ECOSYSTEM FUNCTIONING OF MEDITERRANEAN CONFINED COASTAL LAGOONS:
ECOSYSTEM METABOLISM, MAIN DRIVERS AND PHYTOPLANKTON COMMUNITY

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Universitat
de Girona

DOCTORAL THESIS

“Ecosystem functioning of Mediterranean confined coastal lagoons:

ecosystem metabolism, main drivers and phytoplankton community”

MARIA BAS SILVESTRE

2021

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“Ecosystem functioning of Mediterranean confined coastal lagoons: ecosystem metabolism, main drivers and phytoplankton community”

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Doctoral Programme in Water Science and Technology

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Presented to obtain the degree of PhD at the University of Girona

Dr Xavier de Quintana Pou from the University of Girona,

declare:

That the thesis entitled "*Ecosystem functioning of Mediterranean confined coastal lagoons: ecosystem metabolism, main drivers and phytoplankton community*", submitted by Maria Bas Silvestre to obtain a doctoral degree has been completed under my supervision and meets the requirements to opt for an International Doctorate.

For all intents and purposes, I hereby sign this document.

Dr Xavier de Quintana Pou
Universitat de Girona
Girona, 8th June 2021

"La cura para todo es siempre el agua salada:
el sudor, las lágrimas o el mar"

Karen Blixen

"You can't connect the dots looking forward; you can only connect them looking backwards. So you have to trust that the dots will somehow connect in your future. You have to trust in something — your gut, destiny, life, karma, whatever. Because believing that the dots will connect down the road, will give you the confidence to follow your heart, even when it leads you off the well-worn path. And that would make all the difference"

Steve Jobs

A les iaies,

perquè ja no hi són però continuen sent el meu referent

GRÀCIES! - ¡GRACIAS! - THANK YOU!

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LIST OF ABBREVIATIONS

A_GPP$_{MAX}$ - Ratio between A (primary production per quantum unit of light (i.e., photosynthetic efficiency)) and GPP$_{MAX}$ (gross primary production standardized to maximum light conditions) used as a proxy for the relative importance of *r*-*K* strategies

AIC - Akaike information criterion

alg_bact - Ratio between algavorous and bacterivorous zooplankton

AUTOhigh - Group composed of diatoms and prasinophytes

BASE - BAyesian Single-station Estimation

CDOM - Coloured, dissolved organic matter

COI - Cone of influence

CRYPTO - Cryptophytes

CYANlarge - Cyanobacteria (identified by microscopy)

DIATlarge - Diatoms (identified by microscopy)

DIN - Dissolved inorganic nitrogen

DINO - Dinoflagellates

DO - Dissolved oxygen

DOsat_var - Daily variance of dissolved oxygen saturation values

EC - Electrical conductivity

ER - Ecosystem respiration

ER$_{20}$ - Ecosystem respiration standardized to 20°C

ER$_{m}$ - Monthly ecosystem respiration

ER$_{m20}$ - Monthly ecosystem respiration standardized to 20°C

ER_TOC - Ratio between ecosystem respiration and total organic carbon (ER/TOC)

ESD - Equivalent spherical diameter

EUGLE - Euglenophytes

FCM - Flow cytometry

FL2 - Orange fluorescence

FL3 - Red fluorescence

FSC - Forward scatter

GAMMs - General additive mixed models

GLM - General lake model

GPP - Gross primary production

GPP_{20} - Gross primary production standardized to 20°C

GPP_{m} - Monthly gross primary production

GPP_{20MAX} - Gross primary production standardized to 20°C and to maximum light conditions

GPP_{m20MAX} - Monthly gross primary production standardized to 20°C and to maximum light conditions

GPP_chla - Ratio between gross primary production and chlorophyll-*a* used as an estimation for the production to biomass ratio (P/B)

HAPThigh - Haptophytes with high red fluorescence

HAPTlow - Haptophytes with low red fluorescence

HPLC - High-performance liquid chromatography

MSSA - Multivariate singular spectrum snalysis

NEP - Net ecosystem production

nMDS - Nonmetric multidimensional scaling

N_{org} - Organic nitrogen

N_P - Ratio between dissolved inorganic nitrogen and phosphate (DIN/PO_4^{3-})

orgN_inorN - Ratio between organic nitrogen and dissolved inorganic nitrogen (N_{org}/DIN)

orgP_inorP - Ratio between organic phosphorus and phosphate (P_{org}/PO_4^{3-})

PAR - Photosynthetically active radiation

P/B - Production to biomass ratio

PERMANOVA - Permutational multivariate analysis of variance

PHOTOB - Photosynthetic bacteria

PICOEUK - Picoeukaryotes

phyto_zoo - Ratio between phytoplankton and zooplankton biomass

P_{org} - Organic phosphorus

PRASIlarge - Prasinophytes (identified by microscopy)

RDA - Redundancy analysis

redN_oxN - Ratio between reduced form of nitrogen (ammonium) and oxidized form of nitrogen (nitrate) (NH_4^+/NO_3^-)

SCI - Sites of Community Importance

SPA - Special Protection Areas

SSC - Side scatter

SYNEC - Cyanobacteria (*Synechococcus*)

T - Temperature

TN - Total nitrogen

TOC - Total organic carbon

TOC_TN - Ratio between total organic carbon and total nitrogen (TOC/TN)

TOC_TP - Ratio between total organic carbon and total phosphorus (TOC/TP)

TP - Total phosphorus

VIF - Variance inflation factor

WL - Water level

WTC - Wavelet transformation coherence

XWT - Cross wavelet transformation

LIST OF PUBLICATIONS DERIVED FROM THIS THESIS AND CONTRIBUTIONS

CHAPTER I

Bas-Silvestre, M., Quintana, X. D., Compte, J., Gascón, S., Boix, D., Antón-Pardo, M., Obrador, B. 2020. **Ecosystem metabolism dynamics and environmental drivers in Mediterranean confined coastal lagoons.** *Estuarine, Coastal and Shelf Science* 245: 106989. https://doi.org/10.1016/j.ecss.2020.106989

Contribution: *Conceptualization of the study, samplings and data collection from October 2017, raw data processing, data analysis (including the modification and application of BASE model and statistical analyses) and writing of the first draft of the paper.*

CHAPTER II

Bas-Silvestre, M., Casamitjana, X., Meredith, W., Khan, H., Compte, J., Antón-Pardo, M., Boix, D., Gascón, S., Obrador, B., Quintana, X. D. **Hydrodynamics influence on ecosystem metabolism in Mediterranean confined coastal lagoons.** *Submitted.*

Contribution: *Conceptualization of the study, samplings and data collection from October 2017. Hydrological variables (application of GLM) were provided by other researchers and MBS was in charge of processing both hydrological and metabolism data, analysing the data and writing of the first draft of the manuscript.*

CHAPTER III

Bas-Silvestre, M., Antón-Pardo, M., Boix, D., Gascón, S., Compte, J., Bou, J., Obrador, B., Quintana, X. D. **Phytoplankton community structure in Mediterranean confined coastal lagoons: using ecosystem metabolism for trait quantification.** *Submitted.*

Contribution: *Conceptualization of the study, samplings and data collection. From all the lab work, MBS was in charge of phytoplankton analysis (microscopy, flow cytometry, HPLC) whereas zooplankton samples were processed by other researcher. Processing, analysing the data and writing of the first draft of the manuscript.*

ABSTRACT

Understanding the ecosystem functioning of Mediterranean confined coastal lagoons is essential for their management and conservation. These shallow, dynamic and productive water bodies, located in the land-sea interface, provide many services to humans and play an important ecological role. Despite these features, they have experienced severe degradation from anthropic pressure. One of the most important descriptors of ecosystem functioning is ecosystem metabolism, the use of which has increased recently due to the development of the diel free-water oxygen technique with affordable and reliable sensors. Quantifying metabolic rates and identifying the factors that contribute to their temporal and spatial variation are very important for assessing ecosystem behaviours and their response to future changes. Considering that this methodology and new modelling approaches have not been fully applied to coastal lagoons, the aim of this thesis was to estimate the ecosystem metabolism of a set of Mediterranean confined coastal lagoons, identifying the main drivers of ecosystem metabolism variation, and its applicability in phytoplankton community studies for a better understanding of these ecosystems functioning. The water bodies studied are located in the protected salt marsh of La Pletera (Baix Ter Wetlands, NE Iberian Peninsula), where a restoration project has been developed, including the creation of new lagoons.

Initially, we estimated the ecosystem metabolism of two confined water bodies and tested whether nutrients or other environmental variables (temperature, conductivity, light and water level) had an effect to metabolic variation in these coastal ecosystems (Chapter I). In addition, as these environments are highly dependent on hydrology, we analysed the influence of hydrodynamics on metabolism (Chapter II). The main results highlighted a marked seasonal pattern in dissolved oxygen measurements and other environmental parameters in response to the Mediterranean climate and the flooding-confinement dynamics present in La Pletera salt marsh. Dissolved oxygen ranged from supersaturation to anoxia, with anoxic conditions extending to the surface and lasting for several days, especially during the summer season. Accordingly, the metabolic rates (gross primary production (GPP) and ecosystem respiration (ER)) showed higher values and higher variability during summer. Metabolic rates were among the highest rates published for aquatic ecosystems. Both lagoons showed slight heterotrophy during the study period, and although GPP:ER values close to 1 could indicate some balance between the rates, there was an underestimation of heterotrophy while using the diel oxygen method, as it does not include anaerobic respiration. Temperature was the primary driver of metabolic variation, and nutrients showed no significant relationship with metabolism at the monthly scale, which agrees with a lower functional response of primary producers to nutrient addition when nutrient concentrations are high. When metabolic rates were standardized to temperature and light, dynamics showed higher potential productivity of these lagoons during winter. This finding suggests that potential productivity (standardized rate) is more sensitive to winter inputs, and in contrast, the actual productivity is more related to summer concentration processes due to confinement. When analysing the relationship between metabolism and modelled hydrological parameters at a weekly scale, metabolic rates showed only a significant strong seasonal oscillation, while standardized rates showed significant responses at higher frequencies that could be related to water exchanges (i.e., sea storms), hence suggesting some response of metabolism to nutrient supplies. Despite these results, the amount of unexplained variation was also high, so the unpredictability of Mediterranean hydrological events, in addition to coastal lagoon variability, make the identification of hydrological parameters as drivers of metabolism dynamics in these water bodies a challenge.

Finally, we investigated the phytoplankton community dynamics in a selection of created and natural lagoons La Pletera salt marsh during a hydrological cycle. Moreover, different environmental variables and traits were analysed, utilizing new versions of Margalef's mandala as a reference, to identify the most important factors explaining phytoplankton dynamics in these lagoons (Chapter III). We were particularly focused on the quantification of productivity-related traits using ecosystem metabolism estimations, such as the production to biomass ratio (P/B), using the ratio between GPP and chlorophyll-*a* concentration as an estimation for it, and a proxy for the relative importance of *r*-*K* strategies, using the ratio between photosynthetic efficiency and maximal GPP obtained under no light limitation, variables that are commonly used only in theoretical frameworks. The main results highlighted differences in phytoplankton composition between seasons due to the dominant hydrological pattern of flooding confinement in the salt marsh and between lagoons caused by their different origins and hence different nutrient availability. Salinity and grazing pressure were the most important variables explaining phytoplankton composition. Our results are consistent with a decrease in the P/B ratio and a prevalence of *K*-strategists with seasonal succession. In general, ecosystem parameter estimations are rarely combined with organism studies or trait-based approaches, but this complementarity could provide a better understanding of how these aquatic ecosystems work. Knowledge of ecosystem functioning is essential for understanding and predicting how aquatic ecosystems, and more specifically, these highly variable coastal lagoons, will respond to changes in physical and chemical characteristics and planktonic assemblages associated with global change.

RESUMEN

Comprender el funcionamiento ecosistémico de las lagunas costeras mediterráneas de tipo confinado es fundamental para su gestión y conservación. Estos cuerpos de agua poco profundos, dinámicos y productivos, ubicados en la transición entre la tierra y el mar, proporcionan multitud de servicios ecosistémicos y desempeñan un papel ecológico muy importante. A pesar de eso, han estado sufriendo un fuerte retroceso provocado por la presión antrópica. Uno de los descriptores más importantes del funcionamiento del ecosistema es el metabolismo ecosistémico, cuyo uso se ha incrementado recientemente debido al desarrollo de la técnica de oxígeno diario en agua libre con sensores asequibles y fiables. La cuantificación de las tasas metabólicas y la identificación de los factores que contribuyen a su variación temporal y espacial son muy importantes para evaluar el comportamiento de los ecosistemas y su respuesta a futuros cambios. Si consideramos las pocas aplicaciones de esta metodología con el uso de nuevos modelos en lagunas costeras, el objetivo de esta tesis fue estimar el metabolismo ecosistémico de un conjunto de lagunas costeras confinadas mediterráneas, identificar los principales factores de variación del metabolismo y su aplicación en el análisis de la estructura de la comunidad de fitoplancton, para mejorar la comprensión sobre el funcionamiento de estos ecosistemas. Las lagunas donde se llevó a cabo el estudio se encuentran situadas en la marisma de La Pletera (Aiguamolls del Baix Ter, NE Península Ibérica), donde se han creado nuevas lagunas en el marco de un proyecto de restauración.

En primer lugar, se estimó el metabolismo ecosistémico de dos lagunas de tipo confinado y se examinó si los nutrientes y otras variables ambientales (temperatura, conductividad, luz y nivel del agua) eran responsables de la variación metabólica en estos sistemas costeros (Capítulo I). Además, debido a la gran dependencia de estos ecosistemas del régimen hidrológico, se analizó la influencia que la hidrodinámica ejerce sobre el metabolismo (Capítulo II). Los principales resultados destacaron un patrón estacional muy marcado en los valores de oxígeno disuelto, así como en otros parámetros ambientales, acorde con la dinámica de inundación confinamiento, característica del clima mediterráneo, presente en la marisma de La Pletera. El oxígeno osciló de la sobresaturación a la anoxia, con condiciones anóxicas que se extendían hasta la superficie con varios días de duración, especialmente durante el verano. En consecuencia, las tasas metabólicas (producción primaria bruta (GPP) y respiración (ER)) mostraron valores más altos y con mayor variabilidad durante el verano. Las tasas metabólicas registradas se encontraron entre las tasas más altas publicadas para ecosistemas acuáticos. Ambas lagunas mostraron una leve heterotrofia durante el período de estudio, y aunque valores del cociente GPP:ER cercanos a 1 podrían indicar cierto equilibrio entre las tasas, había una subestimación de la heterotrofia debido a que la técnica utilizada que registra los cambios diarios de oxigeno no considera la respiración anaeróbica. La temperatura resultó ser el principal factor de variación de las tasas metabólicas y los nutrientes no mostraron relaciones significativas con el metabolismo a una escala mensual, de acuerdo con una menor respuesta funcional de los productores primarios a la adición de nutrientes cuando su concentración es alta. Cuando se utilizaban las tasas metabólicas estandarizadas por temperatura y luz, sus dinámicas mostraron una mayor producción potencial de estas lagunas durante el invierno. Estos resultados sugieren que la producción potencial (tasa estandarizada) es más sensible a las entradas invernales y, por lo contrario, la producción real está más relacionada con los procesos de concentración que ocurren en verano ligados al confinamiento. Al analizar la relación entre el metabolismo y los parámetros hidrológicos modelados a escala semanal, las tasas metabólicas sólo mostraron una fuerte oscilación estacional significativa, mientras que las tasas metabólicas estandarizadas mostraron relaciones significativas a frecuencias más altas y que podían relacionarse con intercambios de agua (temporales de mar), lo que sugiere cierta respuesta del metabolismo al aporte de nutrientes. A pesar de estos resultados, la cantidad de variación no explicada también fue alta, por lo que la imprevisibilidad de las perturbaciones hídricas, típica del clima mediterráneo, junto con la propia variabilidad ambiental de las lagunas costeras, hacen que la identificación de parámetros hidrológicos como factores determinantes de la dinámica del metabolismo en estas masas de agua sea todo un reto.

Finalmente, se determinó la dinámica de la estructura de la comunidad de fitoplancton en una selección de lagunas naturales y de nueva creación de la marisma de La Pletera durante un ciclo hidrológico. Además, se analizaron diferentes variables ambientales y atributos utilizando como referencia una nueva versión del mandala de Margalef, para identificar los factores más importantes a la hora de explicar las dinámicas

de fitoplancton en estas lagunas (Capítulo III). Nos centramos de forma particular en la cuantificación de los atributos relacionados con la productividad utilizando las estimaciones del metabolismo ecosistémico, como el cociente P/B, utilizando la ratio entre la GPP y la concentración de clorofila-*a*, y una variable *proxy* de la importancia relativa de las estrategias *r-K,* usando la relación entre la eficiencia fotosintética y la GPP máxima obtenida sin limitación de luz, conceptos utilizados dentro de marcos teóricos, pero pocas veces cuantificados. Los principales resultados mostraron diferencias en la composición de fitoplancton entre estaciones, debido al patrón hidrológico de inundación confinamiento dominante en la marisma, y entre lagunas, como consecuencia de sus diferentes orígenes y, por tanto, diferente disponibilidad de nutrientes. La salinidad y la presión de depredación fueron las variables más importantes a la hora de explicar la composición de fitoplancton. Nuestros resultados son consistentes con una disminución del cociente P/B y una prevalencia de la estrategia *K* con la sucesión. En trabajos anteriores, las estimaciones de parámetros ecosistémicos rara vez se han combinado con estudios de organismos o con aproximaciones basadas en atributos, pero esta complementariedad podría proporcionar una mejor comprensión sobre el funcionamiento de estos sistemas acuáticos. Conocer el funcionamiento ecosistémico resulta indispensable para comprender y predecir cómo responderán los sistemas acuáticos, y más específicamente las lagunas costeras, a los cambios en las características físicas y químicas y en la comunidad planctónica asociados al cambio global.

RESUM

Entendre el funcionament ecosistèmic de les llacunes costaneres mediterrànies de tipus confinat es fonamental per la seua gestió i conservació. Aquests cossos d'aigua de poca fondària, dinàmics i productius, situats a la transició entre la terra i el mar, proporcionen multitud de serveis ecosistèmics i tenen un paper ecològic molt important. Tot i això, han estat patint una important regressió deguda a la pressió antròpica. Un dels descriptors més importants del funcionament de l'ecosistema és el metabolisme ecosistèmic, l'ús del qual s'ha incrementat recentment degut al desenvolupament de la tècnica d'oxigen diari en agua lliure amb sensors assequibles i fiables. La quantificació de les taxes metabòliques i la identificació dels factors que contribueixen a la seua variació temporal i espacial són molt importants per avaluar el comportament dels ecosistemes i la seua resposta a futurs canvis. Si considerem la manca d'aplicacions d'aquesta metodologia amb l'ús de nous models en llacunes costaneres, l'objectiu d'aquesta tesi va ser estimar el metabolisme ecosistèmic d'un conjunt de llacunes costaneres mediterrànies confinades, identificar els principals factors de variació del metabolisme i la seua aplicació en l'anàlisi de l'estructura de la comunitat de fitoplàncton, per millorar la comprensió sobre el funcionament d'aquests ecosistemes. Les llacunes on s'ha portat a terme l'estudi es troben situades a la maresma de La Pletera (Aiguamolls del Baix Ter, NE Península Ibèrica), on s'han creat noves llacunes en el marc d'un projecte de restauració.

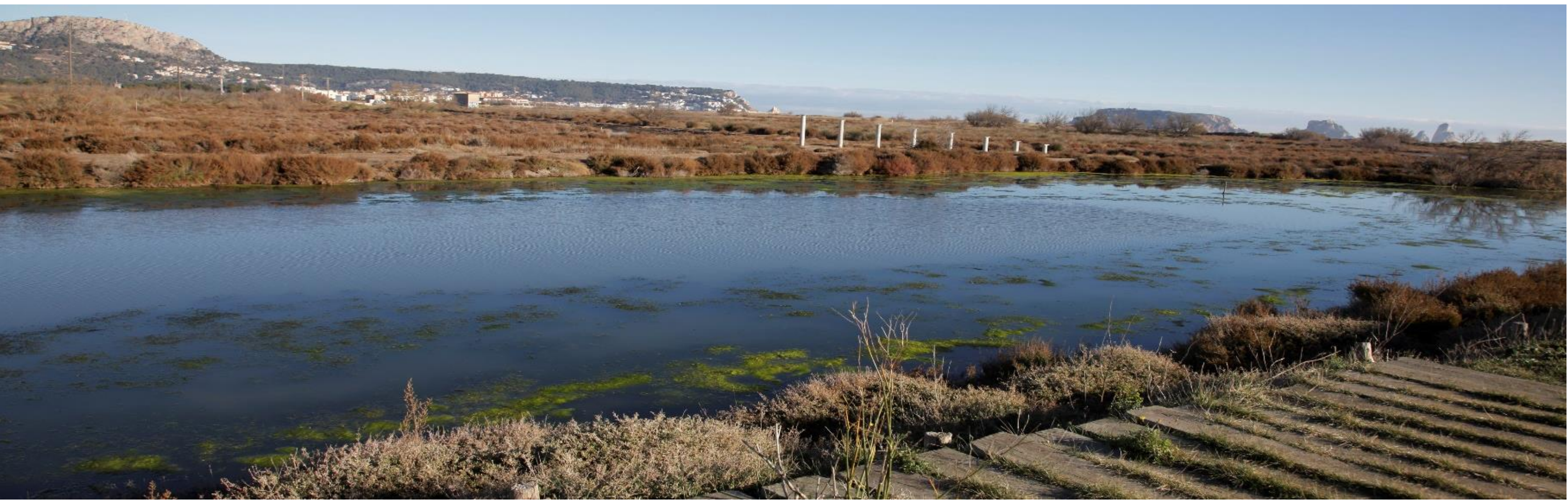
En primer lloc, es va estimar el metabolisme ecosistèmic de dos llacunes de tipus confinat i es va esbrinar si els nutrients i altres variables ambientals (temperatura, conductivitat, llum i nivell d'aigua) eren responsables de la variació metabòlica en aquests sistemes costaners (Capítol I). A més, degut a la gran dependència d'aquests ecosistemes al règim hídric, es va analitzar la influència de la hidrodinàmica sobre el metabolisme (Capítol II). Els principals resultats van destacar un patró estacional molt marcat als valors d'oxigen dissolt així com a altres paràmetres ambientals, d'acord amb la dinàmica d'inundació confinament, característica del clima mediterrani, present a la maresma de La Pletera. L'oxigen va oscil·lar de la sobresaturació a la anòxia, amb condicions anòxiques que s'estenien fins la superfície i duraven diversos dies, especialment durant l'estiu. En conseqüència, les taxes metabòliques (producció primària bruta (GPP) i respiració (ER)) van mostrar valors més elevats i amb major variabilitat durant l'estiu. Les taxes

metabòliques registrades es van trobar entre les més altes publicades per a sistemes aquàtics. Ambdues llacunes mostraren una lleu heterotròfia durant el període d'estudi, i encara que els valors del quocient GPP:ER propers a 1 podien indicar cert equilibri entre les taxes, hi havia una subestimació de la heterotròfia degut a que la tècnica utilitzada que registra els canvis diaris d'oxigen no considera la respiració anaeròbica. La temperatura va resultar ser el principal factor de variació de les taxes metabòliques i els nutrients no van mostrar cap relació significativa amb el metabolisme a una escala mensual, d'acord amb una menor resposta funcional dels productors primaris a l'adició de nutrients quan la seua concentració és alta. Quan les taxes metabòliques van ser estandarditzades per temperatura i llum, les seues dinàmiques van mostrar una major producció potencial d'aquestes llacunes durant l'hivern. Aquests resultats suggereixen que la producció potencial (taxa estandarditzada) és més sensible a les entrades hivernals i, pel contrari, la producció real està més relacionada amb els processos de concentració que es donen a l'estiu lligats al confinament. Al analitzar la relació entre el metabolisme i els paràmetres hidrològics modelats a escala setmanal, les taxes metabòliques només van mostrar una forta oscil·lació estacional significativa, mentre que les taxes estandarditzades van mostrar relacions significatives a freqüències més altes i que van poder ser relacionades amb intercanvis d'aigua (temporals de mar), suggerint, per tant, certa resposta del metabolisme a l'aportació de nutrients. Malgrat aquests resultats, la quantitat de variació inexplicada va ser també alta, de manera que la imprevisibilitat dels fenòmens hidrològics típica del clima mediterrani, juntament amb la pròpia variabilitat ambiental de les llacunes costaneres, fan que la identificació de paràmetres hidrològics com a factors de variació de la dinàmica del metabolisme en aquestes masses d'aigua sigui tot un repte.

Finalment, es va determinar la dinàmica de l'estructura de la comunitat de fitoplàncton en una selecció de llacunes naturals i de nova creació de la maresma de La Pletera durant un cicle hidrològic. A més, es van analitzar diferents variables ambientals i trets utilitzant com a referència una nova versió de la mandala de Margalef, per tal d'identificar els factors més importants a l'hora d'explicar les dinàmiques de fitoplàncton en aquestes llacunes (Capítol III). Ens vam centrar particularment en la quantificació de trets relacionats amb la productivitat utilitzant mesures del metabolisme ecosistèmic,

com el quocient P/B, utilitzant la ràtio entre la GPP i la concentració de clorofil·la-*a*, i una variable *proxy* de la importància relativa de les estratègies *r*-*K*, utilitzant la relació entre la eficiència fotosintètica i la GPP màxima obtinguda sense limitació de llum, conceptes que són sovint utilitzats en contextos teòrics, però poques vegades quantificats. Els principals resultats van mostrar diferències en la composició de fitoplàncton entre estacions, degut al patró hidrològic d'inundació confinament que domina a la maresma, i entre llacunes, com a conseqüència dels seus diferents orígens i, per tant, per la seva diferent disponibilitat de nutrients. La salinitat i la pressió de depredació foren les variables més importants a l'hora d'explicar la composició de fitoplàncton. Els nostres resultats són consistents amb una disminució del quocient P/B i una prevalença de la estratègia *K* amb la successió. En treballs anteriors, les estimacions de paràmetres ecosistèmics es combinen molt poques vegades amb estudis d'organismes o amb aproximacions basades en trets, malgrat que aquesta complementarietat podria proporcionar una millor comprensió del funcionament d'aquests ecosistemes aquàtics. El coneixement del funcionament dels ecosistemes resulta fonamental per comprendre i predir com respondran els ecosistemes aquàtics, i més concretament les llacunes costaneres, als canvis en les característiques físiques i químiques i en la comunitat planctònica associats al canvi global.

1. GENERAL INTRODUCTION



1.1. Coastal Lagoons

Coastal lagoons are shallow, lentic water bodies occupying approximately 13% of the world's coastlines, and of these lagoons, 5.3% occur in Europe (Barnes, 1980; Kjerfve, 1994). In the Mediterranean region alone, there are approximately 400 lagoons covering 6400 km^2 of its littoral area (Pérez-Ruzafa et al., 2019). These lagoons have been defined by the Water Framework Directive (2000/60/EC) as transitional ecosystems due to their location and the influence of inland and marine waters on them (Pérez-Ruzafa et al., 2011a; Schubert and Telesh, 2017). Thus, these ecosystems share many traits and processes with estuaries and other aquatic ecosystems in the transition between the land and sea (Kjerfve, 1994; Pérez-Ruzafa et al., 2019). Coastal lagoons can be connected to the ocean or intermittently closed off by depositional barriers (Kjerfve, 1994; Roselli et al., 2013) and can vary greatly in size, but they rarely exceed a few metres in depth (Pérez-Ruzafa et al., 2011a). They are very dynamic ecosystems, presenting a wide range of morphological, geological and hydrological characteristics (Basset et al., 2013; Guelorget and Perthuisot, 1983; Kennish and Paerl, 2010). Due to their shallowness, lagoon bottoms are usually well irradiated, and currents and hydrodynamics are driven mainly by the wind and bottom topography, usually affecting the entire water column and promoting the resuspension of materials and nutrients (Pérez-Ruzafa et al., 2019; Schubert and Telesh, 2017). Their relatively small volumes increase the effect of climatic conditions, and lagoons usually present high and extreme variations that are either daily or seasonal (Nidzieko et al., 2014; Pérez-Ruzafa et al., 2019). Characterised by long residence times, their salinity can range from oligohaline to hyperhaline conditions (Kennish and Paerl, 2010).

Kjerfve (1986) divided coastal lagoons into three types (leaky, restricted and choked) based on the characteristics of their potential hydrodynamic exchange properties with the open sea. The intensity of the water exchange in lagoons is a function of not only the relative width of the opening but also the real mixing regime, which involves the relationship between the freshwater inflow, the total water volume and the residence time of the water in the lagoon (Schubert and Telesh, 2017). 'Leaky' lagoons have multiple wide entrance channels to the sea and are therefore characterised as having a high marine influence, near-ocean salinities, strong tidal variability and occasionally significant wave energy, where nutrient dynamics depend on nutrient inputs from flooding. ‘Choked’ lagoons have only one narrow channel to the sea, resulting in delayed and dampened water exchange with the sea, long water residence times, high salinities and a strong wind influence, where nutrient dynamics depend more on internal loading processes. ‘Restricted’ lagoons present the intermediate situation that of choked and leaky extremes, and these lagoons usually have multiple channels to the sea and relatively good water exchange with a net transport of water to the ocean (Kjerfve, 1986; Kjerfve and Magill, 1989; Roselli et al., 2013; Schubert and Telesh, 2017).

Other terms, such as ‘confinement’, have been applied to many systems, particularly in Mediterranean lagoons, and this term also expresses the degree of lagoon connection to the sea or other sources of water (Guelorget and Perthuisot, 1983). Confined coastal lagoons are shallow water bodies that have no continuous water connections and can be found along the Mediterranean coast where the tidal influence is minimal (Badosa et al., 2007; Britton and Crivelli, 1993; Quintana et al., 1998). Although these lagoons receive water inputs from the sea, runoff or groundwater, they can also be isolated for long periods of time, especially during the summer months (Quintana et al., 2018). Water exchanges, such as rainfall and sea storms, are often sudden events, and as a result, these particular habitats fluctuate greatly and register high spatial and temporal variability in salinity levels. Moreover, similar to ‘choked’ lagoons, the nutrient dynamics of confined lagoons are more related to internal processes; hence, lagoons play an important role in nutrient cycling and concentration of conservative elements, and their study is essential to understand many ecological processes.

The biological and geomorphological characteristics in addition to the high productivity of coastal lagoons enable them to offer many ecosystem services. On the one hand, they are ecosystems with important ecological values, containing important biodiversity and providing refuge, nursery areas and feeding grounds for many freshwater and marine species, including water birds or migratory birds (Basset et al., 2013; Kennish and Paerl, 2010; Pérez-Ruzafa et al., 2019). Additionally, they act as buffers of the land-sea interface, enabling nutrient recycling, organic matter decomposition, removal of contaminants, etc. and protecting the coasts against erosion (Brito et al., 2012a; Costanza et al., 1997; Kjerfve, 1986). On the other hand, coastal lagoons supply many goods and services to humans, and support a wide range of human activities, including those related to economic sectors, such as fisheries and aquaculture, as well as to leisure and tourism. Therefore, these ecosystems provide not only livelihoods but also numerous benefits to human health and welfare (Newton et al., 2018). However, despite being very valued habitats, they rank among the most threatened ecosystems in the world (Pérez-Ruzafa et al., 2019) and several EU directives include them as transitional waters for protection and conservation of their good or high ecological status (Water Framework Directive, 2000/60/EC; Habitats Directive, 92/43/EEC). Overexploitation, urbanization, pollution, eutrophication and other pressures associated with climate change, such as higher temperatures, sea level rise and changes in precipitation patterns, will compromise the ecosystem functioning of these habitats (Arévalo et al., 2013; Basset et al., 2013; Brito et al., 2012a; Schubert and Telesh, 2017). Moreover, the synergy between climate change and anthropic pressure could be especially important for Mediterranean confined coastal lagoons that already have experienced intense degradation due to human activities (Quintana et al., 2018). Overall, we expect changes in the composition and diversity of their natural communities in addition to an alteration to their sensitivities to threats and capabilities to support goods and services. Global change is therefore particularly important, and lagoons can be considered "sentinel" systems in terms of their sensitivity but also can act as a reservoir of genetic adaptations to stressed environments that can cope with the changes that may occur in the immediate future with more effectiveness (Newton et al., 2018; Pérez-Ruzafa et al., 2019).

Despite the ecological role coastal lagoons play and the insights provided by the studies conducted during the last decades, our knowledge about coastal lagoons is still limited and fragmented (Pérez-Ruzafa et al., 2020, 2019). Currently, studies regarding coastal lagoons functioning have increased (Pérez-Ruzafa et al., 2020), but the application of powerful techniques, such as high-frequency measurements (Marcé et al., 2016; Seifert-Dähnn et al., 2021) that have been fully deployed in other aquatic ecosystems (e.g., deep lakes, rivers), is still scarce in coastal lagoons, wetlands, ponds, etc. The application of such techniques could help to better understand these highly variable environments, which are compromised by numerous pressures and near-future disturbances, and provide a tool for their management and conservation.

1.2. Ecosystem metabolism

Ecosystem metabolism is a fundamental ecological process involving the major carbon pathways. Often, it is described as an integrative indicator of ecosystem functioning that measures the overall activity of a system, ranging from individual organisms to whole ecosystems, and determines the efficiency of fixation and/or decomposition of organic matter (Brown et al., 2004; López-Archilla et al., 2004; Winslow et al., 2016). The three main components of aquatic metabolism are gross primary production (GPP), ecosystem respiration (ER) and net ecosystem production (NEP) (Figure 1.1). GPP is the rate of organic matter production within an ecosystem through photosynthesis, whereas ER represents the degradation of organic matter. Thus, GPP and ER reflect the major rates at which carbon is gained and lost by biological components of an aquatic ecosystem due to the physiological activities of organisms (McNair et al., 2013). NEP (NEP= GPP-ER) or the ratio GPP:ER is usually calculated as a measure of the net gain or loss of biomass (as carbon) of an ecosystem, allowing the classification of a system as an autotrophic (GPP>ER) or heterotrophic (GPP<ER) habitat. These two parameters indicate the extent to which ecosystems are subsidized by allochthonous sources of carbon and the role water bodies play as net sources (GPP<ER) or sinks (GPP>ER) of atmospheric carbon (Hanson et al., 2008, 2003). Currently, with ongoing global change, the importance of understanding aquatic ecosystems as sources or sinks of carbon and hence their role in the global carbon cycle is more important than ever (McNair et al., 2013). Although coastal ecosystems represent a small proportion of global surface area, understanding their productivity and carbon fluxes is essential for constraining global budgets (Murrell et al., 2018).

In recent decades, reliable measurements of the uptake and release of carbon by aquatic ecosystems have been a challenge, and an increasing number of methods have been developed. While no unique methodology has emerged as completely perfect, the variability in the methods allows their application in a wide range of research scenarios (rivers, lakes, estuaries, open ocean, etc.) (Staehr et al., 2012a). Currently, one of the most popular techniques used to calculate metabolic rates is from dissolved oxygen (DO) concentrations (Staehr et al., 2010a). DO in aquatic systems is a highly informative metric that is strongly representative of ecosystem conditions, functionality and behaviour and is used in environmental management and assessment across the globe (D'Autilia et al., 2004; Jankowski et al., 2021; Nidzieko et al., 2014). However, a main distinction should be made between discrete measurements of DO and high-frequency or continuous DO. Although discrete or point measurements are easy to obtain, they represent a highly limited description of ecosystem function that omits important deviations that may occur at higher frequencies (Batt and Carpenter, 2012; Jankowski et al., 2021). For example, a coastal ecosystem exhibiting high fluctuations at a daily scale and presenting hypoxia or anoxia at night would be misinterpreted when measures are only taken in the morning or at midday. Thus, the diel free-water DO technique has been used widely in recent years, allowing the registration of DO changes at high frequencies and providing an understanding of the spatial and temporal variability in metabolism in aquatic ecosystems (e.g., Laas et al., 2012; Nidzieko et al., 2014; Staehr et al., 2012b). The development of this technique has been fuelled by technological advances and the availability of more reliable and affordable DO sensors. The use of this methodology overcomes some of the disadvantages of older methodologies, such as the incubation of small bottles or the use of chambers in light and darkness, which were pioneering efforts on coastal plankton metabolism that have persisted over time (Kemp and Testa, 2011; Staehr et al., 2012a).

Although the free-water DO technique offers several advantages over other techniques, different assumptions and challenges should be addressed, and some of them can be more important depending on the type of ecosystem under study. Deep lakes, where the vast majority of these techniques has been applied, host large-amplitude vertical motions, which can significantly impact the oxygen record, hindering the calculation of metabolic rates. In shallow, productive lakes or lagoons, where the application of this method has not been fully developed, metabolic rate calculations are relatively straightforward after accounting for gas exchange with the atmosphere because oxygen changes driven by biological processes are larger than vertical transport (Fernández Castro et al., 2021; Figure 1.1). However, other disadvantages that affect these techniques are associated with this type of habitat, such as high spatial heterogeneity (Andersen et al., 2017; Obrador and Pretus, 2013).

Overall, recent advances in high-frequency sensing and statistical and modelling approaches have allowed the expansion of the diel free-water DO method in a wide range of aquatic ecosystems. Accordingly, metabolic estimations have become a common tool for research as a proxy of ecosystem functioning, enabling the assessment and management of trophic status and the health of aquatic ecosystems.

1.3. Drivers of aquatic ecosystem metabolism

Recent advances and improvements in free-water methodologies have allowed ecosystem metabolism estimations to become popular in aquatic ecosystem ecological status assessments. With their wide application in many types of ecosystems, new insights and questions have emerged about the temporal and spatial variations in metabolic rates and the factors that govern them (Obrador et al., 2014; Staehr and Sand-Jensen, 2007; Tonetta et al., 2016). This interest dates back many years but has increased in recent years as research on global carbon cycles has expanded (Solomon et al., 2013).

A good understanding of the main drivers affecting GPP, ER and NEP is important for understanding ecosystems functioning. Moreover, many of the physical, chemical and biological factors that contribute to changes in metabolism dynamics may be affected by ongoing global change (Pesce et al., 2018; Figure 1.1), and it is of great importance to understand how these factors are related to metabolism to predict and manage future changes in aquatic environments.

Many variables are related to the spatial and temporal variability in metabolic processes. Temperature (Alfonso et al., 2018; Caffrey, 2004, 2003; Carmouze et al., 1991; Laas et al., 2012; Murrell et al., 2018; Russell and Montagna, 2007), light (Giordano et al., 2012; López-Archilla et al., 2004; Obrador et al., 2014; Staehr et al., 2016; Tonetta et al., 2016), salinity (Caffrey, 2004; Russell and Montagna, 2007), nutrient (Caffrey, 2004; López-Archilla et al., 2004; Staehr et al., 2016) and organic matter (Giordano et al., 2012; Tonetta et al., 2016) contents, hydrology (Alfonso et al., 2018; Barnes, 1980; Murrell et al., 2018; Russell and Montagna, 2007), wind (Alfonso et al., 2018) and depth (Giordano et al., 2012; López-Archilla et al., 2004) are among the most reported factors driving metabolic rates in lentic ecosystems (Figure 1.1). All these drivers and their effects on metabolic rates could differ among the different aquatic systems, with significant differences occurring due to the size and depth of water bodies as well as to other conditions that could be related to local or regional variations. Therefore, comparisons across different sites and ecosystems with different conditions usually become challenging, especially when the standardization of protocols and methodologies is sometimes minimal (Hoellein et al., 2013).

Long-term and high-frequency estimates of GPP and ER are rare in coastal lagoon literature; hence, information about their main metabolic drivers is still limited. This limitation could be of great concern, as some of these ecosystems, such as confined coastal lagoons, could become even more susceptible than other aquatic systems to global change due to the relationship between metabolic drivers and the system's own properties. The location of these lagoons between the land-sea interface in addition to their shallowness and low volumes make these ecosystems very dependent on climatic conditions. Thus, changes in temperature, light and salinity could greatly affect them. For example, higher temperatures could enhance ecosystem respiration rates and generate more frequent anoxic crises (Duarte et al., 2002). Additionally, their response to nutrients may be different from those of other aquatic systems, as explained by Glibert et al. (2010), who noted that when nutrient loading is high, the physiological response of the ecosystem is low as a result of a saturation curve between ecosystem productivity and nutrients. Therefore, in these nutrient-enriched waters, physical conditions become the main drivers of metabolic rates (Beck and Bruland, 2000). However, concentrations of nutrients and organic matter would be crucial when assessing eutrophication, an important threat to these environments (e.g., Derolez et al., 2020a; Rabalais et al., 2009). Moreover, due to their high dependence on water flow conditions, these lagoons may also experience modifications in their dynamics as a result of the expected changes in hydrology, with consequences over other driving factors of metabolism in coastal waters, such as nutrient dynamics, water residence time and water level (Kemp and Testa, 2011).

