**Reviewer #1**

Summary
Perez-Santos et al. revised the manuscript significantly by including more details on data analysis in the Methods and improving figure presentations. However, the manuscript still lacks significance of the study. The authors provided intensive literature reviews in the Introduction, but did not identify a research question in larger context. Various data sets having both spatial and temporal mismatches are presented, which present challenges in comparing interdisciplinary data and interpreting the results. By identifying a clear objective in the Introduction, I believe that the authors can identify the appropriate data sets to answer a specific research question. In addition, I still have a major concern on the acoustic data analysis provided. Even though the 38 kHz is not relevant to examine zooplankton (as stated in the first review), some of the results are still based on 38-kHz data sets alone. This manuscript is not yet ready for publication.

**General comments**
Introduction heavily focuses on literature reviews on physical processes and hypoxia effects on zooplankton, but fails to identify the significance of this study. It is important to identify the goal of the study, which will help identifying data sets needed to answer the question.

- The introduction section was significantly improved. New references, two scientific questions and hypotheses were added.

Too many data sets collected at different sites, time, equipment, and sampling strategies were analyzed in this manuscript. These temporal and spatial mismatches combined with different sampling strategies make it difficult to compare between data sets and fail to identify seasonal and inter-annual variability. To compare the effects of environmental properties on zooplankton distributions, it is critical to analyze the biological and physical data which were collected simultaneously.
- We organized the data collection section and table 1 was updated. It now shows only the information belonging to each field campaign that was presented throughout the text.

I still have major concerns on the analysis of echosounder data to extract zooplankton backscatter. As I stated in the first review, zooplankton species found in their net samples (e.g., copepods, euphausiids) cannot be detected at 38 kHz, because they are too small to be significant backscatterers compared to the wavelength of 38 kHz. Yet, Fig. 5 uses only 38 kHz data to isolate zooplankton which is impossible: high backscatter shown as zooplankton (Fig. 5a, d) are likely fish. There is no description in the Methods about how the authors separated zooplankton vs. fish when only 38 kHz data were collected (e.g., January 2014). Low correlations between Sv and zooplankton taxa can be explained by the use of 38 kHz to detect them (lines 403-407).

We understand and share most reviewer concerns. It was very unfortunate we did not have the chance to conduct dual-frequency surveys at both campaigns. Given this constrain and the lack of additional sampling gears suitable for sampling fish or other targets at different strata, we must acknowledge that we do not have the data/methodology to claim an accurate discrimination between fish and macrozooplankton for single-frequency data. Doing that in the original manuscript was motivated by the low intensity and scattered pattern of fish echoes observed later in the dual-frequency survey and elsewhere in the study area. We also agree that small zooplankton was surely under-represented in the 38 kHz echograms.

After all previous considerations, we have re-analyzed the first (single-frequency) campaign data by pooling all filtered scattering >110 dB (free from noise and bad data) into a single class, addressed and treated as "biological backscattering". We have, then, used this data to describe spatial/vertical distribution patterns and responses to hypoxia and turbulence for this whole group. We strongly believe this approach provides useful information about overall biological responses to physical forcings, without compromising the accuracy of our results.
Regions outside of the usable acoustic data range (i.e., deeper than 250 m) should be identified in Figs. 6 and 7.

-We added a horizontal red line in Figs. 6 and 7 to denote the outside regions of the usable acoustic data.

What animals fall into “blue noise” organisms in your study site?

As was proposed by Ballón et al., (2011) to the blue noise organisms belong: the fish larvae, gelatinous and gas-bearing siphonophores. Our in-situ zooplankton sampling no detected theses species therefore was impossible to correlate the blue noise echograms with the macrozooplankton detected in our study site.

We added some sentence to clarify this information: “Overall the in-situ zooplankton sampling and the echograms showed good agreement with the FL group (Fig. 9a-d). Furthermore, the elevated abundance of macrozooplankton groups (euphausiids and chaetognaths) found between 100-150 m depth during daytime hours (Fig. 9b-f) matched well with acoustic data for the fluid-like group (Fig. 8a), but in the case of BN group the macrozooplankton species were not clearly identified in the in-situ zooplankton sampling”.

How does “Tidal regime” (Section 4.5) related to the goal of this study?

- The tidal analysis was added to the manuscript as a recommendation from Reviewer #3 during the first round of the interactive comments. Semidiurnal tidal regime is the principal physical forcing involve in the high turbulence and hence vertical mixing around Jacaf sill. Therefore, we believe significance this information in the manuscript.

No need to explain data that are not analyzed/presented in the manuscript. Remove lines 199-200, and lines 256-257.

-Removed.

The manuscript needs to be carefully reviewed for typos and grammatical errors. For example;

Line 91: Replace “preys” by “prey”.
- We replaced “preys” by “prey”.

Line 215: Insert space between “Using the values” and “epsilon”.

- We inserted space between “Using the values” and “epsilon”.

Lines 316, 318: Replace “flow meter” by “flowmeter”.

- We replaced “flow meter” by “flowmeter”.

Line 350: Remove comma before “(Fig. 2c)’.

- We removed comma before “(Fig. 2c)”.

We have edited the manuscript carefully and have removed all typos and grammatical errors.

Line 365: When describing times, it should be “17:00 (local time)”, instead of “17 h”. Please correct the description of times throughout the text.

- We changed all time description throughout the text.

Line 378: Insert “m” after “20-50”.

- We added “m” to the text 20-50 m.

Line 403: Insert a space between “mL” and “L^−1”.

- We inserted a space between “mL” and “L^−1”.

Line 425: Insert a space between “the” and “elevated”.

- We inserted a space between “the” and “elevated”.

Line 482: Insert space between a period and “Maximum”.

- We inserted a space between a period and “Maximum”.

Line 1031: Insert space between “35 km” and “on”.

- We inserted a space between “35 km” and “on”.

Line 1043: What do you mean by “contined”?

- We changed contined by continued.
Figs. 6 and 7: Clarify which frequencies are used to present (b) and (c).

- We added the following to the sentence “using the combination of 120-38 kHz frequencies in (a, b, c)”
Reviewer # 2

In their revised version of the manuscript, the authors have reorganised their results and rewritten large parts of the introduction and discussion. Despite these efforts, which one has to acknowledge, the paper remains weak in several respects:

The introduction still does not identify relevant research questions. Three topics are introduced that are related by physical-biological interactions on different spatial scales (turbulence, vertical migration, hypoxic zones), but open scientific questions regarding these or the context of addressing these together in a common study are insufficiently specified. Relevant literature is largely ignored. There is quite some published work related to the distribution of zooplankton in oxygen minimum zones including work using acoustics (to name a few: Maas et al. 2014, Hauss et al. 2016, Seibel et al. 2016). The same applies for the discussion, which has a strong focus on Patagonian fjords, but does not address for instance the recent efforts to understand the effect of expanding minimum zones in the world ocean. Doing so, the study remains of local interest.

-We appreciate the new reference recommended by the reviewer. The principal results from these papers that connected with our manuscript were added to the introduction and discussion sections. Also, two main questions and hypotheses were inserted in the introduction section.

The presentation of the data is still confusing. Although the objectives are primarily addressing the biotic-physical interactions as stated in the introduction, the results do not. Physical data from years in which no zooplankton sampling has been conducted is presented without a relationship to the objectives. The zooplankton data still remains poorly explored, selected data is presented (e.g. a minor proportion of the available vertical profiles) and the presentation does not fit the purpose (although the vertical distribution is highlighted as a major aspect, integrated stocks are presented). Correlations and the data on which these are based are not shown or described in the methods.

-We clarify table 1 and eliminated the hydrographic data that were not connected directly with the experiments. Figure 2 was deleted (Hydrographic data from June 2016) and a new figure 2 was created to show the hydrographic background during the experiments (e.g., May 2013 and January-August 2014). The copepod data were eliminated from the text and more vertical profiles of in-situ zooplankton sampling were added to the new figures 3, 4 and 9. Furthermore, two new figures (Fig. 6 and Fig. 12) now show the results of correlations between Sv and
turbulence data with the in-situ zooplankton samples. The correlation method was clarified in section 3.3.

Finally, the conclusion that zooplankton distribution is affected by the vertical structure in turbulence is supported by little data. Correlations are not shown and also not properly explained in the methods.

Summing up, I find the manuscript not acceptable for publication in Ocean Science. It is repetitive and primarily of local interest.

Minor comments

Title:

The title is misleading as primarily macrozooplankton was analysed from acoustic data.

-We changed the title to “Turbulence and hypoxia contribute to dense biological scattering layers in Patagonian Fjord System”.

Introduction:

1st paragraph: 7 out of 12 citations in the paragraph are missing in the references. I haven’t checked this in the remainder of the manuscript, because the first one in the second paragraph is missing as well... Many citations appear to be related to fjords around Southern America, but more general literature should be cited.

-We thank the reviewer for pointing out this shortcoming. All references were added that were missing. We believe that if the reviewer had been able to see the references there would be more indication that the citations relate to fjords and estuaries found around the world. For example, Basedow et al. (2004) studies advection of zooplankton in Kongsfjorden, Svalbard, Govani et al. (1989) studies larval fishes in the Mississippi River plume, Lough and Manning (2001) discuss frontal entrainment of larvae on Georges Bank, Munk et al. (2002) focus on how stratification affects larvae in the North Sea and finally, Rodriguez et al. (1999) studies how upwelling affects larvae off an African Coast. These references make the findings of this work far-reaching and relatable to many other estuarine and coastal areas found around the globe.
The authors imply that all the effects on zooplankton listed here are referenced by Basedow et al. 2004. I have strong doubts on this. Again, the citation is missing in the references. In addition, it is not correct that effects on larvae imply also general effects on zooplankton community composition, biomass, productivity and the rest.

- The reviewer makes a good point. Basedow et al. (2004) discusses how zooplankton are advected in fjord systems and do not focus on larvae. We have re-worded the sentence to read:

“It has been found that residual flows in fjords can retain planktonic larvae inside the system (Dyer, 1997; North & Houde, 2001, 2004; Meerhoff et al., 2015). Another recent study has shown that advection can determine the import and export of zooplankton in a fjord system depending on the depth at which the zooplanktons are located, which can potentially affect the community composition, biomass, productivity and distribution of zooplankton in the fjord (Basedow et al., 2004).”

Line 96: Why however? How are small scale, short term processes (feeding interactions) related to larger scale processes acting on longer time scales (productivity)? I do not understand what the authors actually mean.

- The main message of this sentence is to relate turbulence to processes that can affect biological activity in estuaries and fjords. Turbulence is known to mix freshwater inflows with deep, dense ocean water and controls estuarine circulation (MacCready et al., 2002; Peters and Bokhorst, 2001). It has also been shown that turbulence affects the dynamics of some biology. For example, turbulent eddy diffusivities in estuaries can impact phytoplankton bloom growth and persistent density stratification has been found to be a necessary condition for phytoplankton blooms to develop in many estuaries, which is controlled by the degree of turbulent mixing (Lucas et al., 1998; Cloern, 1991; Koseff et al. 1993). We have added the following sentence (and associated references) in the manuscript to help articulate our point:

“For example, turbulence is known to mix freshwater inflow with deep, dense ocean water, allowing for oxygenation of the deeper layers (MacCready et al., 2002; Peters and Bokhorst, 2001) and turbulent eddies can impact phytoplankton bloom growth (Cloern, 1991; Koseff et al., 1993).”

Line 98: Change wording. ‘inform our understanding’ is not appropriate.
- We changed the sentence to read “Therefore, additional field studies are needed to confirm the relationship between mixing-inducing physical forcing, such as wind or advection, and vertical abundance patterns and species composition in fjords and other estuarine systems”.

Line 101: How are hypoxia related to turbulence?

- We edited the Introduction section and connected the importance of turbulence with the change of water column properties, especially with the dissolved oxygen variable. Intense turbulence around the sill favors the ventilation of deep fjord water and as a consequence reduces the impact of hypoxia.

Line 107: ‘the sensitivity varies among organisms in different environment’: here, ‘different environment’ needs to be explained.

- We are now explicit and the sentence reads:

“The sensitivity of species to tolerate different oxygen concentrations, however, may vary among organisms from different environments, (e.g., coastal upwelling zone, fjords systems and oxygen minimum zones-OMZ.)

Line 118: This chapter is related to Patagonian fjords. No general questions and hypotheses are identified that would make the study relevant beyond local interests.

- We added two scientific questions in the introduction section.

“**One of the principal questions** that will be address during the present study is:

what is the contribution of turbulence to the mixing of fjord water column properties (e.g., Temperature, salinity and dissolved oxygen) and to the aggregation of macrozooplankton at certain depths (scattering layers) along north Patagonian fjords and channels, emphasizing the role of sills in some locations (e.g., Jacaf Channel, Fig. 1)?
"The second question that motivates this study is: How do hypoxic conditions affect the distribution and aggregation of macrozooplankton species?"

