BALANCE OR IMBALANCE? THE INTERPLAY OF HYDROLOGY AND NUTRIENT DYNAMICS IN MEDITERRANEAN COASTAL LAGOONS

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Universitat de Girona

DOCTORAL THESIS

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DECLARES:

That the thesis title "*Balance or imbalance? The interplay of hydrology and nutrient dynamics in mediterranean coastal lagoons*", presented by Warren Meredith 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. Anna Menció Domingo

Girona, 2023

“The earth, the air, the land, and the water are not an inheritance from our forefathers but on loan from our children. So, we have to handover to them at least as it was handed over to us.”

- Gandhi

To Yolima, Sofía and Clive

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TABLE OF ABBREVIATIONS

Abbreviation	Description
AED_2	Aquatic Ecodynamics Model
ANOVA	Analysis of Variance
AP	Alkaline Phosphatase
AutoCAD LT	Auto Computer-Aided Design Laptop
CA	Correspondence Analysis
CTD Profiler	Conductivity, Temperature and Depth Profiler
Cva	Coefficient of variation among lagoons
CVw	Coefficient of variation within lagoons
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DV	Volume Development
EC	Electrical Conductivity
EEA	Extracellular Enzyme Activity
Eh	Redox potential
GLEON	Global Lake Ecological Observatory Network
GLM	General Lake Model
GLU	β-glucosidase
Kw	Light attenuation
LAP	leucine aminopeptidase
m.a.s.l.	Meters Above Sea Level
NMDS	Nonmetric multidimensional scaling
Norg	Organic Nitrogen
NSE	Nash-Sutcliffe efficiency
Porg	Organic Phosphorous
RMSRE	root-mean-square relative error
SIMPER	Similarity Percentage
TIC	Total Inorganic Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorous
Zr	Relative Depth

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SUMMARY

Coastal wetlands and lagoons are some of the most fluctuating and productive ecosystems in the world. As transition zones between marine and continental environments, they provide numerous ecosystem services, including the purification of water and carbon sequestration. Mediterranean coastal wetlands have a great diversity of aquatic environments, and their water permanence gradient depends on sediment type and the balance between surface and groundwater inputs. With a flooding-confinement hydrological pattern, sea storms and strong flooding increase allochthonous inputs of nutrients and organic matter and water levels, followed by periods of confinement and disconnection from the sea and without external surface input. However, recent studies have shown that groundwater can significantly contribute to the overall hydrology of these ecosystems. With increasing anthropogenic activity, significant contributions of nutrients could be entering these subterranean waters and entering coastal lagoons in a gradual, continuous manner.

Under natural conditions, the high intensity disturbances (or pulse type), such as sea storms, would affect the ecosystem intensely, and tend to result in decreasing availability of resources produced after such disturbances, resulting in the ecosystem becoming more resilient to these changes. However, low intensity disturbances of a more gradual nature (continuous type), such as nutrients entering lagoons through subterranean water flow, could affect the nutrient dynamics of these ecosystems, resulting in high productivity and availability of resources over time. It is therefore hypothesized that the community structure is not well adapted to these gradual inputs, since they do not occur naturally. The restored La Pletera salt marshes and lagoons in the Baix Ter wetlands represent Mediterranean ecosystems with a flooding-confinement hydrological pattern and significant contributions of groundwater ensures their permanency year-round. These lagoons are also under pressure from surrounding anthropological activity. Accordingly, the main goal of this thesis was to quantify the different contributions that make up the water balance of the different restored and natural lagoons in the La Pletera (both intense surface inputs and gradual subterranean inputs), and then determine the effects of gradual and intense pulse nutrient entry into the lagoons, and what influence it has on planktonic community structure and ecological functioning. To achieve this, this thesis analyzed the overall hydrological dynamics of new lagoons, along with their dependence on groundwater circulations, and compared them with natural lagoons using the General Lake Model (GLM), as well as incorporating morphology and lithological characteristics as additional parameters (Chapter 1). A field experiment using mesocosms was conducted to simulate four time-dynamics of nutrient inputs to quantify what affect this may have on the biomass contribution of the different planktonic groups and the heterotrophic activity linked to the use of carbon, nitrogen, and phosphorous sources. (Chapter 2). Finally, the effects of different nutrient input regimes on zooplankton community structure, and the severity of these additions as a disturbance was evaluated using an indicator of the severity of the disturbance (Chapter 3).

Hydrologically, the older and natural lagoons exhibited more consistent patterns of confinement; with deeper morphologies, lower evaporation effect, lower water circulation, and more annual patterns of salinity fluctuation. The presence of low-permeability layers also resulted in less fluctuation of higher salinity levels. Conversely, three of the four new lagoons had similar, shallower morphologies and higher evaporation fluxes, but exhibited different water circulation patterns due to the presence or absence of low permeability layers. Also, their salinity fluctuations were more influenced by seasonal mixing than by evaporation, indicating more susceptibility to climatic influence in their annual hydrological pattern than in the natural lagoons.

With the application of four time-dynamic nutrient inputs, low continuous inputs of nutrients had a bottom-up control on functional group succession temporal patterns. In contrast, the pulse inputs favored bacterioplankton in the short term and phytoplankton in the long term, with a general decline in zooplankton biomass, indicating an absence of a bottom-up control. Furthermore, the rate of nutrient loadings over time was as important as the total nutrient additions on all functional group structure and strategies. Additionally, constant nutrient assimilation and gradual increase of all functional group biomass masked continual inputs of inorganic nutrients in the continuous treatments and kept nutrient levels low that otherwise can be detected after pulse inputs. High extracellular enzyme activities (EEAs) indicated higher organic matter mineralization in the continuous treatments, suggesting that changes in nutrient concentrations in the water may not be detected, but a change at the functional level may be occurring.

When evaluating the severity of nutrient inputs to zooplankton community composition, there were notable differences in relative composition in the final conditions of the pulse treatments to the rest of the treatments, where decreased zooplankton

biomass and the low presence of adults with eggs and nauplii indicated impaired reproduction and growth. The initial composition of the sampled zooplankton assemblage, mainly constituted of cyclopoids, suggested an initial external influence in a continuous state, as the maintenance of the dominance of cyclopoids requires a slow and continuous external energy entry. This potentially could be linked to continuous, low concentration nutrient inputs through subterranean waters to the lagoons, especially in summer. Despite compositional differences in the pulse treatments, the smaller scale increases in the indicator of severity in the continuous treatments indicate a gradually increasing severity of disturbance over a longer period. The persistent bottom-up effect of continuous inputs could influence zooplankton community structure into an alternative state that resists recovery to a previous state.

The findings of this thesis highlight the importance of predetermined morphology and underlying sediment patterns when constructing and restoring lagoons. This could ultimately limit or enhance the success of set objectives and overall ecological functioning in a flooding – confinement driven lagoon ecosystem conditioned by irregular and unpredictable climatic events. Furthermore, the difficulties in quantifying the diffuse inputs of nutrient input points and frequency in which they become available to the aquatic communities may lead to erroneous and confounding predictions, which could lead to inadequate ecosystem conservation and management. Specifically, the potential impact of continuous inorganic nutrient inputs on plankton group dynamics in relation to some aspects of disturbance theory brings into focus the possible influence of subterranean waters as a diffuse nutrient source in confined Mediterranean coastal lagoons.

RESUMEN

Los humedales y lagunas costeras son algunos de los ecosistemas más fluctuantes y productivos del mundo. Como zonas de transición entre los ambientes marino y continental, brindan numerosos servicios ecosistémicos, incluida la purificación del agua y el secuestro de carbono. Los humedales costeros mediterráneos tienen una gran diversidad de ambientes acuáticos, y su gradiente de permanencia del agua depende del tipo de sedimento y del equilibrio entre los aportes de agua superficial y subterránea. Con un patrón hidrológico de inundación-confinamiento, los temporales marinos y las fuertes inundaciones aumentan los aportes alóctonos de nutrientes y materia orgánica y los niveles de agua, seguidos de periodos de confinamiento y desconexión del mar y sin aportes superficiales externos. Sin embargo, estudios recientes han demostrado que el agua subterránea puede contribuir significativamente a la hidrología general de estos ecosistemas. Con el aumento de la actividad antropogénica, importantes aportes de nutrientes podrían estar ingresando a estas aguas subterráneas y a las lagunas costeras de manera gradual y continua.

En condiciones naturales, las perturbaciones de alta intensidad (o de tipo pulso), como las tormentas marinas, afectarían intensamente al ecosistema y tenderían a provocar una disminución de la disponibilidad de recursos producida después de dichas perturbaciones, lo que haría que el ecosistema se volviera más resiliente a estos cambios. Sin embargo, perturbaciones de baja intensidad y de naturaleza más gradual (tipo continuo), como la entrada de nutrientes a las lagunas a través de flujos de agua subterráneos, podrían afectar la dinámica de los nutrientes de estos ecosistemas, resultando en una alta productividad y disponibilidad de recursos a lo largo del tiempo. Por lo tanto, se plantea la hipótesis de que la estructura comunitaria no está bien adaptada a estos aportes graduales, ya que no ocurren de forma natural. Las marismas y lagunas restauradas de La Pletera en los humedales del Baix Ter representan ecosistemas mediterráneos con un patrón hidrológico de inundación-confinamiento e importantes aportes de agua subterránea que asegura su permanencia durante todo el año. Estas lagunas también están bajo presión por la actividad antropológica circundante. En este sentido, el objetivo principal de esta tesis fue cuantificar los diferentes aportes que componen el balance hídrico de las diferentes lagunas restauradas y naturales de La Pletera (tanto aportes superficiales intensos como aportes subterráneos graduales), para luego determinar los efectos de los aportes graduales y entrada intensa de nutrientes en las lagunas y qué influencia tiene en la estructura de la

comunidad planctónica y el funcionamiento ecológico. Para lograr esto, esta tesis analizó la dinámica hidrológica general de nuevas lagunas, junto con su dependencia de las circulaciones de agua subterránea, y las comparó con lagunas naturales utilizando el Modelo General de Lagos (GLM), además de incorporar características morfológicas y litológicas como parámetros adicionales (Capítulo 1). Se llevó a cabo un experimento de campo utilizando mesocosmos para simular cuatro dinámicas temporales de entradas de nutrientes para cuantificar qué efecto puede tener esto en la contribución de biomasa de los diferentes grupos planctónicos y la actividad heterótrofa vinculada al uso de fuentes de carbono, nitrógeno y fósforo. (Capitulo 2). Finalmente, se evaluaron los efectos de diferentes regímenes de aporte de nutrientes en la estructura de la comunidad de zooplancton y la gravedad de estas adiciones como perturbación utilizando un indicador de la gravedad de la perturbación (Capítulo 3).

Hidrológicamente, las lagunas más antiguas y naturales exhibieron patrones de confinamiento más consistentes; con morfologías más profundas, menor efecto de evaporación, menor circulación de agua y más patrones anuales de fluctuación de salinidad. La presencia de capas de baja permeabilidad también resultó en una menor fluctuación de los niveles de salinidad más altos. Por el contrario, tres de las cuatro nuevas lagunas tenían morfologías similares, menos profundas y mayores flujos de evaporación, pero exhibían diferentes patrones de circulación de agua debido a la presencia o ausencia de capas de baja permeabilidad. Además, sus fluctuaciones de salinidad estuvieron más influenciadas por la mezcla estacional que por la evaporación, lo que indica una mayor susceptibilidad a la influencia climática en su patrón hidrológico anual que en las lagunas naturales.

Con la aplicación de cuatro aportes de nutrientes dinámicos en el tiempo, los aportes bajos y continuos de nutrientes tuvieron un control ascendente sobre los patrones temporales de sucesión de grupos funcionales. Por el contrario, las entradas de pulsátiles favorecieron al bacterioplancton a corto plazo y al fitoplancton a largo plazo, con una disminución general de la biomasa de zooplancton, lo que indica una ausencia de un control ascendente. Además, la tasa de carga de nutrientes a lo largo del tiempo fue tan importante como las adiciones totales de nutrientes en todas las estructuras y estrategias de grupos funcionales. Así mismo, la asimilación constante de nutrientes y el aumento gradual de la biomasa de todos los grupos funcionales enmascararon los aportes continuos de nutrientes inorgánicos en los tratamientos continuos y mantuvieron bajos los niveles de nutrientes que de otro modo podrían detectarse después de los aportes de pulsátiles. Las altas actividades enzimáticas extracelulares (EEA) indicaron una mayor mineralización de la materia orgánica en los tratamientos continuos, lo que sugiere que es posible que no se detecten cambios en las concentraciones de nutrientes en el agua, pero puede estar ocurriendo un cambio a nivel funcional.

Al evaluar la severidad de los aportes de nutrientes a la composición de la comunidad de zooplancton, hubo diferencias notables en la composición relativa en las condiciones finales de los tratamientos de pulsos al resto de los tratamientos, donde la disminución de la biomasa de zooplancton y la baja presencia de adultos con huevos y nauplios indicaron problemas de reproducción y crecimiento. La composición inicial del conjunto de zooplancton muestreado, constituido principalmente por ciclopoides, sugirió una influencia externa inicial en un estado continuo, ya que el mantenimiento de la dominancia de los ciclopoides requiere una entrada lenta y continua de energía externa. Potencialmente, esto podría estar relacionado con los aportes continuos y de baja concentración de nutrientes a las lagunas a través de aguas subterráneas, especialmente en verano. A pesar de las diferencias de composición en los tratamientos de pulsos, los aumentos en escalas más pequeñas en el indicador de gravedad en los tratamientos continuos indican una gravedad de la alteración que aumenta gradualmente durante un período más largo. El persistente efecto ascendente de los aportes continuos podría influir en la estructura de la comunidad de zooplancton hacia un estado alternativo que se resiste a la recuperación a un estado anterior.

Los hallazgos de esta tesis resaltan la importancia de la morfología predeterminada y los patrones de sedimentos subyacentes al construir y restaurar lagunas. En última instancia, esto podría limitar o mejorar el éxito de los objetivos establecidos y el funcionamiento ecológico general en un ecosistema lagunar impulsado por inundaciones y confinamiento condicionado por eventos climáticos irregulares e impredecibles. Además, las dificultades para cuantificar los aportes difusos de los puntos de aporte de nutrientes y la frecuencia con la que están disponibles para las comunidades acuáticas pueden dar lugar a predicciones erróneas y confusas, lo que podría conducir a una conservación y gestión inadecuadas de los ecosistemas. Específicamente, el impacto potencial de los aportes continuos de nutrientes inorgánicos en la dinámica del grupo de plancton, en relación con algunos aspectos de la teoría de la perturbación, pone de relieve la posible influencia de las aguas subterráneas como fuente difusa de nutrientes en las lagunas costeras mediterráneas confinadas.

RESUM

Els aiguamolls i les llacunes costaneres són uns dels ecosistemes més fluctuants i productius del món. Com a zones de transició entre entorns marins i continentals, proporcionen nombrosos serveis ecosistèmics, inclosa la purificació de l'aigua i el segrest de carboni. Les zones humides costaneres mediterrànies presenten una gran diversitat d'ambients aquàtics, el gradient de temporalitat de les quals, depèn del tipus substrat i de l'equilibri entre les aportacions d'aigua superficial i subterrània. Aquestes zones humides es veuen afectades per un patró hidrològic d'inundació-confinament, on: les tempestes marines i les fortes inundacions augmenten les aportacions al·lòctones de nutrients i matèria orgànica i dels nivells d'aigua; i en els períodes de confinament, en què queden desconnectades del mar i sense aportacions superficials externes, se’n produeix la seva disminució. Tanmateix, estudis recents han demostrat que les aigües subterrànies poden contribuir significativament a la hidrologia global d'aquests ecosistemes. En aquest sentit, degut a l'augment de l'activitat antropogènica, aportacions significatives de nutrients podrien estar entrant en aquestes aigües subterrànies, així com a les llacunes costaneres, d'una manera gradual i contínua.

En condicions naturals, les pertorbacions d'alta intensitat (o de tipus pulsàtil), com les tempestes marines, afectarien intensament l'ecosistema i tendirien a provocar una disminució posterior de la disponibilitat dels recursos produïts, donant lloc a que l'ecosistema es pugui tornar més resilient a aquests canvis. D’altra banda, les pertorbacions de baixa intensitat i de caràcter més gradual (de tipus continu), com els nutrients que entren a les llacunes a través del flux d'aigua subterrània, podrien afectar la dinàmica dels nutrients d'aquests ecosistemes, donant lloc a una alta productivitat i disponibilitat de recursos a llarg termini. Per tant, es planteja la hipòtesi que l'estructura de la comunitat no està ben adaptada a aquests inputs graduals, ja que no es produeixen de manera natural. La zona humida i les llacunes de la Pletera, al Baix Ter, representen ecosistemes mediterranis amb un patró hidrològic d'inundació-confinament i importants aportacions d'aigua subterrània que n'asseguren la permanència durant tot l'any. Aquestes llacunes també estan sota la pressió de l'activitat antropològica circumdant. En conseqüència, l'objectiu principal d'aquesta tesi és el de: 1) quantificar les diferents aportacions que conformen el balanç hídric de les diferents llacunes restaurades i naturals de la Pletera (tant entrades superficials intenses com entrades graduals subterrànies); i 2) determinar els efectes de les pertorbacions graduals i pulsàtils, en l'estructura de la comunitat planctònica i el

funcionament ecològic. Per aconseguir-ho, s’ha analitzat la dinàmica hidrològica global de les noves llacunes, juntament amb la seva dependència dels fluxos d'aigua subterrània, i s’ha comparat amb llacunes naturals mitjançant el General Lake Model (GLM), a més d'incorporar com a paràmetres addicionals la morfologia i les característiques litològiques del substrat de les llacunes (Capítol 1). A més, s’ha dut a terme un experiment de camp amb mesocosmos per simular quatre dinàmiques temporals d'aportacions de nutrients per quantificar els seus efectes en la biomassa dels diferents grups planctònics i l'activitat heteròtrofa vinculada a l'ús de fonts de carboni, nitrogen i fòsfor (Capítol 2). Finalment, s’ha investigat els efectes d’aquests diferents règims d'entrada de nutrients sobre l'estructura de la comunitat de zooplàncton. S’ha avaluat la severitat de la pertorbació que suposen les addicions de nutrients inorgànics sobre l'estructura de la comunitat de zooplàncton, mitjançant l’ús d’un indicador de severitat (Capítol 3).

Hidrològicament, les llacunes més antigues i naturals han presentat uns patrons de confinament més consistents, al presentar morfologies més profundes, un menor efecte d'evaporació, una menor circulació de l'aigua i i una fluctuació de la salinitat que segueix patrons repetitius. La presència de sediments de baixa permeabilitat a la base de les llacunes, també dona lloc a una estabilitat més gran en la seva salinitat. Per contra, tres de les quatre noves llacunes tenen morfologies similars, són menys profundes i presenten fluxos d'evaporació més alts, però s’hi ha observat patrons de circulació d'aigua diferents a causa de la presència o absència de sediments de baixa permeabilitat en el seu substrat. A més, les seves fluctuacions de salinitat s’han vist més influenciades per la barreja estacional, que per l'efecte de l’evaporació, cosa que indica una major susceptibilitat a la influència climàtica en el seu patró hidrològic anual.

A la segona part de la tesi, en què s’ha analitzat l'aplicació de nutrients, amb quatre entrades de tipus diferent, s’ha observat que les entrades amb baixes concentracions i de tipus continu presenten un control en els patrons temporals de successió de grups funcionals de baix cap a dalt. En canvi, les entrades de tipus pulsàtil han afavorit el bacterioplàncton a curt termini i el fitoplàncton a llarg termini, amb una disminució general de la biomassa de zooplàncton, cosa que indica l'absència d'un control de baix a dalt. A més, la taxa d’entrada de nutrients al llarg del temps ha estat tan important com les addicions totals de nutrients, a l'estructura i estratègies de tots els grups funcionals. Així mateix, l'assimilació constant de nutrients i l'augment gradual de la biomassa de tots els grups funcionals han emmascarat les entrades contínues de nutrients inorgànics en els tractaments continus i han mantingut baixos els nivells de nutrients, que d'altra manera es podrien detectar després de les entrades de tipus pulsàtil. Les elevades activitats enzimàtiques extracel·lulars (EEA) observades indiquen una major mineralització de la matèria orgànica en els tractaments continus, cosa que suggereix que no es detecten canvis en les concentracions de nutrients a l'aigua, però sí que es pot produir un canvi a nivell funcional.

Finalment, quan s’ha avaluat la severitat de la pertorbació que suposaven les aportacions de nutrients a la composició de la comunitat de zooplàncton, s’han observat diferències notables en la composició relativa de la comunitat en les condicions finals dels diferents tractaments. En el tractament de tipus pulsàtil s’hi ha observat diferències significatives, respecte a la resta de tractaments, al produir-se una disminució de la biomassa de zooplàncton i una baixa presència d'adults amb ous i nauplis, indicant una reproducció i un creixement alterats. La composició inicial del comunitat de zooplàncton, constituïda principalment per ciclopoides, suggereix una influència externa inicial continuada, ja que el manteniment del domini dels ciclopoides requereix una entrada d'energia externa lenta i contínua. Això podria estar relacionat amb les aportacions de nutrients contínues i de baixa concentració a través de les aigües subterrànies a les llacunes, especialment a l'estiu. Malgrat les diferències de composició en els tractaments de tipus pulsàtil, els augments en menor escala de l'indicador de severitat en els tractaments continus indiquen un augment gradual de la severitat de la pertorbació durant un període més llarg. Les aportacions contínues de nutrients semblen tenir un efecte persistent de baix a dalt que influiria en l'estructura de la comunitat de zooplàncton. Aquest efecte es traduiria en un estat alternatiu que es resistiria la recuperació a un estat anterior.

Així, els resultats d'aquesta tesi posen de manifest la importància de la morfologia de la tipologia de substrat de les llacunes a l'hora de construir-ne o restaurar-les. En última instància, això podria limitar o millorar l'èxit dels objectius establerts i el funcionament ecològic general en un ecosistema de llacuna condicionat per inundacions i confinaments determinats per esdeveniments climàtics irregulars i impredictibles. A més, les dificultats en quantificar les entrades difuses de nutrients, i la freqüència en què es posen a disposició de les comunitats aquàtiques, pot conduir a prediccions errònies i confuses, que podrien donar lloc a una conservació i una gestió inadequades dels ecosistemes. Concretament, l'impacte potencial de les aportacions contínues de nutrients inorgànics en la dinàmica de grups de plàncton posa en evidència la importància de les aigües subterrànies com a font difusa de nutrients a les llacunes costaneres de la Mediterrània.

1. GENERAL INTRODUCTION

1.1. Coastal wetlands and lagoons

Fluctuating, productive, diverse, cradle of biodiversity. These are some of the words that describe coastal wetlands. They are determined by a wide range of criteria established by the Ramsar Convention to encompass as many coherent sites globally as possible, for the interest of protecting them. As a starting point, the Ramsar convention describes wetlands as "...areas where water is the primary factor controlling the environment and the associated plant and animal life. They occur where the water table is at or near the surface of the land, or where the land is covered by water." (http://www.ramsar.org/). They are also unique in that they are transition zones between marine and continental environments. Coastal wetlands provide numerous ecosystem services that include the purification of water, the mitigation of floods and droughts, groundwater replenishment, sediment retention and carbon sequestration. Mediterranean coastal wetlands exhibit a great diversity of aquatic environments which occur on a water permanence gradient, from ephemeral to permanent waterbodies, depending on the soil or sediment type and the balance between surface and groundwater inputs, rainfalls, and droughts (Skinner and Zalewski, 1995; Álvarez-Cobelas et al., 2005). Although they occupy approximately 2 per cent of the land area, they are home to more than 30 per cent of the basin's vertebrate species (Wetland-Based Solutions, 2020). The lack of tides and the low current velocities in the Mediterranean favor the shallowness of the waters, the isolation of waterbodies from the sea and water stagnation, resulting in coastal wetlands becoming more lagunal or isolated in nature (Britton and Crivelli, 1993; Ibáñez et al., 2000; De Stefano, 2004). Two main types of Mediterranean coastal ecosystems could be differentiated on a flooding-confinement gradient: those receiving continuous freshwater inputs (mostly of riverine origin) with a high-water turnover rate (e.g. Serra et al., 1984, Comín and Valiela, 1993) and those which are semiconfined or confined with restricted water inputs, a low flushing rate and high marine influence (Trobajo et al., 2002; Quintana et al., 1998a). The restored La Pletera salt marshes and lagoons, located in the NE of the Iberian Peninsula, is such a coastal ecosystem where the hydrology is based on a flooding-confinement pattern. These coastal lagoons consist of 4 restored lagoons and 2 natural lagoons, and the main inputs occur during strong storm events, when not only surface but also subsurface and groundwater inputs take place.

These episodes restrict to very few days and coincide with sea storms and strong flooding events. After them, lagoons remain disconnected from the sea and from any surface freshwater source. As is typical of Mediterranean climate events, flooding events occur randomly throughout the year, with no clear seasonal pattern, except that they are more frequent in winter and are especially rare in summer. The frequency of sea storms per year, however, has increased during the last decades, from 2-3 per year during the 70s to 5-6 per year in the first decade of the 21st century (Pascual et al., 2012). No more surface water inputs are significant apart from flooding events, but groundwater inputs can feed the lagoons for a longer time, allowing for their persistence during dry periods (Menció et al., 2017, Casamitjana et al., 2019). Since it is difficult to quantify groundwater discharge into coastal lagoons, groundwater is often neglected as a component of water and nutrient budgets (Sadat-Noori et al., 2016; Santos et al., 2008b). A variety of indirect and direct measurements to quantify groundwater seepage to lagoons have been used. While seepage can be a minimal portion of lagoon water budgets (e.g., Stieglitz et al., 2013), groundwater discharge can be the main contributor to water balances, either year-round or during dry months (e.g., Menció et al., 2017; Sadat-Noori et al., 2016). There are various approaches to estimating groundwater inputs to coastal lagoons that include point-scale methods such as seepage meters (e.g., Duque et al., 2018; Leote et al., 2008) and broad-scale techniques such as geochemical tracers like salinity (e.g., Stieglitz et al., 2013), radon (e.g., Maher et al., 2019; Rodellas et al., 2018), radium (e.g., Bejannin et al., 2017; Ji et al., 2013) and strontium (Danish et al., 2020); water budgets; and numeric modelling (e.g., Alcolea et al., 2019). However, the use of volumetric water balances in estimating groundwater inputs to coastal lagoons can be challenging, as all other variables in the water budget need to be well constrained (Coluccio et al. 2021). Another tool that has been previously used is the General Lake Model (GLM) that integrates the effects such as inflows and outflows, mixing, as well as surface heating and cooling, and computes vertical profiles of temperature, salinity, and density (Casamitjana et al., 2019). The GLM was applied in the La Pletera lagoons in previous studies to analyze the groundwater influence in the salt marsh (see Menció et al., 2017) and the water circulation patterns and salinity fluctuations (see Casamitjana et al., 2019). The model allowed a very good estimation of the water balance of the lagoons and quantified water contributions that were previously very difficult or near impossible to obtain. Other methods to test lagoon dependance on groundwater flows is the use of the Gonfiantini isotopic model, as well as the equilibrium chemical-speciation/mass transfer models that analyze the evaporation percentage and the physicochemical processes affecting the lagoons, respectively (Menció et al., 2017).

1.2. Nutrient dynamics

The flooding-confinement pattern strongly determines nutrient dynamics, which is more related to internal loading processes than to external nutrient supplies, as it is characteristic of choked coastal ecosystems (Gilbert et al., 2010). Lagoons concentrate organic matter and total nutrients during summer confinement. However, inorganic nutrients display different patterns (Quintana et al., 1998; Badosa et al., 2006; López-Flores et al., 2006a; 2014): while phosphate and organic matter tends to accumulate with confinement, inorganic nitrogen concentrations peak with flooding events, but disappear fast with confinement. Due to several biogeochemical mechanisms, primary production in aquatic coastal ecosystems is limited more by nitrogen (N) than by phosphorus (P) (Tyrrell, 1999; Lohrenz et al., 1999). According to Howarth et al. (2011), the possible mechanisms for this may include: a) desorption of inorganic P from clay and silt particles carried by rivers to the coastal areas (Froelich, 1988; Némery and Garnier, 2007; Golterman, 2004; Serrano et al., 2017); and/or b) a low N:P of nutrient loads entering fresh waters moving downstream in coastal ecosystems due to high rates of denitrification on continental shelves and in some coastal aquifers (Galloway et al., 2004; Fennel et al., 2006; Puig et al., 2017).

Mediterranean coastal lagoons are highly affected by anthropogenic pressures such as tourism activities and agriculture and are at severe risk of eutrophication from both point and non-point sources of pollution (Badosa et al., 2006; Quintana et al., 2018, Menció et al., 2023a). The intensity of surface inputs during flooding suggests that this would be the main source of allochthonous organic matter and nutrients entering these systems as an unpredictable pulse disturbance (López-Flores et al., 2009). However, it has been recently recognized that groundwater circulation can significantly supply coastal systems with dissolved inorganic nutrients and can match surface runoff contributions in some cases (Atkins et al., 2013; Cyronak et al., 2013; Santos et al., 2012a; Schwab et al., 2017). Recent studies in Mediterranean lagoons have found that groundwater significantly contributes to the overall hydrology of the area, which could be as high as 80% of water contribution during the summer confinement (Casamitjana et al., 2019; Menció et al., 2017; Meredith et al., 2022a, and 2022b). These contributions would be more gradual and continuous in nature than flood episodic inputs. Furthermore, a recent study by Menció et al. (2023) analyzed the surface and ground water surrounding the La Pletera lagoons to determine the origin, occurrence and processes affecting N, and concluded that continuous input of nutrients into the lagoons at low concentrations is possible, given that fertilizers and sewage were the main source of inorganic nitrogen and levels in groundwater surrounding the lagoons can be up to 5mg/L. This brings in to focus the potential effect of different rates of diffuse nutrient inputs to coastal lagoon communities and ecological functioning; not only in an intense and short-lived pulse disturbance driven by storms and runoff from rainfall, but also in a gradual, continuous disturbance through subterranean waters.

1.3. Community structure

Species composition and community structure in confined coastal lagoons are strongly determined by water level fluctuations, salinity variability and nutrient dynamics characteristic of the flooding-confinement pattern (Brucet et al., 2005; López-Flores et al., 2006b; Quintana et al., 2006). Few euryhaline species can tolerate the adverse conditions found in these habitats and they can achieve stable populations, despite the arrival of many marine or freshwater species during flooding events. Species composition of planktonic primary producers is dominated by unicellular Synechococcus-like cyanobacteria, dinoflagellates, haptophytes or cryptophytes, while diatoms and chlorophytes are scarce (Quintana & Moreno-Amich, 2002; López-Flores et al., 2009; 2014). Moving up the trophic level and according to the flooding-confinement gradient, dominances of different species of invertebrates follow one another over time. Flooding periods are dominated by Rotifers (Synchaeta spp.), which are then substituted by calanoid copepods (mainly *Eurytemora velox* or *Calanipeda aquaedulcis*) (Quintana et al., (2021). During stable conditions when water levels are low, dominant species depend on trophic state: rotifers (*Brachionus* spp.), amphipods (*Gammarus aequicauda*) or jellyfish (*Odessia maeotica*). Cladocera are absent or very scarce. Stable conditions are dominated by Calanoids (Brucet et al., 2005). During calanoid dominances, intraspecific competition is reduced because adults and juveniles of the same species play a different trophic role, especially when nutrient availability is scarce (Brucet et al., 2008; Quintana et al., 2015). Benthic community structure, however, is not so dependent on the temporal flooding-confinement pattern (Gascón et al., 2005). The Iberian toothcarp (*Aphanius iberus*) is one of the most characteristic fish species of these confined coastal lagoons. It is an endangered cyprinodontid fish that is endemic to the Iberian Peninsula and is included in the Habitats Directive (Casas et al., 2011; Doadrio et al., 2011). It is adapted to the fluctuating conditions of these waters and tolerates a wide range of salinity. Including habitat reduction, one of the main challenges of Iberian toothcarp conservation is the presence of the mosquitofish (*Gambusia holbrooki*), an invasive North American species, introduced in the Mediterranean area to control mosquitos and malaria (Doadrio et al., 2011). The Iberian toothcarp is now relegated to habitats with high salinity fluctuations that do not favor mosquitofish populations (Alcaraz & Garcia-Berthou, 2007; Ruiz-Navarro et al., 2011). The flooding-confinement pattern driven by the Mediterranean climate results in high intensity disturbances in a pulse manner, such as sea storms in coastal lagoons or flooding in inland temporary lagoons affect the overall ecosystem and the entire aquatic community. After this disturbance, a series of changes occur in the composition of species, until they return to conditions similar to those prior to the disturbance. Organisms and communities that have evolved under these conditions in Mediterranean aquatic systems are expected to be resilient to changes caused by pulsatile disturbances (Anderson et al., 2013; Maxwell et al., 2014). However, with the intensification of human activity, new alterations of a less pulse manner and more of a gradual and continuous nature have been introduced. Changes in water flows mediated by irrigation or nutrient entry through diffuse routes due to the intensification of agricultural and livestock activity have produced changes in the hydrology and nutrient dynamics of these aquatic systems.

1.4. Threats to Mediterranean wetlands and the need for restoration

As coastal areas are a focal point for human activity, increasing anthropogenic activity has resulted in a 48% loss of Mediterranean Wetlands since 1970, and 36% of Mediterranean wetland species are threatened with extinction (Wetland-Based Solutions. 2020). Also, it is estimated that there are twice as many endangered species in wetlands than in all Mediterranean ecosystems combined (UNEP/MAP and Plan Bleu, 2020). It is therefore imperative to stop ongoing losses and degradation to these wetlands. One way to counter this is through restoration. Restoration refers to “the process of halting and reversing degradation, resulting in improved ecosystem services, and recovered biodiversity. This is usually reflected in the implementation of concrete actions to assist nature to re-establish its own functionality and to return wetlands to a more natural state (intended as the situation prior to significant disturbances and alterations due to human activities), thus improving their ecological status. The usual objective of restoration is to emulate a self-regulating natural system that is ecologically integrated into the landscape in which it occurs. However, despite concerted efforts in protecting and restoring

Mediterranean wetlands, currently 60% of transitional and coastal waters are failing good conservation status as defined by the European Water Framework Directive. Also, due to increasing human populations, 23% of wetlands were artificial in 2012 (Mediterranean Wetlands: Outlook, 2012), and estimated to around ~40% in 2020 (Wetland-Based Solutions, 2020). This leads to a situation of an increasing lack of reference sites of “pristine” or “original” ecological functioning prior to degradation.

2. HYPOTHESIS AND AIMS

Disturbances in aquatic systems can be identified both by measuring a physical event (level of nutrients and water from sea storms or by estimating the intensity of the disturbance, i.e. power of the disturbing factor), as well as the magnitude of its effect on the aquatic community (severity) (Turner et al., 1998). The use of severity measures has the advantage of making it possible to compare disturbances from different origins and the effect it has on the community, such as the effects of a sea storm or an alteration in nutrient inflow (Quintana, 2002). In this sense, aquatic ecosystems in coastal wetlands are affected by two main types of disturbances:

- *High intensity disturbances* (or pulse type), such as sea storms or river overflows and floods due to inland storms, affect the ecosystem and tend to result in decreasing availability of resources produced after such disturbances. Thanks to dispersion and trophic niche partitioning, organisms and communities have adapted to these disturbances and, as a result, are more resilient to these changes.
- *Low intensity disturbances* of a more gradual nature, such as alterations due to irrigation and intensification of agricultural and livestock activity, affect the hydrology and nutrient dynamics of these ecosystems and can result in high productivity and availability of resources over time. Therefore, the community structure does not evolve according to the characteristic pattern of its succession, but instead favors opportunistic strategies (e.g., exotic species invasion).

