DEEP WATER MASSES AND ZOOPLANKTON BIOMASS AT THE AVILÉS CANYON DURING MARCH 2012: THE BIOCANT I CRUISE.

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No data can be taken out

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CERTIFICA:

Que el Trabajo titulado**:** **“DEEP WATER MASSES AND ZOOPLANKTON BIOMASS AT THE AVILÉS CANYON DURING MARCH 2012: THE BIOCANT I CRUISE”** presentado por D/Dña **MARÍA ALEJANDRA OCAMPO ROJAS,** ha sido realizado bajo mi dirección y, considerando que reúne las condiciones necesarias, autorizo su presentación.

En Oviedo, 16 de Julio de 2012

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Summary

Coastal submarine canyons are worldwide recognized as nutrient-rich environments, and therefore high productive zones which enhanced diversity in all trophic levels, and are often inhabited by endangered and/or emblematic species. In this sense most canyons complete the criteria that give them high priority to be declared as a marine protected area (MPA). This is the case of the Aviles Canyon (AC) which is under the process of declaration as a MPA; although few studies have been focused on investigate the physical, geological and biological processes taken place within this exceptional geographic feature of the northern Spanish coast. Therefore, researches that complete the base line information for understanding and managing of this zone are required. Thus, with the aim of generating knowledge about the distribution and interaction of the water masses and the features of the canyon, and how this interaction might affects the biological community that inhabits the AC, full-depth physicochemical parameters and zooplankton profiles from 0 to 4700 m. (when possible), were sampled in nine and five localities using a CTD-O multiparmeter instrument and a multiple opening/closing MOCNESS net of 330 µm mesh (respectively), in the course of BIOCANT I CRUISE, performed during the spring bloom of march 2012. By means of a nondestructive automatized digital imaging system, which allows taking different measurements of zooplankton individuals preserving the organisms for further studies, the biomass, metabolic rates and size spectra of Mesopelagic zooplankton in the Áviles Canyon and its surrounding continental shelf were analyzed. Results show that the Canyon exerts increasing mixing water towards the head of the canyon which mix the Eastern North Atlantic Waters and the Mediterranean Waters and re-suspend nutrients, enhancing productivity close to the photic layer, evidenced as the maximum biomass found in most of the stations.

Key words: Water masses, Biomass, metabolic rates, size spectra, Áviles canyon, mesopelagic zooplankton, Cantabrian Sea.

Introduction

The mesopelagic – one of the largest and least explored biomes on earth – is characterized by its unique biota as organisms living in extreme conditions in terms of low temperature, high pressure and complex organic matter substrates require unique adaptations and specialized metabolic processes (Steingberg & Hansell 2010, Robinson et al. 2010). The remoteness and inaccessibility of this ecosystem makes it difficult to explore. These difficulties are represented in the great amount of time it took before scientists started to get an insight on the deep sea's biota.

Although humans have been investigating and studying all aspects of life since ancient times, the first scientific investigations on life in the mesopelagic or deep sea only started in the early 19th century for benthos and even only in the late 19th century for the first studies on the pelagic deep sea habitats. The logistical constraints of these studies made that knowledge was gained at a slow pace and that it lasted until the '30's of last century before there was proof of the existence of planktonic organisms in the deepest parts of the ocean (Arístegui *et al*. 2009). And even today, in an era characterized by its huge advances in technology, still little is known about this deep planktonic environment (Koppelmann and Weikert 1992, Koppelmann *et al*. 2003, Arístegui *et al*. 2009).

Regarding all the logistical constraints that come along with the collection of samples from the deep sea (Robinson *et al.* 2010), it is important to preserve them, for different analysis and obtain from them all the possible information (Hernández-León & Montero 2006, Lehette & Hernández-León 2009), that is why the use of new techniques like digitalization of samples into images by scanner or photographic camera have been implemented by scientists around the word (Alcaraz *et al.* 2003, Grosjean *et al.* 2004, Hernández-León & Montero 2006, Bell & Hopcroft 2008, Gislason & Silva 2009, Lehette & Hernández-León 2009, Gorsky *et al.* 2010, Bachiller & Fernandes 2011).

In this sense is possible to avoid the loss of information due to the manipulation of the sample and its natural deterioration (Bachiller & Fernandes 2011). Furthermore, by using non-destructive methods is also possible to reduce the error caused by presence of particles that are not zooplankton, and the differences in the manipulation of the samples and the expertise between investigators (Alcaraz et al 2003). One of the latest uses of these image analyses is the estimation of total biomass by converting the digitized area of an organism into individual biomass, instead of using traditional methods that completely destroy the sample (Hernández-León & Montero 2006, Lehette & Hernández-León 2009).

