Diel vertical migration into anoxic and high-\(pCO_2\) waters: acoustic and net-based krill observations in the Humboldt Current

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A select group of marine organisms can enter the Oxygen Minimum Zones (OMZs) and even anoxic waters, while performing diel vertical migration (DVM). DVM of the euphausiid *Euphausia eximia* off northern Chile in the spring of 2015 was documented based on acoustic measurements using an echo sounder along with net samplings. Dissolved oxygen (DO) concentrations were obtained using a vertical profiler, and water samples were collected to obtain in situ nitrite (NO\(_2^-\)) concentrations as well as pH\(_T\), total alkalinity (AT), and therefore carbon dioxide partial pressure (\(pCO_2\)) was estimated. Krill were found to migrate up to the surface (0–50 m) during the night and returned to ca. 200–300 m depth during the day, spending between 11 and 14 h at these layers. At the surface, DO and NO\(_2^-\) concentrations were 208 and 0.14 \(\mu\)M respectively, while pH\(_T\) was 8.04 and 405 \(\mu\)atm \(pCO_2\). In contrast, at the deeper layers (200–300 m), DO and NO\(_2^-\) were <3 and 6.3 \(\mu\)M respectively, with pH\(_T\) 7.53 and 1490 \(\mu\)atm \(pCO_2\). The pH\(_T\) and high \(pCO_2\) values at depths represent the conditions predicted for open ocean waters in a worst-case global warming scenario by 2150. The acoustic scatter suggested that >60% of the krill swarms enter the OMZ and anoxic waters during the daytime. These frequent migrations suggest that krill can tolerate such extreme conditions associated with anoxic and high-\(pCO_2\) waters. The inferences drawn from the observation of these migrations might have strong implications for the current oceanic carbon pump models, highlighting the need for understanding the molecular and physiological adaptations allowing these migrations.

Climate change is undeniably causing profound changes on Earth\(^1\). The consequences for life forms are far from being understood, but it is expected that the physiological diversity among species will determine a broad array of responses. Oceans are not the exemption and are likely to suffer vast climate change driven alterations. For example, the ocean acidification (OA) and carbon dioxide partial pressure (\(pCO_2\)) levels, which are known to be associated with Oxygen Minimum Zone (OMZ) systems, are likely to intensify for open-ocean and coastal waters under the future scenarios of increasing atmospheric carbon dioxide release\(^2\). Unique biological forms that live in close association with these OMZs offer a unique opportunity to understand the adaptations required for open ocean inhabitants to overcome such projected future changes.

In the northern Humboldt Current System (NHCS), a few euphausiid species (e.g. *Euphausia macronuta*) have been described as entering to OMZs during their diel vertical migration (DVM)\(^3\). This OMZ (upper boundary of ~45 \(\mu\)M or 1 mL dissolved oxygen L\(^{-1}\)) has become one of the shallowest and most intense OMZs in the world’s oceans\(^4\), with dissolved oxygen (DO) concentrations falling below the sensor-specific detection limits (~3 nmol L\(^{-1}\)) in a significant fraction of the OMZ waters. Such extreme oxygen-deficient waters have recently
been redefined as Anoxic Marine Zones (AMZs)\(^4\). These AMZs have also been identified as water masses containing DO and nitrite (NO\(_2^−\)) concentrations ≤2 and ≥0.5 μM, respectively\(^5,6\).

Organisms entering the OMZs, such as euphausiids, do not only have to cope with low-oxygen water. Shallow and extensive OMZs are also recognized as areas of significant CO\(_2\) outgassing to the atmosphere, which is the key predictor of global warming, and a challenge for marine organisms\(^5,9\). The intrusion of sub-surface OMZs into shelf waters is already resulting in coastal acidification events, with even surface waters becoming corrosive to aragonite, as demonstrated in the eastern boundary systems of California\(^9\) and northern and central Chile\(^11\–13\). The low pH and poor DO of upwelled water can reach coastal areas and readily affect the vertical distribution of marine planktonic organisms such as copepods and euphausiids\(^12,14\).

