

## SUMMER CRUISE PLANS (October 2020, off Iberian Peninsula)

	PAGE
CRUISE SUMMARY	2
DEPLOYMENTS & LABORATORY REQUIREMENTS	2-4
PERSONAL	4
STATIONS MAP	5
Annex 1. TENTATIVE SAMPLING DATES	6
Annex 2 . OPERATIONS SCHEDULE	7
Annex 3. PLANKTON AND MICRONEKTON PROTOCOLS	8-11
Annex 4 . SECURITY PROTOCOL FOR THE USE OF FORMALIN	12
Annex 5. PROTOCOLO TRAMPA DE SEDIMENTO	13
Annex 6. SELF-CONTAINED ECHOSOUNDER PROTOCOL	14

## SUMMER CRUISE PLANS (October 2020, off Iberian Peninsula)

### CRUISE SUMMARY

We will depart on September 29 from Barcelona (NE Spain) and we will end in Vigo on October 24 (NW Spain).

Acoustic calibration will be done near Mallorca Island. The survey will have 2 main study sites in eastern Iberia- western Mediterranean sea (oligotrophic region), and 2 more in western Iberia (productive region). In each of the four sites repeated samples will be performed day and night during 3 journeys. From Cape San Vicente to Lisbon latitude the work will focus on the location of Eddies of Mediterranean water (MEDIS), therefore CTDs will be the priority. To study the influence on these MEDIS on mesopelagic populations, plankton hauls will be also performed in particular locations, depending on the oceanographic observations (Annex 1).

Due to the difficulties inherent to deep fishing and the length of the hauls, no definitive number of days per site and number of stations in each site can be given (in case of failed sampling, repeated hauls will be needed), although as mentioned earlier we foresee to spend at least 3 days per study site. Two days (day and night) will be devoted to sample micronekton (small fishes, crustaceans, cephalopods etc) and mesozooplankton in the water column (from the mesopelagic to the epipelagic zone). Then, to obtain more accurate view of the migratory populations, one of the journeys will focus on the study of surface layers.

Because dark period in summer is short, the schedule of operations will be set in order to optimize the coincidence of micronekton and plankton hauls during the night-time (Annex 2).

Thermosalinograph and acoustic ecosound EK80 will be running during the entire cruise.

### DEPLOYMENTS & REQUIREMENTS:

1. **CTD-rosette** cast will be performed at each study site, from 0-1000 m, although in the second journeys of each site it will reach up to 2000 m.

Requirements:

CTD-911Plus (24 hydrographical bottles of 12 L each).

Winch and conductor wire.

One UTM technician per shift.

2. **MOCNESS-1 m<sup>2</sup>** (0.2 mm mesh size). Day and night samples will be performed in order to analyse mesozooplankton vertical migration patterns. Oblique hauls (2 knots) from 700 m to surface. Layers sampled: 0: 0-700 m, 1:700-600 m, 2: 600-500 m, 3: 500-400 m, 4: 400-300 m, 5: 300-200, 6: 200-100, 7: 100-50, 8: 50-0 m. Ship speed 2 knots, which deployment and retrieval speed from 10 to 20 m/min. (See also Annex 3 and 4, Plankton and Micronekton protocols).

Requirements:

MOCNESS-winch and conductor wire.

One UTM technician per shift.

Sink

Microscope (max 50x) and

one bench in the upper deck laboratory.

Small fridge in the upper deck lab.

3. **MESOPELAGOS** net (4 mm mesh size). Two journeys of day and night samples will be performed in order to analyse micronekton vertical migration patterns. Oblique hauls (2 knots) from 700 m to surface. Layers sampled: 0: 0-700 m, 1:700-600 m, 2: 600-500 m, 3: 500-400 m, 4: 400-300

m, 5: 300-200, 6: 200-100, 7: 100-0 m. Ship speed 2 knots, which deployment and retrieval speed 20 and 10 m/min, respectively. One journey will be dedicated to sample the epipelagic layer (0-200 m) (See also Annex 3, Plankton and Micronekton protocols). In each study site during one of the nights hauls will concentrate in the 0-200 m.