A better understanding of the factors that regulate metabolism dynamics, such as nutrient or hydrological changes, becomes crucial for providing information to managers to protect these heterogeneous and vulnerable habitats (Alfonso et al., 2018; Caffrey et al., 2014).

1.4. Phytoplankton community structure and ecosystem metabolism

Phytoplankton constitute a very diverse and widespread group that, despite representing only 1% of the planet's photosynthetic biomass, plays a key role in aquatic ecosystem functioning. These mainly single-celled organisms display many important functions, such as the regulation of CO_2 between air and water; they provide the base of most aquatic food webs and are responsible for approximately half of the primary production worldwide (Falkowski, 2012; Field et al., 1998). Phytoplankton, due to their small size and fast reproduction, respond quickly to environmental changes (Schubert and Telesh, 2017; Yang et al., 2020) and have often been considered a good natural bioindicator for monitoring ecological changes in aquatic ecosystems (Hemraj et al., 2017; Pereira Coutinho et al., 2012). In fact, the European Water Framework Directive (2000/60/EC) proposes phytoplankton community composition, species abundance and biomass, and frequency and intensity of blooms as elements of phytoplankton-based ecological quality assessments in different types of water bodies (Caroppo et al., 2018; Leruste et al., 2018; Montes-Pérez et al., 2020).

Although shallow ecosystems, such as coastal lagoons, are not always phytoplankton dominated and the role of benthic primary producers could also be important, phytoplankton dynamics have also been the focus of many studies and are often used as indicators of environmental disturbances and ecosystem functioning in these habitats (Brito et al., 2012b; Caroppo et al., 2018; Hemraj et al., 2017). Understanding how environmental variability affects the structure and composition of phytoplankton communities is therefore essential (Leruste et al., 2018). However, in these highly variable ecosystems, phytoplankton show strong spatial and temporal variability, which makes it more difficult to relate phytoplankton taxonomic features directly to all the environmental constraints and fluctuations that affect these communities (Leruste et al., 2018; Montes-Pérez et al., 2020). Thus, to overcome the difficulty of linking community composition with ecosystem functioning, different functional approaches have been developed.

Margalef (1978) was the first to show in a tentative plot how phytoplankton life-forms could be related to their functionality in the environment. Since that time, phytoplankton studies have evolved from species-based approaches to a combination of taxonomical and trait-based approaches to better describe phytoplankton adaptations to environmental conditions and their ecological role (Litchman and Klausmeier, 2008; Reynolds et al., 2002). In comparison with other disciplines (e.g., terrestrial ecology), aquatic research has not been fully developed and used these approaches, but their use has increased significantly in recent decades. Thus, new attempts to incorporate more dimensions or modifications to Margalef's mandala have been proposed to understand and address some of the ongoing changes derived from global change (e.g., Allen and Polimene, 2011; Cullen et al., 2007, 2002; Glibert, 2016).

There are two opposite perspectives that differ in the way they approach ecology (Smith and Smith, 2001): reductionism, which studies the ecosystem as a sum of its parts, and holism, which considers the ecosystem as a whole. The 'conflict' between the two of these perspectives can help explain a limitation that is emergent in phytoplankton research. Previous literature based on phytoplankton composition has usually used important concepts, such as 'production' or 'productivity', but these parameters have rarely been quantified in the same studies (e.g., López-Flores et al., 2006a; Pulina et al., 2017, 2012; Villamaña et al., 2019). Thus, studies focused on organisms are rarely combined with ecosystem parameters (e.g., ecosystem metabolism). In contrast, studies describing the ecosystem metabolism dynamics in many aquatic ecosystems have not commonly incorporated organisms data or have only used chlorophyll-*a* estimations, and when interpreting metabolic rates at an ecosystem level, these studies only refer to ecosystem processes (e.g., López-Archilla et al., 2004; Martinsen et al., 2017). Interestingly, with the increasing number of studies focusing on the drivers of phytoplankton dynamics and trait-based approaches, new approximations, such as high-frequency measurements and ecosystem metabolism estimations, could be excellent tools for improving these functional approaches and allowing trait quantification.

A better understanding of how physical, chemical and biological changes will affect coastal lagoons requires a better understanding of how these ecosystems work. Thus, these ecosystems serve as a good scenario for the application of a complementarity assessment of taxonomy, trait-based approaches and ecosystem estimations (i.e., ecosystem metabolism) to study ecosystem functioning.

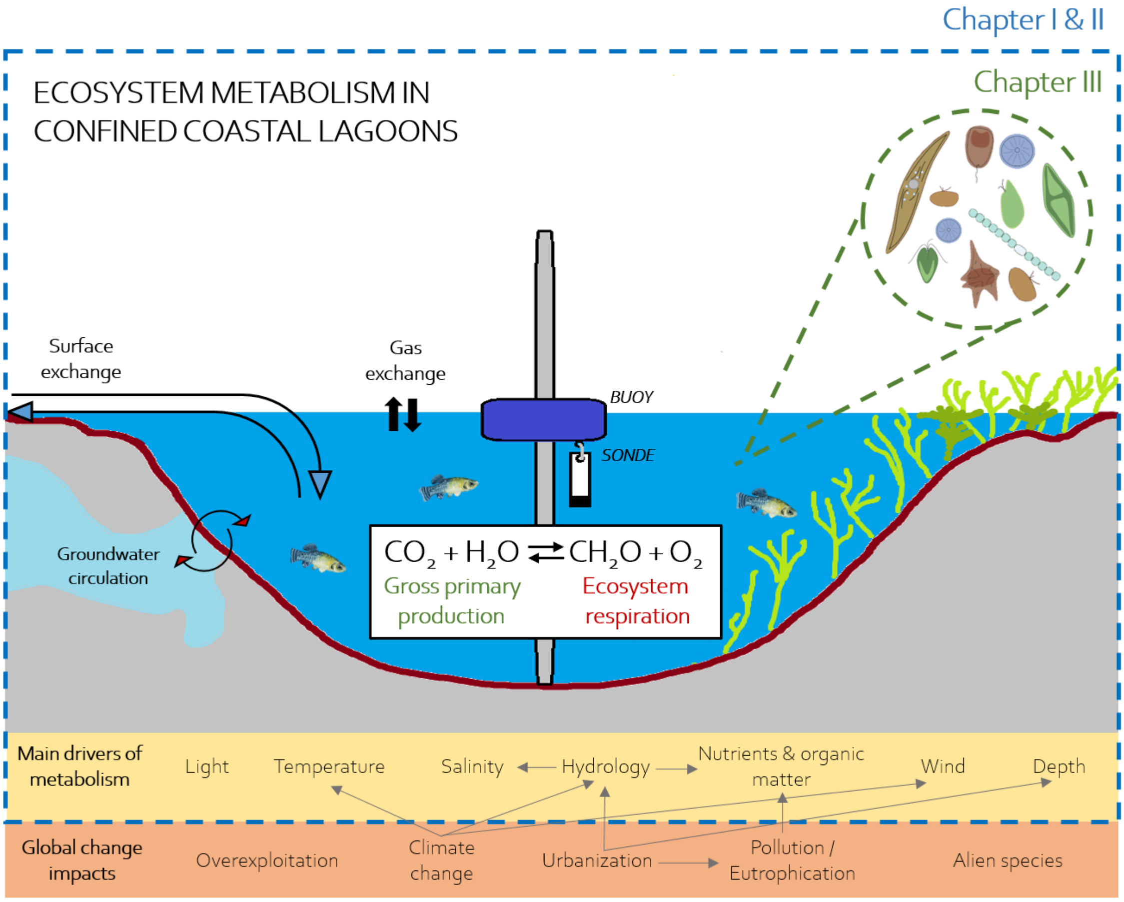


Figure 1.1. Representation of the ecosystem metabolism estimations (gross primary production (GPP) and ecosystem respiration (ER)) in coastal lagoons. Buoy and sonde locations are shown as they have been deployed in this thesis, in addition to some of the physical factors affecting dissolved oxygen measurements in these lagoons. The figure also summarizes the main drivers of metabolism described for lentic water bodies and the influence that some global change impacts may have over them (arrows). Although more relationships could be represented, especially among own drivers, only those of major concern for the present thesis were drawn. Dashed lines outline the different topics that each chapter covers. Adapted from Jankowski et al. (2021).

2. OBJECTIVES

Confined coastal lagoons have experience and are still under strong pressure from human activities. This scenario increases their vulnerability to synergies between anthropic pressures and climate change. Taking into account the important ecological role coastal lagoons play, the services they provide to people, the need to better understand their ecosystem functioning to manage and conserve them in the face of global change, and the consideration of ecosystem metabolism as an integrative indicator of ecosystem functioning with a relatively easy application, the main objective of the present thesis was:

→ To estimate the ecosystem metabolism of Mediterranean confined coastal lagoons, identifying the main drivers of its variation and determining its applicability in phytoplankton community studies to better understand the functioning of these ecosystems.

To attain this main objective, the present thesis was structured with several specific aims, which constitute the three chapters.

Given that high-frequency measurements and new modelling approaches to determine ecosystem metabolism estimates have not been fully deployed in confined coastal lagoons and the information they could provide about how these ecosystems behave has not been obtained, the objectives of chapter I were:

1) to determine the seasonal metabolic dynamics by quantifying the aquatic metabolism from the change of high-frequency dissolved oxygen concentrations during three hydrological years and

2) to identify the primary drivers of primary production in confined coastal water bodies and to ascertain the influence of nutrient concentrations and other environmental parameters (temperature, conductivity, light and water level) on annual metabolism.

Mediterranean confined coastal lagoons, similar to other aquatic ecosystems that are highly dependent on hydrological characteristics, may experience changes under future global change. Given that hydrology could have an important role in metabolism dynamics in these habitats, chapter II aimed:

1) to provide deeper insight into the ecosystem functioning of Mediterranean confined coastal lagoons by understanding the influence of water exchanges on ecosystem metabolism responses.

Ecosystem metabolism measurements could help improve functional approaches that have been increasingly used in phytoplankton research. The application of ecosystem parameters to studies of organisms could provide new insights into how phytoplankton communities respond to environmental disturbances. Thus, chapter III objectives were the following:

1) to analyse the phytoplankton community in a set of Mediterranean confined coastal lagoons during an entire hydrological cycle and

2) to identify the most important environmental variables and traits included in the newest versions of the mandala to explain the phytoplankton dynamics in these lagoons, emphasizing the use of ecosystem parameters (i.e., aquatic metabolism) for the quantification of productivity-related traits.

3. GENERAL MATERIAL AND METHODS

This section outlines the methodology used for the development of the present thesis. Information about the study site is provided in addition to the methodological procedures that are common to the three chapters presented in the '4. Results' section. Specific methodologies and data analyses are provided in the 'Methods' section for each corresponding chapter.

3.1. Study site

Along the coast of Girona (NE Iberian Peninsula) confined coastal lagoons are restricted to two main nuclei, the Alt Empordà and the Baix Ter Wetlands, both included in the Natura 2000 network as Special Protection Areas (SPA) and Sites of Community Importance (SCI). It is known that these ecosystems were much more abundant in the past, similar to other parts of the Mediterranean, but they have been suffering a strong regression with the years due to a high anthropic pressure. Their location behind the coastal sand bar and their isolation from continuous water inputs facilitated the desiccation of these habitats and its replacement with residential areas for touristic purposes during the second half of the 20th century (Quintana et al., 2018 and references herein).

Figure 3.1.1. Location of the six Mediterranean confined coastal lagoons studied in La Pletera salt marsh (Girona, NE Iberian Peninsula). Red dots refer to the position of the high-frequency monitoring sondes.

The present thesis was carried out in a set of permanent water bodies located in La Pletera salt marsh (Girona, NE Iberian Peninsula; Figure 3.1.1). This protected area belongs to the Baix Ter Wetlands, and is situated between the urban centre of L'Estartit (Torroella de Montgrí, Girona) and the Ter River mouth. The salt marsh presents a subhumid Mediterranean climate, with mild winters (mean temperature of 12°C) and summers (mean temperature of 21°C). The average annual rainfall is 585 mm/year and the wind speed is approximately 3 m/s, with a wind influence from the NNW, occasionally reaching values higher than 17 m/s (1966–2019, Estartit meteorological station 0385J, AEMET. Data available upon request in http://meteolestartit.cat/).

Regarding its geology, La Pletera salt marh is located in the Baix Empordà tectonic basin (NE Iberian Peninsula; Figure 3.1.1). This basin was formed during the distensive period of the Alpine orogenesis and is delimited by the Montgrí Range at the north, characterized by Mesozoic limestone formations, and by the Gavarres Range at the south, composed of igneous and metamorphic rocks of Paleozoic age. The basement of this tectonic graben presents Paleozoic and Paleogene sedimentary materials, which were severely affected by the distensive period of the Alpine orogenesis. The basin infilling is mainly composed of Quaternary sediments. These sediments consist of fluvial deposits originated by the Ter River, as well as by some other minor streams, with a maximum depth of 50–60 m in the central part of the basin. Hydrogeologically, the fluvial sediments can be considered as a multilayer aquifer, with the sallowest acting as an unconfined aquifer, in which there is a gaining stream situation along the study area and old paleochannels act as preferential paths for groundwater due to the presence of coarse sediments. This aquifer with a total thickness of 10-30 m was formed by the recent pro-grading alluvial deposits, which near the coast line are substituted by marsh and coastal deposits (Menció et al., 2017 and references herein).

As a result of the high anthropic pressure in the area (urbanisation, tourism, etc.) during the late 1980s La Pletera salt marsh suffered partial urbanisation, causing alterations to the landscape and its hydrology, although two natural water bodies (FRA and BPI) remained. Years later, in the framework of several LIFE projects (from 1999 to 2018), new shallow basins were created among other actions and the salt marsh recovered its ecological functioning (Quintana et al., 2018). More information about the restoration projects is available at www.lifepletera.com. The lagoons included in the present study were the natural ones (BPI and FRA) and from all the created basins during the restoration projects, only four were selected (G02, L01, L04 and M03). FRA and G02 were studied in Chapter I and II, whereas the six lagoons were analysed in Chapter III. G02 was created in 2002 during the first LIFE Project (LIFE99NAT/E/006386) by simply excavating below the sea level, which ensured water permanency all year round; and L01, L04 and M03 were created in the framework of the second LIFE Project (LIFE13NAT/ES/001001) in 2016 (Figure 3.1.1), when the remaining urban features (promenade, accesses, filling material, breakwaters, and debris) were dismantled and substituted by the new lagoons with different orientation and depth to produce lagoons with different properties (Quintana et al., 2018). Therefore, despite the general hydrological features of the salt marsh, these lagoons differ in some characteristics, which partially depend on their different origins. BPI is the most isolated lagoon in the marsh, with no connection with the rest, and has the highest conductivity values. FRA lagoon is the largest and the deepest, and can present some degree of stratification during the winter, presenting higher salinity values with depth (Figure S9.1.1) and anoxia below 1.5 m. The rest of the lagoons are shallow and usually well mixed. By studying all these water bodies, we obtained a gradient from higher salinity, organic matter and nutrient values (BPI and FRA) to lower salinity values, especially G02, and lower nutrient contents (L01).

The coastal lagoons of La Pletera are characterized by the absence of continuous surface freshwater or seawater inflows. The water bodies, located behind a foredune that separates them from the Mediterranean Sea, are free from tidal influence, and water exchange occurs mainly during unpredictable sea storms or intense rainfall events, which are more frequent during the winter (Figure S9.1.2), as typical in the Mediterranean region (Àvila et al., 2019 and references herein; Quintana et al., 2018). The influence of the aquifer and groundwater circulation in the lagoons results in permanent water levels, especially during the summer, and can contribute up to 80% of the water exchange during that season, when surface exchanges are scarce (Menció et al., 2017). Thus, there is a dominant pattern of flooding episodes (winter) with long periods of confinement (summer) (Figure S9.1.1). This results in scarce surface inputs and an increase in salinity when water levels are low (Menció et al., 2017; Quintana et al., 2018). This hydrological flooding-confinement pattern drives the dynamics of nutrients, organic matter and species composition (Brucet et al., 2006; López-Flores et al., 2006a). Due to their characteristics, they have been defined as confined coastal brackish or hyperhaline lagoons (Trobajo et al., 2002) and have been classified as eutrophic systems acting as net importers of nutrients and particulate material. Moreover, as characteristic of other Mediterranean salt marshes, they are rich in phosphorus but poor in inorganic nitrogen (Badosa et al., 2006; López-Flores et al., 2014; Quintana et al., 1998).

As a consequence of salinity, water level and nutrient fluctuations, only a few euryhaline species can live in these ecosystems (Badosa et al., 2006; Brucet et al., 2005; Gascón et al., 2005). The endangered and protected fish *Aphanius iberus* (Valenciennes, 1846) finds refuge in these lagoons, which support an important population (Alcaraz et al., 2008; Alcaraz and García-Berthou, 2007). Regarding macrophytes, *Ruppia cirrhosa* (Petagna) Grande, 1918, meadows are present in the shallowest parts of several lagoons (Gesti et al., 2005). The macroalgae *Ulva intestinalis* Linnaeus, 1753, and *Chaetomorpha* Kütz., 1845, are also present sporadically in some lagoons. Reefs of the alien colonial polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) can also be abundant in some lagoons, such as FRA and G02 (Badosa et al., 2006).

During recent decades, several ecological studies have been conducted in the study area. The role of the hydrological regime was described by Menció et al. (2017) and Casamitjana et al. (2019). Nutrient dynamics were mainly described by Badosa et al. (2006), who stated that inorganic forms of nitrogen are related to water inputs during flooding events and that the internal load of phosphorus, total nitrogen and organic matter are related to the accumulation processes during confinement. Additionally, the low nitrogen/phosphorus ratios of these lagoons were explained by the loss of inorganic nitrogen by denitrification processes and circulation through the sediment to the aquifer. Phytoplankton studies have revealed that hydrological disturbances and nutrient availability control the structure and composition of the phytoplankton community, with top-down control being minimally important and mixotrophs, such as dinoflagellates, being notably present during confinement conditions (Àvila et al., 2019; López-Flores et al., 2014, 2006a). Zooplankton dynamics were also shown to be strongly affected by the hydrological pattern, registering differences between flooding conditions (calanoid copepod dominance) and confinement conditions (rotifer dominance), with no difference occurring between natural and created lagoons and size diversity being mainly related to biotic interactions (Badosa et al., 2007, 2006; Cabrera et al., 2019). Moreover, seagrass reproduction was studied by Gesti et al. (2005), who concluded that the potential reproduction of *Ruppia cirrhosa* is highly plastic as an adaptation to temporary flooding. Gispert et al. (2021, 2020) highlighted the capacity of salt marsh soil as a carbon sink.

3.2. High-frequency measurements

Measurements of different physical parameters were needed in high-frequency for metabolism estimations. Thus, in the selected lagoons MiniDOT data loggers (PME, USA) were deployed on buoys for oxygen and temperature monitoring at a depth of 30 cm approximately from the surface. Each buoy was moored at the deepest and most representative point of each lagoon. Although FRA can be 2.5 m deep, no sondes were installed in deeper waters because there is usually anoxia below 1.5 m (Compte et al., 2018). The sondes measured the oxygen concentration (mg L^{-1}), oxygen saturation (%) and temperature (°C) after every 10-min frequency. Each temperature sensor has a resolution of 0.01°C with an accuracy of 0.1°C, and the oxygen sensor has a resolution of 0.01 mg L^{-1} with an accuracy of ± 5%. The oxygen sensor calibration range is 0 to 150%, and values out of this range were not removed, so it should be considered that values exceeding 150% of oxygen may contain larger errors as we move away from the manufacturer calibration range. We considered all values below 5% saturation as anoxic conditions. Sondes were cleaned monthly to avoid the accumulation of fouling. Usually the fouling was not significant and if drifts were detected in the measurements they were very small and considered negligible (<2%). On a few occasions during the study, significant fouling was detected (in FRA or G02 lagoons), and some drifts or anomalies were detected in the data, so that period of time was discarded from the database instead of being corrected to avoid large errors. Moreover, the proper use of the sensors was controlled twice using fresh water from the laboratory at 100% and we took manual measurements (HACH HQ30d) every month for data verification.

During the first three years of the study period (from July 2015 to July 2018; Chapter I and II) no sondes were available for measuring water level, salinity, wind, and photosynthetically active radiation (PAR) in high-frequency. Conductivity, water level, and density vertical gradients were registered every 10-15 days using a CTD sonde (Aquistar CT2X). The wind speed (m s^{-1}) and solar irradiance (W m^{-2}) were measured at 3-hour and 1-hour intervals, respectively, in meteorological stations located in L'Estartit and La Tallada d'Empordà (Mas Badia), which are located 3 and 10 km away from the lagoons, respectively. Irradiance was converted into PAR (µmols m^{-2} s^{-1}) following Giling et al. (2017a) and references herein. Values of all these parameters were linearly interpolated to obtain 10-min frequency intervals. During the last year of the study period (from July 2018 to August 2019; Chapter II and III), data loggers for water level (CERA-divers), conductivity (CTD-divers), PAR (HOBOpendant), and wind (kestrel5000) were deployed for monitoring at high-frequency, and no interpolations were needed for this period. Water level and conductivity loggers were deployed in each lagoon, whereas light and wind sondes were out of the water and provided data for all lagoons. Light in lux units was converted into PAR (µmols m^{-2} s-^{1}) following Sager and McFarlane (1997). Conductivity to salinity conversions was performed according to APHA (2005). All these sensors were cleaned every month to ensure their proper functioning with manual measurements (DO, conductivity, and water level measurements), meteorological station data verification (light and wind) and the absence of drifts after cleaning. Measurements of pH were taken monthly in addition to the manual measurements, which were used for verification, with a hand-held sonde (model HACH HQ30d). It should be noted that from here on we will only refer to two seasons: summer (May to September) and winter (October to April).

3.3. Aquatic metabolism estimations

Several models have been developed in accordance with Odum's initial method to estimate metabolic rates using the diel, "free-water" changes in DO (Odum, 1956). In the present study, a modification of the BAyesian Single-station Estimation (BASE) program originally developed for stream metabolism (Giling et al., 2017a; Grace et al., 2015; Song et al., 2016) was used for its application in shallow lentic ecosystems, such as ponds and coastal lagoons. The primary equation behind the model is:

$$[DO]_{t+1} = [DO]_t + A \times PAR_t^p - ER_{\bar{T}}\ (\Theta^{T_t - \bar{T}}) - k_t \frac{(DOeq_t - DO_t)}{Z_{mix}} \pm Adv$$

Where $[DO_t]$ and $[DO_{t+1}]$ correspond to the changes in the DO concentrations between consecutive time steps. The subscript t indicates a 10-minute time interval. The GPP is expressed as $A \times PAR_t^p$, where A is a daily constant that represents the primary production per quantum unit of light (i.e., photosynthetic efficiency), PAR is the photosynthetically active radiation (400-700 nm waveband, in µmol m^{-2} s^{-1}) and the exponent p is the coefficient that reflects the ability of the primary producers to use the incident light (Grace et al., 2015). The $ER_{\bar{T}}\ (\Theta^{T_t - \bar{T}})$ term in the equation is the Arrhenius thermo-dependent respiration rate ($ER_{\bar{T}}$), where Θ is the coefficient for temperature dependence, T_t is the temperature at the corresponding time step and $\bar{T}$ is the daily mean water temperature. The expression $k_t \frac{(DOeq_t - DO_t)}{Z_{mix}}$ corresponds to the gas exchanged with the atmosphere, where k_t is the gas transfer velocity, $DOeq_t$ is the equilibrium DO concentration at a given salinity, temperature and barometric pressure, and DO_t is the measured DO concentration. The k_t was obtained from the Schmidt coefficient for oxygen in seawater by considering the water temperature (Wanninkhof, 1992), and based on wind speed equations by Cole and Caraco (1998). The Z_{mix} refers to the depth of the mixed layer. Adv represents other physical fluxes, such as advection or vertical exchange, that were considered negligible (Staehr et al., 2010a).

The barometric pressure was set by default at 1 atmosphere. The PAR was calculated from irradiance values (PAR= Irradiance × 4.6 × 0.45) (Kirk, 1994; McCree, 1981). The DO_{eq} concentrations were determined for the observed water salinity and temperature using Benson and Krause (1984) expressions. All lagoons with the exception of FRA lagoon (during winter) were usually well mixed, and Z_{mix} was equal to the water level. During winter months in FRA, Z_{mix} was calculated from density profiles every 10-15 days. Incomplete days with less than 144 total daily measurements were discarded from the database. No smoothing was applied to any of the data series.

The model estimates whole-ecosystem metabolic rates from single-station measurements in a Bayesian framework using the statistical software R (R Core Team, 2017), which invokes JAGS (Plummer, 2003) to run the Markov Chain Monte Carlo iterations (Grace et al., 2015). To ensure the greatest accuracy, the model was run with

A, p, $ER_{\bar{T}}$ and Θ as estimable daily parameters and the numbers of iterations were fixed to 40000 and 20000 for burn-in (equilibration iterations) (Grace et al., 2015). The estimable parameter distribution priors were set to Gaussian distributions with known physical limits following Grace et al. (2015). Different parameters were used to validate the fit of the model for every day included in the study. The parameter convergence was assessed using Gelman-Rubin statistics ($\hat{R}<0.1$) and the posterior predictive *p*-value (PPP; 0.1-0.9), among other tests. In addition, the models were considered good-fitting when the R^2 was higher than 0.4 (e.g., Giling et al., 2017b). Therefore, days with an $R^2<0.4$ or unconverged chains of the parameters were excluded from the posterior analyses.

The mean daily rates (mg O_2 L^{-1} day^{-1}) were calculated from the estimated parameters as follows:

$$GPP = \sum_{t=1}^{measurements} A \times PAR_t^p$$

$$ER = ER_{\bar{T}} \times 144$$

Lastly, the daily NEP was calculated as the difference between GPP and ER and the GPP:ER as the quotient between GPP and ER.

The mean daily rates were also standardized to remove temperature and PAR effects, thus yielding the potential production and respiration that were not affected by meteorological conditions but only dependent on the water composition. Thus, the GPP and ER were standardized at 20°C as follows:

$$GPP_{20} = GPP \times (\Theta^{20-\bar{T}})$$

$$ER_{20} = ER \times (\Theta^{20-\bar{T}})$$

In addition, gross primary production was standardized using the maximum irradiance value registered during the entire study period and at a temperature of 20°C. The GPP_{MAX} parameter would correspond to the specific photosynthetic rate at optimal illumination (Jassby and Platt, 1976) that has been used in a similar way (P_{max}) in many metabolic models (Hanson et al. 2008), representing the maximum production that autotrophs would have with no light limitation. GPP_{20MAX} was the gross primary production standardized to maximum light conditions and at a temperature of 20°C. For those calculations, the hours of light were set at 12 (i.e., 72 10-minute intervals per day), and we used the estimated median daily values of A, p and Θ because they did not always follow a normal distribution.

$$GPP_{MAX} = A \times maxPAR^{p} \times 72$$

$$GPP_{20MAX} = A \times maxPAR^{p} \times (\Theta^{20-\bar{T}}) \times 72$$

3.4. Nutrient analyses

Water samples for inorganic nutrient analyses were filtered in the field through precombusted (450°C for 4 h) Whatman GF/F filters (0.7 µm pore) and frozen until analysis. The ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) were analysed following the APHA (2005) and the phosphate was measured according to UNE-EN-ISO6878. Unfiltered water samples were also frozen for the analysis of total nitrogen (TN), total phosphorus (TP) and total organic carbon (TOC). The TOC and TN were measured using a TOC analyser (TOC-V CSH SHIMADZU). The TP analyses were performed as described in Grasshoff et al. (2007). Organic forms of nitrogen (N_{org}) and phosphorus (P_{org}) were calculated by taking the difference between TN or TP and the sum of the inorganic forms. The dissolved inorganic nitrogen (DIN) was calculated as the sum of the NH_4^+, NO_2^-, and NO_3^-.

3.5. Pigment analyses

Chlorophyll-*a* concentrations (Chapter I, II and III) along with several diagnostic pigments (Chapter III) were estimated using a high-performance liquid chromatographic (HPLC) method based on a reversed-phase C_8 column and pyridine-containing mobile phases (López-Flores et al., 2006a; Zapata et al., 2000). A variable volume depending on algae density (60-900 mL) was filtered through precombusted (450°C for 4 h) Whatman GF/F filters (0.7 µm pore) with gentle vacuum and the resulting filter was frozen until analysis. For pigment extraction those filters were introduced in Pyrex tubes with 5 mL of acetone 99% and preserved at -20°C for 24 hours. The resulting slurry was filtered with Whatman syringe filters (0.2 µm pore size). Just before analysis, a volume of 600 µL of the extract was mixed with 150 µL of double distilled water to avoid distorsion of earlier eluting peaks (Zapata and Garrido, 1991). All procedures were done protecting samples from light. We used a Waters HPLC with a Waters 996 Photodiode Array Detector.

Chlorophylls and carotenoids were detected by diode array spectroscopy (350–700 nm). The use of standards pigments allowed pigment identification by the retention times and the absorbance spectra. The concentration was estimated after doing a calibration curve for each pigment. The selection of the diagnostic pigments used for taxonomic groups identification was based on Jeffrey et al. (1997), and all of them are summarised in Table 3.5.1.

Table 3.5.1. Distribution of the different diagnostic pigments used in Chapter III in the phytoplankton groups identified. Table adapted from Jeffrey et al. (1997). Code ● = major pigment (>10%); • = minor pigment (1-10%); · = trace pigment (<1%) of the total chlorophyll and carotenoids. Divinyl - Chlorophyll-*a* and Prasinoxanthin were not detected indicating absence of Prochlorophytes and Prasinoxanthin-containing Prasinophytes (e.g., *Ostreococcus*), respectively.

Phytoplankton groups Pigments	Cyanobacteria	Cryptophytes	Chlorophytes	Euglenophytes	Diatoms	Dinoflagellates	Haptophytes
Chlorophyll-*a*	●	●	●	●	●	●	●
Chlorophyll-*b*			●	●			
Divinyl - Chlorophyll-*a*							
Prasinoxanthin							
Lutein			●				
Zeaxanthin	●		•				
Alloxanthin		●					
Fucoxanthin					●		●
19'-hex-fucoxanthin							●
Chlorophyll-*c3*							●
Peridinin						●	

4. RESULTS

This section constitutes the main body of the thesis and contains the three main chapters of the thesis that were structured to address all the specific objectives described in 'Objectives'. The supplementary materials for the three chapters are provided in the 'Supplementary material' section at the end of the manuscript.

4.1. CHAPTER I

Ecosystem metabolism dynamics and environmental drivers in Mediterranean confined coastal lagoons

This is a version of the peer reviewed article:

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4.1.1. SUMMARY

Aquatic metabolism is an important descriptor of ecosystem functioning. The metabolism of ponds and confined coastal lagoons has been poorly studied in comparison to other coastal systems, in which the metabolic dynamics are better understood. In this study, we described the ecosystem metabolism of two confined Mediterranean coastal lagoons located in La Pletera salt marsh (NE Iberian Peninsula), which is dominated by flooding-confinement patterns. We estimated the metabolic rates by applying Bayesian models to three years of high-frequency open water oxygen data. Our aim was to test if nutrients and other environmental variables (the temperature, conductivity, light and water level) that registered as important drivers of metabolism in the literature were the primary drivers of metabolic variation in confined coastal water bodies. We observed clear seasonal patterns in the metabolic rates, with extremely high oxygen variability during the summer season ranging from supersaturation (saturations >200% were recorded) to anoxia (<5%). Despite the high rates of gross primary production registered during the summer, periods of anoxia could prevail for several days during that season. Thus, although the aerobic gross primary production and ecosystem respiration were quite balanced in the lagoons during the study period, these lagoons are probably more heterotrophic since their anaerobic respiration has not been estimated. Because the studied lagoons are rich in nutrients, we expected a low response in the metabolic rates to nutrient increases, since the physiological response of primary producers to nutrient loading is usually low in nutrient-saturated ecosystems; our results supported this hypothesis. The temperature was the primary driver, highlighting the importance of seasonality in these highly productive ecosystems. Our results also showed an uncoupling between the metabolic rates, which were higher in the summer, and the standardized ones, after removing the temperature and irradiance effects, which were higher in the winter and negatively related to the conductivity. This finding suggests that potential productivity (standardized rates) is more sensitive to winter inputs and, in contrast, the actual productivity is more related to summer concentration processes due to confinement.

4.1.2. INTRODUCTION

Coastal lagoons are dynamic water bodies located between inland and marine systems, and they rank among the most productive ecosystems on the planet (Barnes, 1980). These ecosystems rarely exceed a few metres in depth, and they present high levels of primary production and offer nursery, refuge and feeding habitats for a variety of estuarine, freshwater and marine species (Duarte et al., 2002; Giordano et al., 2012). They also play an important role in the biogeochemical cycling of pollutants, nutrients and organic matter as well as in coastal defence in the face of erosion (Brito et al., 2012a; Costanza et al., 1997; Kennish and Paerl, 2010; Kingsford et al., 2016). Occupying ~13% of global coasts and 5.3% of the European coast, they also provide exceptional recreational and commercial value (de Eyto et al., 2019; Pérez-Ruzafa et al., 2011b). However, the co-occurrence of natural and human disturbances (climate change, changes in human land use, and pollutants) makes them among the most heavily impacted ecosystems on earth (Arévalo et al., 2013; Kjerfve, 1994; Morant et al., 2020). They were included in the EU Water Framework Directive (Directive2000/60/EC) for protection as transitional waters, that is, bodies of surface water located close to a river mouth and influenced by freshwater and coastal waters.

Quantifying metabolic rates can help us to understand the energy turnover, nutrient cycling, trophic status and food web dynamics in aquatic systems (Holtgrieve et al., 2010). Over the last century, several methods have been developed for calculating the gross primary production (GPP), ecosystem respiration (ER) and net ecosystem production (NEP) in aquatic ecosystems and have been applied to a wide range of habitat types (e.g., rivers, lakes, estuaries, and open ocean) (Staehr et al., 2012a). Although no unique methodology has emerged as completely satisfactory, the diel oxygen open-water technique proposed by Odum (1956) provides a useful alternative to bottle and chamber incubations because it addresses aquatic metabolism directly at the ecosystem scale (Staehr et al., 2012a, 2010a). The development of reliable oxygen sensors has led to an increase in the use of aquatic metabolism measurements as an integrative indicator of ecosystem functioning worldwide (e.g., Hanson et al. 2008; Obrador and Pretus, 2013; Winslow et al., 2016). Many studies have been published in recent decades about the metabolism of ponds or coastal lagoons (e.g., Carmouze et al., 1991; Delgadillo-Hinojosa et al., 2008; Giordano et al., 2012; Howarth et al., 2014; McGlathery et al., 2001; Thébault et al., 2008). As far as we know, there are only a few studies using high-frequency, long-term datasets on oxygen concentrations. New modelling approaches for estimating metabolic rates that have been used for rivers and deep lakes (Fernández Castro et al., 2021; Grace et al., 2015; Hanson et al., 2008; Winslow et al., 2016) have yet to be fully deployed in the study of coastal lagoons.

Coastal lagoons have a wide range of morphological, geological and hydrological characteristics (Basset et al., 2013; Guelorget and Perthuisot, 1983; Kennish and Paerl, 2010; Pérez-Ruzafa et al., 2005; Roselli et al., 2013) that are shaped by the varying influence of local climate conditions, marine tides and freshwater inputs (Nidzieko et al., 2014). All these factors determine the nutrient inputs that, in turn, are one of the primary drivers of aquatic metabolism. These dynamic ecosystems, with their well-irradiated water columns and high nutrient loading, have high levels of primary production (Duarte et al., 2002). However, a certain slowing of the physiological response of primary producers to nutrient inputs might take place in nutrient-saturated ecosystems, so that additional inputs to these eutrophic systems may not necessarily cause a significant increase in productivity as they would in oligotrophic systems. For this reason, the response of the metabolism to nutrient inputs might be stronger under oligotrophic conditions, whereas a sharp attenuation of this response is predicted in nutrient-rich conditions (Glibert et al., 2010).

In confined coastal lagoons, the surface connections, with their connected freshwater and marine ecosystems, can be restricted to a few days per year (Badosa et al., 2006; López-Flores et al., 2006a; Quintana et al., 1998). These shallow coastal lagoons are found throughout the Mediterranean Sea, which has a very small tidal range (e.g., the average tidal range along the NE Catalan coast is 17 cm (1990-2017, Estartit meteorological station 0385J, AEMET)). Water exchanges are therefore predominantly the result of catchment floods and sea storms, rather than regular tidal refreshment. In these ecosystems, the primary water inputs are limited to those of subsurface or groundwater origin, especially during the winter, but that may account for 15–80% of the water in the lagoons during the summer (Casamitjana et al., 2019; Menció et al., 2017). During the confinement periods, when exchanges with coastal waters and inputs from flooding events are rare, the water level decreases while the salinity increases, along with the organic matter and nutrient accumulation, reaching concentrations similar to those found in eutrophic waters (Àvila et al., 2019; Badosa et al., 2006; López-Flores et al., 2006a; Quintana et al., 1998; Vollenweider and Kerekes, 1982). Therefore, this kind of ecosystem might provide the ideal sites to check for the existence of a saturation-type response in the GPP to nutrient concentrations.

In this study, we measured the metabolism of two confined coastal lagoons (FRA and G02), where long periods of confinement result in high levels of nutrients, organic matter and chlorophyll-*a,* especially during the summer season (Badosa et al., 2006; Quintana et al., 2018). Our first objective was to determine the seasonal metabolic dynamics by quantifying the aquatic metabolism from the change of high-frequency DO concentrations during three hydrological years. The second objective was to identify the primary drivers of primary production in these confined coastal water bodies and to ascertain the influence of the nutrient concentrations and other environmental parameters (temperature, conductivity, light and water level) on the annual metabolism. We hypothesized that increases in nutrient concentrations would not cause a significant response in the metabolism in these ecosystems, suggesting the existence of a saturation-type response of the GPP to the nutrient concentration. Lastly, we discussed the challenges involved in measuring of aquatic metabolism in such productive and highly dynamic ecosystems.

4.1.3. METHODS

4.1.3.1. Sampling and analyses procedure

The study was conducted during three hydrological years from July 2015 to July 2018 (hereafter: 2015/2016, 2016/2017, and 2017/2018) in two different lagoons of La Pletera salt marsh (Figure 3.1.1). From the two lagoons selected, Fra Ramon lagoon (FRA) is a natural lagoon, and G02 lagoon was created in 2002 as part of the LIFE Nature restoration program described in section 3.1 (LIFE99NAT/E/006386). Both are small (Table S9.1.1; Figure 3.1.1), permanent and shallow, free from tidal influences, and with high variability in the environmental parameters summarized in Tables S9.1.1 and S9.1.2 (see also Badosa et al. (2006); López-Flores et al. (2006a); Quintana et al. (2018)). More information about the study site and the lagoons characteristics can be found in section 3.1. DO and temperature measurements every 10 minutes were registered by one optical DO sonde (MiniDOT) deployed in each lagoon. More information about sondes deployment and monitoring of water levels, conductivity, density vertical profiles, wind speed and PAR can be consulted in sections 3.1 and 3.2. Additionally, nutrients, chlorophyll-*a* and other environmental parameters were analysed monthly as described in sections 3.2, 3.4 and 3.5. Aquatic metabolism estimations for both lagoons were computed using a modification of the BAyesian Single-station Estimation (BASE) model described in section 3.3. All details about the model functioning, model selection and standardized metabolic rates calculation are provided in that section.