It is therefore hypothesized that the community structure is not well adapted to these changes, since they do not occur naturally. Although the endemic aquatic communities of the Mediterranean areas are very resilient to pulse disturbances, they respond worse to more gradual and continuous changes in nutrient dynamics due to anthropic activities. The La Pletera salt marshes and lagoons in the Baix Ter wetlands represent Mediterranean ecosystems under pressure from anthropological activity and highlight the importance of restoration efforts to both improve the biodiversity of these environments as well as the adaptation of these aquatic communities to global change scenarios. Accordingly, the main goal of this thesis was first to quantify the different contributions that make up the water balance of the different restored and natural lagoons in the La Pletera (both intense surface inputs and gradual subterranean inputs), and then determine the effects of gradual and intense nutrient entry into the lagoons, and what influence it has on planktonic community structure and ecological functioning.

The following specific objectives were defined:

1) To analyze the overall hydrological dynamics of new lagoons, along with their dependence on groundwater circulations, and compare them with natural lagoons, to assess the restoration efforts based on conservation objectives and quantify the intense and gradual inputs that constitute the overall hydrology.
2) To analyze how different time-dynamics of nutrient inputs to a lagoon may affect the biomass contribution of the different planktonic groups and the heterotrophic activity linked to the use of carbon (C), nitrogen (N) and phosphorous (P) sources.
3) To investigate the effects of different nutrient input regimes on zooplankton community structure using a measure of severity.

These objectives were approached in the three chapters of this thesis whereby the GLM was used to model the hydrological dynamics and water balances of the new and natural lagoons for objective 1 (chapter 1). Morphology and lithological characteristics were incorporated as additional parameters. For objective 2, a field experiment using mesocosms was conducted to simulate four time-dynamics of nutrient inputs: a control (no nutrient additions), a continuous addition over the duration of the experiment, a pulse addition of nutrients (simulating a pulse flooding event; all nutrients added at the beginning of the experiment), and a pulse-continuous addition (to simulate a flooding-confinement regime) (chapter 2). The experiment was performed in a lagoon to be as close as possible to natural conditions. Lastly, objective 3 revisited a multivariate analysis conducted by Quintana (2002), to evaluate the severity of inorganic nutrient additions as a disturbance to the zooplankton community structure. With the aid of functional classification of the disturbances, the aim of this chapter (chapter 3) was i) to evaluate whether the differing input of nutrients affect the composition of zooplankton and ii) if yes, which type of nutrient input has the most severity.

3. GENERAL MATERIALS AND METHODS

3.1. Study site

The research for this thesis was carried out in the La Pletera salt marshes, which are located in the Baix Ter wetlands in the northeast of Catalunya (Figure M1 A) and is south of the urban center of L'Estartit (Torroella de Montgrí, Girona). The climate is sub-humid Mediterranean and has mean temperatures of 25 °C in summer and 10 °C in winter. The average rainfall is 590 mm/year, with the highest rainfall periods in spring (140 mm) and autumn (200 mm; Estartit meteorological station, 1966–2021 period; Pascual, 2021). The La Pletera lagoon system consists of six permanent lagoons, two of which are natural (FRA and BPI, Figure M1 B), which run perpendicular to the coastline, and are remnants of an abandoned river channel. The other 4 lagoons (G02, L01, L04, and M03) were constructed and restored under two LIFE projects (2002 and 2016) and run parallel to the coastline behind an Aeolian dune system. A shallow subterranean plastic clay layer (30-90cm in depth) is present in the SSW of the salt marshes.

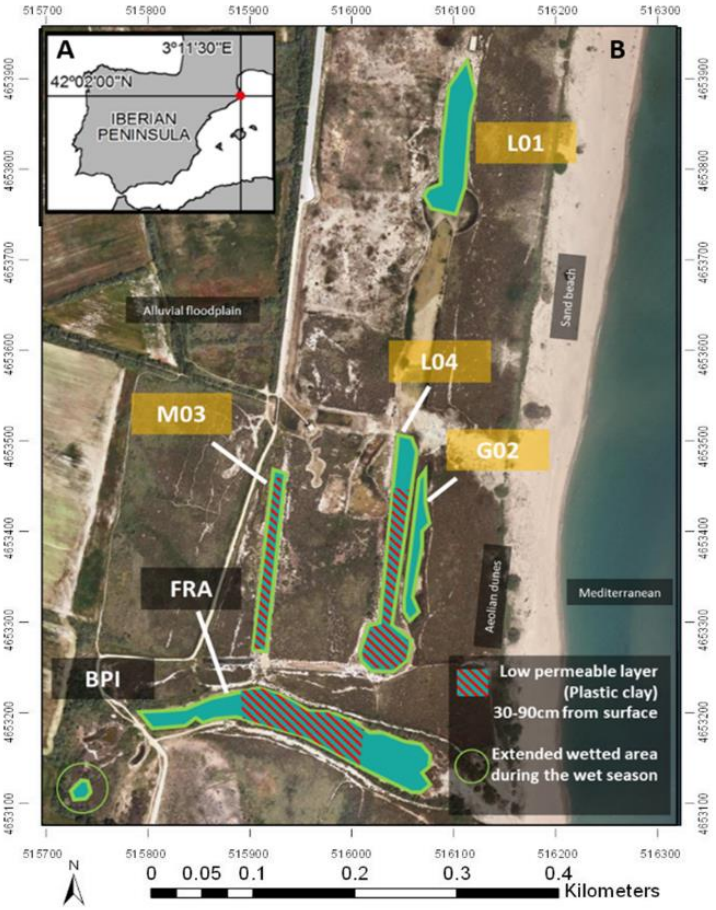

Figure M1. (A) Geographic location of the La Pletera coastal lagoon system map of the study area with, (B) the six studied lagoons. Yellow labels indicate newer constructed lagoons (2016) and an older constructed lagoon (G02 in 2002), black labels indicate natural lagoons.

3.2. Historical background

As is the case in other Mediterranean regions, this area has been affected by high anthropic pressure (such as urbanization and tourism) and the salt marsh was subjected to partial urbanization with alterations to its landscape and hydrology during the late 1980s. This project was later discontinued in the 1990s. Years later, efforts were underway to recover the area´s ecological functioning and two LIFE Nature restoration projects (http://lifepletera.com/en/life-pletera/) were implemented. The first project in 2002 created the lagoon G02. To ensure water permanency, the lagoon was excavated below sea level during construction (Figure M2 A). The second project in 2016 dismantled the remaining urban features (promenade, accesses, filling material, breakwaters and debris, Figure M2 A) and was substituted by a set of new lagoons (L01, M03, L04) with varying depths and shapes to produce lagoons with different salinity levels and permanency characteristics (Figure M1 B; Figure M2 B; Quintana et al., 2018). Quintana et al. (2018) go on to explain that, among different criteria for restoration, the design of the topographic distributions of the lagoons were intended to be a reminder of the failed urbanization process, with the old accesses and promenades converted into lagoons, while the old roundabouts separate the permanent lagoons. The intention was to create a recovered area, with restored ecological functioning, and not a pristine salt marsh. This was because the old morphology was strongly altered and impossible to replicate.

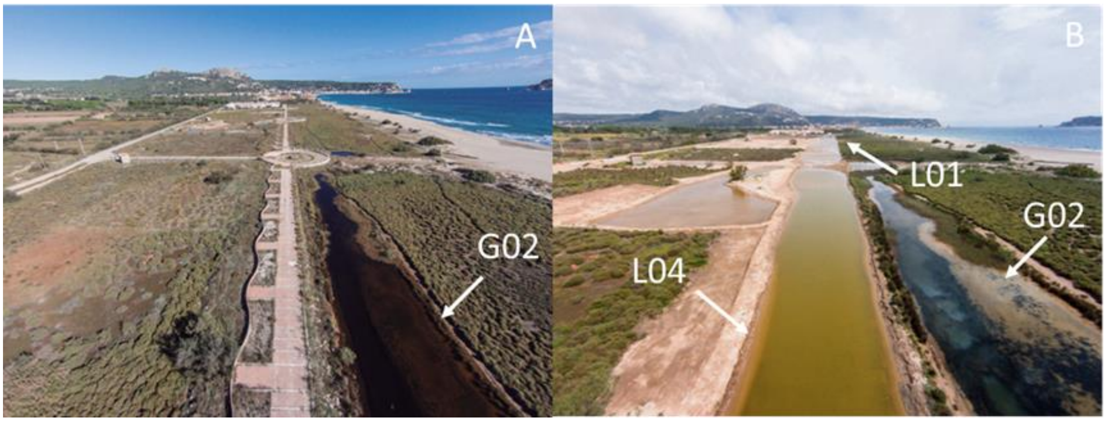

Figure M2. Aerial view of the restored area in La Pletera (Baix Ter wetlands) before (A) and after (B) the restoration in 2016. G02 was constructed in 2002. Figure adapted from Quintana et al. (2018).

3.3. Hydrology

The hydrology of the La Pletera lagoons is characterized by the absence of continuous surface freshwater or seawater inflows. This area has a micro-tidal regime, with a spring tidal range of about 0.15 m. The water bodies are located behind a foredune, with surface water exchanges occurring mainly during winter sea storms or intense rainfall events (Pascual, 2021). These cyclonic storm events associated with strong easterly winds (known as *llevantades*) and can cause sea level rise of more than 1m (Marquès et al., 2001). During these periods, sea waves may enter the saltmarshes, and together with the freshwater surface flow (overland flow), sub-surface flow (lateral percolation through the topsoil) and groundwater inputs, can cause a 0.3–0.9m increase in the level of the salt marsh. Therefore, the hydrology is strongly influenced by the sea, with sudden sea storm flooding, followed by extended periods of decreasing water levels and increasing salinity during confinement (Badosa et al., 2007; López-Flores et al., 2006; Quintana et al., 1998, 2018).

In hydrogeological terms, the La Pletera salt marsh area is connected to the shallowest level of the quaternary sediments that fill the regional basin (Menció et al., 2017). As an unconfined aquifer with a thickness of 10-30m, this unit was formed by recent alluvial deposits which becomes marsh and coastal deposits near the coastline (Montaner, 2010; ICGC, 2011a, b). This results in permanent water levels in the lagoons and contributes up to 80% of the summer water exchange when surface exchanges are scarce (Menció et al., 2017). Furthermore, Menció et al. (2017) also concluded through hydrochemical and isotopic analyses that the water salinity of the lagoons is determined by two main processes: freshwater and seawater mixing (in the lagoon and aquifer) and evaporation. The resulting fluctuations of physical and chemical parameters, such as salinity levels, allow for just a few euryhaline species to establish significant populations in these lagoons.

3.4. Hydrological modelling of the lagoons

3.4.1. Morphometry, lithological and physical characteristics

Bathymetric data were used to calculate the physical characteristics and morphometry of the lagoons. Morphometric parameters such as mean depth, relative depth, and volume development were calculated based on the methods by Hutchinson (1957). This included calculations of the rate of change of area with respect to volume. The geomorphological profiling and geological analysis were conducted in combination with drilling boreholes in the lagoon pre-construction phase (GEOSERVEI, 2016). The main characteristics of the lagoons were estimated from the bathymetric data that were incorporated into AutoCAD LT® software to estimate surface areas, volumes, lengths, and widths.

3.4.2.Hydrological dynamics

Schlumberger water level data loggers (accuracy ± 0.02 m) were used to determine daily water levels from November 2014 to September 2017. Water levels in 2018 and 2019 were obtained biweekly from depth gauge boards installed in the lagoons. A CTD profiler (Sea & Sun Technology) was used to measure biweekly values for temperature and salinity. Daily average relative humidity, precipitation, and maximum and minimum temperatures were obtained from the L´Estartit meteorological station, 2 km from the lagoons (Pascual, 2021). This was used to determine the evaporation and precipitation in these lagoons. Data for solar radiation was obtained daily from Mas Badia (La Tallada, ~10km from the La Pletera) in 2016 and 2017 and *in situ*, in the La Pletera, with radiation sensors in 2018 and 2019.

3.4.3. The General Lake Model (GLM) and application

The now well-documented GLM is an open-source model developed as an initiative of the Global Lake Ecological Observatory Network (GLEON) with several publications documenting simulations using the model (Bueche et al., 2017). Briefly, it is a one-dimensional open-source code designed to simulate the hydrodynamics of lakes, reservoirs, and wetlands (Hipsey et al., 2019) and integrates a Lagrangian layer structure similar to other 1-D lake model designs (Hamilton and Schladow, 1997; Imberger and Patterson, 1981). By integrating the effects such as inflows and outflows, mixing, as well as surface heating and cooling, the model computes vertical profiles of temperature, salinity, and density (Casamitjana et al., 2019). The GLM was applied in the La Pletera lagoons in previous studies to analyze the groundwater influence in the salt marsh (see Menció et al., 2017) and the water circulation patterns and salinity fluctuations (see Casamitjana et al., 2019), and provided, to our knowledge, the first of its application in small water bodies that do not exceed 3m in depth. The work in this thesis is a continuation of the work conducted by Casamitjana et al. (2019) and follows the same methodology in the application of the GLM. This study however extends the period of the natural and old lagoons (BPI, FRA, and G02) to 2019 and introduces 3 new lagoons (L01, L04, and M03) constructed in 2016. The selected study period was from March 2016 to September 2019. The methodology of applying the GLM will be explained briefly below. However, a full description will be given in Chapter 1 of the governing equations of the GLM, the chosen outlet point depths to model the best fit for salinity and temperature, as well as observed meteorological data.

Inflow and outflow measurements were estimated from the water levels of the lagoons. From the bathymetry data, water volume at any single depth was estimated using a polynomial fit; with values of R-squared (R^2) that indicate the goodness of fit (Table M1). This was then followed by the net daily inflow and outflow calculations to fit the volume fluctuations. The modeled inflows and outflows were then set from the net daily inflow and outflow calculations. The modeled volumes were then compared to the observed water volumes. As rain fluxes and evaporation are modeled separately from the inflows and outflows, differing volumes emerged. Through an iterative process, the inflows and outflows were adjusted until the modeled and real volume, temperature, and salinity values showed the smallest possible differences. Many inflows and outflows were compatible with a single water level, due to estimations from the inflows and outflows. However, we followed the hypothesis that there is either inflow or outflow but not both at the same time for a certain day (especially in summer and autumn). Indeed, the real inflow can be higher than the estimated inflow into the lagoons in some situations, especially in periods of heavy autumn rains with small time estimations for water renewal (less than 10 days). Nevertheless, this analysis determined the minimum inflows necessary to accurately model the observed volume levels. Simulation performance was assessed using the commonly applied root-mean-square relative error (RMSRE) and Nash-Sutcliffe efficiency (NSE).

Table M1. Polynomial fit ($V(x)=Ax^5+Bx^4+Cx^3+Dx^2+Ex+F$) for the lagoons BPI, FRA, G02, L04, L01, and M03 where $V(x)$ is the volume in m^3 and x the height above sea level in m. R^2 is the coefficient of determination.

	A	**B**	**C**	**D**	**E**	**F**	**R^2**
BPI	1551.5	627.73	-411.33	77.365	284.6	99.208	1
FRA	-170.88	-577.28	1659.9	6279.7	6811.5	32370.4	0.9992
G02	0	0	20.952	550.22	1205	667.4	0.9998
L04	0	0	-2491.1	7216.7	-2131.7	203.94	0.9913
L01	0	0	0	1887.8	1048.5	190.94	0.9719
M03	0	0	2236.7	-1646.6	1002.4	288.27	0.9981

3.5. Analysis of the effects of nutrient additions through a mesocosm experiment

3.5.1. Experiment design

The experiment was initially planned from 02 March 2020 to 02 April 2020, however COVID-19 restrictions forced the termination of the experiment prematurely by four days (29 March). The experiment was designed to mimic three nutrient loading conditions and analyze what effect they had on a natural community assemblage extracted from the L04 lagoon. We added the same amount of total nutrients to all the nutrient addition mesocosms, but at different concentrations over time to replicate 1) pulse event with total nutrients added at the beginning, 2) continuous additions over the duration of the experiment and 3) pulse-continuous additions to replicate continuous inputs in a flooding-confinement hydrological regime. A total of 20 mesocosms using 8L polyethylene water containers were used. On the initial day (day -3), 4 containers were placed in a produce crate (60x40x18cm), of which there were 5 replicas (Figure M3 A). Each crate consisted of 4 different nutrient addition regimes of a control, continuous, pulse and pulse-continuous. Each crate was fitted with 4 x 1.5L bottles to act as flotation and keep the mesocosm heads above the water line. They were placed within the lagoon to replicate temperature and light fluctuations that occur naturally. The crates were placed approximately 5m apart in the center of the lagoon and attached with rope to one iron rod placed on the bank of the lagoon and one iron rod towards the center of the lagoon for each of the 5 crates (Figure M3 C). The rods were intended to act as a pulley system to move the mesocosms from the lagoon center to the banks and vice versa for nutrient additions and sampling. This was to not enter the lagoon itself and disturb the sediments, which in turn could create excess turbidity in the water column that surrounded the mesocosms. The heads of the mesocosms were left open and fitted with wire mesh (4mm) to prevent the entry of foreign bodies. Each mesocosm was filled with lagoon water with a natural plankton assemblage, which was sampled from a Kayak in the middle of the lagoon at a depth approximately 20-30 cm from the surface. The water was first placed in 4 x 20L basins and randomly poured into the mesocosms until 8L. 3L were then removed to filter for zooplankton and to take initial physicochemical samples (day -3). The containers were left with 5L for 3 days. After 3 days, samples were collected from each mesocosm (day 0). 2L of artificial seawater (distilled water and 3.5% aquarium NaCl salt solution) were added to simulate sea storm disturbance. The salt solution was previously analyzed and found to have no additional nitrate and phosphate concentrations. The mesocosms were then fertilized with nitrate and phosphate according to their nutrient regimes (Table M2, Figure M3 B) and 10ml of distilled water for the control mesocosms. Nutrients were added everyday around midday. Light mixing/stirring was done with a sterilized glass pipette after each addition.

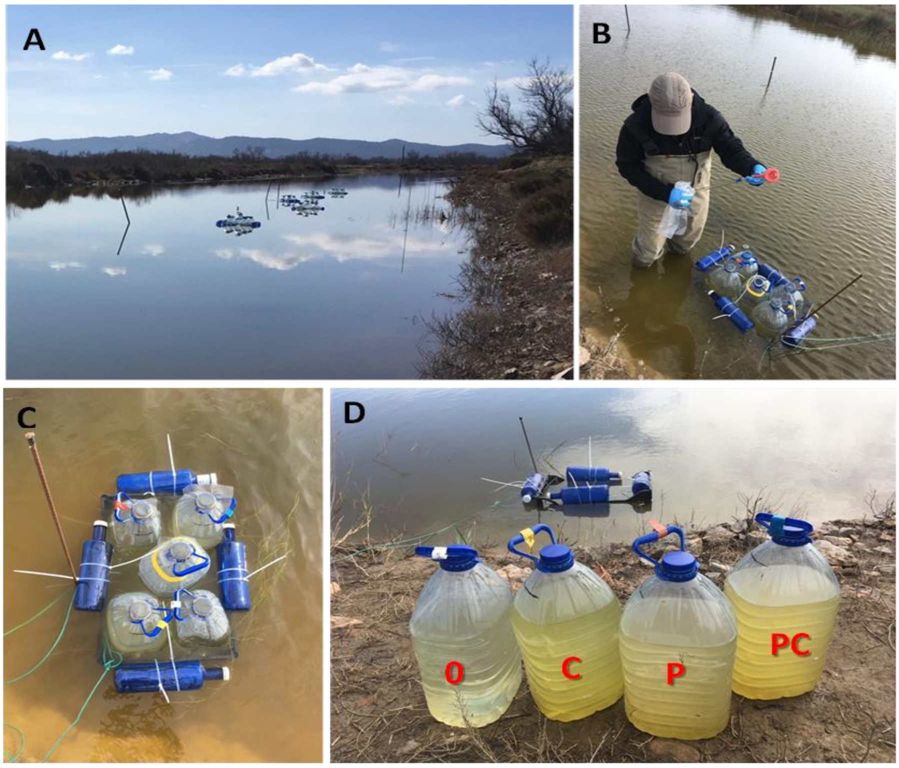

Figure M3. An illustration of the mesocosms placed in a pulley system in the L04 lagoon, within the La Pletera salt marshes. Each crate consisted of 4 treatment mesocosms of which there were 5 replicates. A) mesocosm location within the lagoon; B) nutrients addition in the mesocosms; C) mesocosm arrangements and flotation system; D) visual turbidity differences at the end of the experiment. Note the central mesocosm (5th mesocosm) was for analyzing zooplankton survivability during the experiment (data not shown here). Legend: 0 - control mesocosm; C - continuous nutrient addition mesocosm; P - pulse nutrient addition mesocosm; PC - pulse-continuous addition mesocosm.

3.5.2. Nutrient additions

A summary of the nutrient concentrations and number of additions is shown in Table M2. The N:P ratio was maintained in all treatments at 16:1 to keep with field observations and to not limit phosphorus in the system, as the La Pletera lagoons are not P limited (Boadella et al., 2021). To replicate nutrient additions of nitrate and phosphate, $NaNO_3$ (sodium nitrate) and K_2HPO_4 (dipotassium phosphate) were used. Concentrations were chosen as the maximum concentration of nitrate found in irrigation canals in the area, as well as within the lagoons themselves after a sea storm event (Menció et al. 2023), which was 17mg/L of NO_3^- and 1.48mg/L of PO_4^{3-}. These concentrations were then divided by the intended treatments over 28 days (Table M2).

Table M2: Summary of nutrient additions to the mesocosms over 24 days. Dw indicates distilled water.

Treatments	**Nutrient concentrations added**	**Relative Concentration**	**Addition (ml)**	**No. of Additions**	**Total Nitrate (mg/L)**	
					Day 0	**Daily**
Control	Dw	Dw	10	24	0	0
Pulse [P]	3.266g ($NaNO_3$) + 0.418g (K_2HPO_4) /200ml	[P] + Dw	10	1 + 23 (Dw)	17	0
Continuous [C]	0.583g ($NaNO_3$) + 0.075g (K_2HPO_4) /1000ml	[C]/28	10	24	0.6	0.6
Pulse-Continuous [PC]						
● **Pulse of [PC]**	3.266g ($NaNO_3$) + 0.418g (K_2HPO_4) /200ml	(½ [P])	5 [P]	1	8.5	0
● **Continuous of [PC]**	0.583g ($NaNO_3$) + 0.075g (K_2HPO_4) /2000ml	½[C]/28	10 (Continuous of [PC])	23	0	0.3

Thus, the details of each experiment treatment were:

1) Control (0) daily additions consisted of 10ml of distilled water.
2) Continuous (C) nutrient solution for daily injections was prepared with 0.583g of $NaNO_3$ in 1000ml, along with 0.075g of K_2HPO_4, and both were mixed in 1000ml of distilled water and added as 10ml every day (Table 2). The daily added concentration was 0.61mg/L of $NaNO_3$, and 0.05mg/L of K_2HPO_4. As is the case with the PC additions, the total added nutrient mass would therefore equal the same as the total added concentration of the pulse nutrient solution. Due to COVID-19 restrictions, however, the total concentration levels added were slightly lower than P treatments, as the experiment was terminated 4 days prematurely.
3) Pulse (P) nutrient solution for initial injection was prepared with 3.266g of $NaNO_3$ and 0.418g of K_2HPO_4 and mixed in 200ml of distilled water and added as 10ml in each of the P treatment mesocosms. This was added as a singular nutrient addition on day 0, which resulted in a maximum concentration of 17mg/L of $NaNO_3$ and 1.48mg/L of K_2HPO_4. Distilled water was added in the consecutive days until the end experiment.
4) Pulse-Continuous (PC) solution for initial peak and subsequent injections was prepared the same as P solution for both nitrate and phosphate (3), however, the pulse injection concentration was halved at the beginning of the experiment to facilitate the halved continuous daily contributions of the continuous injection for the intended 28 days. This was done using the same P nutrient addition solution, but only 5ml was used with another 5ml of distilled water to halve the solution. For the continuous nutrient additions of the PC, an additional 1L of the C solution was prepared and then diluted with 1L of distilled water to halve the concentration and added daily as 10 ml solution until the end of the experiment.

4. RESULTS AND DISCUSSION

This section contains three parts that constitute the main body of the thesis. Supplementary material is attached at the end of the thesis. Due to a large number of figures, permutations in analyses, and in the interest of saving paper:

- Supplementary material pertaining to modeled vs observed values for volume, salinity and temperature, as well as rainfall in the La Pletera salt marsh between 2016 and 2019 in Chapter 1 can be found online and the link provided in the section “Supplementary material Chapter 1”.
- Supplementary material pertaining to the ANOVA Post Hoc comparison using Tukey’s HSD in Chapter 2 test are listed the same as it is listed in figshare with the link provided under “supplementary materials”.

Notwithstanding, all materials are available on request with the author.

4.1. Effects of morphology and sediment permeability on coastal lagoons' hydrological patterns.[1]

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[1] Meredith, W., Casamitjana, X., Quintana, X. D., & Menció, A. (2022a). Effects of morphology and sediment permeability on coastal lagoons' hydrological patterns. Journal of Hydrology, 612, 128259. https://doi.org/10.1016/j.jhydrol.2022.128259

4.1.1. BACKGROUND

Coastal lagoons are diverse in their geomorphological and hydrological characteristics and have a wide variety of influences from external factors such as freshwater and saltwater inputs, tidal regimes, and climate dynamics (Basset et al., 2013; Guelorget and Perthuisot, 1983; Kennish and Paerl, 2010; Kjerfve, 1986; Pérez-Ruzafa et al., 2005; Nidzieko et al., 2014). Coastal lagoons within the Mediterranean region are influenced more by storm events than by tidal regimes and their surface connection to the sea and freshwater sources are limited for most of the year. These types of lagoon ecosystems (defined as confined coastal lagoons) are typically shallow (< 5m), and their salinity regimes fluctuate significantly according to the amount of freshwater input, the climate, and the level of connectedness to the sea (Ridden and Adams, 2008; Trobajo et al., 2002; Tyler et al., 2001). Furthermore, these lagoons have also been described as surface representations of shallow aquifers and only recently have groundwater processes been recognized as significant contributors to their hydrological behaviors and biogeochemical compositions (Casamitjana et al., 2019; Menció et al., 2017; Slomp and Van Cappellen, 2004; Windom et al., 2006). As a result, they are vulnerable to minor changes in catchment and groundwater hydrology (Chikita et al., 2015; Menció et al., 2017; Rodellas, et al., 2018; Sadat-Noori et al., 2016). Also, it has been shown that both freshwater inputs and morphological characteristics can control biological roles and determine the level of impact of confined lagoons (Basset et al., 2006; Cancela da Fonseca et al., 2001; Cañedo-Argüelles and Rieradevall, 2010; Félix et al., 2015). It is therefore important from a management perspective to understand the hydrology of these ecosystems, and to quantify the level of impact they may endure due to changes in surface and groundwater inputs.

While coastal wetlands and lagoons are well documented as being the most fluctuating and productive ecosystems in the world, it is also well documented that coastal wetlands are severely threatened due to climate change or shifts in land use (Cvetkovic and Chow-Fraser, 2011; Gabler et al., 2017; Newton et al., 2012; Wingard and Lorenz, 2014). In addition, coastal wetland deterioration can contribute to climate change due to reduced carbon storage capabilities (DeLaune and White, 2012). UNEP/MAP and Plan Bleu (2020) noted a 48 percent reduction in natural wetland habitats between 1970 and 2013 in the Mediterranean basin alone. Thus, the EU habitat management has prioritized restoration and recovery of the ecosystem services of these habitats, which is why projects such as Life

Nature have awarded financial assistance for restoration purposes on some Mediterranean coastal lagoons (Quintana et al., 2018). While this is a great step in mitigating coastal lagoon degradation, aquatic habitat restoration can be challenging and ecological functioning as an end goal is not always fulfilled due to a lack of integrated understanding of the ecosystem being restored (Hobbs & Harris, 2001). Also, comparison of restoration efforts with other natural sites is not always possible, due to a lack of reference sites or pre-existing studies (Antón-Pardo et al., 2013; Drake & Naiman, 2000). While most wetland restoration projects usually focus on eutrophication control, vegetation restoration or water quality improvement, the mechanistic understanding of coastal wetland degradation and ecohydrological processes, especially large scale hydrological and biological connectivity, is still not fully understood (Cui and Yang, 2006; Harttera and Ryan, 2010; Scheffer et al., 1993). In case of the flooding-confinement pattern of Mediterranean coastal lagoons, it has been shown that community structure dynamics are dependent on nutrient dynamics and the variability of water volume and salinity fluctuations (Gascón et al., 2005; López-Flores et al., 2006b; Quintana et al., 2006). While lagoon morphometry and underlying sediment permeability have been studied extensively, combining these disciplines to evaluate lagoon hydrology and salinity dynamics is yet to be fully explored. Therefore, a multidisciplinary approach in understanding these ecohydrological processes will help to evaluate overall ecological functioning within these ecosystem types.

The La Pletera salt marshes is an example of a coastal wetland system affected by a failed urbanization project and was awarded financial assistance from the Life+ project (http://lifepletera.com/es/life-pletera/), which aimed to restore this protected area and to recover its ecological functioning by dismantling unused urban features. Various studies were conducted to assess several structural and functional indicators of restoration of the salt marsh. Among these studies, two were focused on the salinity fluctuations and groundwater dependence of two natural lagoons (BPI and FRA) and one constructed lagoon (G02) in 2002 (see Menció et al., 2017; and Casamitjana et al., 2019). In 2016, three new lagoons (L01, L04, and M03) were created under the second phase of the Life project, and they were constructed by excavating the sediment below sea level, while also considering the underlying sedimentary pattern to ensure the conservation of low-permeability layers present (GEOSERVEI, 2016). The aim was to facilitate higher salinity conditions by decreasing an efficient connection with the aquifer during periods of confinement, to increase favorable refuges for the endangered Iberian toothcarp fish

(*Aphanius iberus*) (Alcaraz et al., 2008; Badosa et al., 2006). These conditions are important to reduce the competition of the invasive mosquitofish (*Gambusia holbrooki*), which is more adapted to freshwater flooding conditions and less salinity variability (Alcaraz & Garcia-Berthou, 2007; Rincón, 2002; Ruiz-Navarro et al., 2011).

The aim of this study was to analyze the overall hydrological dynamics of the new lagoons (including G02), along with their dependence on groundwater circulations, and compare them with natural lagoons, to assess the restoration efforts of increasing salinity fluctuations (higher salinity during the dry period) and ecological functioning. We used the one-dimensional General Lake Model (GLM) to assess water volume fluctuations, salinity variability, and lagoon water circulation (groundwater and surface water inflows, rainfall, outflows, and evaporation) to assess how the lagoons´ hydrological behaviors and their dependence on groundwater and surface water fluxes contribute not only to their salinity fluctuations but their total water budgets. We also combine data of the underlying lithological characteristics and the overall morphometry of the natural and new lagoons, to better understand the interplay of such parameters on the hydrological behavior of each lagoon individually and in comparison with each other.

4.1.1.1. Lithological characteristics of the La Pletera Lagoons

The presence of marsh silts predominates in the areas of the natural lagoons in layers above sea level, while the presence of alluvium and sands are more common in the new lagoons (GEOSERVEI, 2016). BPI is the only lagoon with low-permeability clay layers above sea level. At sea level, the presence of a plastic clay layer becomes evident and extends across the central area of the FRA lagoon, the entire area of the M03 lagoon, and three-quarters of the area of the L04 lagoon, which then tapers away towards the north. The layer reaches a depth of around 90 cm below sea level, which extends deeper in FRA (Table 1.1). G02 does not show a record of a low-permeability layer. At a depth deeper than 1m below sea level, permeable fine sands form the underlying base of the lagoon systems, except for FRA - which still shows the presence of the low-permeability plastic clay layer. Fine sands, however, predominate the deeper sediment profiles of all the lagoons. During the survey, L01 had no low-permeability layers detected throughout its sediment profile. In summary, the lagoons with underlying low-permeability layers are BPI, FRA, M03, and part of L04, while L01 and G02 have higher permeability layers throughout their sediment profiles (Table 1.2).

Table 1.1. Lithological characteristics of the La Pletera lagoons according to the geological survey conducted by GEOSERVEI in 2016.

Height m.a.s.l.	**BPI**	**FRA**	**G02**	**L04**	**L01**	**M03**
> 0m	Marsh Silt/ Fluvial Clay	Marsh Silt/ Alluvium	Marsh Silt	Sandy Silt	Alluvium	Medium Sands
0m	Fluvial Clay	Alluvium	Alluvium	Plastic Clay	Alluvium/ Fine Sands	Alluvium
< 0m > -1m	Fluvial Clay/ Fine Sands	Plastic clay/ Alluvium	Alluvium/ Fine Sands	Plastic Clay/ Fine sands	Fine Sands	Plastic Clay
< -1m	Fine Sands	Plastic Clay/ Fine Sands	Fine Sands	Fine Sands	Fine Sands	Fine Sands
Lagoon Bottom	-0.5m	-1.5m	-1m	-0.3m	-0.2m	-0.3m

Table 1.2. Relative sediment permeability of the unconsolidated deposits of the La Pletera salt marshes, listed according to Lewis et al. (2006) and Freeze & Cherry (1979).

Sediment	**Relative Permeability**
Marsh silt	Moderate-low
Fluvial clay	Low-very low
Plastic clay	Very low
Alluvium	High-low
Medium sands	High-low
Sandy silt	Moderate-low
Fine sands	High-moderate

4.1.2. METHODS

This study used the GLM to model water volumes, salinity and temperature profiles of the six lagoons labelled: BPI, FRA, G02, L01, L04, M03. As this is a confined coastal system, it was not possible to measure inflows or outflows and they had to be calculated manually from the fluctuating volume levels of each lagoon. Table 1.3 is the bathymetry data that calculates the volumes of the lagoons at any single depth using a polynomial fit. Table 1.4 shows the GLM physical parameters used in modelling the La Pletera salt marshes. Because of the small volume and shallow depth of the La Pletera lagoons, the minimum values for volume and thickness of the Lagrangian layers are established one order of magnitude smaller than the typical values. The physical parameters C_K, η_*, C_T, C_S and C_{HYP} are related to the individual mixing process efficiencies and don´t require calibration; their values are based on observations, experiments in the laboratory and theoretical deliberation (Imberger, 1998), and are set to the usual values.

Table 1.3. Bathymetry of the lagoons of the La Pletera salt marshes

Height ASL (m)	Surface Area (m^2)					
	BPI	FRA	G02	L01	L04	M03
1.5		17290	2991			
1.25			2700			
1	5387	16733	2500	8657		8007.08
0.9				7732		
0.8				7059.33		5000
0.7	1383.72	14478	2000	3492.85	9878	4000
0.6						3041.1
0.5	435.83	13605	1550		9060.63	2810.47
0.4						2589.57
0.3				2684.69	7732	2375.15
0.25	288.58	12154	1500			
0.2				2322.05	6399.4	2154.33
0.1				1827.29	4428.9	1906.65
0	213.31	2530.12	1491	1512.18	2950.08	1630.66
-0.1				1068.26		
-0.2	138.12			0	1606.16	500
-0.3					883.58	0
-0.4					585.51	
-0.5	60.12	1516.16	500		217.5	
-0.6	0				0	
-0.75			300			
-1		1056.78	250			
-1.5		259.39	0			
-1.6		0				

Table 1.4. The values of the GLM physical parameters used in modelling the La Pletera salt marshes.

