Convocatoria AEET-SIBECOL de ayudas a proyectos de investigación ERC en ecología (11ª ed., 2021)

1. Datos de identificación.

Nombre de la propuesta	**TH**erm**A**l f**L**uctuations and pulsed resources in a w**A**rmer environment: impact**S** on coa**S**tal microbi**A**l plankton (**THALASSA**)
Modalidad	Consolidando la investigación
Nombre y Apellidos del beneficiario	Marco Jabalera Cabrerizo
Datos de contacto:email y teléfono	marcojabalera@gmail.com/617853363
Centro/Departamento/Área/Grupo investigación/Otros	Fac. Ciencias del Mar/Ecología y Biología Animal/Oceanografía Biología
Dirección/Código postal/Provincia	Campus Lagoas Marcosende s/n, 36310, Vigo (Pontevedra)

2. Memoria Técnica. Actividades y resultados de investigación

2.1. Introducción (Planteamiento, objetivos y justificación)

Resource boosts primary/bacterial production [Lekunberri *et al.*, 2010, Martínez-García *et al.*, 2012; Tsagaraki *et al.*, 2017], respiration [Pulido-Villena *et al.*, 2008], and carbon (C) sequestration [Pabortsava *et al.*, 2017] in plankton communities, but also accentuates the top-down control by mixotrophs on heterotrophs [Cabrerizo *et al.*, 2017], inhibits chlorophyll *a* (Chl *a*) production [Jordi *et al.*, 2012], or picoplankton growth [Rahav *et al.*, 2020]. Temperature differentially stimulates hetero- compared autotrophic processes [López-Urrutia *et al.*, 2006, Liu *et al.*, 2019, Rose & Caron, 2007], increases biodiversity and primary productivity [Yvon-Durocher *et al.*, 2015], and strengthens trophic interactions [Schaum *et al.*, 2018], although reduces C-sink capacity from ecosystems [Yvon-Durocher *et al.*, 2017], and the energy transfer to higher trophic levels [Ullah *et al.*, 2018]. When act together, previous results showed that temperature is who controls the effect of resources on metabolic processes and trophic interactions [Cross *et al.*, 2015; O´Connor *et al.*, 2009]. By contrast, recent findings by Marañón *et al.* [2018] demonstrated that this strong thermal dependence of metabolism is suppressed when resources are limiting, hence such effect could be circumscribed to nutrient-enriched areas (i.e. upwelling systems, coasts). Although these works have improved our understanding about how both drivers impact food webs, they considered that organisms experience constant environments; however, Jensen's inequality states that organisms performance to constant conditions differ from that to variable ones [Jensen, 1906]. Because temperature fluctuations in marine ecosystems can exceed (~10ºC) those estimated in constant warmer environments [Doblin & van Sebille, 2016], and extreme rainfall events [Harris *et al.*, 2018; Fong *et al.*, 2020] and anthropogenic changes in land-use [Rabalais *et al.*, 2009] are pulsing more nutrients toward coastal ecosystems: What are the warming×nutrient impacts on microbial plankton when thermal environment varies beyond mean projected trends?.

General objective

The THALASSA's **general objective** is investigating how the frequency of resource inputs and warming in a thermally varying scenario alter coastal plankton responses over the seasonal succession (i.e. thermal stratification, upwelling, winter mixing, and spring bloom periods).
THALASSA project will test the following specific hypothesis:

H1: The effects of warming and fluctuating temperatures on microbial communities will be strengthened by resource inputs regime when *in situ* nutrients are low (i.e. thermal stratification).

H2: The thermal sensitivity of heterotrophs will differentially increase respect to that of autotrophs with rising frequency of resource inputs, particularly when *in situ* nutrients are high (i.e. upwelling, mixing conditions).

H3: A warmer, variable and nutrients enriched environment will shift the planktonic metabolic balance toward a C-source.

H1-H3 will be tested by addressing the following **specific objectives:**

a.- To determine how interactions between warming, temperature fluctuations and resource input regimes alter the elemental composition, diversity, biomass and size structure in microbial communities.

b.- To evaluate variations in thermal sensitivity of auto- and heterotrophs to warming× fluctuating temperatures when subjected to increased intensity and frequency of resource inputs.

c.- To investigate how a multi-driver's scenario impact the planktonic metabolic balance of marine ecosystems, and whether such changes will be accentuated in contrasted oceanographic conditions.

2.2. Descripción de la ejecución- Metodología

-**Observational approach:** Weekly samplings in a central station at Ría de Vigo over an annual cycle were done (Fig. 1). We used the research vessel from UVigo for monitoring the physical, chemical, and biological properties (see 5.3.3 for a detailed description of them) of the study system.