**Requirements:**

SCANMAR and MARPORT sensors.

Traction multipurpose winch, stern crane (to retrieve).

Deckhands to assist in deck operations, one UTM technician.

Marine weight Balanza Marina POLS

Sink

Microscope (max 50x) and

three benches in the upper deck laboratory. The last (stern) bench in the upper lab for UTM technician.

Small fridge in the upper deck lab.

**Requirements:**

starboard winch,

starboard crane and

deckhands.

4. **NEUSTON PATIN** During one of the nights, repeated surface Neuston, 10- 15 min will be carried out to obtain specimens to establish feeding periodicity.

**Requirements:**

starboard winch,

starboard crane and

deckhands.

5. **WP2** One vertical plankton haul (200-0 m) in each study site will be performed the second night of sampling. It must be done immediately after the night rosette.

**Requirements:**

starboard winch,

starboard crane and

deckhands.

6. **Sediment Trap:** Due to the changes caused by the COVID19 neither the Camera Frame or Snowcatcher could not be used, instead the Sediment trap from ULPGC will be used. It will be deployed before sunrise and recovered the same day by sunset; during the first sampling day in each study site. The trap has a light, a GPS and location system. (see [protocol Annex 5](#))

7. **Acoustics:** In situ Multifrequency lower echosounder (ISMLE), the AZFP and EK80 will be the acoustic equipment's used during the cruise. The ISMLE does NOT need conducting cable and will be deployed by the side, when the vessel was not moving. (see [protocol Annex 6](#))

### **OTHER LAB REQUIREMENTS**

A large number of samples will be kept during the cruise and we will need all the possible space in the **cold room of 4°C** and the freezer and ultrafreezers **-20°C, -80°C**.

Laboratory **Hood:** Plankton samples will be preserved in formalin (using the hood in the upper deck lab) (see Annex 4. **Security protocol for the use of formalin**). Once preserved samples will be kept in a dark room (in general we used the small dark room in the upper deck lab).

In addition to the upper deck lab we will need to use the **termoregulated lab** and the **two analyses labs of desk-1**

### **REQUESTED RV Sarmiento de Gamboa EQUIPMENT**

\* Basic configuration (as indicated from UTM services)

