Journal of
Marine Science and Engineering

Article

VERDA: A Multisampler Tool for Mesopelagic Nets

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Abstract: Different types and systems to discriminate plankton samples at different strata in the water column have been developed in recent decades. For sampling at sufficient depth, opening and closing zooplankton multinets are ideal because there is no contamination of one sample with organisms of the previous one. However, for bigger nets, such as those used to collect micronektonic organism, it is difficult to use multiple net units, and multiple cod ends are preferred because of their simplicity, but with the problem of sample contamination from having a common net passageway. We present here a cod-end Multisampler design, VERDA, that uses a carrousel-like system. Similar to some sediment traps, the system works like a revolver with six or eight compartments whose turning mechanism is triggered when the net arrives to a programmed depth level. This prototype was built with inexpensive and recycled components and electronics similar to Arduino® and Teensy PCB to carry out electronic control. The net we used for testing the equipment was a mid-size midwater trawl of ca. 30 m^2 and total length of 58 m that works with a single towing cable and no doors. The overall system is useful for all type of ships, due to the relatively easy deployment operations and because the Multisampler does not need electrical cable or acoustics. In our case, we used a Marport® (Reykjavik Iceland) and Scanmar (Åsgårdstrand, Norway) sensors for real-time depth monitoring and opening distance.

Keywords: stratified sampling; micronekton; midwater trawls; Mesopelagos

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Citation: Castellón, A.; Olivar, M.P. VERDA: A Multisampler Tool for Mesopelagic Nets. *J. Mar. Sci. Eng.* **2023**, *11*, 72. https://doi.org/10.3390/jmse11010072

Academic Editor: Gualtiero Basilone

Received: 17 November 2022
Revised: 30 November 2022
Accepted: 24 December 2022
Published: 2 January 2023

1. Introduction

The micronekton community is composed of organisms between 2 and 20 cm that swim freely and, unlike plankton, can avoid being carried away by ocean currents. These types of organisms are relatively good swimmers, and are able to avoid plankton nets. Conversely, they are too small for conventional commercial pelagic nets, which results in their extrusion through the large mesh sizes. Another feature of these organisms is that they tend to aggregate in different layers of the water column, with important differences in their vertical location throughout the day cycle. Therefore, for scientific purposes, it is imperative to collect them by discriminating the different layers of the water column. Much effort has been made in this regard for meso- and macrozooplankton [1], and similar prototypes are widely used by the scientific community. There is far less equipment development and a lack of consensus to sample micronekton.

Multinet gears have a rigid frame where various nets are encased, and by means of an electronic or mechanical device, are sequentially dropped (by time or by pressure), trapping the organisms in different collectors, allowing for sampling stratified by depth. Among these types of plankton nets, the most commonly used are MOCNESS [2], BIONESS [3], RMT [4], Tucker [5] or HYDROBIOS [6] multinets. Some of the gears to collect larger micronekton organisms are similar in structure to commercial pelagic gears, with a structure that consists of ropes and twines that hold a mesh net. These nets use doors or hydrofoils, kites, depressors and ballast and floats, which, by offering resistance to water, open the mouth of the gear. Among the latter, the IKMT (Isaacs Kit Midwater Trawl) [7], Cobb Midwater trawl [8], Mesopelagos trawl [9] and others generally do not use multisampling systems, and when they do, it usually consists of a cod-end Multisampler placed at the end of the nets. Sample contamination due to the organisms that may be entangled inside the meshes and eventually released to the cod ends is the main challenge for these type of systems that have a single net passageway.

The objective of our work was to develop a system to sample micronekton organisms at discrete strata and that could be used with midwater trawls of small to medium size and in a variety of vessels. A cod-end Multisampler was designed and tested in combination with an already developed midwater trawl of medium size (Mesopelagos). Here, we present the Multisampler design and discuss some of the results obtained in several surveys.