In this work the zooplankton in the deep submarine Canyon in Aviles (Spain) is investigated. This study is unique in the sense that it is only the second investigation of this canyon that has been carried out and the methods used for collection and analysis of the samples. The relevance of this study also relays on the fact that the Áviles Canyon is one of the deepest of the world, and a place that due to its unique biological and geomorphological features, is one of the marine territories of Spain that has high priority of conservation and is part of the MPA‘s net, that this country is planning to establish before 2020 in order to protect 10% of its marine territory (Louzao *et al.* 2010).

Methods

Study area

The Bay of Biscay, in the North east Atlantic Ocean, is a classic temperate ocean with distinct seasonal patterns (Botas *et al.* 1988), which is characterized by a high stratification and light availability in summer and a sinking of cold surface water during winter. This causes mixing and high concentration of suspended particulates which increases the nutrients but diminish the light availability. In spring and autumn a combination of both nutrients and light occurs, which enables high productivity known as bloom (Botas *et al.* 1988). Sampling coincided with the spring bloom of 2012.

The Bay of Biscay has an average depth of 1,744 m. and a maximum depth of 2,789 m. except for the several canyons that interrupt the continental shelf, which in the French Coast is wider (average 100 Km.) than in the Spanish Coast (average 50 km.). The Aviles Canyon is allocate in Spain, in the middle of the southern Coast of the Bay of Biscay at 7 miles of the Asturian Coast at 43.916°N 6.316°E, with a maximum depth of 4750 m. Figure 1, shows the location of the Canyon and the five stations sampled, three of them forms a transect within the canyon ("C3", "C5" and "C8") and two are outside the canyon ("TP" in the east and "P3" on the west side).

Sampling

Vertical profile samples were collected in the five stations from the 6th to 12th of March 2012 on board of the research vessel "Sarmiento de Gamboa". In each of the sampling localities a CTD was deployed using a computer controlled wire from the Research Vessel, recording measurements of oceanographic parameters every 0.5 seconds. Vertical profiles were used to identify the water masses. depths according to Botas *et al.* (1989) and Llope *et al.* (2006), the fotic layer (fixed at 0-200 m.), the North Atlantic Central Waters (200-500 m. aprox.), Mediterranian waters (500-1300 m. aprox.), and in some stations transition to deep waters (1300-2000 m. aprox.) and deep water (2000-4750 aprox.). Opening and closing depths of the nets was established for each different station with this data.

At each station one oblique stratified haul from the bottom or 2000 m. depth to the surface was conducted using a $1m^2$ MOCNESS net equipped with 5 nets of 330 µm mesh size (Wiebe *et al.* 1985). Immediately after the haul, the cod-end contents were carefully rinsed, fixed in a 4% buffered formalin/seawater mix and stored in a hermetic container at 4°C.

Laboratory Analysis

Sample preparation: Samples were washed with sea water several times to eliminate formalin residuals, an aliquot of variable volume from the sample calculating to obtain around 1000 individuals (minimum 800 maximum 1500). These sub-samples were stained during 24 hours in the dark at 4°C with eosin, a natural compound that dyes the epidermal tissue of the samples with pink color, thus enhancing the contrast between the organism and the background during the scanning process. The dyed samples were washed again with sea water several times in order to eliminate residuals of the eosin, the pink dyed samples were placed in a 12x8x0.5 cm plate to proceed with the digitalization.

Sample scanning: all plates with the pink dyed aliquot of each sample were scanned with a HP Scanjet 8200 at a resolution of 1200 dpi (dot per inch). During this process, it is important to separate manually any overlapping organism and to spread them along the plate avoiding the edges (Grosjean *et al.* 2004, Gorsky *et al.* 2010, Bachiller & Fernandes 2011; Fig. 2). The digital images stored with a code that contains the following information of: Proyect or Campain_Method_Station_percentage of the aliquot_sequence of plate_depth range_resolution eg. BIOCANT-1_MOC_C3_200-0m_1%_1de6_1200.

Image Processing using FLAMINGO: The digital sub-sample images were split into individual particles images by the *Flamingo MATLAB* application developed by

Fernando González-Taboada and Juan Höfer at the University of Oviedo. This application is designed to identify pink-dyed particlers by means of the optical contrast based on two different thresholds, one based on filtering the green channel of the RGB spectrum and the other based on the difference between the red channel and the green one.

