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

Nolwenn Béhagle a,b,⇑, Cédric Cotté c, Anne Lebourges-Dhaussy a, Gildas Roudaut a, Guy Duhamel d, Patrice Brehmer e, Erwan Josse a, Yves Cherel f

aIRD, UMR LEMAR 6539 (CNRS-IRD-IFREMER-UBO), BP70, 29280 Plouzané, France
bCNRS, UMR LOCEAN 7159 (CNRS-IRD-MNHN-UPMC), 4 Place Jussieu, 75005 Paris, France
cMNHN, UMR LOCEAN 7159 (CNRS-IRD-MNHN-UPMC), 4 Place Jussieu, 75005 Paris, France
dMNHN, UMR BOREA 7208(MNHN-CNRS-UPMC-IRD-UCBN-UAG), 43 rue Cuvier, CP 26, 75231 Paris Cedex 05, France
eIRD, UMR 195 Lemar, ISRA-CRODT, Pole de Recherche de Hann, BP221, Dakar, Senegal
fCNRS, UMR CEBC 7372 (CNRS-Université de La Rochelle), 79360 Villiers-en-Bois, France

Article history:
Received 23 September 2015
Received in revised form 13 June 2017
Accepted 21 June 2017
Available online 30 June 2017

Keywords:
Euphausiid
Kerguelen
Myctophid
Southern Ocean
Acoustics

Abstract

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

© 2017 Elsevier Ltd. All rights reserved.

Contents

1. Introduction ......................................................................................................... 277
2. Materials and methods ........................................................................................ 277
  2.1. Acoustic sampling ........................................................................................... 277
  2.2. Biological sampling ....................................................................................... 278
2.3. Bi-frequency method calibration ..................................................................... 279
2.4. Testing the bi-frequency algorithm .................................................................. 280

⇑ Corresponding author at: IRD, UMR LEMAR 6539 (CNRS-IRD-IFREMER-UBO), BP70, 29280 Plouzané, France.
E-mail address: nolwennbehagle@ntymail.com (N. Béhagle).

http://dx.doi.org/10.1016/j.pocean.2017.06.004
0079-6611/© 2017 Elsevier Ltd. All rights reserved.
1. Introduction

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

Acoustic methods have been used in fishery operations and research since 1935 (Sund, 1935). Stock assessment drove a continuous improvement of the methods in order to better investigate the distribution and abundance of targeted marine organisms (Simmonds and MacLennan, 2005). Beyond stock assessment, acoustic data were recorded day and night during the overall dataset was based on 1320 km of acoustic data in oceanic waters off Kerguelen Islands during 14 consecutive days of recording.

2. Materials and methods

The oceanographic cruise (MD197/MYCTO) was carried out during the austral summer 2013–2014 on board the R/V Marion Dufresne II. The overall dataset was based on 1320 km of acoustic data in oceanic waters off Kerguelen Islands during 14 consecutive days of recording.

2.1. Acoustic sampling

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

Transducers were calibrated following the procedures recommended in Foote et al. (1987). Settings that were used during data acquisition are summarized in Table 1. Movies + software (Ifremer development) was used for assessing visually the quality of the data prior further analyses. Depending on this quality assessment, data were filtered using an in-house tool (Béhagle et al., 2016) computed with Matlab (MATLAB 7.11.0.584, Release, 2010b) and Movies3D software (Ifremer development) to remove ADCP (Acoustic Doppler Current Profiler) interference, background noise, and both attenuated- and elevated-signals. Then, an echo-integration by layer, with a threshold set at −80 dB to exclude scatterers which are not representative of micronektonic organisms, was applied on filtered acoustic data with an echo-integration cell size fixed at 3 pings per 1 m depth in order to smooth variability while keeping as much information as possible.

From echo-integration, volume backscattering strength ($S_v$, dB re 1 m$^{-1}$) was used to assess the mean echo level on both 38 and 120 kHz and thus to evaluate differences of relative frequency response of the organisms considered (see Section 2.3 below). Also, the acoustic density of scatterers was estimated by calculating the Nautical Area Scattering Coefficient (NASC, $s_A$, m$^2$ nmi$^{-2}$; MacLennan et al., 2002). NASC was used as a proxy of relative biomass of acoustic targets, assuming that the composition of the scattering layers and the resulting scattering properties of biological organisms are homogeneous (e.g. Simmonds and MacLennan, 2005; Lawson et al., 2008).