Mixing and thermodynamic parameters		
C_K	Mixing efficiency-convective overturn	0.2
η	Mixing efficiency-wind stirring vs convection	1.23
C_S	Mixing efficiency-shear production	0.23
C_T	Mixing efficiency-kinetic requirement	0.51
C_{HYP}	Mixing efficiency-hypolimnetic mixing	0.5
Model structure		
Maximum Lagrangian layers		200 m^3
Minimum layer volume		0.025 m^3
Minimum layer thickness		0.005 m
Maximum layer thickness		0.05 m

When modelling the water balance, salinity and temperature fluctuations, the General Lake Model incorporates inflows/outflows, mixing, and surface heating and cooling, and calculates vertical profiles of temperature, salinity, and density with a flexible Lagrangian layer structure (Imberger & Patterson, 1981; Hamilton & Schladow, 1997). These layers contract or expand according to inflows, outflows, surface mass fluxes and mixing. From the total daily inflow and outflow data and daily averaged meteorological data, the surface momentum, sensible heat, and latent heat fluxes are computed from the following bulk aerodynamic formulae for the stress τ (Nm^{-2}), the sensible heat transfer H (Wm^{-2}), and the evaporative heat transfer E (Wm^{-2})

$$\tau = \rho_A C_D U^2 \qquad [1]$$

$$H = - \rho_A C_P C_H U(T_A - T_S) \qquad [2]$$

$$E = - \rho_A L_V C_W U(q_A - q_S) \qquad [3]$$

where ρ_A= air density; U= wind speed; T= air temperature; q= specific humidity (all daily averaged); and subscripts A for air and S for water surface values. C_H, C_W, and C_D are bulk aerodynamic transfer coefficients, and are determined by the height where the data were taken. C_P is the specific heat of water at constant pressure and L_V is the latent heat of evaporation of water. The water mass evaporation can be calculated from E/Lv of the lagoons and is in kg m^{-2} s^{-1}.

The short-wave radiant flux that is distributed through the water column is calculated using Beer's law formulation:

$$Q(z) = Q_0 e^{-\eta z} \qquad [4]$$

where Q_o= measured radiation at the surface; Q(z)= the intensity at depth z, and η= the light attenuation coefficient. The constant light attenuation factor was set to 1.7 m^{-1}, which is a typical value for eutrophic waters (Armengol et al., 2003)

An integral turbulent kinetic energy model forms the basis for surface layer dynamics [2] and is divided into four discrete processes: wind stirring, convective overturn, interfacial shear production, and Kelvin-Helmholtz billowing. The model calculates the energy available through each of these processes and is a function of the nature of the stratification and the strength of the forcing. This is then compared with the potential energy required to combine the mixed layer with the layer immediately below. The layers are mixed by averaging their properties if sufficient energy is available. This is repeated until not enough energy remains within the present time step to continue the deepening process. This residual energy is then added to the available energy in the next time step. The parameterization for the available turbulent kinetic energy (KE_A) is:

$$KE_A = \frac{C_K}{2}(w_*^3 + \eta_*^3 u_*^3)\Delta t + \frac{C_S}{2}\left(u_1^2 + \frac{u_1^2}{6}\frac{d\delta}{dh} + \frac{u_1\delta}{3}\frac{du_1}{dh}\right)\delta h \qquad [6]$$

and for the required potential energy (PE_R) is:

$$PE_R = \frac{C_T}{2}\left[(w_*^3 + \eta_*^3 u_*^3)^{\frac{2}{3}} + \frac{\Delta\rho g h}{\rho_o} + \frac{g\delta^2}{24\rho_o}\frac{d(\Delta\rho)}{dh} + \frac{g\Delta\rho\delta}{12\rho_o}\frac{d\delta}{dh}\right]\delta h \qquad [7]$$

where u_* and w_* = velocity scales for wind shear and penetrative convection, respectively; u_1 = shear velocity at the surface; Δρ= density jump between the surface layer (with depth h) and the layer immediately below it (with depth dh); ρ_o is a reference density; δ is the Kelvin-Helmholtz billow thickness scale; Δt = the time step; and g= the acceleration due to gravity.

The turbulent diffusivity coefficient, D_z, is used to model hypolimnetic mixing. This depends directly on the dissipation of the turbulent kinetic energy and inversely on the stratification. The formula is based on Weinstock (1981), and is given by the expression:

$$D_z = \frac{C_{HYP}\,\varepsilon}{N^2 + ut_*^2 k_o^2} \qquad [8]$$

where k_0 is the wave number of the largest eddies; ut_* is the turbulent velocity scale; ε is dissipation; and C_{HYP} is a constant related to the mixing efficiency of the turbulence.

4.1.2.1. Data analysis

Bivariate Pearson's correlation coefficient was used to analyze whether a statistically significant linear relationship existed between variables influencing lagoon salinity and volume levels (salinity, volume, evaporation, total inflow, total outflow, inflow salinity, rain, and surface temperature) and to assess the strength of this relationship within the lagoons. Due to highly seasonal patterns, linear mixed models were used to allow for both fixed and random effects within the analysis and aggregate the hierarchical data based on the month and year on lagoons which showed no significance in annual patterns. Stepwise multiple regression models for hydrological parameters affecting salinity and volume were used to analyze the variance among lagoons grouped according to their features: 1) new lagoons (L01, L04, M03, G02), 2) old lagoons (BPI, FRA), 3) presence of low-permeability layers (BPI, FRA, L04, M03) and 4) absence of low-permeability layers (L01, G02). Statistical analyses were done with R software (version 4.1.2; R Project for Statistical Computing, Vienna, Austria) and The jamovi project (2021) (*jamovi* Version 2.2.2, Computer Software, retrieved from https://www.jamovi.org). Uncertainties for empirical and modeled mean values in this study were quantified by the standard coefficient of variation following the methods by (Håkanson, 2005). The CV-value within lagoon variability (CVw) is calculated from time-series data and is related to hydrological and physical conditions. Variations within and among lagoons (CVa) were analyzed using the standard coefficient of variation, CV, to quantify parameter uncertainties as illustrated by Håkanson (2005).

4.1.3. RESULTS

4.1.3.1. The main characteristics and morphometry of the lagoons

The new lagoons (L01, L04, M03) were more homogeneous in their main characteristics and showed similarities in their volumes, surface areas, and mean depths (Table 1.5). They also had little stratification in their surface and bottom salinity levels. L01 was the shallowest of all 6 lagoons and 2.5-fold shallower than FRA. L04 and M03 were similar in their volume development values at ~0.80, while L01 had a value similar to the natural lagoons at ~1.2- 1.36, indicating a typical conical depression shape and easily eroded geology. The new lagoons´ relative depths were similar and lower than 2%.

In contrast, the natural lagoons and G02 were more heterogeneous in terms of their volumes and surface areas, with notable stratification in surface and bottom salinity levels in BPI and FRA. G02 showed little stratification in salinity levels despite being the second deepest lagoon. The natural lagoons and G02 also differed in their mean depths, with BPI showing a notably lower mean depth level in comparison with all the lagoons. BPI also had the lowest volume development value, indicating that it has less of a uniform bottom and is more of a localized deep hole. The FRA and G02 lagoons, however, showed more similarity in terms of their mean depths and have the highest volume development values. They were also the deepest lagoons with higher relative depths (including BPI) in comparison with the other lagoons, with G02 above 3.5% (Table 1.5).

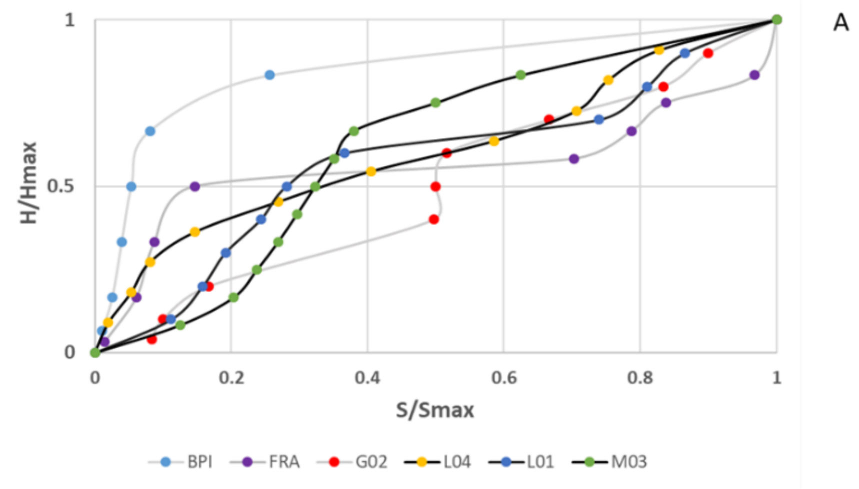
Table 1.5. Summary of the main characteristics and morphometry of the studied lagoons during the studied period March 2016 to September 2019. Salinity (‰) is in parts per thousand. Mean Depth (z) is the average depth of the lagoon. Relative depth (Z_r) is the ratio of the maximum depth as a percentage of the mean diameter of the lagoon at the surface. Volume Development (D_v) is the ratio of the volume of a lake to the volume of a perfect cone with the same surface area and maximum depth. According to Hutchinson (1957), Dv values higher than 1 indicate a typical conical depression shape and easily eroded geology. Lake number (calculated by GLM) is the dimensionless quantitative index of the dynamic stability of the water column, defined as a ratio, at the moment of stabilizing force due to gravity to the moment of turbulence destabilizing forces.

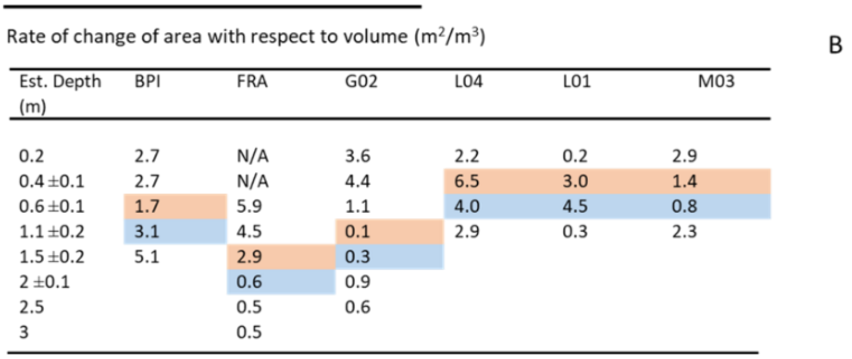
Lagoon	BPI	FRA	G02	L04	L01	M03
Origin	Natural	Natural	2002	2016	2016	2016
Max Depth (m)	1.5	3	2.2	1.6	1.2	1.6
Max Volume ($m^{3)}$	1295	22956	2999	4231	4168	3723
Max Surface area (m^2)	5387	17290	2991	9878	8657	8007
Average Surface Salinity (‰)	43.38	38.74	25.04	21.7	17.01	32.53
Average Bottom Salinity (‰)	77.91	54.49	25.23	22.53	17.26	33.47
Average Surface Temp (°C)	19.08	19.41	18.78	18.08	19.07	18.93
Mean Depth z (m)	0.24	1.33	1	0.43	0.48	0.46
Relative Depth Zr (%)	1.93	2.02	3.56	1.43	1.14	1.55
Volume Development Dv	0.48	1.32	1.36	0.8	1.2	0.89
Lake Number	536	843	1378	179	107	359

When comparing all the lagoons together, little differences in their average surface temperatures were observed. BPI, FRA, and M03 showed 1.5 to 2-fold higher surface salinity levels than the rest of the lagoons. BPI and FRA had the biggest contrast in terms of the volume capacity of all the lagoons, with FRA having a 20-fold greater volume capacity than BPI. L01 showed the lowest surface salinity levels with nearly a 1.5 to 2.5-fold lower difference than the rest of the lagoons. FRA and G02 had the highest lake number values followed by BPI, suggesting more water column stability in these lagoons than the new lagoons L04, L01, and M03.

4.1.3.2. Bathymetric profiles

The normalized bathymetric profiles of the six lagoons, with their respective height to surface ratios are illustrated in Figure 1.1 A. The respective heights and surface areas were normalized by their maximum height and surface area values. The new lagoons were similar in their bathymetric profiles (shown as darker lines). In contrast, the natural lagoons and G02 showed differences in their profiles, with BPI having the biggest surface area over height change the deeper the lagoon becomes. L04 had a slightly higher surface area change with respect to its height. The natural lagoons had more of a conical depression shape (as can be seen with the volume development ratios in Table 1.5) with FRA showing more increase in height over its surface area at around 50% of its height with only an increase of ~15% of its surface area.





Rate of change of area with respect to volume (m^2/m^3)

Est. Depth (m)	BPI	FRA	G02	L04	L01	M03
0.2	2.7	N/A	3.6	2.2	0.2	2.9
0.4 ±0.1	2.7	N/A	4.4	6.5	3.0	1.4
0.6 ±0.1	1.7	5.9	1.1	4.0	4.5	0.8
1.1 ±0.2	3.1	4.5	0.1	2.9	0.3	2.3
1.5 ±0.2	5.1	2.9	0.3			
2 ±0.1		0.6	0.9			
2.5		0.5	0.6			
3		0.5				

Figure 1.1. (A) Bathymetric profiles of the six lagoons normalized by their respective maximum heights and surface areas, with the newer lagoons shown in darker lines and the natural and older lagoons in lighter lines. (B) Rate of change of area with respect to volume (m^2/m^3) and the average depths of all the lagoons. Red indicates summer average depths that correspond with the depth column value and blue indicates all year round. N/A implies the rate of change is very high and non-applicable at this depth. Depths are estimates and can be above or below the indicated level within 0.1m-0.2m.

Figure 1.1 B shows the calculated rate of change of area with respect to volume. Values highlighted in red indicate estimated average depths in summer (June to September). Blue indicates the estimated average depths of the lagoons for the rest of the year (that excludes summer). For the convenience in representation and due to the nature of the bathymetric data and varying depths of each lagoon, depth are estimates that can either be above or below the indicated level within 0.1m-0.2m. L04, L01, and M03 had similar average water depths and were shallower than the natural lagoons and G02 both in summer and for the rest of the year. The difference in the new lagoons′ water levels in summer compared with the rest of the year is ~ 0.2m-0.3m. In contrast, the natural lagoons and G02 showed a ~ 0.5m difference in their water levels from summer to the rest of the year. The rate of change of area with respect to volume shows that the new lagoons had a higher surface area rate of change than the natural lagoons and G02 at shallower depths. This can be seen in the similar bathymetric profiles and steady increase in surface area over the heights illustrated in the darker lines in Figure 1.1 A. FRA and G02 had a less rate of change of area as the lagoons got deeper. However, BPI showed the opposite trend where the rate of change of area increases as the lagoon gets deeper. G02 showed a 65-fold lower rate of change of area compared with L04 in the summer - which also had the highest rate of change on average out of all the lagoons.

4.1.3.3.Model performance

Simulation performance was assessed using the commonly applied Nash-Sutcliffe efficiency (NSE) and root-mean-square relative error (RMSRE) (Table 1.6). For the 4-year study period, the NSE values on average for volume were ~0.95 and ~0.7 for salinity and temperature. The values for RMSRE were ~10% for volume, ~20% for salinity, and ~23% for temperature. Modeled vs observed data graphs are shown in the Data in Brief article, with a link provided in the supplementary material section of this thesis.

Table 1.6. Model performance assessments using the root mean relative square error (RMSRE) and Nash-Sutcliffe efficiency (NSE) for the lagoons BPI, FRA, G02, L04, L01, and M03.

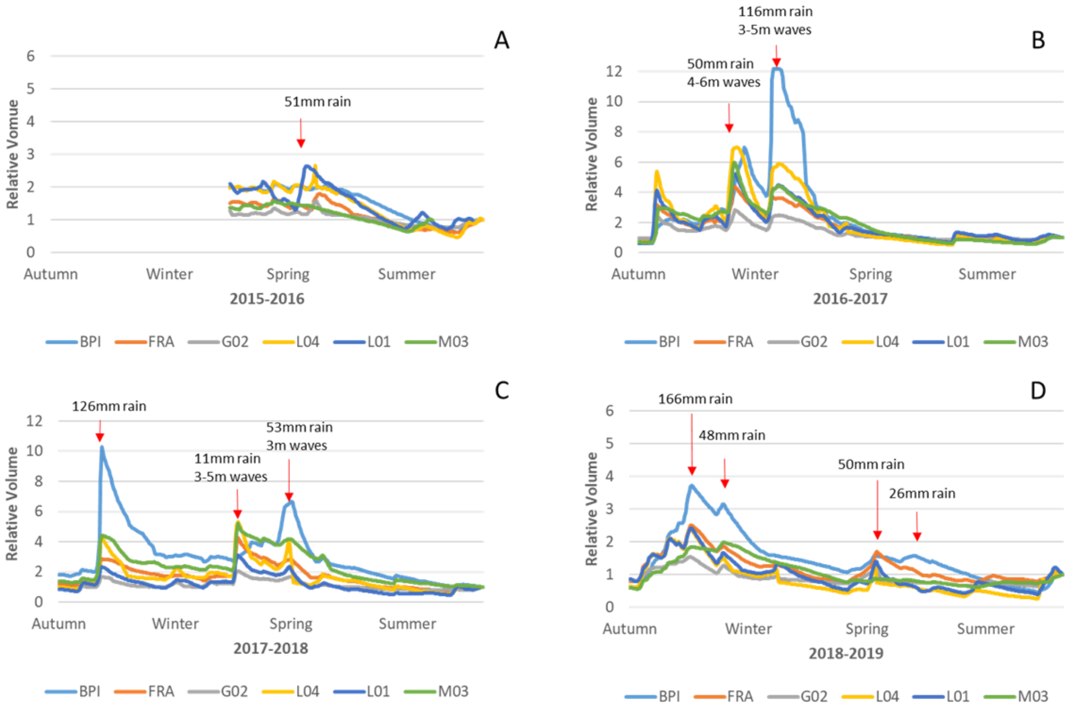
NSE	BPI	FRA	G02	L04	L01	M03
Volume	0.95	0.96	0.94	0.95	0.97	0.98
Salinity	0.71	0.7	0.7	0.71	0.71	0.65
Temp	0.71	0.71	0.68	0.7	0.7	0.65

RMSRE	BPI	FRA	G02	L04	L01	M03
Volume	0.17	0.1	0.07	0.18	0.11	0.11
Salinity	0.25	0.17	0.19	0.17	0.15	0.18
Temp	0.22	0.25	0.23	0.24	0.23	0.23

4.1.3.4. Water volume fluctuations

The relative water volumes of all six lagoons normalized by their initial water volume on the 1st of October, with the hydrological period beginning in October the previous year is shown in Figure 1.2. The year 2015-2016 was evaluated from April to October of the same year due to the availability of data. Similar patterns of mixing and desiccation can be seen in all the lagoons, with levels increasing from the initial volume in autumn and returning to the initial volume level towards the end of summer. The years 2015-2016 and 2018-2019 had no influence of storms and only significant rainfall events (Figure 1.2 A, D), with 2018-2019 showing the most significant rainfall events and changes in volume levels in all the lagoons. All lagoons behaved similarly in 2015-2016, with more fluctuation of volume levels in L01 than in the other lagoons (Figure 1.2 A). The years 2016-2017 and 2017-2018 showed significant storm events with waves higher than 3m on all occasions combined with significant rainfall in 2017 (11mm - 126mm) (Figure 1.2 B, C). When evaluating the individual responses of the lagoons to rainfall and/or seawater inputs, the higher rainfalls (above 100mm) affected BPI more with a 6-fold increase in its relative volume in 2016-2017 and 2017-2018. L04 showed high fluctuations in its volume levels from both rainfall and seawater intrusion. Overall, G02 had a lower response in its relative volumes from rainfall and seawater intrusions over the 4-year study period. The newer lagoons (L04, L01, and M03) showed a 3 to 9-fold increase in their relative volumes due to seawater intrusion and a 2.5 to 3.5-fold increase with significant rainfall events. In contrast, G02 and FRA only had a 1.5 to 3.5-fold increase during similar events.

Figure 1.2. Relative water volumes (normalized to the initial volume of water of the respective lagoons on the 1st of October of the year 2016, 2017, 2018 and 2019 to show periods of mixing through to periods of desiccation in summer (end of September). The hydrological period is from October of the previous year to October of the following year. The year 2015-2016 was evaluated from April to October due to availability of data. Red arrows indicate inputs from either rainfall and/or seawater intrusions from sea storms (wave heights higher than 3m). Note the relative volume axis maximum unit measurement change in 2017 and 2018 (Figure 5 B and C).



4.1.3.5. Calculated GLM water circulation and surface area fluxes

Figure 1.3 shows the modeled fluxes per unit of water volume of the lagoons, calculated during the study period from 2016 to 2019. These fluxes are calculated in cubic meters per day per lagoon volume and are together categorized as water circulation within the lagoons (Inflow/V, Outflow/V, Evaporation/V, Rain/V). For the convenience of representation, calculations are converted to cubic millimeters per day, except for surface area. The time period selected includes the periods of mixing which occur in October, after the autumn rains, and subsequent desiccation that occurs towards September in the summer and illustrates the overall average hydrological behavior of each lagoon. Inflow and outflow were modeled as a singular occurrence and did not occur concurrently. Also, due to the nature of the hydrological activity in Mediterranean lagoons, the sensitivity of mean calculations was considered representative. Modeled results of inflow, outflow, evaporation, and rain were normalized by their respective lagoon volumes at each time step to best represent the overall circulation of each lagoon relative to other lagoons. Surface area flux calculations were also normalized by their respective water volumes and included to compare with evaporation fluxes.

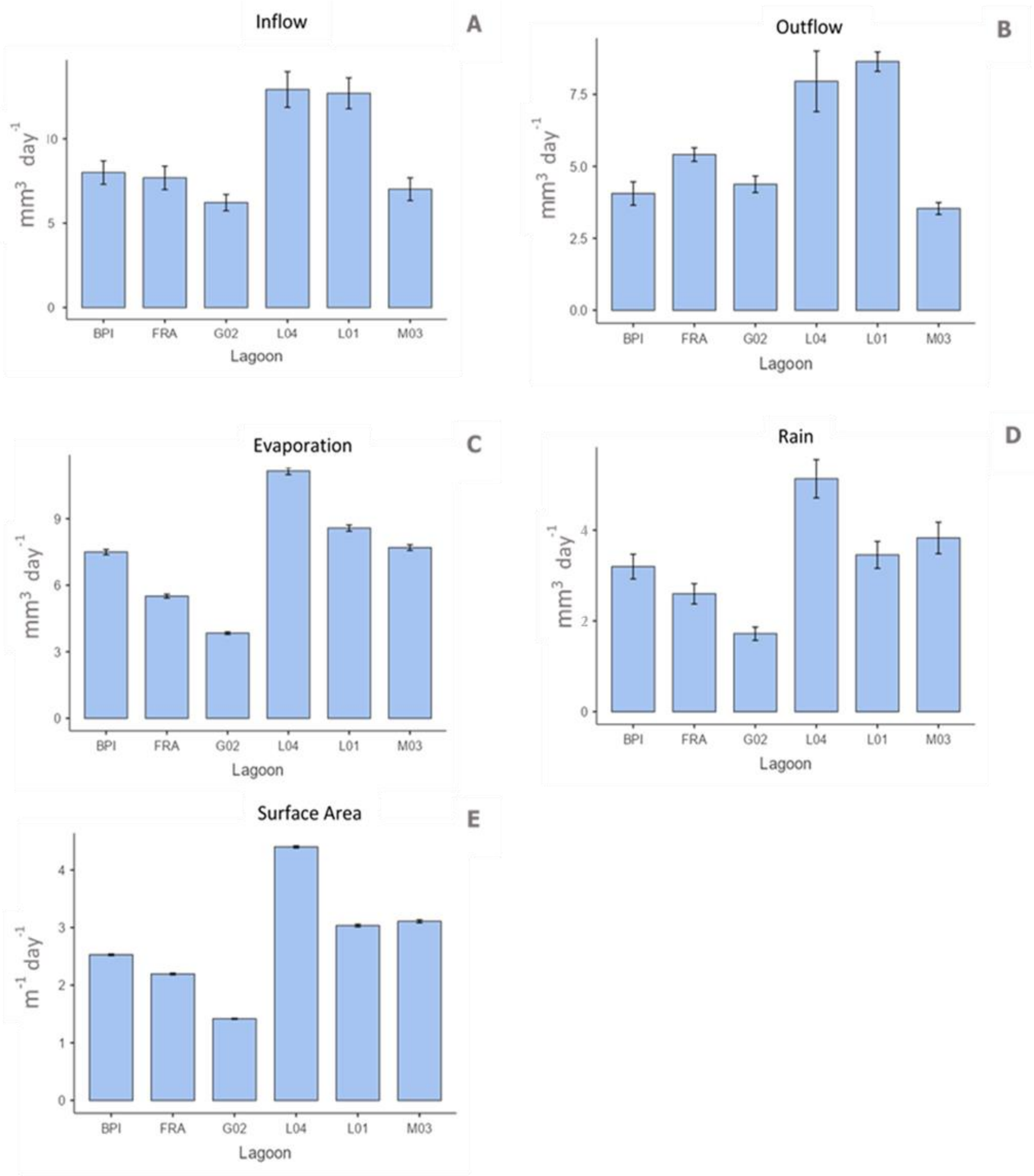


Figure 1.3. Bar plots with error bars of the mean lagoon circulation of the GLM water budget for all the lagoons BPI, FRA, L04 (n = 1309), G02 (n = 1292), L01 (n = 1300) and M03 (n = 1266) for the study period from 2016-2019. Modelled results of (A) Inflow, (B) Outflow, (C) Evaporation, (D) Rain and (E) Surface Area are normalized by their respective daily lagoon volumes. Calculations are in cubic meters per day per lagoon volume and converted to cubic millimeters per day. All parameters are categorized as water circulation within the lagoons (Inflow/V, Outflow/V, Evaporation/V, Rain/V. Evaporation and outflow represent the removal of water in cubic meters per day per lagoon volume. Surface area over volume included to compare the effect of lagoon surface area on evaporation.

Differences in mean water circulation between the lagoons can be seen across the 4-year study period. Despite M03 and BPI having similar mean evaporation (7.69 mm^3 day^{-1} and 7.49 mm^3 day^{-1}, respectively), L04 showed a higher surface area per volume that corresponds with higher evaporation per volume (Figure 1.3 C, E), while G02 had the lowest corresponding surface area and evaporation fluxes, indicating a positive relationship between the surface area to volume ratio and evaporation for all the lagoons combined (Pearson´s correlation, $p = < 0.001$, $R^2 = 0.79$, Table S1.). The new lagoons L01 and L04 showed higher circulation with higher mean evaporation, inflow, and outflow in comparison with M03 and the natural lagoons BPI, FRA, and G02 (Figure 1.3 A, B, C). L01 and L04 had a 1.8-fold higher difference in mean inflow in comparison with M03 (12.9 mm^3 day^{-1}, 12.7 mm^3 day^{-1}, and 7.01 mm^3 day^{-1}, respectively), while L01 showed a nearly 2.5-fold increase in mean outflow compared with MO3 (8.64 mm^3 day^{-1} and 3.53 mm^3 day^{-1}, respectively). The mean effect of rain over volume was greatest in L04 and lowest in G02 with a 3-fold difference between them (5.13 mm^3 day^{-1} and 1.72 mm^3 day^{-1}, respectively), and follow a similar mean pattern to that of the surface area over volume measurements.

4.1.3.6. Modeled salinity inflows

To match observed salinity levels, inflow salinity values were manually entered into the GLM on a daily basis for each lagoon. BPI, FRA, and M03 salinity levels were set above Mediterranean Sea salinity levels (37 ppt) in 2016, 2017 (only for BPI), 2018 and 2019 (only in M03) (Table 1.7). This occurred mainly in the summer and autumn periods, with M03 showing high inflow salinity across the seasons, except in 2017 and the autumn of 2019. G02 registered higher salinity only in the summer of 2018. L04 and L01 showed no inflow salinity greater than sea salinity, with L01 showing the lowest inflow salinities of all the lagoons during summer and autumn. Overall, 2019 showed lower salinity inflow levels in all the lagoons, while 2016 and 2018 had the highest levels.

Table 1.7. Model inflow salinity (ppt) averaged by the season (4 months) for the 4-year study period. Asterisks and highlighted values indicate salinity levels above the Mediterranean Sea level salinity (37ppt).

Year	Season	BPI	FRA	G02	L04	L01	M03
2016	Winter - Spring	**48***	36	21	17	16	**49***
	Spring - Summer	**60***	27	19	11	5	**39***
	Autumn - Winter	25	**44***	23	31	28	**43***
2017	Winter - Spring	18	25	25	15	10	10
	Spring - Summer	**40***	35	23	14	7	28
	Autumn - Winter	28	27	16	15	8	21
2018	Winter - Spring	26	34	21	27	19	**43***
	Spring - Summer	**50***	**45***	**45***	12	3	**60***
	Autumn - Winter	25	**49***	20	26	21	**60***
2019	Winter - Spring	22	33	13	20	13	**60***
	Spring - Summer	22	25	13	20	8	**40***
	Autumn - Winter	22	21	15	20	5	5
	Average	32	33	21	19	12	38

4.1.3.7. Variations among and within lagoons

The within-lagoon variations for all variables, except surface area, mainly depend on seasonal climatic changes such as desiccation in summer and seawater intrusions from winter cyclonic storms. The CV expressing variations among lagoons, CVa, is defined from the coefficient of variation of mean monthly values from different lagoons. Both CVw and CVa were calculated from March 2016 to September 2019. As expected, there were no significant differences between CVw and CVa for surface temperature, with variation around 20% and driven by seasonal temperature changes (Table 1.8). Significant differences in morphological features (surface area and volume) can be seen within and among the lagoons, with BPI showing the greater variance of both within the lagoon and G02 showing the lower variations within. The largest variations among the lagoons were in their volume and surface areas. Circulation parameters among the lagoons showed variation at ~30% for Inflow/V, Outflow/V, Evap/V, and Rain/V, however, the circulation parameters within the lagoons had high variation (due to occasional daily zero values) in Outflow/V, with L04 having a 3-fold higher variation than L01. Salinity variation within the individual lagoons was 1.5-fold lower in both L01 and FRA compared with the rest of the lagoons and 3-fold lower than BPI. The natural lagoons (BPI and FRA) showed the lowest inflow salinity variations, while the constructed lagoons had a 1.5-2-fold higher variation than the natural lagoons.

Table 1.8. Coefficient of variation (CV = SD/MV; SD = standard deviation, MV = mean value) within, CVw, and among, CVa, the lagoons from the study period 2016 to 2019.

	Volume	Surface Area	Surface Temp	Salinity	Inflow Salinity	Inflow	Outflow	Evaporation	Rain
Coefficient of variation within lagoons (CVw)									
BPI	0.85	1.19	0.26	0.71	0.25	3.13	3.51	0.62	3.20
FRA	0.48	0.18	0.24	0.26	0.21	3.37	1.61	0.58	3.28
G02	0.30	0.12	0.24	0.34	0.50	2.84	2.33	0.55	3.19
L04	0.68	0.48	0.26	0.37	0.41	3.11	4.66	0.53	3.10
L01	0.53	0.34	0.25	0.27	0.47	2.56	1.42	0.60	3.16
M03	0.56	0.32	0.23	0.39	0.40	3.43	2.08	0.59	3.25
Coefficient of variation among lagoons (CVa)									
CVa	1.27	1.04	0.06	0.34	0.31	0.32	0.38	0.34	0.35

4.1.3.8. Variables influencing salinity and volume levels

To assess the relationship between variables that influence volume and salinity levels within the lagoons, a series of Pearson product-moment correlations were performed (Table 1.9). When assessing the relationships influencing salinity, the volume levels were negatively correlated with salinity in all the lagoons except for L01, where its total inflow was associated with salinity levels. Evaporation was associated with salinity in the natural lagoons and G02, where it was negatively correlated with salinity in BPI and positively correlated in the FRA and G02 lagoons. Evaporation, however, had no significant correlation with salinity in the new lagoons. Also, inflow salinity was associated with salinity levels only in BPI. As expected, the total inflow calculated by the GLM was positively correlated with volume levels in all the lagoons. Evaporation was negatively correlated with volume levels in FRA and G02. However, evaporation was positively correlated with volume levels in BPI and L04. Inflow salinity was positively correlated with volume levels in L04 and L01.

Table 1.9. Correlation Matrix of Pearson correlation coefficients for all the seasons combined to assess the relationship of hydrological parameters (Evaporation, Total Inflow, Total Outflow, Inflow Salinity, Rain and Surface Temperature) influencing lagoon volume and salinity. Values averaged monthly.

	Salinity						**Volume**					
Lagoon	**BPI**	**FRA**	**G02**	**L04**	**L01**	**M03**	**BPI**	**FRA**	**G02**	**L04**	**L01**	**M03**
Volume	**-0.55***	**-0.77***	**-0.43***	**-0.33***	0.07	**-0.67***	-	-	-	-	-	-
Evaporation	**-0.36***	**0.35***	**0.49***	-0.17	0.27	0.13	**0.89***	**-0.32***	**-0.57***	**0.6***	-0.01	0.08
Total Inflow	-0.24	-0.1	0.07	0.15	**0.45***	-0.07	**0.62***	**0.57***	**0.58***	**0.6***	**0.52***	**0.53***
Total Outflow	**-0.37***	**-0.63***	**-0.38***	-0.21	0.21	**-0.51***	**0.85***	**0.95***	**0.87***	**0.85***	**0.91***	**0.88***
Inflow Salinity	**0.5***	0.11	0.2	-0.03	0.19	0.04	-0.26	-0.04	0.17	**0.34***	**0.45***	-0.1
Rain	-0.3	**-0.41***	-0.15	-0.17	0.13	**-0.38***	**0.77***	**0.6***	**0.64***	**0.72***	**0.71***	**0.6***
Surface Temp	**0.6***	**0.76***	**0.64***	**0.64***	0.27	**0.63***	**-0.59***	**-0.7***	**-0.73***	**-0.69***	**-0.69***	**-0.67***

Asterisks and highlighted indicates significant correlation $p < 0.05$

Table 1.10 shows the analysis of variance among lagoons grouped according to their features using stepwise multiple regression models for hydrological parameters affecting salinity and volume. The lagoons are categorized as new lagoons (L01, L04, M03, G02), old lagoons (BPI, FRA), presence of low-permeability layers (BPI, FRA, L04, M03) and absence of low-permeability layers (L01, G02). G02 was removed from Table 1.10 C & D to improve consistency in lagoon morphology. The new lagoons′ salinity fluctuations were explained more by total inflow and outflow than by rain and evaporation in the regression models (A & C), while volume levels explained more of the variance in the old lagoons (E). A similar effect on salinity was seen in lagoons with low-permeability layers, where volume had a bigger influence (G). However, a combination of inflow salinity and volume helped to explain more of the variance in salinity fluctuations in lagoons without low-permeability layers (I). Total inflow and outflow affected the volume fluctuations more in the new lagoons (D), while rain and evaporation explained nearly all the variance in the old lagoons (F). A similar result was obtained in lagoons with the presence of low-permeability layers, where rain and evaporation explained nearly all of the variation, despite the inclusion of lagoons L04 and M03 (H). However, the model improved greatly when total outflow was added to lagoons without low-permeability layers.

Table 1.10. Stepwise multiple regression models for hydrological parameters affecting salinity and volume according to new lagoons (L01, L04, M03, G02), old lagoons (BPI, FRA), presence of low-permeability layers (BPI, FRA, L04, M03) and absence of low-permeability layers (L01, G02). Asterisk indicates G02 was removed from the new lagoons category due to conflicting morphology (C* and D*). Adjusted r^2 values and the inclusion of parameters at each step are shown. r^2 asterisks indicate significance with a *p*-value below 0.05.

