish *Muraenolepis marmoratus* (31-40 mm long),
213 respectively. The latter corresponds to the pelagic stage of the species (Duhamel et al., 2005),
214 *i.e.* fish were 3-4 cm long and contained a well-developed gas-filled swimbladder, similar to
215 several species of myctophids (*Electrona carlsbergi*, *Krefftichthys anderssoni*,
216 *Protomyctophum* spp.; Saunders et al., 2013). The acoustic characteristics of samples from
217 these two trawl tows were considered as representative of “fluid-like” and “gas-bearing”
218 scatterers, respectively. Only the acoustic characteristics corresponding in time and depth to
219 the two trawls were taken into account to grade the bi-frequency algorithm in order to be sure
220 that they were related to the organisms effectively caught in the net. For doing this, we used
221 the time/depth data provided by the elephant seal tag for extracting the acoustic data from 2 m
222 above the headline up to 10 m below (or 2 m below the footrope) during a limited time period
223 that focused on the acoustically detected aggregations (Fig. 3). The acoustic response at 38
224 and 120 kHz, of each echo-integration cell belonging to the trawl’s path, was represented
225 relative to the 38 kHz frequency (Fig. 4a) to assess the positive *vs.* negative slope of the
226 relative frequency response between discrete 38 and 120 kHz frequencies.

227

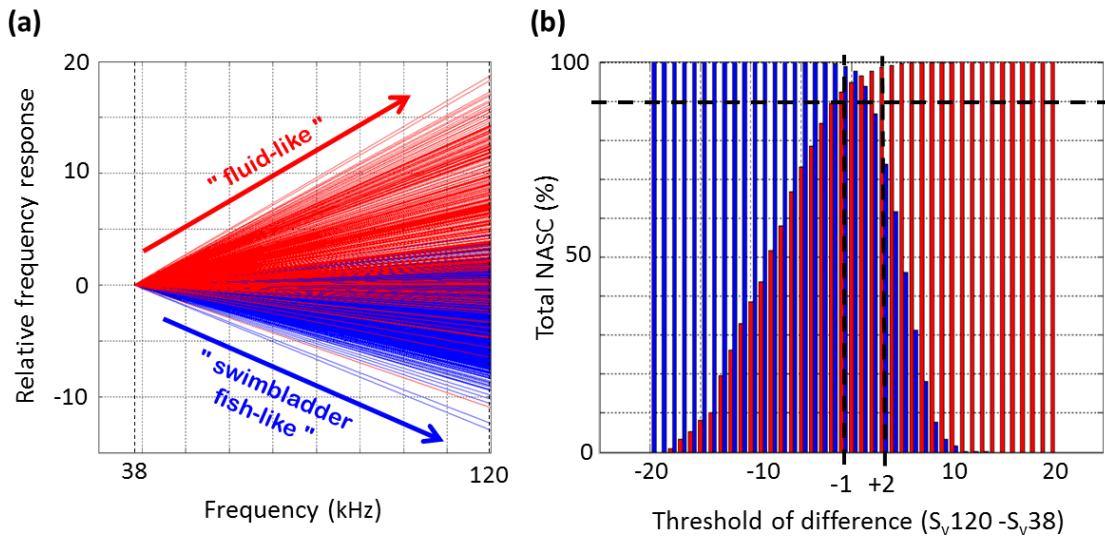


228

229 **Fig. 3.** Acoustic records and the corresponding cruise trawls (T07 and T14) that were used to
 230 fix thresholds of difference in the bi-frequency algorithm. Upper panel: complete trawl
 231 echograms with trawling depths (continuous black line) and limits of data extraction (dashed
 232 black lines). Lower panel: extracted echogram samples focusing on the trawl targeted
 233 aggregates that were selected from acoustic identification estimation. Left: T07 trawl
 234 (euphausiids) sampling on the 120 kHz frequency to discriminate the “fluid-like” group.
 235 Right: T14 trawl (gas-filled swimbladder fish) on the 38 kHz frequency to discriminate the
 236 “gas-bearing” group.

237

238 Secondly, the difference in relative frequency response ($\Delta S_{v,120-38} = S_{v,120} - S_{v,38}$) was
239 evaluated per echo-integration cell using a varying threshold of difference, ranging from -15
240 to 25 dB (Fig. 4b). For each threshold considered one by one, each acoustic sample was
241 classified either in a group “lower than the threshold considered” or in the opposite group
242 “higher than the threshold considered”. The total acoustic density was calculated (on 120 kHz
243 samples for the “fluid-like” group and on 38 kHz samples for the “gas-bearing” group) for
244 each of the lower/higher groups formed and reported in percentage to total acoustic density of
245 the aggregate for each tested threshold (Fig. 4b).



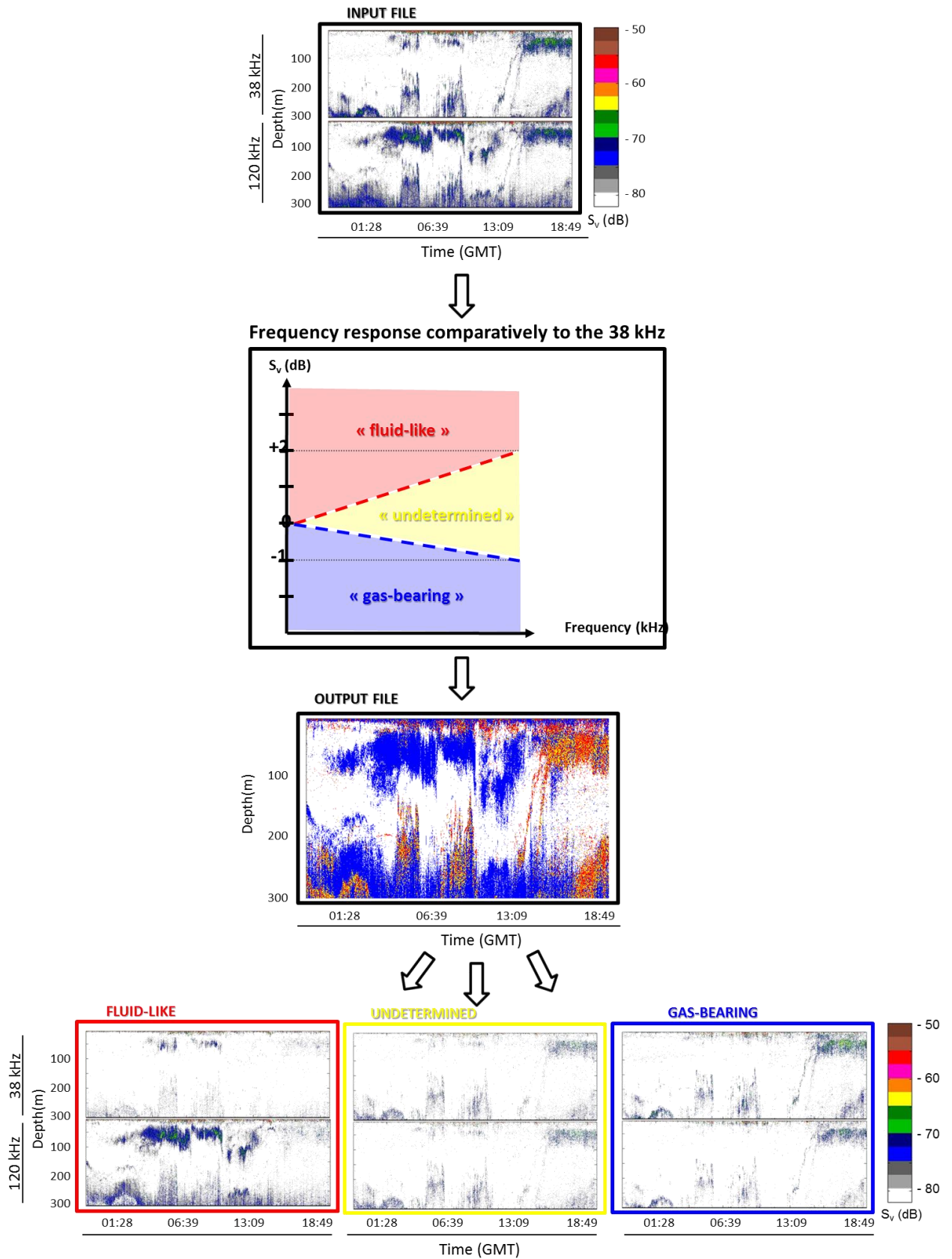
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247 **Fig. 4.** Left panel (a): frequency response of each sample considered relatively to the 38 kHz
248 frequency, with "fluid-like" samples (from the trawl T07) represented in red and "gas-
249 bearing" samples (from the trawl T14) in blue. Right panel (b): bar chart of the percentage of
250 "fluid-like" (in red) and "gas-bearing" (in blue) total NASC, according to a -15 to 25 dB range
251 of threshold of difference, used to define the best thresholds (-1 and +2 dB) delimiting the
252 “undetermined” group by transferring a maximum of 10% of their acoustic energy (total
253 NASC).