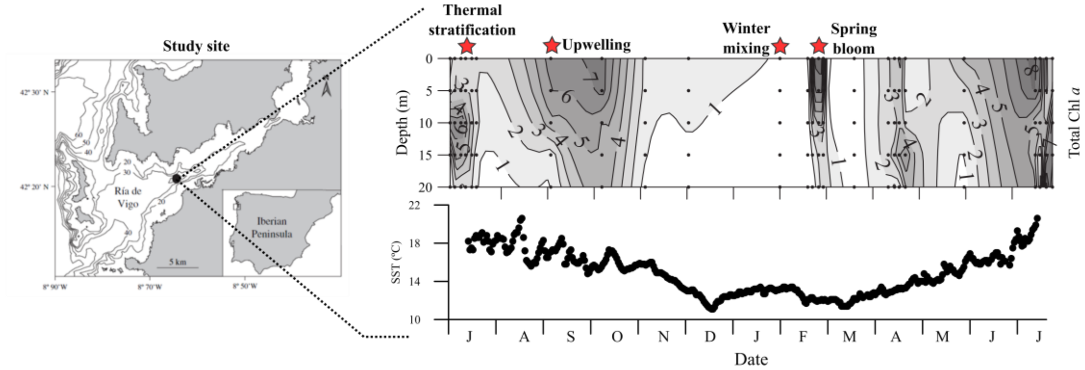


Figure 1.- Chlorophyll *a* concentrations (Chl *a*, upper panel), and sea surface temperatures (SST, lower panel) in Ría de Vigo over the year. Stars indicate the different oceanographic conditions.

-**Experimental approach:** A seasonal sampling in each oceanographic condition (Fig. 1) in which the communities will be exposed to a factorial design (in triplicate) with: *three temperature conditions* [**Control$_{\text{constant}}$** (constant *in situ* temperature), **Control$_{\text{fluctuating}}$** (*in situ* temperature ± 3ºC) and **Warming$_{\text{fluctuating}}$** (*in situ* temperature+3ºC ± 3ºC], and *two resources supply regimes* [**Low** (0.25 µM of NO_3^-; N:P = 16 and N:Si = 1.5) versus **High** (2.5 µM of $NO_3^{-\,-}$; N:P = 16 and N:Si = 1.5).

5-L microcosms (18 in total) were filled with surface planktonic communities (0-5 m depth) sampled in the fixed central station mentioned used for the observational approach. The samples were incubated in an automatized computer-controlled thermostatic system, and exposed under natural solar radiation to the conditions described above for 15 days.

-**Response variables for both approaches:**

-Chl *a*: Samples will be filtered through Whatman GF/F filters, extracted in acetone 90% and measured following Porra [2002].

-Phytoplankton stoichiometry (elemental analysis): C y N will be measured using a Carlo Elba EA 1108 autoanalyzer, and P by molybdate reaction after persulfate digestion.

-Net community production-respiration: Samples will be placed in Winkler bottles, and analysed following Marañón *et al.* [2018].

-Nanoplankton: Cell counting's will be done using lugol-fixed samples and an inverted microscope following Utermöhl technique [Utermöhl, 1958].

-Picoplankton: Samples fixed with a mix of glutaraldehyde and paraformaldehyde will be analyzed using flow cytometry.

-Photosystem II photochemical activity (PSII): *In vivo* Chl *a* fluorescence dynamics in main phytoplankton groups (i.e. cyanobacteria, diatoms/dinoflagellates, chlorophytes and cryptophytes) will be measured using a PhytoPAM II fluorometer.

-Inorganic nutrients: Nitrate/nitrite, phosphate and silicate will be quantified with a Carlo Elba EA 1108 autoanalyzer.

-**Abiotic parameters:** Temperature, salinity, Chl *a* fluorescence, photosynthetically active radiation and dissolved oxygen will be determined using a CTD device.

2.3. Resultados obtenidos (cumplimiento de objetivos)

The results obtained through the THALASSA project can be structured in three sections: meta-analysis, observational and experimental approaches.

2.3.1. Meta-analysis results

Inside the THALASSA framework we posed the question if natural environmental fluctuations could alter the magnitude and direction of the effects of multiple global-change drivers. Traditionally, the global change biology has evidenced that non-additive i.e. interactive effects predominate in marine and terrestrial ecosystems [Crain *et al.*, 2008; Jackson *et al.*, 2016; Villar-Argaiz *et al.*, 2018]. Through a meta-analytical approach containing > 60 works (> 4000 individual estimates) published between 1990-2021 we have evidenced that environmental fluctuations can unmask a negative (or positive) effect of several environmental global-change drivers on different biological targets unnoticed when such fluctuations were not considered (Fig. 2).