Step	Parameter	r^2	Step	Parameter	r^2
New Lagoons Salinity (L01, L04, M03, G02) **A**			New Lagoons Volume(L01, L04, M03, G02) **B**		
1	Rain	0.03*	1	Rain	0.36*
2	Evaporation	0.04	2	Evaporation	0.39*
3	Total Inflow	0.05*	3	Total Inflow	0.39
4	Inflow Salinity	0.36*	4	Total Outflow	0.73*
New Lagoons Salinity (L01, L04, M03) **C***			New Lagoons Salinity (L01, L04, M03) **D***		
1	Rain	0.03	1	Rain	0.44*
2	Evaporation	0.05	2	Evaporation	0.45
3	Total Inflow	0.06*	3	Total Inflow	0.45*
4	Inflow Salinity	0.24*	4	Total Outflow	0.80*
Old Lagoons Salinity (BPI, FRA) **E**			Old Lagoons Volume (BPI, FRA) **F**		
1	Rain	0.02*	1	Rain	0.66*
2	Evaporation	0.09*	2	Evaporation	0.95*
3	Total Inflow	0.10*	3	Total Inflow	0.95*
4	Volume	0.45*	4	Total Outflow	0.97*
Presence in Lagoons Salinity (BPI, FRA, L04, M03) **G**			Presence in Lagoons Volume (BPI, FRA, L04, M03) **H**		
1	Rain	0.04*	1	Rain	0.56*
2	Evaporation	0.06*	2	Evaporation	0.89*
3	Total Inflow	0.08*	3	Total Inflow	0.89
4	Volume	0.30*	4	Total Outflow	0.93*
Absence in Lagoons Salinity (L01, G02) **I**			Absence in Lagoons Volume (L01, G02) **J**		
1	Rain	0.01	1	Rain	0.35*
2	Evaporation	0.03	2	Evaporation	0.46*
3	Total Inflow	0.05	3	Total Inflow	0.47
4	Volume	0.26*	4	Total Outflow	0.70*
5	Inflow Salinity	0.33*			

4.1.4. DISCUSSION

Recent method and technology improvements in data collection have seen a deluge of data generation, and environmental modelling is a way of observing systems coherently with large data sets (Farley et al., 2018; Porter et al., 2012). As with all models, choosing the level of resolution and complexity within a generic model structure, that is both accessible and can confidently predict system process is challenging at best (Bruce et al., 2018; Hipsey et al., 2019). Stress testing the GLM over a global network by Bruce et al. (2018) identified a range of limitations that included warm or cold biased estimations and larger mean errors of temperature, depending on the frequency and location of meteorological data collected. Accurate light attenuation (Kw) and wind speed measurements, as well as the parameterization and classification to physical characteristics were also emphasized to improve model performance. Furthermore, to increase the applicability of the GLM to a wide variety of systems, it was proposed to adopt a Bayesian hierarchical calibration framework and increase the flexibility of assumed globally common parameter values for the core hydrodynamic parameters. Our investigation into shallow coastal lagoons is one of the first (to our knowledge) to model these types of systems that don't exceed 3m in depth. While it was found that shallow, well-mixed lakes performed better overall during stress testing (Bruce et al., 2018), this study contributes to an ever-increasing list of diverse system types modeled by the GLM, and provides insights into shallow, well mixed lagoon systems with higher salinity fluctuations. Also, the diversity of morphologies of the new and natural lagoons, combined with underlying lithological characteristics and different hydrological parameters modeled separately has provided an opportunity to not only quantitatively assess the success of restoration, but also to analyze factors that contribute to lagoon circulation and water volume fluctuations as well as lagoon salinity variability.

4.1.4.1. Shallower lagoon morphometry and lack of low-permeability layers can increase overall water circulation and volume fluctuation

Lagoon circulation and morphometry

Our results indicate differences in the hydrodynamics of the lagoons when assessing overall water circulation and volume fluctuations, in conjunction with differing lagoon morphometry and the presence or absence of low-permeability layers. Differences between the lagoons start with their lithological characteristics and permeability and are defined by their temporal and spatial distributions. Firstly, the accumulation of decomposing plant and organic matter in marsh silts is found at the sediment-water interface in the natural lagoons and not in the new lagoons, due to insufficient time for decay and accumulation (Boadella et al., 2021; GEOSERVEI, 2016). The new lagoons have mostly sandy silts and alluvium deposits at the sediment-water interface. As unconsolidated deposits, the permeability of these sediments differ with marsh silts having moderate to low permeability in the old lagoons, and high to low permeability in alluvium and medium sands in the new lagoons (Freeze & Cherry, 1979; Lewis et al., 2006). The underlying fine sands for all the lagoons have a high to moderate permeability (Freeze & Cherry, 1979; Lewis et al., 2006). Also, the plastic clay layer is distributed in the South and Southwest of the study site, which represents significantly lower permeability (Freeze & Cherry, 1979; Lewis et al., 2006) for the affected lagoons and makes the input of groundwater in the lagoons more inefficient (Solà et al., 2016) for FRA, M03, and part of L04. Although BPI didn´t register the presence of this layer, its lithological characteristics are nevertheless dominated by low-permeability silts and clays. Also, the decision to emulate the new lagoons (L01, L04, and M03) to previous urban developments, such as promenades and rotundas, as well as to preserve the shallow underlying low-permeability layers, provided a guideline during construction and resulted in similar and shallower mean depths and bathymetrical profiles. This led to different morphometric features as well as lithological characteristics between the new and the natural lagoons, resulting in differences in the hydrological patterns between them. The new lagoons have similar summer and yearlong water levels, higher rate of change with respect to volume, and higher surface to volume ratios (Surface/V) in comparison with the old lagoons. Therefore, the new lagoons have a higher evaporation flux in comparison with the old lagoons. A higher Surface/V ratio results in higher evaporation (Casamitjana et al., 2019; McJannet et al., 2008). This is also observed in the rate of change of area with respect to volume, where the new lagoons' surface areas increase with lower water volume.

The strength of the one-dimensional GLM to differentiate inflows, outflows, mixing and surface mass fluxes allows for the distinction of different circulation patterns within the lagoons. This includes inflows separate from rainfall and outflow from evaporation. Due to the nature of the hydrological activity in Mediterranean lagoons, the occurrence of extreme values for inflow, outflow, and rain was common and concurrent with episodes of non-occurrence (i.e. either inflow or outflow occurred, and periods of no rain). This resulted in highly skewed data in its distribution (Figure S1.1 A, B, C, D, E, F; Figure S1.2 A, B, C, D, E). Nevertheless, the NSE and RMSRE values indicate reasonable adjustments despite the sudden changes in volumes (Table 1.6) Also, the sensitivity of mean calculations was considered convenient and representative, as the occurrence of both extreme values and the non-occurrence of daily parameters are equally important in water circulation representations. Distinguishing circulation parameters allowed for two contrasting patterns to emerge between the natural lagoons and G02 and the new lagoons L04 and L01. The circulation is higher in L01 and L04 than the natural lagoons and G02 in all parameters calculated by the GLM. This also coincides with differences in the morphometric features, where mean depth and relative depth are greater in FRA and G02, and their variation of surface area and volume was lower. Both features indicate the deepness of the lagoons and Hutchinson (1957) and Wetzel & Likens (1991) note that those that have a higher relative depth (approaching 4%) usually have smaller surface areas and exhibit greater resistance to mixing. This idea is strengthened with the higher lake numbers for FRA, G02, and BPI. Also, despite the lack of low-permeability layers in G02, the response to inputs is more moderate after the dry period, suggesting water column stability due to deeper lagoon morphometry and resistance to high volume fluctuation. This is supported in Table 1.10, where most of the variance is explained for volume fluctuations in the new lagoons when including total inflow and outflow (D*), whereas most is explained in the old lagoons through evaporation and rainfall, with little affect from total inflow and outflow (F). Combining all these factors has led to two main findings that could be explained by lagoon morphometry. First, the less effect of water volume fluctuations in FRA and G02 can be attributed to more water column stability, due to variations in density with depth and lower evaporation effect due to smaller surface/V ratio; and second, the new lagoons shallower profiles are sensitive to water inflows and outflows due to lower volume capacity and are subject to higher evaporation effect by the higher surface/V ratio. The result is higher water turnover for L04 and L01 and a quicker response to external drivers, such as winter cyclonic storms or long dry periods.

Influence of low-permeability layers on water circulation

Notable differences in the circulation patterns of M03 are observed in comparison with the other new lagoons. Despite sharing similar morphometric features, M03 had different inflow, outflow, and evaporation patterns from L04 and L01. Also, the total coverage of underlying low-permeability layers in M03´s wetted area is unique, where all other lagoons (except for BPI) have a combination of low permeable and permeable layers underlying the wetted area, and the more inland topographical location suggests this lagoon is subject to more confinement than the rest of the lagoons. Furthermore, in the absence of sea storms and with rain inputs only, M03 showed minimal fluctuation in water volumes in comparison with L01 and L04 (Figure 1.2 A), indicating a smaller influence of rising groundwater. Also, sea storm inputs and high rainfall can increase all the lagoons´ water volume 2-12 times the initial volume in autumn. However, there is a lag in declining water volume levels after such events in M03, while L04 and L01's volume levels decline quicker. A similar pattern of lag can also be seen in FRA. M03 and BPI showed similar evaporation patterns over their respective volumes (Figure 1.3 C), despite having similar surface area fluxes as L01 - which has no low-permeability layers. Furthermore, a large part of the variance of volume fluctuations in lagoons without low-permeability layers is explained with the inclusion of total outflows, whereas inflow and outflow contribute only a percentage of the total fluctuations in the presence of these layers (Table 1.10 H). This suggests the underlying low-permeability layers overall effect on hydrological behavior, which influences inflows, outflows, and evaporation patterns, resulting in a more elevated confinement pattern of circulation due to a less efficient connection with the aquifer. Therefore, low-permeability layers and lagoon morphometry can influence water circulation and volume fluctuations, and the interplay of both can create similar hydrological behavior. This is the case with BPI and M03, which behaved similarly in terms of lag to both inflows and outflows, yet can have high increases in their relative volumes due to their lower mean and relative depths.

4.1.4.2. The presence and absence of underlying low-permeability layers influence lagoon salinity variability.

Presence of low-permeability layers

In conjunction with the presence of underlying low-permeability layers, BPI, FRA, and M03 show higher surface and bottom average salinity levels. Furthermore, to fit the GML results to the experimental data when modeling salinity, the inflow salinity levels were set to values higher than sea salinity (Mediterranean salinity ~37ppt), mainly during the summer and autumn seasons. A similar observation was made by Casamitjana et al. (2019), who suggested that these salinity values are similar to those at the bottom of the lagoons (or have a higher salinity with little stratification, as is the case in M03). The study concluded that differences in water input amounts and water salinity may be attributable to the composition and permeability of the lagoons' sediment. While that study showed this effect in the natural lagoons (BPI and FRA having higher salinity inflows, while G02 lower), this was also observed in the new lagoon M03, showing high salinity levels in its inflows, mainly during the summer-autumn period. Although indirect, these results suggest that there is a less efficient connection occurring with the aquifer, which minimizes groundwater inflows and outflow circulations and therefore more water confinement. This idea is supported by a strong negative correlation between volume levels and salinity (Table 1.9), particularly in BPI, FRA, and M03, as well as a negative correlation of total outflows and salinity levels, indicating that outflows are more restricted by the low-permeability layers. This has an effect of increasing overall lagoon salinity when water levels decrease. This idea is strengthened when volume is included in the multiple regression models that affect salinity levels in the presence of low-permeability layers (Table 1.10 G). However, when groundwater levels increase, mostly during autumn and winter cyclonic storm events, the groundwater inflows into these lagoons have salinity similar to summer salinity levels. Additionally, some of the inflowing surface waters can have higher salinities because they flow through small salt deposits formed due to the evaporation of small ponds in between the lagoons and the sea (Casamitjana et al., 2019). These results support similar findings by Sadat-Noori et al. (2016), who observed inputs of shallow brackish hypersaline pore water into the lagoons when groundwater levels rise. Also, Rodellas et al. (2020) observed that the increased hydraulic gradient favors the upward advection of deep hypersaline pore waters in periods of shallow lagoon water depths. Zarroca et al. (2011) went further to explain that textural and mineralogical characteristics condition the retention of salts in sediment, as the low permeability of clays as well as the high capacity of adsorption and absorption contribute to the increase of ion concentrations.

Absence of low permeability layers

While higher salinity levels seem to correspond with the spatial distribution of low-permeability layers, lagoons with lower overall salinity levels (L01, L04, and G02) also correspond with the absence or partial presence of low permeability layers. During rainy and stormy periods (mostly in spring and autumn), the inflow is estimated to be a mixture of groundwater and surface water, while for the rest of the year inflow is mostly from underground sources (Menció et al., 2017). L01 showed significant freshening during the summer period, with higher salinity inflows of seawater entering during autumn storm events. This suggests freshwater is the main input from the aquifer, possibly due to freshwater stratification on top of a saltwater wedge (Menció et al., 2017). Although L04 had the low permeability layer conserved, the partial distribution of this layer has resulted in some connection with the underlying aquifer and consequently more freshening and water circulation. In seemingly contrasting behavior to the presence of low permeability layers, there were no, or weak correlations found for volume levels and salinity in L01 and L04, and outflow had no effect on their salinity levels - suggesting the more efficient connection to the shallow aquifer and susceptibility to the influence of circulating waters (surface and groundwater inflows), despite having the highest evaporation flux of all the lagoons. This idea is further strengthened by the lowest coefficient of variation for inflow and outflow in L01 in Table 1.8. This finding agrees with Rodellas et al. (2018), who determined that water recirculating through permeable sediments in a coastal lagoon could account for more than 60% of the total recharge. In our case, this kind of circulation may explain a significant amount of the water flow that occurs at the beginning of the autumn.

It has long been held that in a flooding-confinement hydrological pattern coastal lagoon surface waters would be more susceptible to evaporation, and salinity would increase as water levels decrease. When analyzing the annual hydrological pattern, there is a positive correlation of evaporation with salinity levels in the natural lagoon FRA and G02 but does not correlate with salinity levels in the new lagoons (Table 1.9) (BPI had a negative correlation due to a smaller surface area at lower volume levels). However, when aggregating the data by seasons using mixed linear models (i.e. Winter, Spring, Summer, and Autumn), evaporation is positively correlated with salinity levels between the winter and summer seasons in both L04 and M03 ($r = 0.44$ and 0.57 respectively; $p < 0.05$; Table S1.2.), but not for L01, where increasing salinity is more dependent on annual total inflows as well as inflow salinities (Table 7, $r = 0.45$; $p < 0.05$) due to sea water inputs. Furthermore, a third of the variance in new lagoons´ salinity can be explained by including inflow salinity, while evaporation is not significant in the multiple regression models (Table 1.10 I). These results imply two findings. First, while evaporation affects the salinity levels in the lagoons, the new lagoons´ salinity is controlled more by seasonal inputs. Second, the absence of low-permeability layers limits salinity variation in a confined lagoon system. It is well documented that evaporation plays a role in changes in salinity levels (e.g. Lécuyer et al., 2012; Ellah and Hussein, 2009). Also, many studies have focused on Submarine Groundwater Discharge (SGD) through permeable sediments and its influence on nutrient loadings and salinity of surface water bodies (Anschutz et al., 2009; Liefer et al., 2013; Rodellas et al., 2015). However, few have quantified parameters affecting salinity fluctuations with known sedimentary patterns and their influence on the overall hydrological pattern from surface and groundwater flows, both from land to sea and vice versa. Our results show that there is an overall influence of low-permeability layers on lagoon salinity variability, despite the strong influences of external hydrological patterns. This can be seen in the similarity of the morphometric features in L01, L04, and M03, where all are relatively shallow and have a high evaporation flux with low water levels in summer, yet their salinity variability varies widely with M03 showing the highest fluctuations with lower circulation. Similarly, FRA and G02 show similar hydrological behavior with similar morphometric features but with different salinity levels. The results of this study show that the new lagoons (especially L01) seem to be more influenced by annual mixing than by evaporation, and possibly due to underlying low-permeability layer distributions that influence the efficient connection with the aquifer. This is supported by Menció et al. (2017), who found that groundwater contributions could be as high as 80% in the dry season. Therefore, lagoon salinity flux is not only limited by lagoon morphology or by evaporation fluxes, but also by the extent of connection with the aquifer.

A lesson learned: the case of G02

As mentioned before, G02 was excavated below sea level to ensure water permanency all year round. However, the underlying lithological characteristics were not taken into consideration and any presence of low-permeability layers were probably removed during construction. At the time, the intention was to increase refuges for the *Aphanius iberus*. However, in the years that followed, G02 showed higher circulations and consistently lower salinity levels than the desired fluctuations, with evidence of a higher connection with the aquifer (Casamitjana et al., 2019). This has resulted in higher populations of *Gambusia holbrooki* (Figure 1.5). Knowledge of this inspired the preservation of low permeability layers during the construction of the new lagoons in 2016, in the hope of reducing an efficient connection with the aquifer and increase salinity fluctuations. As the results of this study suggest, there has been an element of influence from low-permeability layers, and early indications suggest that the *Aphanius iberus* is benefitting from these measures.

4.1.4.3 A tentative conceptual model

Our observations offer some conceptual insight into the La Pletera salt marsh hydrology as summarized in Figure 1.4. In a coastal lagoon system that is highly variable, finding an annual pattern related to hydrological, biogeochemical, and ecological functioning can be challenging. Apart from surface temperature, variation among the lagoons of the La Pletera salt marsh is high, with a range of 30 to 127% for all variables (Table 1.8), illustrating the diversity in lagoon structure and hydrological behavior. Variation within the individual lagoons is also very high, indicating the different ways individual lagoons behave to external climatic and hydrogeological influences. This results in a hydrologically dynamic system with correlating parameters often overlapping. This has been noted in other studies, where the overall hydrology is strongly conditioned by the Mediterranean climate, which is irregular and unpredictable and cause wide fluctuations in lagoon physical, chemical, and biological composition (Álvarez-Cobelas et al., 2005; Beklioglu et al., 2003; Fernández-Aláez, et al., 1999; Quintana et al., 2006; Romo et al., 2004).

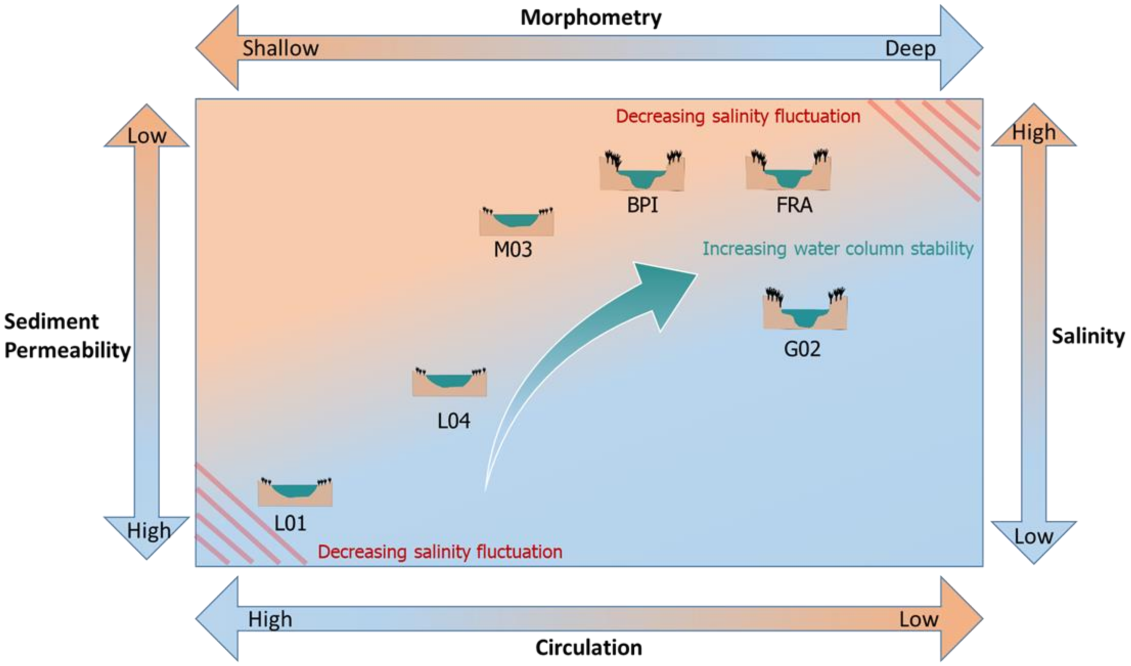


Figure 1.4. A conceptual model describing hydrological stability and salinity fluctuation in a highly variable system. Individual lagoon distributions according to their sediment permeability, morphometric features, circulation, and salinity level relationships reveal more hydrological stability with lower circulation and water level fluctuations, while salinity fluctuations are reduced to either consistently high or low levels according to the depth of their morphometric features and connection with the aquifer.

Despite this unpredictability and variability, our results suggest an emergence of annual patterns of salinity levels and more hydrological stability (less water circulation). These are seemingly determined by morphometric features and distributions of underlying low-permeability layers, despite all having the same climatic limitations. This interaction is better summarized in the conceptual model in Figure 1.4, where all 6 lagoons′ hydrological patterns of circulation and salinity levels are plotted according to their morphologies and the presence or absence of low-permeability layers. Lagoons that are efficiently connected with the aquifer and are shallow in terms of their morphometric features (higher surface area to volume ratios), tend to exhibit higher circulation and less variation in salinity (in our case low salinity as freshening was occurring from groundwater inputs in L01). Conversely, lagoons that are deeper with less surface area to volume ratios and have low permeable underlying layers, tend to exhibit more hydrological and salinity stability, with lower circulation and higher salinity levels (FRA). This illustrates that hydrological variability within the lagoons can be proportional to their physical and geomorphologic variability. When comparing the restoration of the new lagoons to the natural lagoons, we can see that the topographical distribution, morphology, and underlying lithology preservation resulted in distinct hydrological behaviors. The new lagoons' inflows and outflows during high precipitation and sea storm events override the annual patterns of confinement seen in the natural lagoons and are more susceptible to climatic influence in their annual hydrological pattern. Studies have noted that confined coastal lagoons in arid or semi-arid regions with little freshwater inflow, limited water exchange with the sea and high evaporation rates may result in longer turnover times, more stable water columns and become highly saline (Copeland, 1967; Moore and Slinn, 1984; Saccà, 2016). While small in scale, our study has shown the extent of variation among and within the lagoon systems and highlights the importance of morphology and groundwater contributions in a system highly driven by climatic changes. From a restoration perspective, understanding hydrological behavior and parameters that drive them can help to achieve specific ecological functioning objectives outlined in a project.

4.1.4.4. *Aphanius Iberus* Conservation and Ecological Functioning.

In terms of conservation efforts of the *Aphanius iberus* versus the *Gambusia holbrooki*, an important aspect that often influences their population dynamics and proportions is varying climate, i.e., the wet years (which usually results in less salinity in the lagoons) favor the *Gambusia holbrooki*, while the dry years favor the *Aphanius iberus* (with more salinity). Nevertheless, two aspects arise from the conceptual model drawn in Figure 1.4. First, lagoons with less permeable sediments with resulting higher water salinity, and/or less deep-water columns with higher salinity fluctuations appear to be the more suitable conditions for *Aphanius iberus* (brown parts of Figure 1.4). Second, there is a trade-off in these *Aphanius iberus* favorable conditions: shallow lagoons facilitate salinity variability (depending on the connection with the aquifer), but with more risk of desiccation during dry years, while deep lagoons prevent desiccation, but exhibit less salinity variability in wet years. The actual populations and distributions of the two species from 2018 to 2019 can be seen in Figure 1.5, where BPI, FRA and M03 held better proportions and numbers of *Aphanius iberus* versus *Gambusia holbrooki*, despite the significant rainfall and sea storm events. Therefore, a combination of several different water bodies, with different morphologies and water depths, seems to be the best solution to ensure the conservation of *Aphanius iberus* in restored Mediterranean salt marshes.

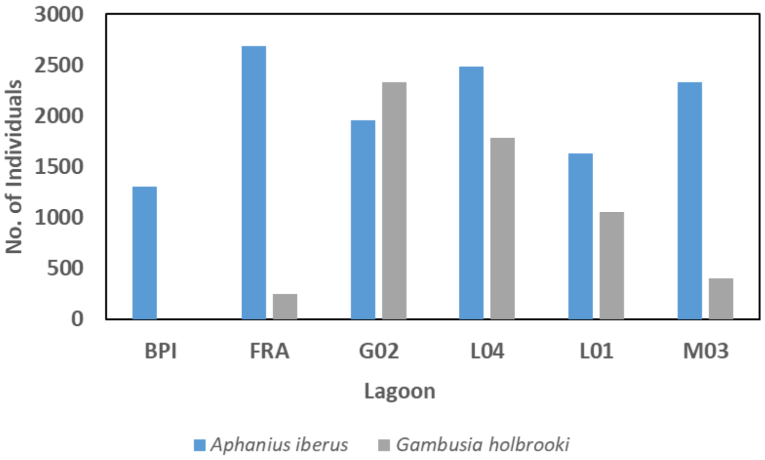


Figure 1.5. Species abundance for *Aphanius iberus* and *Gambusia holbrooki* from 2018 to 2019. Large numbers were capped at 100 for each trap and sampling period during the census. *Aphanius iberus* was introduced into the new lagoons between 2016 and 2018.

When focusing on ecological functioning, the La Pletera salt marshes have seen plant succession towards more mature habitats over the years following the construction of the lagoons and restoration of the area. The intention was to create rich and diverse mosaic of habitats and some halophyte populations have already been established in areas of high salinity, while the perennial and globally distributed *Rupia cirrhosa* has already started to colonize the new lagoons (Bou et al., 2018). This species is of great importance in the La Pletera, as it not only usually grows in deeper waters and tolerates more saline conditions, but it also creates favorable habitats for the *Aphanius iberus* (Bou et al., 2018). Therefore, the management and conservation of both these species could fall within the same favorable conditions, depending on lagoon morphology and the presence and absence of low-permeability layers. Also, investigation into ecosystem metabolism dynamics of the old and new lagoons, from 2016 to 2018, found that although the Gross Primary Productivity to Ecosystem Respiration values (GPP:ER) were close to 1, G02 and FRA were slightly heterotrophy and the potential productivity occurred in winter, when nutrient loading occurs from water inputs (Bas-Silvestre et al., 2020). Another study by Carrasco-Barea et al. (2018) found that carbon storage in sediments were 3-fold higher in BPI, FRA and G02 than in L01, L04 and M03. Although G02 had not reached the same levels as the natural lagoons, it was concluded that lagoon age is an important factor determining carbon storage. A similar idea was hypothesized by Boadella et al. (2021) that investigated microbial heterotrophic functioning. This study suggested that after 15 years of restoration, G02 could achieve functional recovery through organic matter and nutrient cycling, while it was difficult to conclude completed restoration of the new lagoons 1 year after construction (2017). From a hydrological perspective, the magnitude of water inputs and circulation facilitating nutrient loading could be influenced by an efficient connection with the aquifer and/or susceptibility to climate events and surface inputs due to shallower lagoon morphology. This could ultimately influence GPP and ER, as well as the entrance and cycling of organic matter.

Overall, the construction and restoration of the La Pletera salt marshes have been largely successful in terms of restoration criteria established in Quintana et al. (2018). These include the preservation of the flooding-confinement model, increasing existing colonies of the Iberian toothcarp by increasing salinity fluctuations and conserving the ecological functioning of the ecosystem. The different lagoon morphologies and varying permeability layers have not only influenced salinity levels, but also the heterogeneity of water circulations and levels of confinement, which is typical of these ecosystems and to which all the present species are adapted. As the hydrological behavior of the lagoons has already been established by the GLM, further study into water quality by means of aquatic ecology modelling is a logical next step in providing deeper insight into not only the effects of hydrology and hydrogeology on the nutrient cycle, but also the response of the lagoon communities to different scenarios driven by increased or decreased anthropological activities and climate change.

4.1.5. MAIN REMARKS

1. The natural lagoons and G02 exhibited more consistent patterns of confinement; with deeper morphologies, lower evaporation effect, and water column stability.

2. A combination of deeper morphologies and the presence of low permeability resulted in higher salinity levels and with less salinity fluctuation in the natural lagoons, and more stable annual patterns of salinity fluctuation.

3. Despite similar morphology with the natural lagoons, G02 had lower salinity levels due to the lack of low permeability layers.

4. The three new lagoons had similar, shallower morphologies and higher evaporation fluxes, but exhibited different water circulation patterns due to the presence or absence of low permeability layers.

5. The lack of low-permeability layers and shallower morphology in the new lagoon (L01) resulted in lower salinity levels and with less salinity fluctuation.

6. As a result of the new lagoons´ shallow morphometry, their salinity fluctuations were influenced more by seasonal mixing than by evaporation, indicating more susceptibility to meteorological influence in their annual hydrological and salinity patterns than in the natural lagoons and G02.

4.2. Planktonic response to pulse or continuous inorganic nutrient inputs. Part 1: Temporal variations and monitoring implications.[2]

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[2] Meredith, W., Perujo, N., Antón-Pardo, M., Romaní, A., Boix, D., Compte, J., Bas-Silvestre, M., Quintana, X.D., Menció, A. (2023, Under Review). Planktonic response to pulse or continuous inorganic nutrient inputs. Part 1: Temporal variations and monitoring implications. Science of The Total Environment.

4.2.1. BACKGROUND

Mediterranean coastal lagoons have a hydrology that is typically conditioned by irregular meteorological events, that usually result in them becoming flooded by sea storms and/or rainfalls in the autumn and winter, and then become confined and drop their water levels during the summer (Quintana et al., 1998; Brucet et al., 2005; Badosa et al., 2006; Quintana et al., 2018). The intensity of these surface inputs during flooding suggests that this would be the main source of allochthonous organic matter and nutrients entering these systems in an unpredictable pulse manner (López-Flores et al., 2009). Furthermore, it has been recently recognized that groundwater circulation can significantly supply coastal systems with dissolved inorganic nutrients and can match surface runoff contributions in some cases (Atkins et al., 2013; Cyronak et al., 2013; Santos et al., 2012; Schwab et al., 2017). Studies in Mediterranean lagoons have found that groundwater can significantly contribute to the overall hydrology of the area (Casamitjana et al., 2019; Menció et al., 2017; Meredith et al., 2022a, and 2022b). These contributions would be more gradual and continuous in nature than flood episodic inputs. As coastal lagoons act as sinks for all kinds of allochthonous particulate matter and biologically reactive materials, including nitrogen (N) and phosphorous (P) (Boorman et al., 1994; Taylor et al., 1995), they are commonly subject to high anthropic pressure, such as urbanization, tourism and strong agricultural activity (Sardá et al., 2005). Thus, both natural and human stress (i.e., highly variable hydrology-linked nutrient inputs and anthropogenic pressure, respectively) co-occur in Mediterranean coastal lagoons. This was specifically measured in a Mediterranean coastal lagoon (La Pletera), where unpredictable and intense nutrient inputs, in a pulse manner, combined with anthropogenic nutrient inputs in a gradual manner from significant contributions of subterranean waters have been observed (Menció et al., 2023a, 2023b). Therefore, more focus is required to properly understand the functioning and vulnerability of coastal lagoon ecosystems influenced by recirculation-driven nutrient fluxes, which currently is often-overlooked (Rodellas et al., 2018).

The huge nutrient-input variability in coastal lagoons may affect the growth and development of the different plankton groups and this may determine changes in the ecosystem functioning. Also, nutrients leaching into the underlying aquifers, and ultimately into the coastal lagoons, could increase productivity and result in eutrophication or toxic algal blooms (Bricker et al., 2003; Kennish, 2002; He et al., 2022).

In coastal lagoon ecosystems, controversies remain of which environmental factors determine bacterioplankton and/or phytoplankton biomass dominance, with studies suggesting that inorganic nutrient supply governs phytoplankton biomass, while bacterial biomass is influenced by allochthonous inputs of organic carbon (Garnier and Benest, 1990; Revilla et al., 2000). This is not exclusive, however, and bacterioplankton and phytoplankton can compete for inorganic nutrients (López-Flores et al., 2009). Many have proposed explanations to the "paradox of the plankton", stated by Hutchinson (1961), which include that stable equilibrium coexistence is never reached by phytoplankton communities due to external forcing (such as nitrogen and phosphorus loadings) that vary spatially and temporally (Pomati et al., 2022). An example of this would include external forcing of nutrient supply that is either pulsed or continuous and how it influences planktonic community structures. While some studies have investigated the influence of nutrient pulses from runoff or sea upwelling on marine phytoplankton assemblages, few papers have investigated these effects on biomass production and whether there is changeable phytoplankton-bacterioplankton dominance in systems with diffuse nutrient supply that is either pulsed or continuous (or both together) in marine systems (Grover, 1991; Olsen et al., 1989; Yamamoto and Hatta, 2004; Papanikolopoulou et al., 2018). Furthermore, the potential development of zooplankton may exert a top-down control to phyto- and bacterioplankton (Quintana et al. 2021). The final contribution of bacterioplankton vs. phytoplankton vs. zooplankton biomasses may determine the functioning of a lagoon, including the organic matter and nutrient cycling. Specifically, heterotrophic bacterioplankton plays a key role in organic matter degradation and carbon uptake through their extracellular enzyme capabilities. The extracellular enzyme activities (EEA) can be used as a proxy for the microbial needs and acquisition of C, N and P sources. As EEA degrades complex molecules into more assimilable ones, factors affecting their activity or production would impact the entire remineralization pathway and may also affect feedbacks on the carbon cycle and global climate (Hoppe, 1991; Arnosti, 2003; Bardgett, 2008). Measurements of EEA in coastal lagoons appeared to be sensitive to changes in organic matter and nutrient inputs (Boadella et al., 2021). The potential effects of changing nutrient inputs to the biomass of the different planktonic groups and the final heterotrophic functioning might be a result from direct (nutrients driven) and indirect (bacteria-phytoplankton-zooplankton interactions driven). However, this relationship is difficult to disentangle due to the mixed nutrient input sources and their variability (especially in Mediterranean systems), on one hand, and to the food-web interactions between planktonic groups on the other hand. An added conundrum is the question of natural vs. man-made eutrophication that has been acknowledged in the limnological classical literature (Hutchinson, 1973). Elliott and Quintino (2007) elaborated on the idea of *“Estuarine Quality Paradox”* in which estuaries are naturally stressed, highly variable ecosystems that are also exposed to high degrees of anthropogenic stress. This makes it difficult to distinguish natural from human-induced stress. However, establishing methods which can detect anthropogenic stress against a background of natural stress could help establish a signal.

The aim of this study was to analyze how different time-dynamics of nutrient inputs to a coastal Mediterranean lagoon may affect the biomass contribution of the different planktonic groups and the heterotrophic activity linked to the use of C, N and P sources. Specifically, four time-dynamics of nutrient inputs were simulated: a control (no nutrient additions), a continuous addition over the duration of the experiment, a pulse addition of nutrients (simulating a pulse flooding event, all nutrients added at the beginning of the experiment), and a pulse-continuous addition (to simulate a flooding-confinement regime). The experiment was performed in the field by using mesocosms incubated in a lagoon to be as close as possible to natural conditions. We hypothesize that this community structure must be highly resilient to pulse type disturbances and adapted to the diminution of resource availability that occurs after them. However, these communities would be more sensitive to continuous nutrient inputs, usually of anthropogenic origin, and would maintain high productivity and high resource availability over time.