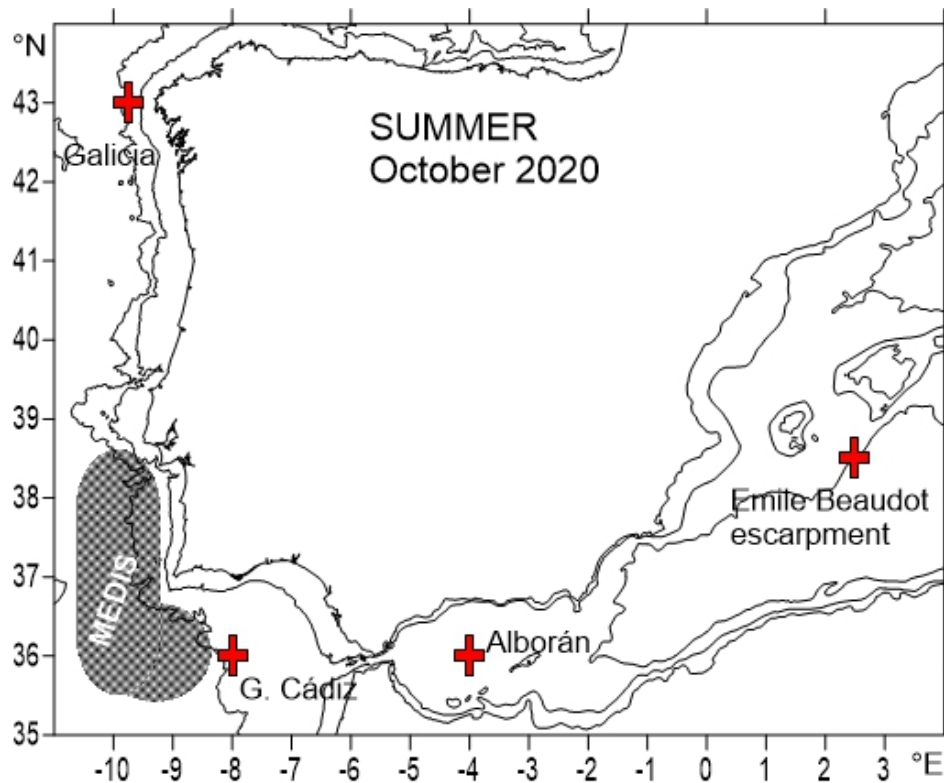
- *Sensor de presión SBE 911 Plus (6800 metros)*
- *Sensor de temperatura dual SBE3 P (6000 metros)*
- *Sensor de conductividad dual SBE 4C (6000 metros)*
- *Bomba SBE 5T*
- *Altímetro (Si queremos llegar al fondo) Teledyne Benthos PSA-916 (6000 metros)*
- *\* Sensor de oxígeno SBE43 (6000 metros)*
- *Sensor de radiación Par QCP-2300 (6000 metros)*
- *Sensor de fluorescencia clorofila y turbidez Wetlabs FLNTU (6000 metros)*
- *Sensor de fluorescencia CDOM Seapoint (6000 metros)*
- *Sensor de transmitancia Wetlabs CSTAR 25 cm (6000 metros)*
- *Sensor de nitratos Deep Suna V2 (2000 metros)*
- *\* Correntímetro doppler LADCP para medir las corrientes hasta 6000 metros.*
- *Termosalinómetro Sea-Bird SEACAT SBE 21*
- *ADCP y L-ADCP*
- *Ecosonda EK 80, a 18, 38, 70, 120 y 200 KHz*
- *Hidrófonos, sensores y MARPORT*
- *Scanmar acoustic communication system*
- *Red múltiple MOCNESS-1m2 con redes de 0.2 mm*
- *Chigres multipropósito*
- *Balanza marina*
- *Espectrofluorómetro Perkin Elmer LS55*
- *Espectrofotómetro Perkin Elmer Lambda 850*
- *Lupa binocular y microscopio*
- *Millipore Milli-Q A10 and Millipore Elix 10 systems*
- *Cámara 4°C y Congeladores de -20 y -80°C*
- *Autoclave*
- *Estufa incubación a 20°C*
- *Incubador Certomat BST Sartorius*
- *Estufa bacteriológica INCUDIGIT 80L JP Selecta*
- *Estufa desecación DIGITRONIC 80L JP selecta*

### **PERSONAL (explantions given to Sarmiento de Gamboa captain)**

The objectives of the project imply the need for day and night samplings, using a variety of equipment and carrying out several replicates at fixed stations. All of these operations will be carried out by 7 UTM y 19 scientists. UTM: Arturo Castellón for Mesopelagos, 1 UTM computing, 1UTM acoustics, 1 UTM\_HID, 3 UTM CTD-MOCNESS. (see file: Lista de Participantes.xls). From the 19 scientists, 10 are from CSIC, 5 from Univ de Las Palmas (ULPGC), 1 from AZTI, 1 from Univ. St Andrews, 1 from Galway Univ. and 1 from Aqua-DTU. Due to the uncertainties caused by COVID, the name of participants and institutions have suffered changes along the previous months.

*Los objetivos del proyecto implican la necesidad de muestreos diurnos y nocturnos , utilizando una diversidad de equipos y realizando varias réplicas en estaciones fijas.*

Estas operaciones se realizarán con la participación de 7 UTM y 19 científicos. Equipo UTM: Arturo Castellon para los lances de Mesopelagos, 1UTM informática, 1UTM acústica, 1 UTM\_HID, 3 UTM CTD-MOCNESS. (ver Archivo: Lista de Participantes). De los 19 científicos, 10 son del CSIC, 5 de la Univ de Las Palmas (ULPGC), 1 de AZTI, 1 de la Univ. St Andrews (UK), 1 de la Univ. de Galway (Irlanda) y 1 del DTU-Aqua (Dinamarca). Debido a las incertidumbres causadas por COVID, la lista de instituciones y personal ha ido cambiando a lo largo de los meses.