### 2.2. Biological sampling

To determine the species and size composition of the dominant scatterers, trawling of micronektonic animals was conducted using the Mesopelagos trawl that was designed by Ifremer (fisheries biology and technology laboratory, LTBH, Lorient, France) (Meillat, 2012). The non-closing trawl vertical and horizontal openings varied between 5 and 6 m and 10 and 12 m, respectively. The trawl has a mesh size of 4 cm in the wings, reducing to 5 mm at the codend during sampling. A terminal rigid collector was fixed on the codend in order to collect micronektonic organisms in good conditions. A Scanmar acoustic device (Åsgårdstrand, Norway) was attached on the net to monitor in real time the depth of trawling simultaneously to acoustic measurements (Williams and Koslow, 1997). The net was also equipped with an elephant seal tag (Sea Mammal Research Unit, UK) that was fixed on the trawl headline. The tag was a multisensor data logger recording pressure (accuracy of 2 dbar) and hence depth, temperature, salinity and fluorescence.
Only depth data were analyzed in the present work, thus providing an accurate time/depth profile for each tow. The trawl was towed for 30 min at targeted depth at a speed of 1.5–2.5 knots. All catches were sorted by species or lowest identifiable taxonomic groups, measured and weighed. While Antarctic krill (*Euphausia superba*) does not occur in the area, collected taxa were representative of the Polar Frontal Zone and Polar Front, including zooplankton-like organisms (i.e. euphausiids, amphipods, large copepods and non-gaseous gelatinous organisms), fish-like organisms (i.e. fish with a gas-filled swimbladder and gaseous gelatinous organisms), and other organisms (i.e. fish without a gas-filled swimbladder and small squids). Most of the 39 pelagic hauls conducted during this survey had mixed catches and were not further considered here. Indeed, to be able to calibrate as correctly as possible a bi-frequency algorithm in this area, we chose to use only mono-specific trawls. Two trawls were suitable for acoustic mark identification, because almost all the catches consisted of one single species in large quantity (see Section 2.3 below).

### 2.3. Bi-frequency method calibration

The acoustic properties of biological organisms vary with the operating frequency of the echo sounder. Therefore, comparing the echo levels of individual scatterers ensonified at different frequencies is likely to provide information on the types of targets that are present in the water column (Madureira et al., 1993a,b; Kang et al., 2002). According to the literature, zooplankton-like and non-gaseous gelatinous organisms have an increasing relative frequency response between 38 and 120 kHz (Stanton and Chu, 2000; David et al., 2001; Lavery et al., 2002; Korneliussen and Ona, 2003), whereas fish with a gas-filled swimbladder and gaseous gelatinous organisms have a stable to decreasing relative frequency response between 38 and 120 kHz, depending on the size of the gaseous inclusion (Warren et al., 2001; Kloster et al., 2002; Korneliussen and Ona, 2003) (Fig. 2). Thus, using the difference of reflectance of well-characterized biological samples collected by trawls, we determined thresholds to obtain the best compromise to separate three acoustic groups of organisms.

Firstly, “fluid-like” organisms were discriminated from “gaseous-bearing” organisms according to trawl sampling and to acoustic properties of scatterers at 120 and 38 kHz, respectively (Simmonds and MacLennan, 2005). Thresholds used in the bi-frequency algorithm to discriminate acoustic groups were fixed using acoustic data from two relevant trawls, which were selected according to: (i) their depth (only trawls between the surface and 200 m depth were considered to minimize as much as possible interference from the saturated outgoing signal at 120 kHz), and (ii) the quality of their acoustic data (mainly depending on the weather; only trawls with more than 50% of clean pings were considered). Two night trawls (T07, 50 m depth and T14, 70 m depth) were mono-specific in their composition, containing almost exclusively adults of subantarctic krill *Euphausia vallentini* (15–24 mm long) and juveniles of the demersal fish *Muraenolepis marmoratus* (31–40 mm long), respectively. The latter corresponds to the pelagic stage of the species (Duhamel et al., 2005), i.e. fish were 3–4 cm long and contained a well-developed gas-filled swimbladder, similar to several species of myctophids (*Electrona carlsbergi*, *Krefftichthys anderssoni*, *Protomyctophum* spp.; Saunders et al., 2013).