254

255 Finally, the calculated “loss” of density for both “fluid-like” and “gas-bearing” groups
256 was used to define two thresholds of differences delimiting the “undetermined” group by
257 transferring a maximum of 10% of their acoustic energy (Fig. 4b) into the “undetermined”
258 group. This group corresponds to an uncertainty zone where scatterers (*i*) have a close-to-flat
259 relative frequency response between discrete 38 and 120 frequencies, (*ii*) cannot be allocated
260 to “fluid-like” or “gas-bearing” organisms according to their S_v difference measured between
261 38 and 120 kHz but (*iii*) are potentially present in the water column and (*iv*) have no
262 biological validation in this work. Preserving such a group allows accounting for organisms
263 that could not be identified during this work from biological sampling but are present in the
264 water column, while being more demanding on well-defined groups. Following this method,
265 thresholds were defined at -1 and +2 dB. Scatterers with $\Delta S_{v,120-38} (i) > +2$ dB are classified in
266 the “fluid-like” group, (*ii*) < -1 dB are classified in the “gas-bearing” group and (*iii*) between -
267 1 and +2 dB are classified in a third “undetermined” group (Fig. 5).

268



269

270 **Fig. 5.** Summary diagram of the bi-frequency algorithm method used in this study.

271 2.4. Testing the bi-frequency algorithm

272 The robustness of threshold values obtained by the bi-frequency algorithm developed
273 in the present work was tested by calculating the theoretical frequency responses of
274 *Muraenolepis marmoratus* and *Euphausia vallentini* using the mathematical models of Ye
275 (1997) and Stanton et al. (1994), respectively. While the Ye model provides an analytic
276 method for studying scattering of “gas-bearing” organisms at low frequencies, the Stanton
277 model focuses on the “fluid-like” organisms’ acoustic properties.

278 The Ye (1997) model highlights a $\Delta S_{v,120-38}$ value of -0.4 dB for fish of 3 to 4 cm
279 length (as sampled during the T14 trawl), and the Stanton et al. (1994) model for randomly-
280 oriented fluid, bent cylinder highlights a $\Delta S_{v,120-38}$ value of +1.9 dB for euphausiids of 15-24
281 mm length (length range of organisms sampled during the T07 trawl). Using the bi-frequency
282 algorithm, the $\Delta S_{v,120-38}$ thresholds amounted to -1 and +2 dB, respectively, and are thus
283 consistent with the results of mathematical models. Our threshold values were even stronger
284 than those of the models ($-1 < -0.4$ and $2 > 1.9$ dB), thus highlighting the selectivity of the
285 algorithm. According to biological samples (see section 2.2. above) and acoustic properties of
286 scatterers at 38 and 120 kHz (see section 2.3 above), three acoustic groups have been defined
287 for micronektonic organisms: (i) “gas-bearing” ($\Delta S_{v,120-38} < -1$ dB), (ii) “fluid-like” ($\Delta S_{v,120-38}$
288 > 2 dB), and (iii) “undetermined” scatterers ($-1 < \Delta S_{v,120-38} < 2$ dB).

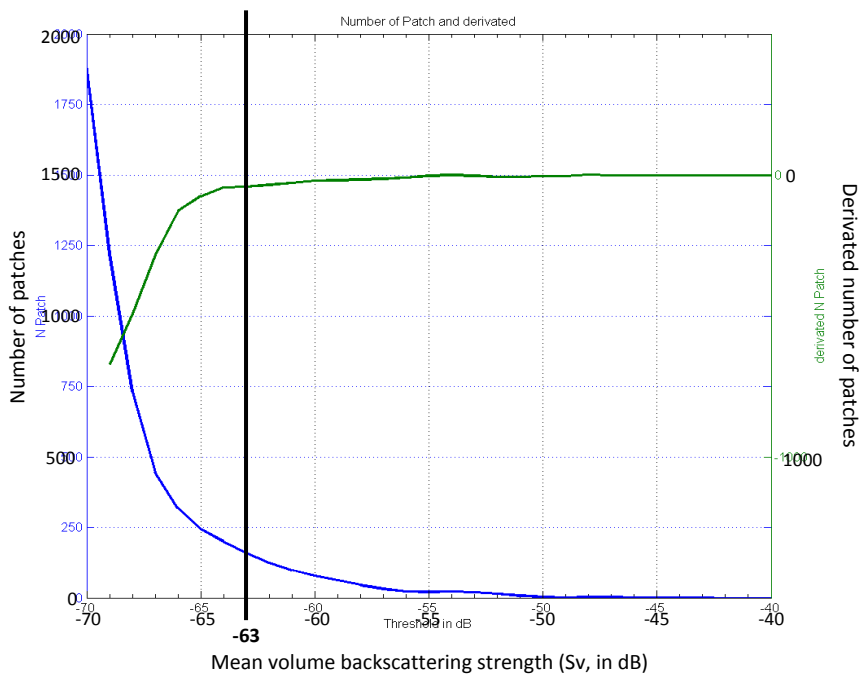
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290 2.5. Data post-processing and statistical analyses

291 Each echo-integration cell was attributed to “fluid-like”, “undetermined” or “gas-
292 bearing” group based on its relative frequency response. Moreover, as living organisms
293 follow non-random and non-uniform distributions (Margalef, 1979; Legendre and Fortin,
294 2004), acoustic data were analyzed separately in terms of patches and layers.