4.1.3.2. Data analyses

To characterize the days with anoxia, a MANOVA analysis was performed, with the temperature, wind and PAR as dependent variables. The explanatory variable was 'anoxia' (1= day with anoxic conditions; 0= day without anoxia) that was included as a factor. We calculated the monthly metabolic rates (hereafter, GPP_m and ER_m) to have the same frequency for the different variables, since nutrient concentrations were measured monthly. We used the median values because the daily metabolic rates were skewed. The relationship between the physical and chemical parameters and the metabolic rates was tested through Spearman correlations. To avoid collinearity among explanatory variables in posterior analyses, the *r* values were used to select them (r<0.7 according to Dormann et al. (2013)) as well as the variance inflation factor (VIF<3) (Zuur et al., 2009). The relative importance of nutrients (DIN, PO_4^{3-}, TOC, $N_{org,}$ P_{org}) and some physical variables (the temperature, conductivity, light and water level) to the monthly metabolic rates (nonstandardized and standardized) was assessed using general additive mixed models (GAMMs). GAMMs were applied with cubic regression splines and Gaussian distributions using the mgcv package in R (Wood, 2011). For all the models, each metabolic rate was included as a dependent variable, and the potential physical and chemical drivers were log-transformed and included as explanatory variables. The optimum model was found to exclude nonsignificant terms through the stepwise backward method. We tested all the models for violations of assumptions of normality, homogeneity and independence. When the homogeneity assumption was breached, different variance structures were applied to the model and then compared using Akaike information criterion (AIC) values following Zuur et al. (2009). We tested the independence of the residuals in the optimum model using an autocorrelation function plot of the residuals. We also applied the same data analysis using different nutrient log-ratios as dependent variables, namely TN:TP, DIN:N_{org}, PO_4^{3-}:P_{org} and DIN: PO_4^{3-}. For all the data analyses, the significance level was set to a $p<0.05$ and all were performed using R (R Core Team, 2017).

4.1.4. RESULTS

High values were found for the temperature, conductivity, chlorophyll-*a* and nutrient concentrations (Figures S9.1.3 and S9.1.4), primarily when the water level was low and coinciding with the summer periods (Figure S9.1.4). Seasonal differences were also observed for the range of variation in DO. During the summer, the oxygen saturation ranged from 0% to >200% over a 24-hour period, and in winter, it ranged from 50% to <150% (Figure 4.1.1). Despite the high DO saturation values, full days with anoxia (oxygen saturation <5%, see '3.2. High-frequency measurements' section) also occurred in the summer months, and it occasionally lasted for several days (Figure 4.1.2). Out of a total of 1057 and 1005 days processed for FRA and G02, respectively, 75 and 72 days showed anoxic conditions. Anoxia occurred primarily during the summer season, and 59% (FRA) and 75% (G02) of those days were discarded by the model of GPP and ER computations mostly because they had $R^2<0.4$. An example of a discarded day due to anoxia with a poor fit ($R^2<0.4$) is shown in Figure S9.1.5. However, some of the days with anoxic conditions displayed good model fit ($R^2>0.4$ and good convergence of the parameters). During those days, the annual minimum GPP rates were recorded (0.002 mg O_2 L^{-1} d^{-1}) (Figure S9.1.6). The MANOVA test revealed that days with anoxic conditions were characterized by higher temperatures ($F_{1,1944}$= 71.79, $p<0.01$) and higher PAR values ($F_{1,1944}$= 13.40, $p<0.01$), but the wind had no effect ($F_{1,1944}$= 0.04, p= 0.85) (Figure S9.1.7).

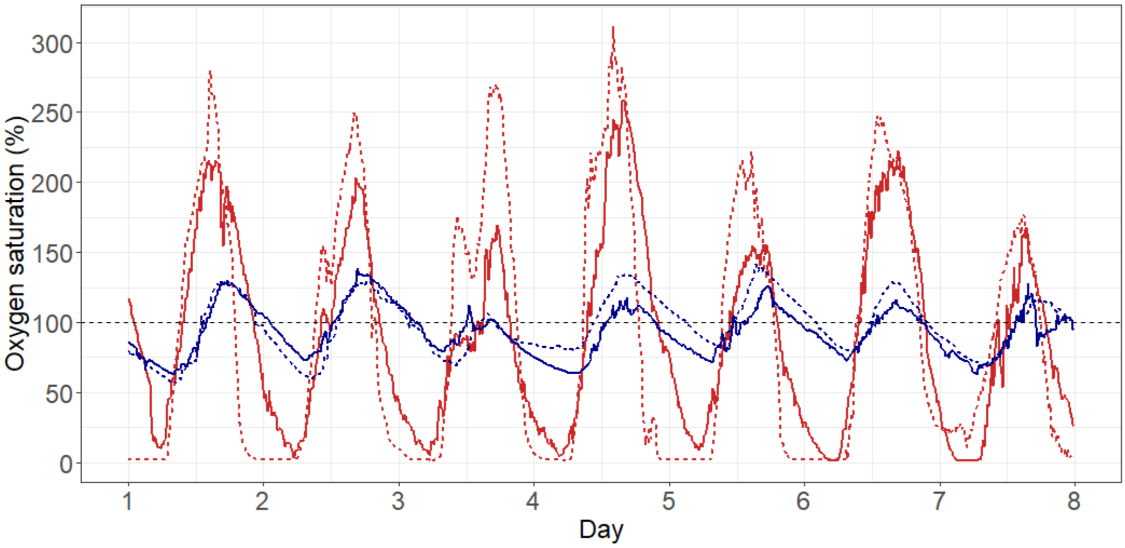


Figure 4.1.1. Example of the seasonal changes in oxygen saturation dynamics over 8 days. The winter period (in blue) ranged from the 1st to 7th February 2016 and the summer period (in red) from the 19th to 25th July 2017. FRA and G02 are represented by continuous and dashed lines, respectively.

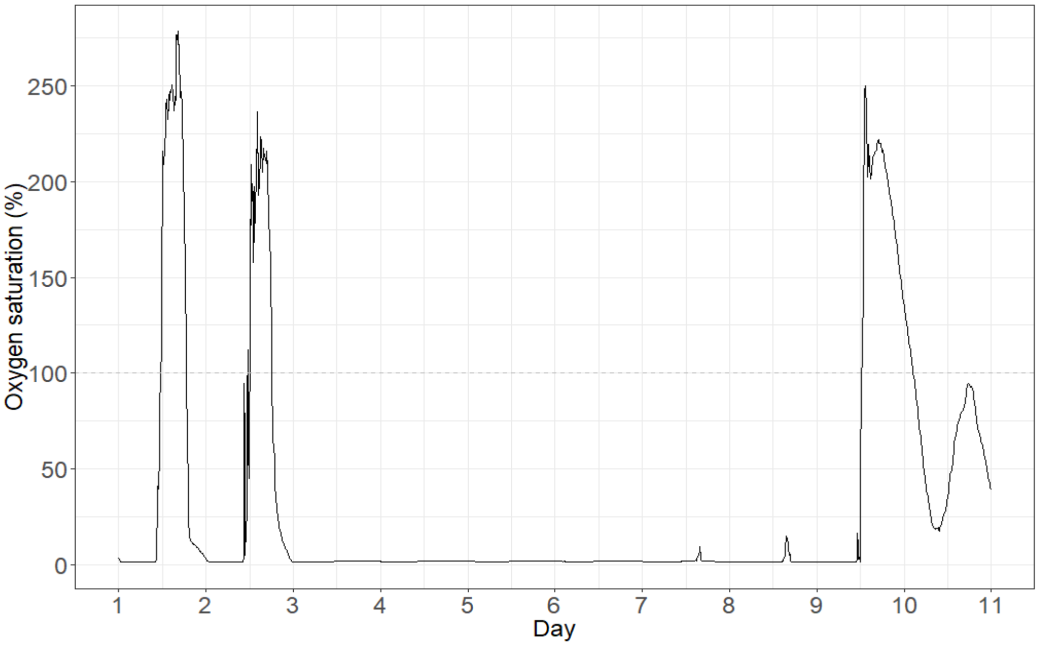


Figure 4.1.2. Variation in oxygen saturation (%) during an anoxic event lasting 6 days. The values correspond to the FRA lagoon from 26th August to 4th September 2015.

A total of 593 and 558 daily ecosystem metabolism rates were selected after modelling (see '3.3. Aquatic metabolism estimations' section) for FRA and G02, respectively. They represented 54 and 51 % of the total sampling days. Two examples of days with good and poor fit and the convergence of the parameters are shown in Figures S9.1.8 and S9.1.9, respectively. Both lagoons showed similar temporal patterns in their metabolic rates during the three years of the study period. Despite some variability, a clear seasonal pattern emerged (Figure 4.1.3). The GPP and ER increased around the spring, reaching the highest values in the summer (maximum GPP and ER of 71.88 and 62.06 mg O_2 L^{-1} d^{-1}, respectively). Both rates declined in autumn and winter (with GPP and ER rates rarely exceeding 30 mg O_2 L^{-1} d^{-1}) and experienced lower variability during this period. The seasonal pattern observed in the daily GPP and ER was not observed in the ln(GPP:ER) dynamics (Figure 4.1.4). Many ln(GPP:ER) values were close to zero with occasional sharp deviations, either positive or negative, with maximum and minimum values of 4.93 and -7.46, respectively.

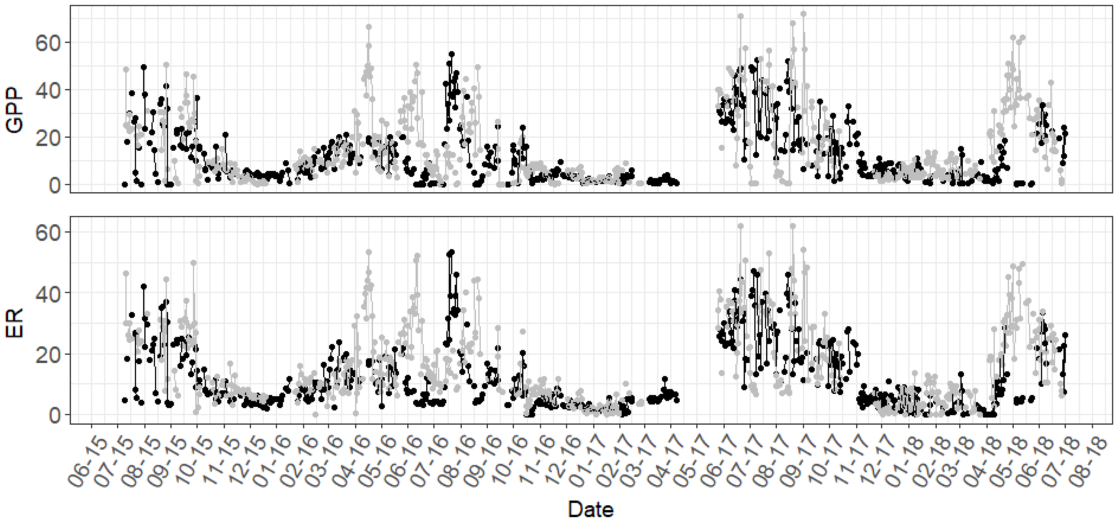


Figure 4.1.3. Daily gross primary production (GPP) and ecosystem respiration (ER) during the study period in the studied lagoons (FRA in black, and G02 in grey). Metabolic rates are represented in mg O2 L-1 d-1. The X-axis labels indicate the month-year.

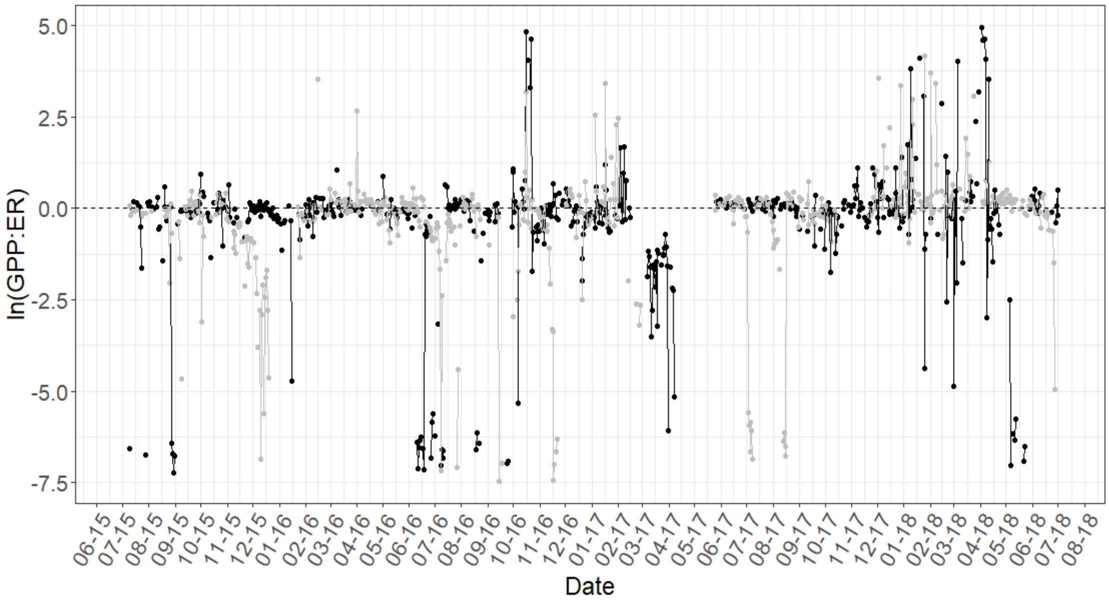


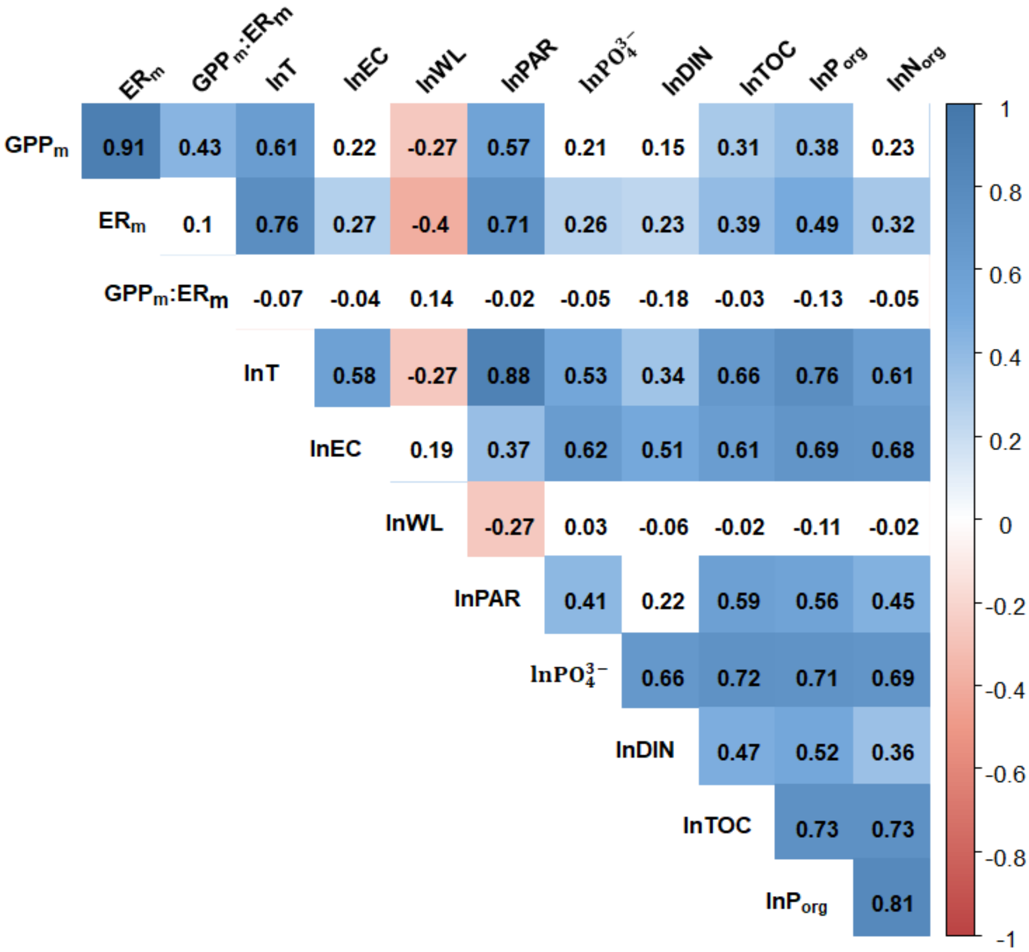
Figure 4.1.4. Daily ln(GPP:ER) ratio during the study period for both lagoons (FRA represented in black, and G02 in grey). The X-axis labels indicate the month-year.

Higher annual GPP and ER values were recorded in G02 than FRA for all hydrological years, with higher interannual variability (Table 4.1.1). During the second hydrological year (2016/2017), the rates were lower for both lagoons. Overall, in both lagoons the GPP was slightly lower than the ER (NEP<0 and GPP:ER<1), but the median GPP:ER was close to 1, indicating a balance between both rates (Table 4.1.1). The only year with GPP higher than ER values (NEP>0 and GPP:ER>1) was 2017/2018 in G02.

Table 4.1.1. Median values of daily ecosystem metabolism parameters for the three hydrological years studied here (from July 2015 to July 2018). The metabolic rates are expressed in mg O_2 L^{-1} d^{-1}. Note that the median values are provided instead of the mean values because the data were not normally distributed. The 5th and 95th percentiles are shown in brackets. GPP: gross primary production; ER: ecosystem respiration; and NEP: net ecosystem production.

	GPP	ER	NEP	GPP:ER
FRA				
2015/ 2016	6.91 (0.006-30.50)	7.97 (3.06-25.30)	-0.86 (-4.39-4.44)	0.92 (0.0014-1.34)
2016/ 2017	3.53 (0.03-45.06)	5.05 (0.70-38.88)	-0.20 (-4.66-7.01)	0.94 (0.0049-2.60)
2017/ 2018	7.94 (0.06-38.94)	8.42 (0.13-34.58)	-0.29 (-5.37-6.46)	0.98 (0.074-21.55)
G02				
2015/ 2016	11.65 (0.35-46.30)	13.09 (4.69-41.73)	-1.62 (-7.05-6.73)	0.91 (0.063-1.48)
2016/ 2017	5.68 (0.01-45.15)	8.04 (0.44-38.89)	-0.47 (-8.45-8.71)	0.90 (0.0013-2.24)
2017/ 2018	10.29 (1.41-51.23)	11.15 (0.43-46.27)	0.97 (-6.64-12.90)	1.10 (0.26-6.43)

The Spearman correlations revealed a significant coupling between the monthly metabolic rates, GPP_m and ER_m (r= 0.91; Figure 4.1.5). High positive correlations were also observed between both GPP_m and ER_m with the temperature and PAR, but both GPP_m and ER_m, showed significant negative relationships with the water level. No significant correlation was found between the GPP_m:ER_m quotient and the physical and chemical variables analysed here. All environmental variables except the water level were positively correlated with the water temperature and conductivity, suggesting a seasonal pattern in water conditions. There was a high correlation between the PAR and P_{org} with the temperature, and the N_{org} and PO_4^{3-} with the TOC (r>0.7; Figure 4.1.5). Therefore, the PAR, P_{org} and TOC were not included in posterior analyses to avoid collinearity in the explanatory variables.



	ER_m	GPP_m:ER_m	lnT	lnEC	lnWL	lnPAR	$lnPO_4^{3-}$	lnDIN	lnTOC	lnP_{org}	lnN_{org}
GPP_m	0.91	0.43	0.61	0.22	-0.27	0.57	0.21	0.15	0.31	0.38	0.23
ER_m		0.1	0.76	0.27	-0.4	0.71	0.26	0.23	0.39	0.49	0.32
GPP_m:ER_m			-0.07	-0.04	0.14	-0.02	-0.05	-0.18	-0.03	-0.13	-0.05
lnT				0.58	-0.27	0.88	0.53	0.34	0.66	0.76	0.61
lnEC					0.19	0.37	0.62	0.51	0.61	0.69	0.68
lnWL						-0.27	0.03	-0.06	-0.02	-0.11	-0.02
lnPAR							0.41	0.22	0.59	0.56	0.45
$lnPO_4^{3-}$								0.66	0.72	0.71	0.69
lnDIN									0.47	0.52	0.36
lnTOC										0.73	0.73
lnP_{org}											0.81

Figure 4.1.5. Spearman correlation coefficients for the monthly metabolic rates, GPP_m (gross primary production), ER_m (ecosystem respiration), the GPP_m:ER_m quotient, and temperature (T), photosynthetically active radiation (PAR), electrical conductivity (EC), water level (WL), dissolved inorganic carbon (DIN), phosphate (PO_4^{3-}), total organic carbon (TOC), organic nitrogen (N_{org}) and organic phosphorus (P_{org}). Significance is indicated by coloured squares. Blue and red colours indicate positive and negative correlations, respectively. High absolute values of correlation are represented by dark colours and low values are indicated by light colours.

The GAMM results showed no significant relationship between the nutrients and metabolic rates (Table 4.1.2). The GPP_m was only significantly related to the temperature while the ER_m was positively related to the temperature and negatively related to the water level, with a significant difference between lagoons due to differences in the depths (Figure 4.1.6). The GPP:ER did not show a significant relationship with any of the physical and chemical variables included in the model. GAMMs including the log-ratios of nutrients as explanatory variables (TN:TP, DIN:N_{org}, PO_4^{3-}:P_{org} and DIN: PO_4^{3-}) did not reveal any significant relationship to any of the metabolic rates.

Table 4.1.2. Results of the generalized additive mixed model analysis (GAMMs) performed to identify the primary drivers contributing to metabolic variations in the study lagoons (FRA and G02). The response variables were the monthly non- and standardized metabolic rates, represented by GPP$_{m}$ (gross primary production), ER$_{m}$ (ecosystem respiration), GPP$_{m20}$ (gross primary production standardized to 20°C), GPP$_{m20MAX}$ (gross primary production standardized to 20°C and to the maximum value of irradiance registered during the study period) and ER$_{m20}$ (ecosystem respiration standardized to 20°C). The GPP$_{m}$:ER$_{m}$ ratio was also included in the analysis as a response variable but none of the explanatory variables showed significant results. The table simply shows the significant explanatory variables resulting from the backward selection procedure. (s) refers to the scaled smoother for each significant explanatory variable; 'edf' is estimated degrees of freedom; AIC: Akaike information criterion; and R^2= adjusted R-squared. T: temperature; WL: water level; EC: electrical conductivity; and PO$_4^{3-}$: phosphate. The number of observations was 72 for all the models.

Response variable: GPP$_{m}$			AIC= 487	R^2= 0.32
	Estimate	**Standard error**	**t value**	**Pr (>|t|)**
Intercept	11.92	1.03	11.64	<0.01
Approximate significance of smooth terms	**edf**		F	*p*
s(lnT)	2.53		22.05	<0.01
Response variable: ER$_{m}$			AIC= 454	R^2= 0.53
	Estimate	**Standard error**	**t value**	**Pr (>|t|)**
Intercept	14.71	1.22	12.11	<0.01
factor (lagoon) G02	-3.97	1.92	-2.07	0.04
Approximate significance of smooth terms	**edf**		F	*p*
s(lnT)	2.50		28.46	<0.01
s(lnWL)	1.00		8.47	<0.01
Response variable: GPP$_{m20}$			AIC= 618	R^2= 0.12
	Estimate	**Standard error**	**t value**	**Pr (>|t|)**
Intercept	16.65	2.21	7.54	<0.01
Approximate significance of smooth terms	**edf**		F	*p*
s(lnEC)	1		12.73	<0.01
Response variable: GPP$_{m20MAX}$			AIC= 702	R^2= 0.22
	Estimate	**Standard error**	**t value**	**Pr (>|t|)**
Intercept	8.88	7.83	1.13	0.26
factor (lagoon) G02	37.81	15.77	2.40	0.02
Approximate significance of smooth terms	**edf**		F	*p*
s(lnPO$_4^{3-}$)	1.36		4.71	0.04
s(lnWL)	2.80		3.25	0.03
Response variable: ER$_{m20}$			AIC= 595	R^2= 0.19
	Estimate	**Standard error**	**t value**	**Pr (>|t|)**
Intercept	17.43	1.78	9.80	<0.01
Approximate significance of smooth terms	**edf**		F	*p*
s(lnEC)	1		18.41	<0.01

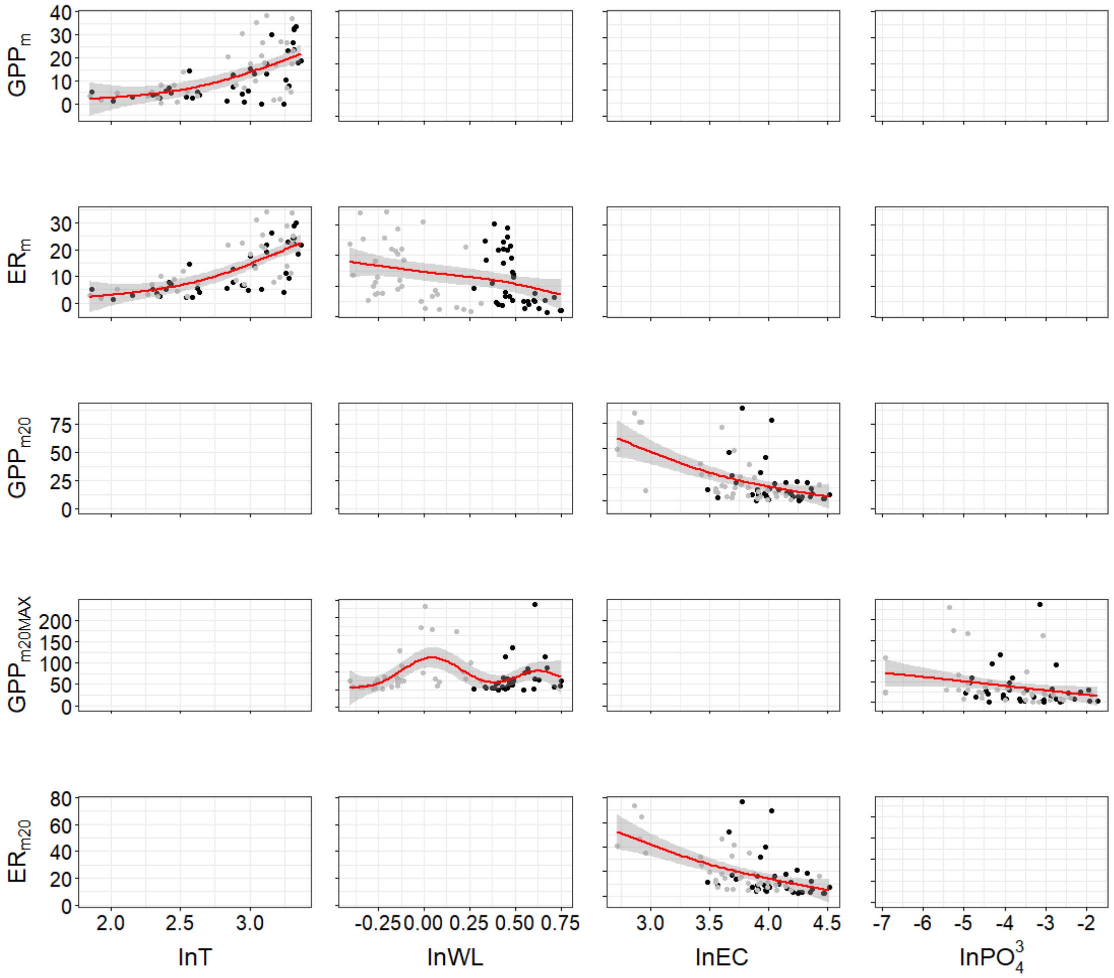


Figure 4.1.6. Selection of significant smoothers for the contribution of explanatory variables (physical and chemical variables) for the optimal generalized additive mixed model (GAMMs) that explains the variation for each metabolic rate (dependent variable). The red line is the smoother and the grey shaded area shows the 95th confidence bands. FRA lagoon is represented in black, and G02 lagoon in grey. Nonsignificant relationships are not shown. T: temperature; WL: water level; EC: electrical conductivity; and PO_4^{3-}: phosphate. The monthly metabolic rates are represented by GPP_m: gross primary production and ER_m: ecosystem respiration, and the monthly standardized rates by GPP_{m20}: gross primary production standardized to 20°C; GPP_{m20MAX}: gross primary production standardized to 20°C and to the maximum value of irradiance registered during the study period; and ER_{m20}: ecosystem respiration standardized to 20°C.

The temperature-standardized rate (i.e., GPP_{20}, GPP_{20MAX} and ER_{20}) dynamics differed from the patterns of nonstandardized ones (Figure 4.1.7). The maximum values for the GPP_{20} and ER_{20} (480.4 and 319.8 mg O_2 L^{-1} d^{-1}, respectively) were observed in the autumn and winter. In addition, the GPP_{20MAX}, which represented the autotroph productivity at 20℃ under maximum light conditions, showed the maximum value (1166.8 mg O_2 L^{-1} d^{-1}) during the winter time. The conductivity arose as the primary factor, with a significant negative relation to the GPP_{20} and ER_{20} (Table 4.1.2; Figure 4.1.6). The model that included GPP_{20MAX} as a response variable revealed a significant positive relationship to the water level and significant differences for both lagoons due to the differences in the depths (Table 4.1.2; Figure 4.1.6). Again, no significant influence from the nutrient concentration on the standardized rates was found, except for PO_4^{3-} with the GPP_{20MAX}, but this relationship was negative (Table 4.1.2).

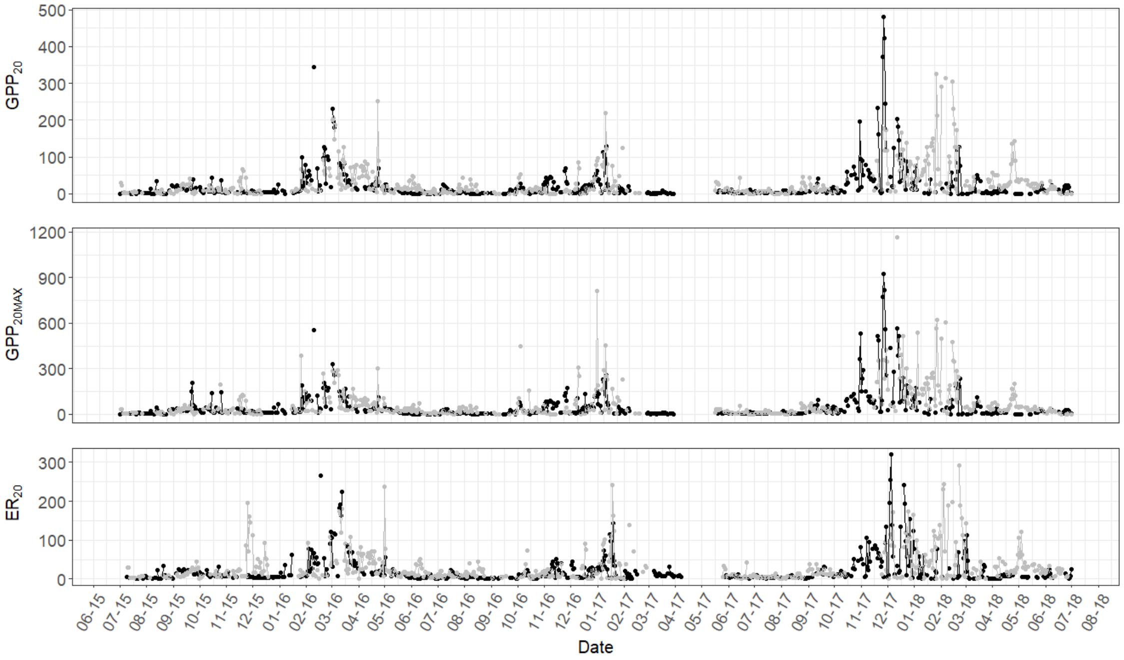


Figure 4.1.7. Daily standardized rates for GPP_{20}, GPP_{20MAX} and ER_{20} during the study period for both lagoons (FRA in black, and G02 in grey). GPP_{20}: gross primary production standardized to 20°C; GPP_{20MAX}: gross primary production standardized to 20°C and to the maximum value of irradiance registered during the study period; and ER_{20}: ecosystem respiration standardized to 20°C. Metabolic rates are represented in mg O_2 L^{-1} d^{-1}. X-axis labels indicate the month-year.

4.1.5. DISCUSSION

The metabolic rates measured in La Pletera are similar in both water bodies studied here and higher than the values reported in other coastal lagoons (Table 4.1.3). When comparing La Pletera lagoons with other types of aquatic systems, we also find higher rates relative to estuaries (Caffrey, 2004, 2003; D'Avanzo et al., 1996; Russell and Montagna, 2007; Thébault et al., 2008) or lakes (Giling et al., 2017a; Staehr et al., 2010a; Staehr and Sand-Jensen, 2007), even the ones in eutrophic and hypereutrophic states (Table 4.1.3). The maximum values for estuaries (28.10 g O_2 m^{-2} d^{-1}; Table 4.1.3) are close to the ones obtained in La Pletera, although they are not the usual values for that type of ecosystem (average value of 7.70 g O_2 m^{-2} d^{-1} for estuaries according to Caffrey (2004)).

Table 4.1.3. Ecosystem metabolism estimations in similar ecosystems. The values are expressed in g O_2 m^{-2} d^{-1}. GPP: gross primary production and ER: ecosystem respiration. The values were converted from carbon units to oxygen in a ratio of 1 except for [a] (photosynthetic quotient= 1.2; respiratory quotient= 1). DO: dissolved oxygen.

Location	Time period studied	GPP	ER	Method	References
FRA, La Pletera, Spain	Annual average range	13.03-14.09	12.84-14.90	Open water (DO)	This study*
G02, La Pletera, Spain	Annual average range	12.05-17.00	13.04-15.70	Open water (DO)	This study*
FRA, La Pletera, Spain	Summer (June-September) average range	19.00-34.64	21.16-32.15	Open water (DO)	This study*
G02, La Pletera, Spain	Summer (June-September) average range	15.82-18.63	16.63-18.69	Open water (DO)	This study*
Ria Formosa, Portugal	Summer (July) average	1.38	0.99	Incubation	Santos et al., 2004
Lower Laguna Madre, Texas, USA	Annual range	2.15-14.10[a]	3.30-12.20[a]	Open water (DO)	Ziegler and Benner, 1998
Ninigret pond, Rhode Island, USA	Summer (August) average	5.40	6.10	Open water (DO)	Nixon and Oviatt, 1972
Saquarema lagoon, Brazil	Annual average	3.40	3.30	Open water (CO_2)	Carmouze et al., 1991
West Falmouth Harbor, Cape Cod, USA	Summer (July-August) average range	4.80-16.00	6.40-17.60	Open water (DO)	Howarth et al., 2014
Everglades peatland, USA	Annual average	3.30	7.04	Open water (DO)	Hagerthey et al., 2010
Albufera des Grau, Balearic Islands, Spain	Summer (July-August) average range	1.40-8.90	3.70-17.00	Open water (DO)	Obrador and Pretus, 2013
Estuaries included in National Estuarine Research Reserves, USA	Annual average range	2.30-28.10	4.40-32.30	Open water (DO)	Caffrey, 2004
Shallow lakes, Northern Highland Lake District, Wisconsin, USA	Summer (3-5 days) average	6.03	6.00	Open water (DO)	Lauster et al., 2006

We found higher GPP and ER values during the summer period, consistent with studies of Ziegler and Benner (1998) in a subtropical seagrass-dominated lagoon and Hagerthey et al. (2010) in the Everglades. Similar studies developed in the Mediterranean climate (Obrador and Pretus, 2012) also reported these seasonal patterns. Similar seasonal patterns in the GPP and ER were described for freshwater shallow lakes (Laas et al., 2012; Staehr and Sand-Jensen, 2007). The seasonal pattern found in the metabolic rates of La Pletera lagoons could be explained by the flooding-confinement dynamics dominating the salt marsh. Confined coastal lagoons have water inputs that are limited to a few days per year and scarce or absent during the summer, when we found higher metabolic rates. During that season, the water level decreases and results in a strong concentration effect (Casamitjana et al., 2019; Menció et al., 2017; Quintana et al., 2018), leading to high conductivity, total nutrients, organic matter and chlorophyll-*a*. These dynamics have already been reported in previous studies in the area and in other nearby Mediterranean coastal systems (e.g., Badosa et al., 2006; Cabrera et al., 2019; Menció et al., 2017; Quintana, 2002).

The small size, low water level and the presence of *Ruppia cirrhosa* and macroalgae in littoral areas might also contribute to the high GPP and ER rates found in La Pletera. Both the minimum and maximum metabolic rates were recorded during the summer time, because of the differences in the observed diel oxygen saturation dynamics. During summer, the oxygen concentration can range from anoxia (less than 5%) at night to supersaturation (more than 200%) during daylight. These supersaturation and hypoxia conditions are rarer in the winter. Large and pulsed oxygen oscillations varying from supersaturation to anoxia on both seasonal and diurnal time scales have already been described in similar ecosystems (Obrador and Pretus, 2013; Viaroli and Christian, 2003). Similar oxygen patterns have also been reported in other eutrophic coastal ecosystems (Beck and Bruland, 2000; Hull et al., 2008; Shen et al., 2008; Ziegler and Benner, 1998), especially anoxic conditions in deep waters as a result of sinking organic matter due to high rates of surface GPP (Kemp and Testa, 2011; Rabalais et al., 2009) or to the metabolic activity of submerged vegetation in macrophyte-rich lagoons (Camacho et al., 2016; Obrador and Pretus, 2013).

Stable anoxia was recorded for consecutive days in La Pletera lagoons, even at shallow depths (30 cm from the surface). These situations typically correspond to days with high temperatures and irradiance. In some systems, anoxia near the surface can result from the upwelling of poorly oxygenated deep waters due to wind influence (Chikita, 2000; Sanford et al., 1990). However, this was not the case at La Pletera, because the periods of anoxia we recorded were not linked to strong winds or anomalous changes in daily temperature fluctuations that were attributable to an upwelling of deep waters at different temperatures. Other authors (D'Avanzo and Kremer, 1994; Thébault et al., 2008; Tyler et al., 2009; Woolway et al., 2017) highlighted calm conditions (such as high water temperature, low wind and cloudiness) as the drivers of severe hypoxic or anoxic periods, when the exchange of oxygen with the atmosphere was scarce or non-existent. In addition, especially for shallow coastal ecosystems, high concentrations of organic matter could drive the system to intensify the ER rates (reducing GPP:ER ratio), and an inhibition of photosynthesis due to high temperatures, a lack of oxygen and/or the presence of chemical reductants originating from anaerobic respiration (such as H_2S) could lead the system to experience prolonged periods of anoxia during the day (Beck and Bruland, 2000; Kemp and Testa, 2011; Thébault et al., 2008). Despite the global concern about oxygen declines in coastal waters at a global scale (Breitburg et al., 2018), it is likely that the well-adapted organisms inhabiting these lagoons are only slightly affected by these hypoxic events; for example, dense populations of the endangered fish endemic species *Aphanius iberus* have been observed in these lagoons in spite of the prolonged periods of anoxia.

In contrast to the GPP and ER rates, the NEP and GPP:ER ratio did not show a clear temporal pattern during the three years of the study, consistent with the results obtained by Howarth et al. (2014) or Giordano et al. (2012) in other shallow coastal lagoons. Contrary to previous observations (Carmouze et al., 1991; McGlathery et al., 2001; Ziegler and Benner, 1998), autotrophy peaks occurred more often during winter rather than summer. The extreme values towards heterotrophy typically corresponded to summer days when an oxygen deficit occurred. A characteristic of shallow coastal lagoons appears to be day-to-day fluctuations in the NEP, oscillating between autotrophy and heterotrophy (Carmouze et al., 1991; Giordano et al., 2012). Seasonal shifts between both states have been observed for coastal lagoons (Carmouze et al., 1991; McGlathery et al., 2001; Obrador and Pretus, 2012) and lakes (Laas et al., 2012). Net heterotrophy has also been described (Delgadillo-Hinojosa et al., 2008; Thomaz et al., 2001; Ziegler and Benner, 1998) as well as in lakes (Staehr et al., 2010b) and estuaries (Caffrey, 2003), but it does not seem to be a general property of coastal lagoons, because annual autotrophy (Giordano et al., 2012) and balanced metabolism (Santos et al., 2004; Thébault et al.,

2008) have also been described. In La Pletera lagoons, high nutrient levels and the presence of seagrasses and macroalgae could support high rates of GPP but also cause the accumulation of labile organic matter, leading to increases in the ER rates and, driving the system to net heterotrophy (Camacho et al., 2016). When considering aerobic metabolism only, both La Pletera lagoons tend to be slightly heterotrophic on an annual basis and the GPP:ER ratios are close to 1, indicating that an equivalent quantity of organic matter was consumed. However, these results should be taken with caution since heterotrophy is underestimated, as we discuss below.

