

Convocatorias 2015  
Proyectos EXCELENCIA y Proyectos RETOS  
Dirección General de Investigación Científica y Técnica  
Subdirección General de Proyectos de Investigación

**AVISO IMPORTANTE**

En virtud del artículo 11 de la convocatoria **NO SE ACEPTARÁN NI SERÁN SUBSANABLES MEMORIAS CIENTÍFICO-TÉCNICAS** que no se presenten en este formato.

**La parte C de la memoria no podrá exceder de 20 páginas.**

**Lea detenidamente las instrucciones para rellenar correctamente esta memoria, disponibles en la web de la convocatoria.**

**Parte A: RESUMEN DE LA PROPUESTA/SUMMARY OF THE PROPOSAL**

**INVESTIGADOR PRINCIPAL 1** (Nombre y apellidos):

FRANZ PETERS

**INVESTIGADOR PRINCIPAL 2** (Nombre y apellidos):

M. MONTSERRAT SALA FARRE

**TÍTULO DEL PROYECTO:** Aportes atmosféricos como fuente de nutrientes orgánicos y microorganismos en ecosistemas marinos

**ACRÓNIMO:** ANIMA

**RESUMEN** [Máximo 3500 caracteres \(incluyendo espacios en blanco\):](#)

El objetivo de ANIMA es cuantificar y caracterizar las entradas atmosféricas de materia orgánica y microorganismos del aire y evaluar su impacto en el marco del ecosistema marino en el Mediterráneo noroccidental. ANIMA combina avances en la caracterización de partículas atmosféricas, vivas e inertes, que depositan sobre el océano y sus interacciones con compuestos químicos y con microorganismos en el mar. Se dará un énfasis especial a la composición y degradación de la materia orgánica, de gran interés en la oceanografía actual ya que afecta el ciclo global del carbono. ANIMA usará tipos de partículas con materia orgánica que se encuentran en aerosoles, tales como el polen o el hollín, y estudiará su degradación y la huella de sustancias disueltas en relación con huellas observadas en el mar. Con diversas técnicas se identificarán microorganismos en el aire, incluyendo hongos, y se analizarán por su capacidad de degradación de materia orgánica en sistemas marinos. La hipótesis global de ANIMA es que la mayor proporción de materia orgánica en aerosoles antropogénicos estimulará preferentemente las bacterias heterótrofas en el agua y cambiará la composición de la comunidad microbiana. También esperamos que una parte de la materia orgánica atmosférica sea refractaria, favoreciendo la acumulación de carbono en aguas superficiales. En escenarios de futuro del Mediterráneo, la deposición de aerosoles debería tener un impacto aun mayor sobre los ecosistemas marinos pues la carga de aerosoles, tanto naturales como antropogénicos, está aumentando mientras que la profundidad de la capa de mezcla está disminuyendo. Por ello, ANIMA ayudará a comprender tanto la actual como la futura respuesta oceánica a la deposición atmosférica.

**PALABRAS CLAVE:** Aerosoles, antropogénico, orgánicos, caracterización, microorganismos del aire, degradación, turbulencia, columna de agua

**TITLE OF THE PROJECT:** Atmospheric inputs as a source of organic Nutrients and microorganisms In MArine ecosystems

**ACRONYM:** ANIMA

**SUMMARY** Maximum 3500 characters (including spaces):

The aim of ANIMA is to quantify and characterize the atmospheric inputs of organic matter and airborne microorganisms and to evaluate their impact in the frame of the NW Mediterranean marine ecosystem. ANIMA combines advances in the characterization of airborne particles, both living and non-living, depositing over the ocean and their interactions with the chemistry and biology in seawater. Special emphasis will be given to organic matter composition and degradation, a subject of high interest in current oceanography as it affects the global carbon cycle. ANIMA will use primary organic matter particles found in aerosols, such as pollen and soot, and study their degradation and the signature of leached dissolved substances in relation to signatures observed at sea. Airborne microorganisms will be mapped for different locations and away from land into the open sea. Microorganisms, including fungi, will be identified using different techniques and scanned for their organic matter degradation capacity in marine systems. The overriding hypothesis of ANIMA is that the larger proportion of organic matter in anthropogenic aerosols preferentially stimulates the heterotrophic bacteria in the water and changes microbial community composition. But we also expect a portion of airborne organic matter to be rather refractory, thus favoring carbon accumulation in surface waters. In future scenarios of the Mediterranean, aerosol deposition should have an even larger impact on marine ecosystems as aerosol loads, both natural and anthropogenic, are increasing while the mixed layer depth is shallowing. Thus, ANIMA will help understand current and future ocean responses to aerosol deposition.

**KEY WORDS:** Aerosols, anthropogenic, organics, characterization, airborne microorganisms, degradation, turbulence, water column depth

## Parte B: INFORMACIÓN ESPECÍFICA DEL EQUIPO

### B.1. RELACIÓN DE LAS PERSONAS NO DOCTORES QUE COMPONEN EL EQUIPO DE TRABAJO

1. Isabel Marín Beltrán. Licenciada en Ciencias del Mar (Universidad de Cádiz), Máster en Ciencias del Mar (Universidad de Barcelona). Contrato en formación.
2. Carolina Antequera. Técnico Superior en Química Ambiental. Contrato de Personal Técnico de Apoyo. Temporal
3. Técnico Contratado. Técnico Superior en Química Ambiental o similar. Persona a contratar con fondos del proyecto durante 3 años.

### B.2. FINANCIACIÓN PÚBLICA Y PRIVADA (PROYECTOS Y/O CONTRATOS DE I+D+I) DEL EQUIPO DE INVESTIGACIÓN

- MINECO: CTM12-34294. DO-RE-MI. Dissolved organic matter remineralization in the ocean: Microbial and biogeochemical constraints. **IP: Cèlia Marrasé**. Participantes: **M Montserrat Sala, Francesc Peters, Laura Arin**. 01/01/2013 – 31/12/2015. 275.000 €. Relación con el proyecto que se presenta: Muy relacionado. Estado: concedido y vigente
- MINECO: CTM2012-37615. PEGASO. Plankton-derived emissions of trace gases and aerosols in the southern ocean. **IP: Rafel Simó**. Participantes: **Marta Estrada, M. Montserrat Sala**. 01/01/2013 – 31/12/2015. 280.000 €. Relación con el proyecto que se presenta: Algo relacionado. Estado: concedido y vigente
- MICINN: CTM2011-23458. ADEPT. Aerosol deposition and ocean plankton dynamics. **IP: Francesc Peters**. Participantes: **Cèlia Marrasé, Marta Estrada, Laura Arin**. 01/01/2012 – 31/12/2015. 222.640 €. Relación con el proyecto que se presenta: Muy relacionado. Estado: concedido y vigente
- Fundación Iberdrola. LITOFUT. ¿Como responderá el plancton al aumento de temperatura y a la presión humana en la costa? Escenarios de futuro. **IPs: Francesc Peters, Estela Romero**. 01/07/2013 - 30/06/2014. 20.000 €. Estado: Concedido.
- MICINN: CTM2011-13137-E. NEMO. Nuevas implicaciones de la Estructura de la Materia Orgánica en el Océano. **IP: M Montserrat Sala**. Participantes: **Cèlia Marrasé, Francesc Peters**. 01/01/2012 – 31/12/2012. 49.500 €. Relación con el proyecto que se presenta: Algo relacionado. Estado: concedido
- MICINN: CTM2009-09352, MAR. STORM. Structure of Organic Matter in the coastal ocean: biogeochemical and ecological implications. **IP: M Montserrat Sala**. Participantes: **Cèlia Marrasé, Francesc Peters**. 01/01/2010 – 31/12/2012. 299.000 €. Relación con el proyecto que se presenta: Muy relacionado. Estado: concedido
- MICINN: CTM2008-03309/MAR. SUMMER. Evaluación in situ de la modulación de la exposición a la radiación solar por mezcla superficial. 2009-2011. **IP: Rafel Simó**. Participantes: **Cèlia Marrasé, Francesc Peters**. Relación con el proyecto que se presenta: No relacionado. Estado: concedido
- Consolider: CSD2008-00077. MALASPINA. Circumnavigation Expedition Malaspina 2010: Global Change and Biodiversity Exploration of the Global Ocean. **IP: Carlos M. Duarte**. Participantes: **M Montserrat Sala, Cèlia Marrasé, Marta Estrada**. 15/12/2008 – 15/12/2014. 698.809 €. Relación con el proyecto que se presenta: Algo relacionado. Estado: concedido
- MEC: CTM2004-04442-C02. VARITEC. Aproximación multiescala a la variabilidad de la turbulencia y su efecto sobre la estructura y la dinámica del ecosistema costero en el Mediterráneo noroccidental. 13/12/2004-12/12/2007. 151.800 €. **IP y Coordinador: Francesc Peters**. Relación con el proyecto que se presenta: Algo relacionado. Estado: concedido

**Parte C: DOCUMENTO CIENTÍFICO. Máximo 20 páginas.**

**C.1. SCIENTIFIC PROPOSAL**

**1. Background and state of the art.**

**The project addresses the role of atmospheric organic matter and micro-organisms in the ocean**

This project builds on two crucial multidisciplinary aspects in oceanography. One is the importance of atmospheric deposition in affecting marine production and dynamics, especially considering that the Mediterranean is an area heavily affected by global change trends in aridity (IPCC 2013) and industrialization, all increasing deposition. The other concerns the dynamics of organic matter in the sea, with a background concentration of slowly degradable material and implications for the regulation of the global atmospheric carbon dioxide pool (Hansell & Carlson 2002). Atmospheric deposition contains organic material, especially when the inputs have an anthropogenic origin, but its role in either stimulating heterotrophic bacterial production or alternatively in adding to the refractory organic carbon pool is largely unknown.