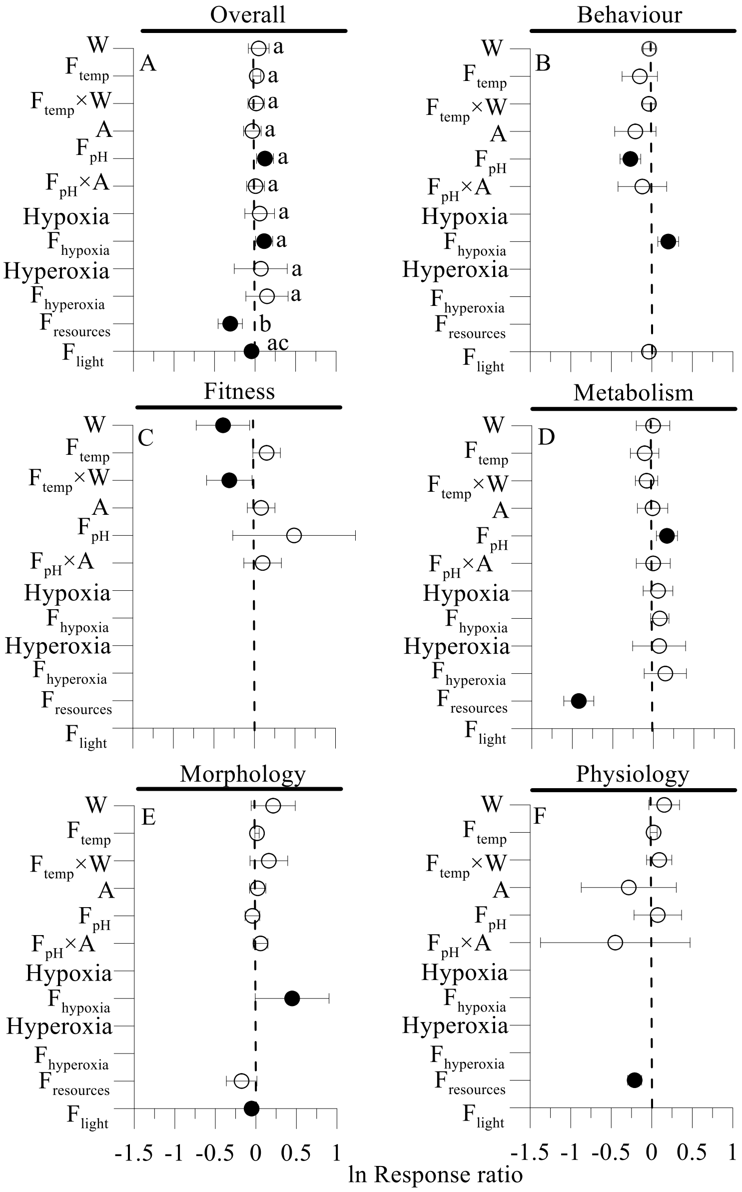
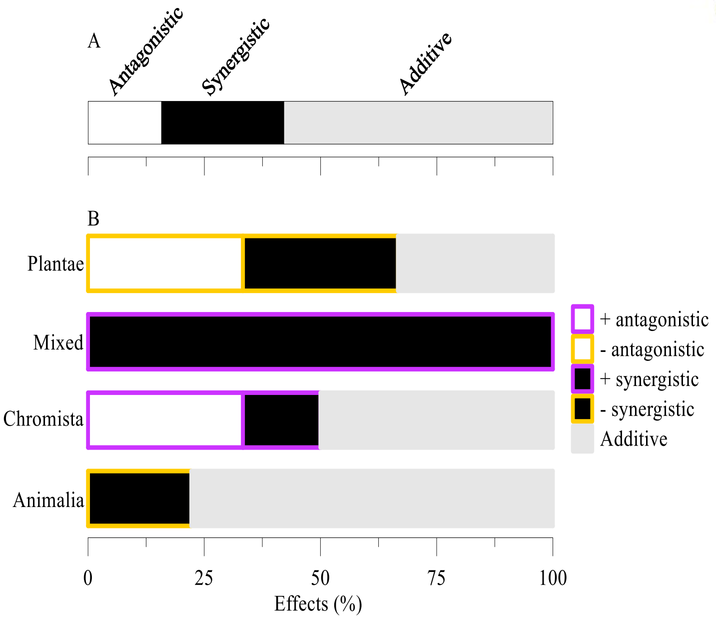


Figure 2. . Overall (A) mean effect categorized by biological traits (B, behavior; C, fitness; D, metabolism; E, morphology; and F, physiology) of warming (W), fluctuating temperature (Ftemp), acidification (A) and fluctuating pH (FpH) and their interactions, and hypoxia, fluctuating hypoxia (Fhypoxia), hyperoxia, fluctuating hyperoxia (Fhyperoxia), fluctuating resources (Fresources), and fluctuating light (Flight). Natural LRR < 0or > 0 indicates a negative (i.e., inhibitory) or a positive (i.e., stimulatory) effect, respectively. Significant effect (black circles only) when the LRR 95% confidence interval does not overlap zero. Different letters (a–c) indicate significant differences by Tukey’s honest significance tests. Ln, natural logarithm.