2. Materials and Methods

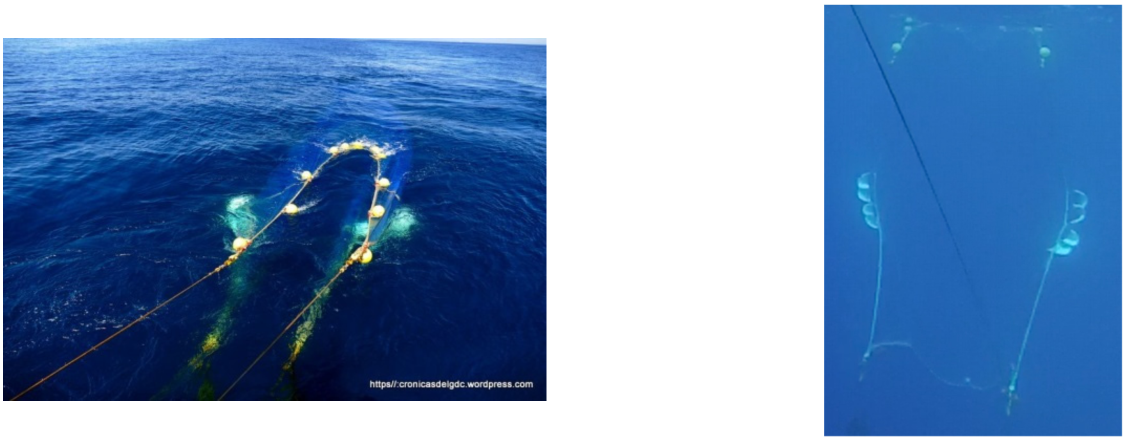
2.1. Midwater Trawl

This investigation is framed within several projects focused on the study of mesopelagic communities (Table 1). It was necessary to determine the appropriate net to collect the target organisms, but also take into account the characteristics of the research vessels used in the different surveys, which do not always permit the use of trawl doors. Commercial fishing trawls use doors for opening the mouth of the net. The operation, maneuvering for launch and recovery of doors need gears on deck and supports on the ship aft. The use of kites instead of doors makes the operations easier, with few gears involved, allowing its use in research ships of all sizes.

Table 1. Acronyms of projects and surveys (Survey). Research vessels (RV). Geographical limits of the survey (Region). Month and year of the survey (Period). Vertical sampling ranges (Depth). Type of acoustic equipment (Echosounder). Number of stations sampled with the system (N. Stn).

Survey	RV	Region	Period	Depth (m)	Echosounder	N. Stn
MAFIA	*Hesperides*	Tropical and Equatorial Atlantic	April 2015	0–800 m	EK60	14
Bathypelagic	*Sarmiento de Gamboa*	Northeastern Atlantic	May–June 2018	0–1800 m	EK60	12
CONECTA	*García del Cid*	Western Mediterranean	July 2016		-	4
WINFISH	*García del Cid*	Northwestern Mediterranean	March 2017	0–200	-	10
SUMMER	*Sarmiento de Gamboa*	Off Iberian Peninsula	October 2020	0–700 m	EK80	19

The Mesopelagos net designed by Ifremer (Fisheries Biology and Technology Laboratory, LTBH, Lorient, France) [9] and manufactured by Le Drezen (https://www.ledrezen.com/, accessed on 1 March 2022) was used in our surveys (Table 1). This gear works with a single traction cable, and uses kites on its wings as well as floats on the headline and a 120 kg ballast on the footrope to open the mouth (Figure 1). The gear has a total length of 58 m, and consists of graded-mesh netting starting with 30 mm and ending with 4 mm. The net sensors Marport® Trawl Explorer (Reykjavik, Iceland) and Scanmar opening distance sensor (Åsgårdstrand, Norway) were used in our surveys to know, in real time, the depth of the equipment and the horizontal and vertical mouth aperture.



(**a**) Surface view (**b**) Underwater view

Figure 1. Surface (**a**) and underwater (**b**) images (Félix Descalzo) of the mouth of Mesopelagos net. Observe the kites in the wings and floats in the headrope.

According to the manufacturer, the nominal opening of the rig is 7 m vertical between headrope and footrope, and 8 to 9 m horizontal between kites [9]. The geometry of the mouth varied depending on the ship and winch speeds, and may be also modified by adding floats, ballast and kites. Other authors give mouth areas of ca. 65 m^2 and vertical (v) and horizontal (h) openings between 5 and 6 m and 10 and 12 m, respectively [10,11]. During our hauls, almost all the records of opening measures were approximately 4 to 5 m horizontal (h) $\times$ 6 to 7 m vertical (v). From the image presented in Figure 1b, taken during a haul at 2 knots, and taking the floats and kites as references, the distance was 5 (h) $\times$ 9 (v) meters. This difference can be attributed to the position and posture of the height sensor that was located on the headrope. A tilt of this sensor produces lower readings as it receives the echo from the sides of the gear instead of from the footrope. Being a very light gear, the sensor location is important, and floats had to be added to compensate for sensor weight.