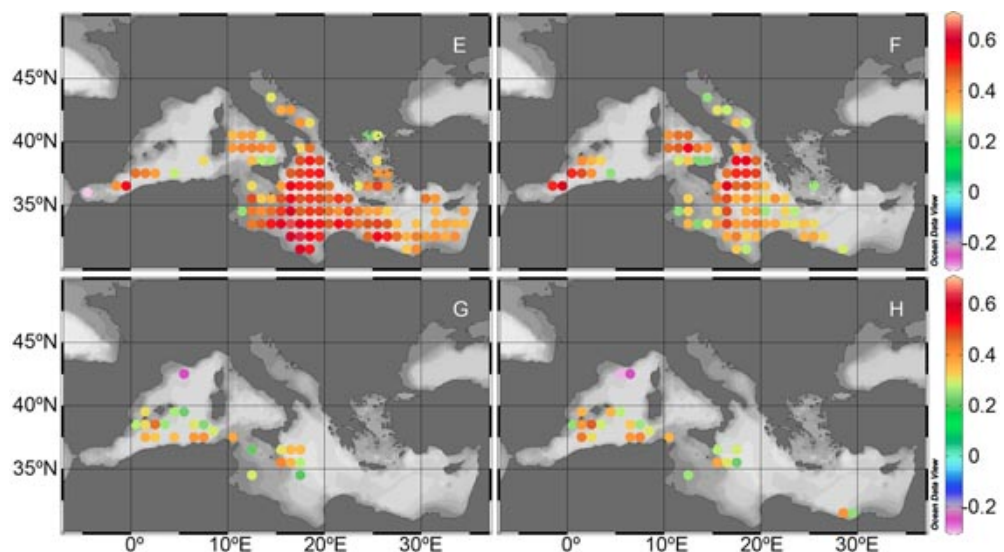


Figure 1. Correlation between chlorophyll concentration and dust deposition. Statistically significant ( $p < 0.05$ ) correlation coefficient ( $r$ ) between chlorophyll concentration and dust deposition (left panels) and between the seasonally detrended chlorophyll concentration and the seasonally detrended dust deposition (right panels) for spring (E,F) and for summer (G,H). From Gallisai et al. 2014.

**Aerosols have major global impacts**

Aerosols have major impacts on weather and climate regulations (Booth et al. 2012, Creamean et al. 2013) and even on crop production (Liu et al. 2013). Atmospheric desert dust may travel large distances from its source and has been proposed to have ocean production regulation effects over geological times scales (Jaccard et al. 2013). The Mediterranean Sea atmosphere is subject to the continuous injection of Saharan and Middle East mineral dust particles (Pey et al. 2013). At the same time, coastal areas, especially in industrialized regions have a large background of deposition of anthropogenic origin, with an important component coming from high temperature combustion processes. Recent studies of our group show that correlations between atmospheric aerosols of Saharan origin and surface chlorophyll depend on location and season of the year (Fig. 1). In local studies of the NW Mediterranean, there are contrasting evidences of enrichment due to phosphorus (Izquierdo et al. 2012) and negative correlations between atmospheric aerosols and chlorophyll (Jordi et al. 2012) pointing to some possible inhibitory effects of trace metals such as copper (Paytan et al. 2009).

**Effects of aerosols in marine systems are still unclear**

**The effect of organic matter from aerosols has been little studied**

The organic fraction of atmospheric deposition has been given less attention by the oceanic research community in relation to the role of inorganic nutrient enrichment and the role of trace metals, but is now recommended a main focus of study (Markaki et al. 2010). In previous experiments (Romero et al. 2011, Marín et al., in prep) we have seen that bacterial production in oligotrophic systems responds faster to aerosol inputs than autotrophic components. This has also been found by other authors (Marañón et al. 2010). Bacterial production also tends to be higher when aerosols are from anthropogenic origin than from mineral crust origin (Fig. 2). When rain washes out the particles in the air, wet deposition may become a large input of stimulating mass for the marine ecosystem. Martínez-García et al (2015) found that rainwater stimulated phytoplankton in the ría de Vigo and that dissolved organic nitrogen (DON) was in part responsible for the stimulation. Rainwater from different environmental backgrounds was assessed, being the rainwater from urban origin the one that produced the strongest responses. Albeit the Mediterranean has less rain events than the NE Iberic Peninsula, large rain events, especially in autumn, may be an important source of organics into the system. Both dry and wet deposition in the Barcelona coastal area amounts to  $200 \text{ mmol C m}^{-2} \text{ y}^{-1}$  (Peters et al., in preparation). All this points to a relevant role of organic carbon and, in general, organic matter of atmospheric origin in the stimulation of marine ecosystems, albeit details remain largely unknown.

**Prominent role in stimulating marine bacteria**

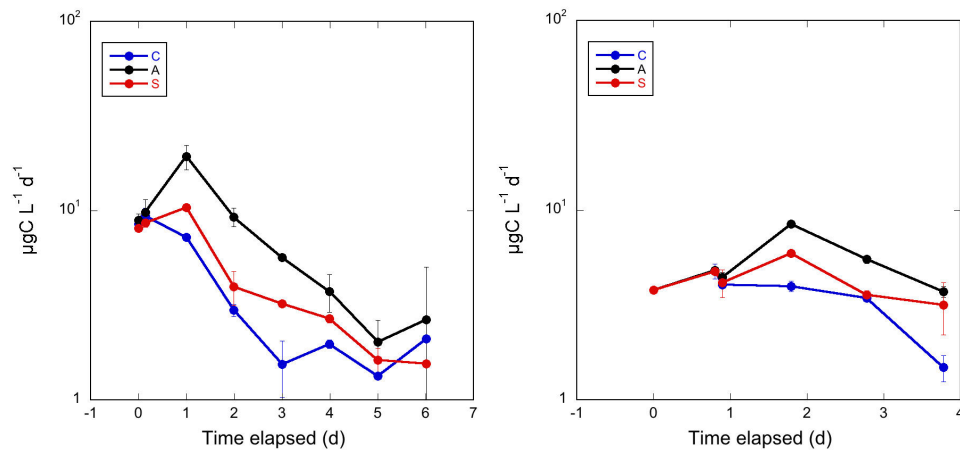


Figure 2. Bacterial production in two experiments with natural seawater, under control conditions (C) and amended with  $0.8 \text{ g L}^{-1}$  of anthropogenic (A) or Saharan (S) aerosols. ADEPT project results. Marín et al. in preparation.

**We have just started to open the box of the fate of different fractions of organic matter from aerosols**

The ADEPT project started to address the dynamics of the subpool of organic matter that has colored properties (CDOM) in experiments with seawater amended with atmospheric aerosols. Despite stimulation of bacterial production (Fig. 2) there is an increase of recalcitrant components of organic matter after aerosol addition that remain largely stable over the ca. week-long experiments. At the same time, we saw increases in fluorescent organic matter (FDOM, a further subpool of chromophoric organic matter) that are larger during Saharan dust deposition, which is puzzling (Fig. 3). In the Barcelona area, Saharan dust events tend to be mixed with local anthropogenic emissions and there may be an interaction. Sorting out the organic matter components in atmospheric aerosols and their reactivity is crucial to advance in this field. The quantification of CDOM in aerosols is also of interest to evaluate the potential role of dust in the exceptionally high CDOM/Chlorophyll ratio recorded in Mediterranean waters (Claustre et al 2002, Para et al 2010, Organelli 2014). The recalcitrant character of the organic matter (OM) atmospheric inputs can induce an increase in the turnover rate of organic matter in the ocean. In this context it is necessary to investigate the degradability of this organic matter



depending on *in situ* and future scenario conditions to evaluate the possible contribution of atmospheric OM deposition as a mechanism of carbon sequestration. ANIMA will continue exploring the dynamics of the chromophoric fractions of organic matter in relation to the atmospheric inputs to seawater, but will also go a step further and use ultra high resolution Fourier transform ion cyclotron resonance mass Spectrometry (FT-ICR MS) (Lechtenfeld et al 2014). This allows to discern a suite of organic matter components independently of their chromophoric properties and look into the dynamics of specific components.

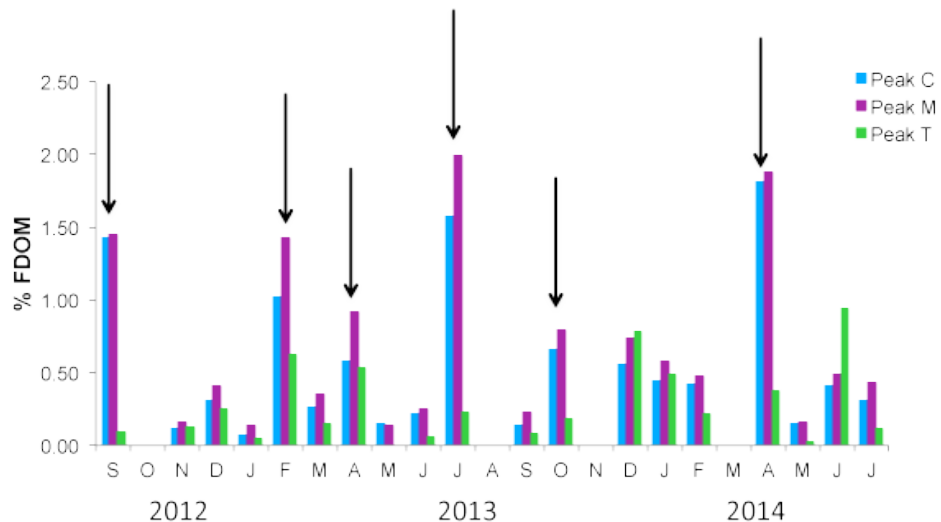


Figure 3. Daily aerosol deposition-derived FDOM flow to the sea surface as a percentage of concentration in Barcelona coastal waters. Humic-like (peak C and peak M) and protein-like (peak T) substances. Humic-like substances are recalcitrant, but quite photolabile, while protein-like substances are more biolabile (Coble, 2007). Arrows indicate Saharan dust events. Sánchez et al. submitted.

**Turbulence to enhance the reactivity of aerosols in seawater**

Large deposition events tend to be accompanied by storms. Increased wind shear over the ocean is transferred into the upper mixed layer in the form of isotropic turbulence (Oakey 1985). Turbulence in water increases the rate of contact of particles and the rate of diffusion of solutes to or away from particles (Peters & Marrasé 2000). Thus, in a solute diffusion limited environment such as the ocean, turbulence may increase the flux of dissolved substances away from deposited particles and the flux of solutes towards osmotrophic organisms. This is especially relevant for 1) high molecular weight substances (low diffusivity) that can be found in organic matter (Malits et al. 2004) or for 2) large osmotrophic organisms (Peters et al. 2006). Overall, turbulence increases the rate of reactivity between the dissolved and the particulate fractions in water, but this has been poorly studied in the context of aerosol deposited material and the consequences for organisms (Romero et al. 2011).