From the above presented results, we also quantified what is the overall frequency of additive and interactive effects over the tree of life, and what is the magnitude (synergistic or antagonistic) and direction (positive or negative) of them for each biological Kingdom. We found that, contrarily to the dominant view in Ecology, additive effects are more frquent than interactive ones when environmental fluctuations act in concert with global change drivers. However, we found that this proportion varies in Plantae and Mixed (i.e. natural communities) where interactive effects dominated over additive ones, whereas additive were more frequent (i.e. in Animalia) or co-dominated (i.e. Chromista) with interactive ones (Fig. 3).



Figue 3. Comparison of the additive and interactive effects of multiple global-change drivers and environmental fluctuations across the tree of life. Frequency distribution across the overall additive, synergistic, and antagonistic effects (A and B) and the particular positive/negative antagonistic, synergistic, and additive effects on Animalia, Chromista, Mixed, and Plantae (C).

2.3.2. Observational results

As stated in the methology proposed in the proposal, we developed an observational study over an annual cycle using as model ecosystem the Ría de Vigo. Over this study, we have developed weekly samplings (i.e. 52 samplings in total), taking samples from surface (0.5-2 m depth) and maximum depth (~30 m depth) of: Chl *a*, phytoplankton (micro-, nano- and picophytoplankton), elemental composition (i.e. carbon, nitrogen and phosphorus), inorganic nutrients (i.e. nitrate + nitrite, phosphate and silicate) and photosynthetical performance (i.e. maximum electron transport rates). Also, we have characterised the physico-chemical and biological properties of the water column (i.e. temperature, salinity, dissolved oxygen, fluorescence as a proxy of phytoplankton biomass, photosyntetically active radiation with depth). On a monthly basis, we have measured the net community production and respiration of the community using the Winkler method (see Methodology).

Through this study we found that Ría de Vigo has two marked periods: one period in which the water column is mixed (i.e. winter and early spring; Fig. 4A – orange/yellow colors), and the second one in which alternates periods of stratification and short-term upwelling pulses, which subsequently supply cold nutrient-enriched water. This hydrodynamism triggers high productivity events which, in some cases, extend over the entire water column (Fig. 4B).

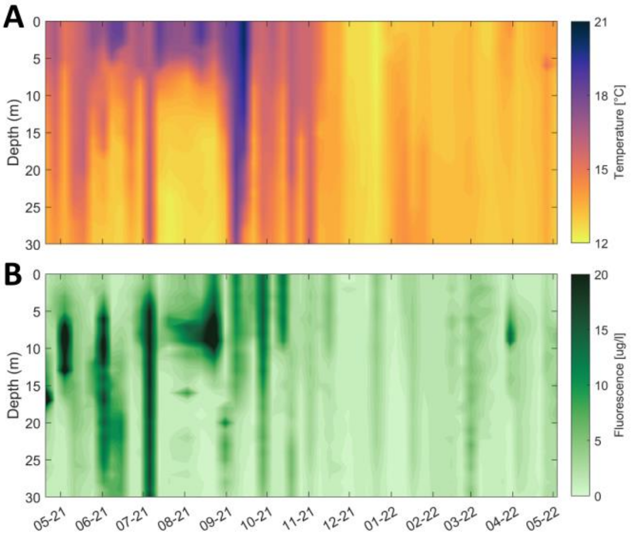


Figure 4. Temporal and vertical variability of (A) temperature and (B) fluorescence over an annual cycle in Ría de Vigo.

Despite seasonality and hydrodynamism of Ría de Vigo triggered high production events, we did not find any significant alteration in the "health status" of phytoplankton community. We found that the maximum photochemical quantum yield (Fv/Fm) ranged, on average, abouth 0.4 (Fig. 5A). By contrast, we found that the maximum electron transport rates (in µmol photons m^{-2} s^{-1}), a measure of the maxima photosyntetic capacity decreased over time. ETRmax rates ranged between 50 and < 20 µmol photons m^{-2} s^{-1} (Fig. 5B).

