Pelagic calcite precipitation in lakes: from a global to a local perspective on its drivers and implications

Hares Khan

DOCTORAL THESIS

Pelagic calcite precipitation in lakes: from a global to a local perspective on its drivers and implications

Precipitació pelàgica de calcita en llacs: una perspectiva local i global a les implicacions i determinants

UNIVERSITAT DE BARCELONA

Department of Evolutionary Biology, Ecology and Environmental Sciences

Thesis for applying for the degree of Doctor of Philosophy in Ecology, Environmental Sciences and Plant Physiology

Kaltsiidi avaveeline sadenemine: põhjused ja tagajärjed globaalses ja kohalikus vaates

Eesti Maaülikool
Estonian University of Life Sciences

Institute of Agricultural and Environmental Sciences

Thesis for applying for the degree of Doctor of Philosophy in Environmental Sciences and Applied Biology

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Areuse, Switzerland, June 2021

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According to the agreement between the University of Barcelona and the Estonian University of Life Sciences for the joint supervisions of the doctoral thesis, signed in May-June 2018, the thesis of Hares Khan has been accepted by the Doctoral Committee for Environmental Sciences and Applied Biology of the Estonian University of Life Sciences and by the Doctoral Committee for Philosophy in Ecology, Environmental Sciences and Plant Physiology of the University of Barcelona.

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Defense of the thesis: September 2021 in University of Barcelona

The Catalan and Estonian in the current thesis were edited by Biel Obrador and Alo Laas.

The publication of this thesis is supported by MANTEL (Management of Climatic Extreme Events in Lakes & Reservoirs for the Protection of Ecosystem Services), a project within the European Union's Horizon 2020 research and innovation program.

ISSN: 2382-7076
ISBN: 978-9949-698-92-9 (pdf)

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Acknowledgments

A journey is always enriching in one way or another, no matter what destination. A journey is made of people, places, learning experiences, struggle, ease, and emotions. It has now been four years since I enrolled in my PhD, and it has proven to be a real journey which I will remember and cherish for a lifetime. I will be ever thankful to the people for what we shared, the places for what they revealed to me, and the experiences for what they taught me. And my deepest gratitude is to the One who arranged and eased this journey and placed therein benefit and knowledge.

During these four years, I had the privilege to be introduced to people whose company has benefitted me immensely. I believe that knowledge and skills are transmitted rather than acquired. I consider myself highly fortunate to have been surrounded by experts in their research fields who were able to transmit to me their expertise as well as their enthusiasm for knowledge and research. I feel honored to have been guided throughout my work by my two supervisors, Biel and Alo. This is by no means a statement of formality, rather, it reflects my sincere appreciation for their constructive and efficient tutelage. Their role has been beyond mere supervision and has extended to what I would call friendship, as they have introduced me to their families and have helped me in all kinds of situations, be it work related or not. It therefore seems to me as the most natural thing to thank them first and foremost. Among them, I would like to include Rafa. Even though he is not officially my supervisor, he has followed my work very closely and has given invaluable inputs of help and knowledge. Each one of them are amazing scientists and personalities. But the magic truly happens when you have all three of them sitting in the same room or on a boat… The science produced during such moments is just as brilliant as the humour.

This PhD is part of the MANTEL ITN project (Management of Climatic Extreme Events in Lake and Reservoirs for the Protection of Ecosystem Services) that gathers experts and Early-Stage Researchers (ESRs) from almost every continent under the same banner: Extreme Events! It is true that we had some extremely fun events! Every now and then we would meet somewhere in the middle of nowhere, for some intensive science training, to sing songs around a campfire, have some nature walks, and enjoy being part of an awesome team. I'm grateful to have sipped on Julio's maté and to have passed it over to Mike, to have hosted Elias, Ewan, Chloé and Maggie, to have had Elias accompanying me and helping me out on several intensive field work campaigns in Sau reservoir, to have Ana troubleshooting my R codes, to have Harriet laughing at my jokes when no one else did, to have Nasime in Barcelona and Estonia during our secondments, to have Jorrits as the genius of the team, to have Truls helping everyone out at the MANTEL meeting in Germany, and to have Alex and Qing joining us on board after having cruised with the MANTEL crew for over a year. That is a heck of a crew! And the boat would have never reached the shore had it not been led by the best captain. Who else could this be other than Eleanor? She is much more than our project coordinator. She has led the project to success and has always shown great care for the well-being and motivation of the ESRs. And at her right and left hands were Alec, Kevin and Gosia who were managing the project with efficiency. I owe a big thanks to the whole MANTEL crew, ESRs, coordinators and experts, for the awesome times we shared together and the quality training we received.

When I started my PhD, I arrived in Barcelona, not knowing much of Spanish and Catalan, and not having any friends or acquaintances. As soon as I stepped into my University Institute, all my worries had dissipated. The welcome I received was warmer than a hot summer day in Barcelona. The first person who comes to my mind here is Silvia. Her sheer kindness put me at ease right from the beginning. I really miss our coffee and lunch breaks with her, Astrid, the two Pol's, Alba, Rebecca, Dani, Veronica, Aida, Rebeca, Cesc, Miguel, Yaiza, Nuria, Andrea, Pau, Neus… Silvia helped me in many matters, and she even accompanied me for field work in Sau reservoir. I also got help from Veronica who analyzed some of my samples along with her batch. These were my first friends in Barcelona University and many of them finished their PhD and dispersed in different places. But then came Amani, the two Laura's and Marta. Amani also came with me to help for field work in Sau reservoir. I enjoyed his company as much as I enjoyed the deep discussions we had. All these people and those who are not mentioned here made it feel like a family. I am thankful to each and everyone of them for what we shared and for making me feel at home in my new home, Barcelona.

My secondment in Estonia lasted a total of eight months. This time was unforgettable, partly due to the people I got to know there. I am still amazed by the hospitality that Alo and his family showed me. I thoroughly enjoyed their company, the local food they were making me taste, and the Estonian culture they were making me discover such as sauna and jumping over the fire at Jaanipäev, even though this nearly ended in a disaster due to my limited jumping skills. I thank them all wholeheartedly for their kindness. At work, I was lucky to share my office with Anu and Ronald. And whenever I needed a break, Burak, Upendra and Nasime were just next door. I was always happy to be able to speak some French with Fabien and to get some of his advice for my research. Every two weeks the whole department would meet at Võrtjärv research center for presentations with coffee and cake. It was always inspiring to listen to those presentations, I always felt integrated and welcome. Therefore, I extend my thanks to the entire department of Limnology.

And then was my one-month secondment in Ireland which turned out to be only two weeks due to the covid-19 outbreak. I wish it had been longer because the place is just as beautiful and charming as its people. I was warmly welcomed by Elvira, her family, and the whole team at the Marine Institute in Mayo. We would always have the 10 o'clock coffee, lunch and 4 o'clock coffee all together. And after lunch we would go for a walk in the rain because the rain loves Irish people and Irish people love the rain. From among the Marine institute team was Tadgh, my awesome flatmate. Every morning he would prepare my Irish breakfast of porridge. And later we would enjoy my vegan biryani or dahl and have endless discussions on the deeper meanings of life. People said we look so similar that they would think we were brothers. At the end of the day, that's maybe what we are. At least that's what it felt like to me. I want to give a warm thanks to Elvira, her family, Tadgh and the entire crew at the Marine Institute.

Being an ITN student enrolled in two Universities (UB and EMU) can be quite hectic to manage for administrations and secretariats. Yet, things worked out smoothly thanks to the people who worked hard behind the scenes. I want to thank the people at the secretariats of UB, Mercé, Anna, Dolors, Marisol and Andreu, and of the secretariats of EMU, Lilian, Diana and Arvi. I also want to thank the committee who has monitored my work, Elvira, Marisol and Rafa, as well as Teresa who is in charge of the Doctoral affairs at our department at UB.

Last but not least, behind every successful venture is the guidance, support, love and inspiration of a family. My father, Saad, who is himself an expert in water related sciences would happily get involved in my work and even accompanied me on field work in several freshwater bodies in Pakistan for the side project named "FunAqua". The support I got from my father, my mother, Brigitte, and my brother, Rehan, is like that of a charger to its phone. No matter the phone model or the number of apps it has, it simply won't work without its charger. Therefore, I owe this thesis to them, with love and gratitude.

This study was funded by MANTEL ITN (Management of climatic extreme events in lakes and reservoirs for the protection of ecosystem services) through European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 722518.

Supervisors' report

Dr. Biel Obrador Sala, Associate Professor at the Department of Evolutionary Biology, Ecology and Environemntal Sciences of the Univeristy of Barcelona, and Dr. Alo Laas, Senior Researcher at Estonian University of Life Sciences as supervisors of the PhD thesis entitled "Pelagic calcite precipitation in lakes: from a global to a local perspective on its drivers and implications",

REPORT

that the research developed by Mr. Hares Khan in his Doctoral Thesis has resulted in three scientific publications already published or in press:

Khan, H., Marcé, R., Laas, A. Obrador, B. 2021. The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs. Limnetica *in press* DOI: 10.23818/limn.41.02

The 2019 Journal Impact Factor of *Limnetica* in Web of Science was 0.918. The journal is ranked in Q3 in the category *Marine & Freshwater Biology*.