Although the application of the diel oxygen method has advantages over other techniques (bottle and chamber incubations), some uncertainties and assumptions of this method that were already described in previous studies (Kemp and Testa, 2011; Staehr et al., 2012a, 2010a) can be magnified in coastal lagoons, especially in confined ones. Firstly, La Pletera lagoons show some degree of horizontal heterogeneity, so the metabolic rate estimations might depend on the sonde location. In the FRA lagoon, there is a large, shallow, macrophyte-rich area far from the deepest part of the lagoon. This type of habitat heterogeneity is very common in coastal lagoons. A sonde installed in the centre of the deepest part might underestimate the total metabolism if it does not detect the littoral metabolism, since production in shallow macrophyte-rich lagoons is usually very high (Barrón et al., 2004; D'Avanzo et al., 1996; Obrador and Pretus, 2013). In trying to avoid errors regarding horizontal heterogeneity, we purposely avoided "whole-lagoon" budgets. However, special attention must be paid during comparisons across sites (Table 4.1.3) due to the fact that it is limited by the number of sondes deployed within each site and the habitat heterogeneity. Secondly, vertical microstratifications in oxygen concentration may appear in the surface layer under calm conditions. Thus, metabolic estimations may depend on the exact depth of the sonde. To quantify the effect of microstratification on the metabolic rates, a test using sondes at different depths was performed during a summer period (July 2017) at FRA lagoon, when the water column was not stratified (Quintana et al., 2018) and the primary mixing forces were assumed to be scarce. The results showed deviations of <15% for GPP and <2% for ER from the metabolic rates calculated from a single-depth sonde. Finally, the diel oxygen method is based on oxygen changes and does not detect anaerobic metabolism, which must be important in the deepest parts of the lagoons and in the entire water column during the observed anoxia events. Many archaea and bacteria can derive energy from the decomposition of organic matter through anaerobic respiration, using other electron acceptors besides oxygen, such as nitrate, sulphate, etc. (Camacho, 2009; Kemp and Testa, 2011). Blooms of the anaerobic bacteria *Chromatium* sp. have been observed, even at the surface of FRA, causing the typical pink colour of the water. To avoid misunderstandings while analysing the NEP or trying to estimate the carbon balances, we should keep in mind that by using the diel oxygen method, we are not accounting for the anaerobic part of the metabolism that seems to be relevant in these ecosystems (especially in FRA) as prolonged periods of anoxia and the presence of *Chromatium* sp. revealed. In any case, the horizontal heterogeneity, vertical microstratification and anaerobic activity contribute to the total ecosystem metabolism, but they are not detected by the diel oxygen method. Thus, the total ecosystem metabolism in confined lagoons may be even higher, highlighting the high productivity of these ecosystems, and they are probably more heterotrophic than the results given by the diel oxygen method.

We found that the GPP and ER were strongly positively related to the temperature and light. These results are consistent with previous studies in coastal lagoons (Carmouze et al., 1991; Giordano et al., 2012; Morant et al., 2020; Ziegler and Benner, 1998), but also in lakes (Laas et al., 2012; Staehr et al., 2010b; Staehr and Sand-Jensen, 2007) and estuaries (Caffrey, 2004, 2003; Russell and Montagna, 2007). Both parameters have already been described as important factors that regulate the coastal ecosystem metabolism and provide the ideal conditions for phytoplankton growth (Beck and Bruland, 2000; Thébault et al., 2008). Light is the ultimate driver of photosynthetic activities, and it is well-known to influence the temperature in many biological processes, such as respiration due to its control of enzyme-catalysed cellular metabolism (Kemp and Testa, 2011). The metabolic rates followed the seasonal temperature and irradiance cycle, reaching the highest rates in the summer time. A high coupling between GPP_m and ER_m was observed, indicating that the production of organic matter by photosynthesis in the ecosystem is consumed or respired in similar proportions. High correlations were also observed in previous studies (Brighenti et al., 2018; Staehr et al., 2010b) that commonly reported weaker coupling in eutrophic than oligotrophic waters (Obrador et al., 2014;

Solomon et al., 2013). In La Pletera, the daily lagoon GPP and ER correlations were lower (*r*= 0.5) than the monthly correlation rates (*r*= 0.91), which is consistent with the low coupling in eutrophic waters. GAMMs highlight the influence of the temperature on the metabolic rates and reveal the poor relationship to nutrient concentrations, with only a weak negative relationship between the standardized GPP_{20MAX} and PO_4^{3-}, indicating that the possible effect of nutrients on metabolic rates are only identifiable when the seasonal effects of temperature and light are removed and suggesting some PO_4^{3-} consumption with an increased GPP. Although many previous studies report an important role for nutrients and organic matter as the primary drivers of metabolism (Brighenti et al., 2018; Caffrey, 2004; Hanson et al., 2003; Morant et al., 2020), our results confirm our hypothesis. This finding is consistent with Glibert et al. (2010), who stated that under high nutrient concentrations, incremental increases in nutrients do not provoke a significant response in metabolism due to the sharp attenuation on increasing GPP in nutrient-rich ecosystems. The exchange of water with the ocean or surrounding systems in La Pletera lagoons is less frequent than in other lagoons or coastal systems. Therefore, the confinement of FRA and G02 causes an accumulation of organic matter and nutrients that remain in relatively high concentrations during the annual cycle. Although the nutrient levels decrease due to a dilution effect during pulsed flooding events and the concentrations follow natural variations throughout the year, the nutrient concentrations seem not to be the significant drivers of metabolism in these lagoons.

In spite of the lack of driving effects of nutrient concentrations on the metabolic rates, an indirect effect from the nutrients should not be discarded. First, the organic and total nutrient concentrations in water are correlated with the temperature caused by the nutrient accumulation during confinement (Àvila et al., 2019; Badosa et al., 2006; López-Flores et al., 2006a). Thus, a possible effect of the nutrient concentrations on the metabolic rates might be masked by their relationship to the temperature. However, when using standardized rates, only a negative relationship between GPP_{20MAX} and PO_4^{3-} emerged, suggesting that the PO_4^{3-} concentration is more of a consequence than a driver of GPP. Second, the total amount of nutrients in the water is not necessarily related to their availability. Serrano et al. (2017) criticized the use of the total phosphorus concentration as a measure of eutrophication in Mediterranean shallow wetlands, arguing that high TP values may be the consequence of a natural concentration caused by the sharp reduction in water volume during confinement. They affirm that the proportion of inorganic vs. organic forms (rather than the total amounts of organic, inorganic or total nutrients) is a better indicator of eutrophication in confined wetlands. Similar amounts of total nutrients but different proportions of inorganic vs. organic nutrients between highly flooded estuaries and long-term isolated confined wetlands have already been reported in confined coastal wetlands (Àvila et al., 2019; Badosa et al., 2007; López-Flores et al., 2006a). In this context, we did not find any significant relationship between the metabolic rates and nutrient ratios. However, coastal wetlands may be N or P-limited (Àvila et al., 2019; Badosa et al., 2006; López-Flores et al., 2014; Quintana et al., 1998), or even both depending on the season (Comín and Valiela, 1993), so no single ratio alone (e.g., DIN:N_{org}, PO_4^{3-}:P_{org}, DIN: PO_4^{3-}, TN:TP) represents the nutrient availability for primary producers. Third, it is remarkable that even after removing the temperature effect from the metabolic rates using GPP_{20}, GPP_{20MAX} and ER_{20}, they peak in winter rather than in summer and correlate negatively with the conductivity and positively with the water level. The relationship of the conductivity and water circulation has been widely described in La Pletera, and water circulation can be easily related to nutrient inputs. Nutrients enter the lagoon during the winter, coinciding with high groundwater circulation, but they concentrate during summer due to confinement, when inputs are scarce (Badosa et al., 2006; Casamitjana et al., 2019; Menció et al., 2017; Quintana et al., 1998). This concentration might mask a weak response in metabolic rates to nutrient inputs, if the water nutrient concentrations are used as a proxy for nutrient inputs, since nutrient inputs and nutrient concentrations are uncoupled unlike other aquatic systems (Morant et al., 2020). Therefore, our data confirm the lack of driving effect by nutrient concentrations on metabolic rates, but the correlation found between standardized metabolic rates and conductivity suggest some response to nutrient inputs, a response that is expected to be weak as predicted by Glibert et al. (2010) for nutrient-saturated habitats. Additionally, we could consider GPP_{20MAX} and ER_{20} as the capacity for production or respiration with no light and temperature limitations, so our data would suggest that water inputs during the winter period provide lagoons with their production capacity, which is not immediately converted to productivity due to low irradiation and temperature. Margalef (1980) defined eutrophy as the capacity for production, suggesting that standardized rates may be a suitable proxy of eutrophy. According to this interpretation, winter flooding inputs would cause eutrophication in La Pletera lagoons, although it will be only evident in the summer, when the light and temperature increase.

4.2. CHAPTER II

Hydrodynamic influence on ecosystem metabolism in Mediterranean confined coastal lagoons

This chapter has been submitted to a journal

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4.2.1. SUMMARY

We analysed the response of ecosystem metabolism to hydrological dynamics in two Mediterranean confined coastal lagoons, in which surface water exchanges are sudden and unpredictable, following a marked flooding (winter) - confinement (summer) pattern, in which water, nutrients, and organic matter enter during flooding but concentrate during confinement. We hypothesize that inputs occurring during winter are the main drivers of metabolic changes in these lagoons, although responses do not emerge until the summer period, when the physical conditions support higher metabolic rates. Wavelet analyses were used to assess the relationships between metabolism and different modelled hydrological parameters (water inflows, outflows, and evaporation). From analyses using raw metabolic rates, we found significant strong seasonal oscillation, but the oscillations were not significant at higher frequencies. However, when using standardized metabolic rates, the results showed significant responses at higher frequencies that could be related to water exchanges (i.e., sea storms). Therefore, standardized metabolic rates showed shorter lagged responses to water inputs than nonstandardized metabolic rates, suggesting some response of metabolism to water and nutrient supplies. This would also indicate limited gross primary production during winter due to physical constraints (e.g., temperature, light) more than a response to the high nutrient concentrations reached during confinement. Despite these results, the amount of unexplained variation was also high, as was the unpredictability of hydrological events in Mediterranean coastal lagoons. Combining long-term hydrological and ecosystem metabolism data provides information about the functioning of these ecosystems and, hence, contributes to management strategies for responding to hydrological changes caused by global change.

4.2.2. INTRODUCTION

Coastal lagoons vary in a wide range of morphological, geological, and hydrological characteristics (Kennish and Paerl, 2010), and several important classifications of coastal lagoons in the literature have been based on their hydrology (Félix et al., 2015; Kjerfve, 1986; Kjerfve and Magill, 1989). Thus, the main drivers defining the nature of these lagoons and their habitat functioning are hydrological characteristics, such as connections to the sea or to freshwater inputs, local precipitation and evaporation regimes, and the surface heat balance (Saccà, 2016). One common category of lagoons is lagoons defined as ‘choked’, which are characterized by limited water exchanges, a lack of significant tides, long residence times, and stable water columns (Kjerfve, 1986). One such example of confined coastal lagoons in the Mediterranean region is the lagoons of La Pletera salt marsh. Although these lagoons are characterized by considerable groundwater circulation (Menció et al., 2017; Quintana et al., 2018), they remain isolated for most of the year, and surface water exchanges only occur during sea storms or flooding events.

Regions with a Mediterranean climate are becoming warmer and drier and experiencing an increase in extreme events (Hallett et al., 2018; IPCC, 2014). The predictions of temperature rise and a lack of precipitation could drive changes in hydrological patterns in these aquatic systems and thereby contribute to eutrophication, salinization, oxygen depletion, algal blooms, etc. Moreover, an increase in the frequency and magnitude of extreme events (such as flooding) would have many consequences, such as habitat loss and erosion (Brito et al., 2012a; IPCC, 2014). Coastal lagoons depend on hydrological characteristics as well as on other environmental variables that drive their dynamics, making these valuable ecosystems even more vulnerable to anthropogenic pressure (habitat loss, hydrological modification, pollution, exotic species, etc.) and to climate change, which may act in synergy (Caroppo et al., 2018; Hallett et al., 2018; Saccà, 2016). Thus, aquatic ecosystems that are strongly dependent on hydrology will experience changes in their ecosystem functioning (Zwart et al., 2017). Our ability to predict how coastal lagoons will respond to future changes depends on a deep understanding of the hydrological dynamics of the lagoons (Badosa et al., 2006; Kjerfve, 1986) and their relationship with ecosystem functioning.

Ecosystem metabolism, a fundamental ecological process representing the capacity of the system to produce and consume organic matter, has been considered a useful indicator of whole-ecosystem functioning (Hoellein et al., 2013; Kemp and Testa, 2011; Staehr et al., 2012a). Previous reports have already recognized the utility of metabolism as an indicator of ecosystem functioning and habitat quality in wetlands (Tuttle et al., 2008). However, despite the possible influence of hydrology on ecosystem functioning and metabolism, to the best of our knowledge, no one has combined long-term monitoring of hydrological data with ecosystem metabolism estimations in lentic environments (but see some application in rivers (Summers et al., 2020)). For confined coastal lagoons, which are dependent on hydrological processes, it can be of great interest to understand how hydrology is related to ecosystem metabolism to better understand their dynamics and assess how coastal ecosystems will respond to future disturbances.

In general, nutrient uptake, photosynthesis, and respiration rates are stimulated by water flow, as is the case in rivers, estuaries, and leaky wetlands, where the positive effects of increasing flow rates on aquatic productivity have been demonstrated (e.g., Cronk and Mitsch, 1994; Tuttle et al., 2008). This can be explained in part by water inputs that supply nutrients, ions, organic matter, etc. The relationship between nutrients and primary production in aquatic ecosystems is well known (e.g., Brighenti et al., 2018; Caffrey, 2004). However, contrary to other aquatic ecosystems, confined coastal lagoons in the Mediterranean region remain isolated from superficial water exchanges for long periods of time, especially during summer, and nutrient supplies occur mostly during flooding periods or sea storms, generally in winter (Figure S9.1.2), when the nutrient concentrations and metabolic rates are the lowest (Quintana et al., 2018; see also Chapter I). This, therefore results in a decoupling of inputs (i.e., nutrient supplies) from nutrient concentrations and GPP (Chapter I). Previous studies in the area showed that nutrient concentrations were not significant drivers of metabolic rates in these lagoons (Chapter I). However, when using standardized metabolic rates, where temperature and light effects are removed, the same studies highlighted a higher capacity of production during winter, when flooding events occur. Thus, the metabolic response may be restricted by physical constraints (such as temperature and light) during the winter period, and a relation of causality between winter inputs and summer metabolic rates cannot be discarded. These results suggest that metabolism could show a lagged response to nutrient supplies rather than dependency on the nutrient concentrations reached during confinement. This, therefore, highlights the need for a deeper understanding of the relationship between hydrology and metabolism in these lagoons.

In this sense, time series analysis could provide a useful way to study the relationship and possible decoupling between hydrology and metabolism in these confined lagoons. Time series analysis and, more specifically, wavelet analysis, has been applied in science and engineering and is becoming a promising tool for analysing time series with important applications in hydrology and ecology (Cazelles et al., 2008; Rodríguez-Murillo and Filella, 2020; Sang, 2013; Schaefli et al., 2007). Wavelet analysis, contrari to classic Fourier analysis, overcomes the stationary assumption that makes most methods unsuitable for many ecological time series (Grinsted et al., 2004).

Mediterranean confined coastal lagoons, similar to other aquatic ecosystems that are highly dependent on hydrological characteristics, may experience changes under a future global change scenarios. These changes may alter the ecosystem functioning of these water bodies, exacerbating their vulnerability. However, the study of hydrology in these lagoons faces the additional difficulty of analysing common response patterns based on unpredictable hydrological events in Mediterranean areas with highly variable ecosystems. The aim of this study was to provide deeper insight into the ecosystem functioning of Mediterranean confined coastal lagoons by elucidating the influence of water exchanges on ecosystem metabolism responses. We hypothesized that water inputs during winter are the main drivers of metabolic changes in these lagoons, although responses do not emerge until the summer period, when the physical conditions support higher metabolic rates.

4.2.3. METHODS

4.2.3.1. Sampling and analyses procedure

The study was carried out in two Mediterranean confined coastal lagoons of La Pletera salt marsh (FRA and G02; Figure 3.1.1) during four hydrological cycles, from July 2015 to September 2019, in which metabolic estimations and hydrological data were collected (more details in sections below). Despite sharing the general hydrological features of the salt marsh, the two lagoons differ in some characteristics (Tables S9.1.1 and S9.1.2; Figure S9.1.3). FRA is the largest and deepest and has some degree of stratification during the winter, with higher salinity values at depth (Figure S9.1.1) and anoxia below 1.5 m (Compte et al., 2018; Quintana et al., 2018). G02 is smaller and shallower and is usually well mixed. G02 also registers lower salinity values and a higher proportion of groundwater circulation (Casamitjana et al., 2019), as the impermeable layer of sediments was probably altered during its creation (Geoservei, 2016). More information about the study site, its hydrology and lagoons characteristics can be consulted in section 3.1. In order to characterize the study period, different parameters were registered (Table S9.1.1; Figure S9.1.3). The water level refers to the height above the average sea level registered in the zone during the years 1990-1995 and is used as a topographic reference in the zone (Martinoy and Pascual, 2018). Chlorophyll-*a* and nutrient analyses protocols can be found in sections 3.4 and 3.5.

For metabolism estimations, continuous, high-frequency monitoring of different physical parameters was needed. All the information about sondes deployment and monitoring of the different parameters is provided in section 3.2. Ecosystem metabolism estimations were computed using a modified version of the BAyesian Single-station Estimation (BASE) model described in section 3.3. All details about the model functioning, model selection and standardized metabolic rate calculation are also provided in that section.

4.2.3.2. General lake model application and hydrological parameters

The general lake model (GLM) is a one-dimensional open-source code designed to simulate the hydrodynamics of lakes, reservoirs, and wetlands (Hipsey et al., 2019). More specifically, the model computes vertical profiles of temperature, salinity, and density by accounting for the effect of inflows/outflows, mixing, and surface heating and cooling (Casamitjana et al., 2019). The GLM was applied in La Pletera lagoons in previous studies to analyse the groundwater influence in the salt marsh (Menció et al., 2017) and the water circulation patterns and salinity fluctuations (Casamitjana et al., 2019) and provided the first record of GLM application in such small water bodies. Further details about model functioning are described in Menció et al. (2017), Casamitjana et al. (2019), or Hipsey et al. (2019).

In the present study, the GLM was used to calculate different daily hydrological variables: inflows, outflows, rain and evaporation. These variables were obtained from measurements of the water level fluctuations, bathymetry and meteorological variables (radiation, cloud cover, air temperature, wind speed, relative humidity, and rain) measured at the Estartit meteorological station (3 km away from the lagoons). For those calculations, water level measurements were interpolated to a daily frequency when they were not available daily (see '3.2. High-frequency measurements' section). The inflows and outflows variables included the different exchanges of water flows from the surface, subsurface, and groundwater in the lagoons, without taking evaporation and rainfall into account. Rain refers to the precipitation that falls directly into the lagoons. Thus, because inflows and rain are related, we considered them to be one variable and referred to them solely as inflows. Outflows and evaporation were kept separate, as they could follow quite different temporal patterns due to the close relationship between inflows and outflows. It should be noted that inflows did not always refer to freshwater inflows. Previous studies in the salt marsh revealed that mixing of seawater and freshwater can occur within the aquifer, and inputs of water with salinities higher than seawater can enter the lagoon, especially during the summer season and the beginning of autumn (with the first cyclonic events), which affected lagoon salinity (Casamitjana et al., 2019; Menció et al., 2017). Each hydrological variable was divided by the lagoons' daily total volume to relativize each variable to the lagoon size. Hereafter, we will refer to these factors as 'Inflows', 'Outflows', and 'Evaporation'.

4.2.3.3. Data analyses

The analyses were performed on two data sets, each consisting of hydrological variables (inflows, outflows, evaporation) and metabolic rate (GPP, ER, GPP_{20MAX}, and ER_{20}) time series. Each data set corresponded to the FRA and G02 lagoons studied during the 4-year period. After discarding days due to BASE model selection or sonde malfunctions (46% and 47% of the data for FRA and G02, respectively), the metabolic rate time series were estimated at a weekly frequency to avoid missing values. Weekly median values were calculated for metabolic rates and for the hydrological variables. Missing values at a weekly frequency were filled using the ‘imputeTS’ package in R (Moritz and Bartz-Beielstein, 2017). This package was designed for univariate time series imputation and includes several algorithms, among which the ‘Kalman Smoothing and State Space’ algorithm was chosen for our study.

To analyse the relationships between metabolic rates and hydrological variables, wavelet analyses were performed. Wavelet transformations provide information about the time series for different frequency intervals, and cross wavelet transformation (XWT) and wavelet transformation coherence (WTC) allow the analyses of dependency between two signals (Cazelles et al., 2008). WTC was performed in pairs between metabolic rates and hydrological variables and can be thought of as a localized correlation coefficient in time-frequency space (Grinsted et al., 2004). Here, we performed WTC computations to avoid some pitfalls already described for XWT (Maraun and Kurths, 2004). These disadvantages would show spurious results if one of the time series is flat and the other exhibits strong spikes, generating spikes in the cross spectrum that may have nothing to do with any relation of the two time series (Maraun and Kurths, 2004; Rodríguez-Murillo and Filella, 2020). The WTC finds locally phase locked behaviour and is equal to 1 when there is a perfect linear relation at a particular time location and frequency between two signals (Cazelles et al., 2008; Grinsted et al., 2004). Therefore, if the series are physically related, we would expect a consistent or slowly varying phase lag (Grinsted et al., 2004). The significance level was determined using Monte Carlo methods. We chose the Morlet wavelet as the type of mother wavelet function to use, as it is reasonably localized in both time and frequency (Grinsted et al., 2004) and is generally used in hydrological studies (Schaefli et al., 2007). For further details of the mathematical and statistical portion of the analysis, see Cazelles et al. (2008), Grinsted et al. (2004) and Torrence and Compo (1998). The biwavelet package (Gouhier et al., 2018) in R (R Core Team, 2017) was used for WTC analyses. As support for the wavelet analyses, periodograms for metabolic rates and hydrological time series were performed using the time series analysis ‘TSA’ package in R (Chan and Ripley, 2020). In these periodograms, a more general view of the entire time series is provided, showing the spectral density of a time series and highlighting the dominant frequencies of oscillation composing it (Khan et al., 2020).

Based on the WTC results, we used multivariate singular spectrum analysis (MSSA) to identify common oscillations between metabolic rates and hydrological time series and isolate the oscillations of interest, such as the seasonal oscillations. Code information was extracted from Khan et al. (2020). We also used Spearman correlations to analyse simultaneous relationships among metabolic and hydrological variables as well as some physical variables (such as temperature, salinity and water level). Considering the high correlations between GPP and ER as well as GPP_{20MAX} and ER_{20} and the similar results obtained from WTC computations, only the WTC results for GPP and GPP_{20MAX} are shown and discussed in this study.

4.2.4. RESULTS

The different physical and chemical parameters collected during the study period (summarized in Table S9.1.1) showed a clear seasonal pattern (Figure S9.1.3). Temperature and salinity as well as nutrients and organic matter showed higher values during the summer season, coinciding with the lowest water levels. Summer periods were also characterized by higher values of GPP and ER but also more variability (Figure 4.2.1) due to the oxygen depletion episodes observed in this period for both lagoons. On the other hand, salinity and nutrient concentrations were low during winter (Figure S9.1.3), when flooding events or sea storms were more frequent (Figures S9.1.2 and S9.1.10). It should be noted that inorganic forms, such as DIN, remained low in the lagoons despite isolated spikes during the flooding periods (Figure S9.1.3).

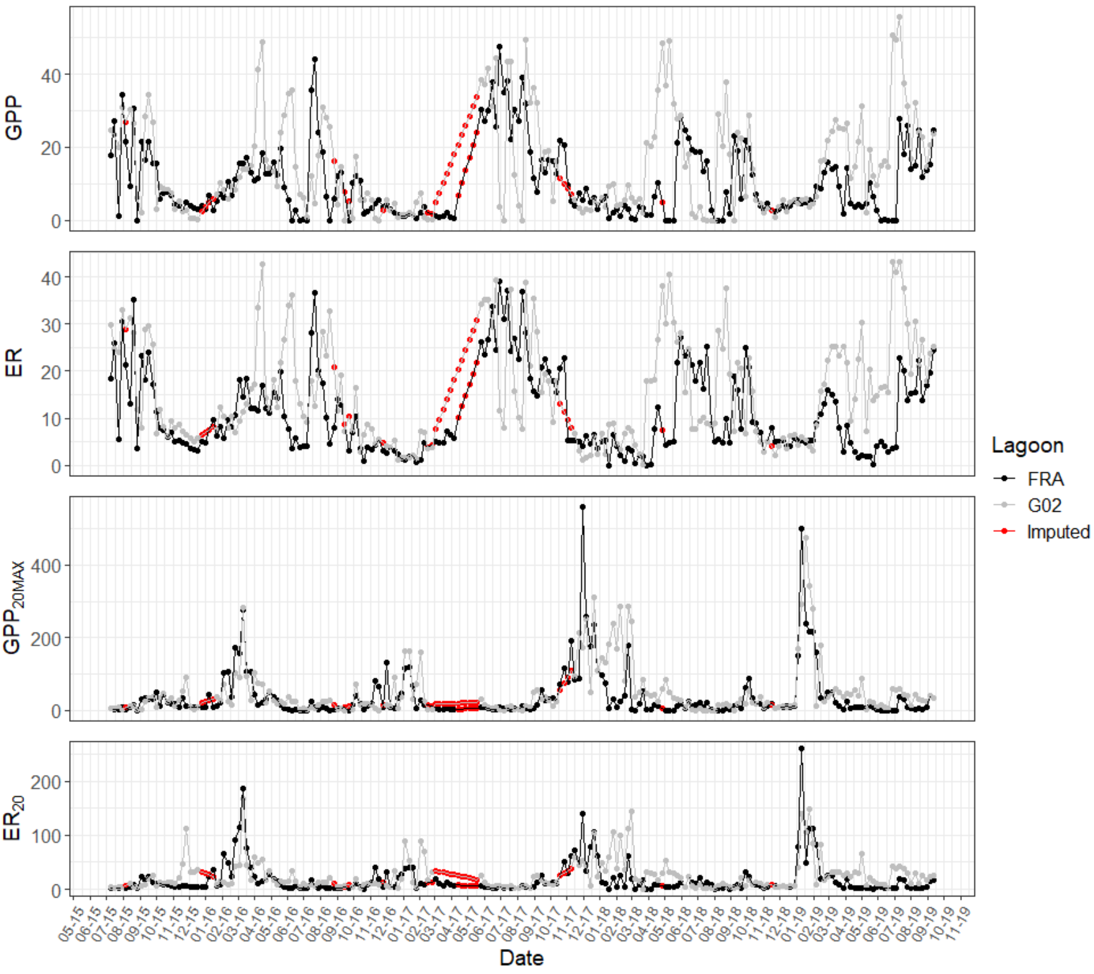


Figure 4.2.1. Temporal changes in gross primary production (GPP) and ecosystem respiration (ER) during the four hydrological cycles (July 2015 to September 2019). The gross primary production standardized to 20°C and to a maximum value of irradiance (GPP_{20MAX}) and ecosystem respiration standardized to 20°C (ER_{20}) are also represented. Metabolic rates correspond to weekly median values in mg O_2 L^{-1} d^{-1}. The values in red are imputed measurements for filling the NAs (modelling discards and sonde malfunctions). For further details, see '4.2.3. Methods' section. The X-axis labels indicate the month-year.

Seasonal patterns in water levels and evaporation were also observed in both lagoons, but the patterns of inflows and outflows were less obvious (Figure 4.2.2). Following Mediterranean climate patterns, sea storms and rainy episodes were concentrated during the winter (Figures S9.1.2 and S9.1.10), but the sudden and unpredictable nature of these events made it difficult to distinguish the seasonal pattern of inflows and outflows in the lagoons (Figure 4.2.2). The second hydrological cycle showed greater water circulation, coinciding with a greater number of sea storms (Figures 4.2.2 and S9.1.10). As shown in Figure S9.1.10, changes in water level and hence hydrological parameters were mainly driven by marine intrusions during sea storms (wave height ≥ 2.5 metres), as intense rainfall did not necessarily provoke large changes in the water circulation of the lagoons.

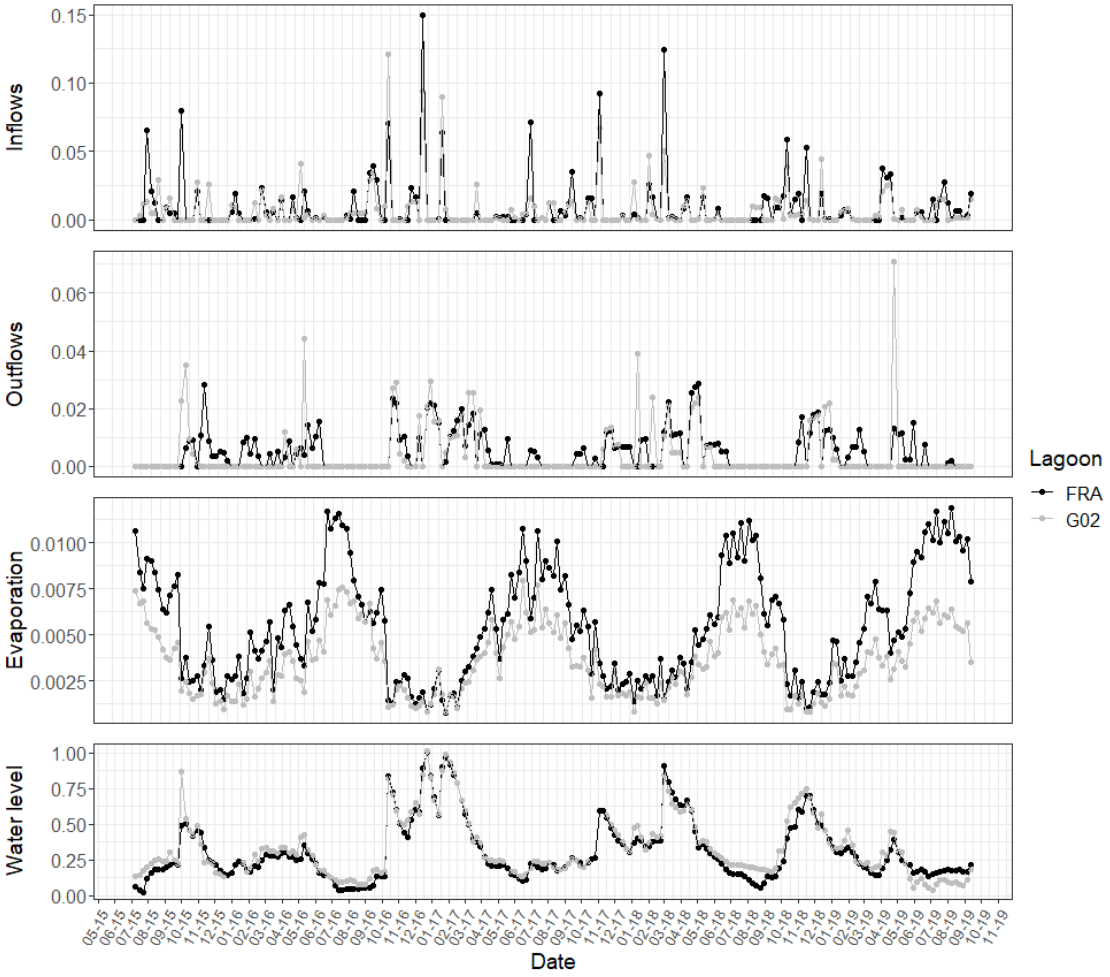


Figure 4.2.2. Temporal changes in the different hydrological parameters included in the study (inflows, outflows, and evaporation) obtained by means of the general lake model (GLM) during four hydrological cycles (July 2015 to September 2019). All hydrological parameters were relative to the lagoon's volume. Additionally, the water level (height above average sea level) is provided (in metres). The values represented are weekly median data. The X-axis labels indicate the month-year.

Spearman correlations for daily data (presented in Table 4.2.1) showed high correlation values between the metabolic rates GPP and ER (*r*= 0.88) and between the standardized rates GPP_{20MAX} and ER_{20} (*r*= 0.84). Inflows showed low and nonsignificant correlation values with the rest of the variables (metabolic rates and physical parameters), even with the outflows (*r*= -0.33). Metabolic rates presented a negative correlation with outflows and a positive correlation with evaporation, while standardized rates showed the inverse pattern.

Table 4.2.1. Spearman correlations between daily values of the hydrological and metabolic variables (in mg O_2 L^{-1} d^{-1}) included in the study, temperature (°C), salinity, and water level (height above or below average sea level, in metres). The hydrological parameters included were previously divided by the corresponding lagoon volume. GPP: gross primary production; ER: ecosystem respiration; GPP_{20MAX}: gross primary production standardized to 20°C and to a maximum value of irradiance; ER_{20}: ecosystem respiration standardized to 20°C. Significant correlations are indicated in bold. The sample size (N) varied, so it was also included in the correlation matrix (grey numbers). Modelled hydrological variables (inflows, outflows, and evaporation) and water level values were complete (N= 3064); standardized and nonstandardized rates values were limited by model validation (N= 1643); and temperature (N= 2885) and salinity (N= 3038) depended on the corresponding sonde functioning.

	GPP	ER	GPP_{20MAX}	ER_{20}	Inflows	Outflows	Evaporation	Water level	Temperature	Salinity
GPP	1	1643	1643	1643	1643	1643	1643	1643	1643	1643
ER	**0.88**	1	1643	1643	1643	1643	1643	1643	1643	1643
GPP_{20MAX}	**0.30**	**0.07**	1	1643	1643	1643	1643	1643	1643	1643
ER_{20}	**0.27**	**0.22**	**0.84**	1	1643	1643	1643	1643	1643	1643
Inflows	**0.06**	0.02	0.05	-0.03	1	3064	3064	3064	2885	3038
Outflows	**-0.28**	**-0.40**	**0.10**	0.04	**-0.33**	1	3064	3064	2885	3038
Evaporation	**0.33**	**0.47**	**-0.35**	**-0.24**	**-0.09**	**-0.32**	1	3064	2885	3038
Water level	**-0.35**	**-0.52**	**0.32**	**0.20**	**0.09**	**0.52**	**-0.71**	1	2885	3038
Temperature	**0.43**	**0.62**	**-0.54**	**-0.42**	0.03	**-0.41**	**0.69**	**-0.68**	1	2885
Salinity	**0.11**	**0.19**	**-0.39**	**-0.37**	**0.05**	**-0.18**	**0.62**	**-0.54**	**0.56**	1

The WTC results for GPP with inflows, outflows, and evaporation highlighted significant signal oscillation at 52 weeks, corresponding to the annual scale, which was quite uniform during the study period in both lagoons (Figure 4.2.3). The rest of the significant signals showed higher frequencies and lower temporal lags and were less predictable from one year to another; thus, they did not follow any interpretable pattern. Thus, the strongest significant signal was the seasonal or annual signal, which presented different phases for the different variables and quite similar responses for both lagoons. GPP and inflows (for FRA) presented an annual oscillation with the arrows in the significant region pointing downwards, indicating a certain lag between the inflows and the GPP, whereby changes in inflows precede changes in GPP. For G02, the arrows pointed slightly left, indicating an anti-phase relationship of GPP with inflows, similar to the relationship between GPP and outflows in both lagoons. This anti-phase indicates that GPP peaked when inflows and outflows were the lowest at 52-week intervals. The WTC, including GPP and evaporation, in both lagoons showed an in-phase relationship (arrows pointing right), which would indicate that GPP reaches its maximum values when evaporation rates are also high. Based on the wavelet analysis results, annual signals were isolated using MSSA (Figure 4.2.3). Similar to the wavelet coherence, the annual oscillation showed certain lag and anti-phase relationships between GPP and inflows and outflows. However, different years displayed differences in magnitude, showing that the third and fourth hydrological years that presented lower GPP values were preceded by lower inflows. Annual oscillation also showed strong coupling between evaporation and GPP. During the time series decomposition process in the MSSA, no further oscillations were identified beyond the seasonal oscillation.

When analysing the GPP_{20MAX} rates, slightly different WTC results were obtained (Figure 4.2.4). The significant annual oscillation was absent in the relationship between inflows and GPP_{20MAX} and was weaker for GPP_{20MAX} with outflows (arrows showing in-phase, right) and evaporation (arrows showing anti-phase, left). In contrast to the analyses using GPP, the WTC with GPP_{20MAX} showed larger significant areas coinciding with regions with major frequencies and intensities of sea storms. It seemed that the two lagoons differed slightly in their responses, but both had significant regions of inflows, showing oscillations of approximately 4-8 weeks during periods when the frequency of the storms was high (November to March of 2017 and January to April 2018) (Figures 4.2.4 and S9.1.10). Despite the significant areas that could be related to the greatest changes in water levels in the lagoons, the metabolic responses to sea storms were variable and were sometimes absent. Moreover, other significant regions were identified during the last hydrological year with oscillations of approximately 16-32 weeks, mainly for inflows and outflows.

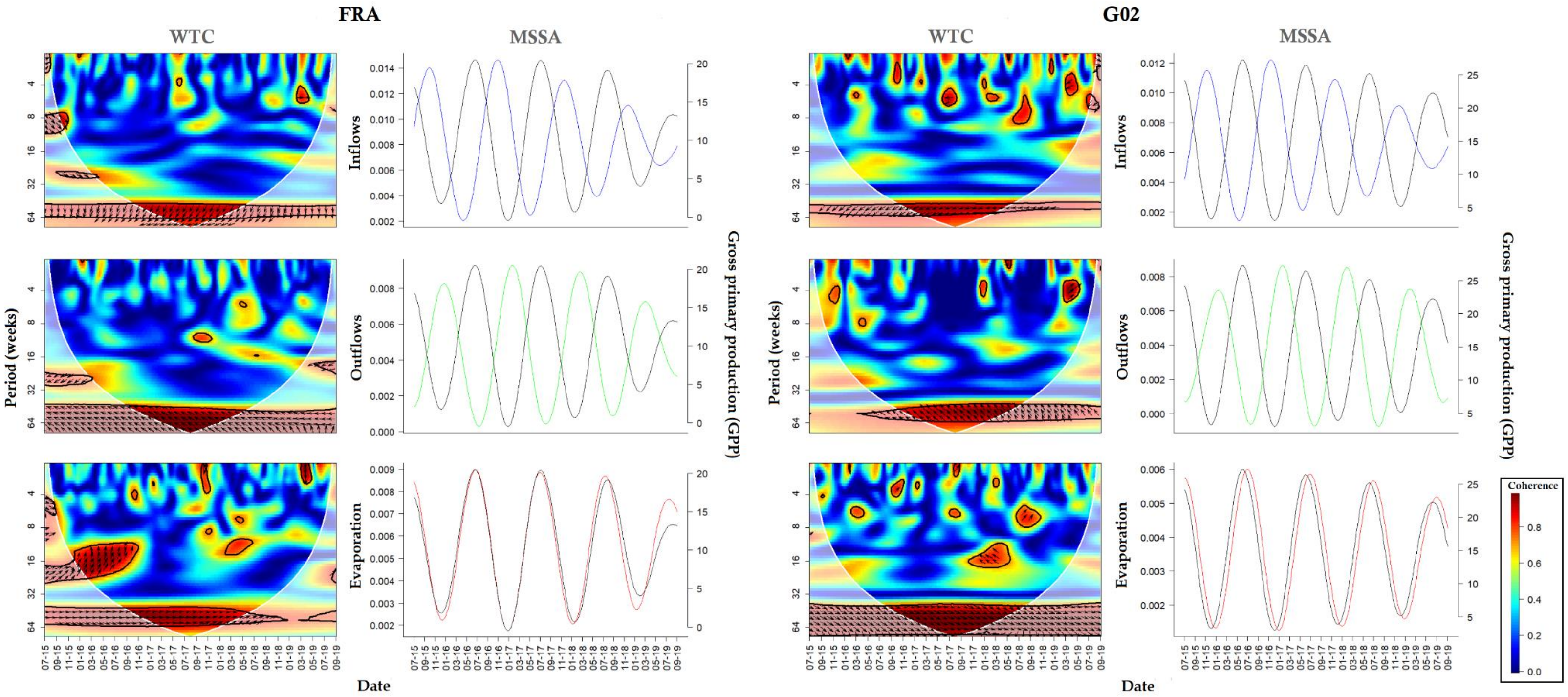


Figure 4.2.3. Wavelet transformation coherence (WTC) analyses between weekly gross primary production (GPP) and inflows (first row), outflows (second row), and evaporation (third row) for both lagoons FRA (left) and G02 (right). The X-axis represents the time of the study (labels as month-year), and the Y-axis represents the period of the oscillation (in weeks). GPP is in mg O_2 L^{-1} d^{-1}, and all the hydrological parameters were divided by the volume of the corresponding lagoon. Colours indicate the measure of coherence between the time series, ranging from 0 (low coherence in blue colours) to 1 (red colours for high coherence). Areas contoured by thick black lines indicate the 5% significance level. The arrows displayed in these areas refer to the phase shift between time series, with in-phase arrows pointing right, anti-phase arrows pointing left, and the arrows of hydrological variables leading or following GPP by 90° pointing straight down or up, respectively. Thick white lines delimit the cone of influence (COI) (region affected by edge effects) in which results cannot be trusted (whitish regions). Annual signals isolated using the multivariate singular spectrum analysis (MSSA) are also represented. The values shown are approximate, as the signals were standardized to 0, and the mean of each variable was summed to show more realistic values. Variables are presented using different colours: black (GPP), blue (inflows), green (outflows), and red (evaporation).

