Dear Editor, Below follows a response to the comments provided by Referee #1. We list the comment of the referee and respond to it after 'Hoving et al'.

Referee #1: This manuscript provided interesting results but it still needs revisions to be acceptable for publication. To improve the quality and readability of this paper, the following remarks and suggestions are to be considered in view:

Referee #1: Abstract: This part is fine and there is no real need for corrections.

Referee #1: Introduction: Line 32: "have been sampled with nets". You might want to add a reference (e.g., Wiebe and Benfield (2003): From the Hensen net toward four-dimensional biological oceanography)

Hoving et al: We added the suggested reference.

Referee #1: Line 33: "a community typically consisting (: : :)" Add a reference.

Hoving et al: We added Benfield et al. 1996 as a reference (comparison MOCNESS to VPR).

Referee #1: Sentence at lines 38-42: "This was particularly true for fragile gelatinous zooplankton.." add some references

Hoving et al: We added and re-organized references to assign references to different delicate faunal groups.

Referee #1: Line 49-50: "pelagic ROV surveys have been applied to study inter and intra-annual variation in mesopelagic zooplankton communities". You can add the following reference: "Hull et al. (2011) Seasonality and depth distribution of a mesopelagic foraminifer, Hastigerinella digitata, in Monterey Bay, California"

Hoving et al: We added suggested reference.

Referee #1: Lines 56-60: I would move the Benfield reference to the first sentence.

Hoving et al: This was moved as suggested.

Referee #1: Line 60: "Examples of instruments include:" You can add the following reference to the Zooglider, an in situ imaging device mounted on a glider (something new compared to the other systems you mention). Reference: Ohman et al. (2018?) Zooglider: An autonomous vehicle for optical and acoustic sensing of zooplankton

Hoving et al: Added suggested reference.

Referee #1: Material and Method: Link at line 123 not working: Hoving et al: The video has been included as ESM as part of the MS

Referee #1: Sub-section 3.4. I am somehow concerned with the way you convert counts/sec to abundances.

Hoving et al: we have split the questions/concerns and address them separately below.

Referee #1: Are Poebius abundant enough for this kind of comparison?

Hoving et al. We specifically chose Poeobius because its abundance ranged from zero to a (given its size) very high abundance of >1 m-3. There is no other species that is as abundant and well identifiable in both instruments and that lacks an escape response.

Referee #1: How do deal with patchiness in this comparison?

Hoving et al: For the sake of the regression, we disregard patchiness as we use the mean abundance (ind m-3) and mean count (ind s-1) encountered during an entire transect (between 9 and 22 min).

Referee #1: The regression that you show in Figure 3 show multiple points where no Poebius were detected with the UVP, while observed with the Pelagios? How do you explain this discrepancy? If you remove those points, do you still have a significant regression?

Hoving et al: The sampling volume is much smaller in the UVP, and it does not record continuous video, but image "slices" with a space in between images. This explains the fact that at low abundances Poeobius may be encountered with PELAGIOS, but not imaged by the UVP. If these points are removed, the regression is still significant and the slope changes from 0.12 to 0.13 (see figure A and B attached). The coefficient of determination decreases from 0.69 to 0.52. In our view, it does not make sense to exclude the "zero" observations from the UVP and/or to force the regression through the offspring, because this offset reflects the "missing" Poeobius that are not observed by the UVP at low abundances.

Referee #1: Regression including "zero" observations in the UVP (Figure A) and with these points excluded (Figure B).

Referee #1: Is there another way to estimate the Pelagios sampled volume, independently from the UVP comparison? It is important to make this point crystal clear as you are making a direct comparison with MOCNESS abundance later on.

Hoving et al: One of the future goals is to improve the quantification of the sampled volume, for example by using a current meter.

We consider the UVP comparison a good comparison but another way of estimating the field of view is by measuring the area of the image with the scale bar at 1 m from the camera. We inserted this in the text "A cross-sectional view field of approximately 0.23 m2 of PELAGIOS can be expected, compared to a theoretical FOV of 0.45 m2 based upon the maximum image dimensions (0.80 m * 0.56 m) at 1 m distance from the lens."

The actual width of view (and hence the field of view) is likely less wide since the view deteriorates to the side. We have moved the PELAGIOS and MOCNESS comparison to the discussion.

Results:

Referee #1: Line 203-223: Do you need to mention every organism that you encountered? Can you somehow make it shorter? It would be nice to have an illustration of the dominant taxa observed by the device (rather than a simple table). It will provide more information for the reader, and potentially raise interest on your device. If you are limited by the number of figures, it could be a supplementary figure.

Hoving et al: We have rewritten this paragraph to be more concise. We have added a figure as suggested, and now have one figure with example gelatinous fauna (Figure 5) and another with observed behaviours (Figure 6).

Referee #1: Line 214: "typical examples of organisms that cannot be captured by nets". Do you have proof of that? (i.e., publication).

Hoving et al: We have changed this sentence to read: "Typical examples of fragile organisms that were not present or identifiable in the MOCNESS samples but which can be efficiently observed by PELAGIOS include (...)" to clarify that we here directly refer to comparative net hauls (specified before as we moved the MOCNESS comparison down).

Referee #1: Line 214: "can be properly quantified by PELAGIOS". Since you don't have a baseline for your quantification, you cannot say that your device "properly" quantifies these organisms. You might actually undersampled them by having a small sampling volume. You can just say "efficiently observed".

Hoving et al: changed according to suggestion.

Referee #1: Line 224-233: Refer to my comment for the Methods section. Everything relies here on your conversion factor. A slight change will affect your abundance estimations and ultimately the comparison with MOCNESS abundances. Also, you say that there is an underestimation by MOCNESS but don't provide any data/proof to the reader.

Can you summarize the information in a table/figure? Also, why only mentioning the example of Beroe? What about the other taxa mentioned previously (e.g., Poebius?). What's the rationale behind the choice of Beroe?

Hoving et al: For intercomparison between two instruments, we need to choose organisms that we can identify in both. Beroe is an example of a comparatively large, sturdy ctenophore that could also be identified in net hauls, but seems to be underestimated as is it often severed in the catch. As for Poeobius, we have never been able to retrieve this organism using nets in the Eastern Tropical Atlantic (not even with a small 200µm multinet), but we can identify it on UVP images, and since it does not have an escape response and falls well in the UVP size range, we assume that UVP observations are quantitative. We have added some considerations on the

accuracy of the sampling volume and area in the results and moved the comparison with MOCNESS to the discussion (lines 379-391).

Referee #1: Sub-section 3.6: Since you made these observations, can you modify Figure 5 (or create a new figure) to provide the visual proof of what you mention in this paragraph?

Hoving et al: We have added a new figure (Figure 6) that illustrates the behaviours observed with PELAGIOS as described in the text.

Discussion:

Referee #1: A general comment regarding this section. There is a lack of references throughout the discussion. We cannot rely only on the author's sayings. I recommend reviewing this section to have clear reference for every/most points you make. Several points are highlighted below. Line 250: "tool that fills a gap in the array of observation instruments that exist". How does the PELAGIOS fill a gap? What gap? You have to develop your point here.

Hoving et al: We have added a couple of sentences to better clarify the need for video observations on transparent, fragile fauna (lines 88-93; 295-306). We also added additional references.