Trawling speed varied between 1.8 knots and 2.5 knots. As much as possible, both the ship's heading and speed were kept constant during the hauls. The ratio between the cable released and depth in the water column varied from a value of 3 for hauls down to 200 m to a value of 1.7 for hauls reaching 2000 m depth. Other authors [10] give a ratio between 2.3 (for 600 m) and 2.6 (for 540 m), at a speed of 2.5 knots. In our case, these ratios are somewhat lower: 2.1 (for 600 m) at a speed of 2 knots. The ratio between the length of the cable and the depth also depends on other factors such as the speed of the cable release, the total weight of the gear, mouth opening, sea state and currents, etc.

2.2. Sampling Protocol

To determine the diel vertical migration processes of micronekton species, it was necessary to sample the water column at different depths. Details of the sampling design are presented in Section 3. In order to sample mesopelagic communities in the water column, both day and night hauls were carried out, discriminating strata that ranged from 20 to 400 m width and from the surface to a maximum of 2000 m depth, depending on the survey's particular objectives.

2.3. Organisms Studied

We present here a brief overview of the main zoological groups collected during our last survey (SUMMER, October 2020), i.e., mesopelagic fishes, decapods, euphausiids and gelatinous organisms. Abundances are analyzed in terms of numerical abundance or biomasses, standardized according to the water filtered. The volume of water filtered was estimated considering an average mouth opening of 30 m^2 multiplied by the distance traveled during each haul.

3. Results

3.1. Design of the VERDA Multisampler

VERDA Vehicle

Our Multisampler consists of a vehicle transporting and encasing several electronic and mechanical devices. It is built in fiber glass and was designed with a funnel shape (Figures 2 and 3) starting with a cylindrical section (250 mm in diameter) that was tied to the end of the Mesopelagos net. From that point on, it widened until reaching a diameter adequate to support the toothed plate that held the collectors. A single 100 mm diameter opening in the stern of the vehicle matched the current collector. An internal net communicated the mouth with the opening of the collector. In this way, it was intended that the water that entered through the mouth could reduce it speed inside the vehicle so as to prevent organisms from being too damaged within the vehicle. Two openings were made in the sides to force the water flow-through. In the lower part of the vehicle, a casing with the motor and the electronics was placed. A pinion transmitted the rotation to the plate. The plate rotation moved the collecting bags so that before each haul, a collector was placed in front of the aft opening (Figure 2).

The buoyancy of the entire vehicle including the casing had to be neutral. During the first trials, the vehicle rotated and caused the net to close at the torsion point, preventing the samples from reaching the collectors. Two wings were installed to prevent this turning. The casing containing electronics, the depth sensor and the motor was placed in the lower part, as a ballast, and two floats were placed in the upper part to prevent the vehicle from turning (Figures 2 and 3). In addition, a sea (drift) anchor was placed to stretch the entire net. In this way, the previous problem was avoided.

An important point is that the geometry of the net cannot twist during the launching of the equipment. To ensure this, the upper part of the net, marked with a green line on the mesh, must coincide seamlessly with the upper part of the vehicle. Another problem that appeared was that the collecting bags, on occasion and due to the turbulence at the stern of the ship, became entangled between the pinion and the plate. To avoid this, all the ends of the collecting bags were tied together and these, in turn, were tied to the rope that held the sea anchor (Figure 2). With this configuration, the problem was solved.

Figure 2. View, in inverted position, of vehicle without the casing. Observe the way collector bags are attached to sea anchor rope.