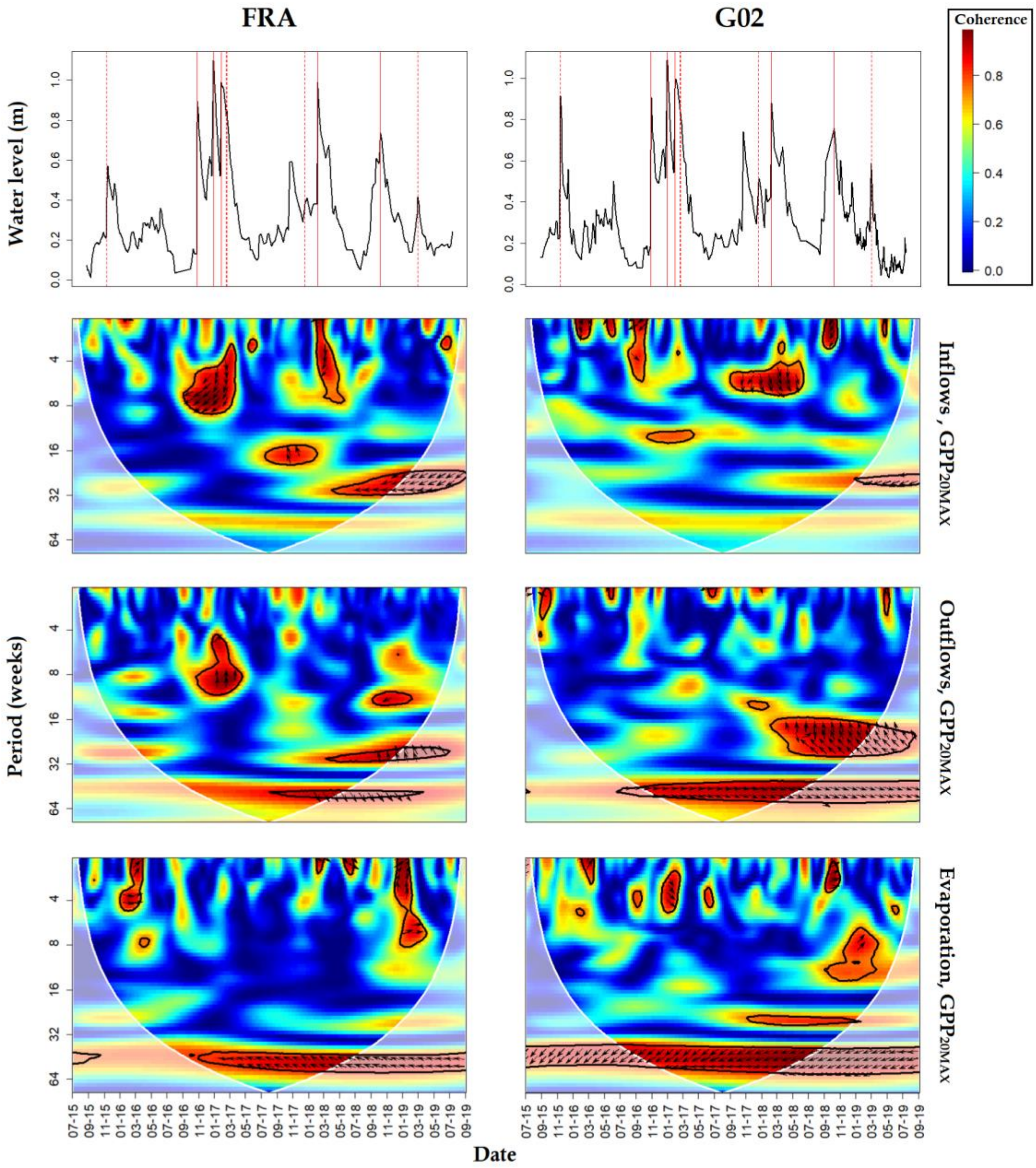


Figure 4.2.4. Wavelet coherence spectra of weekly gross primary production standardized to 20°C and a maximum of irradiance (GPP_{20MAX}) and inflows (second row), outflows (third row), and evaporation (fourth row) for both lagoons FRA (left) and G02 (right). The X-axis represents the time of the study (labels as month-year), and the Y-axis represents the period of the oscillation (in weeks). GPP_{20MAX} is in mg O_2 L^{-1} d^{-1}, and all the hydrological parameters were divided by the volume of the corresponding lagoon. Colours indicate the measure of coherence between the time series, ranging from 0 (low coherence in blue colours) to 1 (red colours for high coherence). Areas contoured by thick black lines indicate the 5% significance level. The arrows displayed in these areas refer to the phase shift between time series, with in-phase arrows pointing right, anti-phase arrows pointing left, and the arrows of hydrological variables leading or following GPP_{20MAX} by 90° pointing straight down or up, respectively. Thick white lines delimit the cone of influence (COI) (region affected by edge effects) in which results cannot be trusted (whitish regions). Water levels (height above or below average sea level) are also represented (first row) for FRA (left) and G02 (right). In these plots, red vertical lines indicate the days when sea storms occurred. We differentiated the storms for which wave height was ≥ 2.5 metres (low intensity) with dashed lines from those with wave height ≥ 3 metres (high intensity) with solid lines.

The periodograms in Figure 4.2.5 corroborate in the results of the wavelet analyses, and a high spectral density is observed at the annual scale for GPP and ER (see the dominant spikes at 52-week frequency) as well as for outflows and evaporation. In contrast, inflows was the hydrological parameter with lowest spectral density at the annual scale, presenting larger spikes at higher frequencies and therefore at lower temporal scales. Standardized rates also showed large spikes at the annual scale, but other frequencies were dominant as well, contrary to the rates that were not standardized.

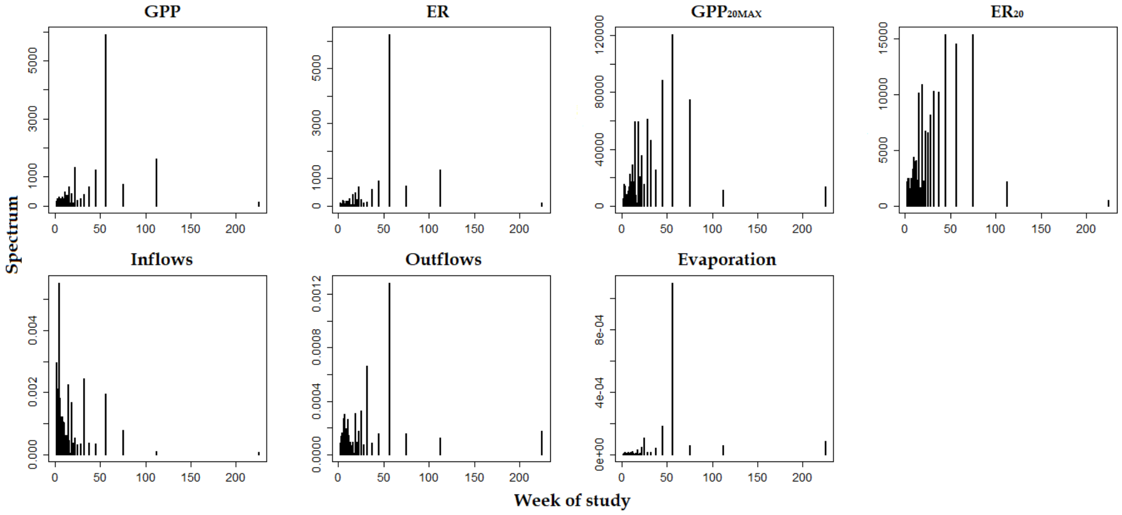


Figure 4.2.5. Example of the periodograms of the different variables included in the study obtained for the FRA lagoon. The X-axis corresponds to the frequency of oscillation (weeks), and the Y-axis is the spectral density or power spectrum, in which higher values indicate important processes occurring at that particular frequency. Notice the dominant spikes at 52 weeks (1 year). Similar patterns were obtained for G02 (plot not shown). Metabolic rates are in mg O_2 L^{-1} d^{-1}, and all the hydrological parameters were divided by the volume of the lagoon.

4.2.5. DISCUSSION

Confined coastal lagoons in La Pletera salt marsh, similar to other coastal lagoons, represent aquatic ecosystems with high dependence on hydrological processes (e.g., Bonilla et al., 2005; Caroppo et al., 2018; Sylaios and Theocharis, 2002). This dependence, along with strong DO signals, make these water bodies ideal locations for analysing the relationship between hydrology and ecosystem metabolism. Physical and chemical parameters registered during the study period, including the metabolic rates, showed a common seasonal pattern as a result of the flooding-confinement processes dominating the salt marsh. Similarly, the main results of the time series analyses highlighted a lack of significance and uniformity in the response of metabolism to hydrological changes, especially at high frequencies, and only a strong annual oscillation corresponding to seasonality during the study period was significant. Seasonality was also an important factor of variation in previous studies in Mediterranean areas (e.g., Badosa et al., 2006; Cañavate et al., 2015; Caroppo et al., 2018; Obrador and Pretus, 2012).

Despite metabolic rates not showing any significant response to changes in hydrology apart from the seasonal pattern, annual isolated signals showed some change in magnitudes during the study period. This could suggest that lower winter inputs could provoke lower responses of summer GPP. However, these low-frequency responses are difficult to consider independent of seasonal variation, and a causal relationship between hydrology and metabolism cannot be firmly established. One of the features widely documented in the literature is the hydrologic effects on nutrient dynamics (e.g., Hagy et al., 2004; Moss et al., 2011; Pesce et al., 2018). The decoupling between nutrient inputs (water exchanges in winter time) and high nutrient concentrations (confinement conditions during summer) present in these lagoons could explain the different responses from other studies that demonstrated that certain water flows enhanced primary production in aquatic systems (e.g., Cronk and Mitsch, 1994; Tuttle et al., 2008). In Chapter I nutrient concentrations were discarded as significant drivers of metabolism in these lagoons based on monthly scale monitoring but suggested a possible metabolic response to nutrient inputs based on standardized metabolic rate dynamics. Similarly, standardized metabolic rates showed a higher production capacity during the winter, when greater water exchange, and hence, nutrient inputs occurred. The lack of response of GPP during winter could be explained by physical constraints (such as light and temperature limitations). Accordingly, wavelet analyses showed different results for the standardized rates in comparison with the nonstandardized rates. Significant oscillations of approximately 4-8 weeks, specifically for the relationship between inflows (leading) and GPP_{20MAX}, were observed and related to intense sea storms that caused significant changes in the water level in the lagoons. However, despite these significant responses, a common and uniform response pattern cannot be described. Additionally, for other significant regions observed, we found no explanation. The absence of patterns could be related to the Mediterranean climate, where flooding events are concentrated during the winter (Figure S9.1.2); however, they are completely unpredictable (Alvarez Cobelas et al., 2005; Beklioglu et al., 2007). Thus, sea storms occur under very different conditions of intensity, temperature, or wind direction, probably resulting in different effects on GPP_{20MAX}.

Based on these results, different explanations could be considered when analysing hydrology as a driver of metabolism in these confined coastal lagoons. First, evaporation could be responsible for metabolic responses since annual signals of evaporation presented perfect coupling with metabolic rates. Evaporation, enhanced during summer due to high temperature and irradiance, is considered a major component of the water balance, especially for closed ecosystems (Hayashi and van der Kamp, 2021) and could act in two ways. On the one hand, evaporation constitutes the most important circulation of water in these lagoons during summer, when calm and anoxic conditions at surface waters can last for several days (Chapter I). Such extreme conditions can enhance GPP through nutrient mobilization or DO dispersion. On the other hand, evaporation contributes to the concentration of nutrients during confinement, even without inputs, as they concentrate when the water level drops (Badosa et al., 2006; Quintana et al., 2018). However, some arguments make it unclear whether evaporation actually drives metabolic responses. In this sense, a lack of significance when relating nutrient concentrations to metabolism has already been described in these lagoons (Chapter I). Moreover, we did not find any coupling between standardized rates and evaporation: if evaporation drives metabolism, we would expect a good coupling between evaporation and GPP_{20MAX} in summer, when there is no light or temperature limitation. A second explanation derived from the results is the key role of water exchanges, particularly inflows, that could cause a lagged response of metabolism. Water inputs during winter provide nutrients but dilute their concentration in lagoon water, since total nutrient concentrations are lower in groundwater than in lagoons (Menció et al., 2017). However, at the same time, the total amount of nutrients concentrated in summer depends on the total winter inputs. This statement can be supported by the lagged or anti-phase response of annual metabolism to inflows and by the high-frequency oscillations of standardized rates observed when sea storms occurred. Similar to evaporation, inflows could cause changes in water, sediment, nutrient, and DO dynamics, and act as a source of nutrients, ions, etc. enhancing GPP. However, a half-year lagged response can hardly be distinguished from an independent response of inflows and metabolism to the annual cycle. Again, some uncertainty is related to the marked seasonality pattern and the lack of common and uniform responses of metabolism typically found in aquatic systems of the Mediterranean region (Hallett et al., 2018). A third explanation could be that hydrology has no direct effect on ecosystem metabolism dynamics, and metabolism could follow a seasonal pattern as a result of the phenology of the primary producers present in the salt marsh, such as seagrasses. Supporting this explanation, a seasonal pattern in seagrass growth is common in Mediterranean coastal lagoons with *Ruppia*-dominated meadows (Menéndez, 2002; Menéndez and Comín, 1989; Obrador and Pretus, 2010), as seen in La Pletera (Gesti et al., 2005). In any case, it is likely that all these factors could be contributing to metabolism dynamics at different temporal scales, making the study of metabolic drivers challenging. The unpredictability of hydrological events, their different intensities, their variability within coastal lagoons, the occurrence of flooding events, the importance of recycling processes for nutrient availability in these lagoons, etc. (Badosa et al., 2006; Quintana et al., 2018, 1998) are several of the factors that could contribute to enhancing the variability of metabolic responses to hydrological changes in these confined water bodies.

There are also difficulties when trying to contrast the different explanations proposed above due to the lack of similar studies combining long-term monitoring data from hydrological parameters and ecosystem metabolism measurements, especially in confined coastal water bodies. Regarding hydrology and metabolism, similarities in freshwater wetlands have been reported in the literature. For example, Tuttle et al. (2008) examined the influence of seasonal hydrologic pulsing on aquatic metabolism in two constructed flow-through riparian wetlands, but their 2-year field data were based on conventional measurements rather than high-frequency observations. Similarly, Cronk and Mitsch (1994) determined the effect of two different hydrologic flows on aquatic metabolism in constructed freshwater riparian marshes, but oxygen measurements were collected manually, and the study was based on 8 sampling dates. Other researchers have examined the effects of episodic events on metabolism in freshwater systems (Acuna et al., 2004; Gallardo et al., 2012; Kobayashi et al., 2015; Shen et al., 2015). However, coastal lagoon studies that examine hydrology, specifically flood pulses, have focused on their influence on the phytoplankton composition (e.g., Bonilla et al., 2005; Caroppo et al., 2018) or other issues (e.g., Bellio and Kingsford, 2013), and the effect on ecosystem metabolism has not been previously assessed.

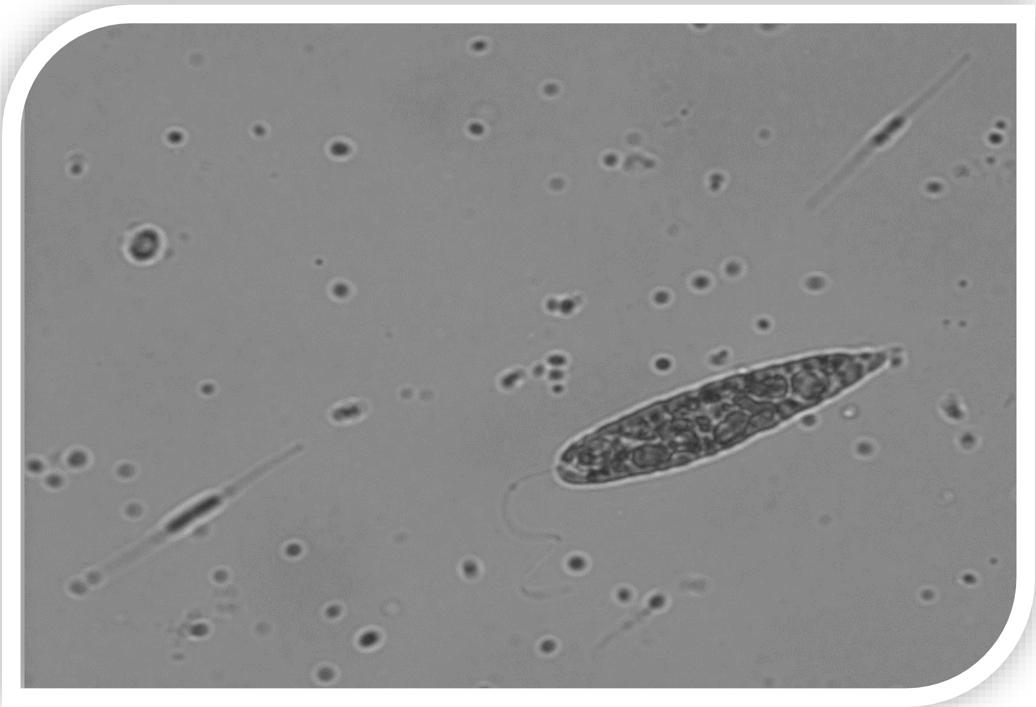
Hydrology determines the nature of coastal lagoons and is considered one of the most important drivers of their ecosystem functioning (Cañavate et al., 2015; Cronk and Mitsch, 1994; Saccà, 2016). Thus, hydrodynamics exert a strong influence on the different physical and chemical parameters, nutrient and organic matter dynamics and, thus, community structure of these aquatic ecosystems (Cañavate et al., 2015; Caroppo et al., 2018; Sylaios and Theocharis, 2002). In Mediterranean confined coastal lagoons in particular, sudden and noncontinuous surface water exchanges (Quintana et al., 2018) and groundwater circulation (Casamitjana et al., 2019; Erostate et al., 2020; Menció et al., 2017) play a key role in lagoon behaviour. Mediterranean regions are expected to have drier and warmer climates along with a high frequency of extreme events that would imply changes in the hydrological regime and therefore changes in nutrient dynamics, community composition, etc. (Hallett et al., 2018; IPCC, 2014; Pesce et al., 2018). Our study suggests that changes in the temperatures and hydrology (e.g., flooding events, sea storms, drought) would alter the seasonal pattern of the lagoons and that a reduction in water exchange would reduce GPP. Moreover, as temperature rises, oxygen depletion will increase along with ER rates. All these changes could drive systems, such as confined coastal lagoons, to present higher heterotrophy, more frequent anoxic episodes, eutrophication, algal blooms, etc. Obtaining a better understanding of the relationship between hydrology and metabolism constitutes a first step in providing information about the functioning of these ecosystems, thereby contributing to better protection of these vulnerable habitats in view of future disturbances.

4.3. CHAPTER III

Phytoplankton community structure in Mediterranean confined coastal lagoons: using ecosystem metabolism for trait quantification

This chapter has been submitted to a journal

Bas-Silvestre, M., Antón-Pardo, M., Boix, D., Gascón, S., Compte, J., Bou, J., Obrador, B., Quintana, X. D. **Phytoplankton community structure in Mediterranean confined coastal lagoons: using ecosystem metabolism for trait quantification.** *Submitted.*



4.3.1. SUMMARY

We investigated phytoplankton community dynamics in a set of Mediterranean confined coastal lagoons during a hydrological cycle using a trait-based functional approach. Phytoplankton groups were identified by combining information obtained from microscopic identification, flow cytometry and high-performance liquid chromatography analyses. Moreover, different environmental variables and traits were used as explanatory variables to identify the most important variables explaining phytoplankton dynamics in these lagoons, utilizing a new version of Margalef's mandala as a reference. We were particularly focused on the quantification of productivity-related traits using ecosystem metabolism estimations, such as two different proxies for the estimation of the production to biomass ratio (P/B) and for the relative importance of *r*-*K* strategies that are commonly used only in a conceptual way but not quantified. Our results highlighted differences in phytoplankton composition between seasons due to the dominant hydrological pattern of flooding confinement in the salt marsh and between lagoons caused by their different nutrient availability. Salinity and grazing pressure arose as the most important variables explaining phytoplankton composition. Our results are consistent with a decrease in the P/B ratio and a prevalence of *K*-strategists with seasonal succession. In general, ecosystem parameter estimations are rarely combined with organism studies or trait-based approaches. This complementarity could provide a better understanding of aquatic ecosystem functioning and thus yield new insights for their protection in the face of future disturbances.

4.3.2. INTRODUCTION

In 1978, Margalef published a conceptual model in a two-dimensional space to explain the influence of physical and nutritional forces on phytoplankton life-form succession (Margalef, 1978). In that tentative plot, named “Margalef’s mandala”, a main distinction was made in the replacement between organisms able to grow in nutrient-rich, turbulent environments (*r*-strategists, as diatoms) and those able to grow under low nutrient concentrations and low turbulence (*K*-strategists, as dinoflagellates). With the presentation of this conceptual model, Margalef had introduced the now familiar ideas about functional groups and trait-based models to predict phytoplankton community composition (Litchman and Klausmeier, 2008). In recent decades, Margalef’s mandala has been continuously expanded and refined as a result of the scientific community’s desire to understand phytoplankton dynamics and how they will evolve in the face of emerging environmental changes (Cullen et al., 2002; Derolez et al., 2020b; Glibert, 2016). Phytoplankton constitute a very diverse and widespread group that play a key role in aquatic ecosystem functioning and have been considered an important biological indicator (Hemraj et al., 2017; Leruste et al., 2018; Seoane et al., 2011). In fact, the study of the drivers that regulate their assembly and dynamics continues to be of great interest to understanding global ecology (Derolez et al., 2020b; Péquin et al., 2017; Pulina et al., 2018; Villamaña et al., 2019).

Contrary to more traditional approaches in which studies provide by a full list of species, the study of phytoplankton based on a functional perspective relies on traits, environmental gradients, species interactions and performance currencies (Litchman and Klausmeier, 2008; McGill et al., 2006; Reynolds et al., 2002). The use of traits, which are measurable features that are directly and indirectly related to fitness (Kruk et al., 2020; McGill et al., 2006), has been tested in phytoplankton groups due to their relative simplicity and well-defined traits (Litchman and Klausmeier, 2008). Thus, trait-based models can contribute to understanding species distributions, as well as ecosystem functioning and processes from a functional and holistic approach (Litchman and Klausmeier, 2008; Smith and Smith, 2001). The development and accessibility of powerful analytical techniques for studying community composition, such as flow cytometry (FCM) and high-performance liquid chromatography (HPLC) (Jeffrey et al., 1997; Sosik et al., 2010), have contributed to improving taxonomic studies, as well as trait-based models (Chase et al., 2020; Leruste et al., 2018; Montes-Pérez et al., 2020; Péquin et al., 2017). Although FCM does not always allow taxonomic identification at the species level, it provides quantification of rare and small phytoplankton in addition to many useful characteristics or indicators that agree very well with trait-based approaches. Detection of some specific populations (e.g., anoxygenic photosynthetic bacteria), quantification and position of populations and individuals and physiological features (such as cell size and pigment content) are some examples of important indicators, which have been summarized by Gasol and Morán (2015). In addition, other methodologies, such as HPLC, are very good complements to FCM or, with the corresponding calibration for habitat type, can be useful techniques to identify phytoplankton (Jeffrey et al., 1997; Zapata et al., 2000).

In addition, linking phytoplankton traits at the community level with ecosystem measurements could improve our knowledge of ecosystem functioning. Ecosystem estimations, such as ecosystem metabolism, could contribute to quantifying productivity and related traits to improve trait-based models for phytoplankton composition studies. In fact, ‘productivity’, one of the most important features of Margalef’s mandala, has been included in revised versions (Derolez et al., 2020b; Glibert, 2016) but has been generally used in a theoretical context and rarely quantified. In recent years, aquatic ecosystem metabolism has gained importance when analysing ecosystem functioning (Hoellein et al., 2013; Staehr et al., 2012b) and has been estimated using a wide range of methods (Kemp and Testa, 2011). One of the most popular techniques (i.e., measuring diel free-water DO (Odum, 1956)) has had generalized usage because of its simplicity and the development of more reliable and affordable sensor technologies. Therefore, despite relying on several assumptions and uncertainties, it has become an excellent monitoring tool for studying ecosystem functioning (Staehr et al., 2012a, 2010a). This type of approximation relies basically on ecosystem processes but has rarely been used in combination with organism studies, trait-based approaches, etc., in phytoplankton composition research (but see Duarte et al. (2006) or Zwart et al. (2015)). Nevertheless, ecosystem metabolism measurements could contribute to bridging the gap between phytoplankton composition and ecosystem functioning, allowing trait quantification to overcome the lack of functional trait diversity quantification (Abonyi et al., 2018).

Coastal lagoons are productive and highly dynamic transitional ecosystems. Their ecosystem functioning and productivity are influenced by strong fluctuations in biological, hydrological, physical and chemical characteristics (Kennish and Paerl, 2010; Pérez-Ruzafa et al., 2019). In particular, Mediterranean confined coastal lagoons are peculiar habitats where metabolic variations and physical changes occur at high rates (Saccà, 2016; see also Chapter I) and are considered a good scenario for the development of the present study. However, the general difficulty of relating taxonomic composition to environmental factors is even more noticeable in these variable ecosystems, making their study and comprehension more challenging (Leruste et al., 2018). Thus, trait-based studies are a promising approach to study these habitats (Derolez et al., 2020b; Leruste et al., 2018; Marchini et al., 2008). Moreover, there is increasing interest in their understanding due to their particular vulnerability to global change (Brito et al., 2012a; Kennish and Paerl, 2010; Saccà, 2016) and how phytoplankton communities will adapt; thus, new approaches to study coastal lagoons may provide excellent tools for their preservation.

Therefore, the main objectives of the present study were 1) to analyse the phytoplankton community in a set of Mediterranean confined coastal lagoons located in La Pletera salt marsh during an entire hydrological cycle and 2) to identify the most important environmental variables and traits included in the newest versions of the mandala to explain the phytoplankton dynamics in these lagoons, emphasizing the use of ecosystem parameters (i.e., aquatic metabolism) for the quantification of productivity-related traits.

4.3.3. METHODS

4.3.3.1. Sampling and analyses procedure

The study was conducted during a complete hydrological cycle from July 2018 to August 2019 in six confined lagoons of La Pletera salt marsh (Figure 3.1.1). Information about the study site and lagoons characteristics is provided in section 3.1. By studying all these water bodies, we obtained a gradient from higher salinity, organic matter and nutrient values (BPI and FRA) to lower salinity values (G02), and lower nutrient contents (L01) (Table 4.3.1; Figures S9.1.11 and S9.1.12). Monthly water samples to analyse water characteristics and plankton composition were collected at a water depth between approximately 5-80 cm. Nutrient analyses were performed as described in section 3.4. Gilvin measurements were also recorded monthly. Gilvin, also known as CDOM (coloured, dissolved organic matter), was measured from 0.7-µm filtered water samples, according to Kirk (1994), as the simple value of absorbance at 440 nm using a spectrophotometer (Shimadzu, UV-1800). This value represents humic substances, more specifically, the soluble yellow substances generated after organic matter decomposition, that contribute significantly to the attenuation of light in fresh and marine waters (Kirk, 1994). Seagrass and macroalgae cover (shown in Table 4.3.1) were calculated using GIS software combined with orthophotographs, from which digital maps were created. All cartographic information was managed using the ESRI ArcGIS Pro programme. Aerial photographs were seasonally taken during the study period (October 18, December 18, May 19, and August 19). As monthly data were not available, these values were only used for lagoon characterization.

Table 4.3.1. Mean value and range (in brackets) of physical and chemical parameters measured in the surface waters of La Pletera lagoons during the study period (July 2018 to August 2019). DIN: dissolved inorganic nitrogen; N_{org}: organic nitrogen; P_{org}: organic phosphorus; TN: total nitrogen; TP: total phosphorus; and TOC: total organic carbon. *Surface values correspond to the mean level of the salt marsh. **Four values are provided for seagrass and macroalgae cover, which correspond to values recorded on October 18, December 18, May 19, and August 19, respectively.

	BPI	FRA	G02	L01	L04	M03
Water temperature (°C)	19.99 (2.30-34.60)	19.72 (1.82-35.41)	19.21 (1.14-35.33)	19.34 (1.62-33.78)	18.64 (1.93-33.27)	20.08 (2.42-35.81)
Salinity	53.27 (10.68-90.53)	45.26 (26.50-73.73)	25.64 (7.66-52.67)	15.43 (5.31-25.67)	23.52 (6.11-40.15)	48.00 (10.19-90.53)
pH	8.55 (8.24-8.95)	8.43 (7.92-8.82)	8.75 (8.19-9.79)	8.74 (8.00-9.59)	9.06 (8.44-9.84)	8.71 (8.00-9.66)
Chlorophyll-*a* (µg L^{-1})	98.89 (2.48-310.23)	71.43 (1.91-250.40)	38.54 (1.13-236.61)	0.73 (0.00-1.88)	13.15 (0.73-45.44)	6.41 (0.00-29.56)
DIN (mg N L^{-1})	0.30 (0.02-1.36)	0.40 (0.10-2.33)	0.15 (0.04-0.60)	0.92 (0.05-6.29)	0.09 (0.02-0.19)	0.16 (0.04-0.45)
PO_4^{3-} (mg P L^{-1})	0.05 (0.00-0.17)	0.04 (0.01-0.15)	0.04 (0.00-0.09)	0.01 (0.00-0.02)	0.02 (0.00-0.04)	0.04 (0.00-0.33)

	BPI	FRA	G02	L01	L04	M03
N_{org} (mg N L^{-1})	12.24 (2.89-30.23)	5.64 (2.33-13.19)	4.12 (1.29-10.89)	1.89 (0.54-3.28)	2.45 (1.10-4.49)	4.28 (1.92-7.65)
P_{org} (mg P L^{-1})	0.32 (0.08-0.63)	0.24 (0.03-0.70)	0.15 (0.01-0.41)	0.02 (0.00-0.08)	0.07 (0.01-0.20)	0.10 (0.01-0.44)
TN (mg L^{-1})	12.54 (3.06-30.31)	6.03 (2.61-13.51)	4.27 (1.33-11.19)	2.81 (1.49-8.89)	2.54 (1.19-4.62)	4.44 (2.03-8.11)
TP (mg L^{-1})	0.32 (0.02-0.64)	0.27 (0.05-0.86)	0.18 (0.02-0.45)	0.03 (0.01-0.10)	0.09 (0.01-0.23)	0.13 (0.01-0.77)
TOC (mg L^{-1})	127.89 (29.9-265.6)	45.48 (25.82-104.5)	36.70 (14.81-73.89)	16.98 (10.12-35.03)	22.10 (11.00-34.31)	30.62 (11.00-68.51)
Depth (m)	0.72 (0.42-1.14)	1.6 (1.41-2.10)	0.87 (0.61-1.35)	0.54 (0.35-1.01)	0.78 (0.57-1.21)	0.62 (0.34-1.05)
Surface (m^2)*	195	10326	2240	3345	5157	1905
Seagrass cover (%)**	0-0-0-0	0-0-0-0	0-41-27-46	14-10-13-85	66-88-30-89	0-0-0-0
Macroalgae cover (%)**	0-0-16-5	4-2-27-17	0-0-0-5	0-2-0-6	10-0-15-8	80-84-65-27

Continuous, high-frequency monitoring of different physical parameters was needed for the metabolism calculations. All the information about sondes deployment and monitoring of the different parameters is provided in section 3.2. Ecosystem metabolism estimations were computed using a modified version of the BAyesian Single-station Estimation (BASE) model described in section 3.3. All details about the model functioning, model selection and standardized metabolic rates calculation are also provided in that section. As phytoplankton samples were collected monthly, we calculated the monthly median value from the daily metabolic rates.

4.3.3.2. Flow cytometry and high-performance liquid chromatography analysis

FCM was used to calculate the abundance and biovolume of nanoplankton (3 - 20 µm) and picoplankton (<3 µm). We identified the different nano- and picoplankton groups according to their position in the FCM diagrams, that is, according to their body size and fluorescence signal, and main blooms were also verified using microscopy. In this sense, it is relevant to note that we observed different species in the same FCM region when they did not differ substantially in body size and pigment content. Thus, the different identified groups are actually not species, but "functional groups", and we will refer to them as "phytoplankton groups" here on assuming that species with similar body sizes and similar pigment contents would have a similar functional role in the aquatic ecosystem (Table 4.3.2).

FCM samples were fixed by adding 0.5 mL of paraformaldehyde (1%)-glutaraldehyde (0.05%) to a total volume of 4.5 mL of the lagoon's water filtered through a 50 µm mesh following Gasol and Morán (2015). The entire procedure used is described in López-Flores et al. (2009). Beads were used as internal standards, and all parameters were normalized to them. Data were analysed using BD CellQuest Pro software. The different parameters provided by the cytometer, such as FSC (Forward Scatter), SSC (Side Scatter), FL2 (Orange Fluorescence) and FL3 (Red Fluorescence), were recorded. As SSC is related not only to the internal structure of the organisms but also to cell size (Gasol and Morán, 2015 and references herein), we estimated cell volumes from SSC signals using a calibration curve. Different known phytoplankton cultures were run on the flow cytometer, and a linear regression was performed between the average SSC value of the phytoplankton population, as normalized to that of the beads, and the population mean volume obtained by inverted microscopy, similar to López-Flores et al. (2009) and references herein. If the same group of phytoplankton had different species detected by FCM (different populations), we found them sufficiently interesting to keep them separately, as they presented different FL3 signals. We added "low" or "high" to the group label to describe their FL3 signal (see Table 4.3.2).

To help with the identification of nano- and picoplankton-dominant species forming blooms, an analysis of pigments was also conducted. We used several diagnostic pigments to identify certain taxonomic groups following Jeffrey et al. (1997). Pigments detected when groups bloomed are listed in Table 4.3.2. Further details on pigment analysis can be found in section 3.5. Chlorophyll-*a* was used as an estimation of primary producer biomass.

4.3.3.3. Phytoplankton and zooplankton counting using inverted microscopy

To estimate the abundance and biovolume of microphytoplankton, which are poorly estimated using FCM (>20 µm) or even filtered during the FCM counting procedure (>50 µm), we used a Zeiss Axio VertA1 microscope. A volume of 125 mL of the water sampled without filtering was fixed with formaldehyde (10% final concentration) in an amber glass bottle. An inverted microscope was used to identify and measure the cells of a 30 mL subsample after sedimentation for at least 10 hours according to Utermöhl (1958). At least 100 individuals or 20 fields at 400x were counted for abundance estimations; for rare big specimens (>50 µm) a quarter, a half or the whole sample was examined at 100x. Measurements of linear dimensions of the cells (25 individuals per group) were used to estimate the biovolume from geometric formulae (Hillebrand et al., 1999). Although some identifications were performed at the genus or species level, phytoplankton cells were classified into the major algal divisions or classes for statistical analyses. Classifying in this way enables homogeneous groups that are comparable to nano- and picoplankton groups to be counted by FCM (see Table 4.3.2). If different species of the same group were identified by different techniques, we added "large" to the group label for those detected by microscopy. Sometimes microplankton lower than 20 µm were counted by microscopy ensuring that they were not counted by FCM.

The total biomass of small ciliates (<50 µm) was also estimated using the microphytoplankton counts described above. Zooplankton (>50 µm) samples were obtained by filtering a total volume of 5 L through a 50 µm mesh and fixed *in situ* in 4% formalin. Identification and counting of the different taxa were performed to the lowest taxonomic level without specimen manipulation using an inverted microscope. For each taxon found in the 50 µm counts, we measured 25 individuals to estimate the organisms' body size and biomass (dry weight). Crustacean biomass was estimated from the allometric relationship between weight and body length (Malley et al., 1989; McCauley, 1984). Rotifer and ciliate biomass was calculated by converting the volume into dry weight (Putt and Stoecker, 1989; Ruttner-Kolisko, 1977). See Cabrera et al. (2019) for more details on zooplankton processing. We distinguished between detritivores or nonselective bacterivorous filter feeders (e.g., harpacticoid copepods or rotifers with maleate or ramate mastax) (Nogrady, 1993) and selective filter-feeding algavores or predators (e.g., rotifers with virgate or incudate mastax or calanoid and cyclopoid copepods) (Einsle, 1993).

Table 4.3.2. Main features of the phytoplankton groups identified by microscopy and flow cytometry (FCM) with the support of high-performance liquid chromatography (HPLC) during the present study. ESD: Equivalent spherical diameter; FL2: Orange Fluorescence; FL3: Red Fluorescence. *Mean and standard deviation (in brackets) of ESD are provided. **Indicates that the corresponding group was composed of different organisms, which did not have the same characteristics; therefore, it should be noted that the attribution of flagella (motility) was based on the most abundant representatives. Classification of phytoplankton groups in Reynolds Functional Groups was performed following Padisák et al. (2009) and according to the last changes summarised by Kruk et al. (2020).

