SUMMIT-MED1 JUNIO 2021

SUMMIT-MED1 9-18 Junio 2021, B/O García del Cid

A. PARTICIPANTES EQUIPO CIENTÍFICO (3 mujeres, 3 hombres)
Rafel Simó Martorell, ICM-CSIC, 35091154P, Jefe Científico, vacunado 2 dosis Pfizer
Queralt Güell Bujons, ICM-CSIC, 39394510H, a vacunar el día 8
Yaiza Castillo de la Peña, ICM-CSIC, 47942162L, a vacunar el día 8
Fran Cornejo Castillo, Station Marine Roscoff, CNRS, 48965512F, vacunado 1 dosis Pfizer
Jeff Mangot, ICM-CSIC, Y3407926F, pasó la covid hace menos de 6 meses, a vacunar con una 2ª dosis en día 8
Marta Masdeu Navarro, ICM-CSIC, 47917217Y, a vacunar el día 8

TÉCNICOS UTM
Gustavo Agudo
Quim Rabadà

B. RESUMEN DEL PLAN DE CAMPAÑA
La campaña consiste en visitas de dos días a tres estaciones del Mar Catalano-Balear que pretenden ser contrastadas: la estación D en aguas profundas de mitad de la cuenca, camino del Canal de Menorca, donde se harán lances de CTD someros (200 m) de día y al anochecer; la estación E en aguas profundas pero algo más productivas al NE, donde se harán los mismos lances de CTD; y la estación S en aguas de plataforma cerca de Blanes, con rutinas parecidas. La mayor parte de las tareas consistirán en experimentos en cubierta, sobretodo en tanques de incubación bañados con agua de superficie. Se utilizarán tanto el laboratorio del buque como el container-laboratorio de popa.

C. PLAN DE CAMPAÑA

Mc 9J: Llegada de bultos y personal científico por la mañana. Carga de equipos, montaje de laboratorios. AL MEDIODÍA NO COMEMOS A BORDO. CENA SÍ.

Jue 10J: DESAYUNO Y COMIDA A BORDO. Montaje de laboratorios y comprobaciones. Salida al atardecer hacia la estación D (40º38'24"N 2º54'22"E)

Vie 11J: (estación D) primer muestreo y pruebas de estrés y grazing, FN2 surf night
- 10:00 – CTD 200 m (botellas de supf, 30 m)
- 11:00 – radiómetro en tanque
- 14:00 – perfil radiómetro 100 m
- 23:00 – CTD 200 m (todas botellas de supf)

Sab 12J: (estación D) Incubs DMS supf día / Grazing / FN2 supf día / B12 day / Incubs DMS supf noche
- 9:00 – CTD supf (todas botellas de supf)
- 10:00 – CTD 200 m (botellas de supf, 30 m)
- 11:00 – radiómetro en tanque
- 13:00 – CTD 200 m (todas botellas de supf)
- 14:00 – perfil radiómetro 100 m
- 23:00 – CTD 200 m (botellas de supf)

Dom 13J: (estación D) Incubs DMS BML día / Grazing / FN2 BML día / Incubs DMS BML noche / FN2

BML noche / traslado a estación E
9:00 – CTD 30 m (todas botellas de 30 m)
10:00 – CTD 200 m (botellas de supf, 30 m)
11:00 – radiómetro en tanque
14:00 – perfil radiómetro 100 m
23:00 – CTD 30 m (todas botellas de 30 m)
24:00 – CTD 200 m (botellas de supf, 30 m)
– salida hacia estación E (dirección NE, aguas profundas frente al Cap de Creus, a definir según mapa de clorofila de satélite y previsión meteo)

Lun 14J: (estación E) Grazing / B12 día / FN2 supf noche
9:00 – CTD 30 m (todas botellas de 30 m)
10:00 – CTD 200 m (botellas de supf, 30 m)
11:00 – radiómetro en tanque
13:00 – CTD 200 m (todas botellas de supf)
23:00 – CTD 200 m (todas botellas de supf)

Mar 15J: (estación E) Grazing / FN2 supf día / isoprene turnover / traslado a aguas de plataforma
9:00 – CTD supf (todas botellas de supf)
10:00 – CTD 200 m (botellas de supf, 30 m)
11:00 – radiómetro en tanque
23:00 – hacia la plataforma, hacia estación S (aprox. 41º35’22”N 2º51’45”E)

Mie 16J: (estación S) Incubs DMS supf día / Grazing / FN2 supf día / B12 día / FN2 supf noche / Incubs DMS supf noche / isoprene turnover
9:00 – CTD supf (todas botellas de supf)
10:00 – CTD 50 m (botellas de supf)
11:00 – radiómetro en tanque
13:00 – CTD 50 m (todas botellas de supf)
14:00 – perfil radiómetro 100 m
23:00 – CTD supf (todas botellas de supf)
24:00 – CTD 50 m (botellas de supf)

Jue 17J: (estación S) ISCA / isoprene turnover / regreso a Barcelona
11:00 – CTD 50 m (botellas de supf)
17:00 – inicio regreso a Barcelona