**Aerosols contain biological particles**

Atmospheric aerosols also contain biological particles. The presence of microorganisms in the atmosphere and their relevance for atmospheric processes have been subject of investigations since the 19th century (see Després et al. 2012 and references therein). Airborne microbes are thought to play important roles in meteorological processes such as the formation of clouds and snow (Christner et al. 2008; Morris et al. 2014), the long-range dispersal of pathogens (Polymenakou et al. 2007) and the maintenance of the diversity in aquatic systems (Hervàs et al. 2009). Most studies of airborne microorganisms focus on public health concerns in hospitals and workplaces and very few have conducted outdoor in urban and rural environments (e.g. Maron et al 2005; Després et al. 2007; Li et al. 2010), and lately also in marine environments (Mayol et al. 2014; Seifried et al.

2015).

**Different techniques provide complementary information on biological aerosols**

Most investigations have used culture-dependent techniques despite the inevitable biases (selectivity of the media, incubation time, incubation temperature). Recent studies have shown that culture-dependent and culture-independent methods produce different results for the same bacterial communities (Cho and Hwang 2011; Ravva et al. 2012) although some findings may overlap (Fahlgren et al. 2010; Urbano et al. 2011). Furthermore, even if viable, only a small fraction of environmental bacterial are culturable (around 0.001-0.01% Colwell 2000). Studies on coastal aerosols have used so far a combination of culture-dependent and culture-independent methods. Bacteria were identified by cloning (Fahlgren et al. 2010; Urbano et al. 2011) or Denaturing Gradient Gel Electrophoresis (DGGE) with subsequently conducted sequencing of bands (Cho and Hwang 2011). Both resulted in few sequences that most likely reflected only the most abundant taxa. However, a high-throughput pyrosequencing approach provides a detailed description of the microbial community including rare taxa (Bowers et al. 2011, Seifried et al. 2015). Although the abundance of aeroalgae was reported less in comparison to other microorganisms such as bacteria or fungi (Tormo et al. 2001) its presence has been acknowledged since 1844 (Ehrenberg 1844). Their main origin is aquatic or terrestrial. The role of these biological particles modifying the natural community composition, and the biochemical or genetic library is an open question.

**ANIMA builds on previous knowledge of the scientific team**

ANIMA builds on the knowledge gained from several previous projects. ADEPT (Aerosol Deposition and Ocean Plankton Dynamics, PI: Francesc Peters) focused on analyzing model data for the Mediterranean in relation to chlorophyll, started time series of deposition measurements, and did experiments assessing the role of atmospheric deposition on plankton communities, with special emphasis on the role of inorganic nutrients. DOREMI (Dissolved Organic matter REmineralization in the ocean: Microbial and biogeochemical constraints, PI: Cèlia Marrasé) focused on the remineralization of organic matter in the ocean, and started looking at the dynamics of different components of the refractory pool of organic matter. STORM (STructure of ORganic Matter in the coastal ocean: biogeochemical and ecological implications, PI: M Montserrat Sala) looked at the structure of organic matter in the ocean from a continuum of dissolved to particulate fractions. The role of small-scale turbulence on the uptake of nutrients by plankton was extensively studied, especially in the context of the European project NTAP (Nutrient dynamics mediated through turbulence and plankton interactions, EU Coordinator: Cèlia Marrasé), and VARITEC (Multiscale approximation to the variability of turbulence and its effect on the structure and dynamics of a NW Mediterranean coastal ecosystem, Coordinator: Francesc Peters).

What we propose in ANIMA is to build on the expertise gained with these projects and expand it to look at the intricate dynamics between the organic matter (living and non-living) of atmospheric origin interacting with the marine ecosystem. We will characterize and address different components of such particulate and dissolved organic matter, including airborne microorganisms, and see their effects on the stimulation of the marine microbial components and the degradability of dissolved organic matter in the ocean. Albeit the focus will be on organic components, our experience with the highly complex matrix of elements in aerosols shows us we will also have to take into account inorganic components and the possible effects on autotrophs in the system.

ANIMA will target the Mediterranean region, with a focus in the area around

**The Mediterranean is subject to global change impacts regarding aerosols and oceanography**

Barcelona since the city provides a large anthropogenic footprint to aerosol composition (Querol et al. 2001). The Mediterranean is one of the areas most affected by global change projections. According to the IPCC panel (IPCC 2013) "the frequency and intensity of drought has likely increased in the Mediterranean" and projections are to continue increasing over the 21st century. This results in more wind erosion and transport of aerosols over the Mediterranean Sea. Aerosol optical thickness shows increasing trends in the Sahara and the Arabian Peninsula. At the same time, it seems that air quality in Europe has improved over the last years, but projections to increase nitrogen load to the ecosystem and industrialization in the southern Mediterranean countries foresee an increase in anthropogenic emissions that may affect the Mediterranean.

**2. Hypothesis and objectives**

The aim of the project is to quantify and characterize the atmospheric inputs of organic matter and airborne microorganisms and to evaluate their impact in the frame of the NW Mediterranean marine ecosystem.

The overall objective has sub-objectives addressed to 1) build Mediterranean databases of atmospheric deposition of organic matter in collaboration with international efforts, 2) identify airborne microorganisms that are depositing over the surface ocean and their role in the degradation of organic matter, and 3) address the specific role of different types of atmospheric inputs, including those of a high anthropogenic footprint, in marine microbial ecology and biogeochemistry.

ANIMA is being applied to the "RETOS de la Sociedad" call of the Spanish Programa Estatal de I+D+i. Specifically ANIMA is included in RETO 2) Seguridad, calidad alimentaria; actividad agraria productiva y sostenible; sostenibilidad de recursos naturales, **investigación marina y marítima**. It tangentially addresses aspects of RETO 5) Acción sobre el cambio climático y eficiencia en la utilización de recursos y materias primas. ANIMA addresses the functioning of marine ecosystems as they are affected by deposition of extraneous living and non-living particles that may come from afar or be heavily modulated by anthropogenic influence. As this deposition is substantially affected by global changes of both natural and anthropogenic origin, understanding its effects in marine systems is crucial to build future scenarios.

The goals of ANIMA are included in several key aspects of the EU HORIZON 2020 program. The program "Fighting and adapting to climate change" mentions 1) Develop technological options and strategies to improve air quality and reduce the carbon footprint of European cities. 2) Create climate change networks to facilitate dialogue among relevant scientific communities, funding bodies and user communities in the EU and 3) Develop climate modelling and science for climate services to help provide trustworthy science-based information to government, public and private decision makers. Under "Food security, sustainable agriculture and forestry, marine and maritime and inland water research and the bioeconomy", there are objectives to better understand the functioning of the ocean to address the global carbon cycle and atmospheric CO<sub>2</sub> regulation and the present and future responses to parameters that affect ocean production. In "Health, demographic change and wellbeing" there are objectives related to assess and secure air quality, including air transported microorganisms. In addition, the gender ratio in ANIMA promotes gender equality in Research and Innovation which is one of the goals of "Science with and for Society" of HORIZON 2020

The aims of the ANIMA are included in large international efforts. SOLAS (Surface Ocean-Lower Atmosphere Study) has "Atmospheric control of nutrient cycling and production in the surface ocean" as a specific priority objective. IMBER (Integrated Marine Biogeochemistry and Ecosystem Research) focuses on marine ecosystem interactions including the extensive use of genomics in relation to nutrient and carbon cycles. LOICZ (Land-Ocean Interaction in the Coastal Zone), especially regarding "Implications of Global Change for Coastal Ecosystems and Sustainable Development" and "Biogeochemical Cycles in Coastal and



Shelf Waters". The French government has specific ENVIMED calls to build international networking regarding environmental processes in the Mediterranean. Francesc Peters participated in an ENVIMED project on building databases of aerosol deposition, and will participate in a second ENVIMED call to continue such efforts.

### 3. Specific Tasks

Workpackage1. Measurements and characterization

This WP contains tasks related to the observation, detection and characterization of different types of aerosols and aerosols deposited in seawater

Task 1.1. Atmospheric deposition measurements. *Task Leader: Peters. Participants: Contracted Technician, Aubert, Marin, Antequera*

Hypothesis: The organic nutrient fraction is proportionally larger in anthropogenically dominated situations than during Saharan dust events.

We have been collecting atmospheric deposition in Barcelona since May 2011, characterizing mainly the input of inorganic nutrients and total organic carbon and looked into the chromophoric properties of organic matter (CDOM and FDOM). Here we plan to expand this time series, constructing another sampling tower identical to that in operation (see picture of the [bulk deposition sampling tower](#) at the roof of ICM) to collect more variables, e.g. dissolved organic compounds, metals, and biological particles. We will determine organic and inorganic dissolved nutrients, trace metals in particulate and dissolved fractions, and characterize organic matter based on fluorescent and chemical properties. All will be collected in 2 l polypropylene bulk samplers with 500 ml sterile artificial seawater. Besides the bulk collector, we will analyze wet deposition in samples using an MTX collector that we have budgeted. The collector has a rain sensor which opens the lid of the collector only when it rains. The time series of deposition measurements gives a backbone of data with seasonal trends and information on events that we will compare to air quality data from monitoring networks (with the collaboration of Dr. Xavier Querol, IDAEA, CSIC) and will it also be used to contextualize experimental results and other type of data and help with their interpretation. At the same time the data will be used in comparisons across the Mediterranean and with deposition data from numerical models.

Task 1.2. Comparison of the organic matter leached from atmospheric particles with other sources of OM in the littoral. *Task Leader: Marrasé. Participants: Contracted Technician, Koch, Romera*

Hypothesis: The proportion of different organic chemicals depends on the source

Besides the routine measurements in Task 1.1, we will take point samples at several times of the year and analyze the dissolved fraction chemically using CDOM and mass spectroscopy. The samples will be obtained from the atmospheric deposition in Barcelona, from the nearby Besòs river mouth, and from a routine monitoring coastal marine station in front of Barcelona. This will allow the identification of specific compounds in the water and use them as signatures for the different origins as well as to identify which compounds appear or disappear from atmospheric and riverine inputs at the coastal station. That is, we will determine two end members and the deviations with respect to pure mixing of the end members at the coastal site, that should take into account the intrinsic marine component. Samples will be analyzed in Germany under the supervision of Dr. Boris Koch.