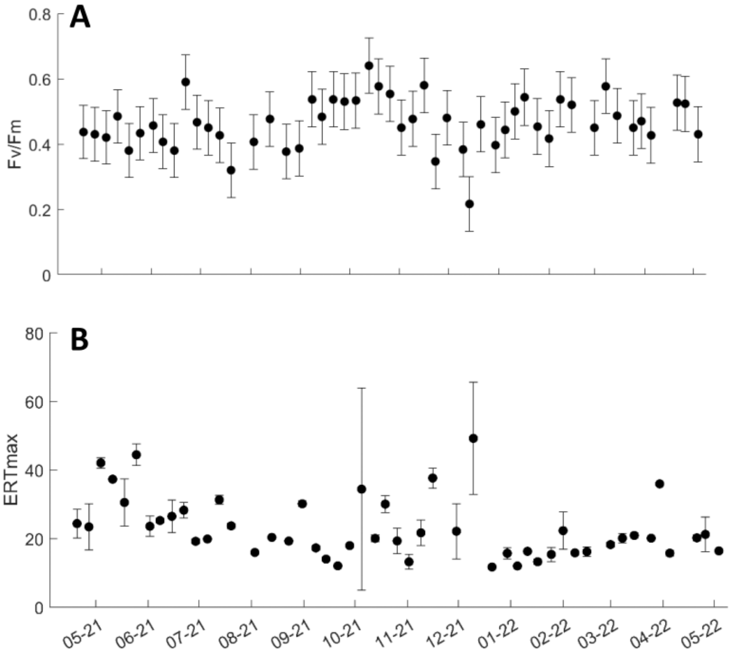


Figure 5. Temporal variability of (A) maximum quantum yield and (B) electron transport rates in surface phytoplankton communities from Ría de Vigo over an annual cycle.

The remaining data related with this section are being currently analyzed by the PI. All data obtained through this observational study will result in one high-impact factor publication (see 2.5). It is intended to submit these results to *Ecology Letters*.

2.3.3. Experimental results

We developed four microcosm experimental studies (15-29 March, 13-27 June, 26 September to 10 October, and 30 November to 15 December 2022). In that studies we followed the experimental approach and methodology described above in 2.2. These studies were carried out in different seasons in order to analyze how different communities over the year respond to interactions between temperature and nutrient supply in a thermally varying environment. All the samples obtained through these experiments are being analyzed in this moment. We will expect to start to analyze this data in March/April 2023, and it will result in an additional high-impact factor publication.

Below, we showed some of the data obtained already available in the last experiment performed. In relation to the experimental setup, we simulated two warming scenarios, one in which the temperature was maintained constant over time (i.e. traditional global-change scenarios (Fig. 6, orange line), and other in which the temperature fluctuated over 24 hs cycles but maintaining the same mean temperature than the scenario one (Fig. 6, grey line).

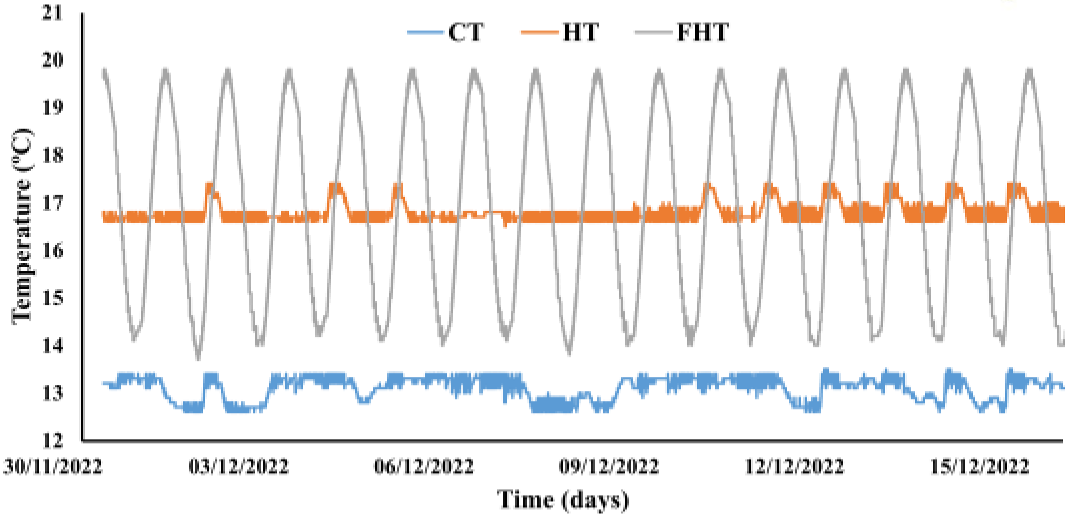


Figure 6. Temporal evolution of temperature in the experimental microcosms over the experimental period. CT refers to constant control temperature (i.e. in situ temperature at the sampling moment), HT to high constant temperature (CT + 4ºC) and FHT to fluctuating high temperature (+4 ± 3ºC).