Line 164 following: Delete this introduction sentences and provide clear scientific hypothesis related to the effects of a) micro-turbulence, b) hypoxia) and c) small scale vertical distribution of zooplankton in fjords in general.

-We added two hypotheses to the text: “According to the information presented in this section, the principal hypotheses of this manuscript are: (1) the pervasive hypoxic layer existing in the Puyuhuapi Fjord limits DVM and overall distribution of macrozooplankton to the first 100 m depth of the water column, reducing the habitat of these species and (2) the higher turbulence originated by the tidal regime around sills favor the mixing of the water column, deepen the hypoxic layer, injects nutrients and, thus, increases primary production. Therefore, macrozooplankton exhibits higher densities and extends deeper in the water column around submarine sills.”

**Study area:**

Line 172 following: A very detailed description of the fjord system that can be shortened. Instead, one expects a short justification of the location of the stations chosen for acoustic measurements and net catches and a description why their features are representative.

-We edited the study area section and added a new paragraph to justify the selection of experiment and station locations.

**Data and methodology**

Line 199 following: Why do the authors refer to results already without having presented the methods? When were CTD measurements conducted? On a seasonal basis, during one cruise? Similarly, when were microstructure measurements taken? With each CTD cast? From 1995-2016? This is still very confusing. Particularly, because ADCP measurements and net catches were temporarily restricted to a few observation periods. This contrasts strongly with the goals of the study which relate primarily to the interaction between physics and biology.
- We clarify the hydrographic information in the text and added the CTD data collected during the field campaigns. Table 1 was updated and we changed figure 2. The new figure 2 shows only the hydrographic profiles from the field campaigns discussed in this study.

Section 3.1 was modified “Hydrographic surveys were conducted during May and November 2013 and January and August 2014 in Puyuhuapi Fjord and Jacaf Channel (Fig. 2, Table 1).”

The section 4.1 Hydrographic features now includes a description of the new figure 2 “

Temperature profiles collected in Puyuhuapi Fjord and Jacaf Channel showed similar structure during the winter and summer campaigns (Fig. 2, a-b). The largest temperature gradients were found between the surface and ~70 m depth, ranging from 8.5°C to 17°C. A thin, fresh layer (salinity values varied from 11 to 29 g kg⁻¹) was found in the first ~10 m of the water column below which salinity varied little (29 to ~34.2 g kg⁻¹), as result of the presence of Modified Sub-Antarctic water (MSAAW, salinity between 31 and 33 g kg⁻¹), the Sub-Antarctic Water (SAAW, salinity between 33 and 33.8 g kg⁻¹) and the Equatorial Subsurface Water (ESSW, salinity>33.8 g kg⁻¹) (Fig. 2, c-d). Hypoxic conditions (dissolved oxygen below 2 mL L⁻¹ and ~30 % saturation) were detected in Puyuhuapi Fjord below 100 m depth, with oxygen concentration between 1-2 mL L⁻¹ (Fig. 2e). Deep water in Jacaf Channel was more ventilated, with dissolved oxygen values above hypoxic conditions throughout the water column (Fig. 2f). The hypoxic layer was located over the depth range of the Equatorial Subsurface Water (ESSW) and oxygen rich water (3-6 mL L⁻¹) was observed at depths occupied by MSAAW and SAAW. Below 10 m depth, high nitrate concentrations were measured in Puyuhuapi Fjord, but concentrations in the winter (August 2014) were higher than in fall (May 2013) and summer (January 2014) (Fig. 2 g). Along with the in-situ hydrographic sampling, in-situ zooplankton samples were collected and will now be discussed.”

Line 237: 300 kHz are given in Table 1. The provision of the table is good for an overview, but reveals that there was no clear strategy in this study to resolve the effects of the interaction of physical structures
with the distribution of organisms. For instance, during the investigation in 2016 in Puyuhapi Fjord and in 2012-2013 in Jacaf Channel no zooplankton samples were taken. This is not consistent with a study that primarily aims to study physical-biological interactions. Communities change with time/area, and a few time points for ‘validation’ are not sufficient.

- We clarify the information of ADCP frequency in table 1 according to the text. The information of 2016 experiment was deleted and only the hydrographic data obtained during the different investigations were added to the table 1 and also to the new figure 2. The data from 2012 and 2013 was used to characterize tidal regime and turbulence conditions as part of other independent research. The turbulence data collected during November 2013 in Puyuhuapi Fjord and Jacaf Channel showed for the first time the high turbulence around the Jacaf sill compare with the interior of Puyuhuapi Fjord. Both datasets were used as a motivation to incorporate the biological measurements and to start the biophysical experiments. Chilean Patagonian is an extreme environment; even we planed collected all the biophysical datasets, some time instruments failed.

Line 288: Table 1 contains information about additional measurements in 2012/13 that are not described here.

- All irrelevant data were removed from Table 1. Perhaps the concern of the reviewer is because the measurements in the period of 2012/2013 did not included acoustic data, which is why they are not discussed in this section.

Line 276-287: The authors are inconsistent here: macrozooplankton is by definition > 2 mm. Why do the authors refer to small copepods here, which contrast with the applicability of the algorithm?

- We deleted “small copepods” from the text.

Line 304: Brierley et al. 2006 is missing in the references.

- We changed the reference Brierle et al., 2004 (error) by Brierley et al., (2006).


Line 322: How long did the sampling last? Making 5 separate net
catches over 150 m is time consuming. What was the hauling speed for both nets?

- We clarify this sentence. The new text reads, “Stratified Tucker tows considered four depth strata: 0-10 m, 10-20 m, 20-50 m, 50-100 m in Puyuhuapi Fjord. In the Jacaf Channel the stratified sampling included five depth strata: 0-10 m, 10-20 m, 20-50 m, 50-100 m and 100-150 m. The hauling speed for both nets was between 2-3 knots”.

Line 327: Please correct: < 1 mm doesn’t represent a 5 mm threshold.

-We eliminated this sentence: “Copepods, the most abundant group, were further classified into <1 mm, 1-5 mm and >5 mm prosome length groups”.

Results:

Line 346: The study aims to investigate the interactions between physics and biology. Why do the authors present physical data for a period (2016) in which no biological data was collected. I suggest to delete this. In the next chapter backscatter data for 2013-2014 is presented. This does not make sense to me.

-We eliminated the hydrographic data from 2016 and also the figure. The new figure 2 shows the physical data collected during the biophysical experiments as was presented before in the Data and methodology comment section.

Line 356: In the methods it is stated that the algorithm integrates particles sizes >0.5 mm. Here data for only > 5mm is presented. The text states with reference to Fig. 3 that copepods < 5 mm were most abundant. This is not shown. Why are selected data shown and not all, especially when the text refers to ‘data not shown’?

-We eliminated this sentence from the text.

-We clarify this paragraph: “A marked change in vertical distribution and in total abundance of the zooplankton groups in the water column was observed from the first sampling hour to the first night sampling time (~18 h), revealing the start of the nocturnal migration to the surface (Fig. 3c) coincident with a DVM pattern as seen in the ADCP-1 backscatter data (Fig. 3a-b)”.

Line 368: This also contrasts with Fig. 4 in which data < 5mm is shown out of the sudden. Analysis of the backscatter requires that the vertical
resolution is shown and not depth integrated data. The methods state 5 depths for sampling, only 3 are shown. Why?

-We eliminated the Copepod data and subplot from figure 4.

-As we mentioned in the section 3.3 Zooplankton sampling.....

“Stratified Tucker tows considered **four depth strata**: 0-10 m, 10-20 m, 20-50 m, 50-100 m in the Puyuhuapi Fjord”. In the depth strata 0-10 m the macrozooplankton groups were not observed; therefore only three strata are visible in the figure. We added a white horizontal bar to represent the **No Data**. We also added two subplots to show better the DVM pattern of macrozooplankton, enhancing the validation process.

Line 377: This description implies that euphausiids migrate from 50m to the surface which is certainly not the case.

-We clarify this sentence: “However, euphausiids showed the clearest diel vertical migration with maximum abundance between 10-20 m depth during night hours, and at ~100 m during the daytime (Fig. 4c-f)”.

Line 384: Fig 3 shows results for the top 50m only, so results cannot match. Furthermore, Fig 4 shows that the surface 80 m were devoid of large zooplankton during daytime. This looks different in Fig 5. Is the NASC for day and night-time significantly different?

-We eliminated the reference of Fig.3 “Most of the backscatter was concentrated in the first 100 m of the water column, matching ADCP-2 results (Fig. 4)”.
-We clarify the sentence: “At the ADCP-2 mooring location (black dot in Fig. 5a), higher backscatter signals were found close to ~100 m depth, showing good agreement between echo-sounder and ADCP-2 data (Fig. 4a and 5a)”.

- The NASC magnitudes were very similar, but the maximum values showed a different position in the water column during day and night-time, evidencing also de DVM observed with ADCP data. Two sentences describe this behavior in the text:

“Average NASC profiles for day-zooplankton showed higher values around 80 m (above the hypoxic layer), with NASC range from 3-3.5 m² n m⁻² (Fig. 5b)”.

“NASC data also show an increase in night-zooplankton abundance (~40 m) but the position of the maximum values was more close to the
surface than the NASC observed during the day-zooplankton sampling (Fig. 5e)“.

Line 404/428: the correlations should be shown? On how many observations are these based?

-We added two new figures (Fig. 6 and Fig. 12) to show the results from the correlation analysis between the $S_v$ values from the 38 kHz acoustic frequency and the in-situ zooplankton sampling. The analysis was carried out using $n=44$ observations during the day and nighttime fixed station in Puyuhuapi Fjord and $n=25$ in Jacaf Channel.

Line 474: This is unclear to me: was this a positive correlation? ‘also’ in the next sentence implies this.

-We deleted this word from the text.

Line 476: The correlation analyses are not described in the methods. Since little data on zooplankton vertical distribution is shown, one wonders on how much data this correlations are based.

- A paragraph was included in the section 3.3 that describes the correlation analyses: “To determine the correlation ($R^2$) between the $S_v$ records from the 38 kHz transducer and the major macrozooplankton groups (Siphonophores, Chaetognaths and Euphausiids), a quadratic polynomial curve was applied between these data sets ($n=44$ for the sampling in Puyuhuapi Fjord and $n=25$ in Jacaf Channel), (further details in section 4.3)”.

Discussion:

Line 494: The ‘clear’ increment of zooplankton related to increased turbulence is not clearly sown. This refers to correlations for which little information on procedures (statistics, analysis) and data is presented (vertical distribution of zooplankton) and needs to be improved.

-The semidiurnal tidal regimen was the principal physical forcing that contributes to the high turbulence measured around Jacaf sill, as was also found by Klymark and Gregg, (2003). Turbulence data collected with VMP-250 microstructure profiler in the same campaign (November 2013) along and across Puyuhuapi Fjord and in Jacaf Channel showed high dissipation rates of turbulent kinetic energy along the Jacaf sill
region. During the biological-physical experiment in August 2014 the VMP-250 presented a technical problem, therefore a Self Contained Automatic Microstructure Profiler (SCAMP) was used. This type of turbulence profiler is not recommended to measure in channels with strong tidal currents such as Jacaf. The turbulence measurements obtained in Jacaf Channel were found to be high compared with turbulence data collected in Puyuhuapi fjord, where there was no sill present. We explored correlations between the $S_v$ data and turbulence measurements collected with the SCAMP, however during the first round of revisions it was recommended by Reviewer #3 to eliminate the SCAMP data from the manuscript. So we did. Moreover, the semidiurnal tide occurs year round in Jacaf Channel, so we can confidently say that high turbulence measured directly with the VMP-250 during November 2013 occurs daily. Further, these results imply that the elevated turbulence injects nutrients to the surface layer, producing an increase in the primary production and hence favors the aggregation and development of high biomass abundance in Jacaf Channel. Therefore, we believe it is very important to show the turbulence data in this study. The information presented in Fig. 11 and 13 are also the first report of turbulence data using shear probe in Patagonian fjords and channels.

The discussion still suffers from a very narrow representation of results obtained in other regions of the world (particularly DVM and zooplankton relationship to hypoxia).

-We have improved the discussion section adding new references about studies conducted around the world where DVM occurred. We also incorporated evidence of the relationship between zooplankton and hypoxic conditions, especially in the OMZ.

Line 498: The study actually shows very little data related to DVM-patterns, several vertical profiles with nets have been obtained. Little of this data is shown.

-We incorporated more vertical profiles from the in-situ zooplankton sampling. The information was added to the new Fig. 3 (May 2013), Fig. 4 (January 2014) and Fig. 9 (August 2014). The new data confirm the DVM-patterns detected with the different acoustic techniques (ADCPs, scientific echo-sounders).

Line 532: This is incorrect. No zooplankton sampling has been conducted in hypoxic conditions, which could give some ideas which organisms actually migrated into hypoxic water (see Fig 9).
The sentence was clarified, “In the current study, acoustic measurements were carried out in hypoxic conditions, revealing that most backscatterers (Sv data) remained above the hypoxic boundary layer (Fig. 5 and Fig. 10).”