To avoid overlap among individual organisms the subsample must contain ca. 1000 individuals (J. Höfer com. pers.). The program saves all the split particles images in a computer folder. After discarding particle images that are not zooplankton, *Flamingo* was used to obtain several measurements and parameters from each particle detected, which were saved as excel files.

Data analysis

Biomass: The biomass estimations were calculated by equation 1 as proposed by Hernández-León & Montero (2006) and Lehette & Hernández-León (2009) from the regression between body area and individual dry mass for subtropical and Antarctic organisms, as a calibration exercise for the estimation of biomass from digitized images.

Model: $\text{Biomass } (\mu g) = \sum \alpha \cdot S^b$ Equation 1

Where: α (the intercept) = 43.38, S is body area in mm^2, and b (the slope) = 1.54 ±0.03. The value must be corrected according the proportion of the aliquots and the volume of water filtered in each sample. And for the total biomass of the water layer this value was multiplied by the depth range of the layer.

Metabolic Rate (Respiration): The metabolic rate (Y oxygen uptake μl O_2) was calculated as a function of biomass and habitat temperature using the model obtained by Ikeda (1985), from a multiple-regression method using plankton data from the Atlantic, Indian and Pacific Ocean, designating biomass (X_1) and habitat temperature (X_2), as the two independent variables.

Model: $\ln Y = a_0 + a_1 \ln X_1 + a_2 X_2$ Equation 2

Where: a_0 = -0.2512), a_1 = 0.7886, a_2 =0.0490, and “In” is the natural logarithm

<u>T/S Diagrams:</u> by means of the software Ocean Data View – ODV (Schlitzer 2011) the depth, the salinity, temperature and density of the water were plotted as a T/S Diagram to identify the waters masses present in the Áviles Canyon and its surrounding continental shelf.

<u>Descriptive statistics:</u> The physicochemical parameters, metabolic rates and size spectra data of all stations were presented by one graph obtained with the program Grapher 4 by Golden Software.

Results

Seven water masses were identified in the Áviles Canyon and its surrounding continental shelf, although for the hauls just four layers were sampled (Table 1). The T-S diagram (Fig. 3), helps to identify the water masses that are present based on the properties of the water. The Surface Water (SW) corresponds to the group of spread dots with high temperature at shallow depths. Right bellow the SW we found the Eastern North Atlantic Central Water (ENACW) where the salinity drops until its lower limit which is the salinity minimum at around 452±62,6 m. The next layer corresponds to the Mediterranean Water (MW) identified as the maximum salinity zone at 1276,8±101,1 m. The Transition to Deep Water (TDW) is characterized by continuous drop in the salinity and temperature until the DW. The Deep Water (DW) from around 1342±8,5 m depth. The Abyssal Water (AW) mass correspond to a homogeneous cold and with low salinity layer, with temperature and salinity values nearly invariant along almost 2000m, close to the bottom of the Áviles Canyon.

In the station C3, the T/S diagram shows evidence of the presence of a water mass with different properties, its maximum salinity (35.712 at 820m.) didn´t reach the minimum salinity (35.799) reported for the MW in this zone (Botas *et al.* 1989) (Fig. 3). Contrastingly, the intermediate water of the station P3 presented the maximum salinity value (35.922) at 985 m deep. And this salinity value is within the range reported for the MW (maximum 35.94 by Botas *et al.* 1989). The depth of this water masses identified in the T/S diagram are presented with the topography of the Aviles Canyon (Fig. 1B and 4), figure 1b shows for each water mass what are the contacting areas of the bottom. In figure 4 this water masses are presented in an axial transect with the canyon, through stations C3, C5 and C8 and a transect in the west shelf through stations P3, P4, P5 the allocation of this stations are shown in figure 1 as black triangles. Each water mass have the same color in all the figures to identified them easily.

Figure 5 presents together the salinity and temperature (°C) profiles with the biomass (µg/L) and the slope of the distribution of size spectra per layers of water masses, all of them share the same *Y* axis which allows comparisons between them according to the depth. The ENACW layer of the station P3 presents the biomass maximum (19103,1 µg/L) for this study, flowed by the surface layer in the station C3 (10890,1 µg/L), the minimum values were in the MW of all the stations except C5. In the statios P3, C5 and C8 the ENACW had higher biomass per liter than in the SW, and this difference remains at evaluating the total biomass (after multiplying by the depth range of the water layer Table 2). This high biomass in ENACW follows an increasing trend from east to west.

