

Thank you for the review of our manuscript. We fully agree with your comment about the importance of data zooplankton sampling for verification of the acoustic backscatter data. The manuscript was revised to address these kinds of questions. We invited Dr. Elena Arashkevich to share with us the Juday net data obtained nearby the profiler mooring. She also contributed her results of analysis of the mesozooplankton species in the samples. We also extend the analysis with the new acoustic data collected most recently (summer-autumn 2020). The manuscript is rewritten in line with yours and the other reviewers' comments. Errors are corrected in the manuscript, the figures are partly replaced, and some materials are brought into the appendix. Below we list your comments together with our responses to them (in blue).

Comment #1: Introduction of the MS did not present lack of the other studies and their innovations to purpose the annual cycle of the SL with the reasons.

Response: Section Introduction is rewritten to review more comprehensively the current status of the mesozooplankton research in the Black Sea and to indicate more clearly the goals of our study.

Comment #2: Material Methods would have data analyses and processing methodology with the methodical terms and study area description for the oxygen and other physical parameters which confine the DVM, e.g. the R and its distinguished importance from the acoustical energy, relationships between the orientation and each transducer of the profiler, acoustical intensity or amplitude calculation, removal of unwanted targets else (fishes, particles, marine snows and some untargeted individuals nearby targeted species relative to frequency) than the zooplankton, detection range of the frequency and dynamic ranges proportion to the aforementioned zooplankton.

Response: The simple method for processing the Nortek Aquadopp data of ultrasonic sounding of the water column at three angles is based on earlier model and laboratory studies (Stanton, Chu, 2000; Roberts and Jaffe, 2007, Roberts and Jaffe, 2008). It allows to distinguish the mesozooplankton sound-scattering layers against the background of vertical flows of settling particles while taking advantage of the fact that the acoustic scattering is isotropic on the settling particles and anisotropic on zooplankton species due to the elongated shape of the animals because their side view area is larger than the head-view area or the tail-view area. For the Nortek Aquadopp acoustic Doppler current meter, the data on sound scattering amplitudes is essentially a by-product. The key to using high-frequency sound in this study is to deploy the acoustical transducer in a manner that gets it sufficiently close to the animal aggregations of interest. Notice, the orientation of the animals in the deep aggregations is poorly explored

(except e.g., Dagg, M. J. (Sinking particles as a possible source of nutrition for the large calanoid copepod *Neocalanus cristatus* in the subarctic Pacific. *Deep Sea Research I.* 40, 1431–1445, 1993), Kiørboe T. (How zooplankton feed: mechanisms, traits and trade-offs. *Biol. Rev.* 86, pp. 311–339. 311. doi: 10.1111/j.1469-185X.2010.00148.x, 2011), see also unpublished report by Ashjian et al.

https://www.researchgate.net/publication/266329080_Spatial_and_Temporal_Variability_of_Zooplankton_Thin_Layers_The_Effects_of_Composition_and_Orientation_on_Acoustic_Detection_of_Layers, 2008)). Our study qualitatively indicates that the mesozooplankton species basically maintain vertical orientation in the deep aggregations in the Black Sea. Unfortunately Nortek Co. does not provide any calibration data on the Aquadopp so one cannot assess relevant acoustic characteristics that you mentioned. However, empirically, we realized that the Aquadopp observational data can be useful for research on the mesozooplankton if the data is processed in the way that we suggested.

Comment #3: Results showed only pure SL moving up/downward and staying at constant depths during the days of the different months. On surface SL, one or two SL migrating daily, and one DSL staying at constant depth in time looking at the R terms which lack of importance and distinguished description of the other scattering energy units... The beam pattern of the organism is needed to outdraw depending on the orientation and shape during the both direction migration and duration at deep depth and surface, shall the three transducers particularly A3, help information on the body shape and orientation.

Response: The beam pattern is shown on Fig. 1. Since the Aquadopp instrument moves up and down along the mooring line, the beam pattern remains unchanged through the water column. Our interpretation of the empirical data is that the orientation of the mesozooplankton species is changed from the random in the upper part of the oxic zone ($[O_2] > 200 \text{ mkm}$) to the vertical in the oxygen-deficient zone.

Comment #4: The 2 MHz was expected to detect particles in size down to 0.2 mm in diameter equivalent to the spherical particles regardless of the beam pattern of the organisms.