Line 536: What about major studies at sites of oxygen minimum zones in the ocean? There are more studies to mention here in the relationship of organisms to oxygen than Ekau et al. (2010). I also think, to make statements for zooplankton in general are not appropriate considering that macrozooplankton data is reported.

We added valuable information regarding zooplankton behavior in different OMZ regions around the world. The zooplankton term was changed to macrozooplankton.

Line 594: Actually, very little data is provided (vertical distribution patterns of zooplankton) to support the statement of ‘evident’ aggregation and to support the conceptual model concluding that vertical mixing influences the vertical distribution of zooplankton and fish. I suggest to omit lines 594-627, because very little own data is presented on this.

As we mentioned before more vertical profiles from the in-situ zooplankton sampling were added to the manuscript to confirm the DVM-patterns detected with the different acoustic techniques (ADCPs, scientific echo-sounders). Lines 603-616 were eliminated from the text, but others remained unchanged due to the significance of this information to the discussion section.

Line 625: Theses statements certainly need some references.

We added some references at the end of the paragraph, “Turbulent mixing also increases primary and secondary production, through enhanced nutrient availability and favors encounters of macrozooplankton with potential prey, increasing growth and survival rates (Visser and Stips 2002; MacCready et al., 2002; Klymak and Gregg 2004; Lee et al., 2005; Visser et al., 2009; Whitney et al., 2014).”
Reviewer #3

This paper has improved significantly compared to the first version and I suggest a publication with minor revisions.

The only thing I am missing in the discussion (and that may be due to an incomplete knowledge about the scientific literature in Fjords): The reason why at sills a higher abundance can be found is not clear/convincing. Can it be that enhanced mixing of e.g. nutrient gradients between the deeper water and the surface water result in an increase of food for the zooplankton which in turn increases zooplankton abundance? If so Figure 12 would have another arrows indicating enhanced mixing of nutrient gradients.

-We added arrows in figure 12, indicating the high concentrations of nitrate and phosphate reported in the subsurface layer due to the oceanic water intrusion. This also showed the low concentrations of nitrate and phosphate in the surface layer due to phytoplankton consumption.

So the question is: Are there nutrients gradients between the hypoxic deep water and the oxic surface water?

- Yes, there are significant nutrient gradients between the hypoxic deep water and the oxygenated surface water. As was mentioned in Section 2, “The principal nutrient (nitrate) is supplied to the fjords by oceanic transport, and particularly through the intrusion of Sub Antarctic water (SAAW), a water mass that may also transport some species of zooplankton (González et al., 2011)“. Furthermore, others manuscripts with study areas in Patagonian fjords showed nutrient gradients between surface and subsurface layers (González et al., 2013; Silva and Vargas 2014; Montero et al., 2017).

Minor remarks (I didn't include minor typos like missing spaces, there are a few of them):

38: kown to date -> known to date
We changed kown by known.

39 condition -> conditions

We changed condition by conditions

75-76: How does horizontal mixing between densities work? Explain better. Densities are stratified in the vertical

-This was poorly worded and has been revised. The sentence now reads:

“Moreover, horizontal mixing of along-channel density gradients has been shown to induce lateral circulation...”

129: ichthyo-plankton in line 79 it is called ichthyoplankton

-We changed ichthyo-plankton by ichthyoplankton

144: severalmonths -> several months

-We changed severalmonths by several months.

185: shallow sill -> add water depth

-We added the sill depth to the sentence “Jacaf Channel is known for its extreme depth (> 400 m at the mouth) and a shallow sill (~50 m depth) located near its head (Fig. 1)”

219 ofε, -> space

-We changed ofε to of ε.

232: measurement -> measurements

-We changed measurement to measurements.

256: ADCP -> ADCP-1?

-We added a new sentence to clarify this information, “During this experiment one RDI Workhorse ADCP with 614.4 kHz frequency (referenced as ADCP-3) was installed at ~30 m depth in the vicinity of the Jacaf sill.”

357: dB re 1 m -1 -> unit
- We added the units to all $S_v$ values (dB re 1 m$^{-1}$) as was recommended by reviewers during the first revision process.

452: the elevated
- We changed the elevated to the elevated

check Bibliography e.g.:
Haren H. and Compton T.J. -> van Haren H.

Turbulence and hypoxia contribute to dense biological zooplankton scattering layers in Patagonian Fjord System.

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Abstract

The aggregation of plankton species along fjords can be linked to physical properties and processes such as stratification, turbulence and oxygen concentration. The goal of this study is to determine how water column properties and turbulent mixing affect the horizontal and vertical distributions of macrozooplankton along the only north Patagonian Fjord known to date where hypoxic conditions occur in the water column. Acoustic Doppler Current Profiler moorings, scientific echo-sounder transects, and in-situ plankton abundance measurements were used to study macrozooplankton assemblages and migration patterns along Puyuhuapi Fjord and Jacaf Channel in Chilean Patagonia. The dissipation of turbulent kinetic energy was quantified through vertical microstructure profiles collected throughout time in areas with high macrozooplankton concentrations. The acoustic records and in-situ macrozooplankton data revealed diel vertical migrations (DVM) of siphonophores, chaetognaths and euphausiids and copepods. In particular, a dense biological backscattering layer of zooplankton was observed along Puyuhuapi Fjord between the surface and the top of the hypoxic boundary layer (~100 m), which limited the vertical distribution of most macrozooplankton and their DVM, generating a significant reduction of habitat. Aggregations of macrozooplankton and fishes were most abundant around a submarine sill in Jacaf Channel. In this location macrozooplankton were distributed throughout the water column (0 to ~200 m), with no evidence of a shallower hypoxic boundary due to the intense mixing near the sill. In particular, turbulence measurements taken near the sill indicated high dissipation rates of turbulent kinetic energy ($\varepsilon \sim 10^{-5} \text{ W kg}^{-1}$) and vertical diapycnal eddy diffusivity ($K_p \sim 10^{-3} \text{ m}^2 \text{s}^{-1}$) values. The elevated vertical mixing ensures that the water column is well oxygenated (3-6 mL L$^{-1}$, 60-80 % saturation), creating a suitable environment for macrozooplankton and fish aggregations. Turbulence induced by tidal flow over the sill apparently enhances the interchange of nutrients, oxygen concentrations, and create a fruitful environment for many marine species, where prey-predator relationship might be favored.

Keywords: turbulence, hypoxia, Acoustic data, macrozooplankton, scientific echo-sounder, Patagonian fjords, sill exchange.
1 Introduction

Spatial and temporal variability of plankton assemblages have been linked to oceanographic features and processes such as water column stratification, tidal mixing and turbulence, frontal structures, advection, and secondary circulation in estuaries and fjords (Govoni et al., 1989; Timothy et al., 1998; Rodríguez et al., 1999; Lee et al., 2005; Lough and Manning, 2001; Munk et al., 2002; Meerhoff et al., 2013; Meerhoff et al., 2015). In fjords, residual flows resemble typical estuarine gravitational circulation with landward flow at depth and seaward flow at the surface. It has been found that residual flows in fjords can retain planktonic larvae inside the system (Dyer, 1997; North and Houde, 2001, 2004; Meerhoff et al., 2015). Another recent study has shown that advection can determine the import and export of zooplankton in a fjord system depending on the depth at which the zooplankton are located, which can potentially affect the community composition, biomass, productivity and distribution of zooplankton in the fjord (Basedow et al., 2004). Moreover, horizontal mixing between interfacial density layers of along-channel density gradients has been shown to induce lateral circulation (Farmer and Feeland, 1983), which in turn affects larval distributions in fjord systems (Meerhoff et al., 2015).

Other recent studies have investigated how tidally induced asymmetries in mixing and therefore tidal variations in stratification affect ichthyoplankton and zooplankton assemblages (Pérez Pérez et al., 1977; Nixon et al. 1979; Oviatt, 1981, Lee et al., 2005). Lee et al., (2005) found that variations in stratification throughout a tidal cycle affected both overall abundance and species composition of zooplankton in the Irish Sea. However, they did not have the tools to relate the hydrodynamic and hydrographic variability of this region to vertical and horizontal distributions of fish larvae and zooplankton. Another study by Oviatt (1981) found that zooplankton concentrations were lower in laboratory tanks than in nature (Narragansett Bay, USA). Since this was not due to the physical action of mixing (induced by paddles in the tank), they hypothesized that tank confinement and turbulence had broken down vertical segregation between adults and juveniles, resulting in increased cannibalism. While vertical segregation of zooplankton groups, probably related to different trophic guilds, has been confirmed by several studies (e.g. Haury et al., 1990), this segregation can be reduced by turbulent processes enhancing contact between prey and predators (Visser and Stips 2002; Visser et al., 2009). For instance, available theoretical models predict optimal prey consumption at dissipation rates of turbulent kinetic energy (ε).
between $10^{-6}$ and $10^{-4}$ W kg$^{-1}$ (Lewis and Pedley, 2001). In fjords, topographic conditions are extremely irregular (Inall and Gillibrand, 2010), inducing high turbulence and enhanced vertical mixing, particularly at sills (Klymak and Gregg 2004; Whitney et al., 2014). However, enhanced productivity, oxygenation, and/or local retention may occur at these same highly turbulent areas. For example, turbulence is known to mix freshwater inflow with deep, dense ocean water, allowing for oxygenation of the deeper layers (MacCready et al., 2002; Peters and Bokhorst, 2001) and turbulent these eddies can impact phytoplankton bloom growth (Cloern, 1991; Koseff et al., 1993). Therefore, additional field studies are needed to inform our understanding of the relationship between mixing-inducing physical forcings, such as wind or advection, and vertical abundance patterns and species composition in fjords and other estuarine systems. One of the principal questions that will be address during the present study is: what is the contribution of turbulence to the mixing of fjord water column properties (e.g., Temperature, salinity and dissolved oxygen) and also to the aggregations of macrozooplankton at certain depths (scattering layers) along north Patagonian fjords and channels, emphasizing the role of sills in some locations (e.g., Jacaf Channel, Fig. 1)?

The dissolved oxygen (DO) is the most important dissolved gas in the ocean as it sustains marine life and ensures ecosystems health. Most of the world’s oceans are oxygenated, however there are some regions of low DO, referred to as hypoxic zones and if their DO concentrations are equal or close to 0 m$\text{L}^{-1}$ they are known as “Dead zones” (Diaz et al., 2001; Ekau et al., 2010; Hauss et al., 2016). Throughout the world’s oceans there exist areas where hypoxic conditions which are permanent and where the DO is significantly lower than well-oxygenated areas (e.g., <20 $\mu$M or 0.31 m$\text{L}^{-1}$). These areas are known as Oxygen Minimum Zones (OMZs) and due to the upwelling associated with them; they experience elevated surface primary production at the surface (Mass et al., 2014; Hauss et al., 2016; Seibel et al., 2016). The major ocean OMZs are located in the Eastern South and North Pacific, the Arabian Sea, Bay of Bengal (Indian Ocean), West Bering Sea, the Gulf of Alaska and the Eastern North Atlantic, covering around 8% of the total ocean (~30 million km$^2$) (Paulmier and Ruiz-Pino, 2009; Fuenzalida et al., 2009; Hauss et al., 2016). The Eastern South Pacific OMZ (ESP-OMZ), present along the Chilean coast, represents an area of 9.8 million km$^2$ (2.6 % of the total ocean) (Fuenzalida et al., 2009). Even the ESP-OMZ decreased and disappeared south of ~$37^\circ$ S, however water with low DO (2-3 m$\text{L}^{-1}$),
associated with the Equatorial Subsurface Water (ESSW), is still present up to 44° S (Silva et al., 2009). The ESSW water mass penetrate and infiltrates into Patagonian fjords and channels and moves northward and southward (41.5°-44° S) depending on the marine topography (Sievers and Silva, 2008).

Hypoxic conditions (< 2 mL L\(^{-1}\)) have been detected in four regions of Patagonian (Puyuhuapi Fjord, Jacaf Channel, Aysén Fjord and the Almirante Montt Gulf), and in each region the oxygen depleted zones are mainly located at the fjords heads and down to 100 m depth (Silva and Vargas, 2014; Schneider et al., 2014). Some of the main contributors to hypoxia in Patagonian fjords and channels—have been found to be (1) The water column stratification causing the separation between the poorly oxygenated bottom water and the oxygenated surface waters, (2) the DO consumption by degradation of organic matter (autochthonous and allochthonous), (3) the low ventilation due to the presence of deep bathymetric micro basins, (4) the advection of the ESSW and (5) the anthropogenic activities such as industrial and sewage discharge, riverine inputs of nutrients, agriculture activities, aquaculture, etc., were mentioned as one of the main causes contributing to hypoxia in Patagonian fjords and channels (Sievers and Silva, 2008; Silva and Vargas, 2014; Schneider et al., 2014).