Referee #1: Viewed from a pessimistic point of view, PELAGIOS can appear as another device wanted by an institution locally, but it will probably never be used outside of this institution. For example, in your introduction, you made the comparison with ROV-video transects. In this case the PELAGIOS appears like an interesting "cost-effective" alternative. Compared to other "well-known" in situ imaging systems (e.g., UVP, VPR), the PELAGIOS does not really provide anything new... You have to better make your point.

Hoving et al: PELAGIOS does provide something new. It allows cost effective observations in a similar way as ROV horizontal transects. It allows the visualization of fauna > 1cm. We have tried to better make our point in the first paragraph of the discussion. PELAGIOS does not cover the same range of planktonic organisms that the VPR or UVP do; there is only a fairly small overlap. We are not aware of a functional instrument that does. We do not attempt to compete with the UVP5 but consider them as complementary tools as we show in our comparison.

Referee #1: Lines 255-257: "The data obtained after annotation of the video can be uploaded into databases (e.g., Pangaea) after publication of the results allowing for efficient data sharing and curation". Any journal requests open-access to published data, you don't have to write this down... Actually, some open-access alternative offers data sharing before publication... (e.g., Ecotaxa, Plankton portal), so it is not even attractive to write such a sentence....

Hoving et al: We have had trouble to obtain raw data from other optical instruments for crosscomparison, so we feel it is valid to point out that data shall be made available on queriable databases (prior to or after publication).

Referee #1: Line 273: "lateral migration of animals towards Senghor seamount at night". Reference?

Hoving et al: We have changed the sentence and added three references.

Referee #1: Line 279: "After annotation, the PELAGIOS video transects may be used to reconstruct species-specific distribution patterns, which can be related to environmental gradients". You have to keep in mind that your device does not provide proper vertical profiles but rather multiple horizontal transects. Compared to other systems (e.g., ISIIS, UVP, VPR, etc.) it does not seem to be the best choice of tool to reconstruct species-specific distribution patterns... You should stress and discuss this point.

Hoving et al: The PELAGIOS is suitable for visualizing plankton and nekton > 1 cm and therefore is not comparable to ISIIS or UVP and we do not attempt to compete with these devices which are highly suitable for quantification of distribution of mesozooplankton and particles. The PELAGIOS video transects are comparable to horizontal ROV transects, and can be used to detect fragile fauna and reconstruct species-specific distribution patterns of larger macrozooplankton, as we show here and in cited publications that use PELAGIOS data. Our deployments were so far typically horizontally since we wanted to have more data from one depth to reconstruct the vertical species distributions. If desired one could deploy PELAGIOS vertically for studies on spatial distribution.

Referee #1: Line 294: "Preliminary comparisons of the data obtained with PELAGIOS and with MOCNESS indicate substantial differences in the documented fauna". See my comments previously. If you don't have further arguments for a robust comparison, you definitely have to stress the uncertainties of your regression.

Hoving et al: We have moved the section on the comparison between PELAGIOS and MOCNESS to the discussion section to emphasize it is an exploration of the obtained data. We particularly refer to the difference in number of taxa in this paragraph, and explore the quantitative difference using the volume from the UVP-PELAGIOS comparison. The uncertainty of this regression is given in the manuscript. Even without the quantitative comparison, and considering only the presence and absence data, substantial differences are obvious. We also state that we are striving to improve the quantitative sampling of the system as part of future development.

Referee #1: Lines 294-306: Not a single reference here. You should include more references in order to provide background information for your argumentation. For example, you did not mentioned Remsen et al. (2004) paper where similar comparison between imaging device and nets were made.

Hoving et al: We have added more references throughout the discussion including Remsen et al 2004

Referee #1: Lines 307-326: I agree with your point that in situ imaging systems can provide useful information for the significance of fragile organisms to pelagic ecosystems & biogeochemical cycles, but your last comparison with the UVP highlights one of the weakness of the PELAGIOS device. Systems like the UVP or the VPR are not the most advanced systems by

far but they have extensive datasets (like you show). It would take decade for a new system like the PELAGIOS before providing extensive datasets enabling studies a large/global scales.

Hoving et al: Even if PELAGIOS does not turn out a standard observation instrument (such as the UVP and VPR, which can be readily integrated to other platforms and have a streamlined image processing pipeline), it is a valuable tool to quantify organisms that are up to now missed by any other quantitative routine observational system, and that are play important roles in the ecosystem and for biogeochemical cycles. We have added sentences in the first paragraph of the discussion to point out where the instrument fills a gap. At the same time, PELAGIOS can be adapted to fit on a CTD or other plankton observation platforms, and with enough effort, large datasets can follow. It should again be mentioned that PELAGIOS collects video transect data and has a different purpose that the UVP and VPR. See earlier comments.

Referee #1: Lines 317-320: "This was illustrated by the discovery of the pelagic polychaete Poeobius sp. during the PELAGIOS video transects in the eastern Atlantic (Christiansen et al., 2018). The observations of the PELAGIOS provided the first evidence for the occurrence of Poeobius sp. in the Atlantic Ocean".

Isn't the Christiansen paper about UVP data? So, does PELAGIOS provide the first evidence of Poebius in the NA? Also, you then mention the distribution patterns of Poebius, revealed by UVP/CTD and not PELAGIOS? what did PELAGIOS brought to this study (apart from the "discovery"?). If you did not have the UVP/CTD system, would PELAGIOS have been able to provide such information?

Hoving et al: Yes, PELAGIOS did provide the first video observation of Poeobius in the Atlantic. Only after this discovery, we checked the extensive UVP image database, found it there as well and created a category for automatic sorting (followed by manual validation) for all available profiles, which then resulted in the dataset presented in Christiansen et al. 2018. The PELAGIOS also provided in situ observations that allowed the estimation of the size of the mucus net for the study. While most of the distribution data came from the UVP5, the discovery was made by PELAGIOS. It was the combination of tools that made an integrative detailed study on the ecology of the species possible.

Referee #1: Line 330: "The joint deployment of the PELAGIOS and UVP also allowed a quantification of the sampled water volume of the PELAGIOS as described above". See my comments above.

Hoving et al: comments noted and responded to

Referee 2

Dear Editor, Below follows a response to the comments provided by Referee #2. We list the comment of the referee and respond to it after 'Hoving et al'.

Referee #2: First, I would like to mention that I'm not an expert in this field and can therefore not comment on the methods. I'm specifically thinking of section 3.4. While I think the manuscript was carefully written, I did find a few things that need to be clarified. Lines 56-57 say: "In the last decades, a variety of optical instruments has been

developed to image and quantify plankton in situ." But then lines 73 -75 say: "However, published descriptions of optical systems, other than ROVs and submersibles, that visualize macrozooplankton and micronekton (>1 cm) in the water column are, to the best of our knowledge, restricted to one (Madin et al., 2006)." This is confusing as it is currently not clear what the difference is between the above mentioned instruments and the ones that have not been described in publications. Maybe mention in lines 73-73 that there are no other instruments capable of capturing such large organisms?

Hoving et al: In the revised version, we have tried to point out the novelty and differentiating characteristics of the instrument and that PELAGIOS is mainly designed to make video observations of large, transparent, fragile organisms, which fills a gap in the current instrument array available.

Referee #2: 75 ff Please be more specific about what makes PELAGIOS different from LAPIS

Hoving et al: We have added information about LAPIS that indicates the difference. For example LAPIS used still imagery, PELAGIOS uses video allowing documentation of behaviour; LAPIS has an illuminated box in which the organisms are photographed, PELAGIOS has forward illumination similar to an ROV. PELAGIOS data can be compared with ROV video transects. There are no additional publications that show LAPIS data and hence the development and application of PELAGIOS is timely.