4.2.2. METHODS

4.2.2.1. Study site

The experiment was performed in the La Pletera salt marsh area (Figure 2.1) The basin is bordered by the Montgrí Range that consists of Mesozoic limestone formations in the north, and by the Gavarres Range with igneous and metamorphic rocks of Paleozoic age in the south (Figure 2.1 A). Furthermore, beach sands, alluvial silty sands and clay sediments result in a multilayer aquifer; with the deep coarser materials acting as a leaky aquifer, and as an unconfined aquifer at the shallowest level (Menció et al., 2023a). It has a a mean temperature of 16°C (1966–2019, Estartit meteorological station 0385J, AEMET. Data available upon request in http://meteolestartit.cat/, Pascual, 2021). The La Pletera itself has several temporary and 6 permanent lagoons (Figure 2.1 B), that are a mix of either natural or newly constructed and restored lagoons under two LIFE projects (LIFE99NAT/E/00 6386 in 2002 and LIFE13NAT/ES/001001 in 2016) (Quintana et al., 2018). The experiment was conducted in one of the permanent lagoons (L04 in Figure 2.1 B). The salt marshes experience a flooding-confinement hydrological pattern (Badosa et al., 2006; López-Flores et al., 2006), with sudden sea storm flooding and strong rainfall in the autumn-winter followed by dry periods in the summer. The permanency of the La Pletera lagoons depends on groundwater inputs, especially during dry periods, when their water salinity increases due to evaporation and high salinity groundwater inputs (a deeper description of these processes is provided in: Menció et al., 2017; Casamitjana et al., 2019; Meredith et al., 2022a).

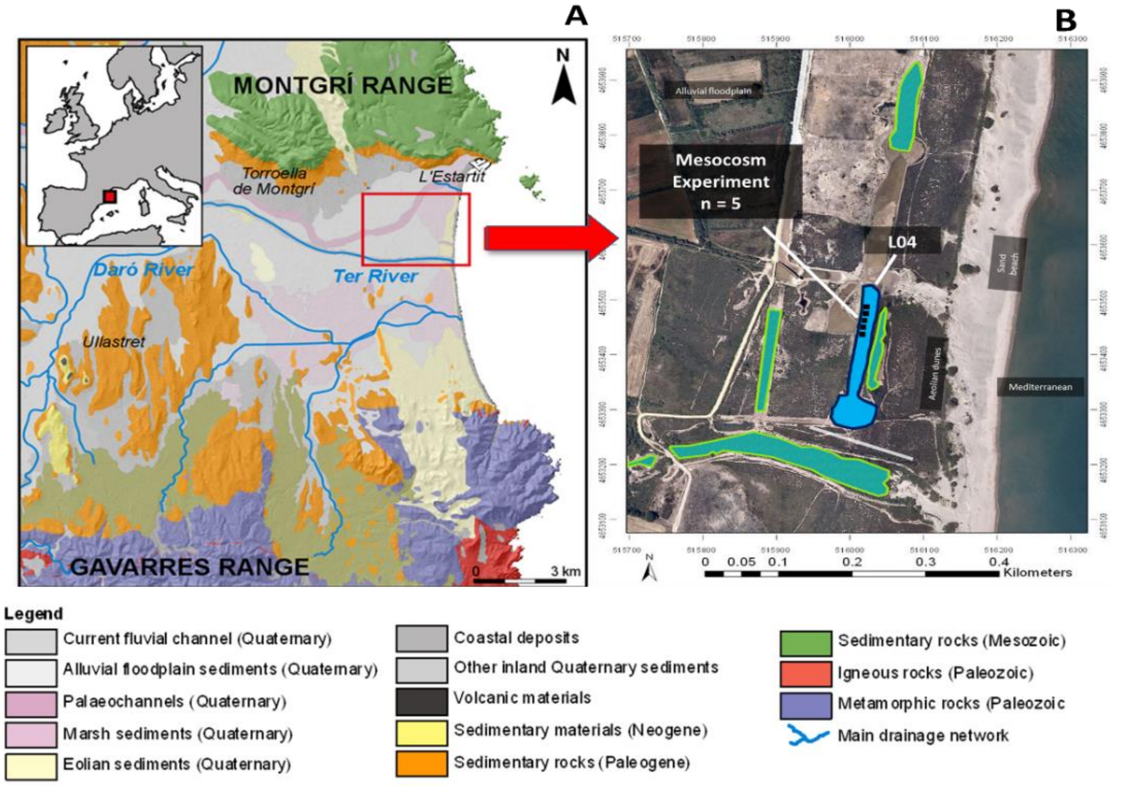

Figure 2.1: Geographical situation of the study area. L04 indicates the coastal lagoon where the experiment was conducted. n = 5 indicates 5 replicas and positions of the mesocosms within the lagoon during the experiment.

4.2.2.2. Sampling and analyses of water characteristics within the mesocosms

Electrical conductivity (EC) and temperature were measured in the field with a Crison CM35 portable conductivity meter with a temperature measurement capability (accuracy EC ≤ 0.5%; temperature ≤0.2 °°C); pH and Eh were also measured *in situ* with a WTW-330i pH/mV meter (accuracy pH ≤ 0.003 pH; Eh ≤ 0.2 mV); and dissolved oxygen (DO) was measured with a Crison OXI45 P portable meter (accuracy DO ≤ 0.5%). Water samples from the mesocosms were taken during 8 field campaigns (day -3, day 0a, day 0b, day 1, day 5, day 11, day 19, day 24), placed in 250 ml amber containers and stored in a fridge at 4°ºC in a dark environment for hydrochemical analysis. Glass pipettes 25ml were used to extract samples and 10ml pipettes were used for nutrient additions. CO_3^{2-} and HCO_3-, used to calculate total inorganic carbon, were determined using Gran titration (their inter-day average precision with percent relative standard deviation, RSD%, was <1%). Unfiltered water samples were frozen for the analysis of total nitrogen (TN) and total phosphorus (TP). Filtered dissolved organic carbon (DOC) was analyzed using catalytic oxidation (RSD% <1%) in the laboratory. Samples for ions and inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}) were filtered with 0.22 μm and 0.45 μm filters, respectively. NH_4^+ was determined using ion chromatography (Thermo Scientific Dionex ICS-5000, RSD% of 2.82%); and NO_2^-, NO_3^-, PO_4^{3-}, and total phosphorus (TP) were determined by spectrophotometry (RSD% of 2.82%, 2.44%, and 3.42%). TN was measured using a TOC analyser (TOC-V CSH SHIMADZU). The quality of the chemical and nutrient analyses was examined by conducting an ionic mass balance, with all samples showing an error lower than 5%. The organic nitrogen (N_{org}) and phosphorous (P_{org}) were calculated from the difference between the total and inorganic nutrient content. The same values for the organic nutrients, including DOC, were then transformed into the molarity of N, P and C and expressed as the organic molar ratio between the 3 elements (C:N, N:P, C:P).

4.2.2.3. Phytoplankton and bacterioplankton biomass and specific growth rate

Water samples were collected in each of the mesocosms to determine the abundance and biomass of pico- and nanophytoplankton (autotrophic organisms) and bacterioplankton (heterotrophic organisms). The abundance and biovolume of picophytoplankton, nanophytoplankton and bacterioplankton were measured with a flow cytometer (FACScalibur by BD Biosciences) with a laser emitting at 488 nm. Samples were filtered through 50 μm mesh, fixed with 1% paraformaldehyde and 0.05% glutaraldehyde (final concentration), deep-frozen in liquid nitrogen and stored at -20°C. For estimating picophytoplankton and nanophytoplankton biomass, samples were thawed and immediately counted on the basis of red autofluorescence from a mixture consisting of 1000 μl sample supplemented with 100 μl of solution of yellowgreen 2 μm Molecular Probes latex beads (10^5 beads/ml final concentration). Bacterioplankton was similarly counted from a mixture consisting of 100 or 10 μl of sample + 100 μl of yellowgreen 1 μm Polysciences latex beads (in any dilution; 10^5 beads/ml final concentration) and completing with water until 1000 μl (900 or 990 μl), stained with 4 μl of Syto 13 (Molecular Probes, 5 μM solution in DMSO). More details of the sample processing and data analysis are found in López-Flores et al. (2009). Phyto and bacterioplankton biomass estimations were performed as described in Troussellier et al. (1999), and processed with CellQuestTM software (BD Biosciences, USA). Specific growth rate (μ d^{-1}) is expressed as the rate of production of biomass per unit time, per unit initial concentration of biomass and calculated as:

$$\mu = \frac{N_1 - N_0}{N_0(t_1 - t_0)}$$

where N_1 is the biomass of phytoplankton or bacterioplankton at time t_1. N_0 is the initial biomass and t_0 is the initial time.

4.2.2.4. Zooplankton collection and biomass:

The collection of zooplankton followed the methodology explained in Quintana et al. (2021). Briefly, zooplankton samples were collected from sampled lagoon water on the initial day before the nutrient additions. Samples were collected again on the final day of the experiment and after physicochemical samples were taken. A total volume of 3L from each mesocosm was filtered *in situ* through a 50 µm net and fixed in 4% formalin. The organisms were counted and identified without specimen manipulation at the lowest taxonomic level possible using an inverted microscope. Zooplankton were classified in several functional groups (i.e., ciliates and rotifers split by taxonomic identity; copepods split by stage (nauplii vs. copepodites & adults) and taking into account if adults are carrying eggs; ostracods split by stage (juveniles vs. adults); gastropods; all of them listed in Table 1). In the case of copepods, because of the difficulty of distinguishing at species level during immature stages, the counts were grouped at order level as calanoids, cyclopoids or harpacticoids. Allometric relationship between the weight and the length of the body were used to obtain biomass for most species (Malley et al., 1989).

4.2.2.5. Extracellular enzyme activity

Three extracellular enzyme activities were measured: β-glucosidase (GLU) (EC 3.2.1.21), leucine aminopeptidase (LAP) (EC 3.4.11.1), and phosphatase (AP) (EC 3.1.3.1–2); which degrade simple polysaccharides, peptides and organic phosphorus compounds, respectively (Romaní et al., 2012). In order to measure these activities, the incubation of the fresh samples was conducted with fluorescent-linked artificial substrates (4-Methylumbelliferone (MUF)-β-D-glucoside, L-leucine4-methyl-7-coumarinylamide (AMC) and MUF-phosphate respectively) at a final saturation concentration of 0.3 mM (Romaní, 2000). In order to control the potential abiotic degradation of the substrates itself, a blank for each artificial substrate was prepared. Samples and blanks were incubated for 1 h at 20 ºC in dark conditions. After 1 h of incubation, glycine buffer (pH 10.4) was added to stop the reaction and maximize MUF and AMC fluorescence. The fluorescence at 365/455 nm (for MUF-based artificial substrates) and 364/445 nm (for AMC-based artificial substrate, i.e. LAP) was measured in a fluorometer microplate reader (Tecan, Infinite M200 PRO). MUF and AMC standards were prepared to link the fluorescence values with extracellular enzyme activity values. Results are given as µmol MUF L^{-1} h^{-1} or µmol AMC L^{-1} h^{-1}. Samples were collected up to day 19. Samples for day 24 were not possible due to Covid-19 restrictions.

4.2.2.6. Data analysis

Differences between the mesocosm treatments on the final day for DO, organic nutrients, pH, temperature, and conductivity were analyzed using one-way ANOVA with the application of a post-hoc Tukey test when differences were detected. For EEA, phyto and bacterioplankton, significant differences between treatments at each sampling date, as well as significant differences between sampling dates were analyzed using two-way ANOVA. Linear regression was used to test if phytoplankton or bacterioplankton biomass significantly predicted zooplankton biomass from the initial and final day of the experiment; that included 5 replicas for phytoplankton, bacterioplankton and zooplankton (N=10), to examine if shifts were caused by bottom-up or top-down effects (McQueen et al. 1986). All variables were log-transformed. Statistical analyses were done with R software (R Core Team, 2021) and the jamovi project (2021) (jamovi Version 2.2.2, Computer Software, retrieved from https://www.jamovi.org).

4.2.3. RESULTS

4.2.3.1. Physico-chemical compositions of the mesocosms on day -3 and day 24

The summary of the hydrochemical data of each of the treatments are shown in Table 2.1 for the initial and last day of the experiment, spanning 24 days. When analyzing changes from the initial to the last day, temperatures were similar in all the treatments and all showed a consistent increase in conductivity of ~6 mS/cm (Figure S2.1). Moderate increases in pH and DO were observed in the control mesocosm. However, the nutrient addition mesocosms had a 2-fold increase in pH compared with the control mesocosms, as well as a significant increase in DO. The control treatments showed moderate alkalinity and TIC decreases, while the rest of treatments had substantial decreases. All treatments showed decreases in NH_4^+ on the final day. Nutrient addition caused a decline in the molar C:N, N:P and C:P ratios compared with the control. Both molar N:P and C:P showed a nearly 3-4-fold decline in nutrient treatments values than the control on day 24. When analyzing differences among the treatments on the final day, DO increases were significant (One-way ANOVA, $p<0.001$). The highest DO increase of 89% occurred in the continuous treatment, then the pulse-continuous with a 64% increase, and the pulse had the lowest increase of 52%. While the continuous and pulse-continuous showed similar levels of dissolved organic carbon (DOC), organic nitrogen (N_{org}) and organic phosphorus (P_{org}) on the final day, the control and pulse treatments showed significantly lower levels (One-way ANOVA, $p<0.001$).

Table 2.1. Summary of the initial (day -3) and final (day 24) conditions of the hydrochemical data (mean ± sd) within each mesocosm treatment of control, continuous, pulse and pulse-continuous (Pulse-Cont). (n=5). I indicates initial day and F indicates final day. Temperature, conductivity (Cond), pH and dissolved oxygen (DO) on the initial day were measured from the same water collected to distribute to the different mesocosms. Results with the same letters are not significantly different between treatments on the final day (day 24) (One-way ANOVA, $p<0.001$).

Treatment/Day	**Control I**	**Control F**	**Continuous I**	**Continuous F**	**Pulse I**	**Pulse F**	**Pulse-Cont. I**	**Pulse-Cont. F**
Temperature (°C)	14.8	13.5 ±0.2	14.8	13.7 ±0.1	14.8	13.7 ±0.1	14.8	13.8 ±0.1
Cond (mS/cm)	15.1	21.9 ±0.2	15.1	21.9 ±0.4	15.1	21.8 ±0.3	15.1	21.8 ±0.2
pH	6.6	8.8±0.1	6.6	10.2 ±0.1	6.6	10.1 ±0.0	6.6	10.1 ±0.1
DO (%)	97.9	105.8 ±3.9^a	97.9	184.8 ±4.9^b	97.90	148.8 ±3.9^c	97.9	160.2 ±5.3^d
NH_4^+ (mg/L)	0.58±0.2	0.03±0.0	0.36±0.2	0.10±0.1	0.28±0.1	0.03±0.01	0.28±0.1	0.03±0.01
Alkalinity (mg/L $CaCO_3$)	275.2 ±8.9	221.7 ±7.3	282.9 ±8.9	148.6 ±6.3	273.34 ±5	145.1 ±6.8	271.9 ±12.7	137.8 ±4.2
TIC (mg/L)	270.3 ±8.5	207.7 ±5.8	276.7 ±9.9	109.2 ±6.4	266.4 ±4.4	111.3 ±5.9	265.9 ±11.6	105.9 ±2.1
DOC (mg/L)	21.9 ±0.7	17.6 ±1.1^a	21.4 ±0.6	20.9 ±0.7^b	22.13 ±0.5	19.9 ±0.4^c	21.5 ±0.9	20.6 ±0.3^b
N_{org} (mg/L)	1.86±0.2	1.36±0.2^a	1.97±0.1	3.14±0.2^b	1.89±0.1	2.75±0.2^c	1.95±0.1	3.15±0.1^b
P_{org} (mg/L)	0.08±0.05	0.06±0.01^a	0.07±0.02	0.29±0.02^b	0.07±0.03	0.25±0.01^c	0.08±0.03	0.29±0.02^b
Molar C:N	13.8 ±1.3	15.2±1.9	12.6 ±0.7	7.9 ±0.6	13.6 ±0.3	8.3 ±0.9	12.8 ±0.8	7.6 ±0.4
Molar N:P	56.6 ±20.5	49.7 ±5.9	65.6 ±16.1	24.2 ±2.1	60.5 ±16.2	23.4 ±1.3	55.9 ±19.0	24.4 ±0.7
Molar C:P	764.4 ±232.5	755.6 ±127.2	820.4 ±170.8	191.3 ±10.1	824.5 ±227.8	194.5 ±21.1	709.6 ±206.6	186.3 ±10.8

4.2.3.2. Nitrate and phosphate concentrations

Nitrate

The control showed fluctuating nitrate levels between 0 and 0.3 mg/L throughout the experiment (Figure 2.2 A). A similar temporal pattern was observed in the three nutrient treatments (Figure 2.2 B,C,D) and were characterized by (i) substantial decreases between day 5 and day 11 (or just before in the case of pulse treatment) of more than 70% in total nitrate; and (ii) low levels were maintained after this decrease with Day 11, 19 and 24 showing values of 1.63, 0 and 0.11 mg/L in the pulse, and 0.03, 0 and 0.22 mg/L in the pulse-continuous, respectively.

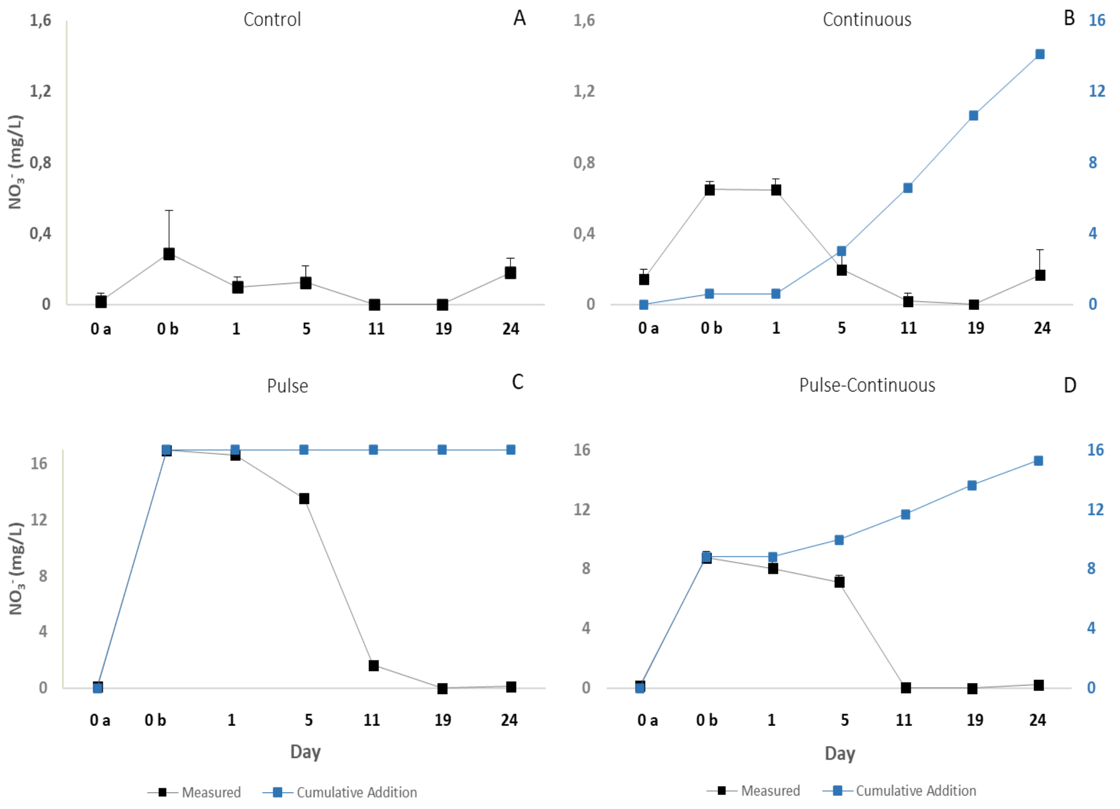

Figure 2.2. Accumulated nitrate additions (blue) -right Y axis- and measured nitrate levels (black) -left Y axis- averaged (mean ± sd) within each of the nutrient treatments of control (A), continuous (B), pulse (C) and pulse-continuous (D). Total nitrate additions for all treatments, except control, were 17mg/L for the duration of the experiment. Day 0a indicates nutrient level before additions and day 0b after additions.

Phosphate

Total planned phosphate level for all treatments was 1.48mg/L for the duration of the experiment. Note measured concentration levels in A and B (black) are 10 orders of magnitude lower than in C and D (black). Day 0a indicates nutrient level before additions and day 0b after additions. The control and continuous treatments showed similar temporal patterns of phosphate levels by day 24 (Figure 2.3 A & B), despite the total mass added to the continuous treatment by this time. In contrast to the nitrate levels, substantial decreases were observed before day 5 in the pulse-continuous treatments, while the pulse treatments had a 65% reduction in phosphate concentration between day 5 and day 11 (Figure 2.3 C D). Low levels were also maintained after these decreases with Day 11, 19 and 24 showing values of 0, 0.004 and 0.018 mg/L in the pulse, and 0.007, 0.005 and 0.03 mg/L in the pulse-continuous, respectively.

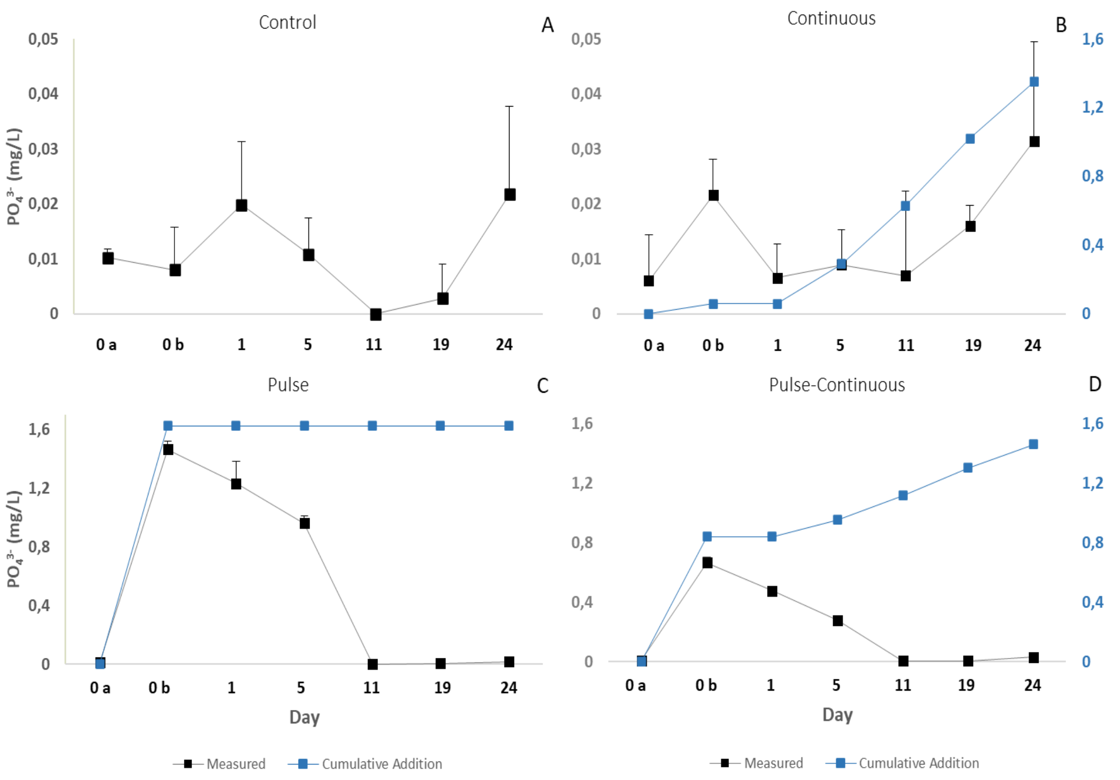

Figure 2.3. Accumulated phosphate additions (blue) -right Y axis- and measured phosphate levels (black) -left Y axis- averaged (mean ± sd) within each of the nutrient treatments of control (A), continuous (B), pulse (C) and pulse-continuous (D) along the duration of the experiment.

4.2.3.3. Phytoplankton and bacterioplankton biomass, specific growth rate and nutrient uptake rates

No significant differences were observed for phytoplankton and bacterioplankton biomass of all the mesocosms on day 0 and day 1 of the experiment (Figure 2.4 A & B, Table S2.2, S2.4). Day 1 saw a decline in biomass in all the mesocosms (both phytoplankton and bacterioplankton). While the control mesocosms showed moderately low fluctuations in biomass, higher concentrations of nitrate and phosphate additions resulted in significantly more similar biomass of phytoplankton in both pulse and pulse-continuous mesocosms at day 11 (Figure 2.4 A & B) (Table S2.4, two-way ANOVA, $p<0.001$), as well as bacterioplankton on day 11 in the pulse and day 19 in the pulse-continuous mesocosms (Table S2.2,). In fact, phytoplankton biomass remained higher in the pulse-continuous treatments than the pulse treatments throughout the experiment. Bacterioplankton biomass declined more than 76% in the pulse and pulse-continuous treatments to biomass similar in the control with no significant differences on the final day (Table S2.2). The continuous treatments saw a steady increase of phytoplankton and bacterioplankton over the experiment duration (Figure 2.4 A & B). Growth rates showed similar trends as biomass in all the treatments (Figure 2.4 C & D), with maximum values on day 11 (except for phytoplankton in the continuous treatment which had the maximum value on day 19. Nitrate and phosphate uptake was also consistent in the continuous treatments, with no significant differences between the sampling days for phosphate and only significant differences from the initial increase of uptake from day 1 to all the other days, and between day 19 and 24 for nitrate uptake (Table S2.8 and S2.6, respectively, Figure 2.4 E). While significantly higher nitrate and phosphate uptake occurred in the pulse treatments in the first 11 days, significantly higher phytoplankton growth rates were observed in the pulse-continuous treatments on day 11. Furthermore, significantly higher phosphate uptake on day 11 coincides with significantly higher growth rates of bacterioplankton in the pulse treatment on the same day (Figure 2.4 D & F, table S2.8 and S2.10, respectively). This indicates a small decoupling of phytoplankton growth and nitrate uptake in the pulse treatment, compared with the continuous and pulse-continuous treatments.

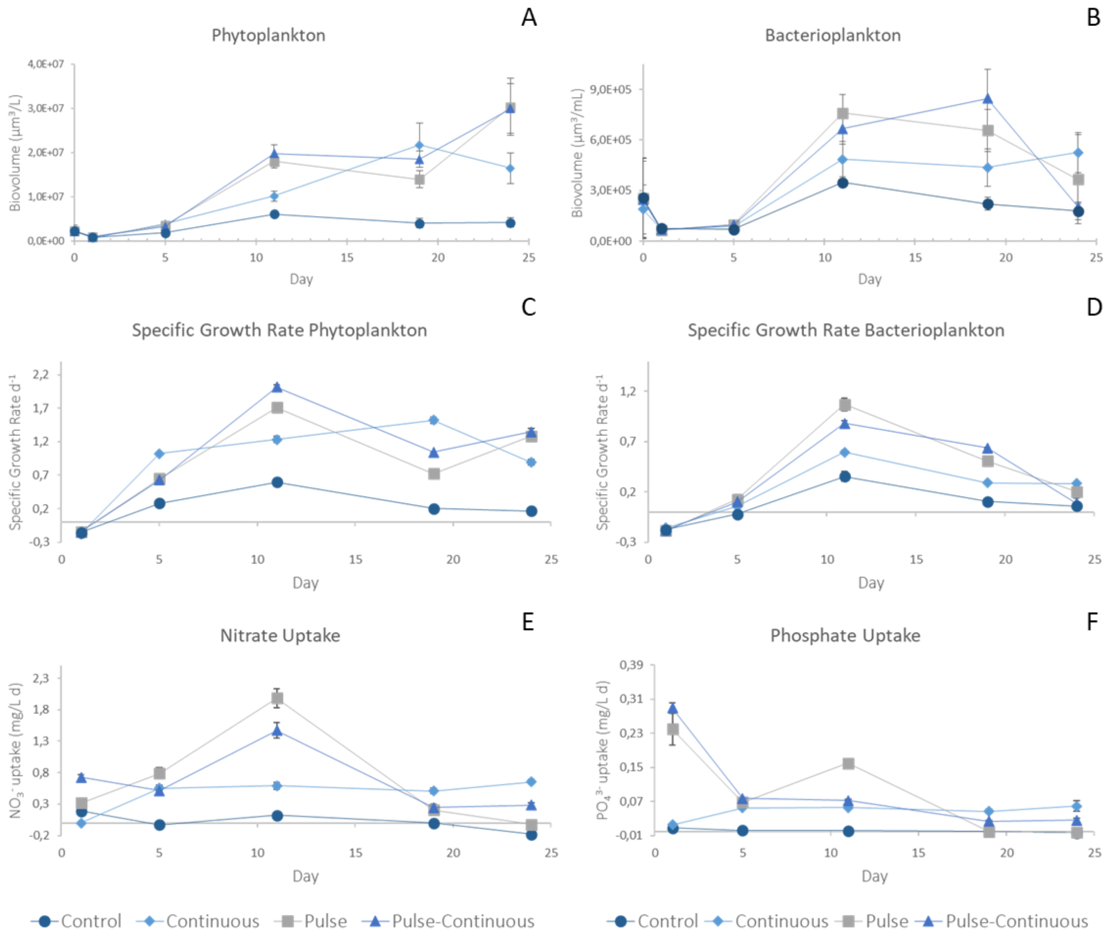

Figure 2.4. Biomass of phytoplankton (A) and bacterioplankton (B) expressed as biovolume from the initial day 0 to day 24. Specific growth rate per day of phytoplankton (C) and bacterioplankton (D), as well as nitrate uptake (E) and phosphate uptake (F) per day according to the nutrient treatment regimes for both phytoplankton and bacterioplankton together in the natural assemblage.

4.2.3.4. Zooplankton biomass.

All treatments showed similar zooplankton biomass on the initial day (Figure 2.5), however, the final day saw a 50 to 67% increase in zooplankton biomass in the control and continuous mesocosms, respectively, and a modest 32% increase in the pulse-continuous mesocosms. In contrast, the pulse mesocosms saw a 58% reduction in biomass on the final day.

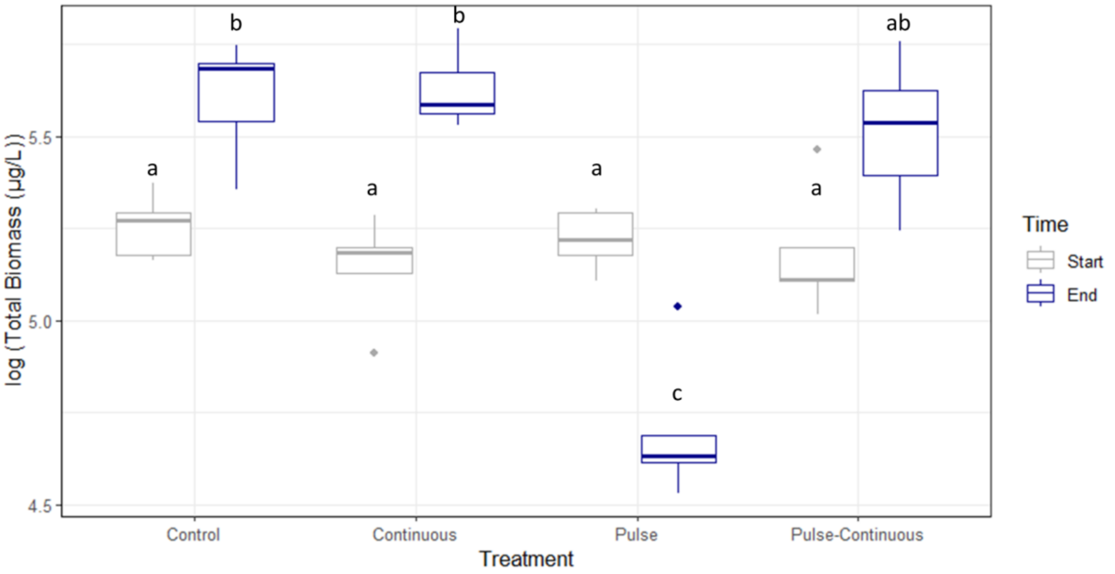

Figure 2.5. Boxplots of the log zooplankton total biomass (µg/L) of each of the treatments of the initial day (Start) and the final day of the experiment (End). Results with the same letters are not significantly different both between treatments and between the initial and last day (two-way ANOVA, $p<0.05$).

4.2.3.5. Bacterioplankton versus phytoplankton biomass relationship

All treatments showed lowest biomass of phytoplankton and bacterioplankton on day 1, with an initial increase of phytoplankton by day 5 (Figure 2.6), along with no significant relationship between phytoplankton and bacterioplankton (Table 2.2). The control showed a linear relationship of increasing bacterioplankton and phytoplankton and is independent of temporal progression in maintaining a linear relationship, with the highest biomass of the two reached in day 11. The continuous treatment showed the strongest bacterioplankton *vs* phytoplankton relationship of the nutrient treatments, where bacterioplankton growth increased along with the phytoplankton growth in a gradual manner, with a significant positive relationship from day 1 to day 24. A weaker linear relationship is observed in the pulse and pulse-continuous treatments (R^2 = 0.26 and 0.18, respectively), with significant positive relationships occurring after day 5 until day 19, whereafter a decoupling of the relationship and a decline in bacterioplankton biomass and an increase in phytoplankton biomass occurred on the final day.

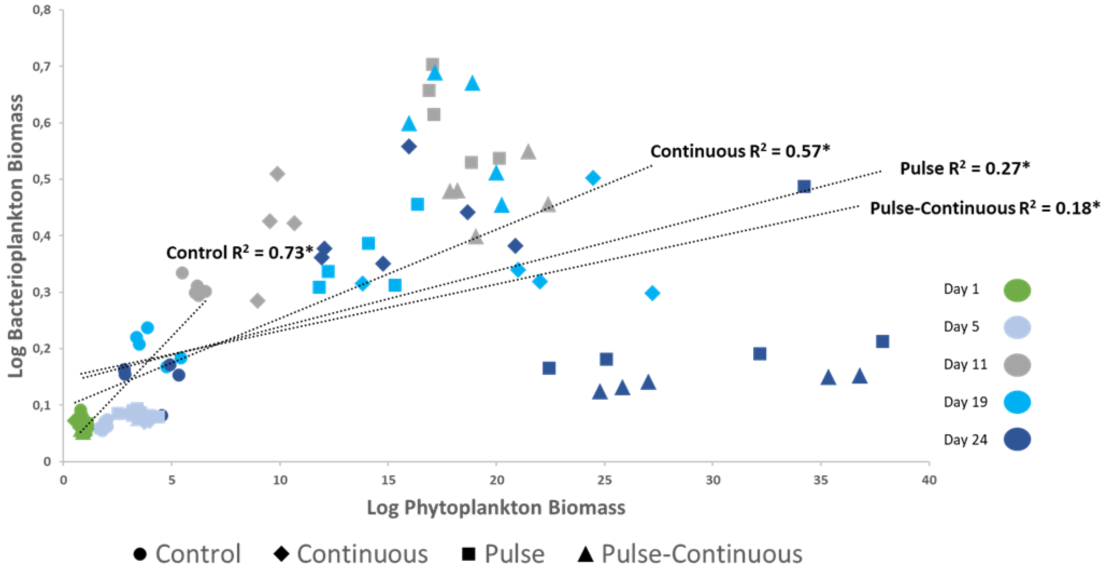

Figure 2.6. Scatterplot of the linear regression model of the log phytoplankton biomass *vs.* the log bacterioplankton biomass relationship according to nutrient additions for 24 days. The coefficient of determination (R^2) is used to identify the strength of the relationships in the graph.