---

**Fig. 3.** Acoustic records and the corresponding cruise trawls (T07 and T14) that were used to define thresholds of difference in the bi-frequency algorithm. Upper panel: complete trawl echograms with trawling depths (continuous line) and limits of data extraction (dashed lines). Lower panel: extracted echogram samples focusing on the trawl targeted aggregates that were selected from acoustic identification estimation. Left: T07 trawl (euphausiids) sampling on the 120 kHz frequency to discriminate the “fluid-like” group. Right: T14 trawl (gas-filled swimbladder fish) on the 38 kHz frequency to discriminate the “gas-bearing” group.
Following this method, thresholds were defined at a column, while being more demanding on well-defined groups. 

work from biological sampling but are present in the water accounting for organisms that could not be identified during this logical validation in this work. Preserving such a group allows that they were related to the organisms effectively caught in the net. For doing this, we used the time/depth data provided by the elephant seal tag for extracting the acoustic data from 2 m above the headline up to 10 m below (or 2 m below the footrope) during a limited time period that focused on the acoustically detected aggregations (Fig. 3). The acoustic response at 38 and 120 kHz, of each echo-integration cell belonging to the trawl’s path, was represented relative to the 38 kHz frequency (Fig. 4a) to assess the positive vs. negative slope of the relative frequency response between discrete 38 and 120 kHz frequencies.

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

Finally, the calculated “loss” of density for both “fluid-like” and “gas-bearing” groups was used to define two thresholds of differences delimiting the “undetermined” group by transferring a maximum of 10% of their acoustic energy (total NASC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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

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

### 2.4. Testing the bi-frequency algorithm

![Fig. 4. Left panel (a): frequency response of each sample considered relatively to the 38 kHz frequency, with “fluid-like” samples (from the trawl T07) represented in red and “gas-bearing” samples (from the trawl T14) in blue. Right panel (b): bar chart of the percentage of “fluid-like” (in red) and “gas-bearing” (in blue) total NASC, according to a -15 to 25 dB range of threshold of difference, used to define the best thresholds (-1 and +2 dB) delimiting the “undetermined” group by transferring a maximum of 10% of their acoustic energy (total NASC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image)

Scatterers with \(\Delta S_{v,120-38} > +2\) dB are classified in the “fluid-like” group, (ii) \(-1\) dB are classified in the “gas-bearing” group and (iii) between \(-1\) and \(+2\) dB are classified in a third “undetermined” group (Fig. 5).

### 2.5. Data post-processing and statistical analyses

Each echo-integration cell was attributed to “fluid-like”, “undetermined” or “gas-bearing” group based on its relative frequency response. Moreover, as living organisms follow non-random and non-uniform distributions (Margalef, 1979; Legendre and Fortin, 2004), acoustic data were analyzed separately in terms of patches and layers.
First of all, in order to get homogenous horizontal sampling at high resolution, filtered data at 38 and 120 kHz have been echo integrated in cells of 10 m (horizontal) by 1 m (vertical). Patches were here defined as isolated groups of echo-integrated cells limited in space (between 10 and 3000 m long) and associated to a mean volume backscattering strength \( S_v/C_21/C_0 \geq 63 \) dB on the mean 38 and 120 kHz echogram. In contrast, layers were defined as continuous and homogenous areas of acoustic detections with a mean \( S_v < -63 \) dB for each echo-integrated cell on the mean 38 and 120 kHz echogram. The \(-63\) dB threshold was defined by the

![Summary diagram of the bi-frequency algorithm method used in this study.](image)
operator after a visual analysis of the number of patches detected along a representative acoustic sample of five hours long and along increasing $S_v$ values from $-70$ to $-40$ dB. The value of $-63$ dB corresponds to a threshold level over which the number of patches did not further increased. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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