Referee #2: Link at line 123 not working:

Hoving et al: The video will be available as ESM in the MS

Referee #2: Line 195: What was the total transect time during the night? Must be the same amount as during the day, if not, did you account for this in your analysis?

Hoving et al: We included the transect time and corrected the comparison since the night transects were in total longer. In Figure 4 the data are corrected for time.

Referee #2: Section 3.5 I find it difficult to read through this section. While it is def. useful to know who lives there, I wonder if there would be a better way to summarize it all in a table and make this section shorter?

Hoving et al: We have rewritten this paragraph to be more concise and improve readability.

Referee #2: 213-215: Do you have a reference for this statement?

Hoving et al: We have added Harbison et al. 1978 as a reference here.

Referee #2: Minor edits Figure captions Figure 2: Why is O2 plotted but never mentioned? Figure 5: Capitalize "Example" Hoving et al: We have integrated the other sensor data in this figure, as an illustration of complementary video and environmental sensor data collection.

1	The Pelagic In situ Observation System (PELAGIOS) to reveal			
2	biodiversity, behavior and ecology of elusive oceanic fauna			
3	Hoving, Henk-Jan ¹ , Christiansen, Svenja ² , Fabrizius, Eduard ¹ , Hauss, Helena ¹ , Kiko, Rainer			
4	Linke, Peter ¹ , Neitzel, Philipp ¹ , Piatkowski, Uwe ¹ , Körtzinger, Arne ^{1,3}			
5				
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11				

12 **1. Abstract**

There is a need for cost-efficient tools to explore deep ocean ecosystems to collect baseline 13 biological observations on pelagic fauna (zooplankton and nekton) and establish the vertical 14 ecological zonation in the deep sea. The Pelagic In situ Observation System (PELAGIOS) is a 15 3000 m-rated slowly (0.5 m/s) towed camera system with LED illumination, an integrated 16 17 oceanographic sensor set (CTD-O₂) and telemetry allowing for online data acquisition and video inspection (Low Definition). The High Definition video is stored on the camera and later annotated 18 using the VARS annotation software and related to concomitantly recorded environmental data. 19 20 The PELAGIOS is particularly suitable for open ocean observations of gelatinous fauna, which is notoriously undersampled by nets and/or destroyed by fixatives. In addition to counts, diversity 21 and distribution data as a function of depth and environmental conditions (T, S, O₂), in situ 22 observations of behavior, orientation and species interactions are collected. Here, we present an 23 overview of the technical setup of the PELAGIOS as well as example observations and analyses 24 25 from the eastern tropical North Atlantic. Comparisons to MOCNESS net sampling and data from the Underwater Vision Profiler are provided and discussed. 26

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28 **2. Introduction**

The open ocean pelagic zones include the largest, yet least explored habitats on the planet (Robison, 2004; Webb et al., 2010; Ramirez-Llodra et al., 2010). Since the first oceanographic expeditions, oceanic communities of macrozooplankton and micronekton have been sampled using nets (Wiebe and Benfield, 2003). Such sampling has revealed a community typically consisting of crustaceans, cephalopods, fishes and some sturdy and commonly found gelatinous fauna (Benfield et al., 1996). Underwater observations in the open ocean via SCUBA diving 35 (Hamner et al., 1975) and later via submersibles (Robison, 1983; Robison and Wishner, 1990) and in situ camera systems (Biard et al., 2016, Picheral et al., 2010) revealed that a variety of organisms 36 are much more abundant in the open ocean than previously estimated from net sampling (Robison, 37 2004)., Haddock, 2004; Biard et al. 2016, Christiansen et al. 2018). This was particularly true for 38 fragile gelatinous zooplankton, a diverse taxonomic group of different phyla, including the 39 40 ctenophores and, medusae (Remsen et al., 2004; Haddock, 2004) as well as siphonophorae, thaliaceans, polychaetes (Christiansen et al., 2018), rhizaria rRhizaria (Biard et al., 41 2016) and pelagic tunicates (Remsen et al., 2004; Neitzel, 2017), larvaceans, which often are too 42 43 delicate to be quantified using nets as they are damaged beyond identification, or they are easily destroyed by the use of common fixatives. 44

Underwater (in situ) observations in the pelagic ocean not only revealed a previously unknown 45 community, they also allowed the collection of fine-fine-scale distribution patterns in relation to 46 biotic and abiotic factors (e.g. Haslob et al., 2009; Möller et al., 2013; Hauss et al., 2016) as well 47 as information on posture, interactions, and behavior (Hamner and Robison, 1992; Robison, 2004; 48 Robison, 1999; Hoving et al., 2017). Submersibles have proven to be valuable instruments to study 49 deep-sea pelagic biology (e.g. Robison, 1987; Bush et al., 2007; Hoving et al., 2013; 2016). Using 50 video transecting methodology, pelagic ROV surveys have been applied to study inter and intra-51 annual variation in mesopelagic zooplankton communities (Robison et al., 1998; Hull et al., 2011) 52 and to explore deep pelagic communities in different oceans (Youngbluth et al., 2008; Hosia et al., 53 54 2017; Robison et al., 2010-). However, due to high costs as well as technological and logistical challenges, regular submersible operations are still restricted to very few institutes and 55 geographical locations. Hence, there is a need for the development of additional more cost-56 57 effective methodologies to explore and document deep-sea communities via in situ observations.

In the last decades, a variety of optical instruments has been developed to image and quantify 58 plankton in situ (Benfield et al., 2007).- The factors that typically differentiate the available 59 plankton imaging technologies are the size fraction of the observed organisms, illumination type, 60 resolution of collected images/video, depth rating, deployment mode (e.g., autonomous, towed, 61 CTD-mounted) and towing speed (Benfield et al., 2007). Examples of instruments include the 62 autonomous Underwater Vision Profiler (UVP5); Picheral et al., 2010), the Lightframe On-sight 63 Key species Investigations (LOKI; Schulz et al., 2009) and towed plankton recorders (ISiiS; 64 Cowen and Guigand 2008; for review see Benfield et al., 2007). These instruments can be deployed 65 66 from ships of opportunity and collect detailed information on fine--scale distribution and diversity patterns of particles and plankton. The data reveal biological patterns on a global scale (Kiko et 67 al., 2017) and of previously underappreciated plankton species (Biard et al., 2016). More recently, 68 optical (and acoustic) instruments have been combined with autonomous gliders, rapidly 69 increasing spatial resolution (Ohman et al. 2019). 70