Phytoplankton group	Size classification	ESD* (µm)	FL2	FL3	Pigment(s) detected when blooming	Typical representatives	Reynolds Functional Groups	Potential phagotrophy	Flagella	Potential toxicity	Label
Euglenophytes	Microplankton	21.1 (1.9)	-	-	-	*Euglena*	W1	No	Yes	No	EUGLE
Dinoflagellates	Microplankton	21.5 (8.1)	-	-	Peridinin	*Oxyrrhis marina, Glenodinium, Gymnodinium, Scripsiella*	Y	Yes	Yes	Yes	DINO
Diatoms	Microplankton	17.3 (5.0)	-	-	Fucoxanthin	*Nitzschia*, *Navicula*, *Cyclotella, Gyrosigma, Achnanthes*	D	No	No	No	DIATlarge
Cyanobacteria	Microplankton	21.0 (9.1)	-	-	-	*Anabaena, Oscillatoria*	H1, T_C	No	No	Yes	CYANlarge
Prasinophytes	Microplankton	16.3 (2.5)	-	-	Chlorophyll-*b*, lutein	*Pyramimonas*	X2	No	Yes	No	PRASIlarge
Cryptophytes	Nanoplankton	15.0 (4.0)	High	High	Alloxanthin	*Hemiselmis, Chroomonas*	X2	Yes	Yes	No	CRYPTO
Haptophytes	Nanoplankton	9.4 (3.1)	Low	Low	Fucoxanthin, 19'-Hexanoyloxyfucoxanthin	*Pavlova*-like cells	X2	Yes	Yes	Yes	HAPTlow
Diatoms/ Prasinophytes	Nanoplankton	5.7 (1.4)	Low	High	Fucoxanthin	*Nitzschia closterium, Cyclotella, Pyramimonas*	D, X2	No	No**	No	AUTOhigh
Haptophytes	Nanoplankton	5.0 (2.1)	Low	High	Fucoxanthin, 19'-Hexanoyloxyfucoxanthin	*Prymnesium*-like cells	X2	Yes	Yes	Yes	HAPThigh
Photosynthetic bacteria	Picoplankton	3.5 (1.1)	Low	Low	-	*Chromatium*	V	No	Yes**	No	PHOTOB

Phytoplankton group	Size classification	ESD* (µm)	FL2	FL3	Pigment(s) detected when blooming	Typical representatives	Reynolds Functional Groups	Potential phagotrophy	Flagella	Potential toxicity	Label
Picoeukaryotes	Picoplankton	1.9 (0.5)	Medium	High	Chlorophyll-*b*, lutein	*Nannochloris*-like cells	K	No	No	No	PICOEUK
Cyanobacteria	Picoplankton	1.9 (1.2)	High	Low	-	*Synechococcus*-like cells	K	No	No	Yes	SYNEC

4.3.3.4. Traits included in the study and their quantification

We quantified several environmental characteristics and traits based on a new version of Margalef's mandala (Glibert, 2016), which are summarized in Table 4.3.3. They included physical and chemical variables, nutrient and organic matter concentrations, productivity-related traits, top-down control ratios and some other properties of the phytoplankton community. Hereafter, we can refer to 'traits' for all groups of variables included in the study (Table 4.3.3), despite environmental variables also being included. They were grouped differently according to the analysis performed, trying to avoid spurious results and circular analyses (see 'Group' in Table 4.3.3). All variables included in group 1 were included in the analyses (see '4.3.4.5. Data analyses' section below), and those included in group 2 were included only as supplementary variables. However, their inclusion as supplementary variables was due to two different reasons: to avoid collinearity problems among variables or because they were considered community-related variables (see columns 'Collinearity' and 'Community-related' in Table 4.3.3, respectively). In the 'community-related' group of traits, we included those that were calculated using phytoplankton data from the study and productivity-related traits quantified using ecosystem metabolism estimations. The 'A_GPP_{MAX}' ratio, included as a productivity-related variable, was used as a proxy for *r*-*K* strategy and needs more extended explanation. The BASE model used in this study estimates GPP by means of daily measured regressions between GPP and solar irradiation arriving at the lagoon surface using a typical photosynthesis-irradiance (P-I) curve. The model outputs include not only daily GPP values but also some parameters that have ecological meaning. One of these parameters is the 'A' parameter, which indicates primary production per unit light (i.e., photosynthetic efficiency) and under a growth curve model could be considered proportional to the growth rate (*r*) (Cullen, 1990; Litchman and Klausmeier, 2008; Zonneveld, 1998). Moreover, 'GPP_{MAX}', which is obtained from the standardization of GPP to maximum light, can also be understood as the carrying capacity of a typical growth model (*K*). Thus, the ratio between these two parameters could be used as a proxy to quantify an *r*-*K* axis for the ecosystem studied. For the phytoplankton/zooplankton ratio (Table 4.3.3), both of their biomasses were transformed to carbon units following Gaedke (1992). High-frequency DO measurements were used to calculate the daily variance of

DO saturation values (Table 4.3.3). Other properties of phytoplankton (‘toxicity’, ‘phagotrophy’ and ‘motility’) were calculated by assigning each property to the different groups of phytoplankton, based on previous literature (Flynn et al., 2013; Jakubowska and Szeląg-Wasielewska, 2015; Reynolds, 2006; Stoecker et al., 2017) (see columns ‘Potential toxicity’, ‘Potential phagotrophy’ and ‘Flagella’ for group assignments in Table 4.3.2) and calculating the corresponding ratios of the abundance (in biovolume) of the different organisms (potential toxics/nontoxics, flagellate/nonflagellated, and potential phagotrophs/nonphagotrophs). Pigmentation, turbulence and growth rate were other traits used in Glibert (2016) that we could not include in the analysis, as we did not measure them. All ratios were log transformed before any statistical analysis to avoid spurious correlations (Pawlowsky-Glahn and Buccianti, 2011).

Table 4.3.3. Summary of the different traits quantified in the present study and included in the redundancy analysis (RDA). In the column ‘Group’, a main distinction is made between the traits included in the RDA (Group 1) and those included in the RDA as supplementary variables (Group 2). The inclusion of the variables as supplementary can be done for different reasons (see ‘Collinearity’ and ‘Community-related’ columns). *For the sake of brevity, we referred to all variables included in the study as ‘traits’, although we also included environmental, physical and chemical characteristics. **Excluding nutrient content. N_{org}: organic nitrogen; P_{org}: organic phosphorus; TOC: total organic carbon; and DIN: dissolved inorganic nitrogen; DO: dissolved oxygen. For more details on some trait calculations and the analyses performed, see the ‘4.3.3. Methods’ section.

Type of traits*	Traits*	Label	Group	Community-related	Collinearity
Physical and chemical**	Temperature	T	1	No	No
	Salinity		1	No	No
	pH		1	No	No
	Depth		1	No	No
	Gilvin		1	No	No
	Daily variance in DO (%)	DOsat_var	1	No	No
Nutrients and organic matter	P_{org}/PO_4^{3-}	orgP_inorP	1	No	No
	N_{org}/DIN	orgN_inorN	1	No	No
	NH_4^+/NO_3^-	redN_oxN	1	No	No
	DIN/PO_4^{3-}	N_P	2	No	Yes
	TOC/TP	TOC_TP	1	No	No
	TOC/TN	TOC_TN	1	No	No
	DIN		2	No	Yes
	PO_4^{3-}		2	No	Yes
	N_{org}		2	No	Yes
	P_{org}		2	No	Yes
	TOC		2	No	Yes

Type of traits*	Traits*	Label	Group	Community-related	Collinearity
Ecosystem productivity-related parameters	Gross primary production	GPP	2	Yes	No
	Ecosystem respiration	ER	2	Yes	No
	GPP standardized to 20°C and light	GPP_{20MAX}	2	Yes	No
	Ecosystem respiration standardized to 20°C	ER_{20}	2	Yes	No
	ER/TOC	ER_TOC	2	Yes	No
	GPP/Chlorophyll-*a*	GPP_chla	2	Yes	No
	A (primary production per quantum of light)/GPP_{MAX} (GPP standardized to maximum light)	A_GPP_{MAX}	2	Yes	No
Top-down control	Algavorous/bacterivorous zooplankton	alg_bact	1	No	No
	Phytoplankton/zooplankton biomass	phyto_zoo	2	Yes	No
Phytoplankton properties	Cell size		2	Yes	No
	Motility		2	Yes	No
	Toxicity		2	Yes	No
	Phagotrophy		2	Yes	No

4.3.3.5. Data analyses

We used nonmetric multidimensional scaling (nMDS), based on the Bray-Curtis dissimilarity matrix, to represent sample differentiation based on phytoplankton abundance (biovolume units). Hellinger transformation was applied to the phytoplankton community data prior to analyses (Legendre and Gallagher, 2001). We also used categorical variables, such as 'lagoon' and 'season' for classification and visualization. Monthly samples were assigned to two different seasons: summer (May to September) and winter (October to April). To test whether the 'lagoon' and 'season' groups presented different phytoplankton compositions, we performed a permutational multivariate analysis of variance (PERMANOVA) and an analysis of multivariate homogeneity of group dispersions (variances). As the results were significant, post hoc Tukey's pairwise comparisons were performed. The relationship between phytoplankton composition and the different variables or traits (Table 4.3.3) was evaluated by redundancy analysis (RDA). The significant variables were selected using a forward selection of variables. The ratio NO_3^-/PO_4^{3-} was discarded prior to analysis, as the variance inflation factor (VIF) was higher than 10. This variable was included in group 2 (Table 4.3.3), as well as productivity-related traits, and was included in the RDA as supplementary variables. All traits were log transformed before analyses. Spearman correlations were computed to analyse the relationship between the different variables included in the study. The level of significance for all analyses was established at $p<0.05$, i.e., 95% confidence interval. Statistical analyses and plots were performed using the vegan and ggplot2 packages in R software (Oksanen et al., 2019; R Core Team, 2017; Wickham, 2016).

4.3.4. RESULTS

4.3.4.1. Physical and chemical characteristics

Apart from their higher water temperature, summer months were characterized by high salinity values (Figure S9.1.12). The lagoons that had higher salinity values were BPI, FRA and M03; in contrast to the L01, L04 and G02 lagoons (Table 4.3.1; Figure S9.1.12). During the summer period (from May to September), the water levels declined (Figure S93.1.12), coinciding with the scarcity of rainy days and other water exchanges. The FRA and G02 lagoons had greater depths, whereas L01 and M03 were the shallowest lagoons. These water level declines resulted in an increase in nutrient concentration, organic matter (Figure S9.1.11) and pigment (e.g., chlorophyll-*a*). Moreover, higher rates of metabolism (GPP and ER) were recorded during the summer period, although high variability of GPP was detected due to high DO variations (Figure S9.1.13). Oversaturation values were accompanied by extended periods of anoxia, especially in BPI, FRA and M03. Seagrass cover showed that *Ruppia cirrhosa* was present in G02, L01 and L04, and was more abundant towards the end of the study period. Macroalgae were more abundant in M03, having less cover at the end of the study period (Table 4.3.1).

4.3.4.2. Phytoplankton composition and seasonal dynamics

A total of 12 phytoplankton groups were identified for the six lagoons during the study period (Table 4.3.2). Temporal variations in the percentages of the different groups are plotted in Figure S9.1.14. The nMDS revealed a differentiation of phytoplankton groups between natural and created lagoons (Figure 4.3.1). In the upper part of the plot, the samples corresponding to the natural lagoons (BPI and FRA) are located. In the upper right are the sites corresponding to winter and with an abundance of PICOEUK. In the upper left are the samples corresponding to the summer and composed mainly of PHOTOB, especially in FRA lagoon. DINO was also present in both lagoons during this period (Figure S9.1.14), appearing on the left side of the plot (Figure 4.3.1). BPI summer samples were more disperse and had other group representations, such as CRYPTO, DIATlarge and PRASIlarge, especially in the summer months (Figures 4.3.1 and S9.1.14). PERMANOVA revealed differences in assemblages among lagoons ($F_{5,78}$= 4.59; *p*<0.01; Figure 4.3.2A) and seasons ($F_{1,82}$= 8.14; *p*= 0.01; Figure 4.3.2B). Tukey's pairwise test did not reveal significant differences (*p*= 0.1) between the natural lagoons. BPI and FRA lagoons presented higher distances to the centroid, followed by G02, the first lagoon to be created in 2002 (Figure 4.3.2A). Created lagoons did not present significant differences among them in Tukey's pairwise comparisons with the exception of L01, which presented significant differences to all lagoons (Figure 4.3.2A). L01 and G02 were both characterized by a high representation of haptophytes (HAPTlow and HAPThigh), while L01 was also composed of SYNEC year round and G02 by DINO, especially during the summer period (Figures 4.3.1 and S9.1.14). The L04 and M03 phytoplankton samples had higher dispersion than L01 (Figure 4.3.1). Both had high percentages of HAPTlow, SYNEC and DINO. Additionally, during late winter and early summer in L04, AUTOhigh reached >30% of the total biovolume (Figure S9.1.14). PHOTOB was also present (>40%) in the first month of the study period in M03. In general, BPI and G02 were the lagoons presenting higher dispersion (Figure 4.3.2A), and the phytoplankton composition between seasons for the created lagoons was not as different as that was for the natural lagoons (Figure 4.3.1).

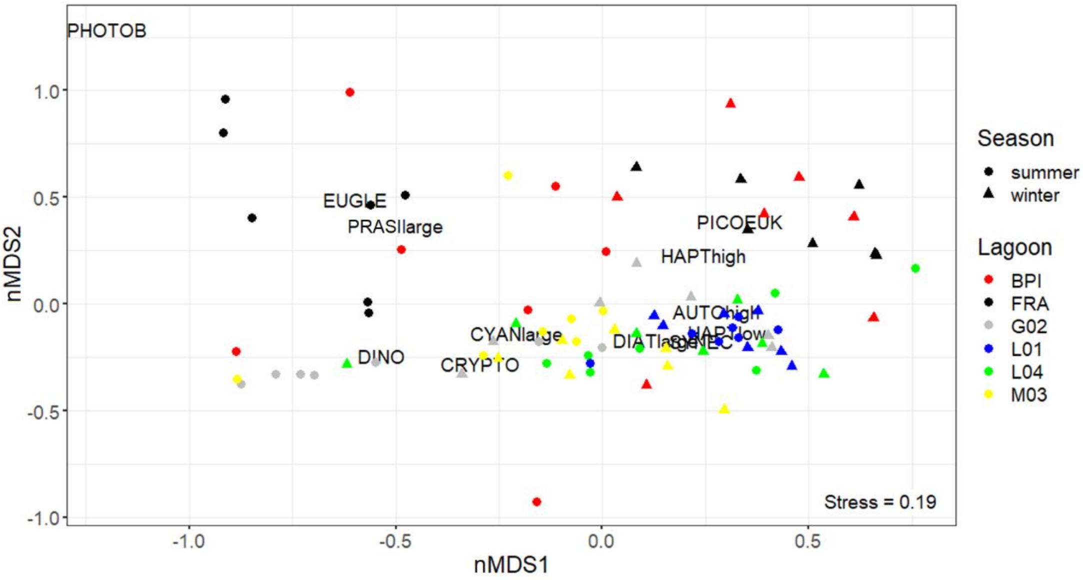


Figure 4.3.1. Results of the nonmetric multidimensional scaling (nMDS) analysis based on the Bray-Curtis dissimilarity matrix, to represent sample differentiation based on phytoplankton groups. For species group label meanings, see Table 4.3.2.

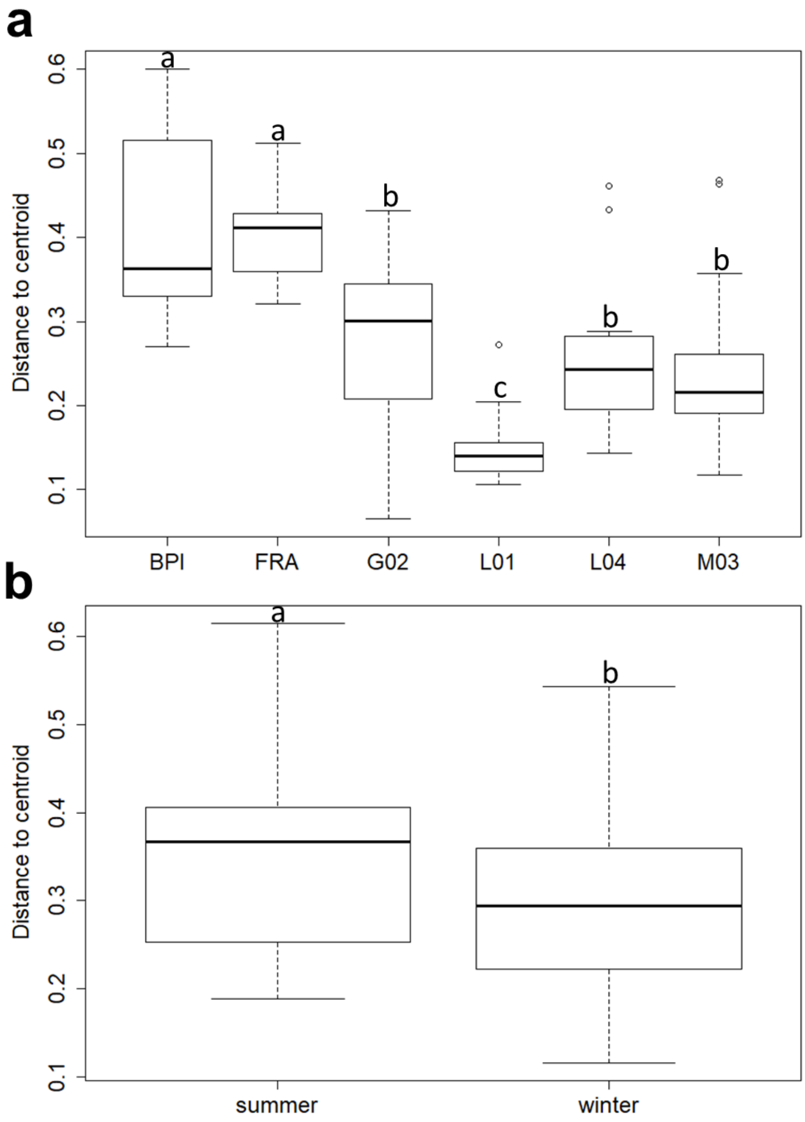


Figure 4.3.2. Representation of the sample distances to the centroid, based on Bray-Curtis dissimilarity, between phytoplankton groups depending on the lagoons studied (A) or the different seasons: summer (May to September) or winter (October to April) (B). Boxplots with the same letter are not significantly different.

4.3.4.3. Phytoplankton composition and traits

The variables included in the RDA (see Group 1 in Table 4.3.3) accounted for 39% of the variation in phytoplankton composition. The first two axes were significant, explaining 74% of the constrained variation. Overall, the first axis is related to physical changes, that is, the flooding-confinement pattern, and the second axis orders the samples according to their origin: natural lagoons (negative values) and created lagoons (positive values). Salinity and 'alg_bact', representing the grazing ratio between algal eaters and bacterivorous zooplankton, were the most significant variables, followed by depth, gilvin, temperature and pH (Figure 4.3.3A). Salinity was positively correlated with the first axis. Our results also indicated that increases in salinity usually coincided with higher water temperatures and in lesser strength with an increase in gilvin values (Figures 4.3.3A and S9.1.15) and that these situations predominantly occurred in summer, highlighting water conditions under confinement. Despite being nonsignificant, the orgP_inorP, orgN_inorN, redN_oxN and DOsat_var ratios (Figures 4.3.3A and S9.1.15) also indicated a higher concentration of organic forms and reduced forms of nitrogen during summer, as well as high variability in DO during confinement conditions. In contrast, during the winter period (flooding conditions), when salinity decreased, an increase in the grazing ratio between algal eaters and bacterivorous zooplankton occurred (Figures 4.3.3A and S9.1.15). Depth was negatively correlated with the second axis and to temperature and pH (Figures 4.3.3A and S9.1.15), indicating a greater depth but lower pH in natural lagoons. GPP and ER were located on the first axis but in opposite directions, showing higher ER rates during confinement (when lagoons also exhibited higher values of temperature, salinity, organic matter, nutrients, etc.) (Figure 4.3.3B). Other productivity-related traits, such as GPP_chla, GPP_{20MAX} and A_GPP_{MAX}, were negatively related to the first axis and to salinity and nutrients and presented higher values in the L04-, L01- and M03-created lagoons (Figure 4.3.3B). Phytoplankton trait motility, phagotrophy, cell size and toxicity were positively correlated with each other (Figure S9.1.15). Motility was represented similarly to temperature, showing a positive relationship with both axes and to other traits, such as the phyto_zoo ratio. Phagotrophy was positively related to the second axis and negatively correlated with salinity and gilvin, representing a higher number of phagotrophs in created lagoons. Cell size and toxicity were positively related to the second axis and negatively correlated with the first axis and with depth, salinity and gilvin; that is, with confinement conditions (Figures 4.3.3B and S9.1.15).

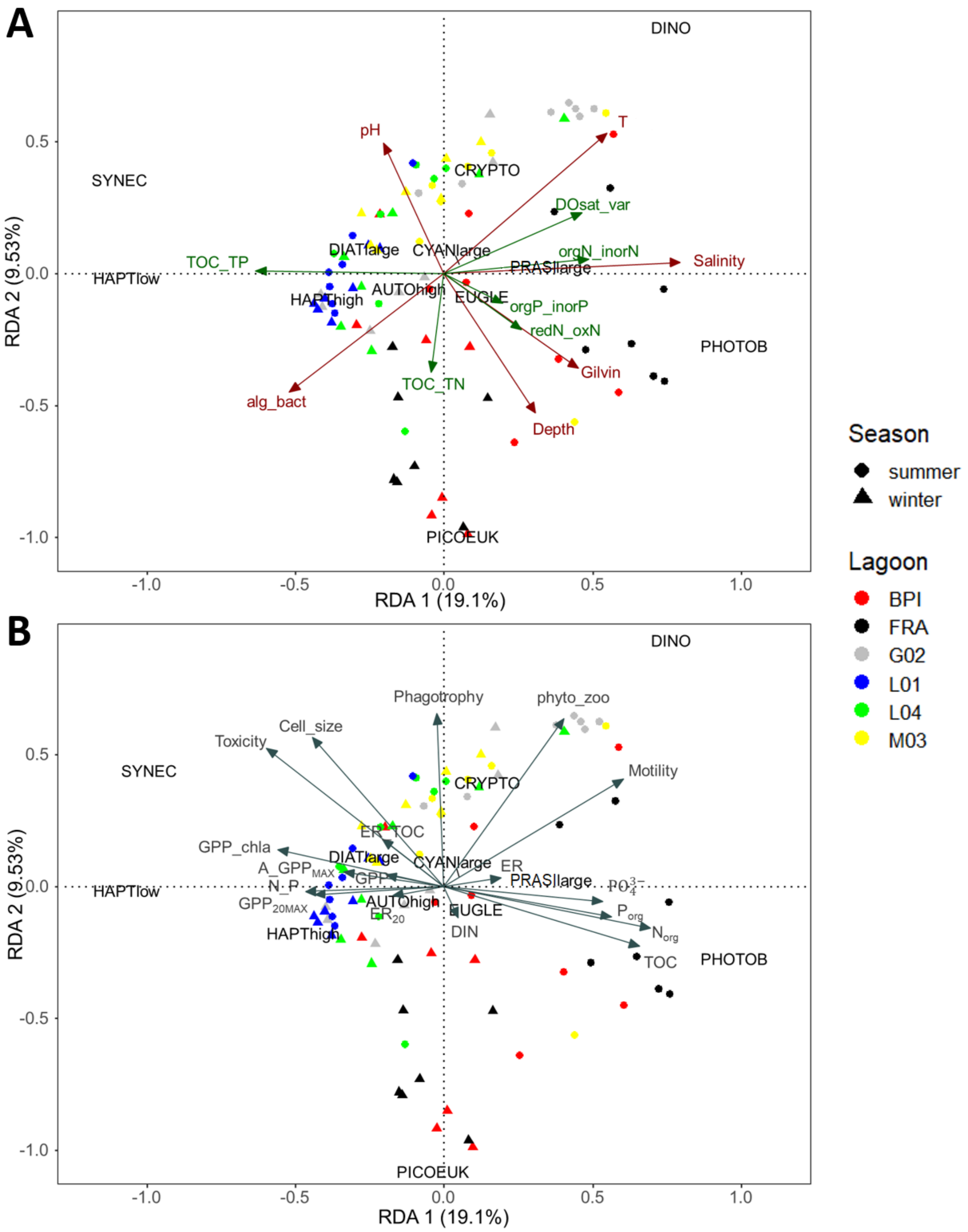


Figure 4.3.3. Results of redundancy analysis (RDA) on the relationships between phytoplankton composition and the first group of variables (A) and supplementary variables included in the second group (B) for the six lagoons in La Pletera salt marsh. See Table 4.3.3 for the variables or traits included in each group and label meanings. In (A), red and green vectors indicate significance and nonsignificance, respectively. In (B), supplementary variables are represented in grey. Species groups are indicated in black; see Table 4.3.2 for label meanings. N_{org}: organic nitrogen; P_{org}: organic phosphorus; TOC: total organic carbon and DIN: dissolved inorganic nitrogen. Eigenvalues (proportion explained in %) of the first two axes are expressed in brackets in the corresponding axes.

Species were displayed in the RDA in a similar way to the nonconstrained ordination (nMDS) (Figures 4.3.1 and 4.3.3), highlighting a positive correlation of the first axis with the DINO and PHOTOB groups; that is, with confinement conditions, and a negative correlation with HAPTlow and SYNEC, which were phytoplankton groups that were more abundant during flooding periods. The second axis showed a positive correlation with DINO and CRYPTO that were more abundant in the created lagoons and a negative relation to PICOEUK, which was mainly present in the natural lagoons.

4.3.5. DISCUSSION

4.3.5.1. Phytoplankton composition in confined coastal lagoons

According to our results, two factors, lagoon and season, play an important role in the phytoplankton composition of La Pletera lagoons. Seasonality was the main factor showing differences in phytoplankton composition between winter and summer as a result of the flooding-confinement pattern already described for this salt marsh. This pattern is related to seasonal changes in water inputs and salinity, with flooding events occurring in winter and dramatic decreases in water level and salinity increases due to evaporation during summer (Badosa et al., 2006; López-Flores et al., 2006a; Menció et al., 2017). The lagoon factor showed that created lagoons presented different phytoplankton compositions than natural lagoons during the studied hydrological cycle. The natural lagoons, BPI and FRA, are characterized by a higher concentration of nutrients and organic matter than created lagoons (Badosa et al., 2006; Cabrera et al., 2019) as a consequence of progressive accumulation during successive flooding-confinement processes. Thus, differences in phytoplankton composition between natural and created lagoons could be related to differences in nutrient availability. Moreover, oxygen monitoring and metabolism showed higher variations in the two natural lagoons during summer, characterized by extended periods of anoxia that could last several days, in which oxygen was lower than 5% (Chapter I). Overall, our results agree with previous studies on coastal lagoons that noted seasonality (Cañavate et al., 2015; Coelho et al., 2015), as well as salinity, temperature, transparency and nutrients, as the most important factor in determining changes in the phytoplankton community (Cañavate et al., 2015; Hemraj et al., 2017; López-Flores et al., 2014; Pulina et al., 2018).

Physical changes, represented as the flooding-confinement pattern, and the differences in nutrient availability between natural and newly created lagoons can be easily related to the two main axes of Margalef's mandala (Margalef, 1978). Taking into account these particularities of the confined Mediterranean coastal lagoons, the dominance of the main phytoplankton groups in La Pletera can be explained by species main ecological characteristics. BPI and FRA have blooms of phototrophic bacteria during the summer as a consequence of oxygen depletion even in surface waters. This situation has already been described in coastal lagoons with extreme organic matter concentrations (Fontes et al., 2011; Lamy et al., 2011; Madigan and Jung, 2009). In these two lagoons, dinoflagellates are abundant and are known to be dominant during stable conditions of maximum confinement, when nutrients are mostly in organic forms (Jiang et al., 2019; López-Flores et al., 2006b; Vandersea et al., 2018). Small picoplankton abound during the winter (flooding period) under high predatory pressure by calanoid grazers dominating during this time (Cabrera et al., 2019), probably favoured by a high growth rate (Bec et al., 2008 and references herein; Witt et al., 1981). The small size of the phytoplankton (PICOEUK, HAPTlow and HAPThigh) and the scarcity of seagrasses in these two natural lagoons could indicate a weaker efficiency in transferring energy and carbon compared with the other created lagoons due to the major complexity of the food web (Pulina et al., 2018). In the created lagoons, nitrogen-fixing cyanobacteria (SYNEC) and potential phagotrophic haptophytes (HAPTlow and HAPThigh) were more abundant, probably favoured by the lower nutrient availability, especially of inorganic nitrogen forms. Phagotrophy or atmospheric nitrogen fixation are frequent nutrient uptake strategies in Mediterranean confined salt marshes, where the imbalanced N/P ratio, specifically the NO_3^-/PO_4^{3-} ratio, is the rule rather than the exception thanks to faster denitrification than nitrification rates (López-Flores et al., 2014; Quintana et al., 1998; Quintana and Moreno-Amich, 2002). Strictly autotrophic, non-N fixers and *Nannochloris*-like picoeukaryotes appear mainly in natural lagoons, which are richer in organic matter, the decomposition of which might provide ammonium as an inorganic nitrogen source (Glibert, 2016; Schulien et al., 2017; Sunda and Hardison, 2007). Strictly autotrophic nano- and microphytoplankton, such as diatoms and chlorophytes, are generally rare in this type of salt marshes, limited by the lack of inorganic nitrogen (Quintana and Moreno-Amich, 2002).

In general, nano- and picoplankton represent important biovolume percentages in the salt marsh. Therefore, small phytoplankton should not be overlooked because, despite being considered important groups only in oligotrophic waters, they have been increasingly reported in brackish and eutrophic ecosystems (Paoli et al., 2007; Pulina et al., 2012, 2018). Despite the valuable diversity and ecological role of coastal ecosystems, studies of the entire phytoplankton community, including picoplankton, are rare (Pulina et al., 2018, 2012). Many studies have focused their attention on bloom formation groups (e.g., Glibert et al., 2001; Lomas et al., 2001; Montero et al., 2017) and, as highlighted by Pulina et al. (2017, 2012), less attention has been given to the entire phytoplankton community (but see Leruste et al., 2016) or to picoplankton (but see Bec et al. (2011, 2005) and Pulina et al. (2017, 2012)). The application of some techniques, such as cytometry and HPLC, may help to correct the bias of not including small-sized groups, which are rather abundant in eutrophic coastal waters (Carrick and Schelske, 1997; Pulina et al., 2017).

4.3.5.2. Phytoplankton traits

Several authors have already pointed out the advantages of using functional or trait-based approaches instead of a traditional taxonomic approach, providing a new perspective on how communities will respond to environmental changes (Kruk et al., 2020; Litchman and Klausmeier, 2008; McGill et al., 2006). This approach can be especially useful for the study of coastal lagoons, which rank among the most dynamic ecosystems on earth (Barnes, 1980; Kennish and Paerl, 2010; Kjerfve, 1994). In this sense, we took several variables and traits from a new version of Margalef's mandala by Glibert (2016) as a reference to study from a more functional approach how phytoplankton behave in confined coastal lagoons. We present an adaptation of the mandala to better visualize our RDA results with two main axes representing physical changes (flooding-confinement hydrological pattern) and nutrient availability (natural and created lagoons) (Figure 4.3.4). Glibert's mandala has an additional advantage when applied to confined coastal lagoons, since most of its traits are based on proportions or relative ratios rather than on absolute abundances. This is important in confined lagoons, where a high concentration of nutrients, organic matter or organisms might be achieved from two opposite processes: an increase in water and nutrient inputs or an increase in evaporation under extreme droughts (Àvila et al., 2019).

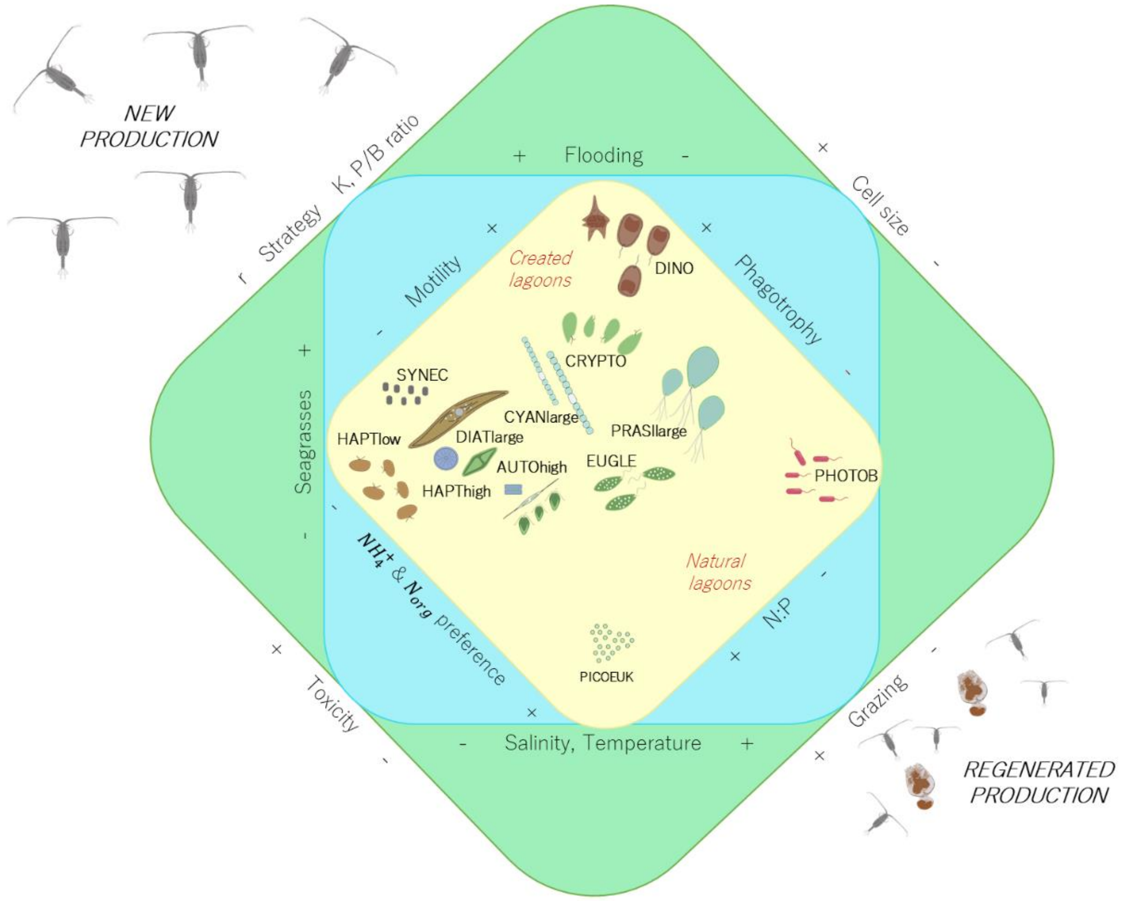


Figure 4.3.4. Conceptual diagram based on the revised version of Margalef’s mandala from Glibert (2016). For further explanation of axes meaning, see ‘4.3.3. Methods’. Groups of phytoplankton are represented following RDA results, but all scales are relative. The organisms’ icons were created with BioRender.com based on the typical representatives of the groups, and their size is relative. ‘Flooding’ and ‘Seagrasses’ were not included in the analysis, as they were not quantified monthly but are represented in the diagram to better understand lagoon conditions and analytical results. All responses within relative space are representative and are not meant to imply that all species or individuals in a given response zone will respond similarly.

Nutrient availability in La Pletera lagoons depends on both the lagoons’ origin and seasonal variability as a consequence of the seasonal flooding-confinement pattern. This highlights the importance of the hydrological pattern on the nutrient composition. Several nutrient ratios are related to the confinement gradient, such as a decrease in the N_P ratio and an increase in reduced forms of nitrogen and organic forms of nutrients with confinement, as shown in previous studies in the area (Àvila et al., 2019; López-Flores et al., 2014, 2006a; Quintana et al., 1998). Thus, whereas results agree pretty well with Glibert’s mandala, the N_P ratio presents discrepancies that could be explained as inputs of inorganic nitrogen into the salt marsh that occur mostly during flooding events in winter, similar to other coastal lagoons, are accompanied by a loss of nitrogen during more stable conditions due to denitrification (López-Flores et al., 2014). In fact, confined waters are characterized by an independent behaviour of nitrogen and phosphorus (Àvila et al., 2016; López-Flores et al., 2014), with more inorganic nitrogen availability when the total nitrogen concentration is low (flooding periods) and phosphorus reaching the highest inorganic and total concentrations under concentration processes (confinement). The lack of diatoms, probably caused by the lack of inorganic nitrogen in La Pletera salt marshes (López-Flores et al., 2006a; Quintana and Moreno-Amich, 2002) is one of the main differences when comparing our results with other coastal lagoons with more water turnover (Derolez et al., 2020b). Since diatoms are typical *r*-strategists, it seems that phytoplankton composition in La Pletera are reduced to more *K* species, with some kind of capacity to obtain nitrogen in less available forms, such as mixotrophy or N_2 uptake. Furthermore, confinement causes the accumulation of nutrients, but also of organisms, causing a decrease of the total amount of nutrients per unit of phytoplankton biomass. This leads to the paradox that nutrients in summer are more abundant, but less available. Thus, the apparent contrasting results of higher *K*-strategists with higher nutrient concentrations when comparing our data with Margalef's mandala and their re-examinations (Derolez et al., 2020b; Glibert, 2016) could be explained in terms of nutrient availability: *r*-strategists dominate with high nutrient availability, but low nutrient concentration, while *K*-strategists dominate under high nutrient contents, but with less availability.

The top-down control variables (alg_bact and phyto_zoo) also appear to be related to the two main features of the mandala. A clear seasonal replacement pattern between zooplankton dominant species has already been described in La Pletera lagoons with the dominance of large-sized, algavorous calanoids during winter (mainly *Eurytemora velox*) and the dominance of the small bacterivorous rotifer *Brachionus plicatilis* during summer, with higher zooplankton densities in the natural lagoons than in the newly created lagoons (Cabrera et al., 2019). Thus, grazing pressure by calanoids could be higher during winter in natural lagoons, which could also affect phytoplankton body size, since a higher predation pressure would favour the dominance of small picoplankton thanks to their faster reproduction rate (Capblancq, 1990; Litchman and Klausmeier, 2008). Regarding grazing within phytoplankton, the percentage of potential phagotrophic organisms strongly correlates with the second RDA axis, suggesting a competitive advantage of a phagotrophic strategy under less nutrient availability, especially under low nitrogen availability during confinement. Thus, under conditions of a severe lack of inorganic nitrogen, some groups could compensate for nitrogen scarcity with predation on bacteria or, as do dinoflagellates, on other phytoplankton (López-Flores et al., 2006a; Raven, 1997), which would agree with the absence of nanoplankton during this period.

4.3.5.3. Productivity-related traits

Ecosystem metabolism estimations have rarely been applied in combination with community composition studies (e.g., Murrell et al. 2018), but DO sensors provide measurable GPP and ER data that can be related to our multivariate species composition analysis to better understand patterns in phytoplankton species composition and to improve trait-based approaches. Furthermore, some terms, such as ‘productivity’, are frequently used in the literature when studying phytoplankton, but they are generally used in a conceptual way and are rarely measured (e.g., Coelho et al. 2015). Metabolic rates can also be combined with other biomass approximations, such as the total amount of chlorophyll-*a*, to obtain a quantifiable proxy for the production to biomass ratio (P/B) ratio, a term with important ecological connotations, but again, sometimes only used in a conceptual way.

When including the productivity-related traits in the RDA, they showed a negative correlation with the first RDA axis. This was not shown only for the metabolic rates but also the standardized rates and the GPP_chla ratio. This would agree with the general ecological theory of a decrease in the P/B ratio along succession, or in the case of phytoplankton, along seasonal succession (Margalef, 1980, 1968; Odum and Barrett, 1971), and suggests that these types of ratios can be helpful as proxies for productivity. However, their use should be taken with caution, since metabolic rates obtained from diel DO changes include not only production from phytoplankton but also from all other ecosystem primary producers. Seagrasses, macroalgae, biofilms and periphyton also contribute to total lagoon GPP, and high production in the water body does not necessarily indicate that phytoplankton production is high. In any case, we found a significant relationship in a shallow water coastal ecosystem, where the relative importance of benthic primary producers could be high (e.g., MacIntyre et al. 1996; McGlathery et al. 2001). Therefore, we can assume that this relationship would be even greater in strictly planktonic open waters, where GPP is limited to phytoplankton.