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

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

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

3. Results

3.1. Horizontal distribution of acoustic groups of micronekton

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

4. Discussion

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

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

4.1. Methodological comments and biological interpretation of the acoustic groups

Methodologically, the frequency-dependent technique based on estimated differences between mean volume-backscattering strength at 38 and 120 kHz has also previously been used to characterize acoustic groups (Madureira et al., 1993a,b; Brierley et al., 1998). The most recent investigations defined two micronektonic groups in Antarctic waters, namely Antarctic krill (macrozooplankton) that was identified using a 2–12 or 2–16 dB $\Delta S_v,120-38$ window (Fielding et al., 2012, 2014), and myctophids (gas-filled swimbladder fish) that were characterized by $\Delta S_v,120-38 < 2$ or $< 0$ dB (Fielding et al., 2012; Saunders et al., 2013). Elsewhere, a threshold at $\Delta S_v,120-38 = 2$ dB was used to discriminate gas-filled swimbladder...
fish (<2 dB) from euphausiids (>2 dB) (De Robertis et al., 2010; Ressler et al., 2015). Using the same overall approach, our $D_{S\text{v},20-38}$ threshold values fit well with theoretical models (Ye, 1997; Stanton et al., 1994). The $D_{S\text{v},120-38}$ threshold value ($C_0$ dB) to discriminate “gas-bearing” backscatters was even lower than the previously used values (0–2 dB). Hence, our identification of “gas-bearing” backscatters is more conservative than in previous investigations, and the method allowed discriminating a third intermediate group of backscatters at $C_0 < D_{S\text{v},120-38} < 2$ dB that cannot be classified as a given type of organism without ground-truthing.

Micronektonic organisms that constituted the three acoustic groups of backcatters can be tentatively defined using a combination of bi-frequency threshold values, acoustic sampling depth (30–300 m), net sampling (Hunt et al., 2011) and predators’ diet (Guinet et al., 1996) within the studied area. (i) The “fluid-like” group ($D_{S\text{v},120-38} > 2$ dB) is likely to correspond primarily to crustaceans, including euphausiids (e.g. Euphausia vallentini, E. triacantha, Thysanoessa spp.) and hyperiids (Themeisto gaudichaudi).

Non-gas-bearing gelatinous organisms (e.g. salps) also occur in the area (Hunt et al., 2011) and they were collected during the cruise, it is here assumed that their acoustic signature was similar to “fluid-like” signature (Wiebe et al., 2010). (ii) The “gas-bearing” group ($D_{S\text{v},120-38} < -1$ dB) includes gas-bearing gelatinous organisms and gas-filled swimbladder fish. Siphonophores occur in the Southern Ocean, but their abundance is relatively low in Kerguelen waters (Hunt et al., 2011). On the other hand, mesopelagic fish were abundant, with most of them belonging to the Family Myctophidae in terms of species, number and biomass (Duhamel et al., 2005). Not all myctophid species contain a gas-filled swimbladder, however, and it is likely that the acoustically detected myctophids were primarily Electrona carlsbergi, Krefftichthys anderssoni and Protomycophylum spp. although it was not possible to differentiate between species (Marshall, 1960; Saunders et al., 2013). Noticeably, all those species are targeted by the myctophid-eater king penguin (Bost et al., 2002; Cherel et al., 2002) and they are known to form school structures (Saunders et al., 2013). Krefftichthys anderssoni was the commonest net-

![Fig. 8. Total density (NASC, in m² nmi⁻², colored on ship track) integrated from 30 to 300 m depth for each acoustic group (“gas-bearing”, “fluid-like” and “undetermined” groups) and for each type of structures (patches and layers).](image)