Vie 18J: Descarga

Nota: Salida del sol 6:15, puesta 21:30

D. MONTAJES A BORDO

-Laboratorio principal: espectrofotómetro, GCMS, sistema MQ, sistemas de filtración
-Laboratorio container: citómetro de flujo, miniFIRE, sistema de filtración en campana
-Tanques en cubierta de popa: 2, con flujo de agua superficial en continuo

E. Tasks

1. Ancillary measurements (Queralt, Yaiza, Jeff, Rafel, Marta, Gus)
Chla, FC, phyto, nutrients, CDOM, TOC, radiometer

2. Chemotaxis & Grazing (Queralt, Yaiza)
-*Do model protists predate preferentially on stressed prey?*
Bottle grazing experiments with cultivated protist (Gyrodinium, Oxyrrhis, Strombidium?) and natural prey assemblage after exposure to light gradients. Flow cytometry, FvFm, DMS/P, 18S, fixation and microscopy
-*Do MZP follow chemotactic responses to phyto metabolites?*
ISCA experiments with several substrates. On deck incubations with coastal waters. 16S and 18S.
ISCA experiments with herbivore-enriched waters (lab incubations with coastal waters from the last day of cruise, after addition of cultivated prey). 16S and 18S.

3. DMSP and DMS cycling (Rafel, Marta, Yaiza)
-*Who is responsible for DMSP uptake, and what genes?*
S-DMSP: daytime & nighttime, vertical profile of DMSP consumption, assimilation vs gene abundance & transcripts
-*Algal vs bacterial DMS production?*
DMS production (DMDS) w/wo GBT – under a light quality gradient
DMS consumption photolysis (filtered seawater)

3. DMSP and N2 fixation (Fran, Jeff)
Do DMSP and acrylate trigger het. N2 fix?
Incubations (within 1 hour) w/wo DMSP additions (and acrylate and glucose).
Sampling mRNA for diversity of nifH transcript amplicons. If evidence of response, then run qPCR of targeted nifH.
Samples from day and night, surface.

4. DMSP and B12 addition experiments (Jeff, Fran)
SF water is added B12 or DMSP or nothing (control) into duplicate carboys, and they ae incubated (1h and 6-8 h) in natural light. Sampling mRNA for metatranscriptomes.

5. Isoprene consumption kinetics (Marta, Rafel) This only aims to complement our previous work. *Is isoprene consumption linear*?
Dark incubations of night-time sample, 40h, 4-5 time points
5'. VOC consumption kinetics (Marta, Rafel) *Consumption rates of halocarbons and other VOCs*, same experiments as for isoprene.

Question	*Method*	*Volume, where*	Variables needed
2.1. Do model protists predate preferentially on	*Bottle grazing experiments with cultivated protist*	3 x 1L bottles x stress treatments x 2 predation treatments	DMS, DMSP, Fv/Fm, chla, FC, phyto, 16s, 18s, lugol protists

stressed prey?	*(Gyrodinium, Oxyrrhis, Strombidium?) and natural prey assemblage after exposure to light gradients. Flow cytometry, FvFm, chla, DMS/P, 18S, fixation and microscopy*	in tank, 1 h stress + 3 h incubation, 14 L total, 5-6 times during the cruise. Stress in Teflon; non-stress in Pyrex; grazing all in Pyrex Teflon: 6 + 1 extra Pyrex: 12 + 1 extra 24 DMSP vials	
2.2. Do MZP follow chemotactic responses to phyto metabolites?	*ISCA experiments with several substrates. On deck incubations. 16S and 18S.* *ISCA experiments with herbivore-enriched waters (lab incubations with coastal waters from the last day of cruise, after addition of cultivated prey). 16S and 18S.*	4 x ISCA in PVC tubes, 1 h, 16 L total (surf), once during the cruise 4 tubes Water collection and dark storage	16s, 18s, FC initial water 16s, 18s, FC
2.3. What are the rates of DMSP consumption, DMS production and DMS consumption? Who is responsible for DMSP uptake, and what genes?	*Between 4 and 6 experiments, depending on time and weather, 4 if only 2 depths, 6 if 3 depths. Experimental design and variables* *(a) Time course (4 times within 1.5h) S-DMSPd loss:* ***rate constant DMSPd consumption****; x[DMSPd] =* ***rate DMSPd consumption*** *(mostly bacterial)* *(b) Time course (3 times within 12h), dark:* ***rate DMSPt consumption by community***	0.5L glass bottle for 35S, 16L for incubations, 12L for metaT Exp 1: surf water from 9:00 Exp 2: surf water from 23:00 Exp 3: BML water (below mixed layer) from 9:00 Exp 4: BML water from 23:00 Exp 5: DCM from 9:00 Exp 6: DCM water from 23:00	Initial water: DMS, DMSPt, DMSPd, chla, phyto, FC, TOC, nutrients, 16s, 18s, metaT