**STATIONS MAP.** Red crosses indicated the four main sampling sites, where repeated hauls will be carried out. Shaded zone indicates the area where Mediterranean water eddies will be searched (MEDIS), and where sampling will be decided according to the observations.

Coarse time estimations: Departure and calibration 2 days. Transit 6.5 days. Time per study site ca. 3 days.

Initial position of each sampling site:

longitude °E	latitide °N	Sites
2.5	38.5	Beaudot (Balears)
-4	36	Alboran
-8	36	G. Cadiz
-10	38.5	SW Iberia-MEDIS
-9.75	43	Galicia

## Annex 1. TENTATIVE SAMPLING DATES DURING THE CRUISE

<b>DATE</b>	
<b>29-sep</b>	Salida
<b>30-sep</b>	calibración
<b>01-oct</b>	calibración
<b>02-oct</b>	Beaudot Site
<b>03-oct</b>	Beaudot Site
<b>04-oct</b>	Beaudot Site
<b>05-oct</b>	tránsito Beaudot>Málaga
<b>06-oct</b>	tránsito Beaudot>Málaga
<b>07-oct</b>	Alborán Site
<b>08-oct</b>	Alborán Site
<b>09-oct</b>	Alborán Site
<b>10-oct</b>	tránsito Alborán>G. Cádiz
<b>11-oct</b>	G. Cádiz Site
<b>12-oct</b>	G. Cádiz Site
<b>13-oct</b>	G. Cádiz Site
<b>14-oct</b>	tránsito G. Cádiz>MEDIS
<b>15-oct</b>	MEDIS Site oceanografía
<b>16-oct</b>	MEDIS Site oceanografía
<b>17-oct</b>	MEDIS Site
<b>18-oct</b>	MEDIS Site
<b>19-oct</b>	MEDIS Site
<b>20-oct</b>	tránsito MEDIS>Galicia
<b>21-oct</b>	Galica Site
<b>22-oct</b>	Galica Site
<b>23-oct</b>	Galica Site
<b>24-oct</b>	Llegada Vigo