The ETR_{max} rates and the alpha exhibited an unimodal response pattern over time, with maximum values at mid-term and lowest at the start and at the end of the experimental period (Fig. 7A, B). ETRmax values tanged between 100 and 200 µmol photons m^{-2} s^{-1}, and those for alpha between 0.2 and 0.6 e^{-} photon. Any clear effect of temperature, nutrients, fluctuating temperature nor its interaction was observed over time, mainly due to the high variability found. By contrast, the acclimation irradiance i.e. the solar radiation level which photosynthesis saturates, increased over the time, with values increasing from ~300 to > 1000 µmol photons m^{-2} s^{-1} (Fig. 7C). In this case, we observed that the interaction between the two global-change drivers considered and under a thermally varying environment exerted a stimulatory effect on I_k respect LN and CT conditions.

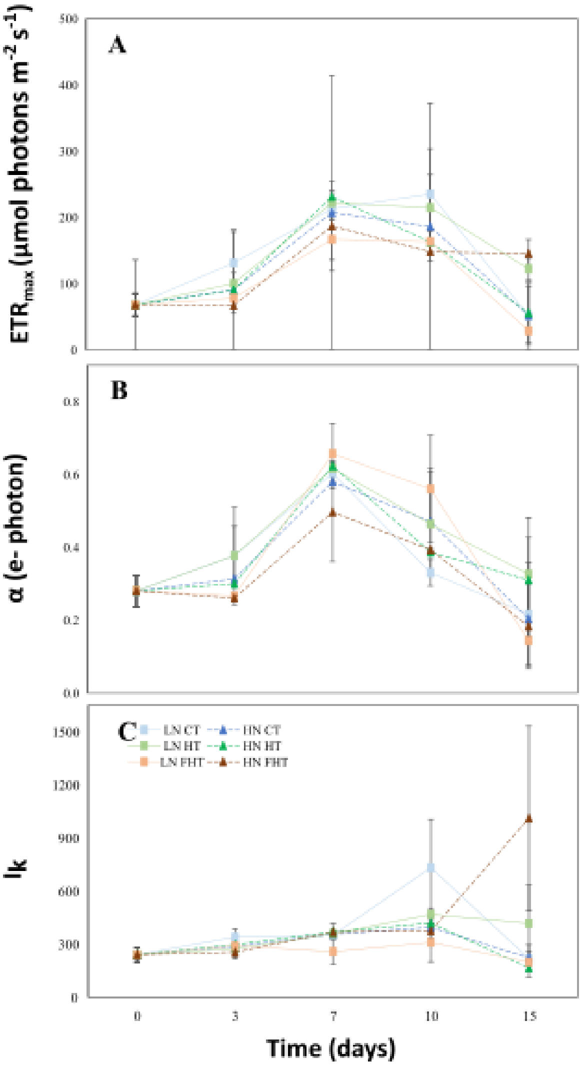


Figure 7. Evolution of (A) maximum electron transport rates, (B) light harvesting efficiency, and (C) acclimation irradiance obtained from rapid light curves with a PhytoPAM II in natural phytoplankton communities from Ría de Vigo exposed to three temperature: CT (control temperature), HT (high temperature) and FHT (fluctuating high temperature), and two nutrient supply (LN, low and HN, high nutrients) regimes over 15 days.

2.4. Conclusiones y valoración de la ejecución

In relation to the conclusions reached through the THALASSA project, we demonstrated, using a meta-analytical approach, that natural environmental fluctuations can counteract the interactive effects of multiple global-change drivers. Also, we found that this pattern was consistent over the different Kingdoms of the tree of life. Therefore, we stress the need of considering the environmental variability in future observational, experimental and modelling studies to better predict the impact of global-change drivers on ecosystems.

In parallel to the meta-analytical approach, we developed an observational and four experimental studies (one per season). From the already available information of both studies, we found that thermal environmental fluctuations seem to attenuate the negative impact of the interaction between temperature and resource supply on primary productivity. These incipient results are consistent with observed in the literature analysis developed as well.

Regarding the execution of the project, it has allowed me to lead, for first time, a proposal and being the person-in-charge in all the steps of it. My personal evaluation about this kind of projects is that it is a much needed option for early career researchers trying to become an independent researcher. The funding amount granted by AEET is appropriate for a 1-year project, although it would be better that the duration of these projects would be extended for at least 6 months i.e. the duration would be 18 instead 12 months. This extension would allow to the PIs to have more time to analyze the data and communicate more results to the funding entity once the projects end.