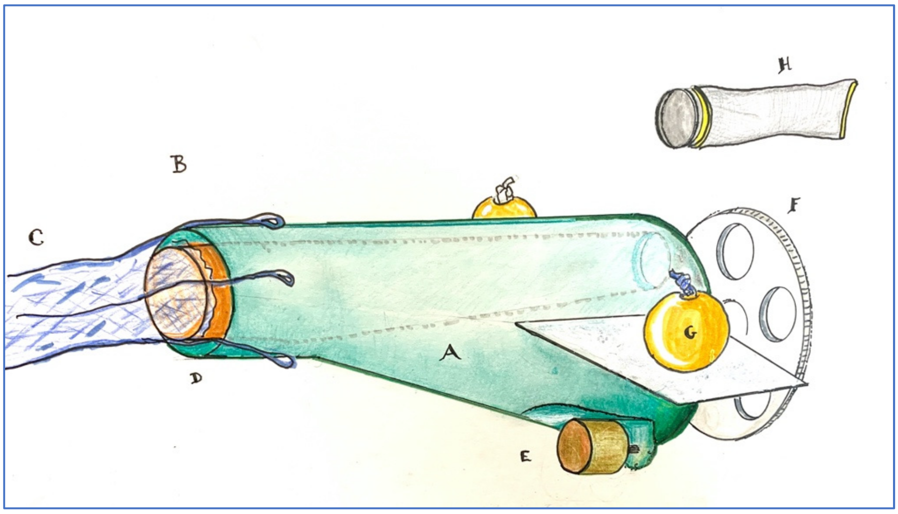


Figure 3. Scheme of the VERDA Multisampler. (A) Fiber glass body. (B) Mouth. (**C**) Mesopelagos net. (D) Attachment. (E) Motor. (F) Plate. (G) Floats. (H) Collector (net).

3.2. Plate and Pinion

Two plates with toothed gears were designed. The diameters of the plates were 400 mm and 398 mm. Both have 264 teeth. The first may contain six collectors (six holes for cylindrical bushings) (Figure 4a). The second was for eight collectors (Figure 4b). In the second case, the collector area was somewhat less than that of the first. In this way, the two plates could be used interchangeably on the same vehicle and the same pinion, avoiding major modifications. The only modification in this case was the number of turns the shaft had to make for positioning the next collector. The pinion has 11 teeth; then, for the first plate, three turns are needed (33 teeth) and for the second, four turns (44 teeth).

(**a**) Round bushings (**b**) Triangular bushing

Figure 4. Plate designs, with round (**a**) and triangular (**b**) bushing.

The non-cylindrical bushings (Figure 4b) of the second plate were made with a 3D printer using PLA filament (polylactic acid with a degradation time of two years) in solid printing. To check their reliability, they were lowered to a depth of 3700 m without any damage due to pressure. However, depending on the PLA filament used (different color), some suffered damage during some sampling, but the result was positive for most of the bushings until the end of the cruise.

3.3. Control Unit

The control unit consisted of a Technicap housing (La Turbie, France https://www.technicap.com/, accessed 1 March 2022) used for the sediment traps and built in aluminum anodized for 5000 m depth. In this case, only the casing and the motor were used. In addition, the carter (casing containing motor and electronics) was designed 10 cm longer. The original electronics were not used and one of our own, described here, was designed for the needs of our sampling. The depth sensor was a Keller Piezoresistive OEM Pressure Transmitter Type PA-20D/100 and 200 bar (Keller AG Winterthur, Switzerland (https://keller-druck.com/en/products/, accessed 1 March 2022)) This sensor has an I2C interface protocol (https://en.wikipedia.org/wiki/I%C2%B2C, accessed 1 March 2022). The casing was machined to house the sensor. The thread pitches of this sensor are the gas/hydraulic standard, so a pressure gauge was used to carry out the tests and calibrations in the laboratory without having to go out to sea or use hyperbaric chambers for haul simulations.

The microcontrollers were: Teensy 3.1/3.2 microcontroller development system (https://www.pjrc.com/teensy/, accessed 1 March 2022) and Arduino® Micro microcontroller (https://www.arduino.cc/en/Main/Products, accessed 1 March 2022). Arduino Micro had the software for controlling the whole system, and the Teensy microcontroller interface with the Keller pressure sensor through I2C protocol sent the data to the Micro using a serial interface. The Teensy I2C line is a 3.3-volt line. The final design was using only Teensy for the whole control.

The system controls the motor electronically with a MOSFET. Technicap's system uses a stem to count the turns of the shaft. In our case, three and four turns were necessary for the pinion to align with the collector openings. A CNY 70 Reflective Optical Sensor with Transistor Output was used to detect stem passages.