Khan, H., Laas, A., Marcé, R., Sepp, M., Obrador, B. 2021. Eutrophication and Geochemistry Drive Pelagic Calcite Precipitation in Lakes. Water 13(5): 597. DOI: 10.3390/w13050597

The 2019 Journal Impact Factor of *Water* in Web of Science was 2.544. The journal is ranked in Q2 in the category *Water Resources*.

Khan, H., Laas, A., Marcé, R., Obrador, B. 2020. Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes. Ecosystems 23: 1566-1580. DOI: 10.1007/s10021-020-00488-6

The 2019 Journal Impact Factor of *Ecosystems* in Web of Science was 4.207. The journal is ranked in Q1 in the category *Ecology*.

We also certify that Mr. Hares Khan has actively participated in all research tasks involved in these publications: setting the objectives and developing the research hypotheses, executing fieldwork, analyses and experiments, conceiving and performing the data handling and statistical analyses, analysing the results, and writing the manuscripts.

Finally, we certify that none of the co-authors of the publications that conform this doctoral thesis, has used or will use these results as part of any other PhD thesis.

Barcelona, 20th May 2021

Dr. Biel Obrador

Universitat de Barcelona

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General summary

Inland waters are relevant components of the global carbon cycle acting as hot spots for biogeochemical processes by which carbon is stored, transformed, and outgassed to the atmosphere. Therefore, less than half of the carbon that is exported from terrestrial ecosystems to inland waters finally reaches the oceans. Lakes and reservoirs have the ability to store and emit carbon in large quantities, comparable in magnitude to other major global carbon fluxes such as fossil fuel combustion, the oceanic carbon dioxide (CO_2) sink and the continental carbon sink. Substantial scientific studies in the last two decades have attempted to quantify these fluxes and identify their drivers so that inland waters are accounted for in carbon budgets and Earth System models.

Much of these studies have given prevalence to organic processes in determining carbon fluxes in lakes. Organic carbon burial has been described as the main form of the sedimentary carbon sink of lakes. Similarly, mineralization of organic carbon resulting from aquatic net heterotrophy has been identified as the main driver controlling CO_2 emissions to the atmosphere. Hence, global carbon budgets and lake models have mostly accounted for the organic components of the carbon cycle in lakes, assuming that inorganic processes such as dissolved inorganic carbon (DIC) reactions or calcium carbonate precipitation play a negligible role. However, geological evidence from sedimentary cores shows that sedimentary carbonates act as a long-term carbon sink in lakes. Furthermore, recent studies suggest that calcite precipitation may be a relevant source of the CO_2 emissions from lakes and that calcite precipitation can have important effects on the DIC equilibria. Yet, calcification, its resulting storage in the sediments, and its associated CO_2 emissions have never been quantified at a global scale. Therefore, the relative contributions of organic to inorganic processes in the carbon budget of lakes is unclear. This stresses the need for a global estimation of this process.

Calcite precipitation is acknowledged to be promoted by primary production through several mechanisms. However, the relationship between primary production and calcification has not been clearly described in freshwaters. Hence, the drivers of calcite precipitation are still unclear. Understanding the drivers of calcite precipitation is needed to be able to parameterize this process for a proper inclusion of inland waters' carbon cycle in Earth System Models. This is particularly relevant in lake models because calcification can cause discrepancies between empirical data and theoretical models that rely solely on metabolic processes for assessing lake carbon fluxes.

The main objective of this thesis is to understand the relevance of calcite precipitation, its drivers, and implications from a global to a local scale on lakes. The main findings are divided into three chapters, each corresponding to a published journal article. The first journal article aims at assessing the global relevance of pelagic calcification and its associated CO_2 emissions in lakes by providing a first estimate of these fluxes at a global scale. This estimate is based on the relationship between calcification rates, reported in the literature, and water alkalinity. The main findings suggest that pelagic calcification in lakes is a globally relevant process, similar in magnitude to organic carbon burial in lake sediments. Its associated CO_2 emissions, however, are minor due to the buffering effect of the carbonate equilibria. Furthermore, pelagic calcification can potentially occur in lakes of alkalinity above 1 meq L^{-1}, representing more than half of the surface area covered by lakes and reservoirs globally. The importance of lake pelagic calcification stresses the need to understand its drivers so that this process can be parameterized and accounted for in lake models and carbon budgets of inland waters. This is addressed in the second journal article that combines *in situ* incubation experiments with high frequency data, obtained from automatic monitoring stations in several European lakes, to understand the drivers of pelagic calcification. A strong relationship between calcification, Net Ecosystem Production (NEP) and calcite saturation was identified, whereby the calcification to NEP

ratio is strongly determined by calcite saturation in water. The effect of NEP on calcification acts at a short temporal scale, whereby an increase in NEP induces calcification. Yet, the efficiency of NEP to induce calcification at a short temporal scale depends on calcite saturation which follows variations at a longer temporal scale, usually reaching its highest values during the summer season. The relationship between the calcification to NEP ratio and calcite saturation can be used as a parameterization for calcite precipitation in lakes and reservoirs. Furthermore, this relationship can also explain the imbalances between inorganic carbon dynamics and lake metabolism often observed in lakes. The third journal article identifies such imbalances in eight Estonian lakes, where large deviations from the metabolic 1:1 stoichiometry between dissolved oxygen (DO) and DIC can occur in lakes of higher alkalinity. The main findings in this article suggest that these deviations are caused by calcification and can be modelled using the calcification to NEP ratio.

This thesis provides a first global estimate of pelagic calcite precipitation and its associated CO_2 release and offers a first parameterization of calcification that can be used to account for calcification in lake carbon budgets. These results should be considered as a stepping stone to be further built upon. Furthermore, this work stresses the need to adjust our current understanding of lake carbon cycling by accounting for organic processes along with inorganic processes such as calcite precipitation in the carbon budgets and models of lacustrine ecosystems.

General summary in Catalan

Les aigües continentals són un component rellevant del cicle global del carboni pel seu paper com a punts calents de processos biogeoquímics mitjançant els quals el carboni s'emmagatzema, es transforma i s'emet cap a l'atmosfera. Com a resultat, menys de la meitat del carboni que s'exporta des dels ecosistemes terrestres a les aigües continentals arriba finalment als oceans. Els llacs i embassaments tenen la capacitat d'emmagatzemar i emetre carboni en grans quantitats, comparables en magnitud a d'altres fluxos globals de carboni com la crema de combustibles fòssils, l'embornal de diòxid de carboni (CO_2) oceànic o l'embornal continental de carboni.

La intensa recerca duta a terme en les darreres dues dècades ha quantificat aquests fluxos i identificat els principals factors determinants per tal d'incorporar les aigües continentals als balanços globals de carboni i facilitar la seva inclusió als models del sistema Terra. Gran part d'aquests treballs han donat prevalença als processos orgànics com a determinants dels fluxos de carboni en llacs. L'enterrament de carboni orgànic s'ha descrit com la forma principal de l'embornal sedimentari de carboni en aquests sistemes. Alhora, la mineralització de carboni orgànic resultant de l'heterotròfia aquàtica neta s'ha identificat com el principal factor determinant de les emissions de CO_2 a l'atmosfera des de llacs i embassaments. En definitiva, els balanços globals de carboni i els models lacustres s'han basat principalment en els components orgànics del cicle del carboni, assumint que els processos inorgànics tals com les reaccions del carboni inorgànic dissolt (DIC) o la precipitació de carbonat de calci tenen un paper negligible. No obstant això, l'evidència geològica en testimonis de sediment mostra que en llacs els carbonats actuen com un embornal de carboni a llarg termini. A més, estudis recents suggereixen que la precipitació de calcita pot ser una font rellevant del CO_2 emès des de la superfície de llacs i embassaments, alhora que la precipitació de calcita pot tenir un efecte important sobre els equilibris del DIC. No obstant això, la calcificació, l'emmagatzemament resultant de carboni als sediments, i les emissions associades de CO_2, no han estat mai quantificades a escala global. Per tant, es desconeix la contribució relativa dels processos orgànics i inorgànics en el balanç de carboni dels llacs. Això posa de manifest la necessitat d'una estima global d'aquest procés.

La producció primària promou la precipitació de calcita a través de diversos mecanismes, si bé la relació entre producció primària i calcificació no ha estat clarament descrita en aigües dolces. Comprendre els determinants de la precipitació de calcita en llacs és un pas necessari per poder parametritzar aquest procés i incloure adequadament els fluxos de carboni en aigües continentals en els models de sistema Terra. Això és particularment rellevant en la modelització de llacs perquè la calcificació pot causar discrepàncies entre les dades empíriques i els models teòrics que només contemplen processos metabòlics per avaluar els fluxos de carboni en aquests sistemes.