Despite the apparently harsh conditions in OMZ-affected areas, DVM is still a common behaviour among zooplankton species\(^15,16\), evolved to avoid predation and coordinated by several signals and cues, such as light, food, temperature, and salinity\(^15,16\). Acoustic record is a powerful tool for documenting and assessing this behaviour; enabling the detection of DVM in zooplankton swarms at high resolution, as zooplankton, euphausiids in particular, are known to avoid nets\(^17\). Euphausiids, like any acoustic scattering group, can be distinguished from other organisms (e.g. fishes) after proper calibration based on their acoustic properties\(^18,19\). For instance, acoustic observations have revealed that some organisms such as fish lack the ability to enter OMZs\(^20\).

Observations on zooplankton moving into and staying in AMZs during daylight hours are scarce and poorly documented, with most of the experiments conducted under controlled or simulated laboratory conditions\(^19,21,22\). Therefore, the primary aim of this study was to characterise the low DO/pH and high-pCO\(_2\) conditions within the AMZ in the upwelling zone off northern Chile, while reporting euphausiid migration into AMZs, using acoustic records complemented and validated with net sampling and hydrographic and chemical profilers.

**Results**

**Environmental setting.** Sampling stations were located close to the coast over areas of abrupt bathymetric changes (Stn L3, seabed ca. 1000 m depth; Fig. 1A). The oceanographic conditions in the water column changed with latitude, with solar radiation causing a slight warming at the sea surface in the northern region of the study area and colder waters in the southern region during the cruise (austral spring) (Fig. 1B). Satellite-derived surface chlorophyll-a (chl-a) concentration was higher in the southern region, most likely as a response to ongoing upwelling events (Fig. 1C).

The continuous north–south measurements of vertical hydrographic conditions confirmed the presence of warmer waters in the northern region, whereas in the southern region cold waters almost reached the surface at 29°S latitude (Fig. 2A). Salinity was also higher in the northern region between the surface and 50 m depth, whereas in the southern region cold waters almost reached the surface. Satellite-derived surface chlorophyll-a (chl-a) concentration was higher in the southern region, most likely as a response to ongoing upwelling events (Fig. 1C).

The continuous north–south measurements of vertical hydrographic conditions confirmed the presence of warmer waters in the northern region, whereas in the southern region cold waters almost reached the surface at 29°S latitude (Fig. 2A). Salinity was also higher in the northern region between the surface and 50 m depth, although lower salinity values were observed around 29°S, associated with freshwater inputs from the Quebrada Los Choros River (Fig. 2B). Seawater density was driven mainly by temperature, showing similar features (Fig. 2C). DO values allowed for distinguishing the OMZ down to 50 m depth, with less than 1 mL DO L\(^−1\) in the entire water column (Fig. 2D). The NO\(_2^−\) accumulation (>0.5 μM) indicated that the waters lack oxygen between 150 and 300 m depth and are linked to the distribution of the AMZ, extending along the coast southward and up to ca. 29°S (Fig. 2D).

The vertical chemical profiles at Stn L3 in the surface layers, i.e. 0 m, showed oxygenated (OXY) waters (208 μM DO), low NO\(_2^−\) concentrations (0.14 μM), pH\(_4\), of 8.04, and pCO\(_2\) of 405 µatm. However, a clear chemocline between 50 and 100 m depth showed an abrupt reduction in DO and pH\(_4\), with an increase in pCO\(_2\) levels at depths. Below 100 m and down to 400 m depth, DO was close to zero (<3 μM). These low oxygen waters were also characterised by low pH\(_4\) (7.53) and high pCO\(_2\) (1490 µatm) as compared to the OXY waters, with the highest concentration of NO\(_2^−\) (6.3 μM) at 300 m signalling the core of the AMZ (Fig. 3).

**Euphausiid composition.** *E. eximia* conformed mono-specific layers, with no other krill species observed. Individuals were adults, mainly ovigerous females, as evidenced by observed mature gonads. Standardized abundance at Stn L3 was >50 ind m\(^−3\) in the upper layer (200–0 m) during nighttime, and >13 ind m\(^−3\) in the deeper layer (200–400 m) during the day. *E. eximia* abundance was 3.8 times higher during the night than during the day.