295 First of all, in order to get homogenous horizontal sampling at high resolution, filtered
 296 data at 38 and 120 kHz have been echo integrated in cells of 10 m (horizontal) by 1 m
 297 (vertical). Patches were here defined as isolated groups of echo-integrated cells limited in
 298 space (between 10 and 3000 m long) and associated to a mean volume backscattering strength
 299 $S_v \geq -63$ dB on the mean 38 and 120 kHz echogram. In contrast, layers were defined as
 300 continuous and homogenous areas of acoustic detections with a mean $S_v < -63$ dB for each
 301 echo-integrated cell on the mean 38 and 120 kHz echogram. The -63 dB threshold was
 302 defined by the operator after a visual analysis of the number of patches detected along a
 303 representative acoustic sample of five hours long and along increasing S_v values from -70 to -
 304 40 dB. The value of -63 dB corresponds to a threshold level over which the number of patches
 305 did not further increased (Fig. 6).

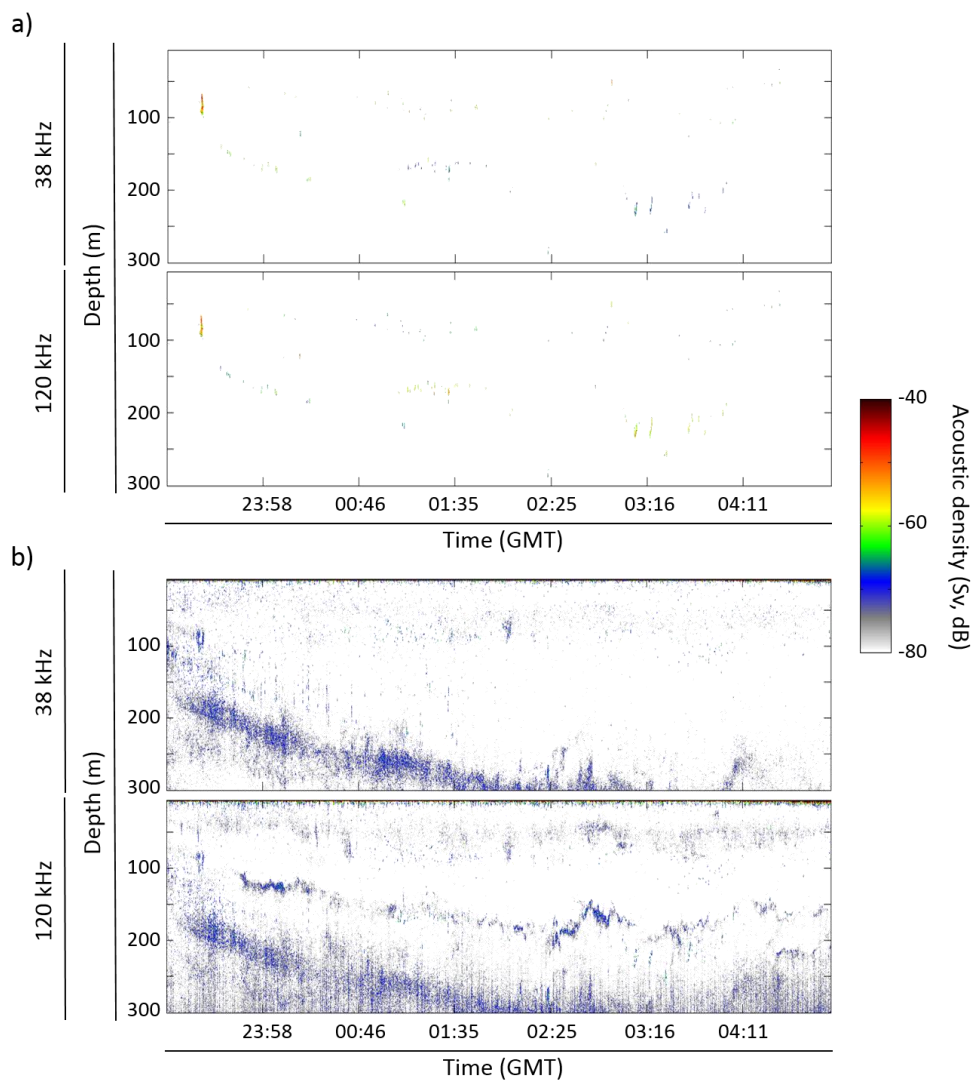
306



307 **Fig. 6.** Representative diagram of the number of patches (in blue) and its derivative (in green)
 308 detected along increasing S_v values from -70 to -40 dB. The value of -63 dB corresponds to a
 309 threshold level over which the number of patches did not further increased.

310

311 Using the Matlab contouring tool, echo-integrated cells having a mean $S_v \geq -63$ dB
312 were extracted from the echo integrated datasets and each contour considered as a detected
313 patch (Fig. 7a). For each patch, mean depth, vertical size, mean S_v and mean NASC values at
314 both frequencies were computed. NASC values were first summed on the vertical and then
315 averaged on the horizontal. Cells that were not considered as patches were considered as
316 layers (Fig. 7b).



317
318 **Fig. 7.** 38 and 120 kHz echograms representing acoustic density (in color, S_v in dB) recorded
319 on the 24th of January 2014 morning from 30 to 300m depth in east waters off Kerguelen for
320 (a) patches- and (b) layers structures.

321 Total-, patches- and layers- datasets were then post-processed following the same bi-
322 frequency algorithm (*see section 2.3* above). Thus, nine datasets were obtained: “fluid-like”,
323 “gas-bearing”, and “undetermined” for layer structures, for patch structures, and for the whole
324 (*i.e.* patches and layers together).

325 Acoustic data were analyzed from 30 to 300 m depth according to the applied offset
326 (*see section 2.1.* above) and the 120 kHz emission range. Day and night data were analyzed
327 separately because many mid-water organisms undergo diel vertical migration. The
328 crepuscular period (45 minutes before and after sunrise and sunset) during which mid-water
329 organisms ascend and descend ([Lebourges-Dhaussy et al., 2000](#); [Benoit-Bird et al., 2009](#))
330 were excluded from the analyses.

331 Statistical analyses were performed within the R environment ([R Core Team, 2014](#)).
332 Differences of distribution between groups were statistically assessed using student t tests.