2.5. Publicaciones resultantes

-Peer-reviewed:

-**M. J. Cabrerizo** & E. Marañón. 2022. Net effect of environmental fluctuations in multiple global-change drivers across the tree of life. *Proc. Natl. Acad. Sci. USA* 32: e2205495119. https://doi.org/10.1073/pnas.2205495119.

-**M. J. Cabrerizo** & E. Marañón. 2022. Fluctuaciones ambientales: Las grandes olvidadas en los estudios sobre los efectos del cambio global. *The Conversation*. https://theconversation.com/fluctuaciones-ambientales-las-grandes-olvidadas-en-los-estudios-sobre-los-efectos-del-cambio-global-187800.

-**M. J. Cabrerizo**, C. Fernández-González, C. González-García, O. Tascón-Peña, M. Pérez-Lorenzo & E. Marañón. Phytoplankton size-structure, metabolism, stoichiometry and photophysiology responses in an upwelling system (Ría de Vigo) to natural environmental variability. *In preparation*.

-M. J. Cabrerizo *et al.* Warming and shifting N:P ratios alter the microzooplankton grazing pressure in marine ecosystems. *In preparation*.

-Non peer-reviewed:

-**M. J. Cabrerizo & E.** Marañón. 2022. Científicos da UVigo demostran a importancia das flutuacións medioambientais para entender os efectos do cambio global sobre organismos e ecosistemas. Diario da Universidade de Vigo. https://www.uvigo.gal/universidade/comunicacion/duvi/cientificos-uvigo-demostran-importancia-flutuacions-medioambientais-entender-os-efectos-cambio

2.6. Otras contribuciones

-Oral communication: *Temperature fluctuation attenuates the effects of warming in estuarine microbial plankton communities.* 2022 Ocean Sciences Meeting. 24 Feb-4 March 2022. Hawaii (USA).

-Participation with a workshop entitled "The color with which the sea is painted" in the Scientific Fair: "**La ciencia que viene tiene nombre de mujer**" (18/03/2022, Vigo)

-Participation in the outreach video: "**Que facemos**?". Grupo de Oceanografía Biológica.

-Supervision of a Collaboration Fellowship granted by the Spanish Ministry of Education and Professional Training and development and defense of two BSc thesis inside the THALASSA project:

-Ecophysiological responses of microbial plankton to environmental variability. BSc Mariana Gª-Boente Soto & 2021 Collaboration fellowship. Score: 9.8. BSc with honors 18/07/2022.

-Effects of temperature fluctuations and resource supply on the structure and functioning of winter-time coastal microbial plankton communities. BSc Inés Cobos Malpesa. Score: 7. 18/07/2022.