Hypoxia is known to have a significant impact on plankton distribution and development, hence on the health of the ecosystem as a whole (Ekau et al., 2010; Mass et al., 2014; Hauss et al., 2016; Seibel et al., 2016). Some species can tolerate hypoxic water, e.g., smaller species, euphausiids and jellyfish can live in under 30% oxygen saturation and dissolved oxygen of 1.6 mL L\(^{-1}\). Other taxa, such as some copepods and fishes, may be more sensitive to hypoxia and have preference for oxygen saturations of 50-100% and DO concentrations of 2.6-5.2 mL L\(^{-1}\) (Ekau et al., 2010; Mass et al., 2014; Hauss et al., 2016; Seibel et al., 2016). The sensitivity of species to tolerate different oxygen concentrations, however, may vary among organisms from different environments, e.g., coastal upwelling zone, fjords systems and OMZ. Although In particular, in coastal upwelling zones along Chile, while some copepods seem to be limited to depth ranges with waters well oxygenated, others seem to be well adapted for residence at depths with minimum oxygen depth (Castro et al., 2007). Hypoxic conditions (< 2 mL L\(^{-1}\)) have been detected in four regions of Patagonia Patagonian (Puyuhuapi Fjord, Jacaf Channel, Aysén Fjord and the Almirante Montt Gulf), and in each region the oxygen depleted zones are mainly located at the fjords heads and down
to 100 m depth (Silva and Vargas, 2014; Schneider et al., 2014)—but no relationship has been determined was made with the zooplankton species that inhabit this ecosystem. Therefore, the second question that motivates this study is: How do hypoxic conditions affect the distribution and aggregation of macrozooplankton species in Puyuhuapi Fjord and Jacaf Channel? This question will be addressed by investigating Puyuhuapi Fjord and Jacaf Channel, two of the four hypoxic systems in Patagonia.

In Patagonian fjords, a comprehensive description of zooplankton distribution patterns has been provided by Palma (2008), considering a total of 220 in-situ plankton samples, from a number of depth strata between the surface and ~200 m. Main zooplankton groups included siphonophores, chaetognaths, cladocerans, copepods and euphausiids. Although a positive north to south gradient in the abundance of major zooplankton species was found, potential relationships between the vertical distributions and environmental variables were not deeply assessed. A later study by Landaeta et al., (2013) investigated the vertical distribution of microzooplankton and fish larvae in Steffen fjord (47.4° S) at four depth strata (200-50 m, 50-25 m, 25-10 m and 10-0 m depth) during November 2008. Copepod nauplii and copepodites of Acartia tonsa together with Maurolicus parvipinnis fish larvae were observed around the pycnocline region, suggesting that the vertical structure of the water column might play a role in larval fish distribution. More recently, studies on zoo- and ichthyoplankton vertical distributions in Reloncaví Fjord revealed that DVM timing might be modified by the tidal regime which is particularly strong in this area (Castro et al., 2014). However, none of these studies provided explicit assessments of the relationships between the vertical distribution of zooplankton and turbulent mixing or water column properties.

Most studies carried out in Chilean coastal waters, including those mentioned above, have relied on plankton nets and other collecting devices (pumps) deployed in single locations (fixed stations). An alternate approach is to use acoustic techniques, which can provide high resolution data on zooplankton DVM patterns (Valle-Levinson et al., 2014; Días-Astudillo et al., 2017) and segregation patterns throughout the water column (Sato 2013; Sato et al., 2016). For instance, DVM patterns of dense krill aggregations have been detected using Acoustic Doppler Current profilers (ADCP) moored around the Antarctic Peninsula, the Kattegat Channel and off Funka Bay, Japan (Buchholz et al., 1995; Lee et al., 2004; Zhou and...
Dorland 2004; Brierley et al., 2006). In Chilean fjords, ADCPs have been used to identify changes in vertical distribution and DVM patterns of zooplankton (e.g., from normal diel to twilight vertical migrations) over several months in Reloncaví Fjord- (Valle-Levinson et al., 2014). These studies, although novel at describing temporal variations in zooplankton patterns, focused mainly on the behavior of particular species, but failed again did not consider how the vertical distribution of zooplankton is modified by water column conditions (e.g., temperature, salinity, oxygen and turbulence).

Compared to ADCPs, scientific echo-sounders are characterized by narrower beam angles, lower frequencies and longer ranges. They have also been used to provide valuable qualitative and quantitative information on various aquatic species and communities, from zooplankton to large predators (Ballón et al., 2011). Overall, macrozooplankton can be acoustically identified and virtually separated from other organisms, such as fish, by considering their acoustic properties (Kloser et al., 2002; Logerwell and Wilson, 2004; Mosteiro et al., 2004; Simmonds and MacLennan, 2005). Although the use of several frequencies does not necessarily increase precision (Horne and Jech, 1999), the use of at least two frequencies (38 and 120 kHz) is currently a standard practice in zooplankton studies as identification methods developed by Ballón et al., (2011) and others can be utilized.

The present study aims to evaluate the effects of water column properties, such as dissolved oxygen and turbulent mixing, upon the horizontal and vertical distributions of dominant macrozooplankton groups along a Patagonian Fjord system. To achieve this goal ADCP and scientific echo-sounder data were combined with biological observations from in-situ stratified zooplankton samples and water column measurements from microstructure profilers and conductivity-temperature-depth-oxygen (CTDO) profilers. According to the information presented in this section, the principal hypotheses of this manuscript are: (1) the pervasive hypoxic layer existing in the Puyuhuapi Fjord limits DVM and overall distribution of macrozooplankton to the first 100 m depth of the water column, reducing the habitat of these species and, (2) The higher turbulence originated by the tidal regime around the Jacaf sills favors the mixing of the water column, deepens the hypoxic layer, injects nutrients and, thus, increases primary production. Therefore, macrozooplankton exhibits higher densities and extends its vertical distribution to deeper in the water column layers around the Jacaf submarine sills.
The remainder of this article will include a detailed description of the study area in section 2, followed by an explanation of the data collected and the data analysis methodology in section 3. The results will be presented in section 4 with a discussion, focusing on differences between two Patagonian fjords, presented in section 5. The main conclusions of the study will be presented in section 6.

2 Study Area

Patagonian fjords extend from 41° S to 56° S, and are typically deep and narrow as a result of their formation during glacial progression. Their hydrography is characterized by two vertical layers, consisting of a low salinity surface layer in the first ten meters of the water column (resulting from rainfall and glacial melt) that overlays a subsurface salty layer originated in the Pacific Ocean (Silva and Calvete, 2002; Pérez-Santos et al., 2014). Fjord systems play an important role in primary production and carbon cycling by providing a zone where energy and particulate material are exchanged between land and marine ecosystems (Gattuso et al., 1998). The principal nutrient (nitrate) is supplied to these fjords by oceanic transport, and particularly through the intrusion of Sub Antarctic Water (SAAW), a water mass that may also transport some species of zooplankton (González et al., 2011; 2013).

Puyuhuapi Fjord and Jacaf Channel are one of the form an atypical representative examples of the Patagonian fjords. The main connection of the Puyuhuapi Fjord with oceanic waters is via its southern mouth. Although a second connection to oceanic water exists via the Jacaf Channel, interchange here is limited by the shallow Jacaf Channel sill, which is 50 m deep and 6 km long, and its main freshwater supply input arrive the fjord mainly from the Cisnes River meets the fjord half way between its head and mouth discharge Jacaf Channel near its head (Fig.1). The Jacaf Channel is well known for its extreme great depth (> 400 m) at the mouth, around its connection to the Moraleda Channel, which contrasts with its very shallow sill (~50 m depth), located 22 to 27 km from Moraleda Channel, near its head that is connected with the Puyuhuapi Fjord (Fig. 1). Seasonal hydrographic measurements along Puyuhuapi Fjord have shown a stratified water column except in late winter, when the water column became partially mixed due to a reduction in freshwater supply from rainfall and glacial melting (Schneider et al., 2014). Hypoxic conditions have been detected in Puyuhuapi Fjord below 100 m depth, where oxygen concentrations were found to be as low as 1-2 mL L⁻¹ (Schneider et al., 2014; 2013).
Pérez-Santos, 2017). This observed oxygen depletion could be caused by limited ventilation due to shallow sills, or by the input of low-oxygen Equatorial Subsurface Water into the fjord (Silva and Vargas, 2014; Schneider et al., 2014). The Puyuhuapi Fjord is the only northwestern Patagonian fjord known to experience such extreme hypoxic conditions. At the same time, it is an area where intense aquaculture activities have been recently developed, which reinforces the need of this study.

The study area offers an excellent opportunity for studying the impact of deep hypoxia upon macrozooplankton distribution and behavior, taking into account considering the continued increase of hypoxic regions around the world (Breitburg et al., 2018). Moreover, the presence of a conspicuous sill in the Jacaf Channel, in the vicinity of its connection to the Puyuhuapi Fjord, opens the possibility to investigate the influence of vertical mixing region (Farmer and Freeland, 1983; Inall and Gillibrand, 2010) upon water quality, especially upon dissolved oxygen concentration, injection of nutrients from subsurface oxygen rich layers, enhancement of primary production and, finally, upon the density of different zooplankton species (Pantoja et al., 2011). Furthermore, the location of an oceanographic buoy in the northern part of Puyuhuapi Fjord (Schneider et al., 2014) is a useful platform to carry out in-situ experiments combined with oceanographic moorings.

3 Data collection and methodology

3.1 Water column properties

Although hydrographic surveys have been conducted from 1995 to 2016 in Puyuhuapi Fjord and Jacaf Channel, only the most recently data, from 2016, is presented in figure 2 to illustrate overall hydrographic background and permanent hypoxic conditions in Puyuhuapi Fjord. Hydrographic surveys were conducted during May and November 2013 and January and August 2014 in Puyuhuapi Fjord and Jacaf Channel (Fig. 2, Table 1). These profiles were obtained with a Seabird 25 CTDO, sampling at 8 Hz with a descent rate of \( \sim 1 \text{ m s}^{-1} \). The data collected, whose nominal vertical resolution was \( \sim 12 \text{ cm} \), were averaged into 1 m bins, following Seabird recommendations. The conservative temperature (°C) and absolute salinity (g kg\(^{-1}\)) were calculated according to the Thermodynamic Equation of Seawater 2010 (COI et al., 2010). Additionally, nitrate samples were taken using a Niskin bottle at various depths and analyzed spectrophotometrically following the methods of Strickland and Parsons (1968). To validate CTDO oxygen measurements, in-situ oxygen samples were analyzed using the
Winkler method (Strickland and Parsons, 1968), carried out using a Metrohom burette (Dosimat plus 865) and an automatic visual end-point detection (AULOX Measurement System).

Microstructure measurements were collected using a Vertical Microstructure Profiler (VMP-250, Rockland Scientific, Inc.). The VMP-250 was equipped with two airfoil shear probes and two fast response FP07 thermistors, which allowed for data recording at 512 Hz with a descending free fall speed of ~0.7 m s\(^{-1}\). The micro-shear measurements permitted the direct measurement of the dissipation rate of turbulent kinetic energy (\(\varepsilon\)) for isotropic turbulence, according to Lueck et al., (2002), Eq. (1),

\[
\varepsilon = 7.5 \nu \left( \frac{\partial u}{\partial z} \right)^2
\]

where, \(\nu\) is the kinematic viscosity, \(u\) is the horizontal velocity, \(z\) is the vertical coordinate axis and therefore \(\left( \frac{\partial u}{\partial z} \right)^2\) is the shear variance.

Using the values of \(\varepsilon\), the diapycnal eddy diffusivity (\(K_\rho\)) was calculated. The most used formulation was proposed by Osborn (1980),

\[
K_\rho = \Gamma \frac{\varepsilon}{N^2},
\]

where \(\Gamma\) is the mixing efficiency, generally set to 0.2 (Thorpe 2005), and \(N\) is the buoyancy frequency. Shih et al. (2005) noted that when the ratio \(\varepsilon/\nu N^2\) is greater than 100, Eq. (2) results in an overestimation. Therefore, they proposed a new parameterization for this case given by:

\[
K_\rho = 2\nu \left( \frac{\varepsilon}{\nu N^2} \right)^{1/2}.
\]

More recently, Cuypers et al. (2011) used Eq. (3) when \(\varepsilon/\nu N^2 > 100\), Eq. (2) when 7 < \(\varepsilon/\nu N^2\) < 100, and considered null eddy diffusivity when \(\varepsilon/\nu N^2 < 7\). This approach was followed in this study. The correlation between the dissipation rate of turbulent kinetic energy and the abundance of major zooplankton groups throughout the water column was accomplished by using a quadratic polynomial curve fit between these data sets (explained in detail in section 4.6). These analyses were only applied to measurements collected at the fixed station in Puyuhuapi Fjord, because the VMP-250 was not available during the measurement campaign in Jacaf Channel.