**Table 2**

Acoustic density (NASC, in m² nmi⁻²) per echo-integration cell of each acoustic group (“gas-bearing”, “fluid-like” and “undetermined” groups). Values are means ± SD. The small size of the 10 m horizontal cells explains both their numbers and very large variances.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Tracks</th>
<th>Total NASC values at 38 or 120 Hz (m² nmi⁻²)</th>
<th>“Fluid-like” NASC values at 120 kHz (m² nmi⁻²)</th>
<th>“Gas-bearing” NASC values at 38 kHz (m² nmi⁻²)</th>
<th>“Undetermined” NASC values at 38 kHz (m² nmi⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day Northern</td>
<td>69,150</td>
<td>446 ± 3402</td>
<td>283 ± 2641</td>
<td>107 ± 1073</td>
<td>56 ± 756</td>
</tr>
<tr>
<td>Southern</td>
<td>37,724</td>
<td>273 ± 1642</td>
<td>209 ± 1507</td>
<td>42 ± 563</td>
<td>22 ± 136</td>
</tr>
<tr>
<td>Both tracks</td>
<td>106,874</td>
<td>385 ± 2907</td>
<td>257 ± 2305</td>
<td>84 ± 372</td>
<td>44 ± 614</td>
</tr>
<tr>
<td>Night Northern</td>
<td>15,252</td>
<td>649 ± 3091</td>
<td>351 ± 2432</td>
<td>225 ± 1487</td>
<td>73 ± 454</td>
</tr>
<tr>
<td>Southern</td>
<td>5065</td>
<td>195 ± 579</td>
<td>73 ± 431</td>
<td>84 ± 372</td>
<td>38 ± 32</td>
</tr>
<tr>
<td>Both tracks</td>
<td>20,317</td>
<td>536 ± 2701</td>
<td>282 ± 2122</td>
<td>190 ± 1303</td>
<td>64 ± 394</td>
</tr>
<tr>
<td>Day and night</td>
<td>84,402</td>
<td>483 ± 3349</td>
<td>295 ± 2604</td>
<td>129 ± 1160</td>
<td>59 ± 711</td>
</tr>
<tr>
<td>Northern</td>
<td>42,789</td>
<td>264 ± 1555</td>
<td>193 ± 1423</td>
<td>47 ± 544</td>
<td>24 ± 128</td>
</tr>
<tr>
<td>Southern</td>
<td>39,135</td>
<td>208 ± 1356</td>
<td>164 ± 1209</td>
<td>47 ± 544</td>
<td>24 ± 128</td>
</tr>
<tr>
<td>Both tracks</td>
<td>127,191</td>
<td>409 ± 2875</td>
<td>261 ± 2277</td>
<td>101 ± 998</td>
<td>47 ± 584</td>
</tr>
</tbody>
</table>
caught myctophid during the cruise and Protomyctophum bolini and P. tenisoni also occurred in significant numbers in trawls (authors' unpublished data). (iii) The “undetermined” group of scatterers (−1 < ΔSN ≤ 2 dB) most likely corresponds to other fish, meaning lipid-filled swimbladder species and fish with no swimbladder (Simmonds and Maclennan, 2005). Again these characteristics point out myctophid fish in the area, including Gymnoscelus braueri that ranked third amongst the net-caught myctophids during the cruise (authors' unpublished data) together with other Gymnoscelus species that constitute the main prey species of fur seals Arctocephalus spp. (Marshall, 1960; Lea et al., 2002; Saunders et al., 2013). Theoretically also, the “undetermined” group can include a combination of “fluid-like” and “gas-bearing” scatterers living in mixed and homogenous layers or patches, thus overall resulting in intermediate SN values.

### 4.2. Horizontal and vertical distribution of the acoustic groups

The acoustic density (NASC) of micronektonic scatterers varied both in time and space, thus showing a complex pattern depending on acoustically-defined groups, time of the day (day/night), depth (30–300 m), the type of structures (patches and layers) and geography (Northern and Southern tracks). Firstly, depth-integrated NASC values of the three acoustic groups were higher in the Northern than the Southern tracks, which may correspond to the Polar Front and Northern Antarctic waters, respectively. This would be consistent with the high abundance of micronekton recorded in frontal areas of the Western Indian sector of the Southern Ocean (Pakhomov et al., 1996; Pakhomov and Froneman, 2000) and deserves a thorough study in combination with hydrographic data. Secondly, the finding of an overall higher biomass at night than during the day is in accordance with a recent large-scale acoustic investigation in the Western Indian Ocean (Béhagle et al., 2016) and the general trend of upward migration of deep-dwelling zooplanktonic and micronektonic organisms at sunset in oceanic waters (Domokos, 2009; Escober-Flores et al., 2013; Béhagle et al., 2014). Finally, other key features of micronektonic distribution were the much higher NASC values in layers (>92% of total NASC values) than in patches, and the almost disappearance of patches (<1%) at night when compared to the daylight hours (Table 4). The latter feature is related to the diel behaviour of mid-water organisms that disperse at night to feed in the epipelagic zone (Hays, 2003). Moreover, in this work, the potential bias in patches detection linked to the increasing acoustic beam with depth is not considered as well as the depth is not a hindrance to our comparisons. Indeed, (i) in most cases, the absence of patches at night makes the comparison between day and night NASC proportions meaningful and independent of depth and (ii) for the only case of night occurrence of patches (along the Northern track for “fluid-like” organisms), the few detected patches were observed at the same depth as during the day which makes comparison possible regardless of any difference in resolution of detecting patches. The only bias could be an underestimation of deep patches detected during the day.