Task 1.3. Characterization of airborne microorganisms. *Task Leader: Sala. Participants: Marrasé, Grossart, Arin, Contracted Technician*

Hypotheses: The composition of the airborne microbial communities will be highly variable and depending on the prevailing atmospheric origin: 1) Land to sea breeze (anthropogenic origin); 2) Sea to land breeze (marine origin); 3) Saharan dust (African origin). We also hypothesize that rain events will wash out the microbes from the atmosphere.

We will identify and quantify eukaryotes, fungi and prokaryotes in atmospheric samples taken at different locations. The main effort will be carried out at the roof of the ICM in Barcelona during an annual cycle and with special emphasis in specific events, but samples will also be

taken in Blanes (70 km north of Barcelona), much less anthropogenically influenced and at sea during a cruise to sample in the NW Mediterranean open ocean (see Task 3.3). Specific deposition events will be targeted using information from operational forecast models. Several methodologies will be applied:

A. Direct exposure of Petri dishes with appropriate culture media for each target group of microorganisms. This will allow isolation and identification of the main culturable groups of airborne prokaryotes and fungi. Agar media for bacteria will be Zobell marine agar and for fungi Seawater Starch Agar (SWSA) and Cornmeal Yeast Extract Seawater Agar (CMYSA), although it is possible that we will need to try other recipes (Nakagiri 2012).

For airborne algae, Bold Basal Medium (BBM) agar plates will be exposed for 10 min and then incubated at 20°C on a 16:8 h light-dark cycle for up to 10 months (Sahu and Tangutut 2015). Further growth in appropriate liquid media is intended. For all organisms, exposition and incubation conditions will be adjusted after initial tests. We will try to isolate some bacterial and fungal strains and, depending on their identity, they might be used in organic matter degradation experiments of Task 2.3.

B. An impingement aerosol sampler (Coriolis micro. Bertin Technologies, Montigny-le-Bretonneux, France) budgeted in ANIMA will be employed to collect samples for the study of airborne microorganisms. This Coriolis sampler is qualified for processing high volumes of air (300 l min<sup>-1</sup>, Dybwad et al. 2015) and accumulating the very small aerosols (0.5 to 10 µm) in a 12 ml sterile phosphate-buffered saline solution. Before each sampling, the sampling device will be cleaned with 70% ethanol. The samples will be processed using different techniques

B1. Transmitted light microscopy for the observation of phytoplankton and protozoa

B2. Epifluorescent microscopy using a generalistic stain (DAPI) for the observation and quantification of prokaryotes, auto and heterotrophic nanoflagellates, spores and fungi and Fluorescent in situ hybridization (CARD-FISH) for broad group identification of prokaryotes

B3. Scanning electron microscopy. Observation of inert particles, phytoplankton, pollen and spores. X-ray microanalysis will be used for particle chemical characterization.

B4. Pyrosequencing for genetic identification of the microbial community. After DNA isolation, samples will be externalized for sequencing using Illumina technologies. Data will be analyzed with the infrastructure of the bioinformatics cluster and expert help of the personnel of the newly established bioinformatics service at the ICM. Dr. Ramon Massana will assess with the interpretation of data on the diversity of the eukaryotic communities, and Dr. Hans-Peter Grossart specifically with that of fungi.

## Workpackage 2. Experiments

This WP contains tasks related to laboratory experiments with addition of different types of aerosols in seawater to assess, among others, system stimulation, microbial community changes, organic matter degradation or interaction with turbulence in water

Task 2.1. Organic matter degradation in seawater after atmospheric deposition. *Task Leader: Marrasé. Participants: Sala, Contracted Technician, Arin, Peters*

Hypothesis: Deposited particles and their leached chemicals will substantially alter the chemical and biological composition of seawater.

We will collect atmospheric particles, including falling organisms, in Barcelona using a new setup that will be built for the project using large polypropylene containers of 29 cm in diameter. We will let the containers collect particles for a period of 3, 7 and 14 days in sterile artificial seawater. In each case we will use three containers, one for extensive analysis of initial conditions and the other two with a natural seawater inoculum (at a 1/1 ratio) that will also have been characterized. We will follow the changes in particle types, bacteria attached to particles, bacterial community composition, bacterial activities and potential to degrade organic compounds, as well as the characterization of the organic matter (DOC, FDOM, CDOM, Mass spectrometry) for several days in the laboratory. Controlled temperature and light conditions will resemble natural conditions. The assessment of the effect of directly deposited aerosols is a substantial advancement with respect to previous experiments that depended on assessing a certain size fraction, determined by the filter type, of atmospheric

aerosols. Using the current approach we assure to test the whole suite of compounds in the deposition.

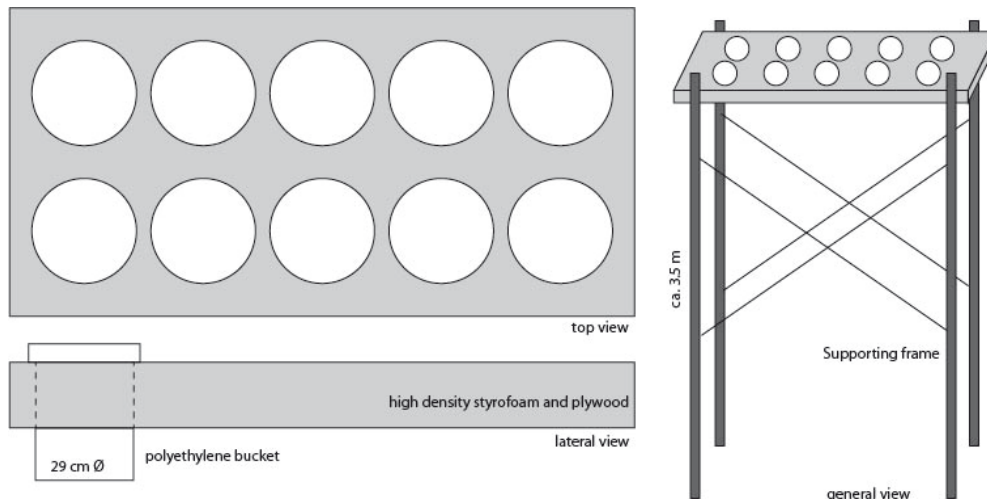


Figure. 4. Scheme of the sampling tower that will be built to hold manyfold large polypropylene containers. This structure will be placed at a ca. 2.5 m height at the roof of the ICM in Barcelona.

Task 2.2. Effects of turbulence on community use of atmospheric aerosols. *Task Leader: Peters. Participants: Contracted Technician, Arin, Sala, Marrasé, Romero, Estrada*  
Hypothesis: Turbulence will favor the larger autotrophs in the system

It has been shown that turbulence accelerates production processes in seawater. We will carry out a series of experiments amending collected atmospheric particles to seawater and tracking several parameters over a time period of 3 to 7 days. Particles will be collected onto a quartz fiber filter using high volume samplers. The inoculum will be obtained from these filters after sonication in artificial seawater. The experiments will be done comparing treatments in still water and water subjected to turbulence (ca.  $10^{-3} \text{ cm}^2 \text{ s}^{-3}$ ). Turbulence will be generated using vertically oscillating grids with an available system that has been profusely used and characterized (e.g. Peters et al. 2002). Experiments will be done in a controlled temperature and light chamber set to resemble ambient natural conditions at the time of experimentation. Although the overriding hypothesis of the project is that heterotrophic microorganisms take advantage of the organic matter, especially in anthropogenic aerosols, turbulence may add an advantage for the larger autotrophs to take up inorganic or organic nutrients. We will track nutrients and organic matter, heterotrophic and autotrophic microorganisms and community composition and microbial activities in order to test the hypothesis that turbulence will favor the larger autotrophs.

Task 2.3. Differential organic matter degradation based on origin. *Task Leader: Sala. Participants: Marin, Arin, Contracted Technician, Antequera*  
Hypothesis: Microbial activity depends of the chemical composition of organic matter.

We will collect organic particles from several different origins, including soot and pollen, which can be found in aerosols and be a source of organic carbon for microorganisms (Rösel et al. 2012, Malits et al 2015) and fungi (Würzbacher et al. 2014). Soot (black carbon) will be collected directly from a high temperature combustion source that will likely be gas heaters and obtained by gas company technicians. Pollen will be collected directly from several plants during their pollen outburst. Outburst forecast and advice will be provided by the Aerobiology group (Dr. Jordina Belmonte) of the Universitat Autònoma de Barcelona that elaborate the pollen calendars in our region. Likely plants will be pine and plane trees (in March), and some grasses (in May-June). We will also collect atmospheric particles based on size with our high volume samplers equipped with appropriate heads to collect total suspended particles (TSP), particles smaller than  $2.5 \mu\text{m}$  (PM<sub>2.5</sub>) and particles smaller than  $1 \mu\text{m}$  (PM<sub>1</sub>). Most Saharan particles are associated with sizes larger than  $2.5 \mu\text{m}$ , while anthropogenic particles tend to be in the smaller fractions. We will characterize particles by scanning electron microscopy and X-ray microanalysis. Then we will add this suite of

particles to 1) artificial seawater and 2) natural seawater and track mainly their chemical leaching and degradation. At endpoints we will also take samples for the abundance and production of microorganisms, their extracellular enzyme activity, and the composition of the microbial community.