3. Referencias.

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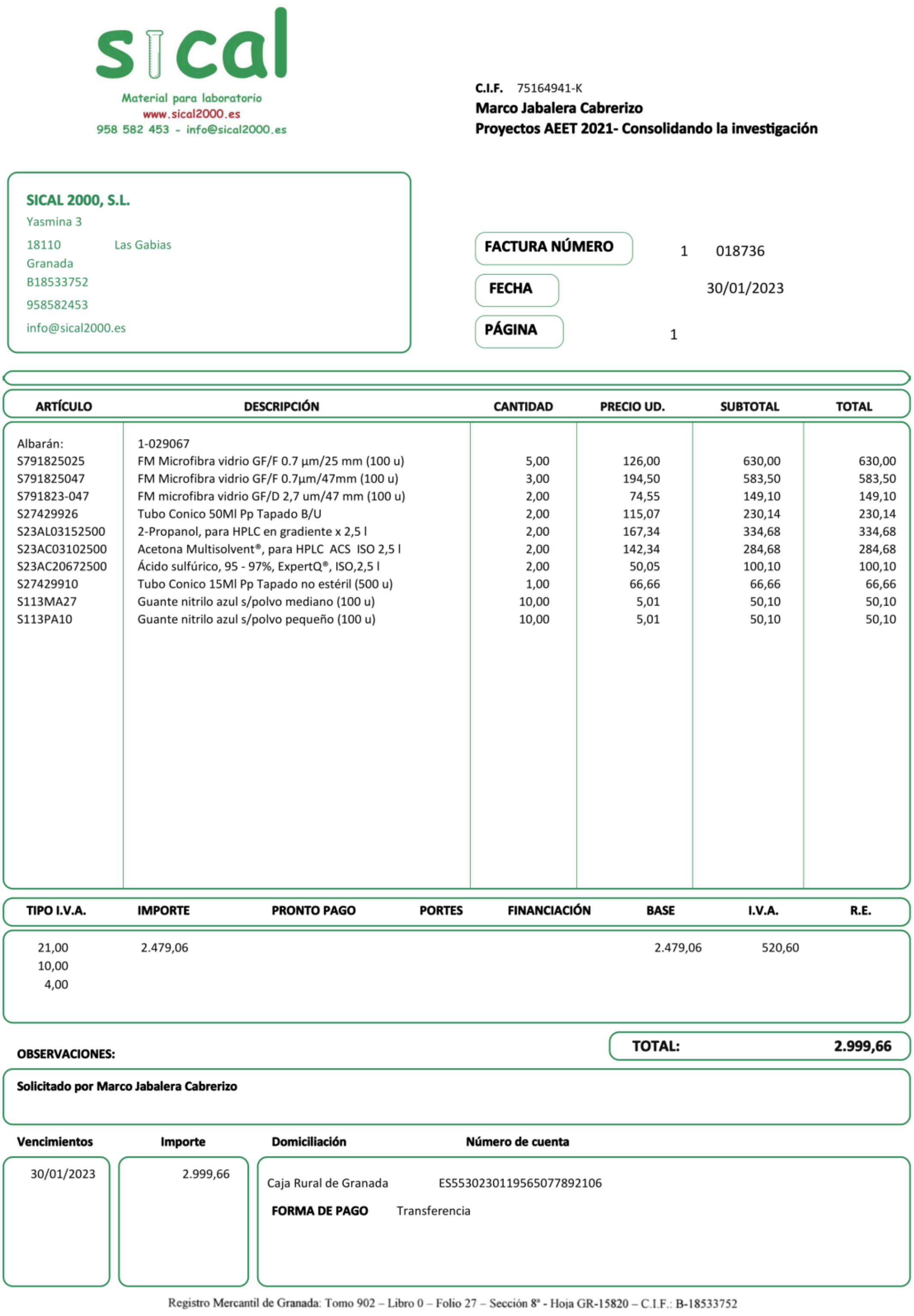
4. **Informe de gastos del proyecto.** Relación de partidas de gastos y sus importes. Se deberán aportar justificantes originales de los pagos realizados (tickets, recibos o facturas).

The expenses of the THALASSA project are described in detail in the image (and attached original pdf file sent through email by the PI to the AEET) presented below:



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C.I.F. 75164941-K
Marco Jabalera Cabrerizo
Proyectos AEET 2021- Consolidando la investigación

SICAL 2000, S.L.
Yasmina 3
18110 Las Gabias
Granada
B18533752
958582453
info@sical2000.es

FACTURA NÚMERO	1	018736
FECHA		30/01/2023
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ARTÍCULO	DESCRIPCIÓN	CANTIDAD	PRECIO UD.	SUBTOTAL	TOTAL
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S791825047	FM Microfibra vidrio GF/F 0.7µm/47mm (100 u)	3,00	194,50	583,50	583,50
S791823-047	FM microfibra vidrio GF/D 2,7 um/47 mm (100 u)	2,00	74,55	149,10	149,10
S27429926	Tubo Conico 50Ml Pp Tapado B/U	2,00	115,07	230,14	230,14
S23AL03152500	2-Propanol, para HPLC en gradiente x 2,5 l	2,00	167,34	334,68	334,68
S23AC03102500	Acetona Multisolvent®, para HPLC ACS ISO 2,5 l	2,00	142,34	284,68	284,68
S23AC20672500	Ácido sulfúrico, 95 - 97%, ExpertQ®, ISO,2,5 l	2,00	50,05	100,10	100,10
S27429910	Tubo Conico 15Ml Pp Tapado no estéril (500 u)	1,00	66,66	66,66	66,66
S113MA27	Guante nitrilo azul s/polvo mediano (100 u)	10,00	5,01	50,10	50,10
S113PA10	Guante nitrilo azul s/polvo pequeño (100 u)	10,00	5,01	50,10	50,10

TIPO I.V.A.	IMPORTE	PRONTO PAGO	PORTES	FINANCIACIÓN	BASE	I.V.A.	R.E.
21,00	2.479,06				2.479,06	520,60	
10,00							
4,00							

TOTAL: **2.999,66**

OBSERVACIONES:

Solicitado por Marco Jabalera Cabrerizo

Vencimientos	Importe	Domiciliación	Número de cuenta
30/01/2023	2.999,66	Caja Rural de Granada	ES5530230119565077892106

FORMA DE PAGO Transferencia

Registro Mercantil de Granada: Tomo 902 – Libro 0 – Folio 27 – Sección 8ª - Hoja GR-15820 – C.I.F.: B-18533752

JABALERA CABRERIZO MARCO - 75164941K

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Fdo: MARCO JABALERA CABRERIZO, en Vigo, a 31 de Enero de 2023