**Vertical migration into anoxic/high-pCO\(_2\) waters.** Algorithms applied to the datasets of 38 and 70 kHz detected high zooplankton abundance in the echograms at both frequencies along the transect. Acoustic records from both 38 and 70 kHz showed active DVM of zooplankton reaching the OMZ and even the AMZ (Figs. 3 and 4). Acoustic records along the coast (23–29°S) denoted a high eco-intensity signal, i.e. volume backscattering strength (S\(_v\), dB re 1 m\(^−1\)) in the deeper layer (mostly at 200–300 m), with an S\(_v\) range from −71 to −65 dB during day, and strong acoustic signals at the surface during night (≤50 m). The echograms obtained on 7 December 2015 at Stn L3 provided a detailed description of DVM patterns of zooplankton (Fig. 3). After sunrise (06:47 h, local time), at 8:30 h the organisms swim down from ~25 m to 300 m at 11:00 h. Integrating vertical acoustic records, the swimming speed of the krill was estimated to be ca. 2.76 cm s\(^−1\) (or 99.28 m h\(^−1\)) during descent. Estimated ascent speeds ranged between 0.69 and 2.06 cm s\(^−1\) (or 24.78 to 73.98 m h\(^−1\)). Averaged echograms showed higher echo intensity signals (S\(_v\) = −71 to −73 dB) and nautical area scattering coefficient (NASC, in units of m\(^2\) n mi\(^−2\)) (100–200 m\(^2\) nmi\(^−1\)) at 175 m depth (Fig. 3).

At Stn L3, NASC values were 1.76 times higher during the day than at night, i.e. 63.7% and 36.3% respectively, indicating the high scatter signal during this time attributed mostly to *E. eximia*, and this was further confirmed by the net catches. During the day, NASC values were higher in the AMZ than in the OXY and OMZ waters, at 61.1%, 31.5%, and 7.4% respectively (Table 1). The NASC values for these layers were statistically different (PERMANOVA test, p < 0.001). The layer considered, i.e. OXY, OMZ and AMZ, was the most significant factor
explaining the differences in NASC values. However, significant interaction between these factors was found when considering the time period along with the oxygen conditions (Table 2).

**Discussion**

Acoustic scatters, confirmed by net samplings, provided clear evidence that *E. eximia* migrate to surface layers (< 50 m) at night, concentrating high abundance and biomass near the surface. The net-based night/day abundance ratio obtained was comparable to the day/night scatter ratio estimated to be 2.7 considering the AMZ layer. These acoustic observations cannot be attributed exclusively to *E. eximia* DVM. Certainly, other migrant organisms could contribute to the observed patterns; however, net catches confirmed that *E. eximia* was the dominant species in both night and day conditions. Therefore, it is plausible to assume that *E. eximia* represented mostly the DVM observed in echograms (see “Methods” section for details on acoustic processing). During the
day, *E. eximia* did not avoid the depth range where anoxia and high pCO₂ prevail. This special physical–chemical condition has been previously reported in other ecosystems, such as Puget Sound estuary (WA, USA), where it was found that the larval development of another krill species, *E. pacifica*, was strongly reduced at pH 7.69. Several studies have attempted to understand the effects of OA on krill species in different regions, mainly based on laboratory conditions. Some findings have predicted severe detrimental effects on embryonic development, larvae and post-larvae growth, reproduction, fitness, behaviour, metabolism, survival and inter-moult period21,30–32,62, while others suggest the capacity of these organisms to tolerate high CO₂ waters33,34. However, all these studies have only manipulated CO₂ levels under normoxic (air-saturated) conditions and, therefore, without truly representing natural OMZ and AMZ conditions. It has been suggested that the efficiency of the aerobic respiration is dependent on the ratio pO₂/pCO₂63, pointing out that the effects of low DO in some oceanic areas could be exacerbated by the increase in CO₂. The physiological basis for the arguments by Brewer and Peltzer63 comes from the fact that haemoglobin has an optimal pH for carrying oxygen (O₂)35. Further, Mayol et al.36 demonstrated that high pCO₂ further challenges respiration in the NHCS. However, recent evidence in fish shows that they are better adapted to tolerate hypoxia under elevated CO₂ conditions as compared to hypoxia alone64. Certainly, the effects of pCO₂ and pO₂ cannot be considered independently, as they co-occur in nature and are both involved in respiratory processes. Although there is no consensus on mechanisms nor on the interactive effects of simultaneous changes of O₂ and CO₂ on animal metabolism, it is clear that *E. eximia* has adapted to survive up to 14 h in these conditions.