#### Workpackage 3 Field and Integrative approach

This WP integrates tasks addressing field surveys at sea, data analysis and comparison across the Mediterranean

Task 3.1. Response to large deposition events along the water column. *Task Leader: Arin. Participants: Peters, Basart, Contracted Technician, Romero, Sala, Marrasé*

Hypothesis: The effect of essential elements coming from atmospheric deposition will decrease with depth under calm conditions

There is a question whether inputs from atmospheric origin have an impact throughout the mixed layer water column or mainly just the upper meters are mostly reactive. In this case we will target some of the large Saharan dust episodes that occur every year, using available operational forecast models and warnings. In particular we will use the CALIMA ([www.calima.ws](http://www.calima.ws)) warnings from the Spanish Ministerio de Agricultura, Alimentación y Medio Ambiente and the forecasts of Barcelona Supercomputing Center NMMB/BSC-Dust and BSC-DREAM8b models that feed to the WMO Sand and Dust Storm Warning Advisory and Assessment System (SDS-WAS) Regional Center for a regional domain comprising Northern Africa, Middle East and Europe (<http://sds-was.aemet.es/>), where it is combined with other dust model forecasts from collaborating institutions (ECMWF, SEEVCC, Met Office, NASA, NCEP, EMA and CNR) and evaluated in near-real time. The NMMB/BSC-Dust model runs operationally (7 days a week, 365 days a year) at the Barcelona Dust Forecast Center (<http://dust.aemet.es/about-us>) with a horizontal resolution of 10 km for the same region. Once events are detected we will sample the long term monitoring coastal station in front of Barcelona (station 1.4 with a water column depth of 40 m), just below the surface, at 1 m depth and at 20 m depth. Physical characterization of the water column will be done with a CTD equipped with PAR, and turbidity sensors. Biological and chemical characterization of the water will be done and bacterial production, extracellular enzyme activities and the utilization of sole-carbon sources measured. This will be compared to periods without a Saharan event, if at all possible, just before the event. With a forecast for an event, we plan to sample before the event and daily up to several days past the event, using the ICM coastal boat Ixasbide. The reason to do it with Saharan dust events is that they are easily identified and of a large magnitude with respect to the background, while anthropogenic deposition is more stable and thus it is more difficult to differentiate events.

Task 3.2. Deposition measurement across the Mediterranean and effect potential. *Task Leader: Peters. Participants: Aubert, Basart*

Hypothesis: Spatio-temporal conditions in the Mediterranean with low in situ nutrients, shallow mixed layers, and high turbulence should show the largest effects of deposition on production

Calculated deposition, inferred from suspended particles in the air and a settling flux, is hard to match real deposition and even more so the final speciation and bioavailability in seawater. Actual deposition data, such as ours in task 1.1, are scarce, especially about organic nutrients and organic matter and are sought after for large area comparisons and model validation. We plan on comparing our deposition measurements with measurements in the Eastern Mediterranean through data summarized in the frame of a French ENVIMED project called Tracomed (PI: Dr. Dominique Aubert) to understand differences across the Mediterranean. Our data will also be compared to modeling data from the Barcelona Supercomputing Center (Drs. Basart and Jorba).

A second part of this task is to provide Mediterranean maps of areas and times susceptible for atmospheric deposition to have a positive impact on system production. On the one hand, in situ essential elements should be relatively low for deposition to have an effect. At the same time, the mixed layer depth (MLD) should be relatively shallow so that the additions are not diluted to depth. What we will do is use MLD reanalysis data for the Mediterranean and



the freely-available output of a Mediterranean biogeochemical model (OPATM-BFM) for essential elements to determine the times are areas susceptible of stimulation by atmospheric deposition, including both inorganic and organic nutrient fractions. We will also incorporate a turbulence component calculated from wind shear reanalysis data (ECMWF40) both to consider it in accelerating processes and to separate effects of turbulence from amendments during storms. This approach is substantially different from the one used in our previous project ADEPT where the physics and chemistry of the receiving water body were not taken into account.

Task 3.3. Distribution of marine airborne microbes. *Task leader: Sala. Participants: Peters, Marrasé, Arin, Contracted Technician, Grossart, Koch, Aubert, Estrada, Romero*

Hypothesis: The importance of marine airborne microbes will increase with distance from the coast

This task is related to Task 1.3, where airborne microbes will be collected at different sites. Since we would like to see if there are differences in the sampled community between the coastal areas and the open sea, we have designed a cruise in the NW Mediterranean to see if there are gradients in the airborne samples with distance to the coast. We will use the Coriolis sampler for this purpose. At the same time we will also collect water column variables including samples for organic matter characterization in order to see if there are gradients of signatures of terrestrial (including atmospheric) matter and trace metals that could be related to atmospheric inputs. Trace metals will be collected away from the ship with an inflatable boat. We know that aerosols of European origin can influence areas of the NW Mediterranean (Jordi et al. 2012), thus, in addition, we plan to carry out anthropogenic aerosol addition experiments with surface water collected during the cruise in order to evaluate microbial community responses and compare them to those obtained in experiments with coastal water. Anthropogenic aerosols will have been collected previously in Barcelona with a high volume sampler. Filters will be weighted for collected mass determination and kept frozen until use in experiments. At sea we will collect subsurface seawater and place in replicate fluorinated containers. Aerosols will be added at  $0.8 \text{ mg L}^{-1}$ . Appropriate control containers will also be prepared. Containers will be incubated onboard at sea surface temperature and partially shaded to simulate light at 5 m depth. Containers will be sampled over several days for a suite of chemical and biological analyses such as in Tasks 2.1 and 2.2.

The relationship between the different scientific tasks can be visualized in Fig. 5. In addition to the scientific workpackages and tasks, there is a logistic workpackage that will take care of coordinating all tasks and efforts within the project, and all the necessary communication channels both within the project and externally to the project.

WP4 Coordination, outreach.

Coordination tasks will be done by the two co-PIs. Peters will take an active role in coordinating deposition sampling efforts, chemical analyses and time series comparisons and Sala will take the lead in coordinating biological and molecular aspects, experimentation and the research cruise.

Task 4.1. Coordination. *Task leaders: Peters, Sala. Participants: all*

The PIs will hold periodic meetings with all project members or different teams for workpackage and task planning, day to day details and results interpretation. Meetings will be presential whenever possible or alternatively skype technology will be used, especially with participants in other countries. In addition, three large workshops are planned. Workshop 1 will be internal and held during the first months of the project to discuss methodological implementations and procedures, details of task development and the like. It will consist of a one day round table presentations and discussions. Workshop 2 will also be internal. It will consist of a 1 day meeting held around month 18 to evaluate project progress and results. It will also serve to introduce changes in the methodology and workplan, if needed. Workshop 3 will consist of a two day meeting around month 35. During the first day there will be internal project presentations and discussions. The second day will consist of



presentations open to the public. All project collaborators will be invited to participate in the Workshop. We will also encourage projects or research groups working on similar or related topics to give presentations during the second day. Mailings will also be done to potentially interested parties.

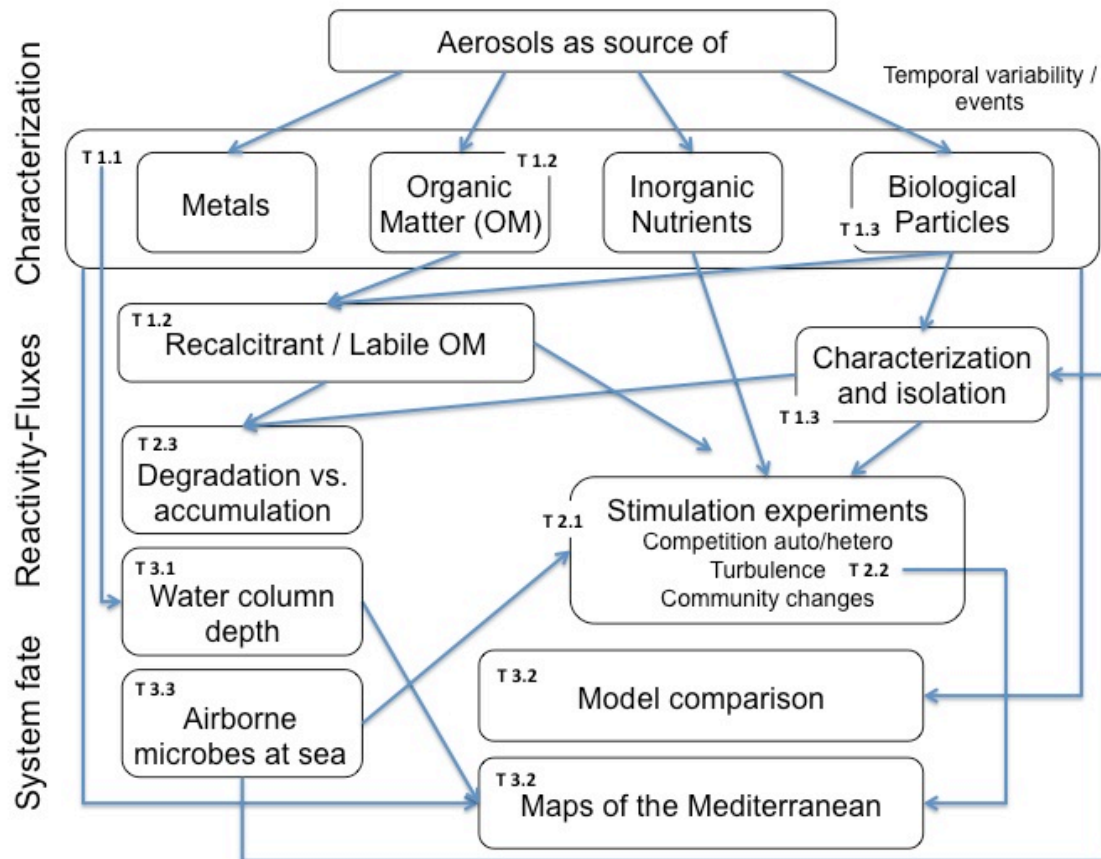


Figure 5. Scheme of the relationships between the different tasks and concepts in ANIMA

Task 4.2. Outreach. Task leaders: Sala, Peters. Participants: all

Besides diffusion of research results at international meetings and peer reviewed journals of high impact, the PIs will seek the endorsement of ANIMA by international projects such as SOLAS and IMBER, that will assure a broader impact of the project outcome. We will implement the Diffusion Plan outlined further below.