Except for the station C3 the slope (in negatives values) was decreasing with increasing depth (Fig. 5) in a range from 1.4 (negative) to 2.4 (negative), for the SW values were higher than 2 (excluding C3), within 1.6 and 1.8 in the ENACW, less than 1.6 in MW not including C3, and the minimum values were found in the DW Figure 5.

Excluding the MW of C3, the pattern of the salinity and temperature profiles were similar in all the stations, warmer in the surface water with slightly dropping temperature by depth until the thermocline (when present), and a relatively high salinity in the surface that drops to the minimum in the ENACW, follow by the maximum in the MW (except for C3). In stations C5, C8 and TP the deep thermocline correspond to the deep pycnocline.

The Table 2 presents the total biomass and respiration calculated for the entire depth range of the water mass, the maximum respiration by layer was found at ENACW in station P3 (628691.3 µl O_2) which correspond to the biomass maximum (3705997,0 µg), followed by MW in station C5 (444367,2 µl O_2) for respiration, and SW in station C3 for biomass (2178017,9 µg). The minimum respiration (76152,8 µl O_2) also corresponds to the minimum biomass (232375,0 µg), ENACW at station TP.

Discussion

Coastal submarine canyons are steep-sided topographical features of the coast that affect the water masses around it (Hickey 1995). In the southern Bay of Biscay the water masses for the first thousand meters have been widely descripted during the last two decades (Botas *et al* 1988, Botas *et al* 1989, Bode *et al.* 1990, Rios *et al.* 1992, Perez *et al.* 2000, González-Pola *et al*. 2005, Llope *et al.* 2006, among others). The SW with the highest temperatures as reported by Botas et al. (1989), but the salinity is reported as lower, while for this work was low just in the mouth and in the middle of the canyon and not for the rest of the locations. Although salinity data from this layer differs from literature reports, this most superficial layer is high variable, because is strongly influenced by atmospheric conditions and sometimes continental fresh water inputs.

For the ENACW the temperature and depth range where the subsurface salinity minimum was found it was similar to those reported for Botas *et al.* (1986), but the minimum salinity value was higher (35.53 by Botas *et al.* 1989, 35.56 by Fraga 1982, and 35.61 in station C8 of this work), which suggests diapycnal mixing with the MW (Van Aken 2000). The sub-superficial salinity maximum at 1276,8±101,1 m identified as MW was deeper than the 1000m depth founded by Botas *et al.* (1989) 1000m by Botas 1989, for the present work) but really close to the 1200 and 1250m depth reported Fraga (1982) and by Van Aken (2000) for the core of this water mass, respectively. The transition to deep water shows a clear mixing with the high salinity MW and the low salinity DW that due to this low salty characteristic can be identify as the Labrador Sea Water (LSW), that flows eastward in the northern coast of Spain, Van Aken (2000) described that in the eastern part of the Bay of Biscay this water mass reach high salinity values caused by diapycnal mixing enhanced by the slope but in the for the study site the core of this water mass is still present and the mix between this two layers is not so strong.

By the other hand, evidence that the Canyon increases mixing water towards the canyon head, which mix the Eastern North Atlantic Waters and the Mediterranean Waters, and re-suspend nutrients enhancing productivity close to the photic layer. Observed in the TS diagram station C3 and C5 and evidenced as the maximum biomass found in most of the stations for the ENACW. This process has been reported also in Monterey (Cartera *et al.* 2005) and Hudson canyons (Hickey 1995).

The process underling this mixing are a production or turbulent kinetic energy, probably related to internal wave breaking and bottom friction near the canyon head. For internal wave braking is required a ratio of less than one between the bottom slope and the slope of the internal tide (Hickey 1995). The station C3 closer to the canyon head showed the highest effect of this mixing, and the station C5 in the middle of the canyon also shows evidence of this process but in lower magnitude, the bottom topography in this station is less inclined than in station C3. Which confirm the situation that could be expected according to this ratio required for an internal wave brake as presented by Hickey (1995; Fig. 4). This mixing was observed in the T-S diagram where stations C3 and C5 did not reach either the maximum or the minimum salinity in shallow depths, this means that the core water of ENACW and MW were mixing with the boundary layers (Brown *et al.* 2001). As C3 is closer to the canyon head the effect was stronger (Cartera *et al.* 2005), followed by C5, which shows a pattern similar to the rest of the stations but without reaching the minimum or maximum salinity, because the core water was mix.