When integrating information from Figs. 3 and 4, it became clear that the *E. eximia* individuals were capable of crossing the OMZ and reaching the AMZ where anoxic/high-pCO₂ waters prevailed. They tolerated this condition for at least 11–14 h during their DVM activity. Some species of zooplankton, such as *E. eximia*, are clearly adapted to inhabit OMZs during the day3,22,29. For instance, Tremblay et al.70 suggested that some Humboldt krill species are able to depress their metabolism based on a regulation index. Our observations indicated that *E. eximia* can tolerate the AMZ in the NHCS for extended periods. During daytime, krill should have little aerobic scope in the OMZ and AMZ; since environmental DO partial pressures (Pcrit) (i.e. pO₂ < Pcrit) to sustain aerobic metabolism. Therefore, our observations suggest that *E. eximia*’s metabolism is suppressed or maintained at its minimum, perhaps fuelled by anaerobic pathways. In their study, Seibel et al.22 found that *E. eximia* exhibited metabolic suppression during DMV, tolerating a pO₂ of 0.8 kPa (or 15 µM DO at 10 °C) for at least 12 h. Our observations and estimations agree with this previous report for *E. eximia*, although the acoustic records and NO₂⁻ measurements at Stn L3 showed that this species can even stay between 11–14 h in anoxic waters. This confirms the ability of *E. eximia* to suppress their metabolism, which might be more advantageous in the long term, than an enhanced anaerobic metabolism, which might still play a crucial role in short term.

**Figure 2.** Hovmöller diagrams of water column variability recorded along longitudinal transect between December 5 and 9, 2015. CTD profiles showing temperature (°C), salinity, density (kg m⁻³) and dissolved oxygen (mL DO L⁻¹), from 0 to 400 m depth along a transect from 21°30’S to 29°S. Black upper arrows indicate locations of oceanographic stations. Figure 2D: The red dotted horizontal line represents the OMZ upper limit (1 mL DO L⁻¹), whereas the white isolines indicate NO₂⁻ concentrations from discrete profiles taken along transect. Stn L3 indicates the location where hydrographic, nutrient and carbonate system profiles were selected. Plots were created using Surfer software (version 16.6.484, https://www.goldensoftware.com/products/surfer).
From the DO vertical profile, a $pO_2$ of 0.23 kPa (or $\sim$3 µM DO at 11.7 °C) was estimated at the AMZ core (300 m). This $pO_2$ value could be even lower or 0, according to NO$_2^-$ measurements (as anoxia proxy), which would make this krill species the first to be reported to temporarily enter such extreme natural conditions. Kiko et al. previously reported that *E. mucronata* survived several hours in anoxia at 13 °C under laboratory conditions, with a $P_{crit}$ of 0.6 kPa at 13 °C. Oxidative stress indicators have been proposed to explain, in part, the ability of *E. eximia* to live in the OMZ. This species has shown an elevated superoxide radical production, but very low lipid peroxidation. These authors suggested that *E. eximia* antioxidant enzyme activity was elevated enough to avoid oxidative damage during highly contrasting oxygen conditions during the species’ DVM.

Other respiratory proxies, such as the electron transport system (ETS) or the electron transport system (ETS), have been reported to be significantly higher in the OMZ during the day than at night in the *E. distinguenda* in the Eastern Tropical Pacific off Mexico, supporting evidence for metabolic suppression. This enhanced enzymatic activity during the day would coincide with the DVM in the OMZ. These authors suggested that higher enzyme activity would allow the organisms to tolerate environments with low oxygen in deep waters.

The DVM speeds estimated from the acoustic scatter were comparable to those in other studies with euphausiids using a similar methodology. By assuming *E. eximia* to represent mostly the DVM observed in echograms, the swimming speeds estimated for *E. eximia* ranged from 24.78 to 99.28 m h$^{-1}$, in agreement with previous reports for *E. mucronata* (60–234 m h$^{-1}$). Using the relationship between swimming speed and oxygen consumption rate proposed by Torres and Childress for *E. pacifica*, we have calculated that *E. eximia* might need to consume between 0.065 to 0.098 µM O$_2$ mg DW$^{-1}$ h$^{-1}$ during its DVM within AMZ and OMZ.