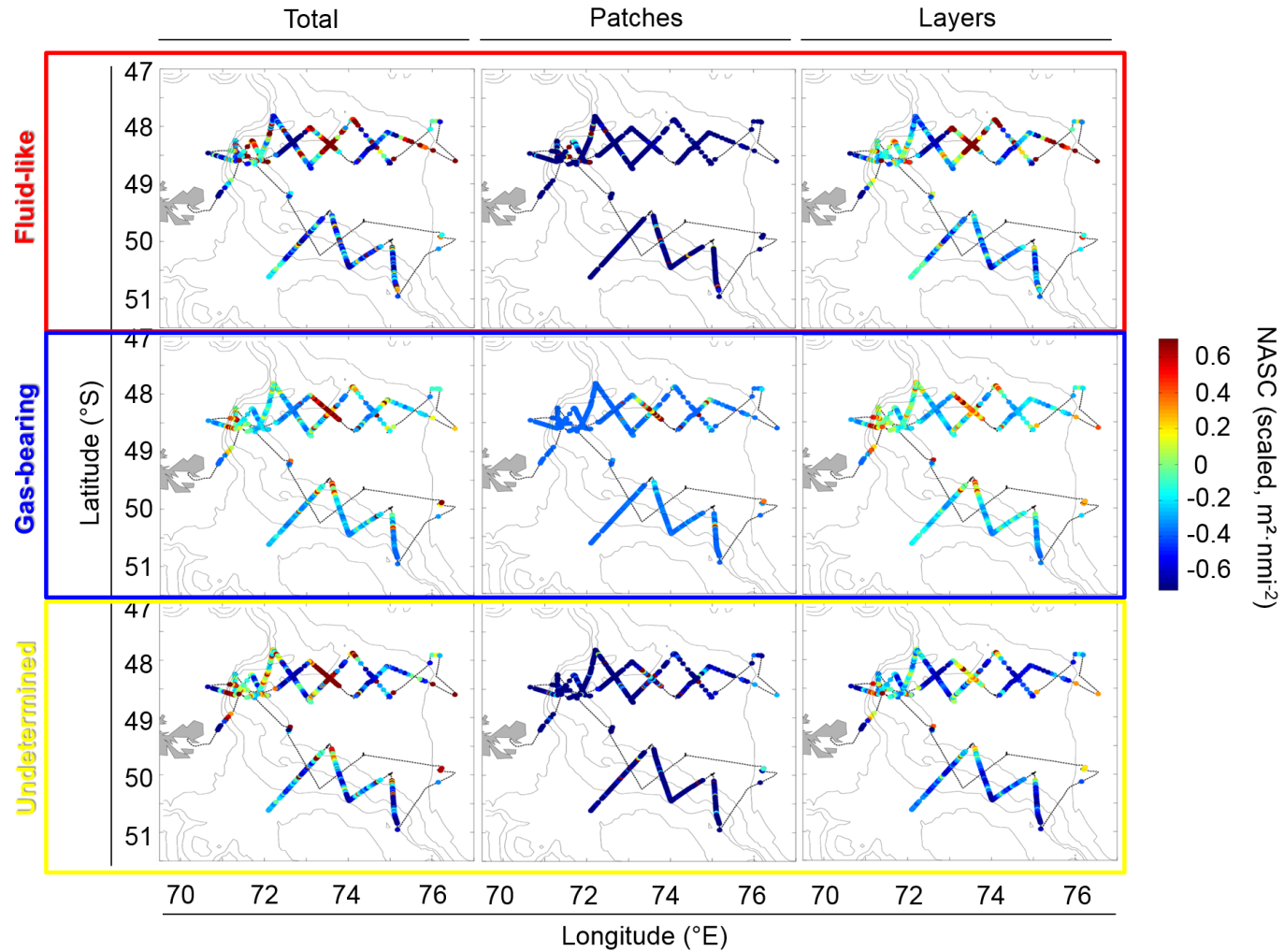
333 **3. Results**

334

335 *3.1. Horizontal distribution of acoustic groups of micronekton*

336 The horizontal distribution of NASC values (on 38 kHz for “gas-bearing” and
337 “undetermined” groups; on 120 kHz for “fluid-like” group) in the 30-300 m depth range
338 varied spatially (Northern and Southern tracks), and according to the daily cycle (day and
339 night), the type of structures (patches and layers) and the three acoustically-defined groups of
340 micronektonic organisms (Fig. 8, Tables 2 and 3). Several features were notable: (i) total
341 integrated NASC values were ~2-3 times higher in the Northern than in the Southern tracks;
342 (ii) with one exception (see below), NASC values of each group were higher at night than
343 during the day; (iii) a much larger proportion of scatterers of the three groups occurred in
344 layers than in patches; (iv) the layers/patches difference was more pronounced at day than
345 during the night, with patches almost disappearing at night (< 1% of the total NASC values);
346 and (v) during the day, a higher NASC proportion of “gas-bearing” (10-26%) and
347 “undetermined” (10-34%) than “fluid-like” (4-5%) scatterers occurred in patches.

348



349

350 **Fig. 8.** Total density (NASC, scaled in $\text{m}^2 \cdot \text{nmi}^{-2}$, colored on ship track) observed during the cruise integrated from 30 to 300 m depth for each
 351 acoustic group (“gas-bearing”, fluid-like” and “undetermined” groups) and for each type of structure (patches and layers).

352 **Table 2.** Acoustic density (NASC, in $\text{m}^2 \cdot \text{nm}^{-2}$) per echo-integration cell of each acoustic group (“gas-bearing”, “fluid-like” and “undetermined”
 353 groups). Values are means \pm SD. The small size of the 10 m horizontal cells explains both their numbers and very large variances.

354

Time period	Tracks	Echo-integration cell per 10 m (n)	Total NASC values at 38 or 120 Hz ($\text{m}^2 \cdot \text{nm}^{-2}$)	"Fluid-like" NASC values at 120 kHz ($\text{m}^2 \cdot \text{nm}^{-2}$)	"Gas-bearing" NASC values at 38 kHz ($\text{m}^2 \cdot \text{nm}^{-2}$)	"Undetermined" NASC values at 38 kHz ($\text{m}^2 \cdot \text{nm}^{-2}$)
Day	Northern	69150	446 \pm 3402	283 \pm 2641	107 \pm 1073	56 \pm 756
	Southern	37724	273 \pm 1642	209 \pm 1507	42 \pm 563	22 \pm 136
	Both tracks	106874	385 \pm 2907	257 \pm 2305	84 \pm 372	44 \pm 614
Night	Northern	15252	649 \pm 3091	351 \pm 2432	225 \pm 1487	73 \pm 454
	Southern	5065	195 \pm 579	73 \pm 431	84 \pm 372	38 \pm 32
	Both tracks	20317	536 \pm 2701	282 \pm 2122	190 \pm 1303	64 \pm 394
Day and night	Northern	84402	483 \pm 3349	295 \pm 2604	129 \pm 1160	59 \pm 711
	Southern	42789	264 \pm 1555	193 \pm 1423	47 \pm 544	24 \pm 128
	Both tracks	127191	409 \pm 2875	261 \pm 2277	101 \pm 998	47 \pm 584