L'objectiu principal d' aquesta tesi és comprendre la rellevància de la precipitació de calcita en llacs, els seus determinants i les implicacions, tant a escala global com local. Les principals conclusions es divideixen en tres capítols, cadascun corresponent a un article publicat en una revista. El primer article té com a objectiu avaluar la rellevància global de la calcificació pelàgica en llacs i les emissions de CO_2 associades, proporcionant una primera estima d'aquests fluxos a escala global. Aquesta estima es basa en la relació entre taxes de calcificació reportades a la literatura i alcalinitat de l'aigua. Els resultats suggereixen que la calcificació pelàgica lacustre és un procés rellevant a escala global, similar en magnitud a l'enterrament de carboni orgànic als sediments dels llacs. Les emissions de CO_2 associades a la calcificació, però, són de menor importància a causa de l'efecte tamponador dels equilibris del DIC. La calcificació pelàgica té lloc potencialment en llacs d'alcalinitat superior a 1 meq L-1, que representen més de la meitat de la superfície global de llacs i embassaments. La importància del la calcificació pelàgica en llacs posa de manifest la necessitat d'entendre'n els factors que la determinen per tal de poder parametritzar aquest procés i incloure'l en els balanços de carboni de les aigües continentals. Aquest tema s'aborda al segon article, que combina experiments basats en incubacions *in situ* amb dades d'alta freqüència obtingudes per estacions automàtiques de monitoreig en diversos llacs europeus, per tal d'entendre els factors que determinen la calcificació pelàgica. Es va identificar una forta relació entre calcificació, producció neta ecosistèmica (NEP) i saturació de calcita, estant el quocient entre calcificació i NEP determinat en gran mesura per la saturació de calcita a l'aigua. L'efecte de la NEP sobre la calcificació es dóna a una escala temporal curta, de manera que un augment de la NEP indueix la calcificació. Tot i així, l'eficiència amb què la NEP indueix la calcificació depèn de la saturació de calcita, que en canvi segueix variacions a una escala temporal més llarga, generalment assolint els valors màxims durant l'estiu. La relació entre el quocient calcificació/NEP i la saturació de calcita pot servir de base per a una parametrització de la precipitació de calcita en llacs i embassaments. A més, aquesta relació també pot explicar els desequilibris sovint observats entre la dinàmica del carboni inorgànic i el metabolisme lacustre. El tercer article analitza aquests desequilibris en vuit llacs d'Estònia, on s'observen grans desviacions de la estequiometria metabòlica 1:1 entre oxigen dissolt (DO) i DIC en els llacs de major alcalinitat. Les principals conclusions d'aquest article suggereixen que aquestes desviacions són causades per la calcificació i que es poden modelitzar mitjançant el quocient calcificació/NEP.

Aquesta tesi proporciona una primera estima global de la precipitació pelàgica de calcita i la conseqüent emissió de CO_2 i ofereix una primera parametrització de la calcificació, que es pot utilitzar per incloure aquest flux en els balanços de carboni en llacs. Els resultats constitueixen un punt de partida sobre el qual aprofundir en aquest tema. Aquest treball ressalta la necessitat de millorar la comprensió actual del cicle del carboni tenint en compte tant processos orgànics com processos inorgànics com la precipitació de calcita, en els models i balanços de carboni dels sistemes lacustres.

General summary in Estonian

Siseveekogud on ülemaailmse süsinikuringe olulised komponendid. Neis toimuvate biogeokeemiliste protsesside tulemusena süsinik kas salvestub, muundub või vabaneb atmosfääri, mistõttu alla poole maismaaökosüsteemidest siseveekogudesse sattuvast süsinikust jõuab lõpuks maailmamerre. Järvedel ja veehoidlatel on võime akumuleerida ja/või emiteerida süsinikku suures koguses, mis on võrreldav teiste suuremate ülemaailmsete süsinikuvoogudega nagu näiteks fossiilkütuste põletamine ja süsihappegaasi (CO_2) neeldumine maailmameres või maismaal. Viimase kahe aastakümne jooksul tehtud olulistes teadusuuringutes on püütud nende voogude suurust hinnata ja tuvastada neid mõjutavaid tegureid, mille tulemusena arvestatakse tänastes globaalsetes mudelites juba ka siseveekogude süsinikuvooge.

Suurem osa neist uuringutest leiab, et peamiselt mõjutavad järvede süsinikuvooge just orgaanilise ainega seotud protsessid. Nii peetakse orgaanilise süsiniku väljasettimist järvede peamiseks süsiniku setteneeluks. Samamoodi nagu järvede CO_2 emissiooni peamiseks põhjustajaks on osutunud orgaanilise süsiniku heterotroofne lagundamine. Nõnda on ülemaailmsetes süsinikubilansi- ja järvemudelites peamiselt arvestatud orgaanilise süsinikuringe komponente, eeldades et anorgaanilistel protsessidel nagu näiteks lahustunud mineraalse süsiniku (DIC) muundumine või kaltsiumkarbonaadi sadenemine, on tähtsusetu roll. Samas geoloogilised setteuuringud näitavad, et sette karbonaatides seotud süsinik jääb ringest välja väga pikaks ajaks. Hiljutised uuringud viitavad ka, et veesambast välja sadenenud katsiidil on veekogus oluline mõju DIC tasakaalule. Siiski ei ole veekogude lupjumist, kaltsiidi ladestumist setetes ja sellega seotud CO_2 emissioonide suurust kunagi ülemaailmselt hinnatud, mistõttu on järvede süsinikubilansi orgaaniliste ja anorgaaniliste protsesside suhteline osakaal ebaselge. Sellest tuleneb tungiv vajadus mineraalse süsinikuga seotud protsesse globaalselt hinnata.

On teada, et kaltsiidi sadenemist veekogudes soodustab primaarproduktisoon erinevate mehhanismide kaudu. Samas pole lubjastumise ja primaarproduktsiooni vahelisi seoseid mageveekogudes seni selgelt kirjeldatud, mistõttu on ka kaltsiidi sadestumise põhjused endiselt ebaselged. Kaltsiidi sadestumist põhjustavate tegurite mõistmine ja väljaselgitamine on aga vajalik selle protsessi parameetrimiseks, et siseveekogude süsinikuringet nõuetekohaselt kaasata globaalsetesse mudelitesse. Seda on väga oluline arvestada ka järvemudelites, kuna lupjumine võib põhjustada lahknevusi empiiriliste andmete ja teoreetiliste mudelite vahel, mis tuginevad järve süsinikuvoogude hindamisel ainult metaboolsetele protsessidele.

Selle töö põhieesmärk on mõista kaltsiidi väljasademise olulisust, seda põhjustavaid tegureid ja tagajärgi järvedele nii globaalses kuni kohalikus mõõtmes. Töö peamised tulemused on jagatud kolme peatükki, millest igaüks vastab teadusajakirjas avaldatud artiklile. Esimese artikli peamiseks eesmärgiks oli hinnata pelaagilise lupjumise olulisust ja sellega seotud süsihappegaasi lendumist järvedes ning anda esialgne hinnang nendele protsesside globaalsele tähtsusele. Antud hinnang põhineb seni kirjanduses avaldatud lubjastumise määradest ja veekogude aluselisusest. Töö tulemused viitavad, et pelaagiline lupjumine järvedes on globaalses mastaabis oluline protsess ja on samas suurusjärgus orgaanilise süsiniku väljasettimisega järvedes. Lupjumisest tulenevad süsihappegaasi heitkogused on tänu järvede karbonaatsel tasakaalul põhinevale puhverdusvõimele siiski väike. Pelaagiline lupjumine saab tekkida vaid üle 1 mekv/l aluselisusega mageveekogudes, kuhu kuuluvad enam kui pooled maailma järved ja veehoidlad. Järvede pelaagilise lupjumise tähtsus rõhutab vajadust mõista selle protsessi liikumapanevaid tegureid, mis omakorda võimaldaks seda protsessi ka parameetrida ning arvestada järvemudelites ja süsinikuringe uuringutes. Nende tegurite olulisust käsitletakse antud töö teises artiklis, kus pelaagilise lupjumise põhjusi uuritakse mitmetes

Euroopa järvedes tehtud *in situ* inkubeerimiskatseid ja automaatsetest seirejaamade mõõtmisandmeid analüüsides. Töö tulemusena tuvastati oluline seos lubjastumise, ökosüsteemi puhastoodangu (NEP) ja kaltsiidi küllastustaseme vahel. Leiti, et lubjastumise ja NEP-i omavaheline suhe määrab kaltsiidi küllastustaseme vees. NEP mõjub lupjumisele lühiajalises skaalas ja võimendab seda. NEP-i efektiivsus lubjastumise indutseerimisel sõltub aga kaltsiidi küllastustasemest, mis saavutab oma tipu tavaliselt suvel. Seost lubjastumise ja NEP omavahelise suhte ning kaltsiidi küllastustaseme vahel saab kasutada kaltsiidi väljasadenemise hindamiseks järvedes ja veehoidlates. Lisaks aitab see seos seletada sageli täheldatud tasakaalu puudumist mineraalse süsiniku dünaamika ja järvede ainevahetuse vahel. Kolmas teadusartikkel tuvastab sellise tasakaalu puudumise kaheksas Eesti järves, kus kõrgema aluselisusega järvedes võivad esineda suured kõrvalekalded metaboolsest 1:1 stöhhiomeetriast, lahustunud hapniku ja DIC vahel. Selle artikli peamised järeldused viitavad sellele, et need kõrvalekalded on tingitud peamiselt lubjastumisest ja et neid on võimalik modelleerida kasutades lubjastumise ja NEP omvahelist suhet.