### 3.2 Acoustic data

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Three types of acoustic data were collected: ADCP, single-frequency echo-sounder and dual-frequency echo-sounder data. ADCP measurements were obtained with two 307.7 kHz Teledyne RDI Workhorse ADCPs, moored upwards at depths of ~50 m (ADCP-1) and ~100 m (ADCP-2), both moored at the same location in north-central Puyuhuapi Fjord but during different time periods (Table 1, Fig. 1. ADCPs moored at the same location). Data were collected hourly with a vertical bin size of 1 m, over periods of austral autumn (ADCP-1: May, 2013) and spring-summer (ADCP-2: January 2014). During the final ADCP-2 mooring deployment, single-frequency data were also collected along the Puyuhuapi Fjord using a SIMRAD EK60 scientific echo-sounder, running a single frequency 38 kHz transducer (ES38B), during daytime and nighttime hours, from January 22-25, 2014 (black line in Fig. 1). These ADCP and single-frequency echo-sounder measurements were complemented by in-situ zooplankton sampling (see section 3.3 for details) was carried out on January 23-24, 2014, at a fixed station close to the ADCP mooring location, over a period of 36 hours (Fig. 1) in order to validate acoustic measurements.

In August 2014, a second scientific campaign was conducted on August 17th and 18th, 2014, which included a dual-frequency echo-sounder survey and a third ADCP mooring (ADCP-3) located in Jacaf Channel was conducted. During this measurement campaign, the echo-sounder survey coverage was extended to eastern Jacaf Channel (Fig. 1, red line) and a second 120 kHz transducer (ES120-7C) was added to the 38 kHz transducer used in the first survey. Several day/night transects were completed from across Puyuhuapi Fjord into and Jacaf Channel, with special attention paid to Jacaf sill (only the most representative echograms were showed in figures 5, 7 and 8). To determine the statistical relationship ($R^2$) between acoustic data from the 38 kHz echo-sounder with hydrographic properties of the fjords (temperature, salinity and dissolved oxygen), a quadratic polynomial curve was also applied between these data sets. During this experiment survey, one two RDI Workhorse ADCP was with 614.4 kHz frequency (referenced two RDI Workhorse ADCP with 614.4 kHz frequency (referenced as ADCP-3) and was ADCP-3) was were installed moored at ~30 m depth in the vicinity of the Jacaf sill. The near-surface placement of ADCP-3 allowed for near-surface currents to be adequately quantified. One 307.7 kHz ADCP was moored close to the bottom (100 m) and another 614.4 kHz one (referenced as ADCP-3) at 30 m. Unfortunately, only data from ADCP-3 was recovered.
Vessel speed during all echo-sounder surveys was maintained between 8 and 10 knots. Echo-sounders were operated using a variable ping rate 0.3-2.0 ping s⁻¹, a pulse duration of 1.024 milliseconds and output powers of 2 kW and 0.5 kW for the 38 and 120 kHz frequencies, respectively. Calibration was made using proper copper spheres and standard procedures (Foote et al., 1987).

3.2.1 Echo-sounder data analysis

Post-processing of echo-sounder data was performed in Echoview (Myriax inc, Tasmania, https://www.echoview.com/), where data was scrutinized to identify and exclude noisy data considered as those collected with weak pings, in regarded as “bad data”, blind areas, in the near field, with background noise or subjected to rainbow phenomenons. These were regarded as “bad data” and were eliminated. After this initial scrutiny and filtering step, all single-frequency echoes (38 kHz, Campaign 1) of intensity >-110 dB were considered and treated as a single “biological backscattering” class, which pooled all biological groups being present in the study area. Dual-frequency echoes, however, were classified into three different groups following the open access version (“FishZpkPeru38&120.evi”) of Ballón et al., (2010). These authors built an algorithm, freely distributed as an Echoview template (“FishZpkPeru38&120.evi”), which combines uses both mean volume backscattering (MVBS) from 38 and 120 kHz, using both differences (ΔMVBS) and summations (ΣMVBS) between 38 and 120 kHz to discriminate and quantify the abundance of macrozooplankton. This algorithm separates raw data into echoes into three different virtual echograms: biological backscattering classes; fish and two macrozooplankton groups (macrozooplankton or “fluid-like” and gelatinous or “blue noise” organisms). The fluid-like group follows a sphere model (Holliday & Pieper, 1995) considered appropriate to represent cylindrical and spherical shapes, including euphausiids and large copepods, which are dominant macrozooplankton groups off Peru and Chile (Ayon et al., 2008). The algorithm is considered to be useful for 38 and 120 kHz data from targets whose radius is ≥0.5 mm and has a dB difference of 2-19 dB (Ballón et al., 2010 and 2011). As implemented, the post-processing file FishZpkPeru38&120.evi is also designed and was used to remove blind areas, near field, background noise and rainbow phenomenons for both single and dual frequency data.
Given physical limitations imposed by near field and sound absorption effects related to selected echo-sounder frequencies used (38 and 120 kHz) across the water column, we defined and limited our analyses to an effective sampling range of the water column up to between 5 and 250 m was expected. Absorption is greater for the 120 kHz frequency, which exhibits the shortest range, but has a greater vertical resolution than 38 kHz echo-sounder. The 38 kHz frequency, on the other hand, exhibits a much longer range (>1000 m), but limited resolution affecting regarding small zooplankton scatterers (e.g. small copepods) detection. Nonetheless, this is the most commonly used frequency, which has proven to be It has been shown, however, to be efficient for studying macrozooplankton distributions of larger groups such as siphonophores, chaetognaths and euphausiids (Mair et al., 2005; Cade and Benoit-Bird, 2015; Ariza et al., 2016).

Volume backscattering strength ($S_v$, dB re 1 m$^{-1}$) values from the single-frequency and from each of the three dual-frequency virtual echograms echo-sounder were integrated using a grid of 20 m (depth) by 50 m (distance), and re-scaled into the customary index “nautical area scattering coefficient” (NASC, in units of m$^2$ n mi$^{-2}$, using a grid of 20 m (depth) by 50 m (distance). Since NASC lies on the linear domain, it can be considered proportional to and suitable for indexing zooplankton targets abundance (Ballón et al., 2011).

To determine Quadratic polynomial models were fit to assess the statistical relationship ($R^2$) between acoustic data from the 38 kHz echo-sounder biological scattering (single-frequency integrated data) with and the hydrographic properties of the fjords variables measured in each fjord (temperature, salinity and dissolved oxygen). A quadratic polynomial curve was also applied between these data sets.

### 3.2.2 Acoustic data analysis from ADCPs

ADCP echo intensity was converted to mean volume backscattering strength ($S_v$, dB re 1 m$^{-1}$), as done for scientific echo-sounder data, following the conversion formula:

$$S_v = C + 10 \log [(T_x + 273.16)R^2] - L_{DBW} - P_{DBW} + 2\alpha R + K_c (E - E_r)$$  \hspace{1cm} (4)

where, $C$ is a sonar-configuration scaling factor (-148.2 dB for the Workhorse Sentinel), $T_x$ is the temperature at the transducer ($^\circ$C), $L_{DBW}$ is log$_{10}$(transmit-pulse length, $L_{trans}=8.13$ m), $P_{DBW}$ is log$_{10}$(output power, 15.5 W), $\alpha$ is the absorption coefficient (dB m$^{-1}$), $K_c$ is a beam-specific sensitivity coefficient (supplied by the manufacturer as 0.45), $E$ is the recorded AGC (automatic gain control), and $E_r$ is the minimum AGC recorded (40 dB for ADCP-1 and 41
dB for ADCP-2). The beam-average of the AGC for the 4 transducers was used to obtain optimal results following the procedure in Brierley et al. (2006). Finally, \( R \) is the slant range to the sample bin (m), which uses the vertical depth as a correction (Lee et al., 2004). Therefore, \( R \) is expressed as,

\[
R = \frac{b + \frac{L + d + ((n-1)d) + (d/4)}{2}}{\cos \zeta} \frac{e_i}{c_i}
\]

where \( b \) is the blanking distance (3.23 m), \( L \) is the transmit pulse length (8.13 m), \( d \) is the length of the depth cell (1 m), \( n \) is the depth cell number of the particular scattering layer being measured, \( \zeta \) is the beam angle (20°), \( e_i \) is the average sound speed from the transducer to the depth cell (1453 m s\(^{-1}\)) and \( c_i \) is the nominal sound speed used by the instrument (1454 m s\(^{-1}\)).

### 3.3 In-situ Zooplankton sampling

In situ mesozooplankton samples were collected with a WP2 net (60 cm diameter mouth opening, 300 µm mesh, flowmeter mounted in the net frame) towed vertically from 50 m to the surface in May 2013, and with a Tucker trawl (1 m\(^2\) mouth opening, 300 µm mesh) used to obtain stratified oblique tows in January 2014 and August 2014 (Table 1). All samples were preserved in a 5% formaldehyde solution. Zooplankton abundances were standardized to individuals per m\(^3\) of filtered seawater. WP2 vertical tows consisted of 5 depth intervals from surface to 50 m, every 10 m (0-10, 10-20, 20-30, 30-40, 40-50 m).

Stratified Tucker tows considered four depth strata: 0-10 m, 10-20 m, 20-50 m, 50-100 m in the Puyuhuapi Fjord. For other side, in the Jacaf Channel, the stratified sampling included five depth strata: 0-10 m, 10-20 m, 20-50 m, 50-100 m and 100-150 m, and also 100-150 m depth in the Jacaf Channel. The hauling speed for both nets was between 2-3 knots. Sampling occurred during a 36-h period every 3 h from January 22-24, 2014 (Puyuhuapi Fjord) and every 5-6 h from August 18-19, 2016 (Jacaf Channel) (Fig. 1, red dots). At all sites and dates, all zooplankters were identified, sorted into functional groups, measured (length) and classified into size-classes using a 5 mm length threshold. Copepods, the most abundant group, were further classified into <1 mm, 1-5 mm and >5 mm prosome length groups. To determine the correlation (\( R^2 \)) between the \( S_v \) records from the 38 kHz transducer and the major macrozooplankton groups (Siphonophores, Chaetognaths and
Euphausiids), a quadratic polynomial curve was also applied between these data sets (n=44 for the sampling in Puyuhuapi Fjord and n=25 in Jacaf Channel) (further details in section 4.3).

3.4 Tidal harmonic analysis

The tidal constituents were computed using HOBO U20 water level loggers and the pressure sensor from ADCP-3 (Table 1-2, Fig. 1). A tidal harmonic analysis was applied to the sea level time series according to Pawlowicz et al., (2002), which considers the algorithms of Godin (1972, 1988) and Foreman (1977, 1978). We classified tides by the dominant period of the observed tide based on the form factor \( F \), defined by the ratio between the sum of the amplitudes of the two main diurnal constituents (larger lunar declinational, \( O_1 \) and luni-solar declinational, \( K_1 \)) and the sum of the amplitudes of the two main semi-diurnal constituents (principal lunar, \( M_2 \) and principal solar, \( S_2 \)), \( F = (O_1+K_1)/(M_2+S_2) \) (Bearman, 1989; where, \( F < 0.25 \) semi-diurnal, \( 0.25 < F < 1.5 \) Mixed semi-diurnal and \( F > 3.0 \) diurnal).

4. Results

4.1 Hydrographic features

Temperature profiles collected in Puyuhuapi Fjord and Jacaf Channel showed similar structure during the winter and summer campaigns (Fig. 2, a-b). The largest temperature gradients were found between the surface and \( \sim 70 \) m depth, ranging from 8.5° C to 17° C. A thin, fresh layer (salinity values varied from 11 to 29 g kg\(^{-1}\)) was found in the first \( \sim 10 \) m of the water column below which salinity varied little (29 to \( \sim 34.2 \) g kg\(^{-1}\)), as result of the presence of Modified Sub-Antarctic water (MSAAW, salinity between 31 and 33 g kg\(^{-1}\)), the Sub-Antarctic Water (SAAW, salinity between 33 and 33.8 g kg\(^{-1}\)) and the Equatorial Subsurface Water (ESSW, salinity>33.8 g kg\(^{-1}\)) (Fig. 2, c-d). Hypoxic conditions (dissolved oxygen below 2 mL L\(^{-1}\) and \( \sim 30 \) % saturation) were detected in Puyuhuapi Fjord below 100 m depth, with oxygen concentration between 1-2 mL L\(^{-1}\) (Fig. 2e). Deep water in Jacaf Channel was more ventilated, with dissolved oxygen values above hypoxic conditions throughout the water column (Fig. 2f). The hypoxic layer was located at the depth range of the Equatorial Subsurface Water (ESSW) and ventilated oxygen rich water (3-6 mL L\(^{-1}\)) was observed at depths occupied by MSAAW and SAAW. Below 10 m depth, high nitrate concentrations were registered in Puyuhuapi Fjord, but concentrations in the winter
period (August 2014) were higher than in fall (May 2013) and summer (January 2014) (Fig. 2.g). Along with the in-situ hydrographic sampling, in-situ zooplankton samples were collected and will now be discussed.