Table 2.2. Multiple regression model with period (day) as a factor or explanatory variable of the log phytoplankton biomass *vs.* the log bacterioplankton biomass relationship. Asterisks indicate significance at 95% confidence interval.

Predictor	Control	Continuous	Pulse	Pulse-Continuous
Intercept ^{a}				
Estimate	0.08048	0.06372	0.05250	0.0639
SE	0.01420	0.02966	0.03273	0.02409
t	56.670	21.484	16.041	2.652
p value	< .001*	0.045*	0.125	0.016*
Phyto				
Estimate	-0.00928	0.00363	0.00793	-7.17e−4
SE	0.00873	0.00530	0.00524	0.00423
t	-10.626	0.6847	15.132	-0.170
p value	0.301	0.502	0.147	0.867
Overall Model Test				
Adjusted R^2	0.920	0.856	0.888	0.945
F	56.2	29.5	39.0	83.0
df1	5	5	5	5
df2	19	19	19	19
p value	< .001*	< .001*	< .001*	< .001*
Period:	*p values*			
Day 1 – Day 5	0.966	0.922	0.925	0.522
Day 1 – Day 11	< .001*	< .001*	< .001*	< .001*
Day 1 – Day 19	< .001*	0.090	0.027*	< .001*
Day 1 – Day 24	0.003*	0.005*	0.780	0.454

4.2.3.6. Phytoplankton and bacterioplankton biomass influence on zooplankton biomass

The control showed significant positive relationships of zooplankton biomass with bacterioplankton biomass and a weak relationship with phytoplankton, suggesting a weak bottom-up effect for zooplankton biomass (Table 2.3). Likewise, a significant positive relationship of both phytoplankton and bacterioplankton biomass on zooplankton biomass was found in the continuous treatments, suggesting a strong bottom-up effect. In contrast, the pulse treatments showed a strong negative relationship of phytoplankton and a weak negative relationship of bacterioplankton to zooplankton biomass. However, the zooplankton biomass decreased from the initial to the final day, which discards the potential top-down and bottom-up effect in this system. The pulse-continuous treatments showed no significant relationships.

Table 2.3. Linear regression analysis to test phytoplankton and bacterioplankton biomass influence on zooplankton biomass from the initial to final day, to examine if shifts were caused by bottom-up or top-down effects in each of the nutrient addition treatments. Asterisks indicate significance at 95% confidence interval.

Zooplankton					
Predictor	Estimate	SE	t	p-value	R^2
Control					
Phytoplankton	0.06	0.03	1.93	0.09	0.32
Bacterioplankton	2.9	0.78	3.7	0.01*	0.63
Continuous					
Phytoplankton	0.03	0.01	2.75	0.03*	0.56
Bacterioplankton	1.33	0.33	4.07	0.01*	0.73
Pulse					
Phytoplankton	-0.02	0.01	-3.59	0.01*	0.65
Bacterioplankton	-1.2	0.7	-1.71	0.13	0.30
Pulse-Continuous					
Phytoplankton	0.01	0.01	1.04	0.33	0.15
Bacterioplankton	2.24	2.46	0.91	0.39	0.12

4.2.3.7. Extracellular enzyme activity

The hydrolytic activities of LAP and GLU increased overall with nutrient additions over the experiment duration and irrespective of nutrient load received after 5 days (Figure 2.7). However, GLU activity was significantly lower in the pulse mesocosms on the last 2 days than the other nutrient addition mesocosms (Figure 2.7 B, two-way ANOVA, $p < 0.05$). Although results are variable over time, the pulse-continuous treatments had higher LAP activity on day 19 than the pulse treatment (Figure 2.7 A, two-way ANOVA, $P<0.001$), and marginally higher in the continuous treatment (Figure 2.7 A, two-way ANOVA, $p=0.06$). GLU activity was also significantly higher in the continuous and pulse-continuous treatments than the pulse on the final 2 days (Figure 2.7 B, two-way ANOVA, $p<0.001$).

The control showed low activity of LAP and GLU from day 5 and till the end of the experiment. Differences between treatments for AP activity appeared on day 5, with higher values in control and continuous treatments than in the pulse and pulse continuous ones. At days 11 and 19, only the control treatment showed a five-fold higher activity than the three nutrient addition treatments.

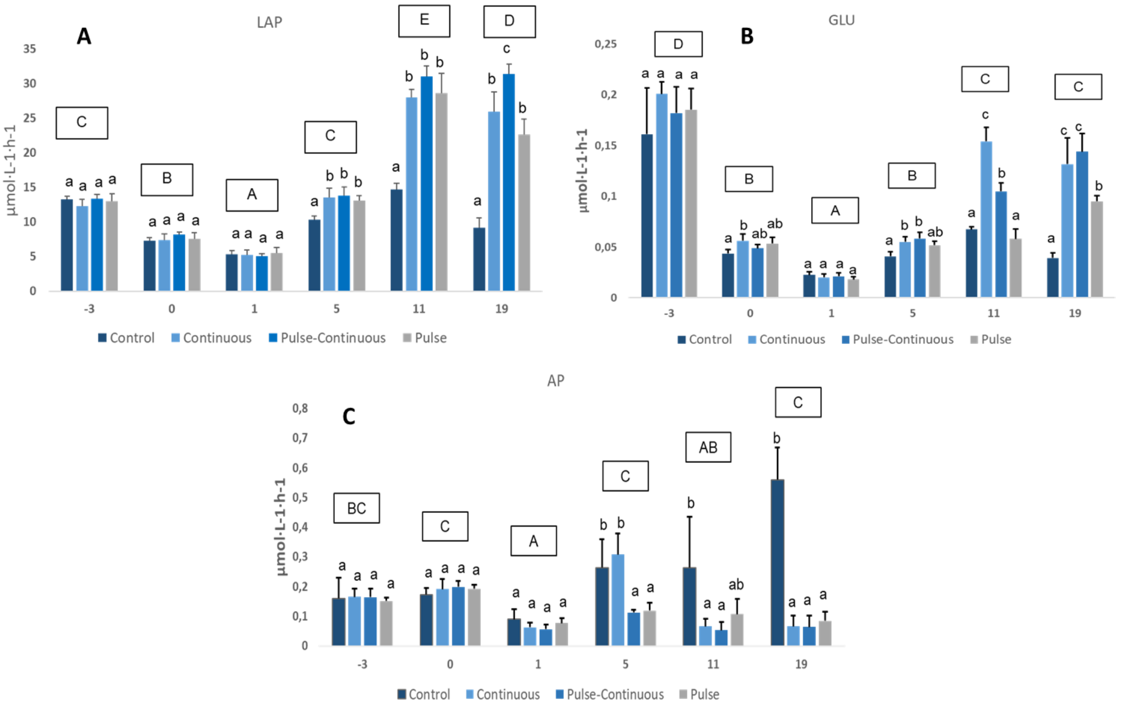

Figure 2.7. Extracellular enzyme activity of leucine aminopeptidase (LAP) (EC 3.4.11.1) (A), β-glucosidase (GLU) (EC 3.2.1.21) (B), and phosphatase (AP) (EC 3.1.3.1–2) (C) of all the mesocosms from the initial conditions prior to nutrient additions (day -3) to day 19 of the experiment. Lower case letters indicate significant differences between treatments at each sampling date ($p < 0.05$), and upper-case letters indicate significant differences between sampling dates ($p < 0.05$), two-way ANOVA.

4.2.4. DISCUSSION

4.2.4.1. Temporal patterns according to nutrient additions: plankton biomass and EEA

While an overall increasing trend in biomass was observed in the initial stages of the experiment, day 11 appeared to be a changing point of all the treatment regimes. The pulse and pulse-continuous nutrient treatments favored an initial increase in bacterioplankton biomass, while the continuous treatment favored a gradual increase of all functional group biomass over 24 days. This suggests that the continuous nutrient inputs influenced steady growth and exerted a bottom-up control. Conversely, bacterioplankton could have benefitted with higher uptake of phosphate, resulting in a decoupling of the bottom-up control in the pulse treatments. While the N requirements are similar, heterotrophic bacteria have a 10-fold higher requirement for P than phytoplankton (Vadstein, 2000). Furthermore, significantly lower DOC levels and low TIC on day 24 in the pulse treatments, which indicate a greater need for carbon and a more rapidly growing community (Sörenson et al., 2020). After total assimilation of both nitrate and phosphate in the pulse treatments, phytoplankton were favored. This may be because some time after the nutrient pulse ceases, the system may adjust the bacterial density to the new/real nutrient concentrations (similar than before the pulse addition), thereby possibly mediating the quorum sensing (Miller and Bassler, 2001). This may also result in the recycling of "exceedance" bacterial biomass which may provide phytoplanktonic communities with a boost of bioavailable nutrients that saw an increase in phytoplankton biomass by day 24. Furthermore, the decline in zooplankton biomass with increasing total nutrient additions and steady phytoplankton biomass was also observed in a study by Butzler and Chase (2009) (and references within), which suggested that nutrient disturbance can influence phytoplankton community characteristics and composition, and may result in inedible or nutritionally deficient phytoplankton, and thereby causing a decrease in zooplankton biomass. This temporal pattern did not occur in the continuous treatments and had less turnover than in the pulse treatments. Ultimately, a gradual and consistent input of low concentration nutrients could lead to eutrophication. This agrees with a previous study by Svensen et al. (2002), who investigated pulse versus continuous nutrient supply to planktonic communities and noted that the continuous supply of nutrients promoted a steady food chain and greater retention of fresh organic matter. In contrast, pulsed nutrients resulted in higher build-up of biomass and higher vertical export of organic matter. The pulse-continuous treatments had a combination of responses seen in the other treatments, suggesting that the nutrient additions had a clear influence on community structure and temporal patterns, distinguishing two very different regimes.

This experiment showed that the increase of LAP and GLU activities in the nutrient treatments could be response to a limitation of C and N, but not P. Within the lagoons themselves, previous work by Boadella et al. (2021) found greater needs for N than for P in the summer that were indicated by high values of LAP/AP ratios. Heterotrophic microbial communities release extracellular enzymes to counteract the lack of nutrients and this functional strategy may represent a competitive advantage over their competitors for nutrients (i.e., phytoplankton). However, phytoplankton can also release some enzymes (mainly phosphatases, Chrost 1991). Results here show that temporal patterns between phytoplankton and bacterioplankton depend/s on the rate and concentration of nutrient inputs. This may be a response to a form of synergy between the two, due to the absence of P as a limiting nutrient, and the interaction does not become competitive (Liu et al., 2012). Furthermore, phytoplankton have always been more related to the use of NO_3^-, while bacterioplankton more to NH_4^+. The increase in LAP activity may be a response to the need of bacterioplankton using other available N sources. This would allow them to compete with phytoplankton for P. In this sense, a lower N:P elemental molar ratio in heterotrophs (N:P 7:1; Cleveland and Liptzin, 2007) compared with autotrophs (N:P 16:1; Redfield, 1958) offers the bacterioplankton mechanisms to compete for P in the case of complementary N sources. As both GLU and LAP activity are related to released peptides and polysaccharides from primary production (Boadella et al. 2021), bacterioplankton and phytoplankton both could have benefited from this synergism that saw an increase in their biomass after nutrient additions up to day 11.

4.2.4.2 Decoupling of growth rate to nutrient load

Our results show that growth rate for the continuous treatments exceeded the pulse and pulse-continuous treatments on day 5 and 19 for phytoplankton and day 24 for bacterioplankton. The apparent decoupling of uptake rates to growth rates between the pulse and continuous treatments could indicate different strategies adopted by phytoplankton and bacterioplankton at specific concentrations of nutrients over time. While the mechanisms and cell quotas behind these results are beyond the scope of this study, we suspect the different strategies of phytoplankton and bacterioplankton could have been related with the availability of nutrients at different rates and at different concentrations over time. Small phytoplankton, typically r-strategists, have a large physiological capacity for luxury consumption and can store nutrients at times of high concentrations and use them for production in the absence of external supplies (Thingstad and Sakshaug, 1990; Svensen et al., 2002). As many as two to five generations may be fueled from stored sources (Barnes and Hughes, 1988). Furthermore, the interface of transport kinetics is important, as is the control of non-limiting nutrient transports, because these processes top-up the quotas and drain the environment of nutrients required by future generations of potential competitors (Flynn, 2005). This strategy would be beneficial in a pulse-confinement setting. However, the fact that the same natural community assemblage had a higher growth rate on lower sequential nutrient concentrations over time indicates other factors affecting higher growth rate. Nevertheless, after nutrient inputs, microbial communities adapt their functional mechanisms, and that change is sustained for some time even after the nutrient input ceases as was seen in the pulse treatments (Figure 2.7 A & B). Due to the flooding-confinement hydrological patterns of the lagoons, the hypothesis of communities adapting to the diminution of resource availability through trophic niche partitioning would be likely. Because of this adaptation, the sensitivity to gradual nutrient inputs would maintain high resource availability over time and high productivity, whereas the functional groups in the pulse treatments appeared to adapt their strategies according to resource availability.

4.2.4.3. The rate of nutrient loadings over time is as important as total nutrient loadings on functional group structure and strategies.

The variable concentrations and rates of nutrient loadings over a 24-day period provided deeper insights into the temporal profile of the stressor (i.e., nutrient addition profile) and the effect it had on the temporal profile of the impact (i.e., changes in water quality, functional activity (i.e., EEAs) and structural composition (i.e., biomass)). As all additions were essentially "pulses" divided into daily or once off, the frequency of nutrients becoming available influenced community strategies and brings into focus the concept of temporal heterogeneity in functional group composition and turnover. The initial responses of phytoplankton and bacterioplankton were similar in all treatments, irrespective of the nutrient load. However, the temporal profile of nutrient additions resulted in different functional group structure and strategies after the initial days and had more influence than by comparing similar total nutrient additions at 24 days. Furthermore, the introduction of total nutrient additions at the beginning of the experiment favored higher bacterioplankton growth rate and biomass production and resulted in significantly lower organic nutrient levels and DO compared with the continuous mesocosms in the long term (Table 2.1). In fact, any pulse of significant concentration and/or followed by a small concentration of nutrients following the pulse addition led to an increase of bacterioplankton biomass composition to phytoplankton biomass of ~2.5% in the pulse and pulse-continuous treatments from day 11 to 19 compared with the continuous treatments (Figure S2.2). This indicates that two different concentration loadings above a certain threshold at the same time resulted in similar functional group compositions. As bacterioplankton are known to be significant consumers of organic matter in aquatic ecosystems, they would play a major role in its accumulation, export, and transformation (Azam, 1998; Hansell & Carlson, 1998). This percentage increase of bacterioplankton biomass over phytoplankton was significant enough to affect water quality of the pulse mesocosms over 24 days in similar conditions of temperature, pH, and salinity. Interestingly, the pulse-continuous treatments did not see this reduction of organic nutrients and instead with higher DO levels, possibly due to the influence of continuous nutrient additions after the initial pulse addition. It was only at day 11 did the effects of the total nutrient additions start to become evident in the pulse treatments, after total assimilation of the added nutrients. In contrast, the continuous treatments were at maximum uptake of nutrients available from the first day and had a quicker growth rate on day 5. This suggests that frequent nutrient inputs are likely for the microbial community to be functionally ready to use or recycle high nutrient loads, with potential implications through the trophic chain. Even though there is a delay in community response to nutrient additions, the temporal aspect of nutrients entering the system seems to influence the response of the community and it adapts accordingly until the next input of nutrients. A similar finding was made by Butzler & Chase (2009), who concluded that the rate of nutrient additions at different nutrient concentrations sometimes affected biomass and community composition more than that of the total amount of nutrients. While that study also included macrophytes and sediments in the mesocosms, the biggest effect was seen at the constituent functional group level at just over half the total nutrient concentrations to our experiment (6000 µg N/L and 200 µg

P/L, Butzler & Chase (2009)). This highlights the influence of inorganic nutrient input variability on the natural assemblage of functional group interactions and structure within the water column, more so than by total inorganic nutrient loads, and brings into focus the temporal profile of the stressor (inorganic nutrient loadings) and the way it enters or becomes available to organisms within ecosystems. Tilman (1982) and Tilman et al. (1982) proposed the Resource Supply Ratio Theory which states that spatial heterogeneity in the environment influences plankton diversity. However, as water is the medium, aquatic ecosystems would exist in more of a homogeneous environment. Therefore, temporal heterogeneity might play a more relevant role in species diversity (Yamamoto and Hatta, 2004). This becomes particularly relevant in the monitoring and management of coastal ecosystems, especially in a flooding-confinement hydrological regime with a strong influence of groundwater inputs. It is commonly accepted that an increase in nutrient loadings increases planktonic community biomass and organic matter loading, resulting in eutrophication in coastal environments (Nixon, 1995; Smith et al., 1999). However, this effect might have different implications on ecological functioning if the gap between nutrient loadings differs from the recovery time of the considered biological response variable (Turner et al., 1993). In our case, the planktonic community adapted to pulse disturbances in a flooding-confinement hydrological regime that is subjected to continuous inputs of low nutrient loads from subterranean waters due to elevated anthropic activities.

4.2.4.4. Implications:” the lagoon quality paradox”

Despite a slight increase of nitrate and phosphate concentration levels after the first additions on day 0b and day 1 in the continuous treatments, concentration levels remained low and comparable with the control treatments throughout the experiment. However, plankton biomass increased with high EEA activity. The total addition of ~14 mg/L of nitrate and ~1.2 mg/L of phosphate over 24 days remained largely undetectable in comparison with the pulse and pulse-continuous treatments after 5 days, and even 11 days for nitrate in the pulse mesocosms. This result indicates a system in a high state of production (both in biomass production and its recycling) and masks the inputs of inorganic nutrients that otherwise can be detected after natural pulse inputs. Previous field studies and models of the lagoons indicated contributions from subterranean water, and their morphology and underlying lithological characteristics can influence the extent of water turnover (Meredith et al., 2022a and 2022b). Combined with this, a recent study by Menció et al., (2023a, 2023b) analyzed the surface and ground water surrounding the La Pletera lagoons to determine the origin, occurrence and processes affecting N, and concluded that continuous input of nutrients into the lagoons at low concentrations is possible, given that fertilizers and sewage were the main source of inorganic nitrogen and levels in groundwater surrounding the lagoons can be up to 5mg/L due to denitrification. The significance of these results combined is two-fold. Firstly, with the recent discovery of significant inputs of subterranean water to the lagoons, any inorganic nutrients present (at low concentrations) in this flow would not be detected in the lagoons themselves, however the community would be under constant influence from such inputs with rapid assimilation, and possibly functioning at elevated production. This was seen in the results of this study, where the functional response took longer to recover than the nutrient water concentrations. This would mean that, depending on the temporal profile of nutrient inputs, we may not detect changes in nutrient concentrations in the water, but a change at the functional level may be occurring. Secondly, as the lagoons are strongly conditioned by unpredictable meteorological events that influence the physical and chemical characteristics of the water (Quintana et al., 1998), these systems are inherently stressed and the long-term anthropogenic effect of gradual infiltration of inorganic nutrients would be extremely difficult to detect in such a highly variable system (a low signal to noise ratio). This would then be an extension from the proposed *Estuarine Quality Paradox* (Elliott and Quintino, 2007) to the eco-hydrological functioning of the La Pletera lagoons. One of the proposals of Elliot and Quintino (2007) (and references within) was to develop different methods that could detect anthropogenic stress against a background of natural stress, to break out of this paradox. The results in this study have shown that the rate in which nutrients enter a system over time seems to have a stronger influence over biomass production and greater bottom-up influence on functional group structure than by the "natural" pulse treatments. Various studies of the area have investigated the influence of environmental factors on phytoplankton-bacterioplankton interactions. These include chemical characteristics and physical properties of the water, as well as interactions between phytoplankton and the rest of the community (bacterioplankton, zooplankton) (see Quintana et al., 1998; López-Flores et al., 2009; López-Flores et al., 2014; Quintana et al., 2021; Boadella et al., 2021) where nutrient availability, salinity, DOM and seasonal shifts can strongly influence these interactions. Nevertheless, the productivity and functioning of an ecosystem is known to be strongly influenced by limiting nutrients such as nitrogen and phosphorus (reviewed in Vitousek et al., 1997; Carpenter et al., 1998), and the results here could be the low signal of community response. Therefore, the state and ecological functioning of the lagoons that are currently evaluated on the effects of detectable nutrient inputs, i.e., pulse disturbances, primary production and ecosystem structure could be in a possible alternative stable state to the flooding-confinement pattern of nutrient inputs perceived in a natural setting. The proposed decoupling of growth to detectable nutrient levels would need to be considered when evaluating overall ecological functioning. This idea extends to the effectiveness of environmental policies, and highlights that in many instances currently, the magnitude, the temporal (e.g., frequency and time between stressor events) and spatial components of the stressor (chemical, physical and biological) are rarely taken into account for effect predictions (Sabater et al., 2019; Perujo et al. 2021). In this case, a lack of consideration of all the diffuse nutrient input points and frequency in which they become available to the aquatic communities could lead to erroneous and confounding predictions, which could lead to inadequate ecosystem conservation and management.

4.2.5. MAIN REMARKS

1. The rate at which nutrients enter a system over time is just as important as the total concentration levels in overall functional group response.
2. Pulse treatments had comparatively better water quality and more recycling of surplus bacterial biomass as the functional groups adapt their strategies according to resource availability.
3. Continuous, low concentration inputs of nutrients influenced functional groups into steady biomass growth, which resulted in more organic nutrients and dissolved oxygen production.
4. Continuous, low concentration inputs can be largely undetected and a decoupling of nutrient levels to ecological functioning should be considered, depending on the varying diffuse nutrient inputs that exist.

4.3. Planktonic response to pulse or continuous inorganic nutrient inputs. Part 2: The effects on zooplankton community structure.[3]

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[3] Meredith, W., Menció, A., Antón-Pardo, M., Romaní, A.M., Perujo, N., Boix, D., Gascón, S., Compte, J., Bas-Silvestre, M., Sala, J. & Quintana, X.D. 2023 (Under Review). Planktonic response to pulse or continuous inorganic nutrient inputs. Part 2: The effects on zooplankton community structure. Science of The Total Environment.

4.3.1. BACKGROUND

Allochthonous organic matter and nutrients enter Mediterranean confined coastal lagoons through irregular meteorological events in a pulse manner, such as flooding by sea storms and/or rainfalls in the autumn and winter, and then become confined and drop their water levels during the summer (Quintana et al., 1998a, 1998b; Badosa et al., 2006). This hydrological pattern would suggest that organic matter and nutrients enter these systems as an unpredictable pulse disturbance, which strongly condition biogeochemical processes and require aquatic communities to constantly adapt to an unstable environment (Gascón et al. 2005; Brucet et al., 2005; López-Flores et al., 2006, 2009). The Mediterranean salt marshes of the La Pletera, located in the Baix Ter wetlands in the northeast of Catalunya, Spain, are such systems with a flooding-confinement pattern where surface water inputs are limited to sudden storm events or intense rainfall, when runoff provide most of the nutrient supply. After these pulse events, these types of coastal lagoons would experience long periods of confinement without external surface inputs. As groundwater can significantly contribute to the overall hydrology, the total amount of groundwater circulation also increases during pulse storm events and decreases during confinement and its extent depends on the presence of underlying low-permeable layers (Menció et al., 2023a, 2023b; Meredith et al., 2022a, 2022b). Moreover, nutrient contents of this groundwater are lower than that of surface runoff that can enter the lagoons on a continuous basis (Menció et al., 2023a, 2023b). Thus, it is expected that most nutrient inputs come from pulse type disturbances, as should do in aquifers without any anthropogenic pressure. Since this is a regular pattern in confined coastal water bodies, we would expect that aquatic species inhabiting these habitats are adapted to this pattern, such as the zooplankton community investigated here.

The zooplankton community structure would depend on the occurrence of these disturbances as external energy, and previous studies related the disturbance with changes observed in the structure of the community (Quintana et al., 1998b). The disturbance intensity can be measured by observing the response of a specific community to the specific environmental change and is estimated from a measurement of the displaced distance of the community from before and after the disturbance (Rojo and Alvarez Cobelas, 1993; Sommer, 1993). Previous studies by Quintana et al. (1998b) analyzed the composition of the zooplankton community in a similar confined salt marsh and identified six environmental situations with a regular community structure using the correspondence analysis (CA) and modelled the entry of external energy, the complexity of the zooplankton community, and the water confinement. The disturbance was therefore measured as divergent displacements in CA factor space for pulse and continuous (or desiccation) disturbances. Based on the results of this CA analysis, Quintana (2002) proposed the variable Z_2 (the score of the second principal dimension of the CA) as a measurement of severity of the disturbance on the zooplankton community, that is, of the damage caused by the nutrient inputs. Temporal patterns in severity on zooplankton found by Quintana (2002) suggest that zooplankton composition should be more adapted to pulse type nutrient inputs than to a continuous nutrient supply.

Nutrients N and P are essential for the growth and reproduction of zooplankton, but they can also have negative impacts if they are too abundant or imbalanced. For example, nutrients can stimulate primary production which increases the food availability for zooplankton and can lead to higher biomass and diversity of zooplankton (Bess et al., 2021). However, increased nutrient concentrations and altered nutrient ratios can also lead to eutrophication and disrupt nutrient and energy transfer through the planktonic food webs, and potentially affecting the growth and reproduction of zooplankton (Sterner and Elser, 2002; Purcell et al., 2007; Dickman et al., 2008). While the effect of nutrient concentrations and ratios have been well documented, nutrient supply that is either pulsed or continuous and what effect it has on planktonic community structures has not been adequately investigated to date. Furthermore, despite mounting evidence that individual nutrient limitation does not describe what occurs in nature adequately, there is a lack of experiments in the literature currently that test the influence of individual and multivariate nutrient limitation in zooplankton using natural phytoplankton and zooplankton communities in general marine systems (Thomas et al., 2022). With this is in mind, a mesocosm experiment was conducted in one of the lagoons of the La Pletera to mimic pulse, continuous and pulse-continuous nutrient loadings to analyze what effect it had on a natural assemblage of functional groups extracted from a lagoon that has an efficient connection with the aquifer (Chapter 2; Meredith et al., 2022a, 2023 b). The same mass of total macronutrients was added to all the nutrient addition mesocosms, but at different concentrations over time. We used the multivariate analysis and Z_2 scores to evaluate the severity of disturbance of the inorganic nutrient additions to the zooplankton community structure. As this study is based on the same zoopankton community (i.e. from the same lagoon system) as in Quintana et al. (1998b), and with the aid of functional classification of the disturbances, the aim of this study was i) to evaluate whether the differing input of nutrients affect the composition of zooplankton and ii) if yes, which type of nutrient input has the most severity. The hypothesis proposed was that the lagoon community must be highly resilient and adapted to the declining resources over time after the pulse disturbance, while continuous inputs would make these communities more sensitive to gradual nutrient inputs, resulting in high productivity due to high resource availability over time (Chapter 2).

4.3.1.1. Study site

The experiment was performed in the La Pletera salt marsh area and the mesocosms were placed in one of the recently restored, permanent lagoons (L04) that has an efficient connection with the aquifer due to high permeable underlying sediments. Comparable zooplankton communities of the previous study in Quintana et al. (1998b) were sampled from a group of temporary basins situated along the coast of the Aiguamolls de l'Empordà Natural Park and are ~25km North of the La Pletera salt marshes. The flooding-confinement hydrological pattern of these salt marshes is similar to the salt marsh studied by Quintana et al. (1998b).

4.3.2. METHODS

4.3.2.1. Data analysis

To determine differences in patterns in community structure with respect to treatment type of control, continuous, pulse and pulse-continuous, a non-metric Multidimensional Scaling (NMDS) was performed from the initial and final days of the experiment. Estimation used Bray-Curtis dissimilarities between treatments. Significant relationships between environmental variables and the NMDS ordination of community structure were tested using the *envfit* function in R. A two-way analysis of similarity (ANOSIM) was used to test significant differences in divergence between the initial and final day, as well as differences between the treatments. A similarity percentages analysis (SIMPER) was performed to calculate the contribution of each species (%) to the dissimilarity between pairs of two treatments. In this case, the control was compared with the continuous, pulse and pulse-continuous treatments and was calculated from the Bray-Curtis dissimilarity matrix.

Calculations for Z_1 and Z_2 were based on the results of the scores of the first and second principal dimensions of the correspondence analysis (CA) of similar zooplankton assemblages (dominated by characteristic species or taxon) collected in a previous study by Quintana et al. (1998b). The multivariate results are applicable to data included in this analysis for the same ecosystem and species composition (Table 3.1). The calculations included coordinates of explanatory taxa in Z_1 and Z_2 axes of the CA analysis listed in Quintana et al. (1998b) and Quintana (2002) and were multiplied by the relative abundance contribution of each taxon to generate Z_1 and Z_2 scores for the same taxa in this study. The variable Z_2 is interpreted as a complexity gradient of the zooplankton community (Quintana, 2002) and, therefore, a measurement of severity of disturbance (the effect of nutrient inputs in this study). Z_1 is related to hydrological disturbances. Significant differences between the Z_2 scores of each replicate of the different treatments on the initial day and the final day were analyzed using one-way ANOVA. An equal variances Tukey HSD post hoc test for multiple comparisons was performed to differentiate treatment effects. Statistical analyses were done with R software (R Core Team, 2021) and the jamovi project (2021) (jamovi Version 2.2.2, Computer Software, retrieved from https://www.jamovi.org).

4.3.3. RESULTS

4.3.3.1. Zooplankton community composition and dissimilarities between treatments

Zooplankton community was dominated by copepods and rotifers for the duration of the experiment and across all the treatments (Table 3.1). Predominant shifts from the initial to final day occurred with Cyclopoida and Calanoida (both with nauplii, N_CyC and N_CAL) and *Hexarthra* spp. Both Calanoida and Cyclopoida with eggs were absent in the pulse treatments on the final day. Indicator species account for an abundance greater than 89% for all the treatments on the final day (Table 3.1).

Table 3.1. Relative species composition (%) (± sd) of the zooplankton community on the initial and final day of the experiment in the different treatments. The same codes are used in the figures. “I” indicates initial day and “F” indicates final day. Indicator species contribution to total abundance refers to the relative abundance of species used for the computation of Z_2 (Quintana, 2002); all species listed were present in the study of Quintana et al. (1998b) and Quintana (2002) except ciliates, which were not determined in these previous studies. 0: control; C: continuous treatment; P: pulse treatment; PC: pulse-continuous treatment.

	Functional Group	**Code**	**Indicator Species**	**0 I**	**0 F**	**CI**	**CF**	**PI**	**PF**	**PCI**	**PCF**
Rotifera	*Synchaeta* spp.	**SYN_SP**	Yes	1.5±1.5	0	0.01±0.01	0.06±0.1	0.6±0.4	0	0.3±0.5	0
	Brachionus plicatilis	**BRA_PLI**	Yes	0	0.08±0.1	0	0.3±0.5	0	0.3±0.5	0	3.8±3.3
	Hexarthra spp.	**HEX_SP**	Yes	13.2±6.3	60.04±7.3	18.3±7.6	47.9±18.26	21.9±12.5	50.8±16.4	9.1±2.6	50.5±17.1
Copepoda	Copepoda Calanoida	**C_CAL**	Yes	7.31±5.97	5.34±3.88	8.26±2.36	5.99±2.13	7.11±3.71	1.65±1.79	16.14±9.01	6.10±3.45
	Copepoda Cyclopoida	**C_CYC**	Yes	8.32±5.46	26.01±2.04	10.46±6.97	27.91±11.14	13.94±15.4	20.5±8.51	10.17±6.82	25.9±13.8
	Nauplius Calanoida	**N_CAL**	Yes	1.23±1.34	2.80±3.28	1.99±1.1	6.81±4.70	0.67±0.49	0.48±0.83	1.38±0.39	1.08±1.07
	Nauplius Cyclopoida	**N_CYC**	Yes	61.03±16.16	19.21±3.1	50.05±13.6	3.85±1.84	45.36±12.18	0.93±2.08	48.3±11.6	2.29±1.21
	Copepoda Calanoida + eggs	**Co_CAL**	No	0	0.3±0.1	0	0.01±0.01	0.02±0.03	0	0	0.1±0.09
	Copepoda Cyclopoida + eggs	**Co_CYC**	No	0.01±0.03	0.1±0.1	0	0	0	0	0	0
	Copepoda Harpacticoida	**C_HAR**	No	0	0.03±0.06	0	0	0	0.2±0.1	0	0
	Nauplius Harpacticoida	**N_HAR**	No	7.4±1.9	5.1±1.2	10.5±2.3	1.3±0.6	10.2±2.9	0.7±1.03	14.6±3.2	0.3±0.3
Ciliata	Mixotrophic ciliate	**CIL_MIX**	No	0	0.03±0.01	0	0	0	0	0	0
	Scuticociliatia	**CIL_SCU**	No	0	0.07±0.06	0	5.8±9.9	0	0	0	9.9±6.8
Ostracoda	Juveniles Ostracoda	**N_OSTRA**	No	0	0	0	0	0	0.2±0.2	0	0
	Ostracoda	**OSTRA**	No	0	0	0	0	0	0	0	0
	Larva Gasteropoda	**GASTER**	No	0	0	0	0	0	0	0	0
Indicator species contribution to total abundance					94.3±1.13		92.9±9.7		98.8±1.6		89.7±7.02

Differences in zooplankton community structure were noted between the control and nutrient addition treatments (SIMPER). Table 3.2 shows the species contributing most to the dissimilarity observed between the nutrient treatments and the control. The indicator species (those with an abundance greater than 89%; Table 1) dominated the highest contributing species to dissimilarity between the treatments. A general pattern regarding the community structure was observed between the treatments with copepods and rotifers accounting ~50% of the contributing species of each of the treatments. Significant dissimilarities between control and nutrient treatments were mostly in copepod nauplii and adults with eggs. While calanoid abundance was similar in both the continuous and pulse-continuous treatments, abundance was significantly lower in the pulse treatments. Furthermore, calanoid nauplii abundance was significantly higher only in the continuous treatments. The pulse treatments favored higher abundance of Rotifers. Dissimilarities were significant between control and pulse treatment, mainly due to the higher abundances of adult copepods with eggs and nauplii of cyclopoids and calanoids in the control than in the pulse treatment.