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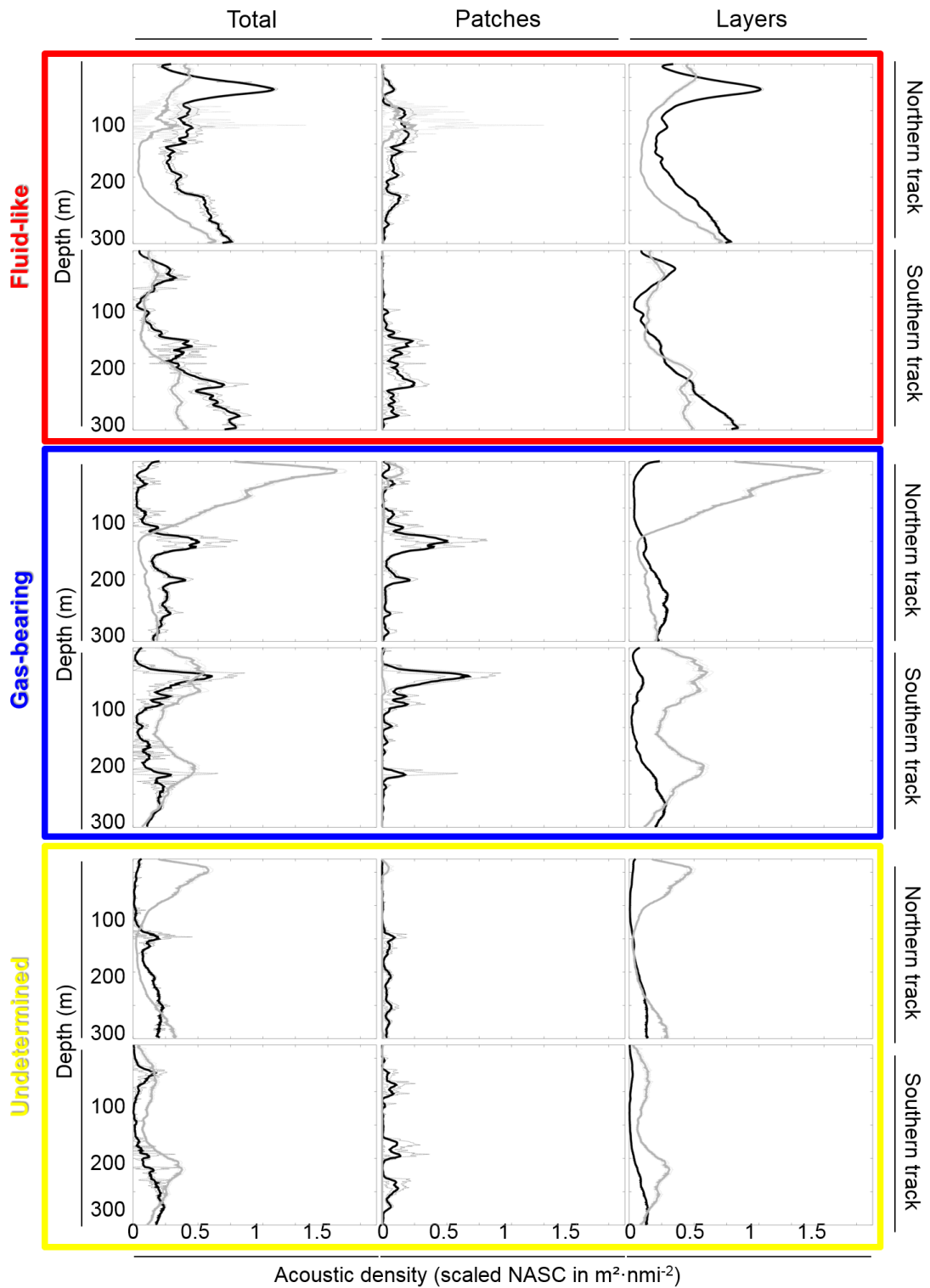
361 **Table 3.** Acoustic density (NASC, in $\text{m}^2 \cdot \text{nmi}^{-2}$) summed in the 30-300 m depth range and
 362 percentage contributions (between brackets) of each acoustic group (“gas-bearing”, “fluid-
 363 like” and “undetermined” groups) as patches and layers (see text for definitions). Daytime and
 364 nighttime were considered separately, as the Northern and Southern tracks were.
 365

Tracks	Groups	Day			Night		
		Total ($10^6 \text{ m}^2 \text{ nmi}^{-2}$)	Patches (%)	Layers (%)	Total ($10^6 \text{ m}^2 \text{ nmi}^{-2}$)	Patches (%)	Layers (%)
Northern	Gas-bearing	11.68 (25.9)	10.3	89.7	5.64 (32.0)	0.8	99.2
	Fluid-like	29.30 (65.0)	4.7	95.3	10.41 (59.1)	0.8	99.2
	Undetermined	4.10 (9.1)	9.7	90.3	1.56 (8.9)	0.7	99.3
	Total	45.08 (100.0)	6.6	93.4	17.61 (100.0)	0.8	99.2
Southern	Gas-bearing	2.23 (12.9)	26.1	73.9	0.68 (44.4)	0.5	99.5
	Fluid-like	14.21 (82.2)	3.4	96.6	0.66 (43.2)	0.0	100.0
	Undetermined	0.85 (4.9)	34.0	66.0	0.19 (12.5)	0.2	99.8
	Total	17.30 (100.0)	7.9	92.1	1.53 (100.0)	0.2	99.8
Total	Gas-bearing	13.92 (22.3)	12.8	87.2	6.31 (33.0)	0.8	99.2
	Fluid-like	43.51 (69.8)	4.3	95.7	11.07 (57.8)	0.8	99.2
	Undetermined	4.95 (7.9)	13.9	86.1	1.75 (9.2)	0.6	99.4
	Total	62.38 (100.0)	6.9	93.1	19.14 (100.0)	0.8	99.2

366 3.2. Vertical distribution of acoustic groups of micronekton

367

368 The vertical distribution of NASC values of the three acoustically-defined groups of
369 micronektonic organisms varied spatially (Northern and Southern tracks), temporally (time of
370 the day), and according to the type of structures (patches and layers) (Fig. 9, Table 4).
371 Overall, “fluid-like” organisms were structured in layers and their NASC values showed: (i) a
372 peak at shallow depths (< 100 m) during the day with an intermediate inter-quartile range
373 revealing a rather unimodal vertical distribution; and (ii) a progressive increase with depth
374 from 150 to 300 m. The pattern was similar at night, but with significantly lower values ($t =$
375 17.5, $p < 0.001$) and higher inter-quartile range, highlighting a consistent distribution in the
376 range 30-300 m. “Gas-bearing” scatterers showed a different vertical pattern with a well-
377 defined change between day and night. While most scatterers were structured in layers, they
378 were more patchily distributed during the day with a main mode at ~150 and ~70 m in the
379 Northern and Southern tracks, respectively. Patches almost completely disappeared at night
380 during which “gas-bearing” organisms occurred in more diffuse layers with a unimodal
381 distribution in the north at ~30 m and a bimodal distribution in the south at ~65 and ~200 m.
382 The distribution of “undetermined” organisms showed no obvious patterns, with discrete
383 small patches during the day and more obvious layers at night, especially at shallow depths in
384 the Northern track (Fig. 9, Table 4).