The most recent hydrographic surveys carried out in Puyuhuapi Fjord (June 2016) showed hypoxic conditions (dissolved oxygen <2 mL L⁻¹) below 120 m depth coherent along the channel (Fig. 2d). The lowest DO values were detected at the head of the fjord (35-50.5 km) with 1.5 mL L⁻¹ and 25% of oxygen saturation (Fig. 2d and 2e). The hypoxic layer was located at the depth range of the Equatorial Subsurface Water (ESSW). Ventilated water (3-6 mL L⁻¹) was observed at depths occupied by Modified Sub-Antarctic water (MSAAW) and Sub-Antarctic Water (SAAW), characterized by a salinity range from 31 to 33 g kg⁻¹, colder water temperature and a weaker temperature gradient (Fig. 2b).

4.2 ADCP Acoustic data and in-situ zooplankton samples

Volume backscatter ($S_v$) from the ADCP-1 (50 m depth, May 2013) showed large variability, ranging from high (-90 to -75 dB re 1 m⁻¹) to low (-115 to -100 dB re 1 m⁻¹) $S_v$ values (Fig. 3a). The highest $S_v$ values (-90 dB re 1 m⁻¹) were recorded during the night hours (~18:00 h to ~07:00 h, local time; with all remaining times for in-situ sampling expressed in local time), while minimum $S_v$ values were observed in the daytime (~07:00 h to ~18:00 h, local time h) suggesting the incorporation of vertically migrating organisms from deeper waters layers (below ADCP-1 mooring depth of 50 m) migrate upwards during nighttime hours. From the in-situ measurements of macrozooplankton collected at various depth strata in May 2013, the most abundant macrozooplankton—groups were copepods (<5 mm), siphonophores, chaetognaths and medusae (Fig. 3c-f). A marked change in vertical distribution and in total abundance of the macrozooplankton groups in the water column was observed from the first sampling hour (Fig. 3c)—late afternoon (~17 h, not shown) to the first night sampling time (~18 h), revealing the start of the nocturnal migration to the surface (Fig. 3d) coincident with a DVM pattern as seen in the ADCP-1 backscatter data (Fig. 3a-b).

Data from the ADCP-2 mooring (positioned deeper but at the same location as ADCP-1) from January 22-24, 2014 also showed a strong macrozooplankton DVM pattern, which extended down to ~100 m depth (Fig. 4a). During daylight hours (8:00-18:00, local time h), dense
zooplankton aggregations were observed between 80-100 m depth, which started to ascend from 18:00 to 21:00 (local time) h, concentrated close to the surface at night, and began to descend at ~06:00 (local time) h. In-situ stratified sampling showed the most abundant macrozooplankton In-situ stratified sampling showed the most abundant macrozooplankton groups were copepods, euphausiids, siphonophores, chaetognaths, decapods and medusae (Fig. 4 b-d). Groups were euphausiids, siphonophores, chaetognaths, decapods and medusae (Fig. 4 b-f). Euphausiids and siphonophores showed higher abundance close to surface layer (10-20 m) during night hours (Fig. 4c and Fig. 4f) and at deeper layers during the daytime (Fig. 4d and Fig. 4e). However, euphausiids showed the clearest diel vertical migration with maximum abundance between 10-20 m layer during night hours, and at 20-50-100 m depth during the daytime (Fig. 4c-f). The in-situ zooplankton samples were complemented by echo-sounder measurements collected along the fjord systems during the summertime and the wintertime. These measurements will now be discussed.

4.3 Acoustic data from scientific echo-sounder data

4.3.1 Summertime single-frequency surveys

The volume backscatter during the summer months overall showed DVM of all macrozooplankton species and a downward migration limit of ~100 m depth due to presence of hypoxic conditions below this depth. Summer daytime $S_v$ values along the Puyuhuapi Fjord averaged -89.1 ±7 dB re 1 m$^{-1}$ and ranged between -110 and -77.3 dB re 1 m$^{-1}$, from the mouth to the head of the Puyuhuapi fjord (Fig. 5a). Most of the biological backscatter was concentrated in the first 100 m of the water column, matching ADCP-2 results, which showed an increase in backscattering towards 100 m depth (Fig. 3 and 4). At the ADCP-2 mooring location (black dot in Fig. 5a), higher backscatter signals were found between 50-100 m (Fig. 3, 4a and 5a). Average NASC profiles for highest daytime NASC values - day-zooplankton showed higher values between 50 were found around 480-500 m (above the hypoxic layer), with NASC range reaching values of 3-3.5 m$^2$ n m$^{-2}$ both during day and night (Fig. 5b and e). Although some NASC values were observed, some backscatter occurred within the hypoxic layer (below ~120 m depth), all dense aggregations were observed above it (Fig. 5g-5e). Daytime $S_v$ signals attributed to fishes were typically detected at 50-50 m depth, being much higher (~60 dB re 1 m$^{-1}$; Fig. 5c) than $S_v$ signals attributed to zooplankton.
Summer nighttime $S_v$-values showed maximum $S_v$ and NASC-values near the surface (Fig. 5d and 5f), suggesting an ascending vertical migration of both zooplankton and fishes. All biological backscatter scatterers (Fig. 5a vs. 5d and Fig. 5c vs. 5f). The depth of fish aggregations in the daytime at the head of the fjord (between 50-100 m depth; Fig. 5e) compared with the depth of fish aggregations near the head at nighttime (~25 m depth; Fig. 5f), shows a distinct vertical migration pattern. NASC profiles also showed both an increase in zooplankton maximum abundances (~40 m) but the and a shift in the vertical position of the maximum values from 60-80 m during daytime to 40-60 m depth was more close to the surface than the NASC in the surface layers (0-60 m) at night observed during the day zooplankton transect during nighttime (Fig. 5b and 5d). Although the water column depth extended to ~300 m, all dense backscatter aggregations were observed above 100 m depth during both day and night time hours (Fig. 5a and 5d). As DO concentrations typically decreased from 2 mL L$^{-1}$ to 1 mL L$^{-1}$ below 100 m depth, zooplankton-biological scatterers in Puyuhuapi Fjord appeared to prefer water with a higher oxygen concentrations (between 3- and 7 mL L$^{-1}$) (Fig. 5e). The correlation between $S_v$ values and the observed density of in-situ different zooplankton groups (in-situ samples, (species >5mm) was high. Such correlations reached values of and the $S_v$-records from 38 kHz frequency was high with $R^2=0.50$. for $S_v$ vs. for siphonophores (Fig. 6a), $R^2=0.64-48$ for $S_v$ vs. chaetognaths (Fig. 6b), and $R^2=0.78-72$ for $S_v$ vs. euphausiids (Fig. 6c). The wintertime sampling showed similar findings but was able to capture more activity in the water column due to the use of two acoustic frequencies. A much weaker relationship ($R^2=0.22$) was found between $S_v$ and copepods >1 mm.

4.3.2 Wintertime dual-frequency $S_v$surveys

Wintertime dual-frequency surveys data, carried out along Puyuhuapi Fjord and Jacaf Channel on August 17th (~35 km total transect length, Fig. 1) using two frequencies (38 and 120 kHz), allowed separation of total backscatter into Fish, Fluid like (FL) and Blue noise (BN) groups (Fig. 6a-b). Total backscatter ($S_v$) in the Puyuhuapi Fjord (0-18 km) reached higher showed elevated values in the first 100 m of the water column, but at slightly deeper locations-depths (50-100 m) than in summer (Fig. 5), possibly due to bad weather conditions encountered on the sampling day. Greater intensity $S_v$-values (~80 to -60 dB re 1 m$^{-1}$) and vertical distribution range (0-220 m) of biological backscattering values ($S_v$>110 dB) were observed in Jacaf Channel (~18-32 km), particularly around the submarine its sill.
(between km 18 and 32; which is 6 km long and occurs between km 22 to 27, with the shallowest point (50 m depth) occurring at ~24 km (Fig. 6). At this site, $S_v > 110$ was found up to ~24 km. Particularly high intensities were attributed to the blue-noise BN (Fig. 6b7b) and the fish FL groups (Fig. 6c7c) around the at either side of the Jacaf Channel sill on both August 17th and 18th surveys (Fig. 7 and 8). An important degree of vertical segregation between BN and FL groups was also observed along the Jacaf Channel, with the first group to be concentrated between 100 and 140 m, while the second was between 120 and 200 m (Fig. 7 and 8).

Continuous acoustic sampling repeated over the Jacaf Channel sill confirmed the presence of intense backscatter signals (Fig. 6, 7 and 8), and presence of two backscattering layers: one denser layer between 100-150 m and a second, less dense layer from 200 to 250 m (Fig. 7a, 8a, showed only the best echogram). The in-situ zooplankton sampling along the Jacaf Channel sill (Fig. 9f) allowed the detection of the major macrozooplankton groups (e.g., chaetognaths, euphausiids and crustaceans) found presented during this experiment (Fig. 9a-d). In general, all sampling stations were carried out during daytime, but station 4 coincided with the ascending moment of macrozooplankton, and highlighting the presence of euphausiids during this time of vertical migration (Fig. 9d). Also, station 1 detected showed the dominance of crustaceans in the at the first strata (0-10 m strata). Overall, during the in-situ zooplankton sampling, acoustic data were collected and the echograms showed good agreements with the FL group (Fig. 9a-d). At the fixed station located in Jacaf Channel detected strong DVM patterns (Fig. 8a9e), where...The 0-150 m depth integrated abundance of zooplankton >5 mm showed increased values during nighttime hours (23:00 h and 22:00 h, local time in Fig 8a9e), in particularly...Further more, the elevated abundance of macrozooplankton groups (euphausiids and chaetognaths) were found in the between 100-150 m depth layer particularly at day-time hours (Fig. 8b9b-f), which matched well with acoustic data for the fluid-like group (Fig. 7a8a), but in the case of BN group the macrozooplankton species were as not clearly identified in the in-situ zooplankton sampling.

A moderate correlation was found between the in-situ zooplankton sampling (species >5mm) and the 38 kHz $S_v$ values from Jacaf Channel. In particular, a positive medium correlation was again obtained and zooplankton density calculated from in situ samples (>5 mm), with $R^2=0.42$ for $S_v$ vs. chaetognaths (Fig. 6d) and $R^2=0.41$ for $S_v$ vs. euphausiids (Fig. 6d).
Now the relationships between water column properties such as temperature, salinity and DO will be compared to the acoustic and in-situ macrozooplankton measurements.

### 4.4 Relationships between acoustic records, biological scattering and water column properties

To examine relationships between zooplankton behavior, the distribution of biological scattering and water column properties, $S_v$ values quantified from the continuous 38 kHz acoustic profiler $S_v$ values obtained in summer and winter at Puyuhuapi Fjord and Jacaf Channel, respectively, were matched to the consecutive time at which CTD and DO data were captured. This was done in Puyuhuapi Channel and Jacaf Channel during the summer and winter seasons, respectively. The polynomial relationship between water temperature and 38 kHz $S_v$ values showed a rather weak correlation during summer ($R^2=0.30$) and winter ($R^2=0.41$), predicting maximum $S_v$ values occurring between 8 and 10°C (Fig. 9a and 10a). Also, a positive but rather and also weak correlation relationship was found between $S_v$ and salinity in Puyuhuapi Fjord ($R^2=0.29$, Fig. 9c) and Jacaf Channel ($R^2=0.35$, Fig. 9d), with higher $S_v$ values predicted found in more the MSAAW and SAAW oceanic water masses (salinity >31 g/kg, MSAAW and SAAW). Both in Puyuhuapi Fjord and Jacaf Channel $S_v$ with both DO and oxygen saturation showed the highest $R^2$ values of the series ($R^2=0.6$, Fig. 9e-h). Hence, only 20.4% of total $S_v$>-110 dB re 1 m$^{-1}$ were located in the hypoxic layer of Puyuhuapi Fjord, while just 1.2% were located in the hypoxic layer in Jacaf Channel (Fig. 9e-h). Now the TKE dissipation will be discussed to relate macrozooplankton assemblages to vertical mixing in the water column.