This mixing and re-suspension of particles, therefore high availability of nutrients, is probably the cause of the high biomass in the ENACW in most of the stations, although the MW get a high concentration of nutrients as well, is possible that because it is a deeper layer and due to the increase of suspended particles in the water the amount of available light is reduced. This reduction of light by turbidity would be the reason why in the ENACW in station C3 the biomass was lower. Although the vertical migrations of zooplankton might generate misleading interpretations (Ribera et al. 1999), in this work the biomass was measure for the entire community without taking into account groups.

Although this work does not have a big number of samples, it gives an overview of the distribution and interaction of the water masses and the features of the canyon, and how this interaction might affects the biological community that inhabits it. Therefore, more samples need to be collected in order to evaluate the interpretations that based on theoretical knowledge were given here. Villareal et al. (2004) evaluated the interaction of the Aviles Canyon with currients, and found that with the curriens take an upcanyon direction over the canyon, therefore upwelling enhanced, as has been evualeted in several canyons of the American east and west coast (Allen et al. 2011, Hickey Banas 2003, Glenn andGredgg 2002, Hickey 1995). But, in this study there was no evidence of upwilling therefore not upwilling enhanced.

Conclusion

During the sampling period, the interaction between the canyon topography and the water masses produced turbulent kinetic energy, probably related to internal wave breaking and bottom friction near the canyon head mixing water towards the canyon head canyon which mix the Eastern North Atlantic Central Water and the Mediterranean Water and re-suspend nutrients, enhancing productivity measured as biomass, close to the photic layer, evidenced as the maximum biomass found in most of the stations.

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Tables

Table 1. Depth ranges sampled in each station, metabolic rates (Biomass and respiration) in each station according to the identified water masses.

	slope		canyon			
Station	**P3**	**TP**	**C3**	**C5**	**C8**	**mean(±SD)**
	depth range (m)					
SW	0 - 200	0 -200	0 - 200	0 - 200	0 - 200	
NADW	200 - 394	200 - 475	200 - 444	200 - 487	200 - 547	200(±0)-452(±62,6)
MW	394 - 1300	475 - 1300	444 -1100	487 - 1348	547 - 1336	452(±62,6)-1276,8(±101,1)
DW				1348 - 1800	1336 - 2000	1342(±8,5)-1900(±141,4)
Total	0 - 1300	1 - 1300	0 - 1100	0 - 1800	0 - 2000	
	areal biomass (g m^{-2})					
SW	0,716	0,536	2,178	0,872	0,566	0,973(±0,686)
NADW	3,706	0,232	0,745	1,418	1,836	1,587(±1,334)
MW	0,665	0,621	0,690	1,700	0,647	0,864(±0,467)
DW				0,590	0,801	0,695(±0,149)
Total	5,087	1,389	3,613	4,579	3,850	3,086(±1,974)
	volumetric biomass (mg m^{-3})					
SW	3,579	2,678	10,890	4,358	2,830	4,056(±3,657)
NADW	19,103	0,845	3,054	7,088	5,291	5,897(±6,993)
MW	0,734	0,752	1,052	1,793	0,820	0,859(±0,578)
DW				1,304	1,207	1,256(±1,256)
Total	5,087	1,389	3,613	4,579	3,850	3,086(±1,974)
	areal respiration (gC m^{-2} d^{-1})					
SW	0,181	0,146	0,440	0,213	0,151	0,226(±0,122)
NADW	0,629	0,076	0,188	0,300	0,411	0,320(±0,212)
MW	0,215	0,194	0,204	0,444	0,199	0,251(±0,108)
DW				0,137	0,181	0,158(±0,0309)
Total	1,024	0,416	0,832	1,095	0,941	0,717(±0,425)

Figure legends

Figure 1. Sampling stations map.

Figure 2. Slide of a scanned sample.

Figure 3. Plate of individuals of zooplankton.

Figure 4. T-S Diagram of water masses found in the Áviles and its surrounding Canyon continental shelf during march 2012.

Figure 5. Plots of biomass (bold line) and the slope of size spectra (dash line) to the left, and temperature (bold line), salinity (dash line) to the right for each MOCNESS station.