385

386 **Fig. 9.** Mean vertical NASC profiles from 30 to 300 m depth of each acoustic group (“gas-
 387 bearing”, fluid-like” and “undetermined” groups) for day (black lines) and night (grey lines)
 388 and for each type of structures (patches and layers). Dashed lines indicate the 95% confidence
 389 intervals.

390 **Table 4.** Maximum acoustic density (NASC) depth, median acoustic density and inter-
391 quantile range proportion of acoustic groups (calculated on the 38 kHz for the “gas-bearing”
392 and “undetermined” groups and on the 120 kHz for the “fluid-like” group) according to the
393 tracks (Northern and Southern), time of the day (day and night) and type of structures
394 (patches and layers).

Timing	Tracks	Groups	Maximum NASC depth (m)	Median NASC values (m ² nmi ⁻²)	Inter-quantile range (%)	
Total						
Day	Northern	Gas-bearing	148	0.16	24.6	
		Fluid-like	66	0.36	23.9	
		Undetermined	147	0.09	50.0	
	Southern	Gas-bearing	72	0.11	19.3	
		Fluid-like	275	0.23	47.7	
		Undetermined	270	0.05	54.5	
	Night	Northern	Gas-bearing	42	0.13	27.3
			Fluid-like	295	0.20	50.0
			Undetermined	45	0.10	33.3
Southern		Gas-bearing	65	0.23	34.7	
		Fluid-like	294	0.13	54.3	
		Undetermined	214	0.12	25.0	
Patches						
Day	Northern	Gas-bearing	147	0.03	10.4	
		Fluid-like	109	0.05	17.1	
		Undetermined	143	0.02	30.0	
	Southern	Gas-bearing	68	0.01	9.7	
		Fluid-like	163	0.02	36.4	
		Undetermined	194	0.01	27.8	
	Night	Northern	Gas-bearing	45	0.00	7.1
			Fluid-like	122	0.00	0.0
			Undetermined	41	0.00	0.0
Southern		Gas-bearing	94	0.00	0.0	
		Fluid-like	38	0.00	0.0	
		Undetermined	58	0.00	0.0	
Layers						
Day	Northern	Gas-bearing	230	0.13	59.3	
		Fluid-like	65	0.31	32.9	
		Undetermined	297	0.04	53.8	
	Southern	Gas-bearing	263	0.07	54.2	
		Fluid-like	292	0.21	39.7	
		Undetermined	297	0.02	46.2	
	Night	Northern	Gas-bearing	42	0.14	27.7
			Fluid-like	294	0.22	45.3
			Undetermined	45	0.09	36.6
		Southern	Gas-bearing	65	0.28	33.3
			Fluid-like	294	0.19	53.7
			Undetermined	214	0.10	27.6

395 **4. Discussion**

396

397 Historically, most of the acoustic investigations conducted in the Southern Ocean
398 since the 1960s focused on Antarctic krill (Demer and Conti, 2005; Fielding et al., 2014), due
399 to its high and variable biomass (Atkinson et al., 2009), key role in the high-latitude pelagic
400 ecosystem (Ainley and DeMaster, 1990) and developing commercial fisheries (Nicol et al.,
401 2012). More recently, the concept of a distinct Antarctic open-ocean food chain where
402 Antarctic krill is absent pointed out the importance of other micronektonic organisms,
403 including mid-water fish (Rodhouse and White, 1995). Hence, different groups were
404 acoustically characterized in the Antarctic krill zone (Fielding et al., 2012; Saunders et al.,
405 2013), but, to our knowledge, little acoustic information is available in Northern waters of the
406 Southern Ocean where Antarctic krill is ecologically replaced by other micronektonic
407 organisms, namely euphausiids, a few hyperiid amphipods and myctophid fishes.

408 The present study focused on productive waters off eastern Kerguelen Islands, where
409 numerous top predators target micronektonic organisms different from Antarctic krill (Guinet
410 et al. 1996). It provides a first depiction of horizontal and vertical (30-300 m) distribution and
411 abundance of three different acoustic groups of micronektonic organisms from a bi-frequency
412 processing of acoustic data (38 and 120 kHz).

413

414 *4.1. Methodological comments and biological interpretation of the acoustic groups*

415

416 Methodologically, the frequency-dependent technique based on estimated differences
417 between mean volume-backscattering strength at 38 and 120 kHz has also previously been
418 used to characterize acoustic groups (Madureira 1993a,b; Brierley et al., 1998). The most
419 recent investigations defined two micronektonic groups in Antarctic waters, namely Antarctic

420 krill (macrozooplankton) that was identified using a 2-12 or 2-16 dB $\Delta S_{v,120-38}$ window
421 (Fielding et al., 2012, 2014), and myctophids (gas-filled swimbladder fish) that were
422 characterized by $\Delta S_{v,120-38} < 2$ or < 0 dB (Fielding et al., 2012; Saunders et al. 2013).
423 Elsewhere, a threshold at $\Delta S_{v,120-38} = 2$ dB was used to discriminate gas-filled swimbladder
424 fish (< 2 dB) from euphausiids (> 2 dB) (De Robertis et al., 2010; Ressler et al., 2015). Using
425 the same overall approach, our $\Delta S_{v,120-38}$ threshold values fit well with theoretical models (Ye,
426 1997; Stanton et al., 1994). The $\Delta S_{v,120-38}$ threshold value (-1 dB) to discriminate “gas-
427 bearing” backscatters was even lower than the previously used values (0-2 dB). Hence, our
428 identification of “gas-bearing” backscatters is more conservative than in previous
429 investigations, and the method allowed discriminating a third intermediate group of
430 backscatters at $-1 < \Delta S_{v,120-38} < 2$ dB that cannot be classified as a given type of organism
431 without ground-truthing.