### 4.5 Tidal regime

The harmonic analysis carried out with the sea level time series obtained in Puyuhuapi Fjord and Jacaf Channel, denoted the dominance (in terms of amplitude) of the semi-diurnal constituents ($M_2$ and $S_2$; Table 2). Diurnal constituents ($O_1$ and $K_1$) were also important, specifically at the Jacaf ADCP-3 station located close to the Jacaf sill region (Table 2 and Fig 1). The contribution of diurnal constituents added the mixed character to the tidal regimen in the study area. The spectral analysis implemented at all sea level stations showed maximum energy in the semi-diurnal band (Table 2), with the highest spectral energy (57.29 m$^2$ cph$^{-1}$).
4.6 Mixing process

Turbulence measurements collected with the VMP-250 microstructure profiler showed high dissipation rates of turbulent kinetic energy ($\varepsilon$) in the upper first 20 m depth of the water column in Puyuhuapi Fjord and Jacaf Channel (Fig. 4011). In this layer $\varepsilon$ ranged from $10^{-7}$ to $10^{-5}$ W kg$^{-1}$. However, below this surface layer (<20 m depth) the highest values were obtained around the Jacaf sill region ($\varepsilon=1.2\times10^{-7}$ W kg$^{-1}$), as shown on 21 November 2013 at 140 m depth (Fig. 40-11 a). In Puyuhuapi Fjord TKE dissipation between the shear estimates obtained between 20-180 m were less intense and therefore $\varepsilon$ was weak ($10^{-10}$ to $10^{-7}$ W kg$^{-1}$), (Fig. 40c-11c and 40d11c). The dissipation rates of turbulent kinetic energy are obtained by integrating the velocity shear spectrum at each respective depth bin up to the noise limit. The noise limit is determined by comparing the measured spectra to the theoretical Naysmyth Spectra and determining where the measurements begin to deviate from theory. To display how the estimates of $\varepsilon$ were obtained at the Jacaf sill depth, the shear spectra are shown for VMP profiles collected at the Jacaf sill region (21 November 2013 at 140 m depth; Fig. 10b11b), and in Puyuhuapi Fjord on 22 November 2013 (at 140 m depth; Fig. 10d11d) and on 23 January 2014 (at 140 m depth; Fig. 10f11f).

In Puyuhuapi Fjord the correlation between $\varepsilon$ and zooplankton $S_v$ data (38 kHz, fixed station, January 2014) was high ($R^2=0.65$, Fig. 12a). In the same campaign, the in-situ macrozooplankton density (>5 mm) was also highly positively correlated with $\varepsilon$ values ($R^2=0.79$ for $\varepsilon$ vs. siphonophores, $R^2=0.66$ for $\varepsilon$ vs. chaetognaths, and $R^2=0.77$ for $\varepsilon$ vs. euphausiids) (Fig 12b-d). Unfortunately, VMP data was not collected in Jacaf Channel in wintertime. In order to confirm the relationship between $\varepsilon$ and various zooplankton species, additional turbulence measurements were collected in November 2013 along the Jacaf sill (Fig. 44a13a). Results showed strong velocity shear in the horizontal velocities (Fig. 44b13b) accompanied by high $\varepsilon$ values ($10^{-7}$ to $10^{-5}$ W kg$^{-1}$; Fig. 44e11c). Maximum $\varepsilon$ was measured at the Jacaf-Puyuhuapi confluence (10 km along transect) at ~63 m depth where $\varepsilon= 1.9 \times 10^{-5}$ W kg$^{-1}$, (Fig. 44b13b; St. 164). The diapycnal eddy diffusivity ($K_\rho$) was also high in the same area with values of $10^{-4}$ to $10^{-3}$ m$^2$ s$^{-1}$ (Fig. 44e13c).
5 Discussion

This study represents one of the first attempts to combine measurements of acoustics, stratified plankton sampling, microstructure profiles, and standard hydrographic profiles to investigate both the vertical distribution patterns of macrozooplankton and why these patterns exist in northwest Patagonian Fjords and other subantarctic latitudes. Three main findings resulted from this effort. First, DVM patterns of macrozooplankton became evident from all methodological approaches, at all study periods: May 2013, January 2014 and August 2014 (Fig. 3-5 and Fig. 7-9). Second, strong evidence arose showing macrozooplankton avoidance of hypoxic layers. And, third, a clear increment of macrozooplankton and fish aggregations around the Jacaf sill could be related to increased turbulence in this area.

5.1 Diel vertical migration patterns

Consistent evidence from multiple echo-sounder surveys, ADCP moorings and semi-continuous in-situ zooplankton measurements supported the existence of major circadian displacements of macrozooplankton during night hours between mid-depth (20-120 m) and subsurface waters in our study area. Similar DVM patterns have been found in Reloncaví Fjord (41.5° S), from 300 and 600 kHz ADCP data, by Valle-Levinson et al., (2014) and by Días-Astudillo et al., (2017) using a 75 kHz acoustic device. Given its larger resolution, the later work was able to confirm that the DVM affected the whole water column of the fjord (~200 m). These studies found the presence of euphausiids, decapods, mesopelagic shrimps, copepods and other groups in the Reloncaví Fjord in July and November, 2006 (Valle-Levinson et al., 2014), as well as in July 2013 (Días-Astudillo et al., 2017). DVM is a common feature of many zooplankton groups, observed around the world using different ADCP and echo-sounders frequencies, e.g., at the Kattegat Channel (Buchholz et al., 1995), the northeast Atlantic (Heywood, 1996), the northwest coast of Baja California, Mexico (Robinson and Gómez-Gutiérrez, 1998), the northeastern Gulf of Mexico (Ressler, 2002), the Antarctic Peninsula (Zhou and Dorland, 2004), the Arabian Sea (Fielding et al., 2004), the Funka Bay, Japan (Lee et al., 2004), the south Georgia, in the Atlantic sector of Southern Ocean (Brierley et al., 2006) and Saanish Inlet, British Columbia, Canada (Sato et al., 2013). The scattering layers observed in these studies highlight the abundances of the major zooplankton species, represented by: amphipods, euphausiids, siphonophores,
chaetognaths, pteropods, crustaceans, small fish and gelatinous plankton. While most DVM patterns reported in these studies occurred between 0 and ~300 m depth, the deepest DVM patterns were observed in the North-Atlantic Ocean, reaching depths ~1600 m (Van Haren and Compton, 2013).

DVM patterns of zooplankton are expected to be associated with visible light intensity, in particular, diel changes in visible light within the photic zone (from surface to ~100 m). Thus, zooplankton can avoid predators during daytime hours and have safe-feeding conditions at night. However, while only small irradiance levels, around <10^7 times surface levels, can be still detected at beyond 600 m or deeper in some areas (Van Haren and Compton, 2013; Sato et al., 2013 and 2016), requiring zooplankton DVM to reach can reach depths below 500 m (Van Haren and Compton, 2013). Moreover, zooplankton DVM patterns of zooplankton have been observed in Arctic fjords (e.g., the Kongsfjorden, Kongsfjordena, and Rijpfjorden fjords) even during the polar night, suggesting the influence of high sensitivity to very low levels of solar and/or lunar light (Berge et al., 2009). Since both the Puyuhuapi Fjord and the Jacaf Channel are not deeper than 300 m, enough light should be able to reach the bottom layer and stimulate zooplankton DVM across the whole water column. However, our results show that zooplankton DVM did not go below (and distribution as discussed in the next section) was limited by the hypoxic boundary layer present in the Puyuhuapi Channel (~100 m; Fig. 5), providing indirect support to the idea that hypoxia may limit DVM in low-ventilated Patagonian fjords and elsewhere (Ekau et al., 2010; Mass et al., 2014; Hauss et al., 2016; Seibel et al., 2016).

5.2 Macrozoooplankton avoidance of hypoxic waters

In Puyuhuapi Fjord, hypoxic conditions have been reported below ~100 m depth, all year round (Schneider et al., 2014; Silva and Vargas 2014), with sporadic deep ventilation events that increase the DO concentration from 1.4 to 2.8 mL L^-1 (Pérez-Santos, 2017). These pervasive hypoxic conditions seem related to ventilation conditions; however, they are not common in all Patagonian fjords. For instance, seasonal hydrographic data from Reloncaví Fjord showed well ventilated conditions along the fjord, with deep, deep-near-bottom DO values between 3-3.5 mL L^-1 (Castillo et al., 2016).

In the current study, acoustic measurements and zooplankton sampling were carried out in hypoxic conditions, revealing that most echo intensity biological backscattering (S,
data) zooplankton remained occurred above the hypoxic boundary layer (Fig. 5 and Fig. 9). Thus, lower DO values in Puyuhuapi Fjord acted as a barrier to DVM and the macrozooplankton distribution throughout the year. Similar findings were reported in Oslofjord, Norway, where hypoxic conditions dominated the water column beneath ~60 m depth, and no fish or krill were observed below this depth (Røstad and Kaartvedt, 2013).

Moreover, in Eastern South Pacific OMZ, it has been previously reported that in other Chilean waters for a number of copepod species and life-stages avoid hypoxic waters (Castro et al. 1993, Escribano et al. 2009), as well as for most gelatinous zooplankton groups (Pages et al. 2001; Giesecke and Gonzalez 2005; Escribano et al. 2009). In the same OMZ region, but further north in Peruvian waters, two diurnal scattering layers were observed, one over the OMZ and other, mainly composed by adults euphausiids, in the core of the OMZ (Ballón et al., 2011). Euphausiids, salps and myctophid fish were also observed in the core of Eastern Tropical North Pacific OMZ (Mass et al., 2014). Seibel et al., (2016--) reported Euphausia eximia and Nematoscelis gracilis tolerance to hypoxic water and suggest this tolerance would enable these species to reduce their energy expenditure in at least 50% during their daytime migration.

In Puyuhuapi Fjord, the highest S values observed in the Puyuhuapi Fjord occurred at DO concentrations ranging from between 2 to and 5 mL L⁻¹ while in the Jacaf Channel with DO concentrations between 3 to 6 mL L⁻¹. This indicates that DO values of 3.5 mL L⁻¹ and 4.5 mL L⁻¹ seemed to represent appropriate conditions for most macrozooplankton species in the Puyuhuapi Fjord and the Jacaf Channel (Fig. 9), respectively, which are similar to the values indicated by Ekau et al. (2010) for zooplankton. Our results also showed that macrozooplankton preferred -oceanic waters with salinity values >31 g/kg, and temperatures between 8 and 10° C (Fig. 9). Nonetheless, it must be considered that these preference values were estimated from observational data, and therefore limited sampling, rather than from controlled experiments.

In our study, hypoxic conditions regulated the depth limit of zooplankton DVM. This implied that hypoxic boundary layers are an important environmental factor promoting the generation of a dense zooplankton scattering layers in Puyuhuapi Fjord.

The Vertical overlapping observed between fish and macrozooplankton abundances at nearly the same layers suggests also that the prey-predator relationships interactions might be enhanced under these hypoxic conditions, as evidenced in the macrozooplankton and fish
echograms (Fig. 5). In the context of pollution and climate change, are hypoxic conditions
continually expanding the extent of hypoxic waters around the world, both in coastal waters and open oceans (Breitburg et al., 2018). While the links between recent anthropogenic perturbations, such as the salmon aquaculture expansion, and hypoxia in the Patagonian Fjords is still under debate, it is important to recognize that this impact upon habitat reductions and enhanced prey-predator relationship enhancement interactions under consideration, which in turn, as it might cause changes in zooplankton groups’ distributions and abundance, particularly those that do not tolerate low DO concentrations.

The fact that some biological backscattering occurred was detected within the hypoxic water layer in our study indicates that hypoxia does not affect all macrozooplankton species equally and that some of them can inhabit this deeper layer, e.g., euphausiids species (Mass et al., 2014; Seibel et al., 2016). Hypoxia tolerant species residing below and within minimum DO layers have been reported, in fact, further north along the Chilean coast during the upwelling season, leading to support hypotheses on predation evasion and horizontal transport aimed to explain such behavior (Castro et al., 2007). Within this context Euphausia pacifica has been reported to exhibit the highest abundance of zooplankton species present in hypoxic waters in Hood Canal, Washington (Sato et al., 2016). Other euphausiids have also been reported to be present in other hypoxic systems in Chile (Escribano et al., 2009; Gonzalez et al., 2016). It has been shown that Euphausia vallentini is a dominant euphausiid species, known to carry out extensive vertical migrations in Patagonian fjords, hence we speculate it might be one of the species occurring in the less oxygenated waters of our study. Unfortunately, due to sampling gear restrictions, we were unable to sample the hypoxic layer, nor to identify firmly the species occurring at this depth. Therefore, future research will be necessary to understand the relationship of the deep, yet scarce, macrozooplankton within the hypoxic waters in Puyuhuapi Fjord. As vertical mixing is a mechanism that could reduce the presence of hypoxic zones in fjords, values of TKE dissipation were compared to the depth strata of macrozooplankton.