Another interesting feature arises from the BASE model used to calculate the metabolic rates in the present study (Giling et al., 2017a; Grace et al., 2015; see also Chapter I). Some parameters derived from ecosystem metabolism estimation can be useful for a community structure perspective, such as the ratio A_GPP_{MAX} used here (see '4.3.3. Methods' section), which provides a proxy to quantify the relative importance of the *r*-*K* strategy, a concept widely used in ecology but rarely quantified. In our results, A_GPP_{MAX} lined up with the first RDA axis, suggesting a gradual change in the *r*-*K* strategy related to the physical flooding-confinement gradient and agreeing with an increase in *K*-strategists during seasonal succession. Thus, automated oxygen sensors could provide a high-frequency, easy-to-obtain proxy for the relative importance of the *r*-*K* strategy in our aquatic community. However, again some issues should be taken into account. Some of them are in regard to the particularities of confined coastal lagoons because the flooding-confinement pattern represents the main seasonal gradient and, therefore, the main gradient of stability, but other short-term, low-intensity disturbances, may also affect the proportion of *r*- and *K*-strategists. Moreover, high organic matter accumulation under maximum confinement may lead to anoxic conditions (Chapter I), which are far from stable conditions. Other issues have taken a theoretical point of view because the relationship between P-I curves and growth model parameters can only be observed under certain assumptions: steady-state photosynthesis rates, photosynthesis rates adapted to growth irradiance, zero maintenance costs and fixed carbon:cell and chlorophyll:biomass ratios (Cullen, 1990; Litchman and Klausmeier, 2008; Zonneveld, 1998). However, none of these assumptions are actually true in natural conditions.

5. GENERAL DISCUSSION

5.1. Metabolism in Mediterranean confined coastal lagoons and the flooding-confinement pattern.

In recent decades, the affordability of reliable sensors has allowed ecosystem metabolism estimates based on high-frequency DO measurements to be included in a wide range of aquatic ecosystem studies (Jankowski et al., 2021; Staehr et al., 2012a). Ecosystem metabolism provides very useful information about habitat functioning and trophic status and can guide management and conservation policies (Kemp and Testa, 2011; Murrell et al., 2018; Staehr et al., 2010a). Thus, ecosystem metabolism measurements could be excellent tools for studying coastal lagoons, habitats with high variability, to provide a deeper understanding of coastal lagoon behaviour and enable their preservation. Despite these benefits, sensors and new modelling approaches have not been fully deployed in these shallow coastal ecosystems, while they could be especially informative when trying to determine ecosystem processes in these heterogeneous habitats (Beck and Bruland, 2000; Caffrey et al., 2014).

In the present thesis, the application of the diel free-water DO technique in a set of Mediterranean confined coastal lagoons of La Pletera salt marsh revealed important information about lagoon functioning and showed the benefits of using continuous DO measurements in this type of environment (Jankowski et al., 2021). Monitoring the diel flux of DO allows the estimation of metabolic rates (GPP, ER and NEP) and gas exchange with the atmosphere at subdaily and daily scales. Moreover, recording the DO minima occurring at night or just before dawn and the extended periods of anoxia in some of the water bodies was possible due to high-frequency measurements rather than discrete observations. This approach was particularly important, as periods with oxygen depletion were shown to be quite common during summer periods in these lagoons, often associated with *Chromatium* blooms. Although high temperatures during calm days seemed to be related to those anoxic periods (Chapter I), the specific conditions that trigger these processes and how the ecosystem recovers from them are still unknown.

In general, lagoons of La Pletera salt marsh show high coupling between GPP and ER, indicating that organic matter is produced and consumed in similar proportions. Despite a relatively balanced metabolism, shifts to autotrophy and heterotrophy are common, as described for other shallow ecosystems (e.g., Laas et al., 2012; McGlathery et al., 2001), but with no clear temporal pattern (e.g., Giordano et al., 2012; Howarth et al., 2014; see also Chapter I). La Pletera lagoons also exhibit high productivity values, which is consistent with the high production described for estuaries and coastal ecosystems (Caffrey et al., 2014; Kennish and Paerl, 2010; Murrell et al., 2018; Thébault et al., 2008). However, in practice, the results are difficult to scale appropriately, owing to the high spatial and temporal variability in these types of environments (Murrell et al., 2018). In fact, the present thesis has several limitations (Chapter I), such as no quantification of horizontal heterogeneity and anaerobic metabolism (Figure 5.1). Thus, metabolic comparisons across sites could sometimes be controversial, as estimations could vary considerably depending on the methodology used, the different periods of time and the properties of the aquatic systems under study (Cloern et al., 2014; Murrell et al., 2018). Taking all these factors into account, this type of study that incorporates the use of new technologies to better understand metabolism dynamics could improve active research on the role these small and shallow habitats play in global carbon budgets, especially with ongoing global change (Beck and Bruland, 2000; Caffrey et al., 2014).

An important part of the research on ecosystem metabolism has been focused on the drivers that govern metabolism dynamics, especially when some affect the influence of global change (Hallett et al., 2018; Solomon et al., 2013; Staehr et al., 2010b; Staehr and Sand-Jensen, 2007; Tonetta et al., 2016; Figure 5.1). The present thesis determined that the most important drivers of metabolism in La Pletera lagoons are temperature, light, water level, salinity and hydrology (Chapter I and II; Figure 5.1), which are the same as the most reported metabolic drivers for lentic ecosystems in the literature (Alfonso et al., 2018; Caffrey, 2004; López-Archilla et al., 2004; Murrell et al., 2018; Russell and Montagna, 2007). However, the results from Chapter I also indicated a lack of significance related to nutrient and organic matter contents in these lagoons, often described as important factors of metabolic variations (Caffrey, 2004; Staehr et al., 2016), and different explanations could be considered feasible. First, a saturation response of GPP to nutrient concentrations may occur in nutrient-rich habitats where changes in nutrient loading does not cause any increase in GPP values (Glibert et al., 2010; see also Chapter I). Second, certain lagged responses of metabolism to nutrients cannot be discarded but are difficult to prove at a monthly scale. Finally, for nutrient-enriched waters, physical conditions may become the most important variables influencing metabolic rates (Beck and Bruland, 2000); for instance, differences in irradiance or temperature will affect metabolism and may weaken statistical relationships with nutrients and other parameters (Staehr and Sand-Jensen, 2007). Certainly, in these particular coastal lagoons, seasonality is a very important factor, as in other Mediterranean regions, and the resulting dominant hydrological pattern of flooding (winter) confinement (summer) drives physical, chemical and biological variations in the salt marsh, as also shown in previous studies in the area (e.g., Badosa et al., 2006; Cabrera et al., 2019; López-Flores et al., 2006; Quintana et al., 1998). Thus, it is difficult to explain processes beyond this pattern, such as the response of metabolism to nutrient supplies, that may be masked by seasonality, as seen when analysing the relationship between hydrology and metabolism in Chapter II. Studying ecosystem metabolism and its main drivers in the Mediterranean confined coastal lagoons of La Pletera showed a high dependency of ecosystem processes on the flooding-confinement pattern. As hydrology may be altered by global change, understanding metabolism dynamics has become very important for predicting and assessing the future management of these water bodies.

5.2. The use of standardized metabolic rates in Mediterranean confined coastal lagoons.

In recent years, an increasing number of studies have analysed aquatic metabolism using the diel free-water DO methodology (Hanson et al., 2008; Staehr et al., 2012a). In conjunction with its development and application, several authors have addressed the different assumptions or difficulties of applying this technique (no component rates, air-water exchange, physical processes, metabolic quotients, etc.) (e.g., Kemp and Testa, 2011; Staehr et al., 2010a). Others have highlighted more specifically the limitations or problems of deploying sensors in shallow aquatic systems (spatial heterogeneity, macrophyte-rich areas, etc.) (e.g., Andersen et al., 2017; Obrador and Pretus, 2013). However, several aspects of rate calculation and standardization have been addressed less frequently in the literature and deserve some mention here.

As typical in Mediterranean regions, the metabolic data in the present thesis were very dependent on temperature, and the seasonal pattern sometimes masked the other processes under study; therefore, using standardized rates was particularly useful. One of the most common standardizations when working with metabolic rates is the standardization of a reference temperature, generally 20°C (e.g., Giling et al., 2017a; Holtgrieve et al., 2010; Solomon et al., 2013). In this study, apart from standardizing the temperature, GPP was also standardized to the maximum value of irradiance registered in the salt marsh during the study period, simulating conditions without light limitation. This type of standardization cannot be found very often in the literature (but see Hanson et al. (2008), Obrador et al. (2014) or Martinsen et al. (2017)), where an estimable P_{max} was already included in the model), but it was considered useful here to constrain rates to fixed values of temperature and irradiance, the two main drivers of seasonality and metabolism in these lagoons.

Standardization of metabolic rates has several advantages and could enable comparisons among metabolic estimates and avoid temperature effects at daily or seasonal scales (Holtgrieve et al., 2010). For the present study, standardizing allowed a more direct comparison of ecosystem changes over time and across water bodies. Standardized rate dynamics, such as GPP_{20MAX}, showed a higher production capacity during the winter, which may be explained by higher water exchanges. Moreover, they were considered particularly useful for their application when studying phytoplankton community structure. As an example, the combined use of GPP_{MAX} and another metabolic parameter derived from the model (A, photosynthetic efficiency) allowed us to estimate a popular dimension of Margalef's mandala, the *r-K* strategy axis, a parameter of high ecological relevance that has rarely been quantified at an ecosystem scale in previous studies.

Despite all these applications, some limitations also emerge from standardized rate calculations. There are three related but different ways of standardizing for temperature: van't Hoff Q_{10}, Arrhenius activation energy E_a and the temperature coefficient ϴ derived from a simplification of the Arrhenius equation (Demars et al., 2015). Regardless of which is more accurate, the main concern is that there is no fixed or universal value for 'Q_{10}', 'E_a' or 'ϴ', and in many studies, a constant is used without any justification (Demars et al., 2015; Holtgrieve et al., 2010). In this thesis, the Bayesian approach permitted us to have an estimated value of 'ϴ', so a modelled value could be used instead of a fixed value. However, some other limitations related to using this standardization must be mentioned. On the one hand, the estimated parameter 'ϴ' has some temperature dependence, as some seasonality could be observed in their distribution when using the estimated ‘Ѳ’ rather than a fixed value of ‘Ѳ’. On the other hand, although ‘Ѳ’ describes the temperature dependence of ER, it was also used for standardizing GPP, similar to previous studies (Giling et al., 2017a). It is known that GPP and ER differ in their temperature sensitivity, and recent studies have suggested higher activation energy for ER than GPP. Although the search for the temperature dependence of GPP and ER is in fact an active field of research (Demars et al., 2015 and references herein), it is likely that the model used here could be improved by adding complexity from other developed models (e.g., Padfield et al., 2017; Schindler et al., 2017). In any case, standardized rates were useful and revealed important information about the functioning of these ecosystems.

5.3. Lagoon restoration and future perspectives under global change.

Mediterranean confined coastal water bodies are heterogeneous habitats in the transition zone between the land and sea that have suffered intense degradation due to high anthropic pressure in the Mediterranean area. Specifically on the Catalan coast (NE Iberian Peninsula), there are still some confined coastal lagoons, but their number and surfaces have been reduced drastically over the years, as these habitats desiccate easily and are replaced with residential areas (Quintana et al., 2018). Given this critical situation and the value of these environments, some restoration projects have been developed to recover the ecological functioning of these ecosystems and enhance the biodiversity that depends on them, among many other goods and services. The Baix Ter Wetlands in Girona are a good example of an area where several restoration actions have been implemented. In La Pletera, two LIFE Nature restoration projects were developed (LIFE99NAT/E/006386, 1999-2003 and LIFE13NAT/ES/001001, 2014-2018). During the last project, the salt marsh of La Pletera recovered its functionality, substituting an abandoned urbanization from 1987 and creating new basins. In the present thesis, the two natural lagoons of La Pletera that were not urbanized (BPI and FRA) were studied in addition to four lagoons that were selected from the created basins (G02, created in 2002; L01, L04, M03, created in 2016).

The study of the phytoplankton community in these lagoons revealed two main factors of variation: season and lagoon (Chapter III). As previously noted, the flooding-confinement pattern dominant in La Pletera salt marsh exerts a strong influence on the ecosystem functioning; therefore, phytoplankton composition and ecosystem metabolism also showed differences between winter (flooding) and summer (confinement) periods. These results agree with those of previous studies in the area that showed that nutrient, phytoplankton and zooplankton dynamics are very dependent on the hydrological pattern (e.g., Badosa et al., 2006; Cabrera et al., 2019; López-Flores et al., 2006; Quintana et al., 1998). However, although seasonality could be considered the main factor governing the dynamics of the salt marsh, differences between natural and created lagoons were also detected, contrary to results from zooplankton studies (Cabrera et al., 2019). Interestingly, the phytoplankton composition of the first created lagoon (G02) was more similar to that of the natural lagoons (BPI and FRA). Thus, based on these results, two different types of succession could be occurring here. One type of succession could be 'seasonal succession' which lagoons experience each hydrological cycle, and the other type is related to 'temporal succession', showing the differences due to the origin or age of the water bodies. The evolution of the G02 lagoon would suggest similar evolutions for the remaining lagoons. As stated by Badosa et al. (2006), a progressive accumulation of organic contents will take place during successive confinement events over years, especially when there are no continuous surface freshwater inputs, rain is low and irregular, and waters remain confined.

However, how restoration will be implemented and how these confined coastal lagoons will respond in the next decades are still unknown. As seen previously, if conditions are similar to current conditions, then created lagoons would show similar phytoplankton composition to that in the natural lagoons (with general dominance of picoeukaryotes and anaerobic bacteria), registering higher depletion of oxygen and a substitution of seagrasses by macroalgae, as a result of confinement processes. However, natural and temporal succession will be conditioned by future changes expected from climate change (IPCC, 2014).

Many aquatic ecosystems will be endangered, as these alterations will imply higher temperatures and sea level rise and an increasing number and magnitude of extreme events (Harley et al., 2006; He and Silliman, 2019; IPCC, 2014; Lu et al., 2018). The studies conducted in these confined coastal lagoons seem to indicate that these lagoons will probably respond differently to other aquatic ecosystems as a result of their own nature and the extreme conditions that influence them. They are adapted to the Mediterranean climate, in which the flooding events that occur during winter follow no pattern, and summer months result in lagoons that are isolated for long periods of time, with a significant part of their surface being dry. Thus, the biodiversity they hold would present certain plasticity to future perturbations and would adapt more easily than that in other areas (Pérez-Ruzafa et al., 2019). As an example, high salinities and perturbations allow endangered and endemic Iberian toothcarp (*Aphanius iberus)* to remain dominant in the water bodies of La Pletera over the exotic eastern mosquitofish (*Gambusia holbrooki*), which is less tolerant to salinity changes. Thus, a future increase in sea water level would increase water salinity in the lagoons, favouring the Iberian toothcarp over mosquitofish. Additionally, some disadvantages derived from climate change could be compensated by other changes that may reduce their consequences, such as a higher number of flooding events, offsetting the accumulation of organic contents and oxygen depletion in these lagoons caused partially by higher temperatures. From a global change point of view, in comparison to climatic changes, man-made alterations arise as more important threats to the conservation of these habitats. In fact, saltmarsh surface reduction and/or eutrophication caused by man-made changes in lagoon morphology and hydrology are the main causes of losses or degradation of confined coastal habitats, and these impacts have already occurred in these habitats for decades. Thus, the greatest problem these lagoons face is the interaction between anthropic pressure and climate change. The high level of pressure from urbanization and the degree of eutrophication these lagoons experience may compromise their capability to respond to climate change. For instance, problems due to sea level rise would be more related to urbanization, as lagoons are restricted and cannot occupy larger areas, rather than to the water level rising *per se*.

Addressing global change will require more research and major efforts related to the management and conservation of these particular ecosystems. In our attempt to preserve these heterogeneous habitats and the biodiversity and services they contain, the present thesis provides a better understanding of the ecosystem functioning of these lagoons based on the application of new technologies and ecosystem approaches.

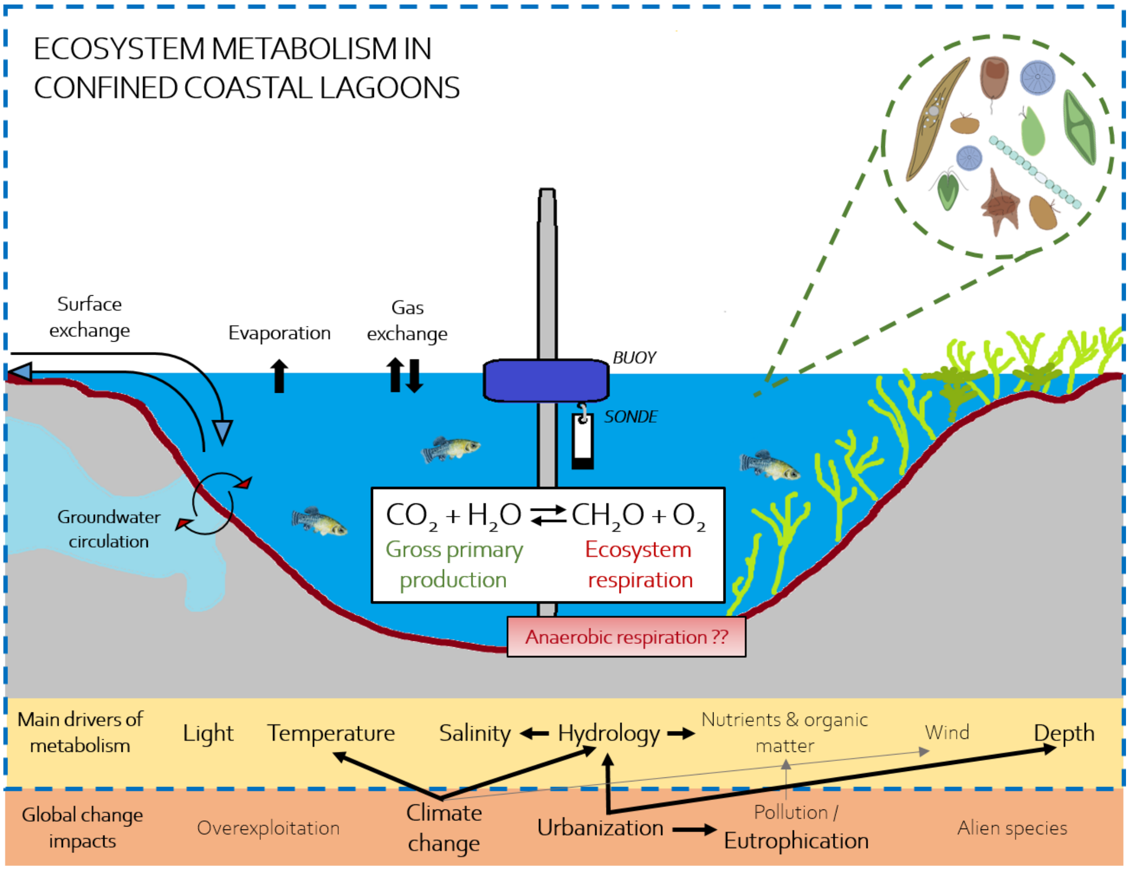
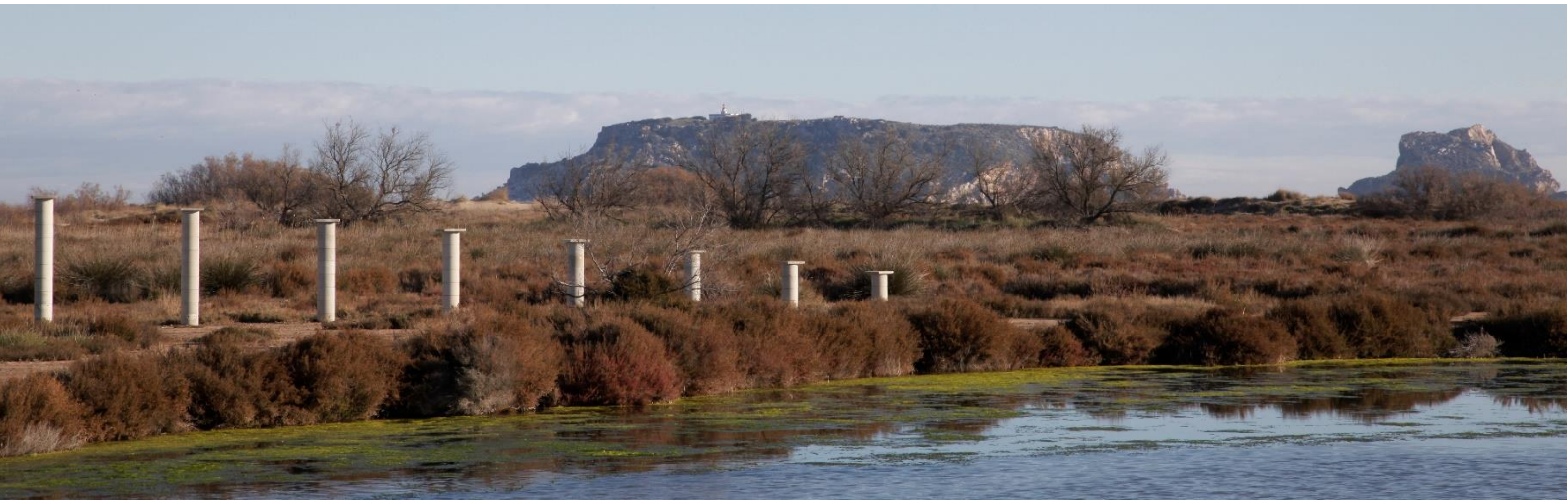


Figure 5.1. Redrawn of Figure 1.1 representing the ecosystem metabolism estimations (gross primary production (GPP) and ecosystem respiration (ER)) in coastal lagoons. Here, we highlighted (in bold) the drivers that this study showed to be important in the ecosystem metabolism of La Pletera and the global change impacts that may influence them (arrows in bold). In the red squares, we highlighted the anaerobic metabolism that was not analysed in the thesis. Adapted from Jankowski et al. (2021).

6. CONCLUSIONS



- In La Pletera lagoons described here, the DO and other environmental parameters, such as temperature, conductivity and chlorophyll-*a*, varied seasonally in response to the Mediterranean climate and the flooding-confinement dynamics present in the salt marsh.
- The DO ranged from supersaturation to anoxia, with anoxic conditions extending to the surface and lasting for several days, especially during the summer season. Accordingly, the metabolic rates (GPP and ER) showed higher values and higher variability during summer. However, the standardized rates showed that the higher potential productivity of these lagoons occurs during the winter, when water inputs enable nutrient loading. This characteristic might be a limitation due to the low temperature that turned out to be the primary driver of variations in metabolic rates.
- Both lagoons showed slightly heterotrophy during the study period. Although GPP:ER values close to 1 could indicate some balance between the rates, there is an underestimation of heterotrophy while using the diel oxygen method, since it does not include anaerobic respiration. Despite that underestimation, the production measurements revealed that the metabolic rates in these lagoons rank among the most productive aquatic ecosystems for which there are published data.
- Owing to the high concentrations of nutrients, no significant relationship between metabolism and nutrients was found, which supports the idea that primary production was at saturation when nutrient concentrations are high, as is the case of the coastal lagoons of La Pletera.
- Wavelet analyses showed a lack of significance and uniformity in the response of metabolic rates to hydrological changes, especially at high frequencies, and only the strong annual oscillation corresponding to seasonality during the study period was significant and robust. Although the annual oscillation could indicate a lagged response of metabolism to the water exchanges during winter, a causal relationship cannot be firmly established.
- Standardized rates showed different results, and GPP_{20MAX} presented significant responses at higher frequencies (i.e., to sea storms), indicating a high production capacity during winter, that is limited by physical constraints (namely,

temperature and light). Therefore, GPP_{20MAX} presented a certain dependency on water exchanges (and nutrient supplies), although the responses did not always follow the same pattern and could be absent.

- Despite the difficulties in studying hydrology as a driver of ecosystem metabolism in Mediterranean confined coastal lagoons, the combination of two types of long-term data provides new insights into the functioning of aquatic ecosystems and, hence, information for deciding upon management and protection measures prior to the occurrence of hydrological modifications caused by global change.
- Season and lagoon origin were observed to be important factors determining the phytoplankton community composition in these confined coastal lagoons. The former factor concerns the flooding-confinement pattern dominating this salt marsh. The latter factor is due to the difference in nutrient availability in the natural and created lagoons. These influencing factors (physical change and nutrient availability) were easily related to the two axes of Margalef's mandala.
- Anoxic conditions during confinement periods in the natural lagoons were characterized by blooms of phototrophic bacteria, while picoeukaryotes were abundant in winter. Created lagoons with lower nitrogen availability were characterized by the presence of nitrogen-fixing cyanobacteria as well as phagotrophs, such as dinoflagellates and haptophytes.
- Physical characteristics and productivity-related traits were determinant for predicting community composition. We proposed the use of parameters obtained from ecosystem metabolism estimations, such as the quantification of the GPP_chla ratio, as an estimation for the P/B ratio and the ratio A_GPP_{MAX}, as a proxy for the relative importance of the *r*-*K* axis, as tools for the improvement of trait-based models, providing an ecosystem-functioning point of view. A decrease in the P/B ratio and a prevalence of *K*-strategists with seasonal succession has been stated using these ratios.
- A better understanding of ecosystem functioning could provide new insights into how aquatic ecosystems, and more specifically these highly variable coastal lagoons, will respond to changes in physical and chemical characteristics and planktonic assemblages associated with global change.

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9. SUPPLEMENTARY MATERIAL

9.1. SUPPLEMENTARY MATERIAL (CHAPTER I, II AND III) - FIGURES

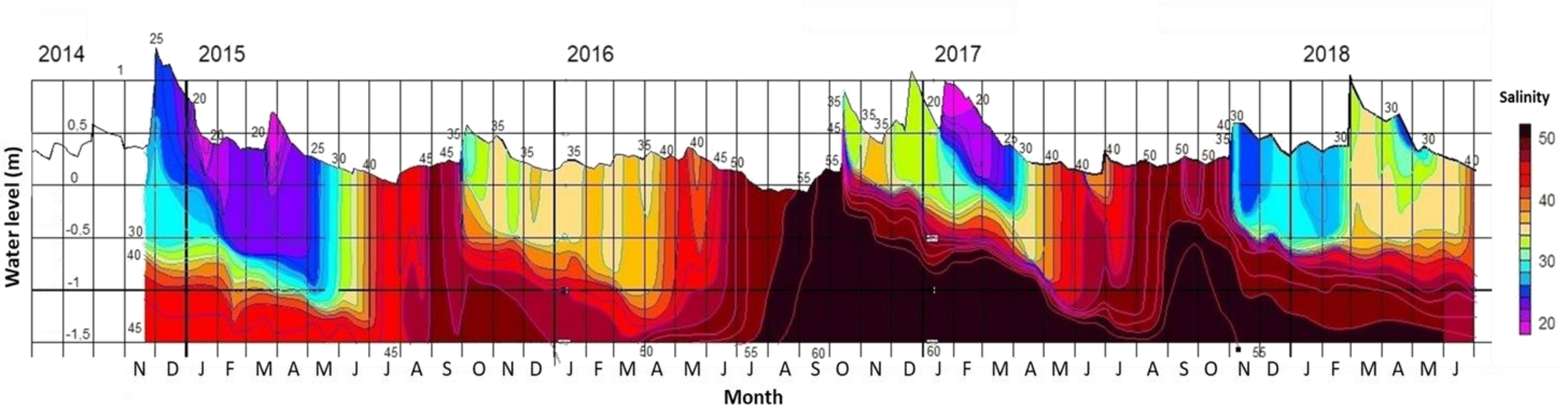


Figure S9.1.1. Temporal changes of water level and salinity in FRA lagoon from November 2014 to June 2018. Note the salinity values decrease when water level increases (flooding events) and the high values of salinity during summer months (confinement conditions). Also, it should be appreciated the stratification of the water, especially during winter time, with an increase in salinity with depth. Thick black lines separate different years. Adapted and updated from Quintana et al. (2018).

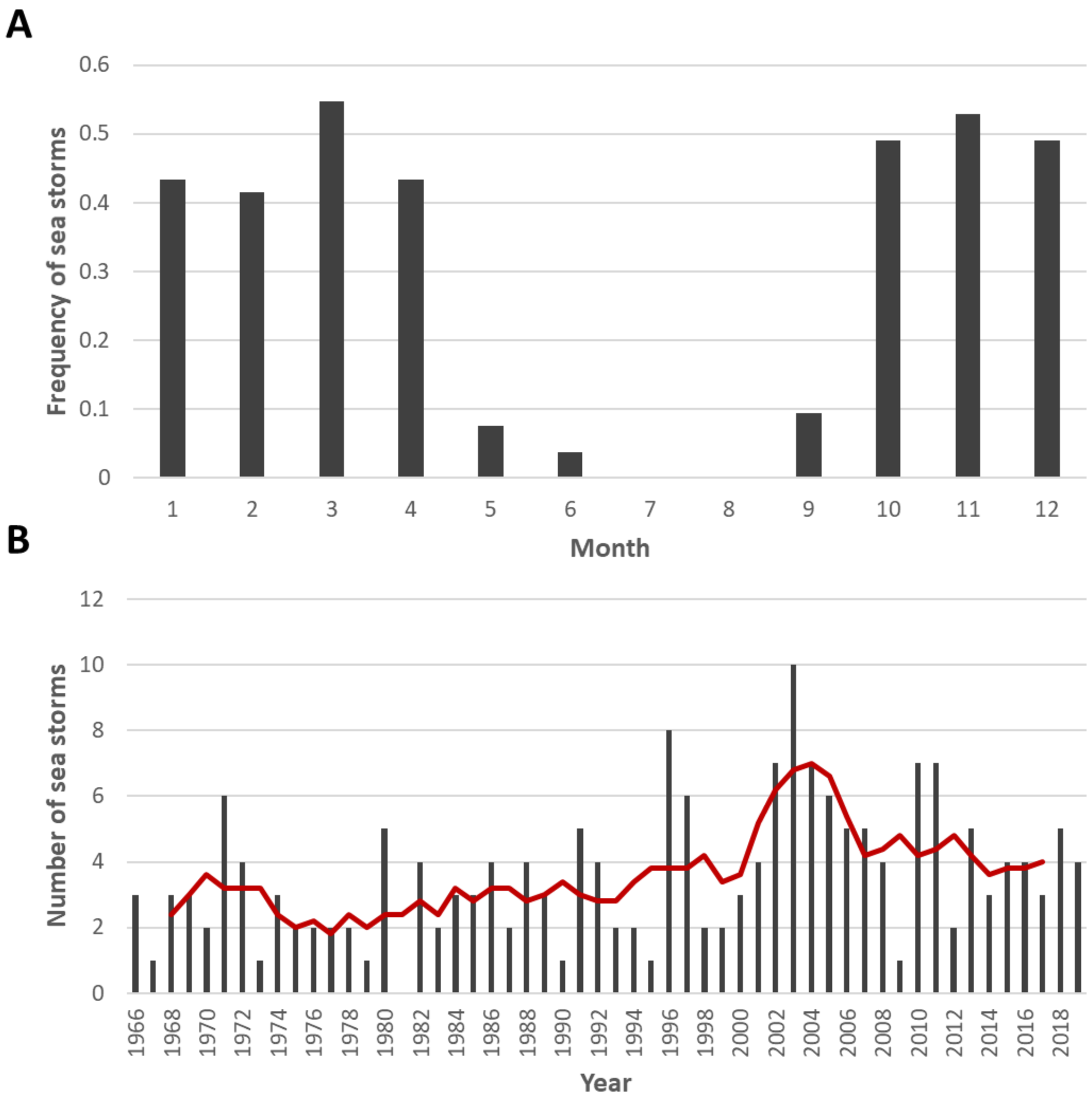


Figure S9.1.2. Frequency of sea storms per month (A) and number of sea storms per year (B) in L’Estartit (Torroella de Montgrí, Girona) from 1966 to 2019. The red line in (B) represents a 5-year moving average. Only sea storms of ≥3 metres are considered here. Figure adapted from Pascual (2020).

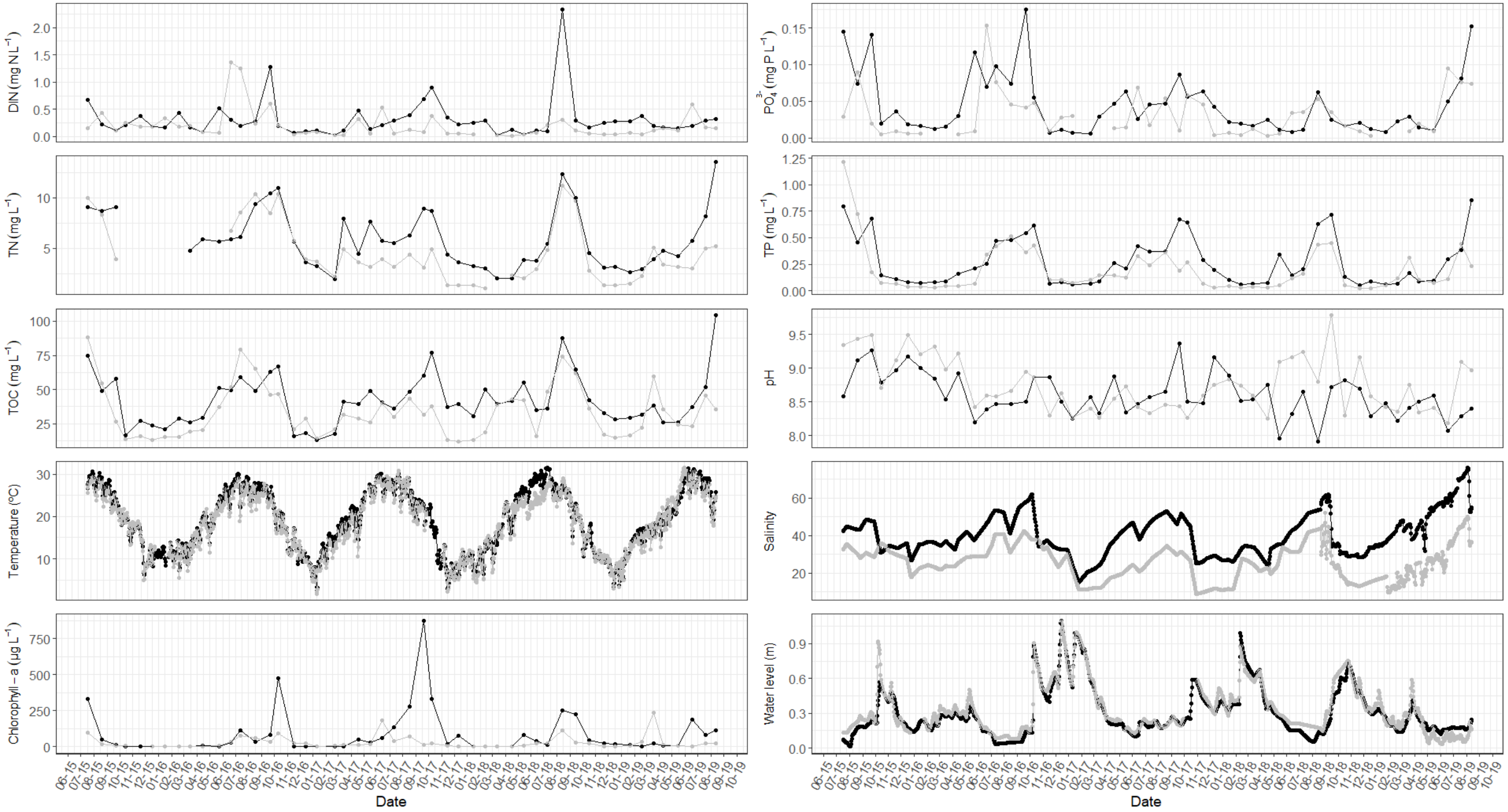


Figure S9.1.3. Temporal variation in different parameters registered from July 2015 to September 2019 in FRA (black) and G02 (grey) lagoons. The values for salinity, temperature and water level (height above average sea level) are daily, and the rest of the variables represent monthly measurements. When daily values of salinity were not available daily values were obtained by interpolation. DIN: dissolved inorganic nitrogen; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus. The X-axis labels indicate the month-year.

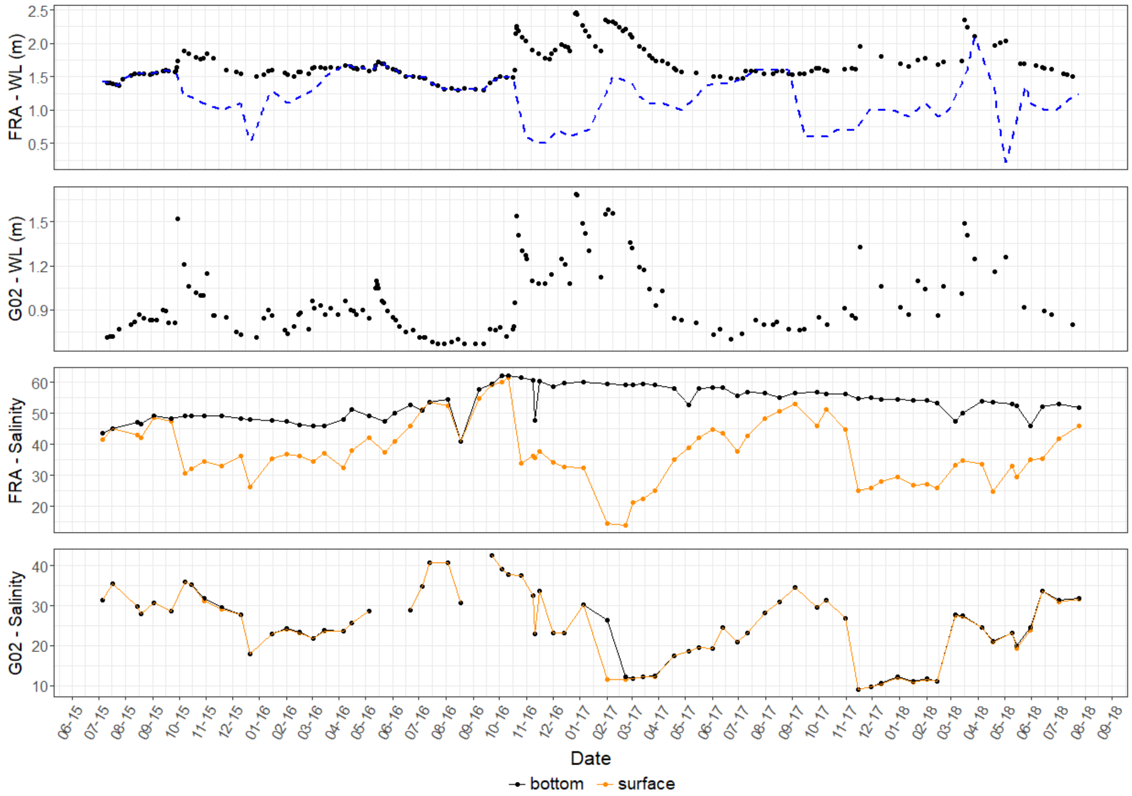


Figure S9.1.4. Water level (WL) and salinity values measured manually every 10-15 days are represented for both lagoons. The Z_{mix} for FRA (dashed blue line) is also shown. Note that during the winter, more differences in the salinity between the surface (orange) and bottom (black) are observed. The X-axis labels indicate the month-year. See Casamitjana et al. (2019) for more details in salinity stratification.

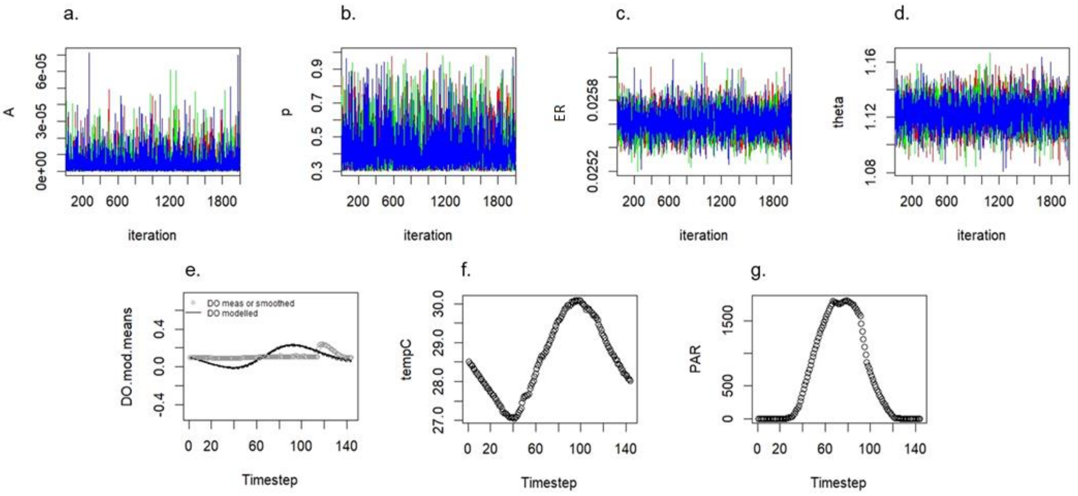


Figure S9.1.5. Example of a BASE program output used for model validation. It shows an anoxic day (<5% of oxygen saturation) with good parameter convergence (A, p, theta, and ER) but was discarded due to poor fit ($R^2<0.4$). The different estimated parameters are represented in the upper graphs (a. to d.) with converged and stationary chains. 'A' refers to the daily constant that represents the primary production per quantum unit of light (a.), 'p' is the coefficient that reflects the ability of primary producers to use the incident light (b.), 'ER' corresponds to the ecosystem respiration (c.) and 'theta' is the coefficient for temperature dependence (d.). The lower graphs show the measured (empty circles) and predicted (black line) dissolved oxygen (DO) curve for each diel period (e.), the measured temperature (tempC, (f.)) and PAR (photosynthetically active radiation, (g.)) data. Thus, every 'Timestep' in (e.), (f.) and (g.) corresponds to 10 minutes (144 for a day).