Other krill species co-inhabiting the central and NHCS, such as *E. mucronata*, have been shown to contribute significantly to the carbon fluxes by migrating into the OMZ, especially through the export of carbon contained in faecal pellets. Many studies have reported that euphausiids feeding on a diatom-rich diet at the base of the oxycline may have a significant role in carbon export flux. Acoustics-based observations should be treated...
Figure 4. The relative backscatter measured by echo-sounder during the LowpHox cruise at 38 kHz (upper panel) and 70 kHz (lower panel) between 7 and 9th of December 2015 (23.5°S to 31.5°S). Horizontal red segmented and dotted purple lines indicate the Oxygen Minimum Zone upper border (defined here by 1 mL DO L⁻¹ or ~40 µM O₂) and the Anoxic Marine Zone upper border (defined here by a value of NO₂⁻ > 0.5 µM, Thamdrup et al.⁶, Ulloa et al.⁵). Arrows indicate DVM patterns. Horizontal bars indicate nighttime (black) and daytime (yellow), respectively. Sunrise and sunset were 06:47 h and 20:23 h, respectively, for sampling date and location. Echograms were produced using Echoview software (version 9.1, https://www.echoview.com).

Table 1. Relative krill abundance as Nautical Area Scatter Coefficient (NASC m² nmi⁻¹) in relation to time and oxygen condition at Stn L3 during Lowphox cruise, December 7, 2015. OXY, oxygenated layer (> 1 mL O₂ L⁻¹); OMZ, oxygen minimum zone (< 1 mL DO L⁻¹); and AMZ, anoxic marine zone (> 0.5 µM NO₂⁻). LQ and UQ indicate lower and upper quartiles, respectively. Number in parenthesis = n.

| Time     | Oxygen condition | Median | LQ–UQ | Sum    | %     |
|----------|------------------|--------|-------|--------|-------|
| Daytime  | OXY (12)         | 75.1   | 54.3–162.0 | 1175.0 | 31.5  |
|          | OMZ (12)         | 9.7    | 3.9–21.0    | 275.9  | 7.4   |
|          | AMZ (60)         | 7.3    | 1.1–76.5    | 2281.4 | 61.1  |
| Nighttime| OXY (9)          | 138.6  | 71.2–151.8  | 1093.2 | 51.4  |
|          | OMZ (9)          | 7.9    | 4.5–28.5    | 200.3  | 9.4   |
|          | AMZ (43)         | 0.6    | 0.1–9.3     | 831.5  | 39.1  |
acoustic confirmation of the results of previous net-based studies showing that krill migrate to a particular depth. Immediately transferred to a 25 mL thermoregulated cell at 25 ± 0.1 °C for standardisation, and pH was measured with a high-resolution Auto Analyzer (SEAL Analytical, model AA3HR).

Furthermore, a large proportion of krill swarms (> 60% NASC) stay in the AMZ, which has strong implications for carbon flux and further modelling. Krill migrating into AMZ are suitable sentinels of the effects of the ongoing climate change in this “natural lab”, as this ecosystem is characterized by extreme pO2/pCO2 conditions. Further studies will be required to better understand the physiological and genetic plasticity as well as adaptability of these krill species, which allow them to survive such extreme environmental conditions. Future experiments should consider environmentally relevant exposure times, i.e. when organisms stay under extreme environmental conditions, rather than unrealistic exposure times defined a priori. The study of the reaction norms, as described as the phenotypic expression of a single genotype across a range of environmental conditions (e.g. temperature, pO2, and/or pCO2) might be a useful experimental tool for addressing such scientific questions.

Although limited in both spatial and temporal terms, our observations during the cruise, showed that euphausiid species inhabiting the NHCS, such as E. eximia, migrate into anoxic and high-pCO2 waters. These water masses already have pH and pCO2 values that are predicted for open ocean waters in the future (for the year ~ 2150–2200) and are based on the trajectories of atmospheric CO2 release by anthropogenic activities. OMZs have expanded over the past 50 years, and this expansion is predicted to continue as the climate becomes warmer worldwide. During our research cruise, DO concentrations were close to zero within the OMZ core, where even high NO2− concentrations established an anoxic condition. The vertical extension of the zooplankton epipelagic community is usually limited by the presence of the OMZ, but our findings not only offer acoustic confirmation of the results of previous net-based studies showing that krill migrate to a particular depth. E. eximia can tolerate sub-critical oxygen levels for many hours and, but also, can temporally tolerate naturally anoxic and high-pCO2 conditions. Furthermore, a large proportion of krill swarms (> 60% NASC) stayed in the AMZ, which has strong implications for carbon flux and further modelling.