Various towed camera platforms have been developed that can obtain video transect observations 71 above the deep sea floor. Examples are the TowCam (WHOI), the DTIS (Deep Towed Imaging 72 system, NIWA), the WASP vehicle (Wide Angle Seafloor Photography), OFOS (Ocean Floor 73 74 Observation System, GEOMAR), and the more recent version OFOBS (Ocean Floor Observation and Bathymetry System)-(; Purser et al., 2018). All these instruments are used for video or photo 75 transects of the seafloor, with a downward looking camera, and typically a set of lasers for size 76 77 reference. However, published descriptions of optical systems, other than ROVs and submersibles, that visualize macrozooplankton and micronekton (>1 cm) in the water column undisturbed by a 78 filtering device or cuvette are, to the best of our knowledge, restricted to one (Madin et al., 2006). 79 80 The Large Area Plankton Imaging System (LAPIS) is the only towed system that was developed

for the documentation of larger organisms in the water column (Madin et al., 2006). LAPIS 81 visualizes organisms between 1 and 100 cm, it combines a low lighta -camera high--resolution 82 color digital CCD camera using progressive scanning interline-transfer technology with red 83 illumination flashing strobes, and it is towed at 1 knot via a conducting fibre optic wire. LAPIS 84 collects still images, illumination is sideways, and organisms have to enter an illuminated volume 85 86 to be visualized. Deployments in the Southern Ocean enabled the reconstruction of depth distributions of the pelagic fauna (salps, medusae) but also allowed some behavior observations, 87 e.g. the moulting of krill (Madin et al., 2006). More publications of data collected with LAPIS are 88 89 unavailable to our knowledge. Other than LAPIS, we wanted to develop a towed pelagic observation system that collects video during horizontal transects (with forward projected light), 90 in a similar way as pelagic ROV video transects, in order to document behaviour in addition to 91 diversity, species-specific distribution and abundance data of pelagic fauna. 92

To establish a baseline in abundance, distribution and diversity of the pelagic fauna in its natural 93 environment, we developed an ocean observation platform for pelagic video transects. The 94 functional requirements for the instrument were the ability to: (1) visualize organisms > 1 cm in 95 waters down to 1000 m with high-definition video, (2) deploy the instrument from ships of 96 97 opportunity in an autonomous or transmitting mode, (3) make it lightweight and practical so it can be deployed easily and safe with 2-two deck persons and a crane-winch operator, (4) enable 98 correlation of observations with environmental parameters (S, T, O₂) and other sensor data, and 99 100 (5) make observations comparable to ROV video transects in other reference areas. We present a description of the Pelagic In situ Observation System (PELAGIOS), examples of the kind of 101 102 biological information it may gather, as well as biological discoveries that have resulted from 103 deployments on research cruises in the eastern tropical North Atlantic.

104 **3**.

3. Pelagic In Situ Observation System

105 **3.1 Technical Specifications**

The PELAGIOS consists of an aluminum frame (length = 2 m) that carries the oceanographic 106 107 equipment (Figure 1). White light LED arrays (4 LEDs produced at GEOMAR, 2 LED arrays (type LightSphere of Deep-Sea Power and Light [©]) which illuminate the water in front of the system 108 are mounted on an aluminum ring (diameter = 1.2 m). Power is provided by two lithium batteries 109 110 (24V; 32 Ah) in a deep-sea housing. High-definition video is collected continuously by a forward viewing deep-sea camera (type 1Cam Alpha, SubC Imaging [©]) which is mounted in the center of 111 the ring. We used the maximum frame rate of 50 frames s^{-1} but a lower frame rate is possible. A 112 113 CTD (SBE 19 SeaCAT, Sea-Bird Scientific [©]) with an oxygen sensor (SBE 43, Sea-Bird Scientific 114 ©) records environmental data. A deep-sea telemetry (DST-6, Sea and Sun Technology ©; Linke et al., 2015) transmits video and CTD data to a deck unit on board allowing a low resolutionlow-115 resolution preview (600_x_480 lines) of the high definition video that is stored locally on the SD 116 117 card (256 GB) of the camera. The power from the batteries is distributed to the LEDs via the 118 camera. The 1Cam Alpha camera is programmable in such a way that there is a delay between 119 providing power to the camera (by connecting to the battery) and the start of recording and 120 switching on the LEDs. This enables the illumination to be turned on only underwater, and 121 prevents overheating of the LED arrays while out of the water. During a cruise with the German 122 research vessel MARIA S. MERIAN (MSM 49) we mounted a steel scale bar in front of the camera at a distance of 1 m. The distance between the centers of the white marks on the bar measured 5 123 124 cm.

The PELAGIOS is towed horizontally at specified depths of 20-1000 m. The standard towing 126 speed over ground is 1 knot (0.5 m/s), and the speed is monitored via the ship's navigational 127 system. A video transect at a particular depth can take as long as desired and is terminated by 128 lowering the PELAGIOS to the next desired depth. Maximum deployment time with full batteries 129 130 is approximately 6 hours. The typical transect duration is 10-30 min. The depth of the PELAGIOS can be monitored via online CTD data. Figure 2 shows the trajectories of the PELAGIOS at 131 different depths in the water column during a video transect down to 700 m. The deployment from 132 133 deck into the water and the reverse is fast and typically takes only about 5 min (see video clip in 134 https://www.wissenschaftsjahr.de/2016-17/das-wissenschaftsjahr/diethe ESM÷ forschungsflotte/forschungsschiff-blogs/unerforschte-meeresgebiete.html). It is possible to deploy 135 PELAGIOS in 'blind mode', where only the depth is monitored using an online depth sensor (e.g., 136 Hydrobios ©) and the video (without transmitted preview) is recorded locally on the camera. The 137 system can be operated completely blind (i.e., with no communication between deck and 138 underwater unit) where the target depth is estimated from the length and angle of the wire put out, 139 and the actual depth is recorded on the system by CTD or an offline pressure sensor e.g. SBE 140 Microcat ©. 141

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143 **3.3 Video analysis and curation**

After a deployment, the video (consisting of individual clips of one hour) is downloaded from the camera. Synchronisation between video and CTD data is done by setting all instruments to UTC prior to deployment, which allows the data and video to be linked during analysis. The video is annotated using the Video Annotation and Reference System VARS developed by at the Monterey

Bay Aquarium Research Institute (Schlining and Jacobsen, 2006). This annotation program allows 148 for frame grabs from the video including time code. A Knowledge Base allows for inserting 149 taxonomic names and hierarchy, and a Query allows for searching the created database. While 150 many kinds of annotation software are available (for review see Gomes-Pereira et al., 2016), we 151 consider VARS the most suitable for our purposes since it combines the features of high resolution 152 video playback with a user friendly annotation-interface and the automatic creation of an 153 annotation database which can easily be accessed through the various search-functions and tools 154 of the Query. The taxonomic hierarchy and phylogenetic trees in the database are directly 155 156 applicable to our video transects. Since this software was developed by MBARI, which also maintains the most extensive databases of deep pelagic observations, it makes communication 157 about and comparison of observations and data practical. Videos are transported on hard drives 158 159 after an expedition. At GEOMAR, videos and are transferred for long term storage on servers maintained by the central data and computing centre at GEOMAR, providing instant access to 160 videos and images with metadata description via the media server ProxSys. 161

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163 **3.4 Sample volume**

To estimate the sample volume of the PELAGIOS we compared video counts from the PELAGIOS with concomitantly obtained abundance data from an Underwater Vision Profiler (UVP5; Picheral et al., 2010). Four deployments from the R/V Maria S. Merian cruise MSM 49 (28.11.– 21.12.2015, Las Palmas de Gran Canaria/Spain – Mindelo/Cape Verde) were used for the comparison where a UVP5 was mounted underneath the PELAGIOS (Figure 1). The UVP5 takes between 6-11 images per second of a defined volume (1.03 L) and thus enables a quantitative assessment of particle and zooplankton abundances. Objects with an equivalent spherical diameter (ESD) >0.5–5 mm are saved as images, which can be classified into different zooplankton,
phytoplankton and particle categories. For the comparison between PELAGIOS and the UVP5,
we used the pelagic polychaete *Poeobius* sp., as 1) this organism could be observed well on both
instruments, 2) *Poeobius* sp. is not an active swimmer and lacks an escape response -and 3) it was
locally very abundant, thus providing a good basis for the direct instrument comparison.