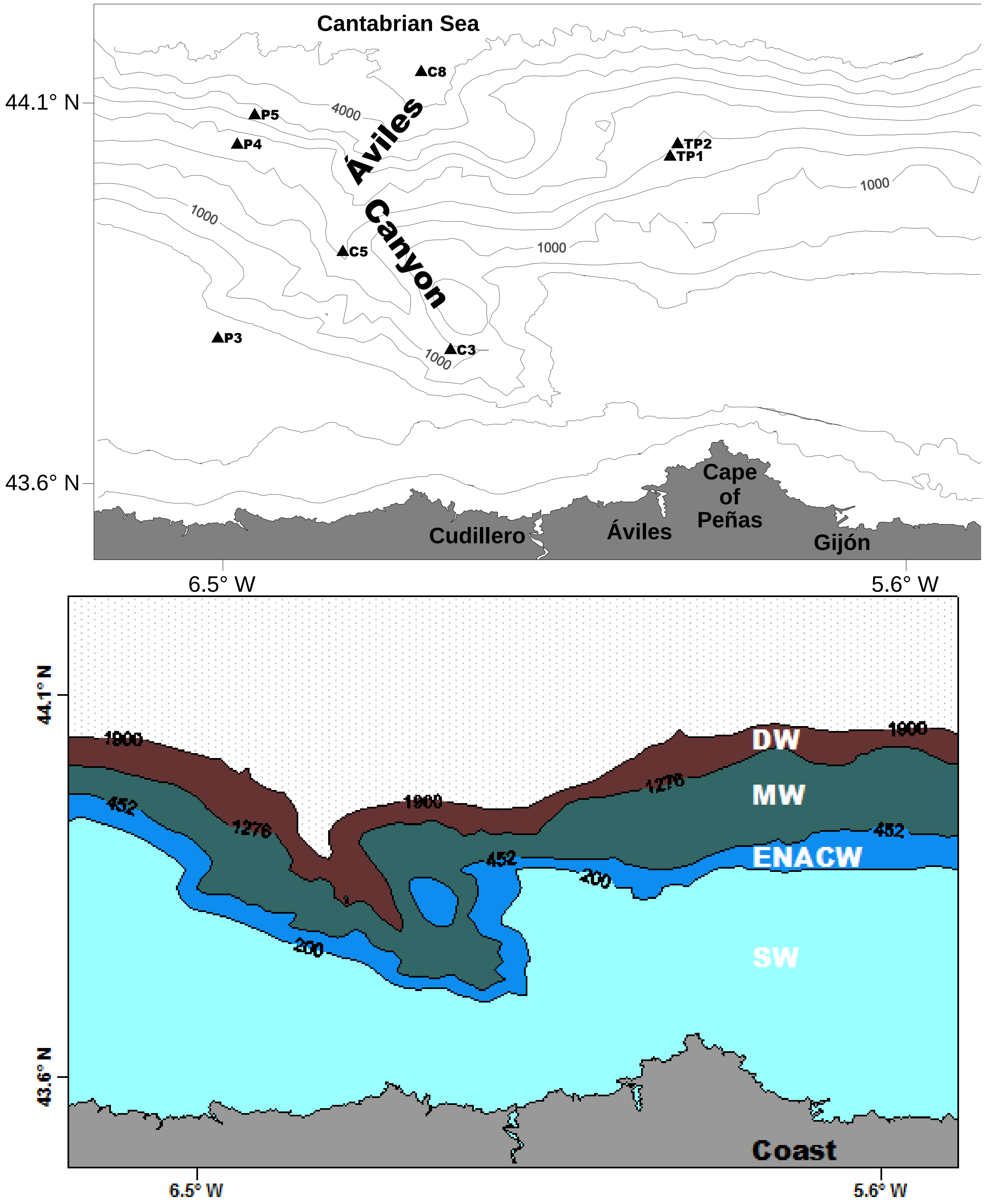

Cantabrian Sea
44.1° N
43.6° N
6.5° W
5.6° W
C8
P5
P4
4000
Áviles
Canyon
TP2
TP1
1000
C5
P3
C3
Cape of Peñas
Cudillero
Áviles
Gijón
1900
1276
452
200
DW
MW
ENACW
SW
Coast

Figure 1.

Figure 2.