See väitekiri annab esmase üldhinnangu kaltsiidi pelaagilisele väljasadenemisele ja sellega seotud süsihappegaasi eraldumisele ning toob välja tegurid, mida saab kasutada lubjastumise arvestamiseks järve süsinikubilansi hindamisel. Neid tulemusi saab pidada hüppelauaks edasistesse teadusuuringutesse. Käesolevas töös rõhutatakse ka vajadust kohandada meie praegust arusaama järve süsinikuringest, et arvestada orgaanilisi süsinikuga seotud protsesside kõrval ka mineraalse süsinikuga toimuvat, näiteks kaltsiidi väljasadestumist, mis on oluline nii mageveeökosüsteemi süsinikubilansi hindamisel kui ka erinevates järve ainevahetuse mudelites.

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1. General Introduction

1.1 Inland waters and the global carbon cycle

While there are 117 million lakes on Earth, they cover only a small fraction (~3%) of the Earth's continental surface (Verpoorter et al., 2014). Yet, lakes profoundly shape human communities by providing key ecosystem services as a source of drinking water and food (irrigation, fisheries, and aquaculture), a means of transportation, a source of energy (hydropower), a source of well-being and recreation that can promote local tourism (Sterner et al., 2020). Lakes also provide diverse habitats, support high levels of biodiversity and other vital ecosystem services such as nutrient cycling, flood control, pollution dilution and climate regulation (Grizzetti et al., 2019; Sterner et al., 2020). Besides providing numerous direct benefits, lakes are important components of biogeochemical cycles, acting as active hotspots of biogeochemical processes (Cole et al., 2007). Increasing interest has been given to their important role in carbon cycling in the last two decades.

Initially, inland water carbon cycling studies focused on lakes as isolated ecosystems. Later, lakes were considered as recipients of imported carbon, thereby expanding the scale of research to their watershed, accounting for interactions between terrestrial ecosystems and inland waters. Recent studies, however, describe lakes as significant players in the global carbon cycle, focusing on fluxes in and out of lakes and exchanges with other inland waters, terrestrial ecosystems, the atmosphere and the sediments (Tranvik et al., 2018). In this view, lakes and more generally inland waters, are important sites that receive, process, emit and store carbon in quantities that are significant on a global scale (Tranvik et al., 2018). The collective contribution of inland waters to global carbon fluxes is substantial in comparison to terrestrial and marine ecosystems, whereby the combined emissions and burial of carbon from inland waters are similar in magnitude to the total global net ecosystem production (Battin et al., 2009; Tranvik et al., 2009). Furthermore, global organic carbon (OC) burial rates in inland water sediments have been estimated between 0.2 to 1.6 Pg C yr^{-1}, exceeding the carbon burial rates of the entire oceanic sediment floors (0.2 Pg C yr^{-1}) (Mendonça et al., 2017). Hence, the traditional representation of the carbon cycle must integrate these fluxes by accounting for all vertical and lateral fluxes of carbon over regional and global scales for appropriately managing CO_2 and climate change mitigation (Battin et al., 2009).

The "active pipe" hypothesis that was first advanced by Cole et al. (2007) is widely accepted and suggests that lakes, streams, rivers and other inland waters are active hotspots of biogeochemical processes, acting as a major component of the global carbon cycle. Because of these biogeochemical processes, only a fraction of the carbon that enters into rivers through soil respiration, leaching, chemical weathering and physical erosion, finally finds its way into the oceans (Aufdenkampe et al., 2011). This active pipe hypothesis is relatively recent and marks a departure from the previous school of thought where inland waters were depicted as passive pipes with a merely transportive function that drains terrestrially produced carbon to the oceans (Cole et al., 2007). This shift in paradigm has strongly influenced our understanding of the role of inland waters in the global carbon budget. Most of the carbon influx from terrestrial ecosystems is emitted from inland waters to the atmosphere in the form of CO_2 or buried in the sediments of lakes, impoundments floodplains and other wetlands (Aufdenkampe et al., 2011). Scientific research has therefore aimed at understanding carbon fluxes from inland waters and quantifying their contributions as sources of CO_2 to the atmosphere and carbon sinks in the sediments (Table 1).

Similarly, scientific efforts have aimed at quantifying methane (CH_4) emissions from different inland water components as CH_4 is also a key potential climate regulator (Tranvik et al., 2009). However, the processes that control CH_4 production are different to those of CO_2, and the high spatial and temporal variability that characterize CH_4 emissions implies extensive and intensive fieldwork and deployment of a large number of devices to be able to assess CH_4 emissions without major underestimations or overestimations (Natchimuthu et al., 2016; Wik et al., 2016).

Aquatic systems have this dual capacity of simultaneously acting as a net carbon source to the atmosphere, yet accumulating and storing sedimentary organic matter received by their terrestrial inputs (Cole et al., 2007). In this sense, inland waters are way more complex than merely transport canals that drain the continental materials to the oceans (Cole et al., 2007). The most recent estimates indicate that 1.7 to 2.7 Pg C yr^{-1} (petagram of Carbon (=10^{15} g C) per year) emigrates from continental soils to inland waters, out of which only 0.9 Pg C yr^{-1} reaches the oceans, whereas the major part, 1 Pg C yr^{-1} (2 Pg C yr^{-1} according to Raymond *et al.*, 2013a), is emitted to the atmosphere in the form of CO_2 and another fraction, 0.2 Pg C yr^{-1}, is buried in aquatic sediments of inland waters (Figure 1.1) (IPCC, 2013). This suggests that inland waters play a major role in carbon cycling and greenhouse gas emissions. Besides CO_2, CH_4 is also an important component in carbon fluxes from inland waters. Lakes, reservoirs, and rivers emit 0.13 Pg C yr^{-1} of CH_4 (equivalent to 4.85 Pg C yr^{-1} of CO_2, considering the radiative forcing of 28 of CH_4), out of which 0.02 Pg C y^{-1} (0.75 Pg C-CO_2 yr^{-1}) are emitted by streams and rivers (Stanley et al., 2016) and 0.11 Pg C yr^{-1} (4.1 Pg C-CO_2 yr^{-1}) by lakes and reservoirs (DelSontro et al., 2018). Hence, CH_4 emissions are of high relevance in the carbon emissions of inland waters. The combined emissions of CO_2 and CH_4 results in a total global carbon emission of 2.2 Pg C y^{-1} (equivalent to 5.1 Pg C y^{-1} of CO_2) (Bastviken et al., 2011; Raymond et al., 2013b; Stanley et al., 2016). Therefore, carbon emissions from inland waters are on par with other major atmospheric sources of greenhouse gases such as fossil fuel combustion and industry (9.6 ± 0.5 Pg C y^{-1}) and land use change (1.5 ± 0. Pg C y^{-1}) (Friedlingstein et al., 2019) (Figure 1.1). Furthermore, carbon fluxes from inland waters are comparable in magnitude to the oceanic CO_2 sink (2.5 ± 0.6 Pg C yr^{-1}) and the continental carbon sink (3.2 ± 0.6 Pg C yr^{-1}) (Friedlingstein et al., 2019) (Figure 1.1).

The relevance of inland waters in greenhouse gas emissions must be comprehended within the context of climate change. The three main long-lived greenhouse gases in the troposphere, that is, CO_2, CH_4, and nitrous oxide (N_2O), have increased since pre-industrialisation by 147%, 259% and 123% respectively (IPCC et al., 2013; World Meteorological Organization and Global Atmosphere Watch, 2019). This alarming raise in the concentration of these greenhouse gases stresses the need for a comprehensive understanding of their global sinks and sources for correctly monitoring their evolution and managing climate change mitigation programs accordingly.