#### 4. Methodologies

**Inorganic nutrient analyses** (nitrate, nitrite, ammonium, silicate and phosphate) will be determined with an Alliance Evolution II autoanalyzer following Hansen and Koroleff (1999) and will be carried out at the ICM analytical service. **TDN and TDP** will be analyzed following Markaki et al (2010). **DOP and DON** will be calculated from TDP and TDN after subtracting DIP and DIN respectively. **DOC** will be measured with a Shimadzu TOC-V organic carbon analyzer also in the ICM analytical service. **CDOM** determinations will be measured in a Varian Cary spectrophotometer. Spectral scans will be collected between 250 and 750nm. Single measurements of peaks of humic and protein-like substances will be performed in a Perkin Elmer LS 55 luminescence spectrometer described elsewhere (Nieto-Cid et al 2006; Romera-Castillo et al 2010). **Metal analyses**. The sample for dissolved metals will be filtered through 0.45 µm cellulose acetate membranes and HNO<sub>3</sub> added for acidification until analysis using an ICP-MS Agilent 7700X (Dominique Aubert at the University of Perpignan). **Mass spectroscopy**. After organic matter extraction following Dittmar et al 2008, Ultrahigh resolution mass spectrometry (FT-ICR MS) analyses will be performed at German Research Center for Environmental health, Neuherberg, Germany with the supervision of Dr. B. Koch. **Bacterial production** will be measured by adding <sup>3</sup>H-leucine to 1.2 ml vials and determining radioisotope incorporation into protein after ca. 2 h. (Kirchman et al. 1985). **Extracellular enzyme activities**. Activities of several extracellular enzymes (α-glucosidase, β-glucosidase,

fucosidase, xylosidase, chitinase, aminopeptidase, alkaline phosphatase and esterase) will be determined with fluorogenic substrates according to Sala et al. (2010). **Utilization of sole carbon sources.** Using Biolog GN plates for prokaryotes, and Biolog FF for fungi, following a similar procedure as in Sala et al. (2006). **Abundance of microorganisms and particles.** Samples will be filtered onto 0.2  $\mu\text{m}$  and 3  $\mu\text{m}$  black polycarbonate membranes, stained with DAPI and counted with an epifluorescence microscope (Porter and Feig 1980). **CARD-FISH:** Catalyzed reporter deposition Fluorescence In Situ Hybridization will be used to assess the abundance of single bacterial groups (Pernthaler et al. 2002). **Diversity of microorganisms.** After PCR amplification of DNA, sequencing of the 16S rRNA gene for prokaryotes and of the 18S rRNA for eukaryotes will be performed by Illumina MiSeq platform, following published protocols (Caporaso et al. 2012). **Flow cytometry.** For abundances of microorganisms (virus, prokaryotes, picoeukaryotes, nanoeukaryotes), according to Gasol and del Giorgio (2000). **Culture plates:** Petri dishes will be filled with culture media specific for the target microorganisms (bacteria, algae, fungi) under sterile conditions. **Scanning electron microscopy** observations will be carried out at the microscopy service of ICM. X-ray microanalyses will be done following Segura-Noguera et al (2012).

### 5. Infrastructure and other equipment.

The ICM houses most of the infrastructure needed to accomplish the objectives of the project, e.g. high volume air samplers, a deposition sampling tower, temperature controlled chambers, systems to generate turbulence, spectrophotometers, spectrofluorometers, epifluorescence and inverted microscopes, CTDs, clean rooms for culturing organisms, and an equipped molecular biology lab. Also the ICM holds services for chemicals analyses (inorganic nutrients and organic matter), flow cytometry, bioinformatics, scanning electron microscopy (including X-ray microanalyses), boat for coastal sampling. Samples for mass spectrometry and metals will be analyzed by members of the working team: Dr. B. Koch and Dr. Dominique Aubert, respectively. A second deposition sampling tower will be setup. Also, a dust collection frame to hold manyfold bulk collectors (Task 2.1) will be designed and externalized for construction and setup. We will need to acquire a Coriolis microbial air sampler, heads for particle size fractionation for our all purpose MCV high volume samplers, and an MTX dry/wet deposition sampler, as well as some other small lab and computer equipment.

### Risks of failure

The influence of atmospheric deposition on ocean processes over geological times is unquestioned. Studies relating atmospheric deposition to microbial processes at ecological times scales do show a wide range of results, from significant to non-significant, and there are no clear trends. We will look mainly into the organic matter (so far, less studied) of atmospheric deposition (mainly of anthropogenic origin) with novel techniques that will discern different chemical components. There is a risk of obtaining non-significant responses of the seawater microbial community subjected to anthropogenic aerosols at ecological times scales but this will be a result in itself anyhow.

The characterization of the airborne microorganisms is a current hot-topic in microbial ecology. A broad spectrum of approaches both for sampling collection and for sample analysis are being applied, without a clear consensus on a standard procedure. Since organisms are present at very low concentrations in the atmosphere the main caveat is the detection limit of the techniques. The choice of the sampling device and the proper standardization of the protocols of sampling are crucial. In order to solve these initial shortcomings we plan to devote the first 6 months of the project to fine-tune the methodologies of sampling and of observation and identification of airborne microorganisms.

Sampling in coastal waters before and after the large Saharan deposition events of Task 3.1 depends on the events actually happening, on our ability to foresee them a few days in advance, and on logistics (sea state and others). If days previous to the event can not be sampled we will have to use an averaged reference for days previous to events.

The ANIMA consortium is aware of these risks and will large efforts in minimizing them.

6. Chronogram

Tasks	Centre	Persons	First Year (*)	Second Year (*)	Third Year (*)
Task 1.1. Deposition measurements	ICM, CEFREM	Peters, Cont. Technician, Aubert, Marin, Antequera	█	█	█
Task 1.2. Characterization of organic matter	ICM, AWI, RSMAS	Marrasé, Cont. Technician, Koch, Romera	█	█	█
Task 1.3. Airborne microorganisms	ICM, IGB	Sala, Marrasé, Grossart, Arin, Cont. Technician	█	█	█
Task 2.1. Amendment experiments	ICM	Marrasé, Sala, Cont. Technician, Arin, Peters	█	█	█
Task 2.2. Turbulence experiments	ICM, UPMC	Peters, Cont. Technician, Arin, Sala, Marrasé, Romero, Estrada	█	█	█
Task 2.3. Organic matter degradation	ICM	Sala, Marin, Arin, Cont. Technician, Antequera	█	█	█
Task 3.1. Water column measurements	ICM, UPMC	Arin, Peters, Basart, Cont. Technician, Romero, Sala Marrasé	█	█	█
Task 3.2. Deposition across Mediterranean and effect potentiality	ICM, CEFREM, BSC	Peters, Aubert, Basart	█	█	█
Task 3.3. Distribution of airborne microbes at sea	ICM, AWI, IGB CEFREM	Sala, all	█	█	█

## 7. Contracted personnel

We are asking for a full time technician at the level of Técnico Superior en Química Ambiental or similar. This contract is crucial for the ANIMA project as there is a vast amount of daily media and solution preparations, filtering, sample preparation and analysis, labware cleaning, routine sampling and analysis of aerosols, preparation and support during experiments and much more. These tasks do not require a trained researcher, so we ask for a lab technician. The ANIMA consortium will train the technician in the details of the different routines and techniques, which will give him/her an edge for future employment.

## References

- Booth BBB et al (2012) Aerosols implicated as a prime driver of twentieth-century North Atlantic climate variability. *Nature* 484: 228-232.
- Bowers RM et al (2011) Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J* 5: 601-612.
- Caporaso J et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 6: 1621-1624.
- Cho BC, Hwang CY (2011) Prokaryotic abundance and 16S rRNA gene sequences detected in marine aerosols on the East Sea (Korea): prokaryotes above the East Sea. *FEMS Microbiol Ecol* 76: 327-341.
- Christner BC et al (2008) Ubiquity of biological ice nucleators in snowfall. *Science* 319: 1214-1214.
- Claustre H et al (2002) Is desert dust making oligotrophic waters greener? *Geophys Res Lett* 29, 10.1029/2001GL014056
- Coble PG (2007) Marine optical biogeochemistry: the chemistry of ocean color. *Chem Rev* 107: 402-418.
- Colwell RR (2000) Viable but nonculturable bacteria: a survival strategy. *J Infect Chemother* 6: 121-125.
- Creamean JM et al (2013) Dust and Biological Aerosols from the Sahara and Asia Influence Precipitation in the Western U.S. *Science* 339: 1572-1578.
- Després VR et al (2007) Characterization of primary biogenic aerosol particles in urban, rural and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. *Biogeosciences* 4: 1127-1141.
- Després VR et al (2012) Primary biological aerosol particles in the atmosphere: a review. *Tellus B* 64: 1555-98.
- Dittmar T et al (2008) A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. *Limnol Oceanogr-Meth* 6: 230-235.
- Dybwad M et al (2014) Comparative Testing and Evaluation of Nine Different Air Samplers: End-to-End Sampling Efficiencies as Specific Performance Measurements for Bioaerosol Applications. *Aerosol Sci Tech* 48: 282-295.
- Ehrenberg GG (1844) Bericht Ueber die Zu Bekanntmachung Geergneten Verhandunger der Konigl Preuss. Acad Wiss Berlin 9: 194-197.
- Fahlgren C et al (2010) Annual variations in the diversity, viability, and origin of airborne bacteria. *Appl Environ Microb* 76: 3015-3025.
- Gallissai R et al (2014) Saharan Dust Deposition May Affect Phytoplankton Growth in the Mediterranean Sea at Ecological Time Scales. *PLoS ONE* 9: e110762
- Gasol JM, del Giorgio PA (2000) Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Sci Mar* 64:197-224.
- Hansell DA, Carlson CA (2002) *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, 774 pp.
- Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Ehrhardt M, Kremling K (Eds.) *Methods of Seawater Analysis*. Verlag Chemie, Weinheim, pp. 161e228.
- Hervàs A et al (2009) Viability and potential for immigration of airborne bacteria from Africa that reach high mountain lakes in Europe. *Environ Microbiol* 11: 1612-1623.
- IPCC, 2013: *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker TF et al (eds.)]. Cambridge Univ. Press, 1535 pp.