The UVP5 images were classified as described in Christiansen et al. (2018). Poeobius sp. 176 abundance (ind m⁻³) was calculated for 20 s time bins and all bins of one distinct depth step (with 177 durations of 10-11 minutes at depths ≤ 50 m, 19-22 minutes at depths ≤ 350 m and 9-11 minutes 178 at depths ≥ 350 m) averaged. These mean abundances were compared to the PELAGIOS counts 179 (ind s⁻¹) of the same depth step. A linear model between the PELAGIOS counts as a function of 180 UVP5 abundance provided a highly significant relationship (linear regression: p < 0.001, adjusted 181 $r^2 = 0.69$; Figure 3). The linear regression slope b (0.116 m³ s⁻¹, standard error 0.01 m³ s⁻¹) between 182 the PELAGIOS-based count ($C_{PELAGIOS}$, ind s⁻¹) and mean UVP-based abundance (A_{UVP} , ind m⁻³): 183

184

$$C_{PELAGIOS} = b * A_{UVP} + a$$
 (Equation 1)

was used to estimate the volume recorded per time in $m^3 s^{-1}(b)$ and the field of view in m^2 (*b*/towing speed) recorded by PELAGIOS.

- 187 From this calculation it can be derived that PELAGIOS recorded an average volume of $0.116 \text{ m}^3 \text{ s}^-$
- 188 ¹ at a towing speed of 1 knot (= 0.5144 m s^{-1}). A cross-sectional view field of approximately 0.23
- 189 m^2 of PELAGIOS can be expected, compared to a theoretical field of view (FOV) of 0.45 m^2 based
- 190 upon the maximum image dimensions (0.80 m * 0.56 m) at 1 m distance from the lens.-
- 191 We can now calculate the individuals observed by PELAGIOS per time to individuals per volume.
- 192 <u>To do so we use the number of individuals in one transect and divide this number by the duration</u>
- 193 of the transect to obtain individuals/minute, and divide this by 60 to get the individuals/second.

194 From the UVP-PELAGIOS comparison we derived a conversion factor of 6 to calculate the
 195 number of individuals per second to number of individuals per m³. This value is then multiplied
 196 by the conversion factor 6, and again multiplied by 1000 to go from m³ to 1000 m³.

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198 **3.5** Abundance, size and diversity at an example station "Senghor NW"

To provide an example of the type of data that can be obtained with the PELAGIOS, we report 199 here on day and night video transects down to 950 m in the Eastern Tropical North Atlantic, on 200 the northwestern slope of Senghor Seamount (17°14.2'N, 22°00.7'W; bottom depth of 201 202 approximately 1000 m). The results from the video annotations show that faunal abundances depend on the depth of deployment, and time of the day. During two transects of 11 minutes at 203 204 400 m, 22632 individuals (1066 Ind/1000m³) were encountered during the day (the three dominant organism groups are were fish, euphausiids and appendicularians) compared to 208-196 205 206 individuals (591 Ind/1000m³) during the night (the four dominant organism groups are fishes, 207 chaetognaths, medusae and ctenophores). Overall abundance of chaetognaths, decapods and mysids, and somewhat for fishes was higher during the night. The peak of euphausiids' abundance 208 209 at 400 m shifts to the surface at night (Figure 4). The higher abundance of decapods, mysids and chaetognaths at night may indicate lateral migration or daytime avoidance. The vertical migration 210 that was observed for fishes and crustaceans was much less clear for the gelatinous zooplankton 211 212 groups including the medusae and appendicularians (Figure 4). Ctenophores and siphonophores 213 were abundant in the surface at night (but we did not perform transects at 20 and 50 m during the day) and the thaliaceans migrated vertically and were most abundant in shallow waters at night. 214 The total number of annotated organisms for the daytime transects (total transect time 1872 215 minutes; max. depth 950 m) was 835 compared to 1865 organisms for the longer nighttime 216

217 transects (total transect time 292 minutes; max depth 900)-. Remarkable is the enormous 218 abundance of gelatinous zooplankton (1289) annotated organisms (899 Ind/1000m³) belonging to the three dominant groups of Ctenophora (53), Siphonophorae (2132) and Thaliacea (44) in the 219 220 topmost layer (20 m) at night. Below this layer, the depth profile shows a minimum in numbers of annotated individuals at 100, 200, and 300 m water depth with a smaller peak of 576 gelatinous 221 222 organisms (299 Ind/1000m³) in 450 m. Compared to this, the depth distribution at day time shows a more regular, almost Gaussian shape with a maximum of 31 (254 Ind/1000m³) 47 and 54 (254 223 224 Ind/1000m³) gelatinous organisms at 200 and 400 m water depth, respectively.

225 <u>We compared PELAGIOS video transects with MOCNESS net (opening 1 m²) abundance data by</u>

226 <u>integrating the PELAGIOS counts over the respective depth strata of the MOCNESS. The diversity</u>
 227 of the gelatinous zooplankton in the total MOCNESS catch is much lower (8 different taxa) ., than

228 in the pooled video transects (53 annotated taxa) on the same station. The ctenophore Beroe is an

229 example of a gelatinous organism captured in MOCNESS hauls and also observed on PELAGIOS

230 <u>transects. Normalization and subsequent standardization of the encountered *Beroe* in MOCNESS</u>

and PELAGIOS transects shows that on the same station and the same depths, PELAGIOS
 observes 3.3-4.7 times more *Beroe* at the three depths where they were encountered by both
 instruments. Additionally, the PELAGIOS also repeatedly observed *Beroe* at depths where they
 were not captured by MOCNESS at all (although there were also depths where PELAGIOS did
 not observe any *Beroe*).

236

The faunal observations at station Senghor NW include a wide variety of taxa (Table 1<u>; Figures 5</u>
 and 6), spanning in size from radiolarians to large siphonophores (such as *Praya dubia* and
 Apolemia). The smallest annotated specimens belonged to the radiolarians. Chaetognaths were

240 the dominant faunal group. Typical examples of fragile organisms that were not present or 241 identifiable in the MOCNESS samples from the same cruise (Christiansen et al 2016; Lüskow et al in prep.) but which can be efficiently observed by PELAGIOS include large larvaceans 242 243 (probably Bathochordaeus and Mesochordaeus), pelagic polychaetes (Large larvaceans tentatively identified to belong to the genus Bathochordaeus and Mesochordaeus were also 244 observed. Pelagic polychaetes of the genus Poeobius, Tomopteris) (Figure 5), and smaller 245 siphonophores (such as *Bargmannia* and *Lilyopsi*; the latter can be easily distinguished by their 246 247 fluorescent body parts).- and lobate ctenophores (such as Thalassocalyce inconstans, Leucothea, 248 Bathyceroe, see Harbison et al., 1978 for differences in robustness among ctenophores)(Figure 5). can be easily distinguished and are up to 23 mm long (Christiansen et al., 2018). Other pelagic 249 worms are tomopterid and alciopid worms, the latter can reach 1 m in length. The faunal group 250 with the largest specimens, attaining up to several metres in length, are the siphonophores, 251 252 including Praya dubia and Apolemia. Siphonophores of the genus Bargmannia and Lilyopsis were also observed. Lilyopsis can be easily distinguished by their fluorescent body parts. Observed 253 medusae belonged to the genera Periphylla, Halitrephes, Haliscera, Crossota, Colobonaema, 254 Solmissus and Solmundella (Figure 5). Lobate ctenophores such as Thalassocalyce inconstans, 255 Leucothea, Bathyceroe are typical examples of organisms that cannot be captured by nets but 256 257 which can be properly quantified by PELAGIOS. Venus girdles (Cestum spp.), Beroe, and cydippids are otherand lobate ctenophores t(such as Thalassocalyce inconstans, Leucothea, 258 259 Bathyceroe, see Harbison et al., 1978 for differences in robustness among ctenophores) hat were encountered at Senghor NW (Figure 5). Cephalopod observations are-were rare but small 260 individual eranchid cranchiid squids were observed in the upper 50 m at night. Mastigoteuthid 261 262 squids were observed with their mantle in a vertical orientation and with extended tentacles in