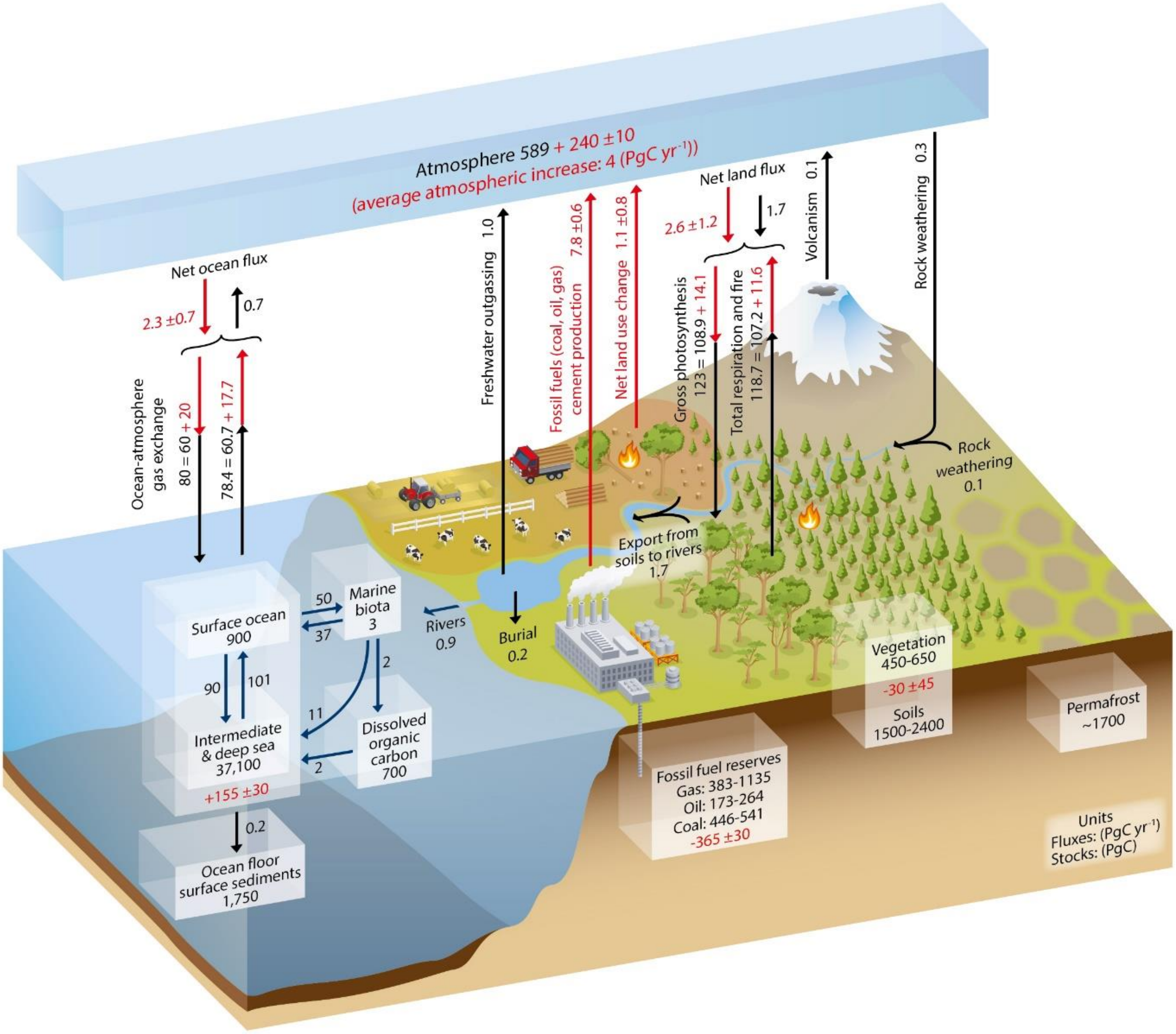

Figure 1.1. Simplified schematic of the global carbon cycle and its anthropogenic perturbations. Numbers represent reservoir mass, also called 'carbon stocks' in PgC (1 PgC = 1015 gC) and annual carbon exchange fluxes (in PgC yr^{-1}). Black numbers and arrows indicate reservoir mass and exchange fluxes estimated for the time prior to the Industrial Era, about 1750. Red arrows and numbers indicate annual 'anthropogenic' fluxes averaged over the 2000-2009 time period. These fluxes are a perturbation of the carbon cycle during the Industrial Era post 1750. Red numbers in the reservoirs denote cumulative changes of anthropogenic carbon over the Industrial Period 1750–2011. By convention, a positive cumulative change means that a reservoir has gained carbon since 1750. Uncertainties are reported as 90% confidence intervals. Figure source: (IPCC et al., 2013).

The results of these global estimations have stressed the important role of inland waters in the global carbon budget and the need to integrate their different components in local or global carbon budgets and Earth system models (Deemer et al., 2016; Holgerson & Raymond, 2016; Keller et al., 2020; Marcé et al., 2019; Mendonça et al., 2017), be it lakes, reservoirs, rivers, ponds, wetlands, or even dry inland waters (Table 1.1). It is still quite uncertain how these fluxes respond to local or global drivers such as eutrophication, land use change or climate change. It is therefore necessary that scientific research aims at understanding the processes that control carbon fluxes and the parameters that affect such processes. This thesis is essentially dedicated to the study of CO_2 emissions and inorganic carbon (IC) dynamics within lakes and reservoirs. More specifically, this thesis addresses the contributions of IC to the sedimentary carbon sink and atmospheric carbon source, through the process of calcite precipitation in lakes and reservoirs.

Table 1.1. Compilation of inland water surface areas, carbon emissions to the atmosphere and carbon storage in the sediments.

Inland water components	Surface Area x10^6 km^2	Annual carbon emissions Tg C y^{-1}	Annual CO_2 equivalent Tg $CO_{2,\ eq}$ y^{-1}	Storage in sediments Tg C y^{-1}
Lakes	3.7 - 4.5 ^{a,e,f}	70 - 860 ^{a,h,j,k}	256 - 3148	90 (organic carbon) ^{o}
Reservoirs	0.3 - 1.5 ^{c,d,e}	37 - 273 ^{b,d}	135 - 999	60 (organic carbon) ^{o}
Small ponds	0.15 - 0.86 ^{h}	19.4 - 152.5 ^{h}	71 - 558	NA
Wetlands	8.6 - 26.9 ^{g}	-1280 ^{l}	-4685	830 ^{l}
Rivers	0.3 - 0.6 ^{a,i}	1800 ^{a}	6588	NA
Dry inland waters	0.29 ^{m}	120 - 220 ^{m,n}	439 - 805	NA

Small ponds correspond to non-running water bodies with a surface area smaller than 0.001 km^2.

Uppercase letters correspond to the following references: ^{a} Raymond & Cole, 2003, ^{b} Deemer *et al.*, 2016, ^{c} Lehner *et al.*, 2011, ^{d} St. Louis *et al.*, 2000, ^{e} Downing & Duarte, 2009, ^{f} Verpoorter *et al.*, 2014, ^{g} Melton *et al.*, 2013, ^{h} Holgerson & Raymond, 2016, ^{l} Bastviken *et al.*, 2011, ^{j} Tranvik *et al.*, 2009, ^{k} Aufdenkampe *et al.*, 2011, ^{l} Mitsch *et al.*, 2013, ^{m} Marcé *et al.*, 2019, ^{n} Keller *et al.*, 2020, ^{o} Mendonça *et al.*, 2017

1.2 Organic versus inorganic drivers of dissolved inorganic carbon dynamics in inland waters

Globally, most lakes are supersaturated in CO_2 and act as carbon sources to the atmosphere (Sobek et al., 2005). Their CO_2 emissions have widely been attributed to the prevalence of net heterotrophy in aquatic systems whereby the oxidation of organic matter through the process of respiration exceeds photosynthetic production (Duarte & Prairie, 2005). This supports the hypothesis that excess CO_2 in lakes is the result of the breakdown of allochthonous OC that is originally fixed by the vegetation of terrestrial ecosystems (Del Giorgio et al., 1999). Dissolved organic carbon (DOC) has been described as the key variable that controls CO_2 partial pressure (pCO_2) in lakes (Larsen et al., 2011). Lateral exchanges of carbon between land and aquatic ecosystems are therefore considered to play a crucial role in determining the metabolic status and the gas exchanges with the atmosphere (Duarte & Prairie, 2005; Hutchins et al., 2020). Scientific research has described the fate of allochthonous OC by quantifying the amount of OC that is either stored, remineralized or passed to downstream ecosystems, and have identified key parameters that control the fate of DOC such as lake morphometry, residence time and temperature (Hanson et al., 2011; Vachon et al., 2021). Furthermore, mineralization of DOC, as a process that generates CO_2, has been seen as the key driver process controlling potential CO_2 emissions from lakes to the atmosphere (Vachon et al., 2017). At a global scale, carbon budgets have quantified OC burial but neglected the burial of IC, emphasizing the central role of OC in the carbon cycle of lakes (Mendonça et al., 2017). Hence, lake models and carbon budgets have largely relied on the assumption that OC is the main driver that controls lake carbon fluxes.

While this assumption has been given precedence in the scientific literature, recent findings have challenged the notion that net heterotrophy stands as a universal driver of CO_2 emissions in lakes and reservoirs. Most studies that directly link CO_2 supersaturation to net heterotrophy are conducted in lakes of boreal latitudes that have non calcareous lithology, relatively low alkalinity and high DOC values (McDonald et al., 2013). While this paradigm is often valid for such low-alkalinity lakes, it has recently been contested for lakes and reservoirs of higher alkalinity, which represent a large fraction of the world's lakes (Marcé et al., 2015). Cases have been reported where lakes are simultaneously in an autotrophic state, yet they emit CO_2 to the atmosphere (Bogard & Giorgio, 2016; Perga et al., 2016; Stets et al., 2009). According to a recent model, this could be the case for approximately 35% of the lakes and reservoirs in the contiguous United States (McDonald et al., 2013). In such cases, lake metabolism alone cannot explain CO_2 emissions. This may be related to the fact that apart from net heterotrophy, major drivers of carbon dynamics in lakes include direct exogenous inputs of dissolved inorganic carbon (DIC) from soil respiration and mineral weathering of the catchment, interactions with the DIC equilibria and calcite precipitation (Maberly et al., 2012; Marcé et al., 2015; McDonald et al., 2013; Weyhenmeyer et al., 2015; Wilkinson et al., 2016). Mineral weathering and IC dynamics is estimated to be a major driver of lake CO_2 emissions in 57% of the world's lakes (Marcé et al., 2015). Hence, calcite precipitation is potentially a major source of CO_2 emissions from lakes and reservoirs on a global scale (Marcé et al., 2015). The third chapter of this thesis puts into perspective the role of calcite precipitation in the global carbon budget of lakes by offering a first estimate of global calcification rates and its resulting CO_2 emissions.