432 Micronektonic organisms that constituted the three acoustic groups of backscatters can
433 be tentatively defined using a combination of bi-frequency threshold values, acoustic
434 sampling depth (30-300 m), net sampling (Hunt et al., 2011) and predators’ diet (Guinet et al.,
435 1996) within the studied area. (i) The “fluid-like” group ($\Delta S_{v,120-38} > 2$ dB) is likely to
436 correspond primarily to crustaceans, including euphausiids (e.g. *Euphausia vallentini*, *E.*
437 *triacantha*, *Thysanoessa* spp.) and hyperiids (*Themisto gaudichaudii*). Non-gas-bearing
438 gelatinous organisms (e.g. salps) also occur in the area (Hunt et al., 2011) and they were
439 collected during the cruise, it is here assumed that their acoustic signature was similar to
440 “fluid-like” signature (Wiebe et al., 2010). (ii) The “gas-bearing” group ($\Delta S_{v,120-38} < -1$ dB)
441 includes gas-bearing gelatinous organisms and gas-filled swimbladder fish. Siphonophores
442 occur in the Southern Ocean, but their abundance is relatively low in Kerguelen waters (Hunt
443 et al., 2011). On the other hand, mesopelagic fish were abundant, with most of them
444 belonging to the Family Myctophidae in terms of species, number and biomass (Duhamel et

445 al., 2005). Not all myctophid species contain a gas-filled swimbladder, however, and it is
446 likely that the acoustically detected myctophids were primarily *Electrona carlsbergi*,
447 *Krefflichthys anderssoni* and *Protomyctophum* spp. although it was not possible to
448 differentiate between species (Marshall, 1960; Saunders et al., 2013). Noticeably, all those
449 species are targeted by the myctophid-eater king penguin (Bost et al., 2002; Cherel et al.,
450 2002) and they are known to form school structures (Saunders et al., 2013). *Krefflichthys*
451 *anderssoni* was the commonest net-caught myctophid during the cruise and *Protomyctophum*
452 *bolini* and *P. tenisoni* also occurred in significant numbers in trawls (authors' unpublished
453 data). (iii) The “undetermined” group of scatterers ($-1 < \Delta S_{v,120-38} < 2$ dB) most likely
454 corresponds to other fish, meaning lipid-filled swimbladder species and fish with no
455 swimbladder (Simmonds and MacLennan, 2005). Again these characteristics point out
456 myctophid fish in the area, including *Gymnoscopelus braueri* that ranked third amongst the
457 net-caught myctophids during the cruise (authors' unpublished data) together with other
458 *Gymnoscopelus* species that constitute the main prey species of fur seals *Arctocephalus* spp.
459 (Marshall, 1960; Lea et al., 2002; Saunders et al., 2013). Theoretically also, the
460 “undetermined” group can include a combination of “fluid-like” and “gas-bearing” scatterers
461 living in mixed and homogenous layers or patches, thus overall resulting in intermediate
462 $\Delta S_{v,120-38}$ values.

463

464 4.2. Horizontal and vertical distribution of the acoustic groups

465

466 The acoustic density (NASC) of micronektonic scatterers varied both in time and
467 space, thus showing a complex pattern depending on acoustically-defined groups, time of the
468 day (day/night), depth (30-300 m), the type of structures (patches and layers) and geography
469 (Northern and Southern tracks). Firstly, depth-integrated NASC values of the three acoustic

470 groups were higher in the Northern than the Southern tracks, which may correspond to the
471 Polar Front and Northern Antarctic waters, respectively. This would be consistent with the
472 high abundance of micronekton recorded in frontal areas of the Western Indian sector of the
473 Southern Ocean (Pakhomov et al., 1996; Pakhomov and Froneman, 2000) and deserves a
474 thorough study in combination with hydrographic data. Secondly, the finding of an overall
475 higher biomass at night than during the day is in accordance with a recent large-scale acoustic
476 investigation in the Western Indian Ocean (Béhagle et al. 2015) and the general trend of
477 upward migration of deep-dwelling zooplanktonic and micronektonic organisms at sunset in
478 oceanic waters (Domokos, 2009; Escobar-Flores et al., 2013; Béhagle et al., 2014). Finally,
479 other key features of micronektonic distribution were the much higher NASC values in layers
480 (> 92% of total NASC values) than in patches, and the almost disappearance of patches (<
481 1%) at night when compared to the daylight hours (Table 4). The latter feature is related to the
482 diel behaviour of mid-water organisms that disperse at night to feed in the epipelagic zone
483 (Hays, 2003). Moreover, in this work, the potential bias in patches detection linked to the
484 increasing acoustic beam with depth is not considered as well as the depth is not a hindrance
485 to our comparisons. Indeed, (i) in most cases, the absence of patches at night makes the
486 comparison between day and night NASC proportions meaningful and independent of depth
487 and (ii) for the only case of night occurrence of patches (along the Northern track for “fluid-
488 like” organisms), the few detected patches were observed at the same depth as during the day
489 which makes comparison possible regardless of any difference in resolution of detecting
490 patches. The only bias could be an underestimation of deep patches detected during the day.

491

492 “Fluid-like” scatterers occurred predominantly within layers with a bimodal
493 distribution at shallow and deep depths (Fig. 9). A similar bimodal vertical distribution was
494 previously observed from acoustic-based records at the Polar Front area westward (Pakhomov

495 [et al., 1994](#)). A prominent feature of “fluid-like” scatter occurrence in Kerguelen waters was a
496 well-defined layer at ~60 m depth during the day, which likely corresponds to some key
497 crustacean species collected with nets (*E. vallentini*, *Thysanoessa* spp., *T. gaudichaudii*;
498 [Pakhomov and Froneman, 1999](#); [Hunt et al., 2011](#); [this study](#)). Noticeably, those crustacean
499 species form the bulk of the food of the most abundant diving air-breathing predator from the
500 area, the macaroni penguin (*Eudyptes chrysolophus*), which predominantly forages at 20-60
501 m depth during the day ([Sato et al., 2004](#); [Bost and Cherel, unpublished data](#)).

502 Most scatterers of the “gas-bearing” and “undetermined” groups were structured in
503 layers that were more pronounced at night than during the day. Especially obvious was a ~50
504 m-deep layer during the northern track that suggests a high abundance of mid-water fish in
505 the upper epipelagic at night. Indeed, surface layers are invaded at that time by myctophids in
506 Kerguelen waters and elsewhere, with the species including a pool of gas-filled swimbladder-,
507 lipid-filled swimbladder- and swimbladderless myctophids ([Duhamel et al., 2005](#); [Collins et](#)
508 [al., 2012](#); [Saunders et al., 2013](#)). This pattern corresponds well with the night-time diving
509 behaviour of Antarctic fur seals (*A. gazella*) that prey primarily on mid-water fish at 40-60 m
510 depth in eastern Kerguelen waters ([Lea et al., 2002, 2006](#)). A major characteristic of the “gas-
511 bearing” group was the significant amount of scatterers structured in patches during daytime.
512 It is likely that patches corresponded to schools of fish, as already depicted in the Atlantic
513 sector of the Southern Ocean ([Fielding et al., 2012](#); [Saunders et al., 2013](#)), and that the species
514 were mainly myctophids with a gas-filled swimbladder ([Collins et al. 2008](#)). Patch depth
515 observed during the survey was < 180 m, thus suggesting that they were composed of
516 *Krefflichthys anderssoni* and *Protomyctophum* spp., and not of deeper-living species as *E.*
517 *carlsbergi* ([Duhamel et al., 2005](#); [Collins et al., 2008](#); [Flynn and Williams, 2012](#)). Indeed, the
518 survey overlapped the foraging area of the king penguin (*Aptenodytes patagonicus*) that is
519 known to target primarily *K. anderssoni* in the 100-150 m depth range during the day ([Bost et](#)