- Izquierdo R et al (2012) Atmospheric phosphorus deposition in a near-coastal rural site in the NE Iberian Peninsula and its role in marine productivity. *Atmos Environ* 49: 361-370.
- Jaccard SL et al (2013) Two Modes of Change in Southern Ocean Productivity Over the Past Million Years. *Science* 339: 1419-1423.
- Jordi A et al (2012) Copper aerosols inhibit phytoplankton growth in the Mediterranean Sea. *PNAS* 109: 21246-21249.
- Kirchman D et al (1985) Leucine incorporation and its potential as a measure of protein-synthesis by bacteria in natural aquatic systems. *Appl Environ Microbiol* 49: 599-607.
- Lechtenfeld OJ et al (2014) Molecular transformation and degradation of refractory dissolved organic matter in the Atlantic and Southern Ocean, *Geochim Cosmochim Acta* 126: 321-337
- Li KS et al (2010) Comparison of the biological content of air samples collected at ground level and at higher elevation. *Aerobiologia* 26: 233-244.
- Liu X et al (2013) Enhanced nitrogen deposition over China. *Nature* 494: 459-462.
- Malits A et al (2004) Effects of small-scale turbulence on bacteria: A matter of size. *Microb Ecol* 48: 287-299
- Malits A et al (2015) Potential impacts of black carbon on the marine microbial community. *Aquat Microb Ecol* 75: 27-42
- Marañón et al (2010) Degree of oligotrophy controls the response of microbial plankton to Saharan dust. *Limnol Oceanogr* 55: 2339-2352
- Markaki Z et al (2010) Variability of atmospheric deposition of dissolved nitrogen and phosphorus in the Mediterranean and possible link to the anomalous seawater N/P ratio. *Mar Chem* 120: 187-194.
- Maron et al (2005) Assessing genetic structure and diversity of airborne bacterial communities by DNA fingerprinting and 16S rDNA clone library. *Atmos Environ* 39: 3687-3695.
- Martínez-García et al (2015). Impact of atmospheric deposition on the metabolism of coastal microbial communities. *Est Coast Shelf Sci* 153: 18-28
- Mayol E et al (2014) Resolving the abundance and air-sea fluxes of airborne microorganisms in the North Atlantic Ocean. *Front Microbiol* 5:557.
- Morris CE et al (2014) Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Glob Change Biol* 20: 341-351.
- Nakagiri A (2012) Culture collections and maintenance of marine fungi. In *Marine Fungi and fungal-like organisms*, Jones EBG and Pang KL (Eds). Walter de Gruyter GmbH & Co. KG, Berlin/Boston
- Nieto-Cid M et al (2006) Microbial and photochemical reactivity of fluorescent dissolved organic matter in a coastal upwelling system. *Limnol Oceanogr* 51: 1391-1400.
- Oakey NS (1985) Statistics of Mixing Parameters in the Upper Ocean During JASIN Phase 2. *J Phys Oceanogr* 15: 1662-1675.
- Organelli E et al (2014) Seasonal dynamics of light absorption by chromophoric dissolved organic matter (CDOM) in the NW Mediterranean Sea (BOUSSOLE site). *Deep-Sea Res Part I*: 91: 72-85.
- Para J et al (2010) Fluorescence and absorption properties of chromophoric dissolved organic matter (CDOM) in coastal surface waters of the northwestern Mediterranean Sea, influence of the Rhône River. *Biogeosci Discuss* 7: 4083-4103.
- Paytan A et al (2009) Toxicity of atmospheric aerosols on marine phytoplankton. *PNAS* 106: 4601-4605.
- Pernthaler A et al (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl Environ Microbiol* 68: 3094-3101
- Peters F et al (2002) Turbulence and the microbial food web: effects on bacterial losses to predation and on community structure. *J Plankton Res* 24: 321-331
- Peters F et al (2006) Effects of small-scale turbulence on the growth of two diatoms of different size in a phosphorus-limited medium. *J Mar Syst* 61: 134-148.
- Peters F, Marrasé C (2000) Effects of turbulence on plankton: an overview of experimental evidence and some theoretical considerations. *Mar Ecol Prog Ser* 205: 291-306.
- Pey J et al (2013) African dust outbreaks over the Mediterranean Basin during 2001–2011: PM10 concentrations, phenomenology and trends, and its relation with synoptic and mesoscale meteorology. *Atmos Chem Phys* 13: 1395-1410.



- Polymenakou PN et al (2007) Particle size distribution of airborne microorganisms and pathogens during an intense African dust event in the eastern Mediterranean. *Environ Health Perspect* 116: 292-296.
- Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol Oceanogr* 25: 943-948.
- Querol X et al (2001) PM10 and PM2.5 source apportionment in the Barcelona Metropolitan area, Catalonia, Spain. *Atmos Environ* 35: 6407-6419.
- Ravva SV et al (2012) Bacterial communities in urban aerosols collected with wetted-wall cyclonic samplers and seasonal fluctuations of live and culturable airborne bacteria. *J Environ Monitor* 14: 473-481.
- Romera-Castillo C et al (2010) Production of chromophoric dissolved organic matter by marine phytoplankton. *Limnol Oceanogr* 55: 446-454.
- Romero E et al (2011) Coastal Mediterranean plankton stimulation dynamics through a dust storm event: An experimental simulation. *Est Coast Shelf Sci* 93: 27-39.
- Rösel S et al (2012) Effects of pollen leaching and microbial degradation on organic carbon and nutrient availability in lake water. *Aquatic Sciences*. 74: 87-99.
- Sahu N, Tangutur AD (2015) Airborne algae: overview of the current status and its implications on the environment. *Aerobiologia* 31: 89-97.
- Sala MM et al (2006) Seasonal differences in functional diversity of bacterioplankton in contrasting environments of the Catalan coast (NW Mediterranean). *Aquat Microb Ecol* 44:1-9.
- Sala MM et al (2010) The impact of ice melting on bacterioplankton in the Arctic Ocean. *Polar Biol* 33: 1683-1694.
- Sánchez-Perez E Organic matter quality alterations induced by dust inputs. Time series and experimental approaches in the NW Mediterranean. Submitted.
- Segura-Noguera M et al (2012) An improved energy dispersive microanalysis method for analyzing simultaneously carbon, nitrogen, oxygen, phosphorus, sulfur, and other cation and anion concentrations in single natural marine microplankton cells. *Limnol Oceanogr Methods* 10: 666-680.
- Seifried JS et al (2015) Spatial distribution of marine airborne bacteria. *Microbiol Open* 4(3): 475-490.
- Sunyer J et al (2015) Association between Traffic-Related Air Pollution in Schools and Cognitive Development in Primary School Children: A Prospective Cohort Study. *Plos Medicine* 12(3): e1001792
- Tormo R et al (2001) A quantitative investigation of airborne algae and lichen soredia obtained from pollen trap in south-west Spain. *Eur J Phycol* 36: 385-390.
- Urbano R et al (2011) Detection and phylogenetic analysis of coastal bioaerosols using culture dependent and independent techniques. *Biogeosciences* 8: 301-309.
- Wurzbacher, C (2014) Importance of Saprotrophic Freshwater Fungi for Pollen Degradation. *PLoS ONE* 9:e94643

## C.2. EXPECTED IMPACT OF THE RESULTS

### Scientific, social and economic impacts

ANIMA will provide a dataset on deposition of detailed organic nutrients and organic matter, for which there is little information. ANIMA will also study the fate of atmospheric organic matter once deposited over the ocean, and whether it stimulates system production or ads to the refractory carbon pool. The fate of organic carbon in the ocean has far-reaching consequences for the regulation of the global carbon cycle. ANIMA will also monitor and identify airborne microbes, with a special emphasis on fungi, and study their role in degrading organic matter in the ocean. All these issues are current scientific hot topics in marine and environmental sciences and ANIMA will provide significant advances. Project results will be published in the highest impact journals in the fields of oceanography, microbiology and geosciences, and presented at relevant international meetings, including the ASLO Ocean Sciences Meeting and the SOLAS and IMBER Open Science Conferences. Although ANIMA is not focused on technological development, it will provide novel Although ANIMA is not focused on technological development, it will provide novel methodological solutions, i.e. we will build a bulk deposition sampling tower for manyfold large containers, which will enable the collection of enough atmospheric particles to be used directly in

stimulation experiments. International collaboration is built within ANIMAs working group with the aim of establishing firm research networks and applying to international funding calls. The Mediterranean region is most affected by global changes in aridity. This results in increased mineral dust loads. At the same time, the industrialization trends, especially of northern Africa, provide a scenario with higher anthropogenic aerosol loads, which carry a larger fraction of organic matter. Aerosols will deposit over an ocean that has a shallower mixed layer owing to global warming, thus multiplying the potential impact of deposition on ecosystem production and economies that depend on it. Aerosol particles have further social and economic implications in terms of air quality, health and security. Sunyer et al. (2015) found a relationship between air pollution and cognitive development in primary school children. His host institution (CREAL) will be an EPO of ANIMA. Finally, ANIMA will generate a job position for 3 years.

### **Diffusion plan**

The diffusion plan will be implemented through Task 4.2 in the coordination workpage. The members of the consortium normally carry out a wide range of outreach activities, including authoring outreach articles, media interviews, lectures at schools, indoor and outdoor fairs and special events, etc. We plan on setting up a project web page (for which 2000 € are budgeted) where news will be posted together with the data that will be collected. An extra facebook page will be established at no cost. We will also distribute emails to colleagues and potential users of our data and knowledge. ARM (Association for the Advancement of Marine Research) will be a EPO in ANIIMA and will provide another forum for project diffusion. Further diffusion will be assured through the services provided by the ICM Divulga (<http://icmdivulga.icm.csic.es>) and the press release services of the CSIC to spread the news to the mass media. Thus, diffusion of results to the scientific community and the public at large is assured.

### **C.3. FORMATIVE CAPACITY OF THE TEAM**

The researchers of the group take active part as lecturers in several postgraduate programs: the Marine Sciences Program of the Universitat de Barcelona, Universitat Politècnica de Catalunya, Universidad de Las Palmas de Gran Canaria, and the Master of Global Change Master of the Universidad Internacional Menéndez Pelayo. Members of the consortium also organize regularly courses such as the Margalef Summer Course or offer several courses in the platform Barcelona Ocean. The ICM has a regular weekly program of plenary seminars and lectures, on top of which the microbial ecology group adds fortnightly-specialized seminars and discussion workshops in which special attention is given to PhD student's oral presentations. Students are encouraged also to attend selected international meetings. Support is commonly provided for students to attend international courses and have placements abroad.

Owing to its multidisciplinary nature, combining ocean and atmospheric dynamics, microbial ecology, molecular biology and biogeochemistry, the ANIMA project and consortium provide an excellent opportunity for the training of future marine scientists with a broad and global perspective. The consortium has ample experience in student training and former students now hold positions in the industry or in research institutions around the world (C3.3). In addition, several doctoral students of ANIMA researchers are scheduled to defend their dissertations in the next couple of years, and this would be an excellent student replacement time. For all the reasons above, we think that both the ANIMA project and the ANIMA consortium are well suited to train two PhD students and offer them a stimulating and highly productive environment, good research tools, and a dedicated and qualified education on research skills. The subjects for these two fellowships will be as follows:

- 1) *Coastal airborne microorganism diversity and survival in marine waters and its implications in microplankton ecology and in biogeochemistry.* The candidate will be trained in culturing prokaryotic and eukaryotic microbes, including fungi, determining diversity through molecular techniques and also put the biological observations in the context of the biogeochemistry and physics of sea-atmosphere interactions. During the first year, the student will visit Dr. Grossart's lab (Stechlin, Germany) and be trained in the newest molecular techniques for fungal analysis.