Table 3.2. SIMPER analysis for zooplankton community structure according to the control treatments vs. nutrient treatments. Mean abundance of the species in each pair of treatments (ava and avb, respectively) and percentage (%) explained by the taxa contributing most to dissimilarity. Asterisks indicate significance at 95% confidence interval. Note that N_CYC and N_CAL are Nauplius Cyclopoida and Nauplius Calanoida, respectively, and are not shown in Table 1; as they were combined with C_CYC and C_CAL for the relative composition analysis.

	ava	avb	%	*P* value
Control vs Continuous				
C_CYC	0.36	0.51	18.6	0.7
HEX_SP	0.38	0.33	15.6	0.9
C_CAL	0.79	0.73	14.4	1.0
Co_CAL	0.11	0.02	12.1	0.1
N_CYC	0.19	0.10	12.0	0.5
N_CAL	0.06	0.12	8.4	0.02 *
CIL_SCU	0.01	0.04	4.5	0.7
Co_CYC	0.04	0.00	4.1	0.03 *
BRA_PLI	0.01	0.03	3.8	0.9
Control vs Pulse				
C_CAL	0.79	0.57	21.90	0.02 *
C_CYC	0.36	0.55	18.00	0.06
HEX_SP	0.38	0.46	15.80	0.3
N_CYC	0.19	0.09	11.20	0.01 *
Co_CAL	0.11	0.01	8.90	0.04 *
BRA_PLI	0.01	0.09	8.00	0.4
N_CAL	0.06	0.03	3.20	0.9
Co_CYC	0.04	0.00	3.00	0.01*
Control vs Pulse-Continuous				
HEX_SP	0.38	0.29	17.1	0.6
C_CYC	0.36	0.47	15.8	0.8
C_CAL	0.79	0.75	15.1	0.9
N_CYC	0.19	0.08	11.7	0.1
Co_CAL	0.11	0.06	10.6	0.02 *
BRA_PLI	0.01	0.08	8.2	0.6
CIL_SCU	0.01	0.08	8.1	0.01*
N_HAR	0.06	0.03	3.7	0.1
Co_CYC	0.04	0.00	3.5	0.01*
N_CAL	0.06	0.04	3	0.9

4.3.3.2. Variances between treatments and measuring severity

Results of the NMDS show a moderate convergence of the samples (stress = 0.1, Figure 3.1). The treatments were statistically significant for divergence from the initial and final day and the nutrient treatment effect (ANOSIM global R = 0.49, $P < 0.001$; global R = 0.17, $P < 0.004$, respectively). All treatments had similar zooplankton assemblages from the same lagoon water administered on the initial day, with negative values in the first NMDS axis (Figure 3.1). However, differences between the treatments were found on the final day. While the nauplii of cyclopoida and calanoida and copepod adults with eggs dominated initial conditions of the treatments and the final day of the control, cyclopoida and rotifera (*Brachionus plicatilis* and *Hexarthra* spp.) were more associated to the nutrient treatments on the final day. The *envfit* function (Figure 3.1) shows the significant environmental parameters ($P < 0.05$), with higher temperature, DO and DOC values in the initial conditions and in the final conditions of the control. Final conditions in all treatments are characterized by higher nutrient concentrations and phyto- and bacterioplankton biomass. Despite its addition, NO_3^- does not show a significant increase in final treatment samples. Slight increases in salinity were observed in all the treatments throughout the experiment. Statistically significant differences were found between the treatments in the final conditions (ADONIS, $R^2 = 0.57$, $p < 0.001$), however they were not significant in the initial conditions (ADONIS, $R^2 = 0.25$, $P < 0.27$).

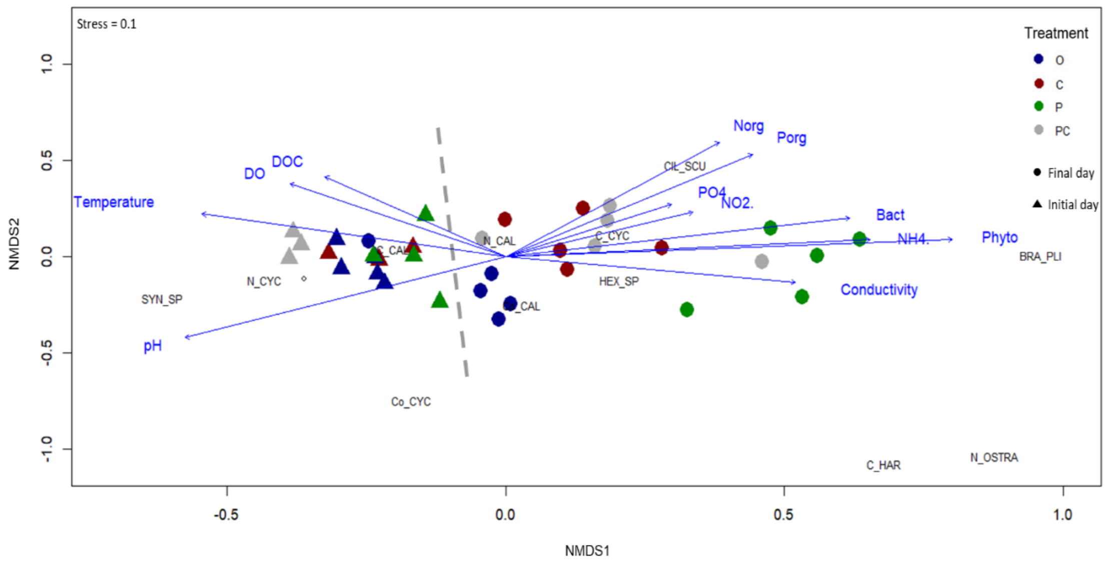

Figure 3.1. Multidimensional scaling (NMDS) plot (Bray-Curtis dissimilarity) for zooplankton assemblages of all the nutrient treatment mesocosms on the initial and final day (taxa codes in Table 1). Significant relationships with environmental variables and community structure ($P < 0.05$) are plotted. Dashed line indicates the division between the initial and final day groupings. Environmental parameters include organic nitrogen (Norg), organic phosphorus (Porg), phosphate (PO_4^{3-}), nitrite (NO_2^-), ammonium (NH_4^+), dissolved organic carbon (DOC) and dissolved oxygen (DO). Other components of the plankton community as parameters included phytoplankton (Phyto) and bacterioplankton (Bact). Treatments represented are control (0), continuous (C), pulse (P) and pulse-continuous (PC).

The positions of the initial and final days of the zooplankton when plotting them in a Z_1 and Z_2 plot are grouped along a similar gradient between the “calanoids” and “cyclopoids situation”, both coinciding with situations of hydric stability with little to low rates of entry of nutrients (Figure 3.2 B and C). Groupings are also maintained at negative values for Z_2 (Figure 3.2 C).

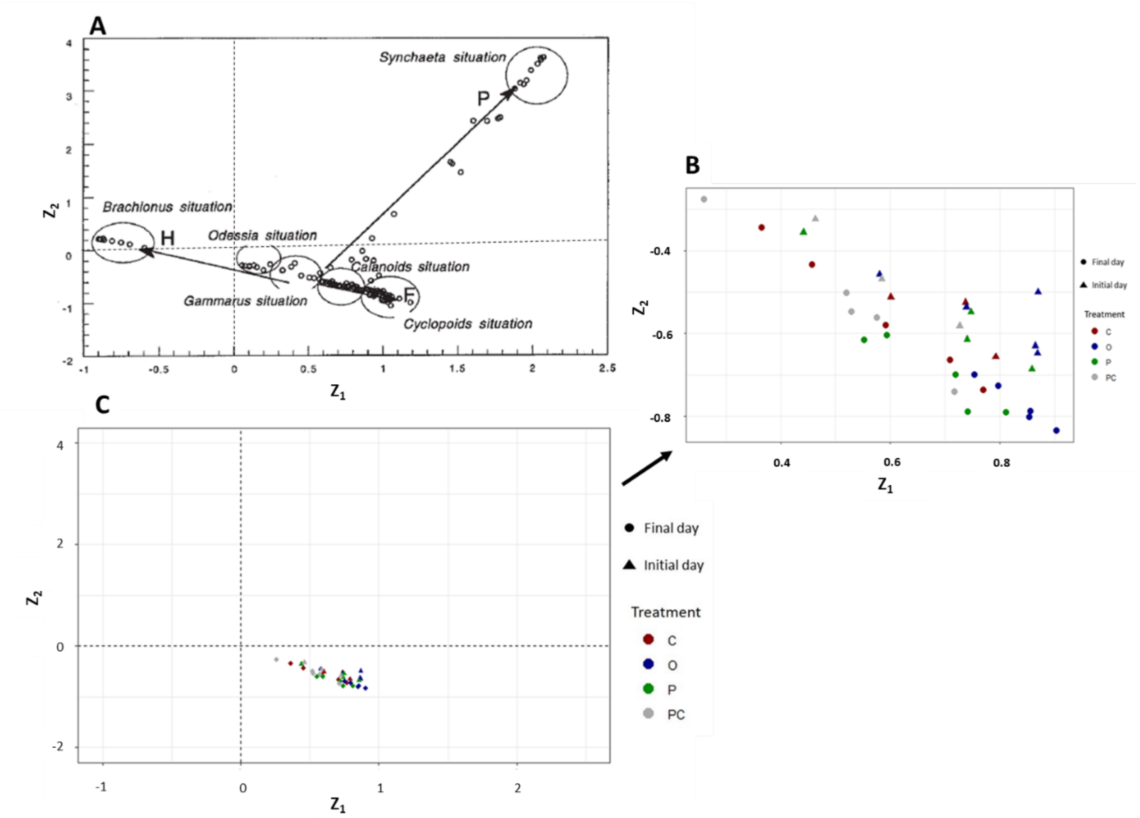

Figure 3.2. Bidimensional representation of samples and species in the CA factor space for the first 2 axes identified in Quintana et al. (1998b). Six environmental situations with a regular community structure (A), where disturbances are indicated by: P = high-intensity pulse disturbance; F = low-intensity continuous disturbance; H = incidents of hypertrophy. Factor space results (Z_1 and Z_2 scores) for zooplankton in the mesocosms experiment according to different nutrient loading regimes (B), and the same results superimposed onto the same factor space as in A (C). Treatments represented are control (0), continuous (C), pulse (P) and pulse-continuous (PC). Figure 2 A adapted with permission from “Nutrient and plankton dynamics in a Mediterranean salt marsh dominated by incidents of flooding. Part 2. Response of the zooplankton community to disturbances” by X. D. Quintana, (1998b). J. Plankton Res., 20, 2109–2127. Copyright 1998 by the Oxford University Press.

Z_2 scores in Figure 3.3 are a small-scale representation of differences in severity of nutrient input disturbance on zooplankton community between the mesocosms during the experiment. While all mesocosms showed similar community structure and Z_2 scores on the initial day, a gradient of severity to the nutrient inputs manifested on the final day according to increasing Z_2 scores. Significant differences in the control and the pulse-continuous treatments established the extremities of the gradient. Lower Z_2 scores in the control showed the least severity to the disturbance, while the nutrient treatments increased the Z_2 scores.

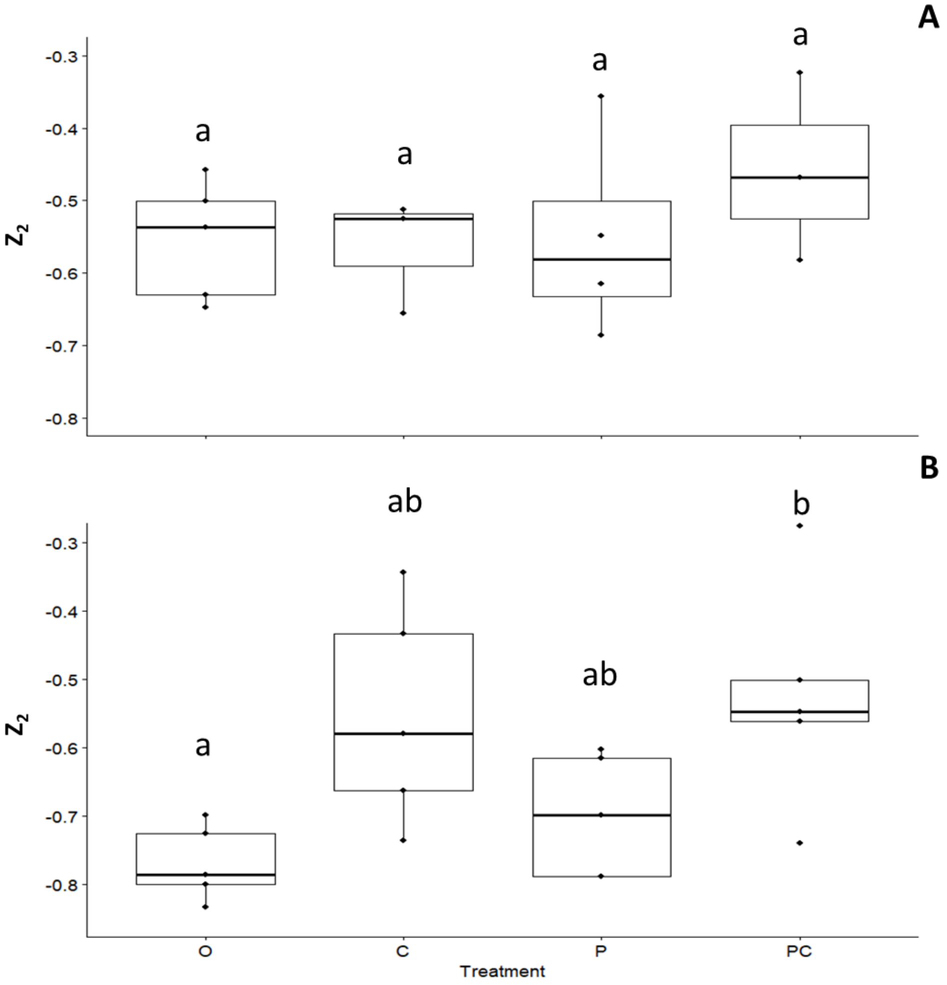

Figure 3.3. Boxplots of the Z_2 scores for each of the nutrient treatments and the control on the initial (A) and final day (B). Results with same letters are not significantly different (one-way ANOVA, $P < 0.05$). Treatments represented are control (0), continuous (C), pulse (P) and pulse-continuous (PC).

4.3.4. DISCUSSION

4.3.4.1. The structure of the zooplankton community according to nutrient treatments

The composition of the zooplankton community found in this study was similar to those typically found in natural lagoon assemblages with medium to high mineralization that occur in Mediterranean coastal lagoons (Aguesse and Marazanof, 1965; Margalef, 1969). The zooplankton community in the Empordà and La Pletera wetlands is dependent on natural and anthropogenic disturbances, as well as the tolerance to variation in salinity (Quintana et al., 1998b). This resulted in similar overall zooplankton composition structures of copepods and rotifers in our study (Table 1) to the study by Quintana et al. (1998b). According to environmental situations in a natural setting, the latter study ordered zooplankton samples along the axis from a low number of species (usually an opportunistic species of rotifers, such as *Synchaeta* spp. or *Brachionus plicatilis*) to a more complex composition of various species (simultaneous presence of calanoids, cyclopoids and other rotifers or crustaceans; Figure 3.2 A). The situation of low complexity (*Synchaeta* situation) is driven by the high pulse disturbances linked with sea storms that increase nutrient loading and water levels in a short period of time, which rapidly increases primary production and causes major disturbances to the zooplankton community. This disturbing factor acts intensively and briefly, after which the community restores itself to the previous situation (Quintana et al., 1998b; Figure 3.2 A). Alternatively, a structured and relatively complex zooplankton community with a greater number of species (cyclopoid situation) is associated with a slow and continuous disturbance. In our mesocosm study, one parameter of disturbance (inorganic nutrients) in the short term (up to 1 month) is analyzed. Therefore, the statistical differences in composition observed in the final conditions were the sole result of different nutrient additions over time. The notable similarity of the initial composition of the zooplankton community structure to the cyclopoid situation of the sampled natural assemblage would suggest an initial external influence in a continuous state. When relating this finding in a natural setting, linkages with nutrient measurements of the surrounding wells, groundwater flow and modelled water balance of the La Pletera lagoons can be made. The newly constructed L04 lagoon (2016) was found to have an efficient connection with underlying aquifer (Meredith et al., 2022a and 2022b) and potentially receiving low concentrations of inorganic nitrate through the subterranean waters (Menció et al., 2023a, 2023b). The maintenance of the observed cyclopoid situation of the initial conditions of the natural assemblage would require a slow and continuous external energy entry (such as in flow cultures), as noted by Quintana et al., (1998b). As this is gradual over time, it would be realistic to make these linkages in our study of nutrient input to zooplankton community structure dynamics at the outset of the experiment. The divergence of relative composition in the pulse treatments in the final conditions to the rest of the treatments, as well as a total zooplankton biomass decrease (Figure S3.1), illustrate the differences between pulse and continuous inputs. Although cyclopoids, calanoids, and *Hexarthra* spp. dominated all the nutrient treatments on the initial and final day of the experiment, the standout compositional differences occurred with the low presence of adults with eggs and nauplii in the pulse treatments compared with the other treatments (especially the control), indicating a disturbance effect that impaired reproduction and growth. It has been noted that intense inputs can destabilize food chain connections, thereby decreasing diversity (Margalef, *1997).* Also, zooplankton reproduction and growth variation can be explained by N and P limitation in the food, even in the presence of abundant and edible phytoplankton (Sterner & Elser, 2002; Peltomaa et al., 2017). Although not significant, the relatively higher C:N and C:P ratios in the water and significantly lower organic N and P in the pulse treatments (Chapter 2) could indicate different nutrient cycling patterns that may have had a consequence on zooplankton reproduction and growth. Also, given the irregularity of Mediterranean aquatic ecosystems, it would be realistic that species' anticipation of suitable growing conditions would be hindered, resulting in opportunistic reproductive patterns, not fixed (Quintana, 2002). Therefore, any long-term changes in environmental conditions would condition the response for reproduction and growth of the zooplankton community accordingly.

4.3.4.2. Variation of Z_2 as a measure of severity

Defining Z_2

Severity can be related to the values of the second principal dimension of the CA (Z_2) using zooplankton samples calculated from the natural assemblage of the mesocosms (Figure 3.2 C). This was originally interpreted as a 'complexity gradient of the zooplankton community' in natural systems in Quintana et al. (2002), who found significant correlation between Z_2 and various measurements related to the community structure. The interpretation and analogies of the values on the CA factor space are related to the functioning of different aquatic systems in terms of input of external or auxiliary energy (further explanation in Quintana et al., 1998b) and Z_2 is a measure of disturbance or severity in the destructuring effect of the zooplankton community. Also, sudden and recurrent changes in physicochemical composition have been shown to increase the value of Z_2 (Quintana, 2002). In our study, the fact that the disturbance occurs in mesocosms as solely inorganic nutrient additions, the focus is more on the severity of the disturbance. This agrees with the concept of severity as 'the damage of the disturbing force' (Sousa, 1984; Pickett and White, 1985; Turner et al., 1998).

Severity of inorganic nutrient disturbance

While previous observations in Quintana et al. (1998b) focused on the hydrological regimes and nutrient loadings related to the meteorological events over a yearly basis, zooplankton community structure changes in the mesocosm experiment can be seen as transitions over the short term. The smaller scale increases in Z_2 in the continuous treatments (C and PC; Figure 3.3) suggest a gradual, higher severity of disturbance to the zooplankton community that can accumulate over a longer period. On a larger scale and comparable to natural settings, the cluster of Z_2 scores for all the treatments in the cyclopoid situation, both in the initial and final conditions, was both surprising and insightful. Both the mesocosm experiment and the analysis in natural systems concluded that the response of the system depends more on the rate of entry of nutrients than on the quantity of these entries (Quintana et al., 1998b; Chapter 2). In a natural setting, pulse disturbances can be cyclic, whereby a quick turnover of nutrients would occur after the intense input, followed by a period of recovery until the next input. However, pulse treatments in the mesocosms had this recovery period for the duration of the experiment. Conversely, a low turnover rate was associated with continuous flooding and nutrient additions in a natural setting, permitting greater production and the maintenance of a greater number of species (Quintana et al., 1998b). A similar effect was observed in the mesocosm experiment and supported by significant positive relationships of all the functional groups′ biomass in the continuous and pulse-continuous treatments, and significantly lower DOC and DO for the pulse treatments (Table 2.3; Table 2.1). If under a persistent regime, the bottom-up effect of gradual and continuous inorganic nutrient inputs could influence zooplankton community structure into an alternative state that resists recovery to a previous state. This could possibly explain the persistent cyclopoid situation and similar Z_2 scores for the control and pulse treatments and a small divergence of the continuous and pulse-continuous treatments. There are several reasons for reaching this interpretation based on the previous findings of Quintana (2002). Firstly, conditions of stability are shown as convergent displacements towards a point near the origin of the Z_2 coordinates in a natural setting. This displacement towards an intermediate position of Z_2, which has been defined as a '*Gammarus* situation' due to the dominance of *Gammarus aequicauda*, represents conditions absent of high intensity pulse or low intensity continuous disturbances (Figure 3.2 A). Secondly, it was noted that composition of the community before the disturbance can affect the final level of severity observed in a natural setting. Thirdly, while samples of community structure were taken on the initial and final day only, the end (or return) state of the community composition of the pulse and control treatments to comparable states of the continuous treatments or "press disturbances" could indicate quicker species turnover in the pulse treatment and a resistance of natural tendency to oligotrophy (Margalef, 1983) in the control. On a small scale, the fact there is a gradient in terms of severity of inorganic nutrient inputs from the control to the continuous treatments, and not the pulse treatment, indicates a lack of recovery and impact that continuous inputs could have on a larger scale and in the long term.

4.3.4.3. Implications

When assessing disturbance intensity, measuring a change in the community runs a risk of confusing the cause with the effect of the disturbance (Sommer, 1993). An example is the selection of only one functional group in the previous studies. Nevertheless, the use of zooplankton as an indicator of the community is advantageous due to the intensity and speed of responses (Quintana et al., 2002). Not only were similarities found in the experiment and in the field, but the Z_2 values of the zooplankton community in the mesocosms were tested directly with the interactions of the phytoplankton and bacterioplankton groups (Figure 3.1) , each responding to the rate of non-limiting nutrient inputs over time. A situation which is lacking in current literature (Thomas et al., 2022). This has not only reinforced observational concepts proposed by Quintana et al. (1998b) but has provided insight into the potential long-term consequences of continuous, low concentration nutrient inputs. Some of which may not be detectable due to rapid assimilation (Chapter 2). While the amount of total nutrient loadings is important in excessive growth and production, this study has also highlighted the rate of entry of nutrients, whereby the relatively slower turnover in continuous inputs could lead to excessive growth and organic matter production and ultimately eutrophication. If persistent, such as contributions from subterranean waters, the bottom-up effect of nutrient inputs could tip zooplankton community structures into alternatives states of growth and functioning, possibly favoring more opportunistic species, and a resistance to a previous state could be enforced (Quintana et al., 2021). Malone and Newton (2020) noted that the main sources of nutrient inputs to coastal lagoons are river discharge, groundwater discharge, atmospheric deposition, and human activities such as agriculture, aquaculture, sewage and urban runoff. However, the relative importance of these sources varies depending on the hydrological and biogeochemical characteristics of each lagoon. Additionally, Maher et al. (2019) found that groundwater inputs of nutrients and dissolved carbon stimulated both primary production (which reduces CO2) and CO2 fluxes to the atmosphere in a coastal lagoon in Australia. With a prediction of an increase of exported anthropogenic N of around 45% to coastal ecosystems by 2050 (Galloway et al., 2004), focus on diffuse nutrient inputs becomes especially important, as the restoration and management of coastal lagoons depends on preserving ecological functioning. Due to potentially rapid nutrient assimilation of the planktonic community, low concentration nutrient inputs could be undetected and not accounted for in primary production analyses. With efficient connections with underlying aquifers and increasing anthropogenic activity, this could lead to confounding predictions and erroneous decision-making in conservation and management. The simplicity of obtaining Z2 values and its logical application has proven itself a valuable tool in management of these types of ecosystems, and the results in this study have shown the level of impact inorganic nutrients have on plankton group dynamics in relation to some aspects of disturbance theory.

4.3.5. MAIN REMARKS

1. The pulse treatments resulted in decreased zooplankton biomass and the low presence of adults with eggs and nauplii, indicating impaired reproduction and growth.

2. The cyclopoid situation of the initial composition of the sampled zooplankton assemblage suggested an initial external influence in a continuous state, which is potentially linked to continuous, low concentration nutrient inputs through subterranean waters to the lagoons, especially in summer.

3. Despite compositional differences in the pulse treatments, the smaller scale increases in Z_2 scores in the continuous treatments indicated a gradual, higher severity of disturbance over a longer period.

4. The persistent bottom-up effect of continuous inputs could influence zooplankton community structure into an alternative state that resists recovery to a previous state.

5. GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

5.1. Lagoon hydrology: revisiting the concept of confinement

Until recently, knowledge of groundwater contributions to coastal ecosystems has been limited, due to temporally variable, patchy, and diffuse nature of groundwater discharge (Sadat-Noori et al., 2016; Santos et al., 2008). This is also true for the La Pletera salt marshes, whereby quantifying inputs (surface and subterranean) is extremely difficult due to a lack of point sources. Through the GLM, the relatively simple methodology of using observed lagoon water levels has proven a useful tool in addressing this problem with a relatively high level of confidence. Another advantage of the confinement pattern is it can help eliminate uncertainty of potential mixing if the lagoons were connected to the sea by tidal regimes, which was not our case. Any positive inputs of water to maintain or increase the water level balance, that do not coincide with rainfall or sea storms, is groundwater contribution. Studies by Casamitjana et al. (2019) and Menció et al. (2017) of these contributions, including the physicochemical properties (such as temperature and salinity), drew attention to a part of the hydrological dynamics that was lacking previously in explaining overall hydrological dynamics. This thesis took this concept further by quantifying the water balance of the newly restored lagoons and integrating morphology and lithology as additional parameters. In doing so, comparisons between naturally occurring lagoons with newly restored lagoons began to illicit how successful the restorations were (to date) according to the set objectives of salinity fluctuations and ecological functioning, and gained insight in the varying contributions of groundwater separated from surface water. All this, despite experiencing the same climatic constraints. Traditionally, confined Mediterranean coastal wetlands have been perceived as having no water inputs between flooding events, especially in the summer. However, the results in chapter 1 have revealed a more dynamic system, with continuous contributions of water year-round. Furthermore, heterogeneity in hydrological functioning between individual lagoons exist, and challenges presumptions that all lagoons behave the same hydrologically in the same area. As has been demonstrated, these groundwater contributions are more gradual and continuous in nature, and ensures the permanency of the lagoons, especially in summer.

5.2. Accompanying nutrients in groundwater inputs

In a similar vein to hydrological confinement, Mediterranean coastal wetlands have generally been considered as net importers of nutrients and organic matter (Ibáñez et al., 2000; Cloern 2001; Newton et al., 2018). This would mean that there is a concentration effect of the salts, nutrients (mainly in organic form) and organisms, and progressive accumulation occurs along successive confinement events over the years. Also, previous studies have attributed the nutrient dynamics of confined lagoons more to internal loading processes than to external nutrient supplies due to the flooding-confinement pattern (Gilbert et al., 2010). The La Pletera lagoons have been documented to accumulate phosphate and organic matter, with inorganic nitrogen concentration peaks after flooding events, and disappear fast with confinement (Quintana et al., 1998a; Badosa et al., 2006; López-Flores et al., 2006a; 2014). While denitrification rates were found to always be higher than nitrification ones (López-Flores et al., 2014), a net loss of inorganic nitrogen would occur if inputs do not compensate it. Therefore, the intense nitrogen losses have been attributed more to the balance between nitrification and denitrification processes (Quintana et al., 2018). As the results in chapter 1 challenge the idea of total confinement of the lagoons, the idea of groundwater as a diffuse source of nutrients feeding into the lagoons has become feasible. The identified sources of N in the alluvial aquifer of the La Pletera area by Menció et al. (2023a, 2023b) lends wait to this possibility. Therefore, groundwater might play more of a role in terms of allochthonous inputs in the absence of surface inputs. The potential N concentrations that can arrive to the lagoons, coupled with the significant amount of groundwater contribution to the lagoons, especially during summer, leads to question again the attenuation processes that exist in the lagoons, especially as N levels remain low between storm events, and by how much would assimilation of N from the planktonic community contribute to the whole N budget in these lagoons? A previous study conducted by López-Flores et al. (2014) analyzed potential denitrification rates of rhizomes and sediments in different regions of Spain, including the Fra Ramon lagoon (FRA) as part of the locations making up the Empordà wetlands. At its peak in summer, the Fra Ramon denitrification rates were estimated at around 0.72 $mgN \cdot d^{-1} \cdot gdw^{-1}$. According to nutrient availability, which was at its maximum in the pulse treatments, assimilation of Nitrate in the pulse mesocosms reached 1.98mg/L d by day 11 (Chapter 2). Although rudimentary in comparison at this stage, it nevertheless reveals potential attenuation processes in isolation. Quick assimilation of N by planktonic communities is realistic, as it is the limiting nutrient in these types of systems, as was demonstrated in Chapter 2. This could ultimately be felt through the trophic levels, possibly altering zooplankton community structure, as demonstrated in Chapter 3.

5.3. Implications

Following the hypothesis proposed earlier, this thesis has demonstrated that gradual, continuous nutrient inputs can promote growth of all functional groups and increases oxygen production, while pulse nutrient inputs, although intense, provide a period of recycling and recovery until the next pulse input (Chapter 2). Coupled with evidence of groundwater contributions to the lagoons, it is possible that ecological functioning is decoupled from measurable nutrient levels, especially N, and the La Pletera lagoons are at greater risk of eutrophication than previously believed. This point even more relevant as the rate at which nutrients are entering can be as important as the total concentration in affecting biomass growth and elevated oxygen levels (Chapter 2). Studies have noted eutrophication in the La Pletera lagoons and surrounding areas, with a saturation of primary production at high concentrations of nutrients (Bas-Silvestre et al., 2020; Serrano et al., 2017). Although potential productivity (standardized rates) was found to be more sensitive to winter inputs, the actual productivity was more related to summer concentration processes, as a result of confinement (Bas-Silvestre et al., 2020). This also coincides with the biggest contribution of groundwater to the lagoons during the year (Chapter 1).

Other case studies of two Llobregat Delta lagoons in eutrophic situations exhibited similar symptoms of higher organic matter content and elevated oxygen levels to the continuous treatments in chapter 2, and this was caused by a combination of continuous freshwater inputs loaded with nutrients from wastewater treatment plants, as well as alterations to their hydrological functioning that resulted in salinization (Cañedo-Argüellesm et al., 2018). There were clear transitions from clear water states to regular phytoplankton blooms and anoxia. Despite altering these nutrient inputs to correct the transitions, these lagoons continue to be in turbid water states, unable to break the hysterical response and unable to reach its full potential in biodiversity development (Cañedo-Argüellesm et al., 2018). Also, concerns of shallow morphology were highlighted, where shallow depth promotes wind induced mixing and favouring the resuspension of the sediments and phosphorus, thereby resulting in internal cycling of nutrients and positive feedback of the dark phase. One suggestion of returning lagoons to clear water states is altering their hydrology by connecting them to the sea (or oligotrophic waters). However, depending on the level of continuous nutrient contributions, constant connection with the sea doesn't guarantee exclusion from eutrophication. A case in point with recent studies revealing significant contributions of submarine groundwater discharge (SGD) high in nitrogen to the Mar Menor hypersaline coastal lagoon, resulting in a eutrophication crisis, despite increased water renewal rates from channel enlargement with the Mediterranean Sea (Álvarez-Rogel et al., 2020). Although hysteresis and eutrophication are complex processes that are influenced by many parameters and balances that maintain the biological productivity that are intrinsic in coastal lagoons, increasing organic and/or nutrient inputs have been shown to increase biological productivity (Pérez-Ruzafa et al., 2018), possibly enhanced further by continuous inputs rather than in pulses. An important result that emerged in this thesis was how deeper or shallower morphologies influence water column stability, and this can determine how much influence meteorological events have on hydrological stability and physicochemical properties such as salinity. This could become an important factor in the face of climate change. For example, longer droughts and intense evaporation could increase salinity and/or desiccate lagoons completely that have an inefficient connection with underlying aquifers, while intense storms could swing water levels dramatically with shallower morphologies, leading to more "flushing" or mixing. This could lead to higher severity of disturbance of planktonic communities and macrophyte colonization. Nutrients present within aquifers could pose a threat to lagoons with efficient connections, and combining this with deeper or shallower morphologies could have different consequences in terms of biological productivity and cycling.

5.4. Future directions

Nutrient inputs and lagoon morphology appear as key elements in lagoon eutrophication, turbidity and salinization that can contribute to ecological hysteresis. The interactions of these processes are complex and finding a "one fit fixes all" solution is challenging at best, especially taking into account the heterogeneity of lagoon systems. The multidimensional approach in this thesis is a starting point in an attempt to quantify these processes. The obvious observation at this stage is to measure the amount of nutrients that are entering the system through groundwater contributions. Due to the nature of the La Pletera system, determining nutrient entry directly into the lagoons is challenging. For this reason, most studies have based results on observed or measurable levels of nutrients in the lagoons themselves. With the calibration of the GLM to the La Pletera lagoons, the intention now is to incorporate the Aquatic Ecodynamics Model (AED/AED2) to the GLM to model the biogeochemistry and aquatic ecosystem dynamics of the lagoons. The model components establish carbon cycling, nitrogen and phosphorus dynamics, as well as oxygen. Furthermore, the model simulates organic matter, as well as different functional groups of phytoplankton and zooplankton, in conjunction with sediment and water column geochemistry. Nutrient levels are required as an input parameter. Although indirect, as was the inflow and outflow estimations (chapter 1), it nevertheless could give a good indication of nutrient inputs as well as of the overall processes occurring in each individual lagoon, according to their unique hydrology. Also, results from chapter 2 and 3 of planktonic dynamics according to nutrient loadings (continuous or pulse) would be considered, to better understand processes unique to the La Pletera, as well as stress test the model in possibly integrating processes not yet considered. To the author´s knowledge at writing, water quality modelling (with AED2) has never been done in coastal water bodies less than 3m in depth. Information gained from such an endeavour could prove important when constructing and restoring lagoons according to predetermined morphology and underlying sediment patterns, as it could ultimately limit or enhance the success of set objectives and overall ecological functioning in a flooding – confinement driven lagoon ecosystem conditioned by irregular and unpredictable climatic events.

Following on from the methodology of establishing inflow and outflow data from observed water levels of the lagoons, results from recent investigations in the area using electrical resistivity tomography (ERT), as well as monitoring newly installed piezometers (data not shown here) have shown quite good agreement with the results of the GLM in this thesis in terms of groundwater flow and salinity gradients. Although the results are preliminary, these investigations could add more validity to the use of the GLM in these types of coastal systems. If so, the intention would be to publicise these validations, in order for other regions to consider incorporating such methodologies that might not have access to specific measuring equipment or face logistical limitations when trying to model confined water bodies.

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7. SUPPLEMENTARY MATERIALS

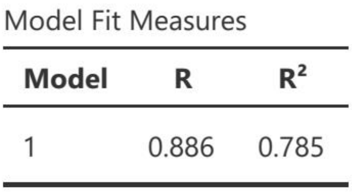
7.1 Effects of morphology and sediment permeability on coastal lagoons’ hydrological patterns.

Table of contents:

Supplementary material pertaining to modeled vs observed values for volume, salinity and temperature, as well as rainfall in the La Pletera salt marsh between 2016 and 2019 in Chapter 1 can be found online as a Data in Brief article at:

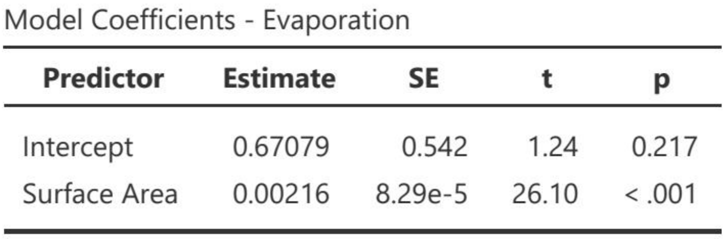
https://doi.org/10.1016/j.dib.2022.108593

Table S1.1. Relationship between lagoon surface area (m^2) and evaporation (m^3 day^{-1}) Significance at $p < 0.05$ at 95% confidence interval using Spearman rank correlation calculations.