1.3 The role of inland waters as carbon sinks

While inland waters are important sources of gaseous carbon to the atmosphere they have also been described as carbon sinks due to their capacity to accumulate and store carbon in their sediments. This role has been quantified in several inland water components such as lakes, reservoirs and wetlands (TABLE 1), although only OC burial has been considered in global and regional estimates (Mendonça et al., 2017; Waters et al., 2019). Therefore, OC has been described as the essential constituent of the carbon sink via OC burial (Mendonça et al., 2017; Waters et al., 2019). Studies have shown that OC burial is usually smaller than CO_2 emissions (Chmiel et al., 2016; Mendonça et al., 2017), although some exceptional cases have been reported where carbon burial surpasses CO_2 emission (Morales-Pineda et al., 2016). Nevertheless, carbon burial represents a significant long-term carbon sink in lakes and reservoirs around the world (Kastowski et al., 2011; Mendonça et al., 2017). Besides organic carbon burial, geological evidence suggests that a large fraction of the carbon stored in hard water lakes is inorganically produced through the process of calcite precipitation (Pentecost, 2009; Roeser et al., 2021; Tucker & Wright, 1990). The inorganic carbon sink is a relevant additional component of the carbon cycle that stores carbon at geological timescales, without being prone to the degradation processes that characterize the organic carbon sink. Metabolic processes cause organic carbon in the sediments to be mineralized to CO_2 or CH_4 by heterotrophic microbes, while only a fraction is buried and stored over geological timescales (Sobek et al., 2009). The efficiency by which sediments store organic carbon has been estimated at an average of 48% of the deposited OC (Sobek et al., 2009). The inorganic carbon sink is therefore more stable than its organic counterpart because it is not affected by mineralization processes, although it may be affected by calcite dissolution in the hypolimnion or sediment pore waters (Bluszcz et al., 2009; Kufel et al., 2020; Roeser et al., 2016). Lake sediments may differ significantly in their ratio between OC and IC due to varying groups of primary producers that differ in their ability to induce calcification (Kufel et al., 2020). In fact, IC in profundal lake sediments was shown to be a better predictor of trophic status of a lake than OC, due to the strong linkages between primary production and calcification that affect sediment carbon composition (Kufel et al., 2020). This inorganic fraction of carbon is not accounted for in the carbon sink estimations of lakes and reservoirs, likely resulting in an underestimation in the total global carbon sink of inland waters.

Freshwater sedimentary records suggest that calcite precipitation, also referred to as calcification, is an important process in lakes (Tucker & Wright, 1990). Calcium-rich hard water lakes can have a considerable fraction of their sediments composed of calcite due to important calcium carbonate precipitation that often occurs in such aquatic systems (Kelts & Hsü, 1978; Koschel, 1990; Stabel, 1986). The most evident cases for this can be found in marl lakes where the content of surface-sediment calcium carbonate exceeds 50% of the dry weight of the inorganic fraction, and can reach over 90% in deeper sediments (Pentecost, 2009). A recent study found that calcite precipitation and storage in reservoirs offers an important ecosystem service by decreasing downstream concentrations of total dissolved solids, alkalinity and salinity (Deemer et al., 2020). Hard water lakes account for almost half of the global volume of inland waters (Wetzel, 2001). The large fraction of calcite often found in hard water lake sediments may provide evidence for the important role that calcite precipitation has as a geological carbon sink in lakes and reservoirs. Yet, this role has never been quantified at a global scale and has been assumed to be negligible in comparison to OC burial which has been widely quantified at local, regional and global scales (Hanson et al., 2015; Mendonça et al., 2017; Sobek et al., 2012). It is therefore necessary to quantify the contribution of calcite precipitation to the global carbon sink of lakes and reservoirs, and to identify parameters that could be used to estimate its role in regional and local carbon budgets. This will also be addressed in the third chapter of this thesis which offers a first global gross estimate of calcite precipitation rates, highlighting its potential role as a global carbon sink to the sediments.

1.4 Calcite precipitation as a source of CO2

Calcification is a relevant process determining CO_2 dynamics in aquatic systems whereby the chemical equilibria of the DIC system implies an effect of calcite processes on the concentration of DIC species, including CO_2 (Zeebe & Wolf-Gladrow, 2001; Stumm & Morgan, 1996). While this role is well acknowledged for marine systems (Frankignoulle et al., 1994, 1995; Gattuso et al., 1999, 1993, 1996; Opdyke & Walker, 1992; Ridgwell & Zeebe, 2005; Suzuki, 1998), it has rarely been studied in lakes. According to its chemical equation (Equation 1.1), calcite precipitation releases one mole of CO_2 per mole of calcite that precipitates. Its chemical equation implies a loss of 1 mole of DIC and 2 equivalents of alkalinity per mole of calcite that precipitates. Despite acting as a long-term carbon sink, it is acknowledged in marine systems that calcification increases CO_2 concentrations in surface waters, thereby acting as a carbon source to the atmosphere (Ridgwell & Zeebe, 2005).

Equation 1.1: $Ca^{2+} + 2HCO_3^{-} \leftrightarrow CaCO_3 + CO_2 + H2O$

The molar ratio between released CO_2 and calcite precipitation (characterized by Ψ (“phi”)) depends on the buffering capacity of water; 1 mole of $CaCO_3$ precipitated releases 0.6 moles of CO_2 in seawater (Ψ = 0.6) and 1 mole of CO_2 in freshwater (Ψ = 1) assuming that pCO_2 is 355 µatm and alkalinity is 2.4 and 0.6 meq L^{-1}, respectively (Frankignoulle et al., 1995). This estimation is robust for changes in pH ranging from 8.1 to 8.3 and from pCO_2 variations ranging from 300 to 400 µatm (Ware et al., 1992). However, in freshwater systems such as lakes and reservoirs, pCO_2, alkalinity and pH values can express large temporal and spatial variability. Most lakes are supersaturated with CO_2 (Sobek et al., 2005) with higher CO_2 and alkalinity values than those that were used to calculate Ψ (Marcé et al., 2015; Wetzel, 2001), resulting in possible discrepancies between the theoretical Ψ and its observed values. pCO_2 is constantly re-equilibrating with the atmosphere and is therefore increasing together with atmospheric pCO_2 as a result of industrial activity and fossil fuel combustion (Caldeira & Wickett, 2003; Kurihara, 2008). Previous research shows that atmospheric CO_2 will continue to increase in the following years (IPCC, 2014), which is confirmed by the rise of CO_2 which is still being observed today. As a result, the value of Ψ is estimated to reach 0.74 by 2050 in oceans which would result in a net source of ~130Mt C yr^{-1} (Frankignoulle et al., 1994) representing the equivalent of up to 2% of fossil fuel fluxes (IPCC, 2013).

Discrepancies were found in seawater between the theoretical Ψ (0.6) and its observed value, that is 0.006 at a community level (Michel Frankignoulle et al., 1994; J.-P. Gattuso et al., 1993) and 0.1 at an organism level for scleractinian corals with zooxanthelle (Frankignoulle & Gattuso, 1993). These important differences can be attributed to a tight coupling between photosynthesis and calcification, where CO_2 uptake by primary producers decreases the CO_2 evasion to $CaCO_3$ precipitation ratio (Frankignoulle et al., 1994). The theoretical Ψ exclusively describes CO_2 released due to calcite precipitation. However, when comparing directly measured CO_2 fluxes with calcite precipitation, it is necessary to account for net changes in CO_2 caused by organic processes (Suzuki, 1998). By considering these organic processes, Suzuki (1998) suggests to redefine Ψ as *(released CO_2 + CO_2 uptake from photosynthesis) / $CaCO_3$ precipitation.* He suggests that the ratio between OC and IC production (R_{oi}) can be used to determine whether an aquatic system acts as a sink or a source of carbon. This highlights the importance to consider both organic and inorganic processes when assessing carbon fluxes. Interestingly, the critical R_{oi} ratio is identical to the Ψ ratio; when OC production is larger than 0.6 times the calcite precipitation ($R_{oi} > 0.6$), pCO_2 decreases allowing the aquatic system to absorb CO_2 from the atmosphere, and inversely when $R_{oi} < 0.6$ the resulting increase in pCO_2 promotes CO_2 release to the atmosphere (Gattuso et al., 1999; Suzuki, 1998). The example of Ψ is mentioned here to stress that CO_2 fluxes cannot be comprehended by calcification alone, nor are they independent from it. Rather, the effect of calcite precipitation on CO_2 fluxes can only be correctly assessed by considering the coupling between calcification and primary production, which is detailed in the following subchapter.