### Methods

#### Physical–chemical measurements.

Samples were collected during the LowpHox I expedition to the OMZ off northern Chile, which was conducted in December 2015, on board the R/V Cabo de Hornos. Hydrographic variables were continuously measured at stations along the transect using a Pump Profiler System (PPS). This equipment pumps water directly from the desired depth (at resolution 1 m) while profiling the water column with an attached conductivity-temperature-depth (CTD) system (Seabird SBE-25) that provides continuous determination of salinity, temperature, and depth. DO was measured by an oxygen sensor (Seabird model 43) attached to CTD. Filtered seawater was collected for nutrient analysis in plastic flasks (30 mL) and was analysed on board for NO2−. Nutrient concentrations were obtained by automated colorimetric methods using a high-resolution Auto Analyzer (SEAL Analytical, model AA3HR).

The pH samples were collected (in triplicate for each station and depth stratum) in 50 mL syringes and immediately transferred to a 25 mL thermoregulated cell at 25 ± 0.1 °C for standardisation, and pH was measured in a Metrohm pH meter equipped with a combined, double-junction, glass Ag/AgCl electrode (Metrohm model 6.0258.600). The electrode was calibrated with 8.089 Tris buffer solution at 25 °C (material supplied by Andrew Dickson, Scripps Institution of Oceanography, San Diego, USA). The pH values were reported on the total scale (pH7). Samples (500 mL) for alkalinity (A7) were poisoned with 50 µL of saturated HgCl2 solution, stored in borosilicate bottles, and kept in darkness at room temperature. A7 was determined by the open-cell titration method using an automatic Alkalinity Titrator Model AS-ALK2 Apollo SciTech. All the samples were analysed on board for NO2−. Nutrient concentrations were obtained by automated colorimetric methods using a high-resolution Auto Analyzer (SEAL Analytical, model AA3HR).

### Table 2. PERMANOVA test applied to the relative krill abundance as Nautical Area Scatter Coefficient (NASC m2 nmi−1) in relation to time and oxygen conditions at Stn L3 during LowpHox cruise, December 7, 2015. OXY, oxygenated layer (> 1 mL DO L−1); OMZ, oxygen minimum zone (< 1 mL DO L−1); and AMZ, anoxic marine zone (> 0.5 µM NO2−).

| Source           | df  | SS    | MS    | Pseudo-F | P(perm) |
|------------------|-----|-------|-------|----------|---------|
| Condition        | 1   | 228.1 | 228.1 | 0.966    | 0.326 ns|
| Layer            | 2   | 11,740| 5689.9| 24.853   | 0.001** |
| Condition × Layer| 2   | 1984.7| 992.33| 4.2015   | 0.013*  |
| Residuals        | 139 | 32,830| 236.19|          |         |
| Total            | 144 | 48,674|       |          |         |

| Condition Groups | T | P(perm) |
|------------------|---|---------|
| Daytime          |   |         |
| OXY, OMZ         | 4.5662 | < 0.001** |
| OXY, AMZ         | 3.6749 | < 0.001** |
| OMZ, AMZ         | 0.3608 | 0.758 ns |
| Nighttime        |   |         |
| OXY, OMZ         | 5.4154 | < 0.001** |
| OXY, AMZ         | 5.5323 | < 0.001** |
| OMZ, AMZ         | 2.6101 | 0.009**  |
| OXY              | Day, Night | 1.2728 | 0.213 ns |
| OMZ              | Day, Night | 0.45326 | 0.692 ns |
| AMZ              | Day, Night | 4.1449 | < 0.001** |
Acoustic analysis. The acoustic information was obtained from the R/V AGS 61 "Cabo de Hornos". This vessel is equipped with the scientific echo sounder model EK60 (Simrad, Kongsberg) with six transducers (Split-beam), previously calibrated at frequencies of 18, 38, 70, 120, 200 and 333 kHz. These transducers were installed on a retractable keel that reaches 9 m below the water surface. The echo-integration system was calibrated following the manufacturer's specifications. The calibration consisted of the insonification of a known target to correct the gain of the transducer for the estimates of target strength (TS) and volumetric backscatter force (Sv) for the echo-integration. The target used corresponded to a copper sphere of known white strength, specific for each frequency, which was located under the vessel in the centre of the acoustic beam and outside the near field of the transducer. The system parameters during the acoustic radiation emission were set according to the CCAMLR standards (Table S1).