	(c) Time course (3 times within 12h), natural light: rate net DMS production *(d) Ibid +DMDS:* ***rate gross DMS production by community*** *(e) Ibid +DMDS +GBT:* ***rate DMS production by phytoplankton*** *(f) d-c =* ***rate bacterial DMS consumption*** *(g) Time course (3 times within 12h, only daytime), 0.2 um filtered seawater, light and dark:* ***rate constant DMS photolysis****; x[DMS] =* ***rate DMS photolysis****, to be use to correct above incubations in the light* *(a) In 30 ml DOC bottles* *(b) In 2L amber glass bottles* *(c) In 2L teflon bottles* *(d) Ibid* *(e) Ibid* *(g) In 1L teflon bottles* *(h)=(d)/(b) =* ***DMS yield by the community*** *(i)=(e)-(d) =* ***DMS production by bacteria*** *(j)=(i)/(a) =* ***DMS yield by bacteria*** *(k) Incubation (12h) with S-DMSPd, light, and filtration:* ***% S-DMSP***		

	assimilation *by 2 size fractions: 0.2-1.2 um and >1.2 um; x(a)* *=* ***DMSPd assimilation rates*** *The idea is to compare (a), (e), (f), (i), (k) with the* ***transcripts of the relevant genes****.*		
2.4. Do DMSP and acrylate trigger het. N2 fix?	*Incubations (within 1 hour) w/wo DMSP additions (and acrylate and glucose).* *Sampling mRNA for diversity of nifH transcript amplicons. If evidence of response, then run qPCR of targeted nifH.* *Samples from day and night, surface.*	2 x 20L carboys x 4 treatments in tank, natural light, 4L subsampling within 1h Total: 162L One CTD cast only Each time: the 8 carboy subsamples filtered in parallel through 47 mm 0.2 um (prefiltration through 20 um during filling)	DMSP, DMS, 16S, 18s, chla, DOC, nutrients, FC, nifH amplicons, (qPCR nifH transcripts?)
2.5. Does B12 arrest DMSP transport? Does DMSP arrest sulfate reduction?	*SF water is added B12 or DMSP or nothing (control) into duplicate carboys, and they are incubated (1h and 6-8 h) in natural light.* *Sampling mRNA for metatranscriptomes.*	2 x 20L carboys x 3 treatments (x 2 times), natural light in tank, twice during the cruise. Total: 122L One extra carboy of each for mRNA amplicons. Use botellon?	DMSP, B12, FC, chla, DOC, nutrients, FC, metaT+metabarcoding
2.6. What is the kinetics of isoprene turnover?	*Dark incubations of night-time sample, 40h, 4-5 time points*	2 x 2L amber bottles, dark in tank, once during the cruise. Total: 5L (surf)	Isoprene & other VOC
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MEASURED VARIABLES

Variable	Where/when	Volume	Sampling	Analysis	Method

DMS and VOCs		0.5 L	Marta/Rafel	On board	P&T GCMS
DMSPt, DMSPd		0.1 L	Marta/Rafel	ICM	P&T GCFPD
Diss. vitamin B_{12}		1.5 L	Jeff	USC Sañudo	HPLC
Nutrients		0.02 L	Marta/Jeff	ICM	autoanalyzer
TOC		0.04 L	Marta/Jeff		OC analyzer
CDOM		0.25 L	Marta	On board	Spectrophot.
Chla		0.1-0.2 L	Yaiza/Jeff	ICM	Fluorimetry
Phyto (microscopy)		0.05 L	Yaiza/Jeff	ICM	Microscopy
Bact, phyto (FC)		0.02L	Yaiza	On board	Flow Cytom.
16S, 18S, nifH amplicons		4 L	Jeff/Fran	Texas, ICM	Illumina
Metagenomes			Jeff/Fran	CNAG, ICM	Illumina
Metatranscriptomes		15 min or 8L	Jeff/Fran	CNAG, ICM	Illumina
Fv/Fm		0.02 L	Queralt/Rafel	On board	miniFIRE
S-DMSP consumption		0.5 L	Rafel/Yaiza	On board / ICM	Dark incub.
DMS(P)+VOC cycling		12 L	Rafel/Marta	On board / ICM	Light/dark incub.
DMS+VOC photochem.		2 L	Marta/Rafel	On board	Light/dark teflon incub.
Isoprene turnover		5 L	Marta/Rafel	On board	Dark glass incub.
T, S, fluorescence, O2, beam atten., vertical mixing	Vertical profiles all CTDs		UTM/Rafel	On board	CTD
Sunlight attenuation	Once a day		UTM/Rafel	On board	Radiometer
Surface irradiance	Continuous		UTM	On board	Pyranometer
Wind speed	Continuous		UTM	On board	Anemometer
SST, surf salinity	Continuous		UTM	On board	Underway TSalinometer

Chemicals (reagents, fixatives, gases, etc)

Chemical	Form	Volume	Storage	Waste
NaOH	pellets	250 ml	lab	No waste
HCl 10%	solution			Diluted into sink
H2SO4 20%	solution	0.1 L		No waste

Paraformaldehyde	solution		fridge	In carboy
Paraformaldehyde + glutaraldehyde	solution		fridge	In carboy
Ethanol	bottle	1 L	lab	In carboy
TCA 5%	solution	0.5 L	fridge	In carboy
Liquid N_2	Dewar	2 x 40 L	outdoors	
UHP He	Gas bottle	1 x 10 L	Lab/outdoors	