1.5 An intimate coupling between calcite precipitation and primary production

Even though primary producers tend to decrease pCO_2 through photosynthesis, they can also promote calcite precipitation and its subsequent release of CO_2 (Dittrich et al., 2004). Detailed experiments showed that some algae (C*hlorococcum* sp.) can induce $CaCO_3$ precipitation when exposed to light, implying that the mechanism that triggers precipitation is directly linked and coupled to photosynthesis (Hartley et al., 1995). A strong linear positive relationship (R^2=0.95) was found between chlorophyll-*a* and calcite saturation, indicating a strong sensitivity of the IC equilibrium to biologically mediated processes (Effler, 1984). This intimate coupling between calcification and photosynthesis has been described as a “chicken and egg” dilemma: is it photosynthesis that causes calcite to precipitate by increasing the carbonate ion activity (Equation 1.2), or is it calcification that promotes photosynthesis by counteracting photosynthetic CO_2 depletion (Equation 1.1) (McConnaughey & Whelan, 1997).

Equation 1.2: $2HCO_3^- \rightarrow CH_2O + O_2 + CO_3^{2+}$

Benthic calcification in lakes by calcifying macrophytes such as *Chara sp.* has been well described in the literature (Kufel et al., 2020; McConnaughey, 1991; Sand-Jensen et al., 2018). Benthic ecosystems dominated by charophytes are often characterized by dense bottom-dwelling calcified vegetation (Andersen et al., 2017; Sand-Jensen et al., 2018). In such systems, daily alkalinity losses of up to 0.57

meq L^{-1} have been observed due to high rates of calcification (Andersen et al., 2017). In comparison to benthic calcification, pelagic calcification has been less addressed in the scientific literature, likely due to the prevalence of calcifying macrophytes in lakes (Vadeboncoeur et al., 2008) and the potentially high calcification rates observed from such organisms (McConnaughey & Whelan, 1997). However, the main reason for the lesser scientific attention given to pelagic calcification is possibly the rarity of planktonic organisms that produce calcareous shells in freshwaters (Kelts & Hsü, 1978), with the exception of the freshwater chlorophyte *Phacotus lenticularis*. This species has shown remarkable contributions to the total suspended calcite precipitation in several hard water lakes (Krienitz et al., 1993; Lenz et al., 2018). Assuming pelagic calcification to be a rare process, based on the rarity of calcifying planktonic species is misleading because biogenic calcification can occur in the absence of calcifying organisms. Biogenic calcification can either be induced or controlled (Dittrich & Obst, 2004). In the case of induced biogenic calcification, photosynthesis raises the pH, influencing the carbonic acid equilibrium and shifting the balance towards oversaturation of carbonates (Dittrich & Obst, 2004). This process facilitates heterogeneous nucleation, that is, the development of nuclei on the surface of foreign solids of small size. This is the predominant pathway of calcite precipitation, especially in the presence of picoplankton and cyanobacteria. These organisms act as ideal nucleation surfaces for calcite formation and have the ability to actively induce calcite precipitation by changing the chemical microenvironment of the water layer adjacent to the cell (Dittrich & Obst, 2004; Lowenstam & Weiner, 1989; Obst et al., 2009; Stabel, 1986). In the case of controlled biogenic calcification, the role of the organism goes beyond inducing an oversaturation of carbonates locally. Rather, the organism controls the processes of calcite mineralization via an organic matrix made of macromolecules that extend from the outer cell membrane to the solution (Dittrich & Obst, 2004; Lowenstam & Weiner, 1989). This is the strategy used by calcifying planktonic species such as coccolithophorids to build sophisticated calcite structures (Dittrich & Obst, 2004).

Because of its strong association with primary production, pelagic calcification is affected by nutrient increase and its resulting eutrophication conditions (Hamilton et al., 2009). Yet, pelagic calcification was shown to have a negative feedback to lake eutrophication, through a process where algae sinks to the lake bottom while being trapped in calcite crystals (Koschel et al., 1983), resulting in round or elliptic holes of 2-3 µm diameter within the crystal structure, caused by the remains of microalgae (Figure 1.2). The potential of calcification to remediate lake eutrophication has been investigated since the 1980's and studies have found that calcification is an effective means of phosphorus removal through the coprecipitation of calcite with phosphates (Hamilton et al., 2009; Kleiner, 1988; Murphy et al., 1983; Walsh et al., 2019). This way, eutrophication can result in calcification events that, in turn, act as a self-remediation mechanism against lake eutrophication. Understanding and quantifying the complex relationship between photosynthesis and calcification is essential for estimating calcite precipitation rates, its contributions in carbon budgets, and its evolution within the context of global change and lake eutrophication. The fourth chapter of this thesis indirectly addresses this need by providing a mechanistic understanding of the relationship between calcite precipitation and primary production and how this relationship is affected by lake eutrophication and geochemical drivers.

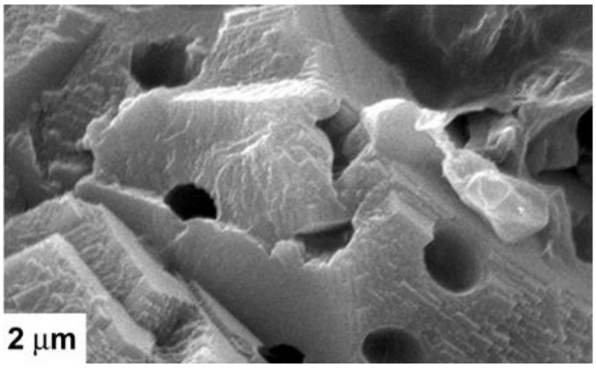

Figure 1.2. image from Scanning Electron Microscopy of calcite crystals. Source: Dittrich *et al.*, 2004

The tight coupling between primary production and calcification has sometimes been described using the calcite precipitation to NEP molar ratio (α). This ratio, as defined by McConnaughey (1991), has rarely been measured in freshwater systems and was shown to range between 0.2 and 1 (McConnaughey, 1991; McConnaughey & Whelan, 1997; Megard, 1968). Compared to marine studies, this range is similar to marine planktonic assemblages (Riebesell et al., 2000) but lower than the ratio of 1.3 found in marine benthic ecosystems (Gattuso et al., 1999). While the 1:1 ratio is often found in calcareous macrophytes, this ratio can increase at pH levels above 9 due to CO_3^{2-} competing with HCO_3^- for protonation (McConnaughey, 1991). Accounting for α in lake models was shown to improve their accuracy in explaining CO_2 supersaturation in lakes (Marcé et al., 2015). Yet, the value of α is not clearly defined and its variation along environmental gradients has not been quantified. This will also be addressed in the fourth chapter of this thesis which aims at quantifying α and the way it responds to environmental gradients in view of parameterizing calcite precipitation and its effect on DIC dynamics.

1.6 Calcite precipitation and carbon dynamics: implications for lake models

High rates of calcite precipitation are often found during the summer season when calcite saturation indices peak due to enhanced primary production that rise the pH by depleting CO_2 concentrations (Fuchs et al., 2016; Müller et al., 2016; Nõges et al., 2016; Trapote et al., 2018). High levels of calcification can result in "whiting events" where the abundance of fine-grained $CaCO_3$ crystals can be seen through satellite imagery and can be monitored using remote sensing (Heine et al., 2017; Nouchi et al., 2019). Such events can have a significant effect on the carbon dynamics of a lake. Because of the CO_2 released from calcification, it is expected that this fraction of inorganically produced CO_2 would result in imbalances between DIC and metabolic rates (Marcé et al., 2015). A study in a reservoir in Spain shows that despite a persistent net heterotrophic state, CO_2 emissions show no clear dependence on the metabolic balance. A significant decrease of surface alkalinity during summers suggests that calcite precipitation may be the main mechanism responsible for the imbalance between IC variability and metabolic rates in this reservoir (López et al., 2011). Several studies suggest that the release/consumption of CO_2 caused by calcite precipitation/dissolution can involve important deviations from the metabolic 1:1 stoichiometry between DIC and O_2 (Barrón et al., 2006; Obrador & Pretus, 2012; Vachon et al., 2020). Such imbalances have been reported from lakes that are in an autotrophic state, yet they emit CO_2 to the atmosphere (Perga et al., 2016; Stets et al., 2009). In the contiguous United States, 35% of the lakes and reservoirs are likely to be in this condition (McDonald et al., 2013). In such lakes, CO_2 emissions have been attributed to hydrologic inputs and CO_2 released by calcite precipitation (Stets *et al.*, 2009; McDonald *et al.*, 2013; Perga *et al.*, 2016, Marcé et al 2015).

The effect of calcite precipitation on carbon dynamics has important implications for modelling carbon fluxes in lakes. Conceptual models that rely on DOC to predict pCO_2, assume that heterotrophy is the main driver of CO_2 release and that inorganic processes such as calcite precipitation play a negligible role (Finlay et al., 2019). This is likely to create discrepancies between theoretical models and empirical data (Lu et al., 2018). A recent study shows that the model performance to predict the effect of DOC on pCO_2 declined by 50% in successive seasons from spring, to summer, to autumn (Finlay et al., 2019). Such decoupling between DOC and microbial metabolism during summer or fall could be caused by calcification among other processes such as groundwater inputs, carbonate buffering or anaerobic metabolism (Finlay et al., 2019). The fifth chapter of this thesis addresses the role of calcite precipitation in causing imbalances between DIC and metabolic rates and how such imbalances depend largely on the geochemistry of a lake.

In view of what has been mentioned, the contribution of calcite precipitation in global carbon budgets, its relationship with primary production, its effects on carbon dynamics and finally, its implications for lake models are relevant topics where research is needed. This thesis is dedicated to these topics and is an attempt to answer some of its most impending questions which will be defined in the following chapter that describe the objectives and hypothesis of this thesis.