waters below 500 m. One <u>large squid</u>, *Taningia danae* was observed during a transit between
transecting depths. Other pelagic molluscs include the nudibranch *Phylliroe* and different pteropod
species. Observed fishes are snipe eels, hatchet fishes, lantern fishes and *Cyclothone*. Fishes are
among the dominant organisms encountered during PELAGIOS transects but it is often impossible
to identify fishes to species level from the video.

We compared PELAGIOS video transects with MOCNESS net (opening 1 m²) abundance data by 268 integrating the PELAGIOS counts over the respective depth strata of the MOCNESS. The diversity 269 of the gelatinous zooplankton in the total MOCNESS catch is much lower (8 different taxa) than 270 271 in the pooled video transects (53 annotated taxa) on the same station. The etenophore Beroe is 272 captured in MOCNESS hauls and also observed on PELAGIOS transects. Normalization and subsequent standardization of the encountered Beroe in MOCNESS and PELAGIOS transects 273 274 shows that on the same station and the same depths, PELAGIOS observes 3.3-4.7 times more Beroe at the three depths where they were encountered by both instruments. Additionally, the 275 PELAGIOS also repeatedly observed Beroe at depths where they were not captured by MOCNESS 276 at all (although there were also depths where PELAGIOS did not observe any Beroe). 277

278

279 **3.6 Individual behavio**^ur

In situ observations by PELAGIOS video may reveal direct observations on individual behavior. Decapod shrimps were observed to release a blue or green bioluminescent cloud after performing their tail flip as part of the escape response (Figure 56d). Potential reproductive behavior was observed for two specimens of krill which were seen in a <u>what could be a</u> mating position, and salps were observed to reproduce asexually by the release of salp oozoids (Figure 56c). Feeding behaviors were observed for large pravid siphonophores and calycophoran siphonophores which had their tentacles extended. *Poeobius* worms were observed with their mucus web deployed to
capture particulate matter (Christiansen et al., 2018) (Figure 6a). Narcomedusae of the genus *Solmissus* were observed with their tentacles stretched up and down, which is a feeding posture
(Figure 5). In situ observations by the PELAGIOS also showed the natural body position of pelagic
organisms. Snipe eels were observed in a vertical position with their heads up, while dragonfishes
and some myctophids were observed in an oblique body position with their head down (Figure 6b).

293

4. Discussion

295 PELAGIOS is a cost-effective pelagic ocean exploration tool that fills a gap in the array of observation instruments that exist in biological oceanography, as transparent and fragile organisms 296 297 (> 1 cm) are up to now undersampled by both net-based and optical systems. The PELAGIOS video transects are comparable to ROV video transects and can be obtained in a cost efficient cost-298 efficienteffective way. The resulting data can provide information on diversity, distribution and 299 abundance of large (> 1cm), fragile zooplankton and some nekton, and also of rare species. Due 300 to the collection of HD colour video, behaviour, colour and position in the water column are 301 documented which may provide additional ecological information. Thus, it the system 302 303 complements gear that are suitable for stratified observations and collections of robust mesozooplankton and micronekton (MOCNESS, Hydrobios Multinet, and others) and optical 304 systems that are suitable for high-resolution sampling of small and abundant organisms (e.g. VPR, 305 306 UVP5) (e.g. Benfield et al., 2007; Picheral et al., 2010; Biard et al., 2015). The instrument can be deployed with a small team and from vessels of opportunity, in transmission or 'blind' mode. The 307 relatively simple design limits technical failures and makes the PELAGIOS a reliable tool for 308

oceanic expeditions. While thus far the system has only been deployed in the open ocean, it can
 be used in any pelagic environment with water that has reasonable clearance and visibility. The
 data obtained after annotation of the video can be uploaded into databases (e.g., <u>the large database</u>
 PangaeaPANGAEA) after publication of the results allowing for efficient data sharing and
 curation.

314 The clear signal-distribution patterns that we observed of the vertical migration in some animal groups (fishesfish, crustaceans and some gelatinous fauna) that we observed during after 315 annotating the video transects confirms that established biological processes such as the 316 317 dailydiurnal vertical migration (e.g. Barham, 1963) can be detected in PELAGIOS data, and that 318 the distribution data that we observe for encountered organisms are representative for the natural situation. It has to be noted, though, that while the observed distribution patterns should be 319 representative, care must be taken with regards to abundance estimates of especially actively- and 320 321 fast-swimming organisms. Some fish and crustaceans react to the presence of underwater 322 instrumentation (e.g. Stoner et al., 2008). Gear avoidance (e.g. Kaartvedt et al., 2012) can lead to 323 an underestimation of abundance, whereas attraction to the camera lights (e.g. Utne-Palm et al,, 324 2018; Wiebe et al., 2004) would result in an overestimation. The large bioluminescent squid Taningia danae seemed to be attracted to the lights of the PELAGIOS, and attraction behaviour 325 of this species has been described in other publications (Kubodera et al., 2007).- Compared to day 326 transects, the high abundance of gelatinous organisms close to the surface during night is likely to 327 be partly an effect of the higher contrast in the videos of the night transects and better visibility of 328 329 the gelatinous fauna than during day transects. Therefore we did not perform transects shallower than 50 m during the day. Many of the observed gelatinous fauna might be as well be present as 330 well at shallow depths during day-light but are not detectable at 'blue-water-conditions'. The large 331

332 difference between encountered taxa during the day and night transect may also be explained by thedue to lateral migration of animals towards Senghor seamount at nighttrapping of organisms at 333 the slopes of Senghor Seamount during the day (Isaacs and Schwartzlose, 1965; Genin, 2004) or 334 335 by other causes for patchiness (Haury et al., 2000). However, from a methodological side it should be noted that while the ship's towing speed is typically 1 knot, the current speeds at the survey 336 337 depths may differ, also between day and night. Currents may result in more or less sampled volume of water and hence a variation in plankton being visualized. Since abundance estimation relies on 338 an accurate determination of the image volume, it needs to be pointed out that it is our aim to better 339 340 technically constrain the image area in future developments (now derived from UVP quantitative observations) and to include flowmeter measurements. Therefore it is recommended to perform 341 future surveys with a current meter to measure the speed through water. 342

After annotation, the PELAGIOS video transects may be used to reconstruct species-specific 343 344 distribution patterns, which can be related to environmental gradients (Neitzel, 2017; Hoving et al. in prep.).- Such data is are valuable for studies on overlap comparison in distribution patterns 345 346 of consumers and food items (see e.g. Haslob et al., 2009; Möller et al., 2012). (e.g. Poeobius and particles, ctenophores and krill). The data can also be used in biological studies that aim to predict 347 348 the consequences of a changing ocean with altering environmental gradients for species' 349 distributions, as it has been done for net sampling of mesozooplankton (Wishner et al., 2013)- One example of changing environmental gradients is the global trend of oxygen loss in the world oceans 350 351 (Oschlies et al., 2018). Oxygen minimum zones (OMZs) are occurring naturally in the mesopelagic 352 zone (Robinson et al., 2010), and in different oceans they have been found to expand horizontally 353 and vertically as a result of climate change (Stramma et al., 2008; Oschlies et al., 2018). Expansion 354 of OMZs may result in a habitat reduction of the pelagic fauna (e.g., Stramma et al., 2012), or

increase the habitat for species with hypoxia tolerance <u>(Gilly et al., 2013)</u>. To predict the potential
consequences of OMZ expansion for pelagic invertebrates we investigated the abundance and
distribution of distinct large gelatinous zooplankton species, including medusae, ctenophores,
siphonophores and appendicularians, in the eastern tropical <u>North</u> Atlantic using PELAGIOS
video transects and correlated the biological patterns to the oxygen gradients (Neitzel, 2017;
Hoving et al., in prep.).