Annex 2. TENTATIVE OPERATIONS SCHEDULE, eg first samplig day

Hours in site	Sampling Day	Local Time (h) GMT+1	ZP	MOCNESS	MESOPELAGOS	WPE	Sediment Trap	CTD Rosette	In situ Acoustic
		6:00	N						
		6:30	N				Launch Trap		
		7:00							
0.5	1	7:30						CTD 1000 m	
1.0	1	8:00						D1	
1.5	1	8:30	D					1h	
2.0	1	9:00	D		Arrangements				Acoustics
2.5	1	9:30	D		PEL_D1				D1
3.0	1	10:00	D		1:20 h down				
3.5	1	10:30	D						
4.0	1	11:00	D						
4.5	1	11:30	D		2:30 h UP				
5.0	1	12:00	D						
5.5	1	12:30	D						
6.0	1	13:00	D						
6.5	1	13:30	D						
7.0	1	14:00	D	MOC-D1	lab				
7.5	1	14:30	D	1h down	lab				
8.0	1	15:00	D	2:30 h UP	lab				
8.5	1	15:30	D		lab				
9.0	1	16:00	D		lab				
9.5	1	16:30	D		lab				
10.0	1	17:00	D		lab				
10.5	1	17:30	D		lab				
11.0	1	18:00	D		lab				
11.5	1	18:30	D		lab				Acoustics
12.0	1	19:00	D		lab				
12.5	1	19:30	D		lab				
13.0	1	20:00	N		PEL_N1				N1
13.5	1	20:30	N		1:20 h down				
14.0	1	21:00	N						
14.5	1	21:30	N						
15.0	1	22:00	N		2:30 h UP				
15.5	1	22:30	N						
16.0	1	23:00	N						
16.5	1	23:30	N						
17.0	1	0:00	N						
17.5	1	0:30	N	MOC-N1	lab				
18.0	1	1:00	N	1h down	lab				
18.5	1	1:30	N	2:30 h UP	lab				
19.0	1	2:00	N		lab				
19.5	1	2:30	N		lab				
20.0	1	3:00	N		lab				
20.5	1	3:30	N		lab				
21.0	1	4:00	N		lab			CTD 1000 m	
21.5	1	4:30	N		lab			N1	
22.0	1	5:00	N		lab			1h	
22.5	1	5:30	N		lab				
23.0	1	6:00	N		lab				
23.5	1	6:30	N		lab		retrieval		
24.0	1	7:00					Trap		
48.5	2	7:30							
49.0	2	8:00							
49.5	2	8:30	D						
50.0	2	9:00	D						
50.5	2	9:30	D						
51.0	2	10:00	D						
51.5	2	10:30	D					CTD 1000 m	
52.0	2	11:00	D					D2	
52.5	2	11:30	D					1.5h.	
53.0	2	12:00	D					Rosetta/Lia	
53.5	2	12:30	D						
54.0	2	13:00	D						Acoustics
54.5	2	13:30	D						D2
55.0	2	14:00	D						
55.5	2	14:30	D						
56.0	2	15:00	D						
56.5	2	15:30	D						
57.0	2	16:00	D						
57.5	2	16:30	D						
58.0	2	17:00	D						
58.5	2	17:30	D						
59.0	2	18:00	D						
59.5	2	18:30	D						
60.0	2	19:00	D						
60.5	2	19:30	D						
61.0	2	20:00	N		PEL_Surf				
61.5	2	20:30	N		1:30h				
62.0	2	21:00	N						
62.5	2	21:30	N						Acoustics
63.0	2	22:00	N						
63.5	2	22:30	N						N2
64.0	2	23:00	N						
64.5	2	23:30	N					CTD 2000 m	
65.0	2	0:00	N					N2	
65.5	2	0:30	N					1:30h	
66.0	2	1:00	N					Rosetta/Lia	
66.5	2	1:30	N						
67.0	2	2:00	N		PEL_Surf				
67.5	2	2:30	N		1:30h				
68.0	2	3:00	N						
68.5	2	3:30	N						
69.0	2	4:00	N						
69.5	2	4:30	N		Neuston hauls (several replicates)				
70.0	2	5:00	N						
70.5	2	5:30	N						
71.0	2	6:00	N						
71.5	2	6:30	N						
72.0	2	7:00							
24.5	3	7:30						CTD 1000 m	
25.0	3	8:00						D3	
25.5	3	8:30	D					1h	
26.0	3	9:00	D		Arrangements				Acoustics
26.5	3	9:30	D		PEL_D3				D3
27.0	3	10:00	D		1:20 h down				
27.5	3	10:30	D						
28.0	3	11:00	D						
28.5	3	11:30	D		2:30 h UP				
29.0	3	12:00	D						
29.5	3	12:30	D						
30.0	3	13:00	D						
30.5	3	13:30	D						
31.0	3	14:00	D	MOC-D3	lab				
31.5	3	14:30	D	1h down	lab				
32.0	3	15:00	D	2:30 h UP	lab				
32.5	3	15:30	D		lab				
33.0	3	16:00	D		lab				
33.5	3	16:30	D		lab				
34.0	3	17:00	D		lab				
34.5	3	17:30	D		lab				
35.0	3	18:00	D		lab				
35.5	3	18:30	D		lab				
36.0	3	19:00	D		lab				Acoustics
36.5	3	19:30	D		lab				N1
37.0	3	20:00	N		Arrangements				
37.5	3	20:30	N		1:20 h down				
38.0	3	21:00	N						
38.5	3	21:30	N						
39.0	3	22:00	N		2:30 h UP				
39.5	3	22:30	N						
40.0	3	23:00	N						
40.5	3	23:30	N						
41.0	3	0:00	N						
41.5	3	0:30	N	MOC-N3	lab				
42.0	3	1:00	N	1h down	lab				
42.5	3	1:30	N	2:30 h UP	lab				
43.0	3	2:00	N		lab				
43.5	3	2:30	N		lab				
44.0	3	3:00	N		lab				
44.5	3	3:30	N		lab				
45.0	3	4:00	N		lab				
45.5	3	4:30	N		lab			CTD 1000 m	
46.0	3	5:00	N		lab			N3	
46.5	3	5:30	N		lab			1h	
47.0	3	6:00	N		lab				
47.5	3	6:30	N		lab				
48.0	3	7:00							

### **Annex 3. PLANKTON AND MICRONEKTON PROTOCOLS**

#### **SAFETY PROTOCOL FOR COLLECTION AND SAMPLE ANALYSIS OF PLANKTON AND MICRONEKTON**

Once the fishing and collection operations are completed, the scientists in charge will go on deck to clean the nets and collect the samples. The scientific staff will be equipped with safety shoes and a helmet, in case of need to help in the process of collecting the gear, a safety harness will also be worn.