**Table 3**

Acoustic density (NASC, in m² nmi⁻²) summed in the 30–300 m depth range and percentage contributions (between brackets) of each acoustic group (“gas-bearing”, “fluid-like” and “undetermined” groups) as patches and layers (see text for definitions). Daytime and nighttime were considered separately, as the Northern and Southern tracks were.

<table>
<thead>
<tr>
<th>Tracks</th>
<th>Groups</th>
<th>Day</th>
<th>Night</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (10⁶ m² nmi⁻²)</td>
<td>Patches (%)</td>
<td>Layers (%)</td>
</tr>
<tr>
<td>Northern</td>
<td>Gas-bearing</td>
<td>11.68 (25.9)</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>Fluid-like</td>
<td>29.30 (65.0)</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Undetermined</td>
<td>4.10 (9.1)</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45.08 (100.0)</td>
<td>6.6</td>
</tr>
<tr>
<td>Southern</td>
<td>Gas-bearing</td>
<td>2.23 (12.9)</td>
<td>26.1</td>
</tr>
<tr>
<td></td>
<td>Fluid-like</td>
<td>1.42 (82.2)</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Undetermined</td>
<td>0.85 (4.3)</td>
<td>34.0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17.30 (100.0)</td>
<td>7.9</td>
</tr>
<tr>
<td>Total</td>
<td>Gas-bearing</td>
<td>13.92 (22.3)</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>Fluid-like</td>
<td>43.51 (69.8)</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Undetermined</td>
<td>4.95 (7.9)</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>62.38 (100.0)</td>
<td>6.9</td>
</tr>
</tbody>
</table>

A similar bimodal vertical distribution was previously observed from acoustic-based records at the Polar Front area westward (Pakhomov et al., 1994). A prominent feature of “fluid-like” scatter occurrence in Kerguelen waters was a well-defined layer at ~60 m depth during the day, which likely corresponds to some key crustacean species collected with nets (E. vallentini, Thysanoessa spp., T. gaudichaudii; Pakhomov and Froneman, 1999; Hunt et al., 2011; this study). Noticeably, those crustacean species form the bulk of the most abundant diving air-breathing predator from the area, the macaroni penguin (Eudyptes chrysolophus), which predominantly forages at 20–60 m depth during the day (Sato et al., 2004; Bost and Cherei, unpublished data).

Most scatterers of the “gas-bearing” and “undetermined” groups were structured in layers that were more pronounced at night than during the day. Especially obvious was a ~50 m-deep layer during the northern track that suggests a high abundance of mid-water fish in the upper epipelagic at night. Indeed, surface layers are invaded at that time by myctophids in Kerguelen waters and elsewhere, with the species including a pool of gas-filled swimbladder-, lipid-filled swimbladder- and swimbladderless myctophids (Duhamel et al., 2005; Collins et al., 2012; Saunders et al., 2013). This pattern corresponds well with the night-time diving behaviour of Antarctic fur seals (A. gazella) that prey primarily on mid-water fish at 40–60 m depth in eastern Kerguelen waters (Lea et al., 2002, 2006). A major characteristic of the “gas-bearing” group was the significant amount of scatterers structured in patches during daytime. It is likely that patches corresponded to schools of fish, as already depicted in the Atlantic sector of the Southern Ocean (Fielding et al., 2012; Saunders et al., 2013), and that the species were mainly myctophids with a gas-filled swimbladder (Collins et al., 2008). Patch depth observed during the survey was <180 m, thus suggesting that they were composed of Krefftichthys anderssoni and Protomyctophum spp., and of deeper-living species as E. caribergi (Duhamel et al., 2005; Collins et al., 2008; Flynn and Williams, 2012). Indeed, the survey overlapped the foraging area of the king penguin (Aptenodytes patagonicus) that is known to target primarily K. anderssoni in the 100–150 m depth range during the day (Bost et al., 2002;
Charrassin et al., 2004; Bost and Cherel, unpublished data). Interestingly, patches occurred at different depths during the northern (~150 m) and southern (~70 m) tracks, which can be related to different species within patches or to physical oceanography in different water masses or to a combination of both. The limited information available shows that myctophids are linked to the physical, chemical and biological characteristics of the water column, with bottom depth, temperature and oxygen content of the water being key environmental factors controlling their distributions (Hulley and Lutjeharms, 1995). Moreover, despite patches were detected only during daylight, variations in light levels could also affect the vertical distribution of mesopelagic organisms as it has been observed for deep scattering layers (Klevjer et al., 2016).