520 [al., 2002](#); [Charrassin et al., 2004](#); [C.A. Bost and Y. Cherel, unpublished data](#)). Interestingly,
521 patches occurred at different depths during the northern (~150 m) and southern (~70 m)
522 tracks, which can be related to different species within patches or to physical oceanography in
523 different water masses or to a combination of both. The limited information available shows
524 that myctophids are linked to the physical, chemical and biological characteristics of the water
525 column, with bottom depth, temperature and oxygen content of the water being key
526 environmental factors controlling their distributions ([Hulley and Lutjeharms, 1995](#)).
527 Moreover, despite patches were detected only during daylight, variations in light levels could
528 also affect the vertical distribution of mesopelagic organisms as it has been observed for deep
529 scattering layers ([Klevjer et al., 2016](#)).

530 In conclusion, the present study highlights the usefulness of combining acoustic
531 records with biological sampling to use reliable bi-frequency algorithms to discriminate
532 groups of backscatters. When validated, the method bypasses the problem of net avoidance by
533 micronekton, especially during the daylight hours ([Kloser et al., 2009](#); [Pakhomov and](#)
534 [Yamamura, 2010](#); [Kaartvedt et al., 2012](#)). Despite uncertainties with species identification
535 and depth limitation in acoustic data, it provides an essential descriptive baseline of the spatial
536 distribution and structure of micronektonic organisms. More at-sea investigations are needed
537 to better define the species-specific acoustic response of crustaceans (*e.g.* [Madureira et al.,](#)
538 [1993b](#)), myctophids (*e.g.* [Gautier et al., 2014](#)) and gelatinous organisms (*e.g.* [Wiebe et al.,](#)
539 [2010](#)). As it stands, however, the method can already help (*i*) to link micronektonic group
540 distribution to physical oceanography both horizontally and vertically to better define their
541 oceanic habitats ([Koubbi et al., 2011](#)), (*ii*) to investigate predator-prey interactions by
542 combining real time acoustic surveys and bio-logging ([Benoit-Bird et al., 2011](#); [Bedford et](#)
543 [al., 2015](#)), and hence (*iii*) to gather useful information on the functioning of the still poorly
544 known oceanic ecosystem. Overall, the distribution of the acoustic groups fit well with the at-

545 sea behaviour of air-breathing diving predators from Kerguelen Islands (see above). More
546 specifically, however, a thorough comparison between net trawling and predator foraging
547 ecology underlines some fundamental mismatches that can be investigated using active
548 acoustic surveys. For example, the subantarctic krill *E. vallentini* is traditionally considered to
549 live deeper than 100 m during the day (Perissinotto and MacQuaid, 1992; Hamame and
550 Antezana, 2010), while it is one of the most important prey items of various diurnal seabirds
551 (e.g. crested penguins) that feed primarily in the top 50 m of the water column (Ridoux, 1988;
552 Tremblay and Cherel, 2003; Sato et al., 2004).

553

554 **Acknowledgements**

555

556 The authors thank the officers, crew and scientists of the R/V *Marion Dufresne II* for
557 their assistance during the research cruise LOGIPEV197. This work was supported financially
558 and logistically by the Agence Nationale de La Recherche (ANR MyctO-3D-MAP,
559 Programme Blanc SVSE 7 2011, Y. Cherel), the Institut Polaire Français Paul Emile Victor,
560 and the Terres Australes et Antarctiques Françaises.

561

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776 **Fig. 1.** 38 and 120 kHz echograms representing acoustic density (in color, S_v in dB) recorded
777 on the 24th of January 2014 morning from 30 to 300m depth in east waters off Kerguelen.

778

779 **Fig. 2.** Schematic description of the relative frequency response, $r(f)$. Horizontal lines indicate
780 typical range positions of selected acoustic categories when measured at frequencies 18-200
781 kHz. Source: [Korneliussen and Ona \(2003\)](#).

782

783 **Fig. 3.** Acoustic records and the corresponding cruise trawls (T07 and T14) that were used to
784 define thresholds of difference in the bi-frequency algorithm. Upper panel: complete trawl
785 echograms with trawling depths (continuous black line) and limits of data extraction (dashed
786 black lines). Lower panel: extracted echogram samples focusing on the trawl targeted
787 aggregates that were selected from acoustic identification estimation. Left: T07 trawl
788 (euphausiids) sampling on the 120 kHz frequency to discriminate the “fluid-like” group.
789 Right: T14 trawl (gas-filled swimbladder fish) on the 38 kHz frequency to discriminate the
790 “gas-bearing” group.

791

792 **Fig. 4.** Left panel (a): frequency response of each sample considered relatively to the 38 kHz
793 frequency, with "fluid-like" samples (from the trawl T07) represented in red and "gas-
794 bearing" samples (from the trawl T14) in blue. Right panel (b): bar chart of the percentage of
795 "fluid-like" (in red) and "gas-bearing" (in blue) total NASC, according to a -15 to 25 dB range
796 of threshold of difference, used to define the best thresholds (-1 and +2 dB) delimiting the
797 “undetermined” group by transferring a maximum of 10% of their acoustic energy (total
798 NASC).

799

800 **Fig. 5.** Summary diagram of the bi-frequency algorithm method used in this study.

801

802 **Fig. 6.** Representative diagram of the number of patches (in blue) and its derivative (in green)
803 detected along increasing S_v values from -70 to -40 dB. The value of -63 dB corresponds to a
804 threshold level over which the number of patches did not further increased.

805

806 **Fig. 7.** 38 and 120 kHz echograms representing acoustic density (in color, S_v in dB) recorded
807 on the 24th of January 2014 morning from 30 to 300m depth in east waters off Kerguelen for
808 (a) patches- and (b) layers structures.

809 **Fig. 8.** Total density (NASC, in $m^2 \cdot nmi^{-2}$, colored on ship track) integrated from 30 to 300 m
810 depth for each acoustic group (“gas-bearing”, fluid-like” and “undetermined” groups) and for
811 each type of structures (patches and layers).

812

813 **Fig. 9.** Mean vertical NASC profiles from 30 to 300 m depth of each acoustic group (“gas-
814 bearing”, fluid-like” and “undetermined” groups) for day (black lines) and night (grey lines)
815 and for each type of structures (patches and layers). Dashed lines indicate the 95% confidence
816 intervals.