Response: The mesozooplankton species composition in the deep aggregations is presented in detail in the revised ms. The typical sizes of the mesozooplankton species are given at the end of subsection 3.1.

Comment #5: The 2 MHz must detect many species but only one or sometimes two SL were observed during the diel movement. As a consequence, some studies showed two scattering

layers belonging to two different species of fluid-like organisms depending on their acoustical reflection coefficients in the Black Sea (copepods, Cheatognatha) as well as significant detection of moon jellyfish using rather lower frequencies than the frequency used in the MS. Main of the results and findings have showed one scattering layer migrating during the day, which could be more number of scarring layer in the present study.

[Response: The sound-scattering layers \(SSLs\) are validated via the net zooplankton sampling in the revised ms.](#)

Comment #6: The SL was compared only with dissolved oxygen and not other physical parameters such as sigma-t of water density which describe the DVM in the Black Sea, and study area was well described for the regional differences such as upwelling or downwelling, rim currents which course the DVM and their speed across oxygen and water density so depth of water column. Daily differences could be ignored using the water density along the rim currents. Some vertical lineations occurred from surface down to greater depth of the SL which could be attributed presumably to the particle sedimentations. The SL amplitude as counts showed daily differences in same months without a reasonable explanation. Mostly, one SL migrated between surface and a certain depth above the minimum oxygenated layer, not reaching the hypoxia layer, but other studies observed different DVM reaching deep layers as well as staying at minimum oxygen layer during diapausing and daytime.

[Response: The SSLs are compared with the water density vertical distribution in the revised ms. The main objective of our study is the temporal \(seasonal\) rather than spatial variability.](#)

Comment #7: The R values are expected to changes in time during the DVM, because mainly of the changes in individual swimming speeds and organisms concentration insonified volume by the acoustics. Therefore, what is importance of the R to denote significance in the intensity of the SL. The swimming speed through the oxygen concentration is one of the recognition parameters identical for some species migrating during the day in the Black Sea. When the DSL arrived non-migrating SL at sub-surface, there was however no aggregation of the SL during the night. Inherently, some scattering layers must occur at sub-surface even if the orientation of the organisms change over there.

[Response: We agree that the estimates of the DVM speed based on the acoustic data does not account for the fact that the different components of zooplankton have different swimming speed also the different species start the DVMs from different depths. So in the revised ms, we decided to remove the relevant part of the discussion.](#)

Comment #8: What is the difference between Fig. 11 and 12 as well as Fig. 9, one was average of amplitude of A1 and A2, the next one is based on their ratio through the same water column denoted with the oxygen concentrations. Indeed, both figures contain zooplankton migrating upward and downward through the same water column in order to show the orientation of the organisms. They had similar information.

Response: Fig. 12 is brought into Appendix and Fig. 9 is deleted.

Comment #9: Breaking apart from the DVM SL, some SL returned back or stopped going down at the middle way of the water column during the DVM as shown in Fig. 10. In general, the echogram data show revealing much information remained unexplained because of lacked one of the ground-truthing methods, discrete layered zooplankton samples.

Response: The net zooplankton sampling data are added extensively in the revised manuscript to validate the acoustic backscatter observations.

Comment #10: Discussions were not well written to justify the findings, observations and acoustical parameters used in the MS. Most of the results were postulated to the assumptions for the justification. One DVM SL was predominated in the present study, seemed to be typical characters of *Calanus euxinus*' DVM from a region of a downwelling zone, not reaching the minimum oxygen layer of the Black Sea. Juday net samples are missing to show the DVM of the zooplankton *Calanus euxinus* and *Pseudocalanus elongates*, which are claimed to be observed by the acoustics, but one DVM SLs are presents overall in the MS. The swimming speed estimated in the MS was discussed with those of the other studies, but there were no data for the swimming speed of the zooplankton in the results MS. Why other zooplankton which contain the similar body material properties to two targeted copepod species did not appear in the SL of the DVM using the very high frequency, even though the results were discussed for the two mesozooplankton.

Response: Section Discussion is rewritten in line with your and the other reviewers' comments.

Comment #11: Such questions could be clarified already in the MS but the English of the MS is not comprehensible to me.

Response: Prior to submission of the preprint it was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English speaking editors at AJE. This certificate can be verified on the AJE website (aje.com/certificate) using the verification code 1418-EF73-F68E-3B50-E63P.