To record the data, a µSD card was used. When the device is deployed, the clock of the system is reset and the pinion is placed in its starting position. Time and Keller sensor data, temperature and pressure were recorded in the µSD card. The motor activation time, the number of each sampling collector and the time of its rotation, as well as the setup files are also stored in this card. The setup file consists of a maximum depth to reach, and the desired depths to be sampled (those where the collectors must turn). The procedure consists of lowering the system to exceed by ca. 20 m the maximum desired sampling depth (i.e., the depth of the first sample) and then starting the ascent. The system is triggered when sensors perceive that the net is rising and the maximum pre-programmed sampling depth is reached. From here on, the different samples are triggered as the depth configured in the setup file is reached.

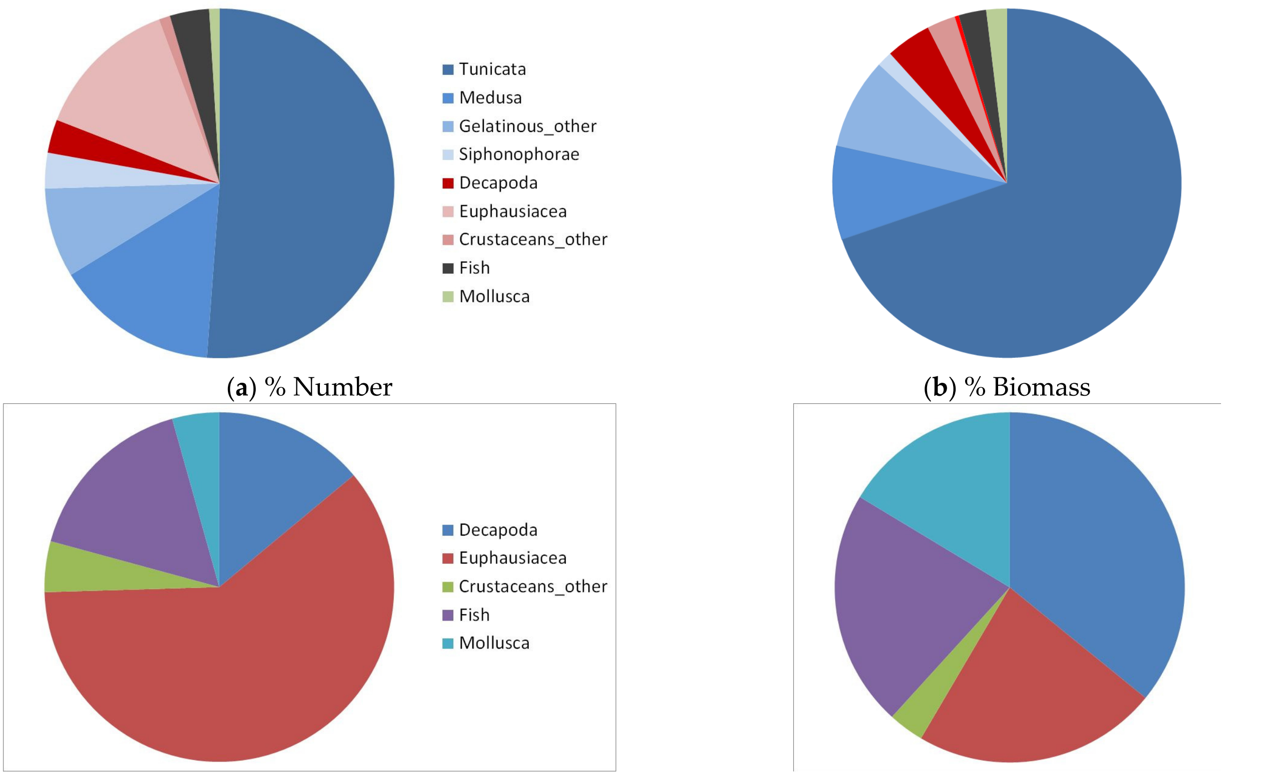
The software loop works by continuously reading the Keller pressure sensor. A buffer stores previous depth data and calculates the average slope of vehicle depth to know if the equipment is descending or ascending. The system uses a system of flags (Booleans):

- In the water: start to submerge (5 m deep);
- Arrived at the maximum depth (in setup);
- Arrived at layer "*n*";
- Sinking or rising.