5.3 Turbulent mixing at the fjord sill
Patagonian fjords and channels cover an area of ~240,000 km$^2$ and feature a complex marine topography, including submarine sills and channel constrictions (Pantoja et al., 2014; Inall and Gillibrand, 2010). Bernoulli aspiration, internal hydraulic jumps and intense tidal mixing are all processes that can be found near a fjord sill (Farmer and Freeland, 1983; Klymark and Gregg, 2003; Inall and Gillibrand, 2010; Whitney et al., 2014). Our data showed elevated values of dissipation rate of turbulent kinetic energy, $TKE_{dissipation}$ in the Jacaf Channel ($\varepsilon = 10^{-5}$ W kg$^{-1}$ and $K_{\rho} = 10^{-3}$ m$^2$ s$^{-1}$) near the sill in the subsurface layer (from 0-60 m depth). These values are similar to those observed at the sill of Knight Inlet in Canada (Klymark and Gregg, 2003). Lower $\varepsilon$ values were found in Puyuhuapi Fjord (Fig. 11). The elevated vertical mixing (high $K_{\rho}$) in Jacaf Channel is probably due to the barotropic tide interacting with the submarine sill (Schneider et al., 2014; Fig. 11, Fig. 13 and Table 2). This was also observed in Martinez Channel (Pérez-Santos et al., 2014), Central Patagonia, where semidiurnal internal tides were found to dominate the estuarine dynamics (Ross et al., 2014). This region is highly influenced by the Baker river, whose discharge enhances stratification and introduces suspended solids that subsequently limit productivity in the water column (González et al., 2010; Daneri et al., 2012; González et al., 2013).

The evident aggregation of macrozooplankton and fish found near Jacaf sill (within ~1 km) matches the area exhibiting the highest $\varepsilon$ values ($\varepsilon = 10^{-5}$ W kg$^{-1}$; Fig. 13). Thin (2-5 m) and thick (10-50 m) regions of enhanced vertical shear layers measured directly with the VMP-250 microstructure profiler contribute to vertical mixing. Subsequently this enhances the exchange between the subsurface rich nutrient layer (Fig. 2) and the photic layer, leading to increased phytoplankton productivity (Montero et al., 2017a; Montero et al., 2017b), as shown in the conceptual model of figure 14. Thus, the acoustic and turbulence measurements collected near the Jacaf sill promote the importance of a sill modulating vertical mixing and also influencing the vertical distribution of oxygen, macrozooplankton and fish on both sides of the sill.

The effect that sills have on zooplankton distribution has been studied in a variety of systems, including fjords (McQuillen 2005, Ianson et al., 2011), using both field sampling (stratified zooplankton sampling, echo-sounding, or in situ imaging) and biophysical modeling (Ianson et al., 2011). The interaction of horizontal flows with a steep sloping surface usually results in a vertical component of the flow inducing turbulence that can be
sensed by zooplankton. Multiple hypotheses have been formulated to explain zooplankton concentration around sills aside from increased productivity. Depending on the species swimming capabilities, areas of increased turbulence may favor zooplankton feeding aggregations by increasing the likelihood of encounters between the zooplankton and their prey (Rothchild and Osborn, 1988). If a rotational flow develops above the sill (e.g., Taylor columns), both zooplankton and their food may also concentrate around the sill, thus increasing plankton retention. Turbulence generated around the sill enhances ventilation of the water column and reduces the extent of hypoxic layers, which allows zooplankton to migrate and accumulate at deeper depths.

A summary of the processes that can contribute to macrozooplankton vertical distribution and aggregation in Puyuhuapi Fjord and Jacaf Channel are presented in a conceptual model (Fig. 14). In Puyuhuapi Fjord, a shallow oxycline around 1000 m depth separates a high nutrient and high production layer (Daneri et al., 2012; Montero et al., 2017a; Montero et al., 2017b) from a hypoxic layer below, which limits species distribution and lacks significant aggregations of zooplankton. Above the hypoxic waters, turbulent mixing favors contact between macrozooplankton predators and their prey (Visser et al., 2009). In Jacaf Channel, the hypoxic layer occurs deeper in the water column than in Puyuhuapi Fjord, which stretches the vertical distribution of macrozooplankton to a deeper range. Turbulent mixing also increases primary and secondary production, through enhanced nutrient availability and favors encounters of macrozooplankton with potential prey, increasing growth and survival rates (Visser and Stips 2002; MacCready et al., 2002; Klymak and Gregg 2004; Lee et al., 2005; Visser et al., 2009; Whitney et al., 2014).

5.4 Other findings and considerations

Results showed similar groups of macrozooplankton (>5 mm) in Puyuhuapi Fjord and Jacaf Channel: euphausiids, chaetognaths, medusae and siphonophores during summer (January 2014) and winter (winter 2014). However, euphausiids were not observed in fall 2013, which was an unexpected result which deserves further confirmation and analysis. In contrast, fall 2013 sampling presented the highest acoustic abundances within the time series (Fig. 3). The elevated accumulation of macrozooplankton species around the sill may impose a significant modification in the amount and quality of carbon exported to deeper waters in
particular zones of the fjords. Future studies on carbon flux quantification in fjords should incorporate sill regions to test this hypothesis, in order to improve ocean pumping assessments in the context of climate change and variability.

6 Conclusions

- A hypoxic layer (DO < 2 mL L\(^{-1}\) and <30% oxygen saturation) was observed below ~100 m depth along Puyuhuapi Fjord whereas in Jacaf Channel it was deeper. This is because the water column in Jacaf Channel was more ventilated due to enhanced vertical mixing caused by tidal flow interacting with the shallow sill.

- Diel vertical migration (DVM) of zooplankton was detected in Puyuhuapi Fjord and Jacaf Channel using ADCP backscatter signal and scientific echo sounders. In Puyuhuapi Fjord most of the larger migrating zooplankton species stopped DVM above the hypoxic boundary layer, therefore implying that they can not tolerate hypoxic conditions. As the hypoxic layer was located deeper in the water column in Jacaf Channel, due to the enhanced turbulent mixing at the sill, the zooplankton DVM patterns penetrated deeper in the water column.

- The most prevalent zooplankton groups detected with \textit{in situ} zooplankton net sampling were siphonophores, chaetognaths, euphausiids, medusae and copepods. A correlation significant non-linear relationships was demonstrated between oceanographic variables and the relative abundance of macrozooplankton biological backscattering (DO, \(R^2=0.6\), salinity, \(R^2=0.3\) and temperature, \(R^2=0.35\)). This highlighted finding suggest the preference of macrobiological dominating scatterers macrozooplankton for well oxygenated water (3-6 mL L\(^{-1}\), 60-80% saturation) and temperatures of 8-10°C, conditions characteristic of the MSAAW and SAAW oceanic water masses. (MSAAW and SAAW), zooplankton for well oxygenated water (3-6 mL L\(^{-1}\), 60-80% saturation) and temperatures of 8-10°C, conditions characteristic of oceanic waters (MSAAW and SAAW).

- Scientific echo sounder records showed high aggregation of zooplankton and fishes around the Jacaf sill, where high dissipation rates of turbulent kinetic energy (\(\varepsilon \sim 10^{-5}\) W kg\(^{-1}\)) and vertical diapycnal eddy diffusivity (\(K_{\rho} \sim 10^{-4} \text{ m}^2 \text{ s}^{-1}\)) were recorded. Turbulence therefore contributed to vertical mixing in Jacaf Channel, which...
promotes enhanced interchange of nutrients, zooplankton feeding, and prey-predator relationships overlapping, and organic carbon cycling.

This paper was aimed to determine how hypoxic conditions affect the vertical distribution of macrozooplankton in fjords and to assess how vertical mixing relates to abundances of macrozooplankton at fjord sills. Results showed that the hypoxic layer in Patagonian Fjords limits DVM and overall distribution of macrozooplankton to the upper ~100 m of the water column, reducing the habitat of these species. The hypoxic zones were found away from underwater sills or areas that would experience enhanced turbulence. When assessing the abundance of macrozooplankton in conjunction with TKE dissipation near a submarine sill it was found that elevated turbulence generated by the barotropic tide interacting with the sharp bathymetric feature enhanced vertical mixing, deepened the hypoxic layer and injected nutrients. In addition, macrozooplankton were found in higher densities and extended deeper in the water column around the submarine sills. This is thought to be due to an increase in primary production that would result from the effects of elevated vertical mixing.

Acknowledgment

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Figure captions

Figure 1. Study area in relation to South America and the Pacific Ocean is the small panel in the top right. The main figure enlarges the study area (Puyuhuapi Fjord and Jacaf Channel) and indicates the instruments used for data collection, fixed point station positions, and the sill location near the head of Jacaf Channel. The contours indicate the depth of the fjords.

Figure 2. (Upper panel) Profiles of temperature (a-b), salinity (c-d), dissolved oxygen (e-f) and nitrate (g) collected during different oceanographic campaigns in the northern central part of Puyuhuapi Fjord and (lower panel) in eastern region of the Jacaf Channel. Hydrographic measurements from Puyuhuapi Fjord. (a) Map of the study area showing the transect (red lines) conducted on June 16, 2016 where black dots indicate locations where profiles were taken, (b) Conservative temperature, (c) absolute salinity, (d) dissolved oxygen and (e) oxygen saturation. For (b) to (e) the black vertical lines denote along-channel profile locations.

Figure 3. (a) Volume backscattering strength ($S_v$) calculated from the ADCP-1 backscatter signal in Puyuhuapi Fjord, deployed at 50 m depth from the 8th to the 26th of May, 2013. (b) Zoom of the $S_v$ data and the times of in-situ zooplankton sampling (black dots) carried out during May 25-26, 2013. (c-d) Vertical abundance of main zooplankton groups (>5 mm length) from the in-situ sampling at 16:00 and 18:00 (local time)-h on May 25th and (e-fd) at 9:00 and 11:00 (local time)-h on May 26th.

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Figure 5. Single frequency Along-fjord transects using a (38 kHz) scientific echo-sounder SIMRAD with 38 kHz frequency transect conducted along the Puyuhuapi Fjord during the Summertime field campaign (January 2014). Distribution indicated by colors representing $S_v$. (a) Daytime transect of echo-sounder measurements ($S_v$) throughout depth (y-axis) from the...
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Figure 6. (a) Dual-frequency (38 and 120 kHz) scientific echo-sounder transects along Puyuhuapi Fjord (0-18 km) and Jacaf Channel (18-35 km) during nighttime on August 17, 2014 using the combination of 38 and 120 kHz frequency. (a) Fluid like and (b) blue noise echogram for zooplankton and (c) the fish echogram. Distribution indicated by colors representing S<sub>v</sub> values. The black arrow in (a) represents the entrance to Jacaf Channel. Horizontal red lines in (a, b, c) denote lower limits of usable acoustic data (250 m).

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Figure 10. Profiles of water temperature (blue line), vertical shear (red line) and dissipation rate of turbulent kinetic energy (black line with green dots) obtained with the VMP-250 microprofiler at the depth of the Jacaf sill (~140 m depth) in (a) Jacaf Channel on 21 November 2013 (c) Puyuhuapi Fjord on 22 November 2013 and (e) in Puyuhuapi Fjord on 23 January 2014. (b, d, f) Representative -spectrum of velocity shear ($\partial u/\partial z$) for shear probe 1 (blue line) and 2 (red line) in wavenumber space in Jacaf Channel on 21 November 2013, Puyuhuapi Fjord on 22 November 2013 and Puyuhuapi Fjord on 23 January 2014, respectively. The black line denotes the dimensional Nasmyth spectrum and the red and blue triangles the cut-off of maximum wavenumber ($k_{max}$) for each shear probe. The shear spectrums were carried out in the same layer (135-145 m) for all turbulence profilers.

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48
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Figure 11. (a) Microstructure profile locations along Jacaf Channel and sill using VMP-250 in November 2013. (b) The color bar showed the dissipation rate of turbulent kinetic energy ($\varepsilon$) and the blue lines depict the velocity shear at each station location along Jacaf Channel (as shown in (a). The horizontal scale (-2 to 2 $s^{-1}$) applied to profiles at stations 160, 162 and 163. Station 164 is located at the confluence of Jacaf Channel and Puyuhuapi Fjord (10.5 km) (c) The diapycnal eddy diffusivity profiles ($K_p$), obtained at each station shown in (a).
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<th>Date</th>
<th>Season</th>
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<th>Instruments</th>
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<td>-WP2 net</td>
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58
Table 2. Harmonic analysis implemented to water level time series in Puyuhuapi Fjord and Jacaf Channel.

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<th>Sea level time series</th>
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<th>Amplitude of principal constituents (cm)</th>
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<th>Tidal regime</th>
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<td>M₂: 83.45, S₂: 28.32, O₁: 14.46, K₁: 22.33</td>
<td>0.32</td>
<td>Mixed semi-diurnal</td>
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<td>Jacaf- ADCP</td>
<td>08/2014-05/2015</td>
<td>57.29</td>
<td>M₂: 60.67, S₂: 61.01, O₁: 57.78, K₁: 42.48</td>
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<td>Puyuhuapi- HOBO south</td>
<td>02-06/2016</td>
<td>44.45</td>
<td>M₂: 81.97, S₂: 31.51, O₁: 13.37, K₁: 18.36</td>
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<td>Puyuhuapi- HOBO north</td>
<td>02-11/2016</td>
<td>49.17</td>
<td>M₂: 89.15, S₂: 31.07, O₁: 11.03, K₁: 17.75</td>
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<td>Semi-diurnal</td>
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