The process of analysing plankton and micronekton samples in the laboratory does NOT involve the use of reagents, and therefore does not require special equipment.

It will only be necessary to comply with special safety regulations when specimens must be fixed in formalin (see Formalin Use Safety Protocol).

#### *PROTOCOLO DE SEGURIDAD PARA LA RECOGIDA y ANÁLISIS DE MUESTRAS DE PLANCTON y MICRONEKTON*

*Una vez finalizadas las operaciones de pesca y recogida de la red, los científicos encargados irán a cubierta a limpiar las redes y recoger las muestras. El personal científico irá equipado con zapatos de seguridad y casco, caso de tener que ayudar en el proceso de recogida de la red, se llevará asimismo un arnés de seguridad.*

*El proceso de análisis de muestras de plancton y micronekton en el laboratorio NO comporta el uso de reactivos y por tanto no requiere equipamiento especial.*

*Únicamente se precisará atender a normas especiales de seguridad cuando se deban fijar muestras en formol (ver Protocolo Seguridad Uso de Formol).*

#### **Zooplankton protocol**

Scientist involved in the on board Zooplankton work:

Vanessa Raya (CSIC)  
José M. Landeira (ULPGC)  
Linda Latuta(Aqua)

#### MOCNESS Zooplankton hauls:

The first and the third journey in each study site, day and night samples will be performed in order to analyse vertical migration patterns. Samples will be taken with a MOCNESS-1 m<sup>2</sup> net that consisted in 8 different nets, of 0.2 mm mesh size. Hauls will be oblique from 700 m to the surface, according to this preliminary sampling scheme:

1. 700-600 m
2. 600-500 m
3. 500-400 m
4. 400-300 m
5. 300-200 m
6. 200-100 m
7. 100-50 m
8. 50-0 m



Zooplankton spreadsheets records:

While the net is in the water one person from this team will be in the operations laboratory filling data on the exact position and time when hauls for each layer start. Although the volume of each net is recorded by the system, as a control measure, we take notes of the filtered volume by each net.

Survey: SUMMER (2020)				RS: Sarmiento de Gamboa					
Site	Net	Haul#	Day (D)/Night (N)	MOCNESS (MOC)					
	MOC								
<small>I:Balears - II:Alborán - III:Cádiz IV:Portugal - V:Galicia</small>				Previous CTD#					
Date dd/mm/yy		____/____/2020							
Moon and/or clouds									
Sea conditions									
Boat speed down		knots							
Boat speed up		knots							
Winch speed down		m/min							
Winch speed up		m/min							
Starting data, at 0 m:		Bottom depth, m	hour UTC (h:min)	lat °	lat ' N	lon °	lon ' E		
MOC	sampling DEPTH, m	to fill in, at the end of each layer							
Layer#	Start	End	Vol m <sup>3</sup>	hour UTC	m CABLE	lat °	lat ' N	lon °	lon ' E
0	0								
1									
2									
3									
4									
5									
6									
7									
8		0							
COMMENTS:									

Zooplankton handling protocol on board:

Once the MOCNESS is on board the net will be washed on the deck and the cod ends brought to the laboratory. One bench of the upper deck laboratory will be used.

The integrated sample will be split in two, half will be frozen and the other half preserved in ethanol. Samples from the different layers will be placed in jars and brought to the same volume. One or two subsamples (ca.10%) will be taken for later biomass measurements using scanner and image analyses. Fishes from the rest of the sample will be sorted out in the laboratory. In samples from particular scattering layers zooplankton organisms will also be sorted out for lipids, isotope, gut

content or DNA analyses, and frozen (-80° and -20°C). While sorting every layer the rest of samples will be maintained in the fridge available in the lab, or kept over an ice bed.