After several trials, it was decided that the best configuration for the VERDA Multisampler was to leave the first hole empty, without the cod end in the first bushing ("0"), so that the vehicle does not fish when sinking. The rest of the holes were used with the corresponding collecting bags. The end of the hauls was set when reaching 10 m on the ascent, and in this moment, the plate rotates to place it back in hole "0". In this way, it is avoided that, in the recovery of the device, with turbulence produced in the stern of the boat, the last sample can be emptied.

3.4. Collection of Organisms

The different surveys in which this system was used (Table 1) provided samples where many of the micronekton groups were represented, from small fishes and crustaceans of sizes ca. 2 to 20 cm, to gelatinous organisms (Figure 5). Gelatinous items were mainly represented by jelly fish, siphonofores and salps (Tunicata), and were the most abundant taxonomic group both in number and biomass in most of the samples. Within crustaceans, euphausiids dominate in terms of number of individuals, while decapods were dominant in terms of biomass. Fishes were represented by several mesopelagic families, among which Stomiiformes and Myctophiformes were the most abundant and frequently represented [12]. Nevertheless, size frequency distributions within species were skewed towards small size classes. On the other hand, fishes of the genus *Cyclothone*, although being abundant in our surveys, were underrepresented by these hauls when comparing to concurrent collection with finer meshes [13]. Finally, cephalopods were poorly represented in the samples, and most of their collections corresponded to paralarval stages.



(**a**) % Number (**b**) % Biomass

(**c**) % Number excluding gelatinous organisms (**d**) % Biomass excluding gelatinous organisms

Figure 5. Relative contribution, in number (**a**) and biomass (**b**), of the main micronekton and macrozooplankton groups collected during the SUMMER-2020 cruise around the Iberian Peninsula.

The vertical resolution of the VERDA system allowed for the discrimination of samples obtained from vertical strata (Figure 6), from several hundreds of meters to just 20 m. In our surveys, samples obtained on a day/night basis at different layers in the water column offered information on the night vertical displacements of fishes (almost all myctophids and some species of stomiiforms) from the mesopelagic layers (200–1000 m) where they are located during the day to the upper 100 m at night [12,13].

Figure 6. Photographs of the results of three hauls. Observe the difference in the catches for the different strata. **Left** and **right**: deeper strata in the front. **Middle**: deeper strata at the bottom.

4. Discussion

4.1. Investigations on Distribution Patterns

Investigations on mesopelagic organisms' distributions and abundances have a number of limitations and constraints related to both the type of organism and their behavior, and the difficulties in accurately sampling them from the different layers of the water column. The system we present here departs from the scientific midwater trawls previously designed [9] combined with the VERDA Multisampler we described in this paper. The system efficiently discriminates samples obtained at different layers of the water column (from several hundreds of m to 20 m), being particularly valuable to investigate daily vertical migratory displacements of mesopelagic organism, and has been the subject of a few earlier publications [12–15].

The system catches a variety of mesopelagic fishes, decapods, euphausiids and gelatinous organisms that were well represented in terms of number of species and relative abundances. For mesopelagic fishes, decapods and euphausiids, our assessment is that they are well represented in terms of diversity [12]. Abundance and biomass estimations are, however, important issues yet to be solved due to net escape and mesh extrusion. Net avoidance has been advised as an important problem in the assessment of biomass of mesopelagic fishes from net hauls [16]; however, there are no studies of the Mesopelagos net selectivity for these organisms, and therefore, our abundance estimations cannot be directly used as the actual abundance at sea. In general, in our hauls, juvenile fish were better represented than the older and larger adults, indicating higher net avoidance from these more advanced developmental stages with higher swimming and vision capability. These comments can be extended to the very low capture of cephalopods, mostly represented by the low swimming paralarval stages. One possibility to reduce net avoidance may be to increase haul speed, but for an efficient Mesopelagos net deployment, there is a speed constraint of 1.8 to 2.0 knots [9]. The use of larger nets will enable the possibility of hauling at higher speeds, which points to the use of a combination of these types of nets to obtain a wide spectrum of sizes [17].