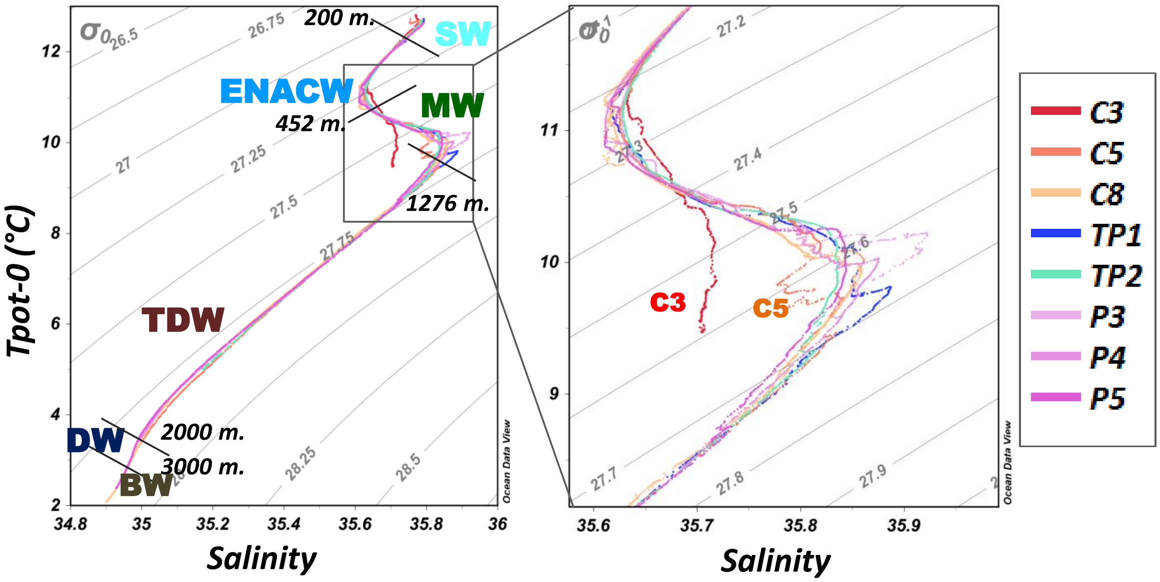

σ0
26.5
26.75
27
27.25
27.5
27.75
28
28.25
28.5
200 m.
SW
ENACW
452 m.
MW
1276 m.
TDW
DW
2000 m.
3000 m.
BW
Tpot-0 (°C)
12
10
8
6
4
2
34.8
35
35.2
35.4
35.6
35.8
36
Salinity
Ocean Data View
σ0
27.2
27.3
27.4
27.5
27.6
27.7
27.8
27.9
11
10
9
C3
C5
35.6
35.7
35.8
35.9
Salinity
Ocean Data View
C3
C5
C8
TP1
TP2
P3
P4
P5

Figure 3.