A careful record of all the specimens sorted out from each sample will be noted in the spreadsheets (indicating whether photos are available).

Once sorting is finished plankton samples will be concentrated in small jars, fixed with buffered formalin (5%), labelled and stored in the dark room.

## **Micronekton protocol**

Scientist involved in the on board Micronekton work:

M. Pilar Olivar	Fishes
Ainhoa Bernal	Fishes
Ana Sabatés	Data record
Roger Villanueva	Cephalopods
Maria Vigo	Micronekton
Fernando Álvarez (Fafa)	Cephalopods/ Data record
Guiomar Rotllant	Decapods
Ferran Palero/ Pere Abelló	Decapods
Oscar Escolar	Gelatinous

### MesoPELagos Micronekton hauls:

Day and night samples will be performed in order to analyse vertical migration patterns, abundance, and to obtain specimen for different purposes.

Samples will be taken with a MesoPELagos net fitted with a Multisampler used to differentiate captures at 7 different layers, according to this preliminary sampling scheme. Mesh size of the net is 4 mm. Hauls will be oblique from 700 m to the surface.

1. 700-600 m
2. 600-500 m
3. 500-400 m
4. 400-300 m
5. 300-200 m
6. 200-100 m
7. 100-0 m

Two journeys will be dedicated to vertical sampling and a third night will focus on the epipelagic layer.

Arturo Castellón will be in charge of all net operations (deployment, retrieval and Mutinet system software)

### Micronekton spreadsheets records:

While the net is in the water one person from this team will be in the operations laboratory filling data on the exact position and time when hauls for each layer start.

Survey: <b>SUMMER (2020)</b>				RS: Sarmiento de Gamboa					
Site	Net	Haul#	Day (D)/Night (N)	<b>MESOPELAGOS (PEL)</b>					
	PEL								
<small>I:Balears - II:Alborán - III:Cádiz IV:Portugal - V:Galicia</small>				Previous CTD#					
Date dd/mm/yy		___/___/2020							
Moon and/or clouds									
Sea conditions									
Boat speed down		knots	mouth opening m <sup>2</sup>						
Boat speed up		knots	width m						
Winch speed down		m/min	high m						
Winch speed up		m/min							
Starting data, at 0 m:		Bottom depth, m	hour UTC (h:min)	lat °	lat ' N	lon °	lon ' E		
<b>PEL</b>	sampling DEPTH, m		to fill in, at the end of each layer						
Layer#	Start	End	bottom depth	hour UTC	m CABLE	lat °	lat ' N	lon °	lon ' E
0	0								
1									
2									
3									
4									
5									
6									
7		0							
COMMENTS:									

Micronekton working protocol on board:

Once the Mesopelagos is on board the net will be washed on the deck and the cod ends brought to the laboratory. Samples will be analysed in the upper deck lab (three benches necessary). While the sample from one layer is analysed the rest will be placed in the cold room 4°C or cold portable containers until analyses.

The first net to be analysed will be the surface one. Samples will be spread on a tray and an overall photography will be taken. The following groups will be carefully studied: Fishes, decapods, cephalopods. They will be sorted out, identified, counted and measured (weighted and length). After this, selected specimens will be kept for lipids, isotope, gut content analyses and DNA, and frozen (-80° and -20°C, according to the desired analyses). The rest of individuals will be preserved in 10% buffered formalin, frozen, ethanol etc (or according to the specialists).

The rest of micronekton groups (cnidarians, tunicate, mollusc, euphausiids, amphipods) will be weighted (and counted if possible, or a subsampled will be counted and weighted) and preserved in buffered formalin. In case some macrozooplankton items such as cnidarians, tunicates, euphausiids, amphipods (from the main scattering layers) were not enough represented from the MOCNESS samples; they may also be sorted out for lipids and isotope analyses.

#### **Annex 4 . SECURITY PROTOCOL FOR THE USE OF FORMALIN**

The bottles of concentrated formalin will be stored in an area ventilated and protected from the sun.