Acoustic data were collected during the LowpHox cruise off northern Chile (Fig. 1A). The cruise track was a coastline parallel transect with a bottom depth ranging from 800 to 1200 m. The vessel speed was between 6 and 12 knots. The full dataset covers 991 km of acoustic recording in oceanic waters from 7 December (23.5°S; 70.8°W) to 13 December 2015 (32.1°S; 71.7°W). Frequencies of 38 and 70 kHz were used to detect and separate zooplankton (euphausiids) from other dispersants in the water column. Analyses were carried out with Echoview software (version 9.1). The flow chart for the process of noise reduction is shown in Fig. S1. We excluded data from the surface (5 m depth from the transducer, i.e. “ringdown” effect) and the bottom echo. Data were filtered to remove all noise sources in an efficient and semi-automated way (user-independent), thereby avoiding the altering of acoustic measurements and biases when manually removing noise sources. To identify and compensate for the attenuated pings, the “attenuated signal filter” algorithm described by Ryan et al. was used. Noise generated by interference from other acoustic equipment was removed using the impulse noise removal function. Background noise was removed using the algorithm-based operator formulated by Robertis and Higginbottom. Finally, the number of pings function was used to resample acoustic signals in order to better visualise daily migrations of the zooplankton swarms. The concept of noise must be understood as any acoustic signal, either biological or mechanical, and/or electrical interference not of interest or representing false measurements.

We defined and limited our analysis to an effective sampling range between 5 and 400 m, given the physical limitations from near-field and sound-absorption effects related to the echo-sounder frequencies used (38 and 70 kHz). Absorption is greater for the 70 kHz frequency, which exhibits the shortest range but has a greater vertical resolution than the 38 kHz echo sounder. The 38 kHz frequencies, on the other hand, exhibit a much longer range (> 1000 m) but with limited resolution for small zooplankton scatters. However, both frequencies have proved to be efficient for studying the macro-zooplankton distributions (e.g. euphausiids). Diaz-Astudillo et al. used 75 kHz frequency to study the zooplankton structure and vertical migration, detecting a high abundance of macro-zooplankton groups in the fjord water of Chilean Patagonia. In this work, a maximum threshold was used to detect krill aggregations and bias when manually removing noise sources. To identify and compensate for the attenuated pings, the “attenuated signal filter” algorithm described by Ryan et al. was used. Noise generated by interference from other acoustic equipment was removed using the impulse noise removal function. Background noise was removed using the algorithm-based operator formulated by Robertis and Higginbottom. Finally, the number of pings function was used to resample acoustic signals in order to better visualise daily migrations of the zooplankton swarms. The concept of noise must be understood as any acoustic signal, either biological or mechanical, and/or electrical interference not of interest or representing false measurements.

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PRIMER-e software (version 7.0.13)37. PERMANOVA is a routine for testing the simultaneous response of one or more variables to one or more factors in an analysis of variance based on a resemblance measure. Datasets were averaged for each 38 and 70 kHz paired cell and fourth root transformed before analysis. The Bray–Curtis similarity of NASC values was used as a resemblance measure among sample groups36. Chemical conditions, considered a categorical factor, were set according to the chemical values in the water column: OXY, >1 mL DO L⁻¹; OMZ, <1 mL DO L⁻¹; and AMZ: >0.5 µM NO₃⁻4,6.

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Author contributions

R.R.-B. designed the shipboard experiment. I.P-S. and N.A. analyzed and interpreted the acoustic data. C.A.V. analyzed pH and pCO₂ data. R.R.-B., I.P-S. and N.A. prepared all figures. M.A.U. wrote the physiological implications of the findings. R.R.-B. did statistical analyses. All authors reviewed and wrote the manuscript. All these authors contributed equally to this work.

Competing interests

The authors declare no competing interests.

Additional information

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