In conclusion, the present study highlights the usefulness of combining acoustic records with biological sampling to use reliable bi-frequency algorithms to discriminate groups of backscatters. When validated, the method bypasses the problem of net avoidance by micronekton, especially during the daylight hours (Kloser et al., 2009; Pakhomov and Yamamura, 2010; Kaartvedt et al., 2012). Despite uncertainties with species identification and depth limitation in acoustic data, it provides an essential descriptive baseline of the spatial distribution and structure of micronek-
tonic organisms. More at-sea investigations are needed to better define the species-specific acoustic response of crustaceans (e.g. Madureira et al., 1993b), myctophids (e.g. Gauthier et al., 2014) and gelatinous organisms (e.g. Wiebe et al., 2010). As it stands, however, the method can already help (i) to link micronektonic group distribution to physical oceanography both horizontally and vertically to better define their oceanic habitats (Koubbi et al., 2011), (ii) to investigate predator-prey interactions by combining real time acoustic surveys and bio-logging (Benoit-Bird et al., 2011; Bedford et al., 2015), and hence (iii) to gather useful information on the functioning of the still poorly known oceanic ecosystem. Overall, the distribution of the acoustic groups fit well with the at-sea behaviour of air-breathing diving predators from Kerguelen Islands (see above). More specifically, however, a thorough comparison between net trawling and predator foraging ecology underlines some fundamental mismatches that can be investigated using active acoustic surveys. For example, the subantarctic krill E. vallentini is traditionally considered to live deeper than 100 m during the day (Perissinotto and McQuaid, 1992; Hamame and Antezana, 2010), while it is one of the most important prey items of various diurnal seabirds (e.g. crested penguins) that feed primarily in the top 50 m of the water column (Ridoux, 1988; Tremblay and Cherel, 2003; Sato et al., 2004).

Acknowledgements

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


tonic organisms. More at-sea investigations are needed to better define the species-specific acoustic response of crustaceans (e.g. Madureira et al., 1993b), myctophids (e.g. Gauthier et al., 2014) and gelatinous organisms (e.g. Wiebe et al., 2010). As it stands, however, the method can already help (i) to link micronektonic group distribution to physical oceanography both horizontally and vertically to better define their oceanic habitats (Koubbi et al., 2011), (ii) to investigate predator-prey interactions by combining real time acoustic surveys and bio-logging (Benoit-Bird et al., 2011; Bedford et al., 2015), and hence (iii) to gather useful information on the functioning of the still poorly known oceanic ecosystem. Overall, the distribution of the acoustic groups fit well with the at-sea behaviour of air-breathing diving predators from Kerguelen Islands (see above). More specifically, however, a thorough comparison between net trawling and predator foraging ecology underlines some fundamental mismatches that can be investigated using active acoustic surveys. For example, the subantarctic krill E. vallentini is traditionally considered to live deeper than 100 m during the day (Perissinotto and McQuaid, 1992; Hamame and Antezana, 2010), while it is one of the most important prey items of various diurnal seabirds (e.g. crested penguins) that feed primarily in the top 50 m of the water column (Ridoux, 1988; Tremblay and Cherel, 2003; Sato et al., 2004).

Acknowledgements

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

References


oceanography across 20°-50°S latitudes in the southwestern Indian Ocean.
Deep-Sea Res. II 110, 20–32.