During various cruises, the UVP5 was mounted underneath the PELAGIOS providing concomitant 361 data on macrozooplankton and nekton (PELAGIOS) as well as particles and mesozooplankton 362 363 (UVP5). The combination of the two instruments provides a great opportunity to assess both the mesopelagic fauna and particles during one sampling event. The joint deployment of the 364 PELAGIOS and UVP5 also allowed an estimation of the sampled water volume of the PELAGIOS 365 as described above. The linear relationship between counts of the non-moving *Poeobius* sp. with 366 UVP5 and the PELAGIOS indicates comparability of the two different methods for animals in this 367 size class and provides a correction factor to estimate organism abundance (ind m⁻³) from 368 PELAGIOS count (ind s^{-1}) data. 369 The field of view (FOV) derived from the UVP5 comparison for the PELAGIOS was estimated to 370 be 0.23 m^2 in comparison to 0.45 m^2 based on measurement of the scale bar at 1 m from the camera. 371 The angle of view of the PELAGIOS is 80° and therefore the field of view (FOV) is much smaller 372 than the FOV of video transects with a wide-angle lens e.g. by ROV Tiburon (Robison et al., 373 2010). When comparing the FOV, it is important to take into account the object that is observed. 374 We provided an estimate of the FOV using *Poeobius* sp., which is a small organism that can be 375 detected only when it is close to the camera. Therefore, the area of the FOV for quantification of 376

377 <u>Poeobius sp. is smaller than when quantifying larger organisms, and the initial identification</u>
 378 <u>distance differs between species (Reisenbichler et al., 2017).</u>

We compared PELAGIOS video transects with MOCNESS net (opening 1 m²) abundance data by 379 integrating the PELAGIOS counts over the respective depth strata of the MOCNESS that happened 380 at the same cruise (Christiansen et al 2016; Lüskow et al in prep.). The diversity of the gelatinous 381 zooplankton in the total MOCNESS catch is much lower (8 different taxa) (Lüskow et al., in prep.) 382 than in the pooled video transects (53 different annotated taxa) on the same station. The ctenophore 383 Beroe is an example of a gelatinous organism captured in MOCNESS hauls and also observed on 384 385 PELAGIOS transects. Normalization and subsequent standardization of the encountered Beroe in MOCNESS and PELAGIOS transects show that on the same station and the same depths, 386 PELAGIOS observes 3-5 times more *Beroe* at the three depths where they were encountered by 387 both instruments. Additionally, the PELAGIOS also repeatedly observed *Beroe* at depths where 388 they were not captured by MOCNESS at all (although there were also depths where PELAGIOS 389 390 did not observe any Beroe). Preliminary comparisons of the data obtained with PELAGIOS and with MOCNESS indicate substantial differences in the documented fauna, a phenomenon also 391 observed in previous comparisons between optical and net data (Remsen et al., 2004). Many more 392 393 gelatinous taxa were observed during PELAGIOS video transects than were captured in 394 MOCNESS catches at the same station (data presented here, Lüskow et al., in prep.) due to , This discrepancy is likely the result of the delicate nature of many ctenophores, medusae and 395 396 siphonophores, preventing their intact capture by nets. A notable exception are the with the exception of the small and robust calycophoran colonies of the families Diphyidae and Abylidae 397 which were also captured by MOCNESS. This discrepancy is likely the result of the delicate nature 398 399 of many etenophores, medusae and siphonophores, preventing their intact capture by nets.

400 AdditionallyIn contrast, avoidaence behavior of strongly and fast swimming jellyfish (e.g. Atolla, 401 *Periphylla*), which may escape from the relatively slowly moving towed PELAGIOS, may explain their increased occurrence in nets compared to video recordings. While PELAGIOS is certainly 402 suitable for visualizing delicate gelatinous fauna, it cannot replace net-sampling since 403 complementary specimen collections are needed to validate the identity of organisms that were 404 observed during PELAGIOS video observations. Therefore, it is desired that net tows with open 405 and closing nets such as Multinet Maxi or MOCNESS are performed in the same areas, or that 406 407 collections during submersible dives are made. An advantage of ROVs over PELAGIOS is the 408 ROV's ability to stop on organisms for detailed close up recording and potentially the collection of the observed organisms. This is not possible with PELAGIOS as the ship is towing the 409 410 instrument.

411 While the imaging processing pipeline is not as streamlined as in other optical systems that use still images such as the VPR or the UVPUVP5, tThe potential of the PELAGIOS as an exploration 412 tool is illustrated by the discovery of previously undocumented animals. An example is the 413 ctenophore Kiyohimea usagi (Matsumoto and Robison, 1992) which was observed seven times by 414 the PELAGIOS and once by the manned submersible JAGO during cruises in the eastern tropical 415 416 North Atlantic. This large (>40 cm wide) lobate ctenophore was previously unknown from the Atlantic Ocean and demonstrates how in situ observations in epipelagic waters can result in the 417 discovery of relatively large fauna (Hoving et al., submitted2018). Since gelatinous organisms are 418 419 increasingly recognized as vital players in the oceanic food web (Choy et al., 2017) and in the biological carbon pump (Robison et al., 2005), in situ observations with tools like the PELAGIOS 420 can provide new important insights into the oceanic ecosystem and the carbon cycle. But small 421 422 gelatinous organisms may also have a large biogeochemical impact on their environment. This

423 was illustrated by the discovery of the pelagic polychaete Poeobius sp. during the PELAGIOS 424 video transects in the eastern tropical North Atlantic (Christiansen et al., 2018). The observations 425 of the PELAGIOS provided the first evidence for the occurrence of *Poeobius* sp. in the Atlantic 426 Ocean. During the R/V Meteor cruise M119, Poeobius was found to be extremely abundant in a mesoscale eddy. Following this discovery, it was possible to reconstruct the horizontal and vertical 427 distribution of Atlantic Poeobius in great detail Using-using an extensive database of the UVP5 428 (956 vertical CTD/UVPUVP5 profiles) in the eastern tropical North Atlantic, it was possible to 429 reconstruct the horizontal and vertical distribution of Atlantic Poeobius in great detail-and to 430 431 establish that the high local abundance of Poeobius was directly related to the presence of 432 mesoscale eddies in which they possibly substantially intercepted the entire-particle export flux that was on the way to the deep sea (Christiansen et al., 2018; Hauss et al., 2016). 433