Diluted formalin will be prepared under a laboratory hood, and it will remain in closed bottles inside the hood enclosure. The samples to be stored in formalin will also be fixed under the extractor hood. Once the sample is fixed, it will be verified that no drops of formalin have fallen out of the bottle (and it will be cleaned, if necessary, with some blotting paper). The samples will be kept in a sample cabinet until the port arrival. In case samples need to be opened, this will be done under the extractor hood.

Any accidental spillage of formalin will be cleaned with blotting paper, which once used, will be deposited in a plastic container that we will take to the ICM where it will be placed in its final residue container.

#### *PROTOCOLO DE SEGURIDAD PARA EL USO DE FORMOL*

*Las garrafas de formol concentrado se guardarán en una zona exterior del barco, ventilada y protegida del sol.*

*Se preparará formol diluido bajo una campana extractora, y se conservará bien tapado dentro del recinto de la campana. Las muestras que deben conservarse en formol se fijarán también bajo la campana extractora. Una vez fijada la muestra, se comprobará que no hayan caído gotas de formol por fuera del frasco (y se limpiará, en su caso, con un poco de papel secante). Las muestras se conservarán en un armario de muestras hasta su llegada a puerto, y no se abrirán más que en caso necesario, y siempre bajo campana extractora.*

*Cualquier derrame accidental de formol se limpiará con papel secante, que una vez usado, se depositará en un bidón de plástico, que será llevado al ICM, en donde se depositará en el contenedor de residuos final.*

## Annex 5. Protocolo TRAMPA DE SEDIMENTO

proceso de trabajo con La trampa de sedimento:

Normalmente, el contramaestre se encarga de La maniobra con Los marineros y nuestra ayuda.

### 1) Lanzado y recogida

El cabo de La trampa se enrolla en el winche de Las redes pequeñas (WP2,...) en el p<sup>o</sup>rtico que hay a La salida a cubierta por estribor. Se engancha La trampa y se va bajando con el winche. Cuando llega al final, se engancha La boya con el GPS, Luces,... y ya se deja en el agua alrededor de 24 horas.

### 2) evaluación del tiempo requerido para el lanzado y recogida (y condiciones de Luz)

Una media hora para poner en el agua y otra para recogerla, pero primero hay que ir a buscarla, localizarla y engancharla con el grampín. Esto suele ser una hora (a veces menos pero...). Las condiciones de luz no importan mucho pero sería deseable que siempre fuese igual, y lo mejor de mañana.

## Annex 6. SELF-CONTAINED ECHOSOUNDER PROTOCOL

The equipment weighs about 85 kg in air.

Deployment will be from the side please, on a non-conducting wire (the equipment is self-powered and self-logging).

We are interested to make acoustic measurements at the depth of Deep Scattering Layers. This depth will be determined from underway echosounder observations (ship's EK80) on approach to the station. Prior to deployment the frame should be stood up vertically in water (one of shipping cases could be used for this), and the wire attached to the eye on the top of the frame with a shackle - which should be seized before deployment. The frame has to be stood in water because we will have the transducers running before deployment, and they should not be run in air.

The frame will be lifted over the rail (this will require Yang + 1 seaman to keep it stable), and lowered to the desired depth. The frame will be evident on the ship's echosounder, and deployment depth will be determined with reference to the ship's echosounder display: there will need to be clear communication between echosounder display space and winch operator.

The frame will be held at the deployment depth for up to 30 minutes. If 2 scattering layers are present, we would like to make measurements at the shallowest for c. 20 mins and then go deeper to the second for another c. 20 minutes. Overall deployment time will be less than an hour.

On one occasion we will need to suspend a calibration sphere beneath the frame (this is what we need the bamboo canes for) to calibrate the echosounder at depth. In that case we would like to make a series of measurements of the sphere (about 10 mins each) at near-surface, 50 m, 100 m, 200 m, 300 m and 500 m. The sphere will be hung beneath the frame on a pyramid of monofilament fishing line (one end of each of 4 lines on each bamboo cane). The sphere will be lowered carefully over the side (taking care not to knock the sphere in to the side of the ship) and in to the water before the frame is lowered in to the water.