- 2) *Chemical characterization of aerosols. Airborne organic matter degradation and its consequences on anthropogenic carbon accumulation in surface waters.* The candidate will be trained in experimental designs for studying the degradability of organic matter of atmospheric origin under different conditions of light, turbulence and inorganic nutrient availability. The candidate will also be trained in combining experimental results with in situ observations to build a broad picture of sea-air interphase ecology and on the fate of organic carbon in surface marine waters. The candidate will learn cutting-edge technologies such as HPLC and mass spectrometry with Dr. Boris Koch (Germany) who accepted to mentor a student placement from the ANIMA project.

The characteristics of the project, in which many different experimental setups are combined with a time series sampling in the natural environment makes the project a very appropriate framework also for the training of marine lab technicians. The contracted technician will be trained in the preparation of samples for biological and chemical analyses, the organization of sampling trips and experiments, and will be introduced to the fundamentals of several techniques, such as flow cytometry, molecular biology, and electron microscopy. He/she will also have the opportunity to attend the regular program of seminars, lectures and workshops mentioned above. Members of ANIMA have past experience in the training of qualified technicians within the framework of research projects, including PTA contracts.

For these reasons we think that both, the ANIMA project and the ANIMA consortium are well suited to train a technician and offer her/him a stimulating environment, good research tools, and a dedicated and qualified education on technical skills.

#### PhD Dissertations

##### In progress

Isabel Marín (Peters). Effects of atmospheric deposition on microbial dynamics and composition in two anthropogenically-influenced contrasted coastal sites. Universitat Politècnica de Catalunya. Lectura prevista en 2017

Francisco Luis Aparicio (Marrasé & Mar Nieto, IIM, CSIC). Tracing the dynamics of DOM in relation to bacterial activities in different marine systems. Universitat Politècnica de Catalunya. Lectura prevista en 2016

Encarna Borrull (Sala). Actividad bacteriana asociada a la degradación de materia orgánica en ecosistemas marinos. Universidad de Las Palmas de Gran Canaria. Lectura prevista en 2016.

Rachelle Gallisai (Peters). Potential effect of dust deposition on production in the Mediterranean Sea. Universitat Politècnica de Catalunya. Lectura prevista en 2016

Mireia Mestre (Sala & Josep M Gasol, ICM). Diversidad bacteriana asociada a la estructura de la materia orgánica. Universidad de Las Palmas de Gran Canaria. Lectura prevista en 2016.

Denisse Sánchez (Marrasé & Pascal Conan, CNRS, France). 2015. The role of abiotic and biotic mechanisms controlling the dynamics of the dissolved organic matter in pelagic ecosystem (NW Mediterranean). Université Pierre et Marie Curie.

##### Finished

Cristina Romera (Marrasé). 2011. Optical properties of dissolved organic matter as tracers of microbiological and geochemical processes in marine ecosystems. Universidad de Las Palmas de Gran Canaria.

Julia Boras (Sala & Dolors Vaqué, ICM). 2010. Dynamics of virioplankton and its contribution to changes of composition of the bacterioplankton communities of the NM Mediterranean. Universitat de Barcelona. Outstanding PhD thesis award 2009-2010.

Estela Romero (Peters). 2010. Sources of plankton variability in an urbanized coastal ecosystem. Universitat Politècnica de Catalunya. 22/9/2010.

Òscar Guadayol (Peters & Marrasé). 2007. Influence of turbulence variability on osmotic plankton dynamics in a coastal area. Universitat Politècnica de Catalunya. 23/05/2007.

Bachelor Theses, Practicums, DEAs, MS Theses supervised by senior researchers of the project within the last 10 years:

**Susana Sánchez** (Sala). 2016. Practicum. Bacterial community associated to particles in the ocean. Univesitat de Barcelona. **Sergio González Motos** (Marrasé). 2015. TFG. A la recerca d'un patró general de la relació FDOM-Bacteri en l'oceà profund. Universitat de Barcelona. **Ana Estrella Michel Lois** (Marrasé & Montse Vidal, UB). 2015. Master. Linking stoichiometry with physical structure in marine systems. Universitat de Barcelona. **Sergi Rodríguez López** (Arin). 2015. Practicum. Relationship between the taxonomic composition of phytoplankton and the fluorescent dissolved organic matter in a section of the Atlantic Ocean. Universitat de Barcelona. **Helena Torné** (Peters). 2015. TFG. Turbulence modulation of the growth of bacteria on organic matter of high and low molecular weights. Universitat de Barcelona. **Mireia Llorente** (Sala). 2014. Practicum. Seasonal changes in size and quality of particles and bacterial colonization in the coastal NW Mediterranean. Universitat de Barcelona. **Héctor Romanos** (Peters & Jordina Belmonte, UAB). 2014. Master. Contribution of organic matter to the sea by airborne pollen and spores. Universitat Autònoma de Barcelona. **Estibalitz Txurruca** (Peters). 2014. Master. Análisis de las dinámicas de las poblaciones marinas de presas y depredadores microbianos y los efectos provocados por la deposición atmosférica. Universitat de Barcelona. **Enrique Real García** (Peters). 2013. TFG. Sobre les causes del descens de les poblacions de Callista chione (Linnaeus, 1758) a la costa del Maresme, Catalunya. Universitat de Barcelona. 28/02/2013. **Judit Bellés** (Sala). 2012. Practicum. Detection of bacterial of the genus Vibrio sp. associated to toxic phytobentos blooms. Universitat de Barcelona. **Encarna Borrull** (Sala). Master. 2011. Diversity and activity of the microbial community associated to bloms of toxic phytobentos. Universitat de Barcelona. **Alessandro Scandurra** (Sala). 2009. Master. Bacterioplankton response to distinct sources of organic matter in an estuarine bay. University of Trieste, Italy. **Rachele Gallisai** (Peters). 2010. Master. Potential effect of dust deposition on production in the Mediterranean Sea. Universitat de Barcelona. **Estela Romero** (Peters). 2006. DEA. Identificación de variables de estado del ecosistema costero sensibles a la turbulencia y a la carga de nutrientes. Universitat Politècnica de Catalunya. **Rodrigo Almeda** (Peters). 2005. DEA. Efecto de la turbulencia sobre la dinámica del microzooplankton. Universitat de Barcelona. **Julia A. Boras** (Peters). 2005. DEA. Effects of turbulence on viral infection of bacteria. Universitat de Las Palmas de Gran Canaria.

### [3. Breve descripción del desarrollo científico o profesional de los doctores egresados del equipo de investigación.](#)

Òscar Guadayol had postdoctoral stays at Oregon State University (Dr. Tim Cowles) and at the Hawaii Institute of Marine Biology (Dr. Florence Thomas) both in the United States. He now holds a position at with Dr. Stuart Humphries at the University of Lincoln (UK).

Julia Boras was awarded the "Outstanding Doctorate Award 2009-2010 " at the University of Barcelona. Currently, and since 2010 she works in the company Provitalgroup SA as R&D Project Manager in the development of new natural active ingredients for cosmetics.

Andrea Malits had a postdoctoral position with Dr. C. Marrasé at ICM (April 2011 to October 2012 ). Estudio de la ecología viral de partículas en el océano costero. Presently, she has a research position at the National Scientific and Technical Research Council (CADIC) - Centro Austral de Investigaciones científicas, Ushuaia, Argentina.

Estela Romero has been contracted by the group of Dr. Josette Garnier (Université Pierre et Marie Curie, Paris), through several projects, and has also led her own project through an Iberdrola Foundation contract.

Cristina Romera has been contracted by Dr. Dennis Hansell (Rosenstiel School of Marine and Atmospheric Science at Miami University, USA) after two years of postdoctoral fellowship with Dr. Rudolf Jaffé (Department of Chemistry and Biochemistry, Florida International University, USA).

#### **C.4. ETHICAL AND/OR BIOHAZARD IMPLICATIONS**

There are no ethical or biohazard implication to be addressed.



## C.5. ANNEX. LETTERS FROM COLLABORATORS



Dr. Oriol Jorba  
Atmospheric Composition Group Lead  
Earth Sciences Department  
Barcelona Supercomputing Center  
Nexus II Building  
Carrer Jordi Girona, 29  
08034 Barcelona (Spain)

July 17, 2015

Dear Administrators and Selection Committee,

I have learned of the ANIMA (Atmospheric inputs as a source of organic nutrients and microorganisms in marine ecosystems) project proposal to the Spanish Ministerio de Economía y Competitividad from one of its principal investigators, Dr. Francesc Peters. The project includes the estimation of the atmospheric deposition, with special emphasis on organic components and airborne microorganisms in the NW Mediterranean. My research group has ample experience in atmospheric particle load and transport and is interested in the deposition data that will be collected, which may contribute to the EMEP (European Monitoring and Evaluation Programme) network and in the comparison of actual deposition measurements with deposition inferred from airborne particle load. I will collaborate in the project by providing PM10 and PM2.5 data as well as chemical analysis expertise of atmospheric aerosols.

Sincerely yours,

Dr. Xavier Querol Carceller  
Institut de Diagnòstic Ambiental i Estudis de l'Aigua  
Consejo Superior de Investigaciones Científicas

Barcelona, 16 July 2015

To whom it may concern,

I have learned of the ANIMA (Atmospheric inputs as a source of organic nutrients and microorganisms in marine ecosystems) project proposal to the Spanish Ministerio de Economía y Competitividad from one of its principal investigators, Dr. Francesc Peters.

The ANIMA project will evaluate atmospheric deposition data with dust deposition modelling efforts in the Mediterranean. If funded, we are interested in collaborating with this project on the one side providing aerosol deposition output data from our models BSC-DREAM8b and NMMB/BSC-Dust for use in the project ANIMA and on the other side in using atmospheric deposition observational measurements from ANIMA to further validate and calibrate our models.

Do not hesitate to contact me should you need further details.

Yours sincerely,

Dr. Oriol Jorba