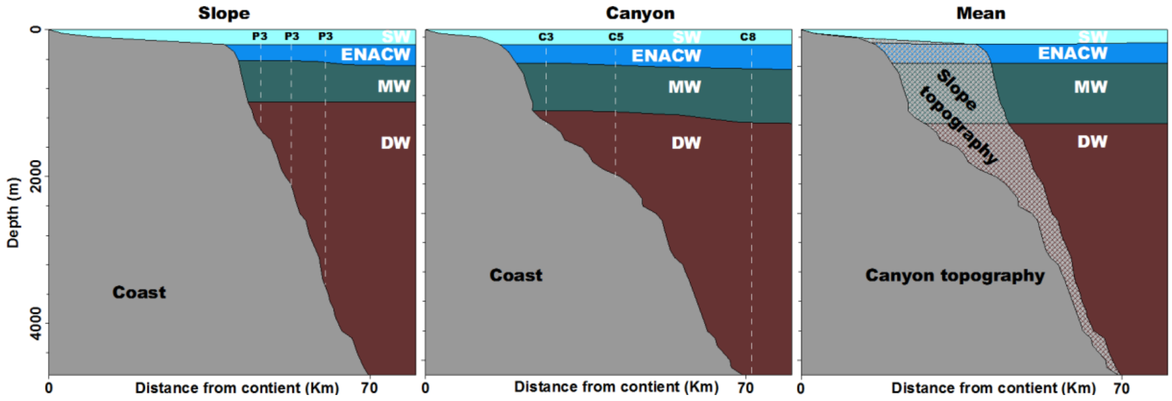
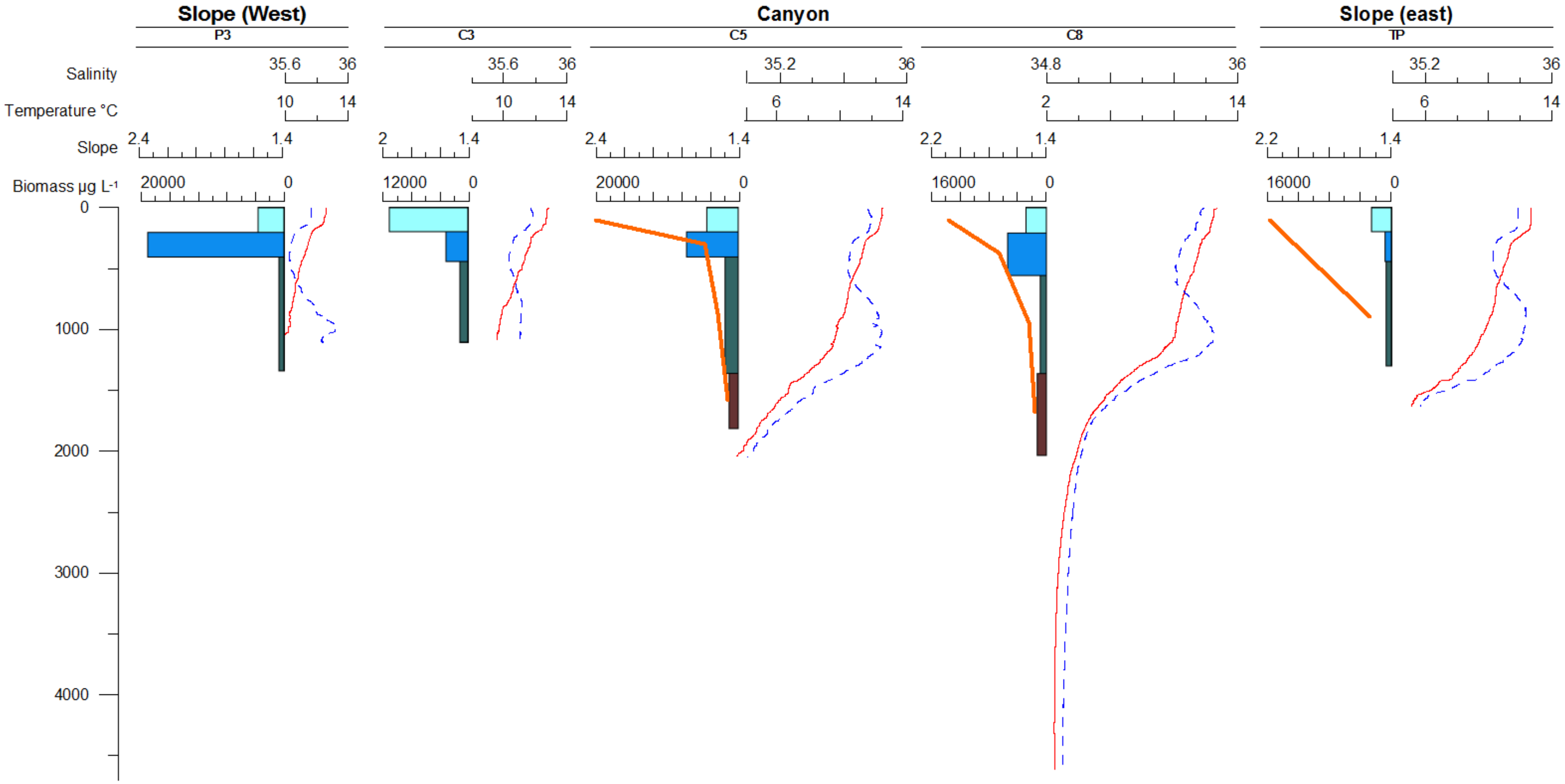

Slope
Canyon
Mean
Depth (m)
0
2000
4000
P3
P3
P3
C3
C5
C8
SW
ENACW
MW
DW
Coast
Coast
Slope topography
Canyon topography
Distance from contient (Km)
0
70

Figure 4.



Slope (West)
Canyon
Slope (east)
P3
C3
C5
C8
TP
Salinity
Temperature °C
Slope
Biomass µg L-1
35.6
36
10
14
2.4
1.4
20000
0
35.6
36
10
14
2
1.4
12000
0
35.2
36
6
14
2.4
1.4
20000
0
34.8
36
2
14
2.2
1.4
16000
0
35.2
36
6
14
2.2
1.4
16000
0
0
1000
2000
3000
4000

Figure 5

SIGILLUM REGIS UNIVERSITATIS OVETENSIS

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