Model Fit Measures

Model	R	R^2
1	0.886	0.785



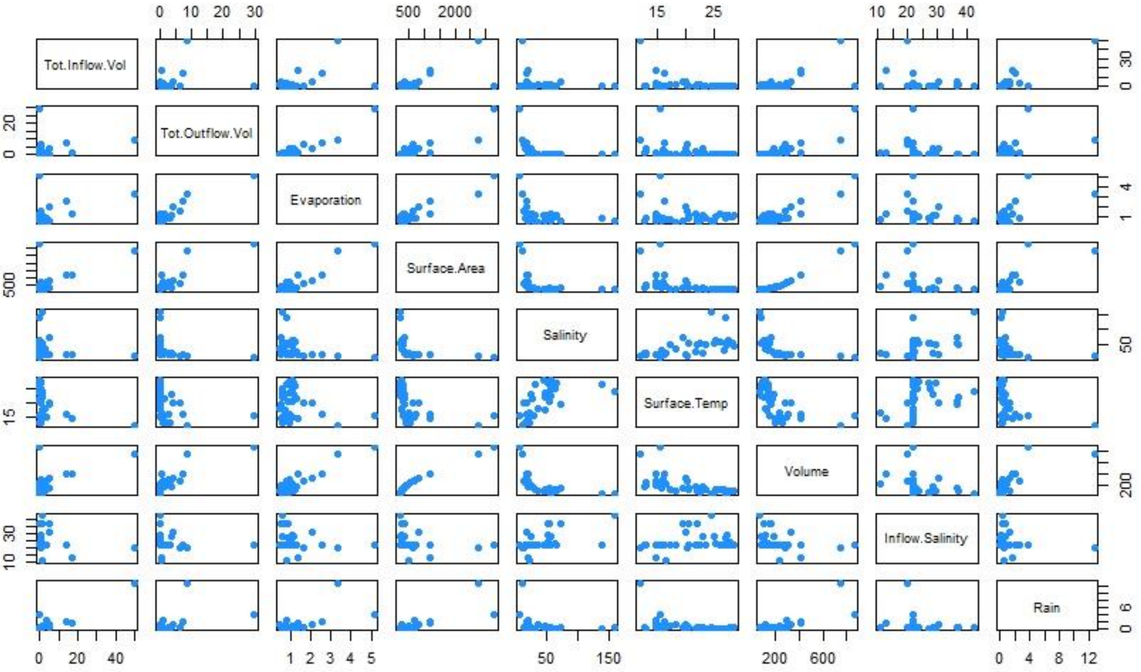
Model Coefficients - Evaporation

Predictor	Estimate	SE	t	p
Intercept	0.67079	0.542	1.24	0.217
Surface Area	0.00216	8.29e-5	26.10	< .001

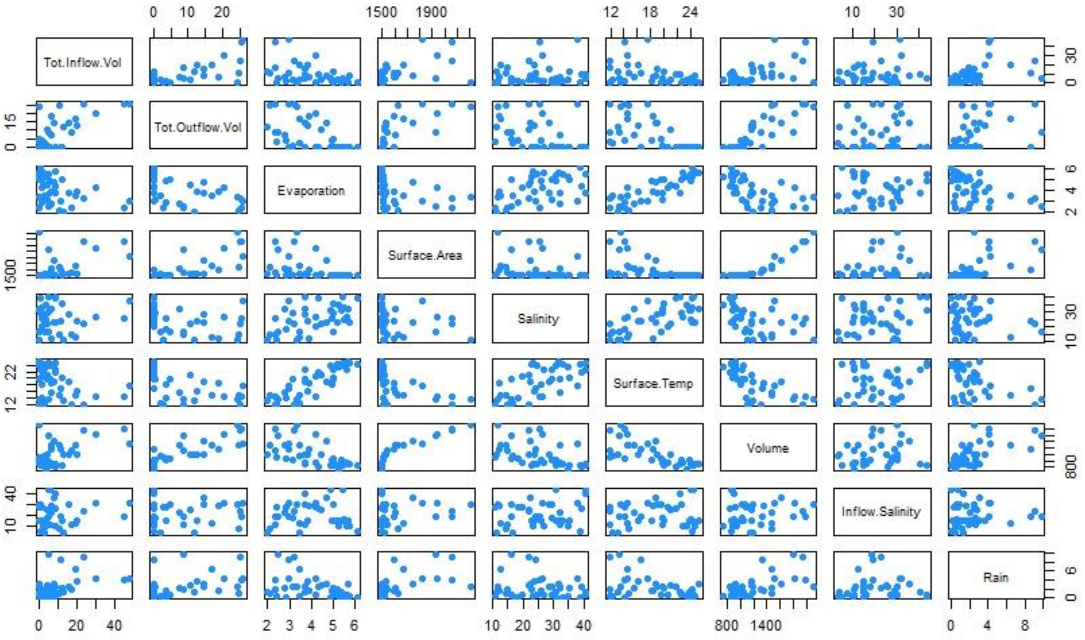
Table S1.2. Relationship between evaporation (m^3 day^{-1}) and salinity (ppt) and lagoon volume (m^3 day^{-1}). Significance at $p < 0.05$ at 95% confidence interval using linear mixed models to allow for both fixed and random effects within the analysis and aggregate the hierarchical data based on the month and year on lagoons which showed no significance in annual patterns.

	Salinity			Volume		
	L04	**L01**	**M03**	**L04**	**L01**	**M03**
Evaporation (Winter vs Summer)	0.44*	0.18	0.57*	0.83*	0.83*	0.8*

Lagoon BPI **A**

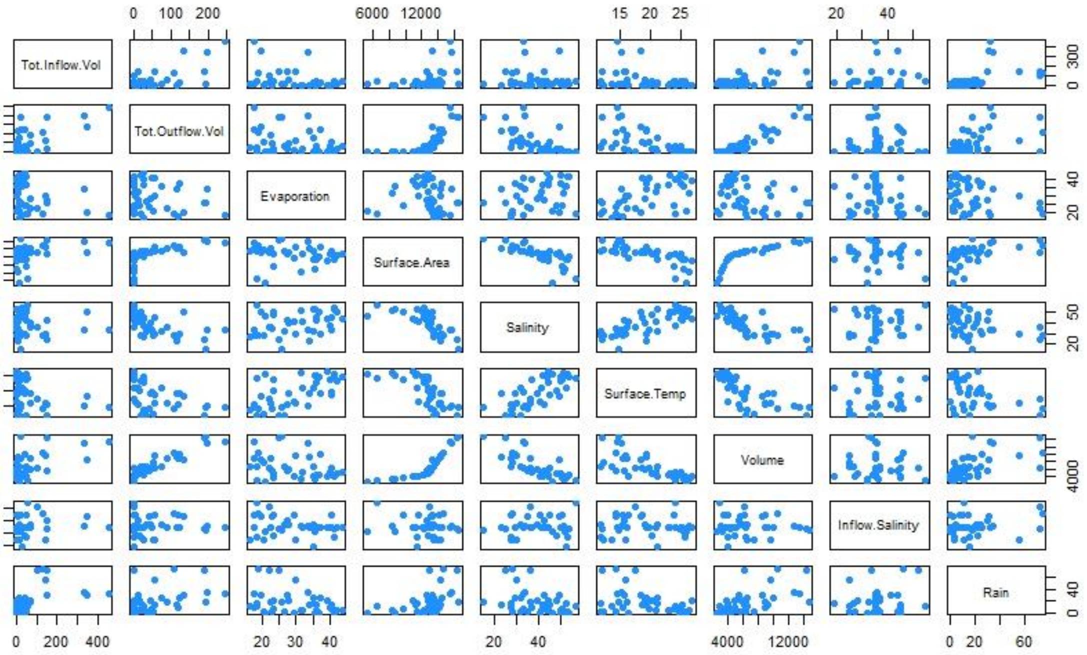


Lagoon FRA **B**



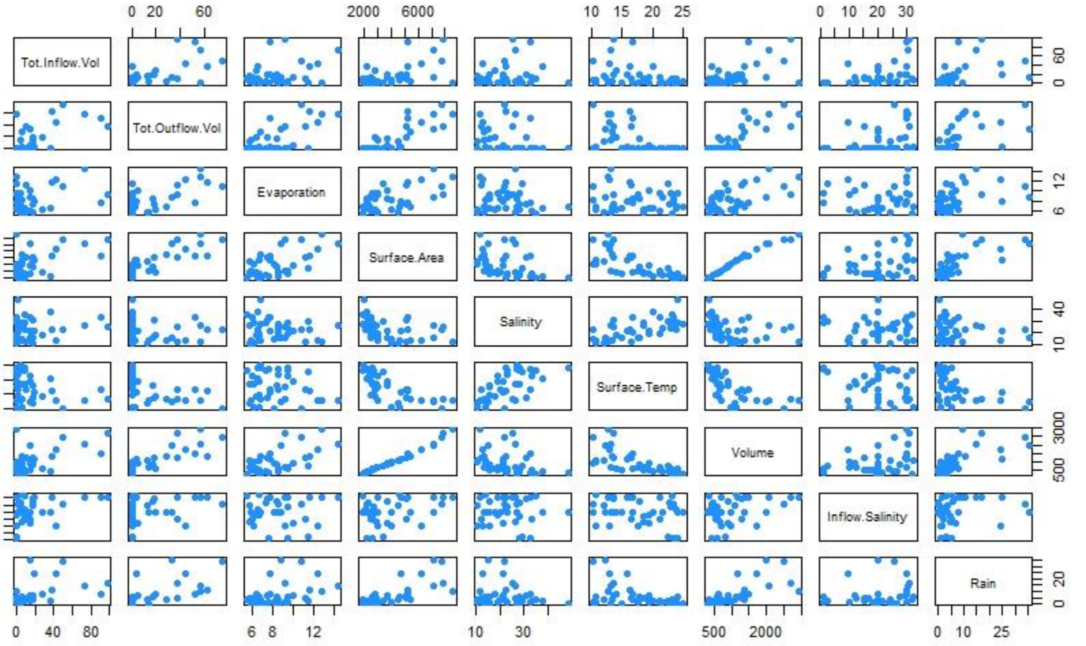
Lagoon G02

C

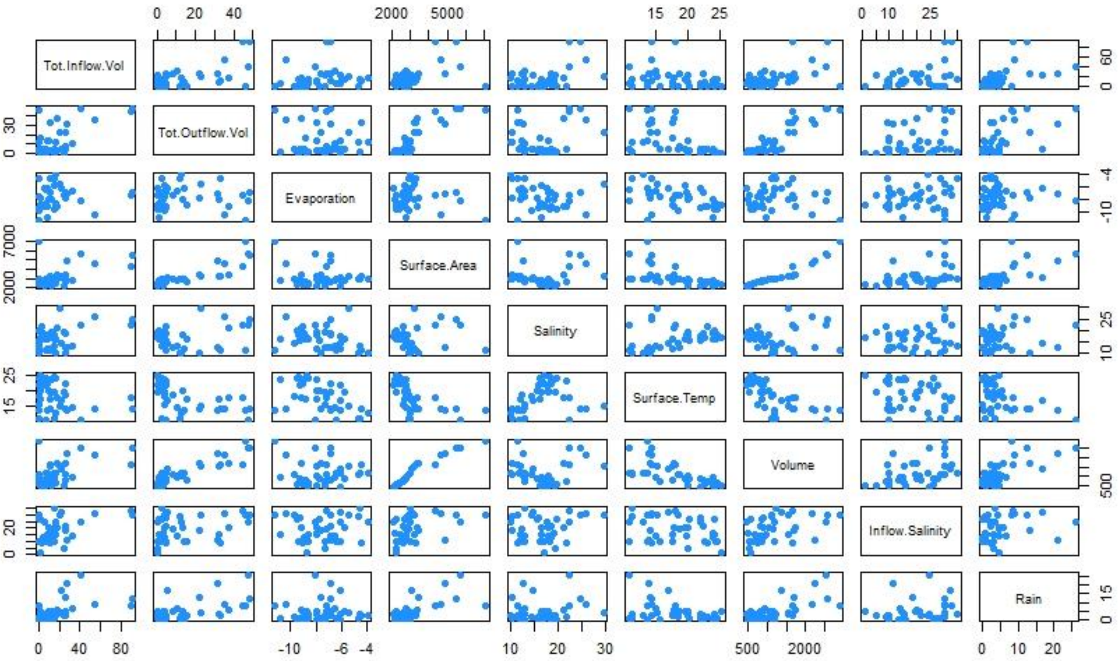


Lagoon L04

D



Lagoon L01 **E**



Lagoon M03 **F**

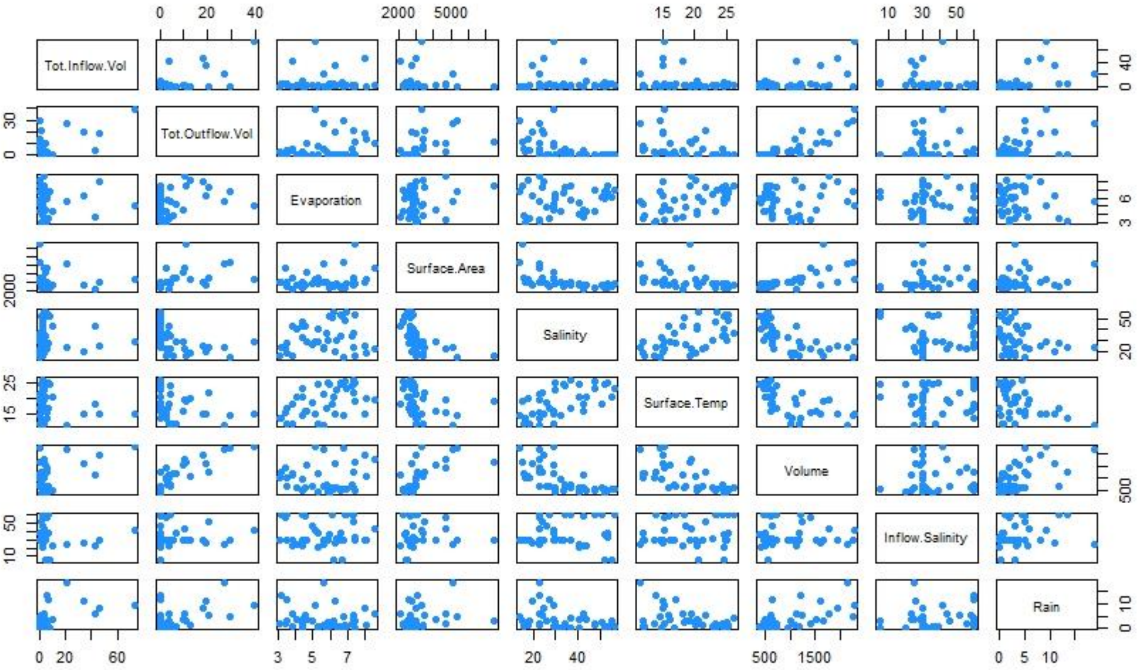
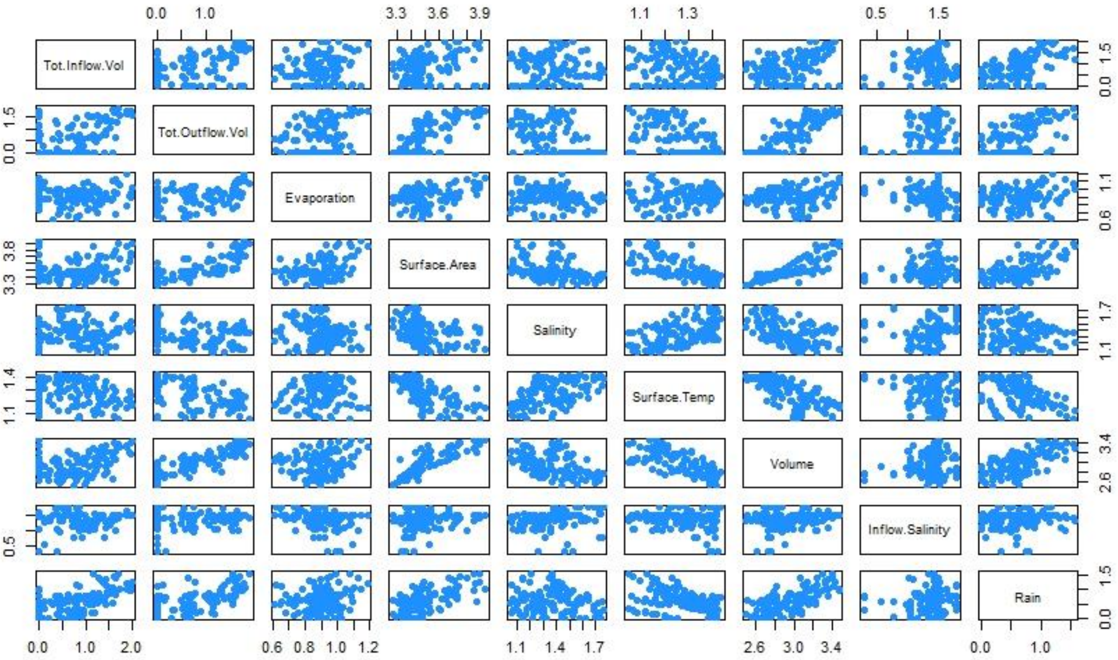
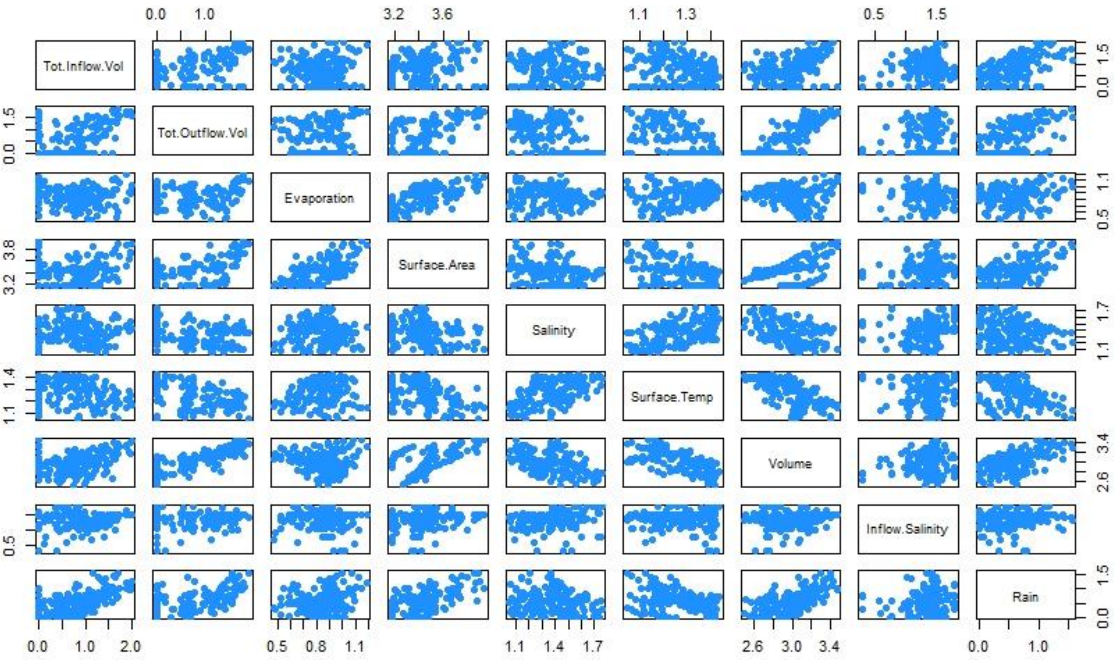


Figure S1.1. Scatterplots of all the variables associated with circulation, salinity and overall water balance of all the La Pletera lagoons. Significant and relevant relationships are shown in Table 7, which represent relationships within the lagoons.

New Lagoons (L01, L04, M03) **A**

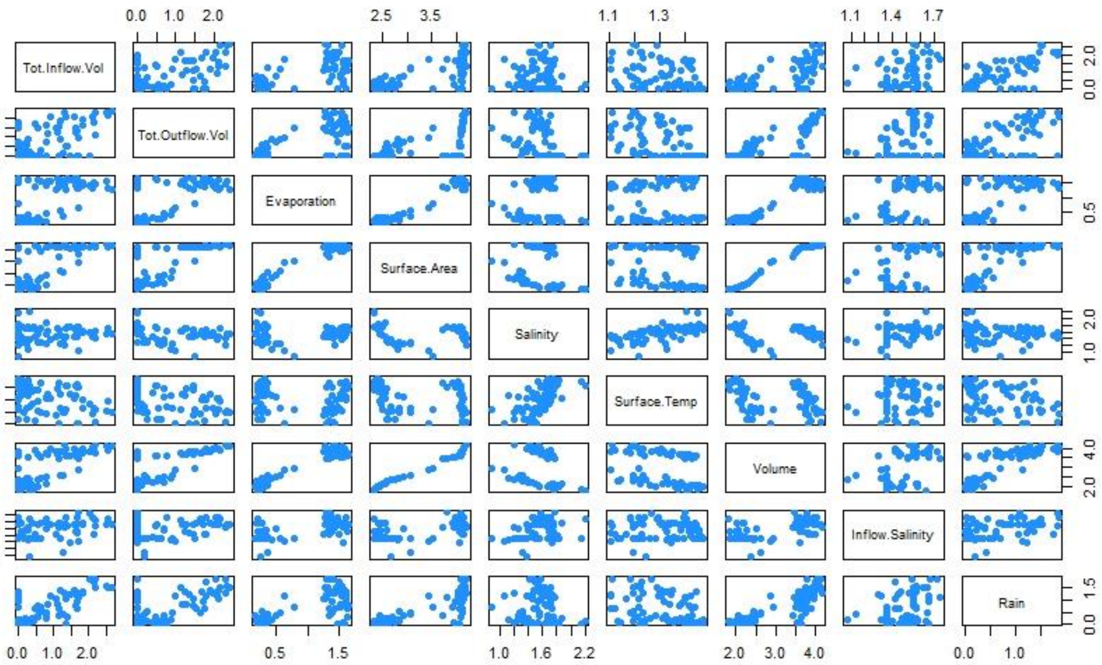


New lagoons with G02 **B**



Old lagoons (BPI and FRA)

C



Presence of low-permeability layers (M03, L04, FRA, BPI)

D

Absence of low-permeability layers (G02, L01)

E

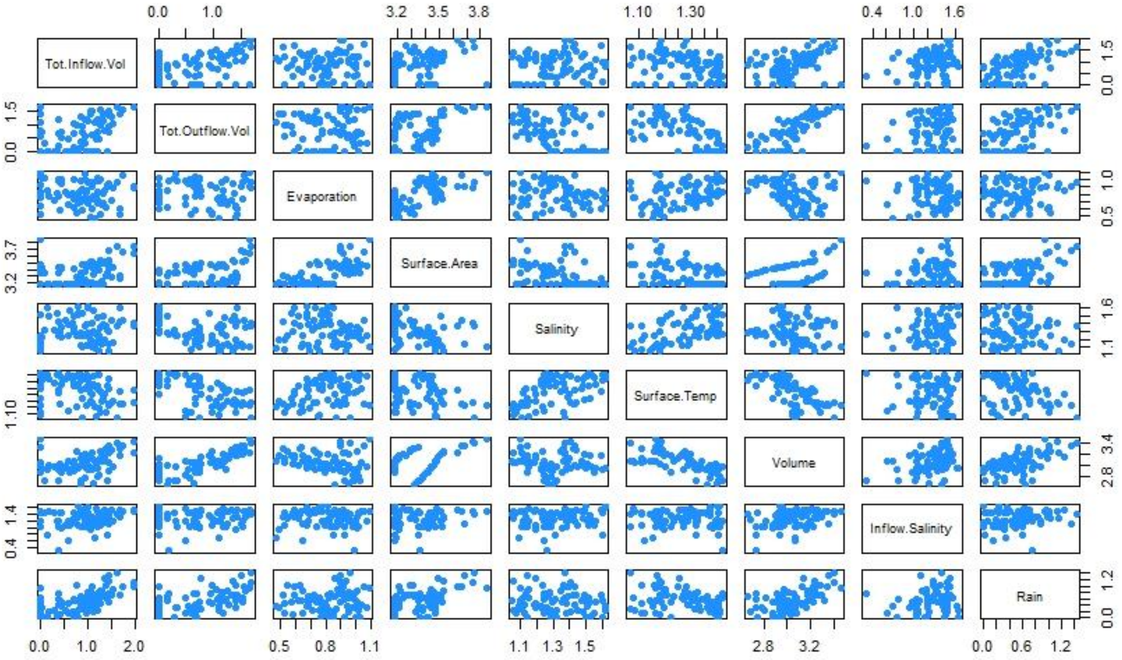


Figure S1.2. Scatterplots of all the variables associated with variables that influence volume and salinity levels for grouped lagoons according to new (L01, L04, M03), new with G02, old (BPI, FRA), presence of low-permeability layers (M03, L04, FRA and BPI) and absence of low-permeability layers (G02, L01).

7.2. Planktonic response to pulse or continuous inorganic nutrient inputs. Part 1: Temporal variations and monitoring implications

Table of contents:

Supplementary material pertaining to the ANOVA Post Hoc comparison using Tukey's HSD test are stored in pdf formats and are listed the same as it is listed in the link below:

https://figshare.com/articles/thesis/Chapter_2_ANOVA_Analysis/24168258

The separate pdf tables are also available on request.

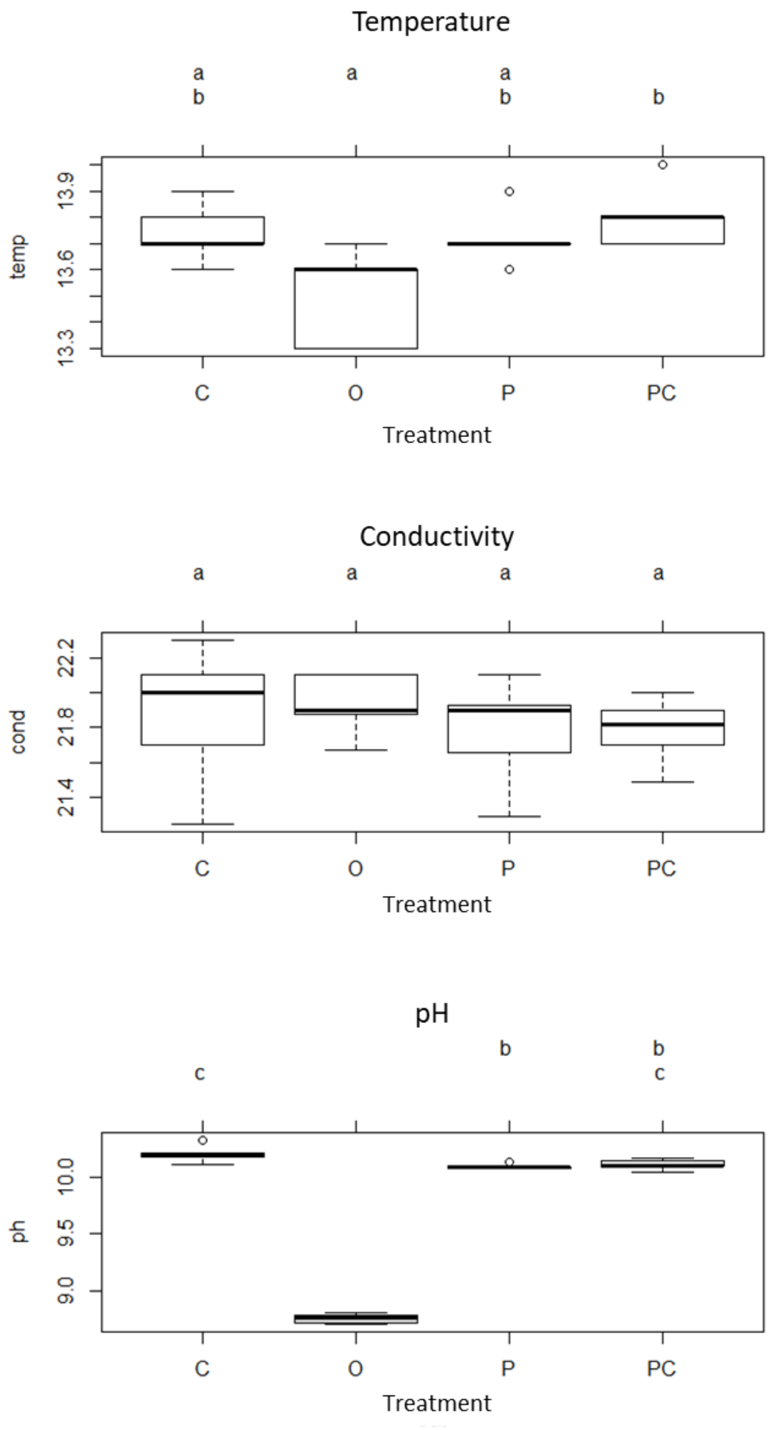


Figure S2.1. Differences between the mesocosm treatments on the final day for temperature, conductivity and pH using one-way ANOVA.

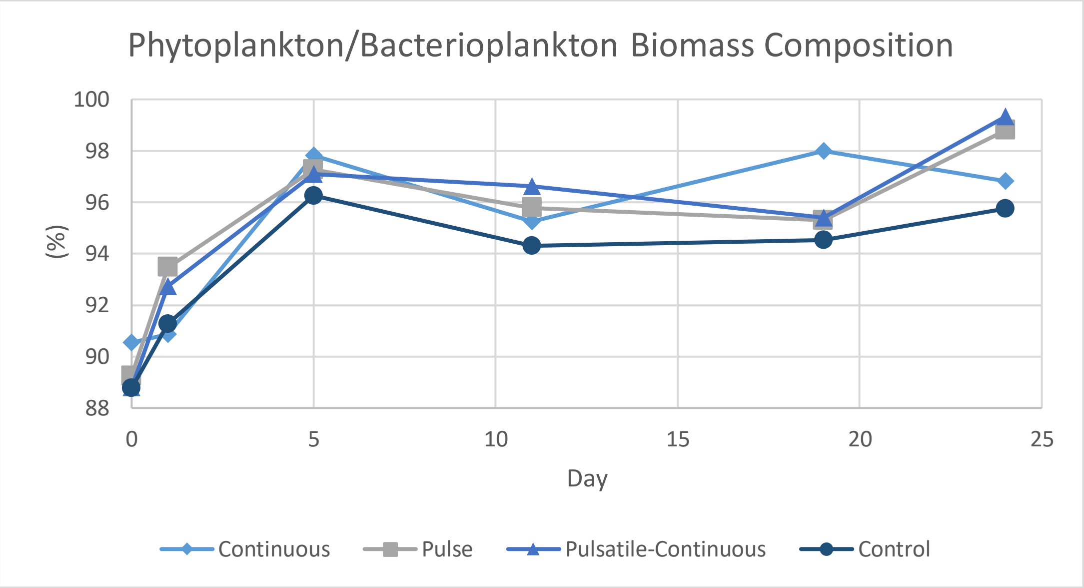


Figure S2.2. Phytoplankton over Bacterioplankton biomass composition expressed as a percentage.

Table S2.1. ANOVA analysis summary of bacterioplankton biomass according to treatments and sampling days.

ANOVA – Bacterioplankton Biomass

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	5.82e+11	3	1.94e+11	12.95	< .001
Day	4.67e+12	5	9.34e+11	62.26	< .001
Treatment ✻ Day	1.47e+12	15	9.80e+10	6.54	< .001
Residuals	1.44e+12	96	1.50e+10		

Table S2.2. Post Hoc comparison using Tukey’s HSD test for bacterioplankton biomass according to treatment and day (double-click the pdf icon to access the table).

Bacterioplankton
Biomass ANOVA

Table S2.3. ANOVA analysis summary of phytoplankton biomass according to treatments and sampling days.

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Day	6.45e+15	5	1.29e+15	235.5	< .001
Treatment	1.55e+15	3	5.16e+14	94.2	< .001
Day ✻ Treatment	2.32e+15	15	1.55e+14	28.2	< .001
Residuals	5.26e+14	96	5.48e+12		

Table S2.4. Post Hoc comparison using Tukey’s HSD test for phytoplankton biomass according to treatment and day (double-click the pdf icon to access the table).

Phytoplankton
Biomass ANOVA

Table S2.5. ANOVA analysis summary of nitrate uptake according to treatments and sampling days.

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	6.624	3	2.20815	639	< .001
Day	9.676	4	2.41900	700	< .001
Treatment ✻ Day	9.836	12	0.81970	237	< .001
Residuals	0.277	80	0.00346		

Table S2.6. Post Hoc comparison using Tukey's HSD test for nitrate uptake according to treatment and day (double-click the pdf icon to access the table).

Nitrate Uptake
ANOVA

Table S2.7. ANOVA analysis summary of phosphate uptake according to treatments and sampling days.

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Day	0.23362	4	0.0584	601	< .001
Treatment	0.15056	3	0.0502	516	< .001
Day ✻ Treatment	0.24860	12	0.0207	213	< .001
Residuals	0.00778	80	9.72e-5		

Table S2.8. Post Hoc comparison using Tukey’s HSD test for phosphate uptake according to treatment and day (double-click the pdf icon to access the table).

Phosphate Uptake
ANOVA

Table S2.9. ANOVA analysis summary of growth rate for bacterioplankton according to treatments and sampling days.

	Sum of Squares	**df**	**Mean Square**	**F**	**p**
Treatment	1.1629	3	0.388	1476	< .001
Day	9.3213	4	2.330	8874	< .001
Treatment ✻ Day	1.3880	12	0.116	440	< .001
Residuals	0.0210	80	2.63e-4		

Table S2.10. Post Hoc comparison using Tukey’s HSD test for growth rate for bacterioplankton according to treatment and day (double-click the pdf icon to access the table).

Growth Rate
Bacterioplankton ANC

7.3. Planktonic response to pulse or continuous inorganic nutrient inputs. Part 2: The effects on zooplankton community structure

Table of contents

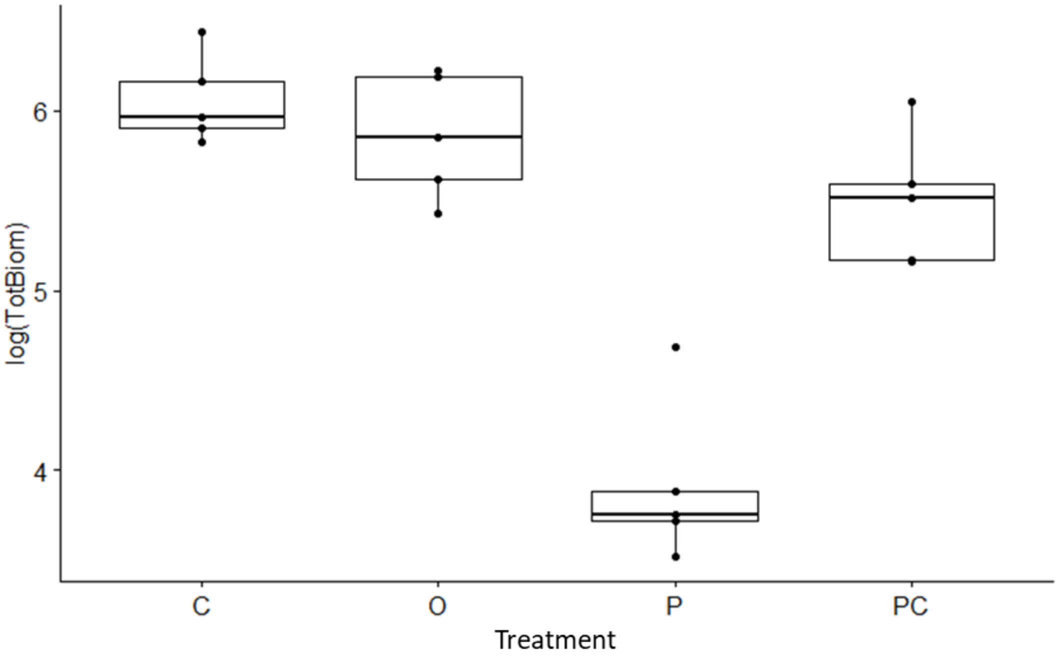


Figure S3.1. The log total biomass of zooplankton for all treatments on the final day.