During various cruises, the UVP 5 was mounted underneath the PELAGIOS providing 434 concomitant data on macrozooplankton and nekton (PELAGIOS) as well as particles and 435 mesozooplankton (UVP5). The combination of the two instruments provides a great opportunity 436 to assess both the mesopelagic fauna and particles during one sampling event. The joint 437 deployment of the PELAGIOS and UVP also allowed a quantification of the sampled water 438 volume of the PELAGIOS as described above. The linear relationship between counts of the non-439 moving Poeobius sp. with UVP5 and the PELAGIOS indicates comparability of the two different 440 methods and provides a correction factor to estimate organism abundance (ind m⁻³) from 441 PELAGIOS count (ind s⁻¹) data. The field of view (FOV) for the PELAGIOS was estimated to be 442 0.23 m². The angle of view of the PELAGIOS is 80° and therefore the field of view (FOV) is much 443 smaller than the FOV of video transects with a wide-angle lens e.g. by ROV Tiburon (Robison et 444 445 al., 2010). When comparing the FOV, it is important to take into account the object that is

observed. We provided an estimate of the FOV using *Pocobius* sp., which is a small organism that
can be detected only when it is close to the camera. Therefore, the area of the FOV for
quantification of *Pocobius* sp. is smaller than when quantifying larger organisms, and the initial
identification distance differs between species (Reisenbichler et al., 2017).

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Future effort should be focused on improving the assessment of the sample volume by integrating 451 technology that can quantify it (e.g. current meters, a stereo-camera setup or a laser-based system). 452 A stereo-camera set up would also allow for size measurements of the observed organisms, which 453 454 could be beneficial to estimate the biomass of the observed organisms from published size-toweight relationships. It might also be possible to obtain similar information based on structure-455 from-motion approaches that proved successful in benthic video imaging (Burns et al., 2015). The 456 457 PELAGIOS system can also be a platform for other sensors. For example, Tthe PELAGIOS was used to mount and test the TuLUMIS multispectral camera (Liu et al., 2018). Future developments 458 459 include the preparation of the system for deployments down to 6000 m water depth. The integration of acoustic sensors would be valuable to measure target strength of camera observed organisms, 460 to estimate gear avoidance or attraction and to estimate biomass and abundance of organisms 461 outside the field of view of the camera. We strongly encourage the use of complementary 462 instruments to tackle the relative importance of a wide range of players organisms in the oceanic 463 pelagic ecosystem. 464

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466

467 **Author contribution**

469	This instrument was designed, tested and applied by Henk-Jan Hoving and Eduard Fabrizius.
470	Rainer Kiko and Helena Hauss developed the idea of combining the PELAGIOS with the UVP5.
471	Philipp Neitzel and Svenja Christiansen analyzed the data in this manuscript in consultation with
472	Henk-Jan Hoving, Rainer Kiko and Helena Hauss. Arne Körtzinger, Uwe Piatkowski and Peter
473	Linke added valuable input to the further development of the instrument and its application
474	and/or the data interpretation. All authors contributed to writing the paper. All authors approved
475	the final submitted manuscript.

Data availability

The datasets generated and/or analysed during the current study will be available in the Pangaea
PANGAEA repository: https://doi.pangaea.de/10.1594/PANGAEA.902241. A link will be
provided when the paper is accepted.

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- 674 Figure 1: a) The Pelagic <u>Iin Ssitu Oobservations Ssystem (PELAGIOS)</u> with the battery (1),
- 675 CTD (2), telemetry (3), camera (4), LEDs (5), depressor (6), during deployment from R/V
- 676 POSEIDON in February 2018.
- 677

Figure 2: Stairwise trajectory of PELAGIOS through the water column, to the desired depths with concomitantly measured environmental data.







691	the same transects at two	stations on cru	ise MSM 49 on	RV MARIA S.	MERIAN.
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Figure 4: Day and night comparison of faunal observations obtained by PELAGIOS at the North
West flank of Senghor seamount A: fishes, krill, chaetognaths and decapods B: gelatinous
zooplankton groups



Figure 5: Eexamples of organisms encountered during pelagic video transects with PELAGIOS
during cruise MSM49 in the eastern tropical Atlantic. (a) a medusa *Halitrephes* sp. (b) a
siphonophore *Praya dubia* (c) a tomopterid worm (d) the ctenophore *Thalassocalyce inconstans*(e) the medusa *Solmissus* (f) the ctenophore *Cestum*. The distance between the white bands on the
horizontal bar on the bottom of the images is 5 cm.



- Table 1: Taxonomic groups which were encountered during pelagic video transects in the eastern tropical Atlantic.

Phylum	Class	Order	Family	Genus
Cercozoa	Thecofilosea			
Radiozoa				
Cnidaria	Hydrozoa	Narcomedusae	Solmundaeginidae	Solmundella
			Aeginidae	Aegina
				Aeginura
			Cuninidae	Solmissus
		Trachymedusae	Halicreatidae	Halicreas
				Haliscera
				Halitrephes
			Rhopalonematidae	Colobonema
				Crossota
				Rhopalonema
			Geryoniidae	Geryonia
				Liriope
		Siphonophorae	Agalmatidae	Halistemma
				Marrus
				Nanomia
			Apolemiidae	Apolemia
			Diphyidae	
			Forskaliidae	Forskalia
			Hippopodiidae	Hippopodius
				Vogtia
			Physophoridae	Physophora
			Prayidae	Craseoa
				Lilyopsis
				Praya
				Rosacea
			Pyrostephidae	Bargmannia
			Resomiidae	Resomia
	Scyphozoa	Coronatae	Atollidae	Atolla
			Nausithoidae	Nausithoe
			Peryphyllidae	Periphylla
Ctenophora	Nuda	Beroida	Beroidae	Beroe
	Tentaculata	Cestida	Cestidae	Cestum
				Velamen
		Cydippida	Aulacoctenidae	Aulacoctena
			Pleurobrachiidae	Hormiphora
		Lobata	Bathocyroidae	Bathocyroe
			Eurhamphaeidae	Kiyohimea
			Leucotheidae	Leucothea
			Ocryopsidae	Ocyropsis
		Thalassocalycida	Thalassocalycidae	Thalassocalyce

Chaeotognatha	Sagittoidea			
Annelida	Polychaeta	Phyllodocida	Tomopteridae	Tomopteris
		Canalipalpata	Flabelligeridae	Poeobius
Arthropoda	Malacostraca	Amphipoda		
		Decapoda		
		Euphausiacea		
		Isopoda	Munnopsidae	Munnopsis
Mollusca	Cephalopoda	Octopoda	Amphitretidae	Bolitaena
			Octopodidae	
		Teuthida	Cranchiidae	Helicocranchia
			Mastigoteuthidae	Mastigoteuthis
			Octopoteuthidae	Octopoteuthis
				Taningia
			Ommastrephidae	Sthenoteuthis
	Gastropoda	Nudibranchia	Phylliroidae	Phylliroe
		Pteropoda		
Chordata	Appendicularia	Copelata	Oikopleuridae	Bathochordaeus
				Mesochordaeus
	Thaliacea	Doliolida		
		Pyrosomatida	Pyrosomatidae	Pyrostemma
		Salpida	Salpidae	Cyclosalpa
	Actinopteri	Anguilliformes	Nemichthyidae	
		Myctophiformes	Myctophidae	
		Stomiiformes	Gonostomatidae	Cyclothone
			Sternoptychidae	