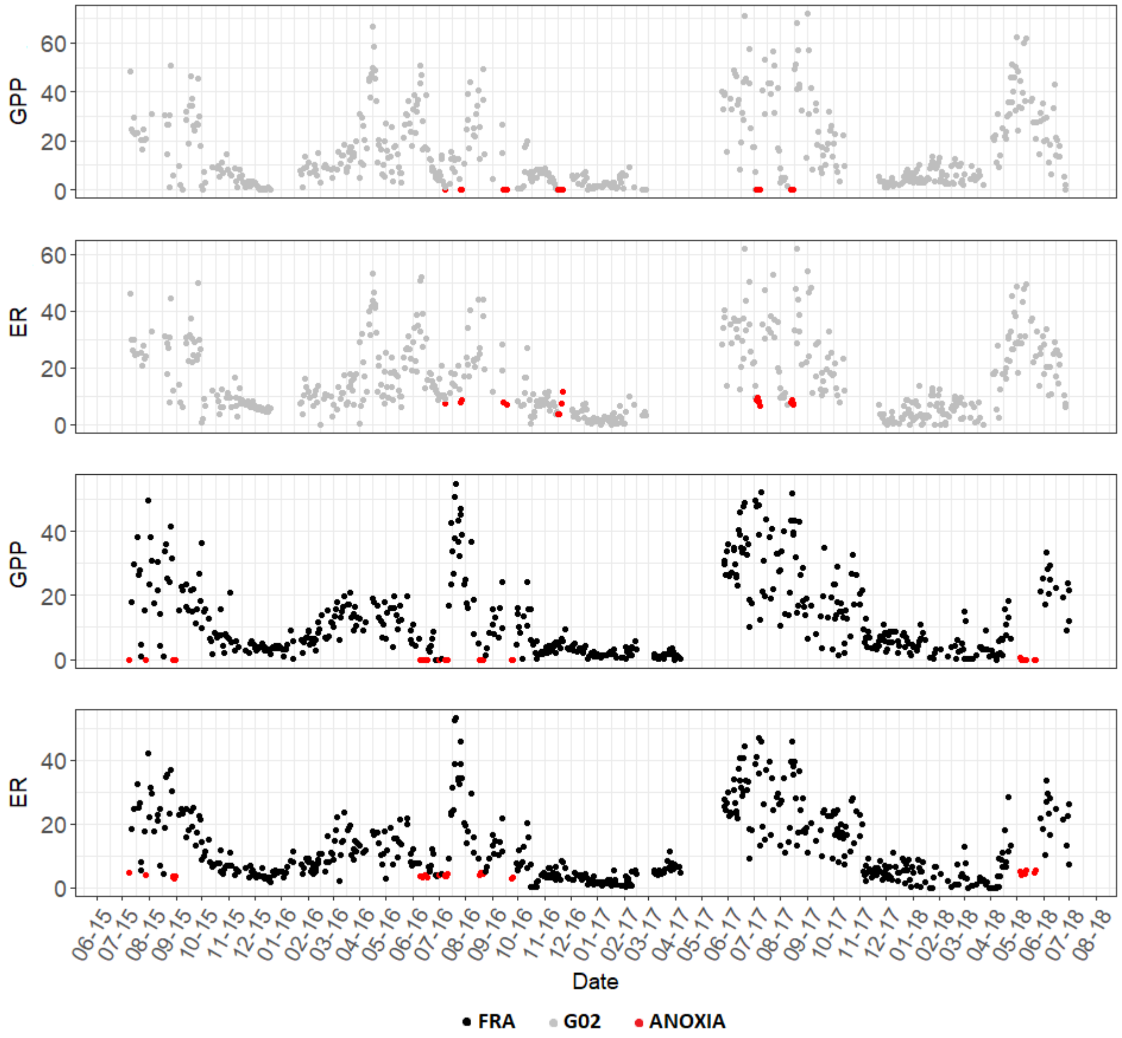


Figure S9.1.6. Daily gross primary production (GPP) and ecosystem respiration (ER) for both lagoons over the study period. Days with <5% of oxygen saturation (anoxia) but with a good fit (R^2>0.4 and good convergence of parameters) are represented in red. They primarily occur during the summer periods. Rates are shown in mg O_2 L^{-1} d^{-1}. The X-axis labels indicate the month-year.

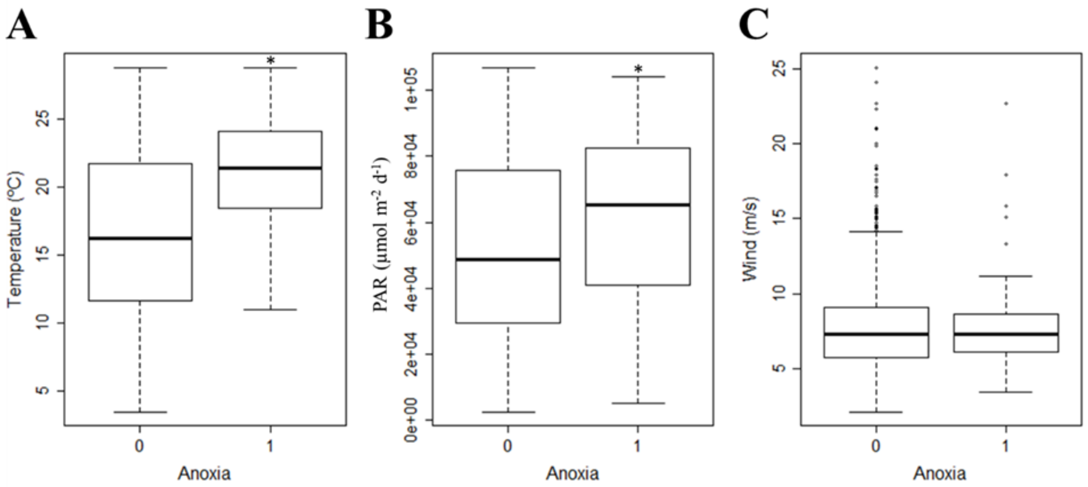


Figure S9.1.7. Mean daily values for the temperature (A), photosynthetically active radiation (PAR) (B) and wind (C) on days with anoxia (value of “1” in X-axis) and without anoxia (value of “0” in X-axis). Significant results (*p*<0.05), indicated by (*), showed that days with anoxia are characterized by high temperatures and PAR. The PAR values represented in the figure are the accumulated data.

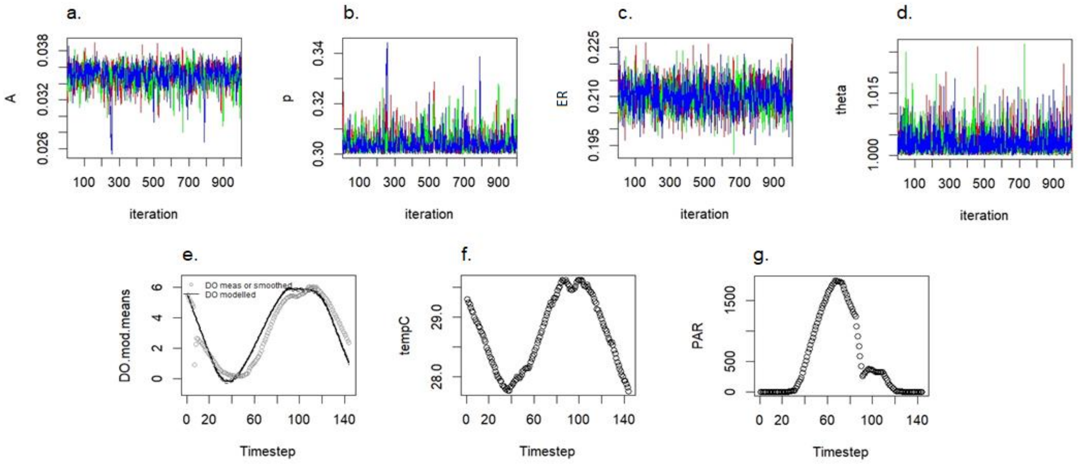


Figure S9.1.8. Example of a BASE program output used for model validation. It shows a day with good parameter convergence (A, p, theta, and ER) and a good fit (R^2>0.4). The different estimated parameters are represented in the upper graphs (a. to d.) with converged and stationary chains. ‘A’ refers to the daily constant that represents the primary production per quantum unit of light (a.), ‘p’ is the coefficient that reflects the ability of primary producers to use the incident light (b.), ‘ER’ corresponds to the ecosystem respiration (c.) and ‘theta’ is the coefficient for temperature dependence (d.). The lower graphs show the measured (empty circles) and predicted (black line) dissolved oxygen (DO) curve for each diel period (e.), the measured temperature (tempC, (f.)) and PAR (photosynthetically active radiation, (g.)) data. Thus, every ‘Timestep’ in (e.), (f.) and (g.) corresponds to 10 minutes (144 for a day).

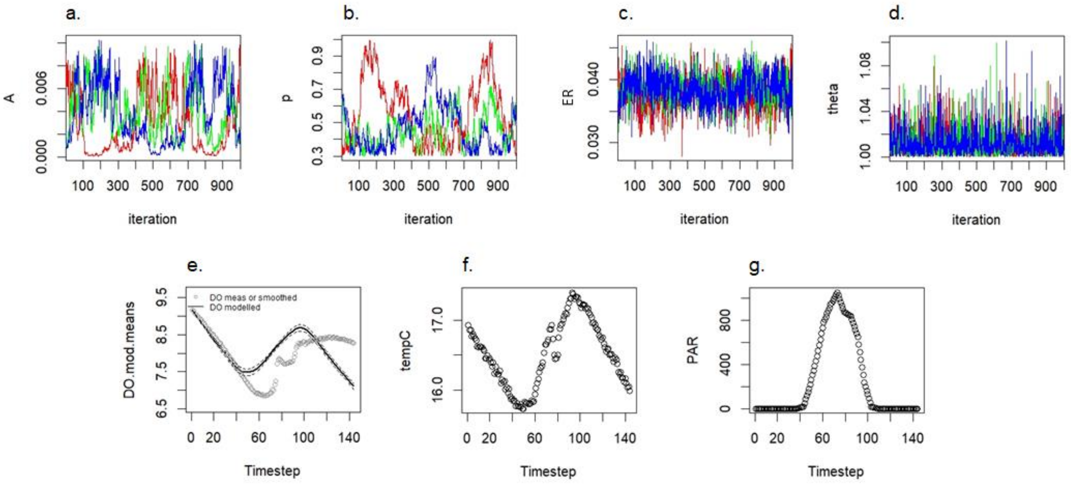


Figure S9.1.9. Example of a BASE program output used for model validation. It shows a day which was discarded with no parameter convergence (A, p, theta, and ER) and a poor fit (R^2<0.4). The different estimated parameters are represented in the upper graphs (a. to d.) with unconverged chains with poor mixing. ‘A’ refers to the daily constant that represents the primary production per quantum unit of light (a.), ‘p’ is the coefficient that reflects the ability of primary producers to use the incident light (b.), ‘ER’ corresponds to the ecosystem respiration (c.) and ‘theta’ is the coefficient for temperature dependence (d.). The lower graphs show the measured (empty circles) and predicted (black line) dissolved oxygen (DO) curve for each diel period (e.), the measured temperature (tempC, (f.)) and PAR (photosynthetically active radiation, (g.)) data. Thus, every ‘Timestep’ in (e.), (f.) and (g.) corresponds to 10 minutes (144 for a day).

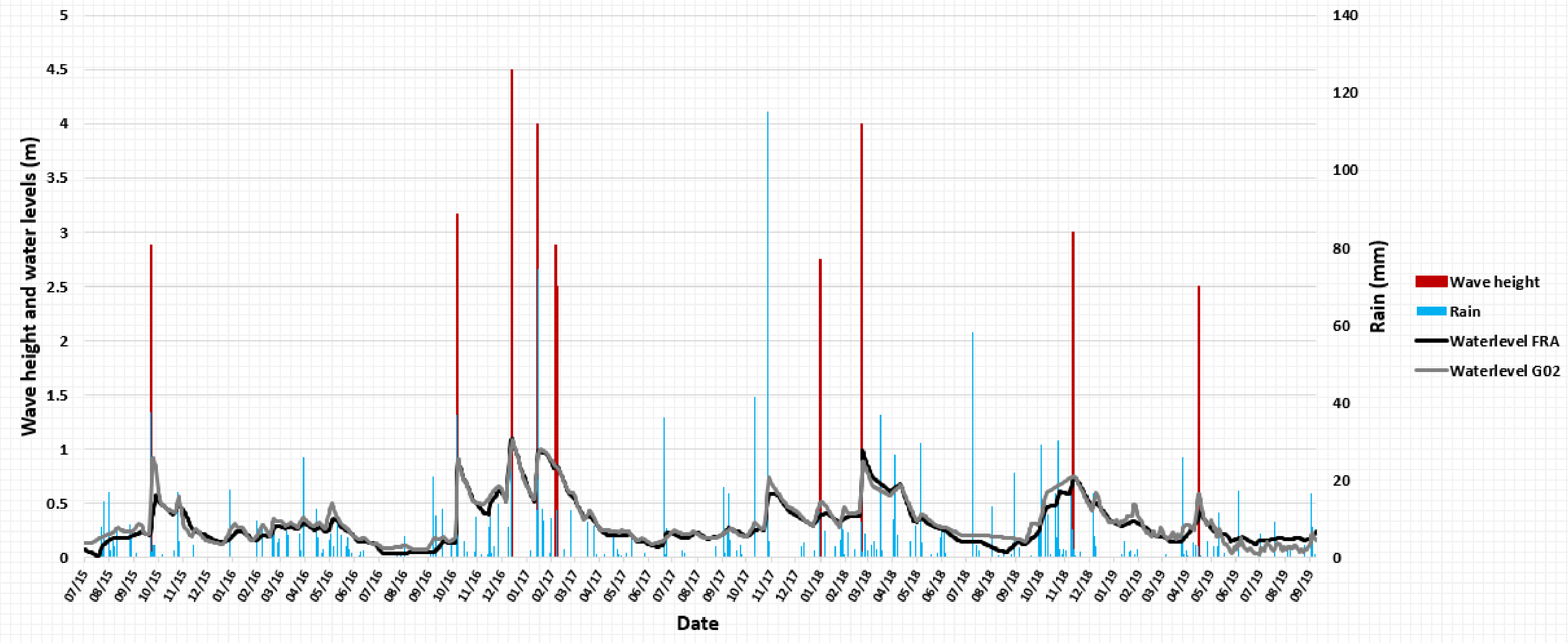


Figure S9.1.10. Representation of water levels (height above or below average sea level) for FRA and G02 with rain and wave height values. Note that the large changes in the water level of the lagoons are due to the increase in wave height during sea storms. In the present study, sea storms were considered when the wave height was ≥2.5 metres and lower values of wave height were not represented here. The X-axis labels indicate the month-year.

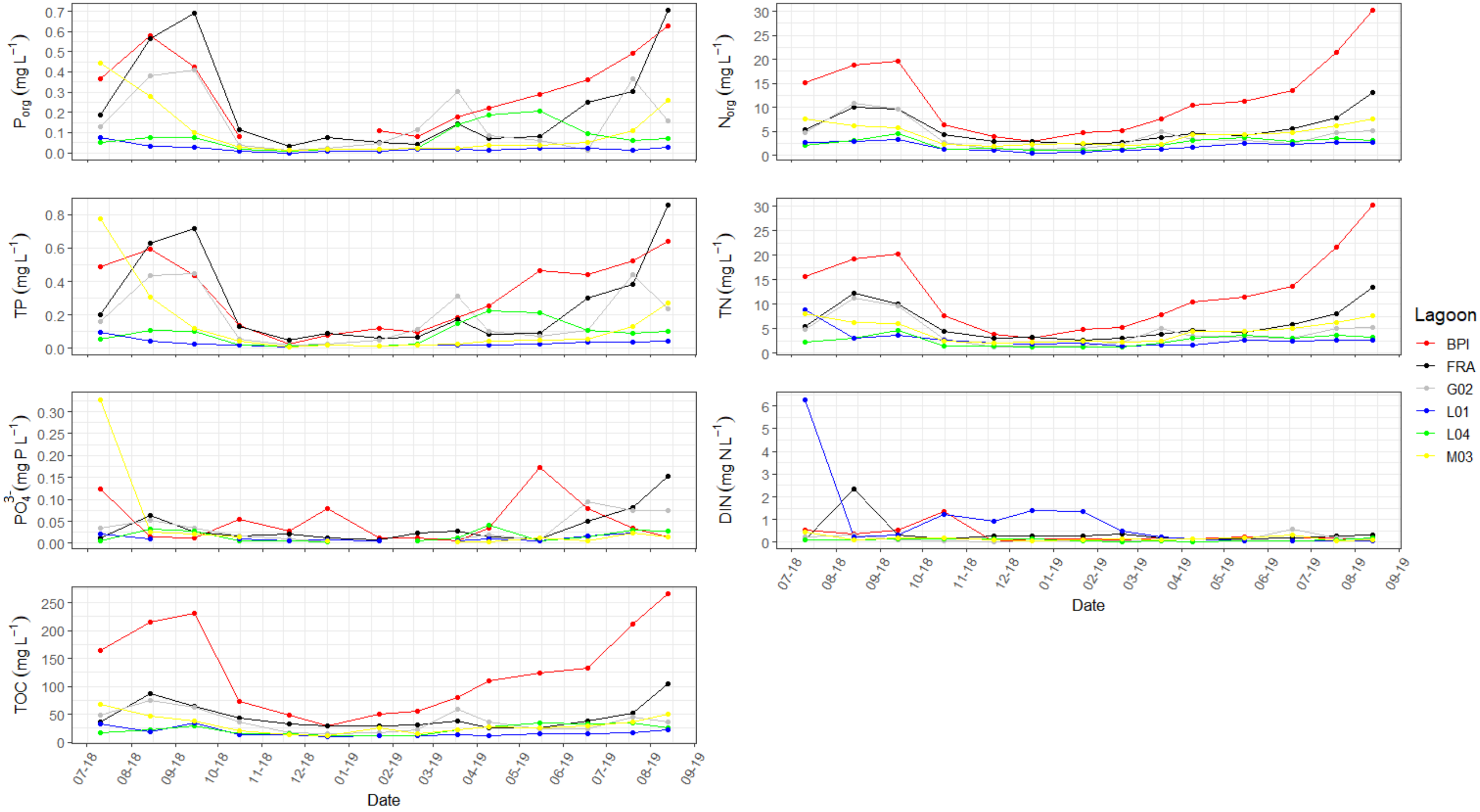


Figure S9.1.11. Monthly measurements of total organic carbon (TOC) and nutrients in the six lagoons during the study period (July 2018 to August 2019). DIN: dissolved inorganic nitrogen; N$_{org}$: organic nitrogen; P$_{org}$: organic phosphorus; TN: total nitrogen; TP: total phosphorus. The X-axis labels indicate the month-year.

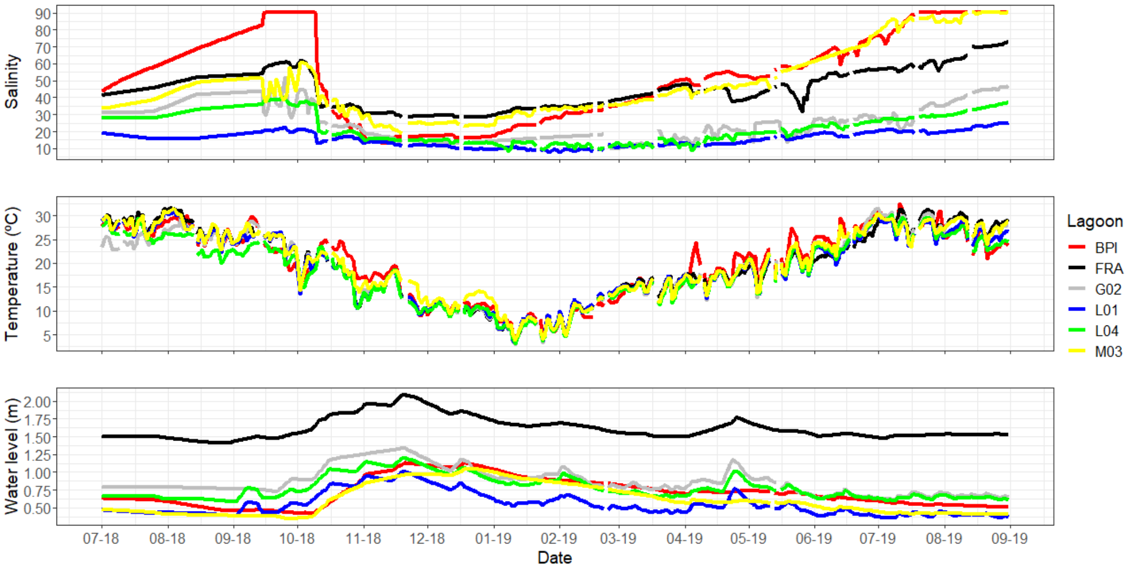


Figure S9.1.12. Daily values of salinity, temperature, and water level during the study period. The X-axis labels indicate the month-year.

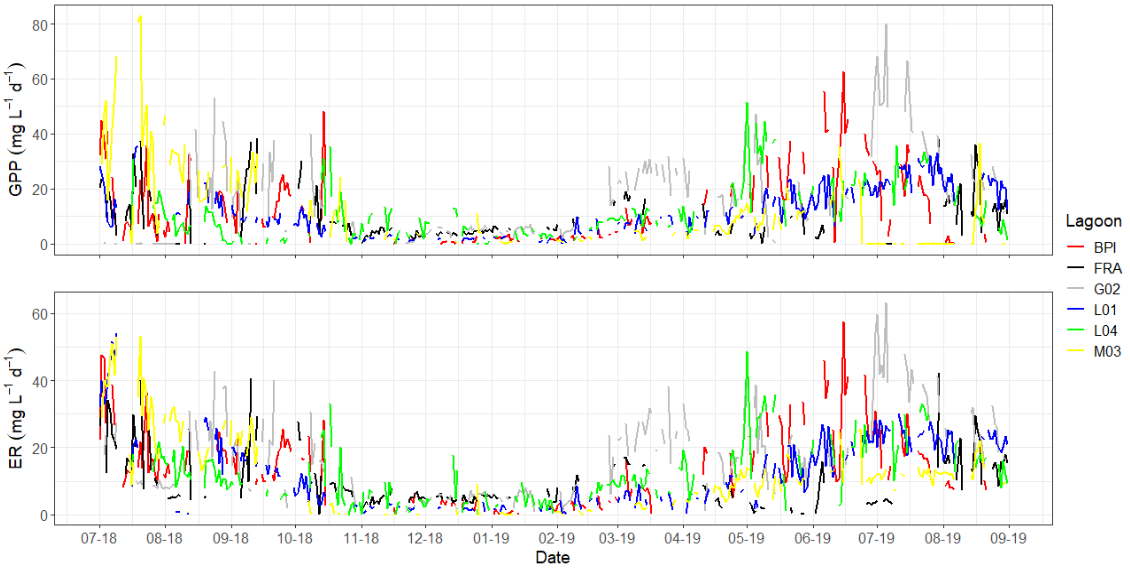


Figure S9.1.13. Daily values of gross primary production (GPP) and ecosystem respiration (ER) during the study period. The X-axis labels indicate the month-year. Gaps in the data series correspond to days that were discarded by the model due to no parameter convergence (A, p, theta, and ER) or/and a poor fit (R^2<0.4). Further details can be found in section 3.3.

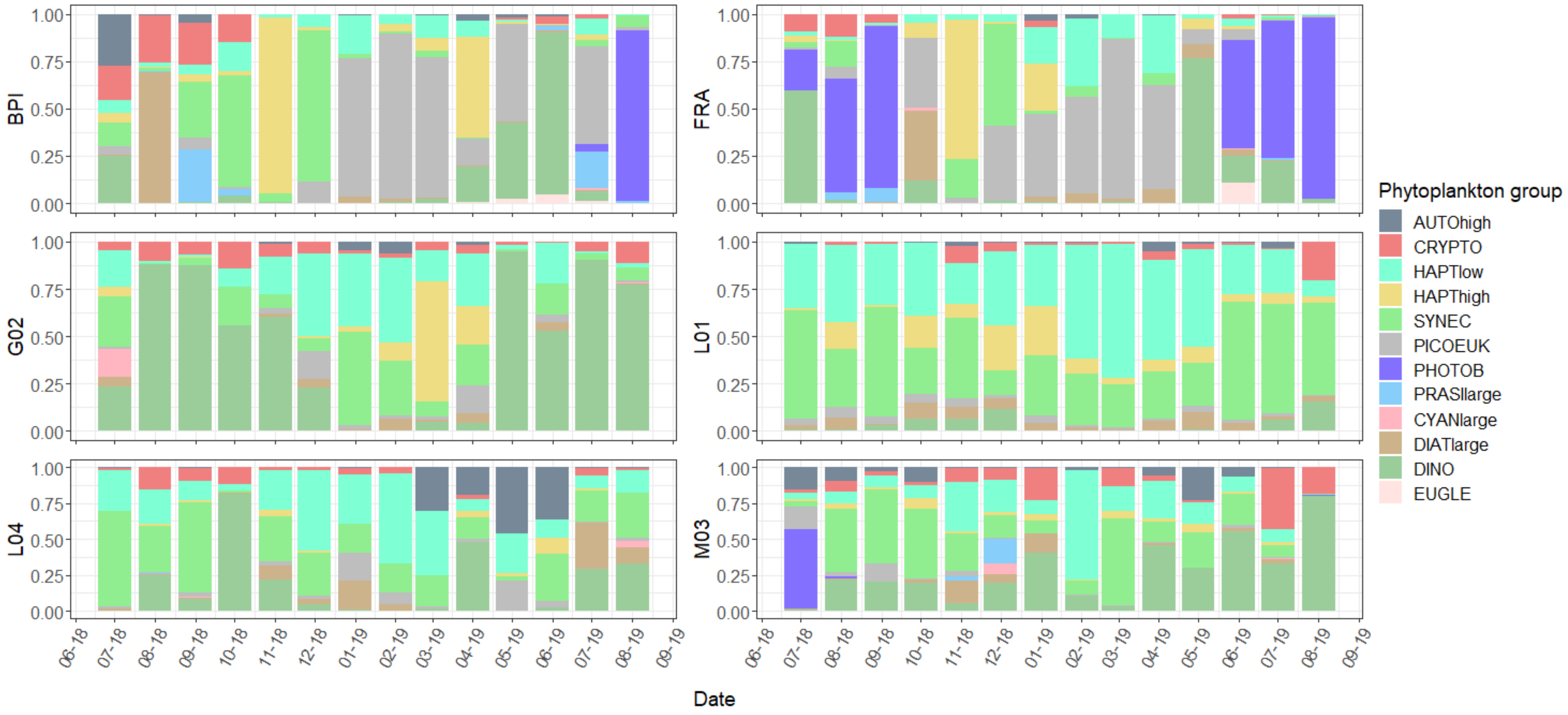
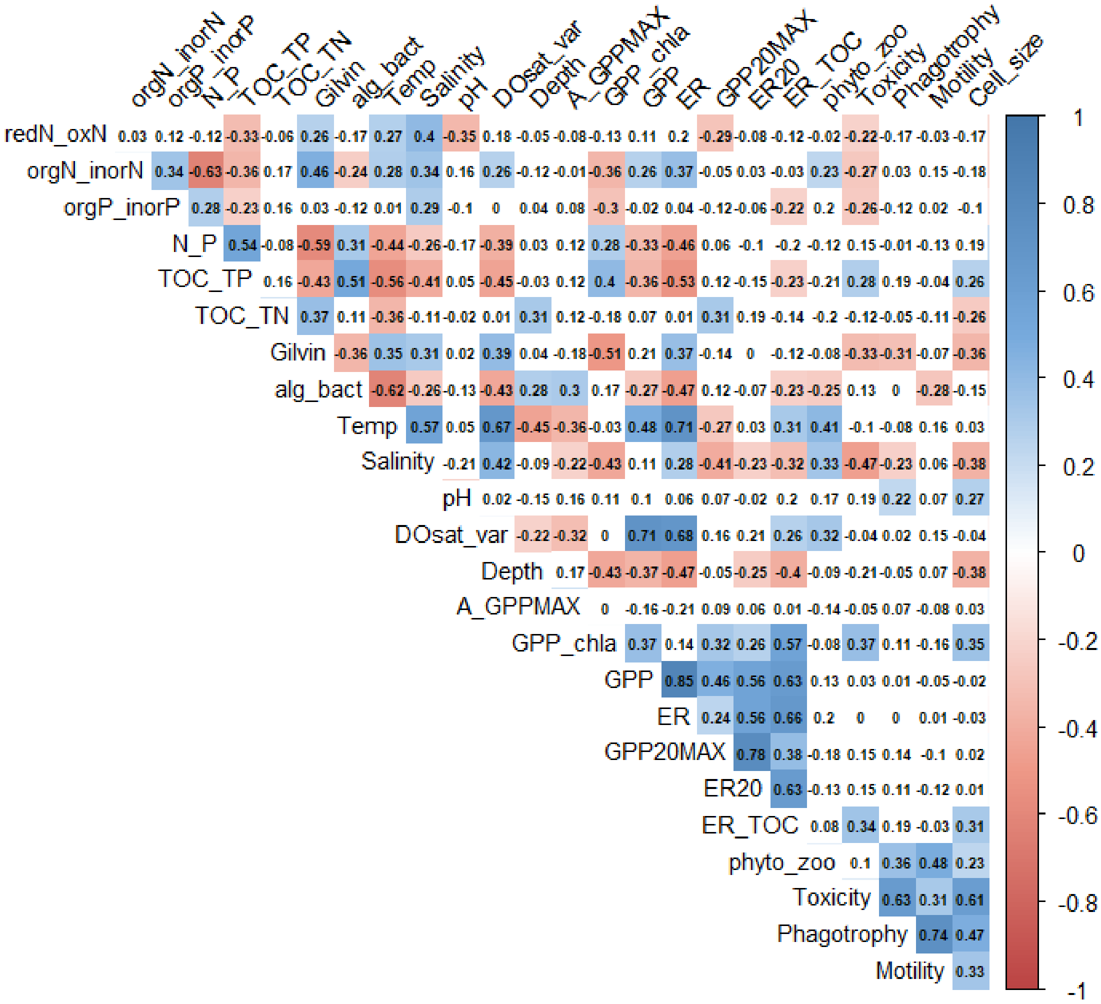


Figure S9.1.14. Temporal changes in phytoplankton group biovolume (in µm^3/mL), expressed as percentages (%). See the label meanings and more details of the group characterization in Table 4.3.2. The X-axis labels indicate the month-year.



	orgN_inorN	orgP_inorP	N_P	TOC_TP	TOC_TN	Gilvin	alg_bact	Temp	Salinity	pH	DOsat_var	Depth	A_GPPMAX	GPP_chla	GPP	ER	GPP20MAX	ER20	ER_TOC	phyto_zoo	Toxicity	Phagotrophy	Motility	Cell_size
redN_oxN	0.03	0.12	-0.12	-0.33	-0.06	0.26	-0.17	0.27	0.4	-0.35	0.18	-0.05	-0.08	-0.13	0.11	0.2	-0.29	-0.08	-0.12	-0.02	-0.22	-0.17	-0.03	-0.17
orgN_inorN		0.34	-0.63	-0.36	0.17	0.46	-0.24	0.28	0.34	0.16	0.26	-0.12	-0.01	-0.36	0.26	0.37	-0.05	0.03	-0.03	0.23	-0.27	0.03	0.15	-0.18
orgP_inorP			0.28	-0.23	0.16	0.03	-0.12	0.01	0.29	-0.1	0	0.04	0.08	-0.3	-0.02	0.04	-0.12	-0.06	-0.22	0.2	-0.26	-0.12	0.02	-0.1
N_P				0.54	-0.08	-0.59	0.31	-0.44	-0.26	-0.17	-0.39	0.03	0.12	0.28	-0.33	-0.46	0.06	-0.1	-0.2	-0.12	0.15	-0.01	-0.13	0.19
TOC_TP					0.16	-0.43	0.51	-0.56	-0.41	0.05	-0.45	-0.03	0.12	0.4	-0.36	-0.53	0.12	-0.15	-0.23	-0.21	0.28	0.19	-0.04	0.26
TOC_TN						0.37	0.11	-0.36	-0.11	-0.02	0.01	0.31	0.12	-0.18	0.07	0.01	0.31	0.19	-0.14	-0.2	-0.12	-0.05	-0.11	-0.26
Gilvin							-0.36	0.35	0.31	0.02	0.39	0.04	-0.18	-0.51	0.21	0.37	-0.14	0	-0.12	-0.08	-0.33	-0.31	-0.07	-0.36
alg_bact								-0.62	-0.26	-0.13	-0.43	0.28	0.3	0.17	-0.27	-0.47	0.12	-0.07	-0.23	-0.25	0.13	0	-0.28	-0.15
Temp									0.57	0.05	0.67	-0.45	-0.36	-0.03	0.48	0.71	-0.27	0.03	0.31	0.41	-0.1	-0.08	0.16	0.03
Salinity										-0.21	0.42	-0.09	-0.22	-0.43	0.11	0.28	-0.41	-0.23	-0.32	0.33	-0.47	-0.23	0.06	-0.38
pH											0.02	-0.15	0.16	0.11	0.1	0.06	0.07	-0.02	0.2	0.17	0.19	0.22	0.07	0.27
DOsat_var												-0.22	-0.32	0	0.71	0.68	0.16	0.21	0.26	0.32	-0.04	0.02	0.15	-0.04
Depth													0.17	-0.43	-0.37	-0.47	-0.05	-0.25	-0.4	-0.09	-0.21	-0.05	0.07	-0.38
A_GPPMAX														0	-0.16	-0.21	0.09	0.06	0.01	-0.14	-0.05	0.07	-0.08	0.03
GPP_chla															0.37	0.14	0.32	0.26	0.57	-0.08	0.37	0.11	-0.16	0.35
GPP																0.85	0.46	0.56	0.63	0.13	0.03	0.01	-0.05	-0.02
ER																	0.24	0.56	0.66	0.2	0	0	0.01	-0.03
GPP20MAX																		0.78	0.38	-0.18	0.15	0.14	-0.1	0.02
ER20																			0.63	-0.13	0.15	0.11	-0.12	0.01
ER_TOC																				0.08	0.34	0.19	-0.03	0.31
phyto_zoo																					0.1	0.36	0.48	0.23
Toxicity																						0.63	0.31	0.61
Phagotrophy																							0.74	0.47
Motility																								0.33

Figure S9.1.15. Spearman correlations among the different monthly variables included in the study. For label meanings see Table 4.3.3. Significance is indicated by coloured squares. Blue and red colours indicate positive and negative correlations, respectively. High absolute values of correlation are represented by dark colours, and low values are indicated by light colours. Variables were log transformed before analysis.

9.2. SUPPLEMENTARY MATERIAL (CHAPTER I, II AND III) - TABLES

Table S9.1.1. Mean and range (in brackets) values for some of the parameters registered from July 2015 to September 2019 in FRA and G02 lagoons. DIN: dissolved inorganic nitrogen; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus.

	FRA	G02
Temperature (°C)	18.90 (1.38-35.41)	18.43 (0.10-35.33)
Salinity	45.03 (14.04-76.74)	26.47 (7.67-52.67)
pH	8.60 (7.92-9.37)	8.76 (8.20-9.79)
Chlorophyll-*a* (µg L^{-1})	82.79 (0.58-875.46)	29.58 (0.41-236.61)
DIN (mg N L^{-1})	0.32 (0.02-2.33)	0.21 (0.02-1.36)
$\mathbf{PO_4^{3-}}$(mg P L^{-1})	0.045 (0.01-0.18)	0.03 (0.00-0.15)
TN (mg L^{-1})	5.91 (2.00-13.51)	4.53 (1.05-11.19)
TP (mg L^{-1})	0.27 (0.05-0.86)	0.19 (0.02-1.21)
TOC (mg L^{-1})	42.29 (13.29-104.50)	33.46 (12.28-88.21)
Depth (m)	1.61 (1.30-2.46)	0.92 (0.60-1.69)
Surface (m^2)	10326	2240

Table S9.1.2. Means and standard deviations (in brackets) of the nutrients measured during the three hydrological years of the study period (from July 2015 to July 2018) for both lagoons. The concentrations are in mg L^{-1}. PO_4^{3-}: phosphate; DIN: dissolved inorganic nitrogen; N_{org}: organic nitrogen; P_{org}: organic phosphorus; TN: total nitrogen; TP: total phosphorus; and TOC: total organic carbon.

	DIN	PO_4^{3-}	N_{org}	P_{org}	TN	TP	TOC
FRA							
15/16	0.29 (0.18)	0.06 (0.05)	6.72 (1.83)	0.20 (0.21)	7.02 (1.86)	0.26 (0.25)	37.88 (17.91)
16/17	0.26 (0.34)	0.05 (0.05)	6.16 (2.75)	0.23 (0.18)	6.43 (2.90)	0.28 (0.21)	39.40 (19.12)
17/18	0.31 (0.26)	0.04 (0.02)	4.31 (2.10)	0.24 (0.20)	4.62 (2.33)	0.28 (0.21)	45.85 (13.12)
G02							
15/16	0.29 (0.35)	0.03 (0.05)	6.72 (2.65)	0.21 (0.35)	7.24 (2.57)	0.24 (0.37)	30.99 (23.16)
16/17	0.29 (0.36)	0.04 (0.02)	5.46 (2.76)	0.21 (0.14)	5.75 (2.90)	0.24 (0.16)	37.37 (19.34)
17/18	0.09 (0.10)	0.02 (0.02)	2.47 (1.21)	0.10 (0.10)	2.55 (1.28)	0.12 (0.11)	28.25 (12.87)

9.3. SUPPLEMENTARY MATERIAL - PHOTOGRAPHIC RECORD

BPI in July 2019. BPI is the most isolated lagoon in the salt marsh, and the low water level when the photograph was taken should be noted. Author: Maria Bas.

Aerial photograph of BPI in May 2019. Author: Maria Bas.

FRA lagoon in January 2018. The highest surface and depth values have been recorded for this lagoon. Note, the presence of macroalgae at the edges of the lagoon. Author: Jesús Ortiz.

Photograph of FRA lagoon during a *Chromatium* bloom in September 2018. Author: Maria Bas.

Photograph of the created lagoons G02 (right) and L04 (left) in January 2018. Author: Jesús Ortiz.

L01 in February 2019. Note the presence of *Ruppia cirrhosa* patches. Author: Maria Bas.

M03 lagoon in January 2018. Author: Jesús Ortiz.

Aerial photograph of La Pletera salt marsh in 2014 (before the restoration project). Only BPI, FRA and G02 (created in 2002) are present. Author: Institut Cartogràfic de Catalunya.

Aerial photograph of La Pletera salt marsh in 2017 (after the restoration project). Note that when the photograph was taken, there were high water levels in the lagoons that allowed a connection between some of them. Author: Institut Cartogràfic de Catalunya.


nutrients
high-frequency
hydrology
phytoplankton
seasonality
lagoons
evaporation
salt marsh
Mediterranean
traits
time series
anoxia
La Pletera
drivers
restoration
metabolism
oxygen
salinity
wavelet analysis
ecosystem functioning
global change
temperature
mandala