A word of caution must also be given regarding the collection of small and thin fishes, such as those of the genus *Cyclothone* (which has one of the greatest abundances of individuals of any vertebrate genus [18]), as well as for the assessment of metamorphic stages of most lanternfish, which are thinner that the mesh size of the Mesopelagos net and are extruded through the meshes. In our surveys, we overcame this by using a combination of the Mesopelagos midwater trawl and a smaller plankton net, MOCNESS-1 m^2 (mesh size 0.2 mm) [12,19].

4.2. Future Research Directions

The use of this system, Mesopelagos + VERDA Multisampler, in combination with acoustic sampling using the on-board biological echo sounders (as in some of our surveys), may be used to decide, beforehand, the layers to be sampled, and eventually to correlate the acoustic information with the biological samples. The next step is to use an acoustic/USBL (Ultra Short Base Line) modem to be able to position the equipment on the echo sounder's echogram. Through the acoustic modem, it will be possible to "trigger" the VERDA Multisampler remotely to obtain samples on selected scattering layers observed on the live echogram. In this way, we trust that the acoustic information (Target strength, especially) can be correlated with the fished individuals. However, this has a difficulty since the fished individuals travel almost 60 m from the mouth of the gear until they reach the VERDA Multisampler, and this time/distance must be taken into account.

With the present system, the gear depth is currently obtained in real time using the Marport Trawl Explorer acoustic sensor. This sensor is located in the mouth of the gear. With the USBL, which will be located in the vehicle, the depth of the VERDA Multisampler (vehicle) will be obtained through the modem. With this information, it is possible to know what the geometry of the gear is and if the vehicle goes higher or lower than the mouth of the gear. Being able to have real-time information on the relative location of the vehicle and the mouth of the net could be useful to analyze the behavior of the entire system, and will allow placing the equipment more reliably in the echograms or even to sample, for example, near the seabed.

The next step in the control electronics will be to incorporate software to receive and send information through an acoustic modem. On the one hand, it will allow us to receive the "fire" order from the ship and also to send control data to the ship to monitor the operation of the system. A microcomputer (Raspberry Pi zero) and Python programming will be included to achieve a more user-friendly interface.

Finally, another improvement will consist of designing a larger plate and holes and cod ends of a larger size to be able to house the gelatinous organisms in particular.

5. Conclusions

This gear uses kites on its wings as well as floats on the headline and a 120 kg ballast on the footrope to open the mouth; therefore, it is an easy gear to deploy on any vessel that has a suitable winch and cable (Figure 1). It works with a single traction cable, so there is no need for heavy fishing doors to open the mouth.

Ideally, the vehicle should be at the same height as the mouth. The use of a sea (drift) anchor helps this purpose.

The final VERDA Multisampler design allows us to sample at seven different strata with a resolution from 10 to 20 m. The system works well both for horizontal and oblique hauls. Our recommendation, however, is to prevent long sampling times when gelatinous organisms are dominant, such as in the presence of jelly fish and salps. The accumulation of large quantities of these heavy organisms in the collectors compromises the plate rotation.

Author Contributions: A.C. was responsible for the design, development and validation of the VERDA Multisampler system and necessary software, as well as optimization of the entire sampling system. M.P.O. was responsible for the investigation of micronekton distribution patterns, decision of sampling schemes and data curation and analyses. Both authors contributed to the draft preparation. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the projects "Migrants and Active Flux in the Atlantic Ocean" (MAFIA, CTM2012-39587-C04) and "Biomass and Active Flux in the Bathypelagic Zone" (Bathypelagic, CTM2016-78853-R) from the Spanish Ministry of Economy and Competitiveness, and SUMMER (grant agreement number 817806) from the European Union (Horizon 2020 Research and Innovation Programme), and the support through the "Severo Ochoa Centre of Excellence" accreditation (CEX2019-000928-S).

Institutional Review Board Statement: The study was approved by the Ethics Committee of the Spanish National Research Council (CSIC) on the bases that this investigation did not include any procedure with animal experimentation and in accordance with the provisions set forth in Royal Decree 53/2013 (1 February 2013).

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We would like to thank to the crew of R/V Hespérides, R/V Garcia del Cid and R/V Sarmiento de Gamboa, scientists and technicians of the Marine Technology Unit (UTM.CSIC) for their support and help during the cruises.

Conflicts of Interest: The authors declare no conflict of interest.

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