2. Objectives and Hypotheses

The general objective of the present thesis is to understand the role of pelagic calcite precipitation in lakes, addressing its drivers, its effect on carbon dynamics and its global implications. The thesis is divided into three chapters, each corresponding to a separate publication that addresses one of the following three research questions.

Question 1 (third chapter): *Is pelagic calcification a relevant flux in the global carbon budget of lakes and can it be predicted by lake alkalinity?*

Hypotheses: Pelagic calcification occurs in lakes of higher alkalinity. Its potential role as a sedimentary carbon sink and as a neglected source of CO_2 to the atmosphere is not negligible at a global scale.

Question 2 (fourth chapter): *How does planktonic metabolism drive calcification and what controls its intensity?*

Hypothesis: Primary production is a main driver of pelagic calcification. Its efficiency in driving pelagic calcification depends on geochemical parameters such as water alkalinity. The ratio between calcification and primary production can increase with higher alkalinity.

Question 3 (fifth chapter)*: How does calcification affect the response of DIC variability to lake metabolism along a gradient of water alkalinity?*

Hypothesis: In lakes of low alkalinity, lake metabolism is the main driver of DIC variability, but in lakes of higher alkalinity, imbalances between metabolic rates and DIC variability are to be expected. Such imbalances are caused by calcification which acts as an important driver of carbon dynamics in such lakes.

3. The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs

Original publication (Appendix A):

Khan, H., Marcé, R., Laas, A. Obrador, B. 2021. The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs. Limnetica *in press* DOI: 10.23818/limn.41.02

3.1 Abstract

Calcite precipitation acts as a carbon sink in the sediments and a short-term source of carbon dioxide (CO_2) to the atmosphere, as widely acknowledged in marine studies. However, pelagic calcite precipitation has received limited attention in lakes. Here we use the relationship between lake water alkalinity and reported calcification rates to provide the first global estimate of pelagic calcification in lakes. Global gross calcification rates amount to 0.03 Pg C yr^{-1} (0.01 – 0.07) comparable to rates of organic carbon burial, whereas its related CO_2 release is largely buffered by the carbonate equilibria. Calcification occurs at water alkalinity above 1 meq L^{-1} corresponding to 57% of global lake and reservoir surface area. Pelagic calcification therefore is a prevalent process in lakes and reservoirs at the global scale, with a potentially relevant role as a sedimentary inorganic carbon sink, comparable in magnitude to the total calcite accumulation rates in ocean sediments.

3.2 Introduction

Lakes and reservoirs are important components of the continental carbon (C) cycle due to large emissions of methane and CO_2 to the atmosphere and burial of organic carbon (OC) in their sediments (Cole et al., 2007; Mendonça et al., 2017; Raymond et al., 2013a). OC production, decomposition and burial have been described as the main drivers of C fluxes (Duarte & Prairie, 2005). Most studies relating C fluxes to OC metabolism in lakes have been conducted in systems with low water alkalinity (i.e. less than 1 meq L^{-1}) and high dissolved OC concentrations (McDonald et al., 2013). However, increasing evidence suggests that inorganic carbon (IC) may be equally relevant in lakes of higher alkalinity (Marcé & Obrador, 2019), comprising 57% of world's lakes area (Marcé et al., 2015; McDonald et al., 2013). While the role of benthic calcification is acknowledged in such systems, calcification in the pelagic zone is assumed to be negligible due to the rarity of planktonic organisms that produce calcareous shells in freshwaters (Kelts & Hsü, 1978). However, recent evidences stress the important role of pelagic calcite precipitation in lakes as an alkalinity sink and CO_2 source (Müller et al., 2016). This double-edged role is widely acknowledged and quantified in marine studies (Michel Frankignoulle et al., 1994; Ridgwell & Zeebe, 2005), but its relevance in lakes has been assumed to apply only to saline systems. Evidence suggests that calcification contributes to the large CO_2 emissions found in saline lakes, yet those systems represent only a fifth of the Earth's lake surface (Duarte et al., 2008). The biogeochemistry of carbon in saline lakes usually differs from those of freshwater lakes, because of the large carbon pools supporting high CO_2 exchange rates and chemical enhancement processes that are usually less relevant in freshwater systems (Duarte et al., 2008). It is therefore necessary to study pelagic calcification in freshwater lakes separately to assess the relevance of this process in these systems representing the vast majority of the Earth's lake surface. Moreover, the majority of freshwater lakes are above the alkalinity threshold of 1 meq L^{-1} where calcification is likely to occur (Khan et al., 2020; Marcé et al., 2015; McConnaughey & Whelan, 1997). While the occurrence of pelagic calcification in freshwater lakes has often been reported, it has never been quantified at a global scale, nor has its derived CO_2 emissions.

High rates of calcite precipitation are often found during the summer season of lakes when calcite saturation peaks due to enhanced primary production that rises the pH by depleting CO_2 concentrations, thereby shifting the carbonic acid equilibrium towards oversaturation of carbonates (Müller et al., 2016). Yet, spontaneous calcification is unlikely to occur, rather it is biologically mediated by picocyanobacteria that provide ideal nucleation sites that further facilitate calcite precipitation in the pelagic zones of lakes (Obst et al., 2009). Calcite precipitation in lakes has been presented as a natural process that can reduce summer eutrophication (Rodrigo et al., 1993). Because of this seasonal association and its occurrence during high primary production, most studies that have quantified calcite precipitation in lakes have focused on the summer season. Using summer pelagic calcification rates from the literature (n=26, Supplementary information, available at http://www.limnetica.net/en/limnetica), here we identify a clear association with water alkalinity.

Using water alkalinity as the driver variable with obvious mechanistic links with calcification, we provide a first gross global estimate of calcification rates and its derived CO_2 emissions. We intentionally excluded rates measured in other seasons than summer or averaged over the year, rates for benthic calcification caused by calcifying macrophytes, rates in brackish systems and saline lakes.

3.3 Methods

Data on pelagic calcite precipitation was obtained from published literature reporting areal or volumetric rates from either sediment traps, sediment cores, C or Ca^{2+} mass balances, incubations, or modelling (Supplementary information, available at http://www.limnetica.net/en/limnetica). Most rates found in the literature are average rates covering only the summer season, therefore we excluded mean annual rates from this analysis. When reported per unit area, areal rates were divided by the mixed layer depth (Zmix) to obtain rates per unit volume, assuming that most pelagic calcification occurs in the epilimnion (Hartley *et al.*, 1995; Obst *et al.*, 2009). If Zmix of a specific lake was not specified in the literature, it was calculated using FLAKE Global model (Kirillin et al., 2011). The strong relationship found between alkalinity and calcification rates was used in the upscaling to global rates, using the 5%, 50% and 95% quantiles to obtain a range of uncertainty. The volumetric rates obtained from this relationship were converted to areal rates by multiplying the volumetric rates with an average Zmix value of 6.72. The average Zmix was calculated from a dataset of 628 lakes (Woolway & Merchant, 2019), using a hyperbolic function between Zmix and lake fetch (Lewis, 2011). The areal rates by alkalinity level were multiplied by the global surface area of lakes corresponding to each level of alkalinity (alkalinity intervals of 0.05 meq L^{-1}) (Marcé et al., 2015). The sum of these rates was multiplied by the average number of days of measurements (94 days) from which each reported daily rate in the literature was averaged. These calculations are presented in the supplementary Data file.

For upscaling CO_2 emissions, the 5%, 50% and 95% quantiles of the relationship between alkalinity and calcification was used to build a model that simulates the resulting range of lake CO_2 partial pressure (pCO_2) increase at different daily calcification rates that are dependent on the initial alkalinity value. The model is based on the chemical dissolved inorganic carbon (DIC) equilibria whereby 1 mole of calcite that precipitates results in a loss of 1 mole of DIC (because 2 moles of HCO_3^{2-} are lost in the reaction and 1 mole of CO_2 is released) and a loss of 2 equivalents of alkalinity. The fate of the CO_2 released by calcification and its interaction with the DIC equilibria is therefore taken into account in this model. The initial pCO_2 value was set at the atmospheric equilibrium value of 410 ppm. The model is freely available online in the following link (http://hdl.handle.net/2445/172489). The global upscaling was done similarly to the upscaling of calcification rates, using existing data of the global surface area of lakes per alkalinity levels (Marcé et al., 2015). CO_2 flux was calculated using Fick's Law of gas diffusion (Millero, 1979) using the global average Piston velocity (k_{600}) value of 1m d^{-1} with an uncertainty range between 0.74 and 1.33 (Raymond et al., 2013a). The sum of the fluxes correspond to the global annual CO_2 emissions caused by planktonic calcification. It is a first estimate of the CO_2 emissions related to calcification and should therefore be understood as an approximate estimation that can be further refined by research, rather than an ultimate value. All calculations are available in the Supplementary Data File.

3.4 Results and Discussion

A significant positive linear relationship ($R^2=0.4$, n=26, $p<0.001$) was found between water alkalinity and calcite precipitation rates, suggesting that the importance of this process is significantly higher with increasing alkalinity (Figure 3.1a). No significant effect of chlorophyll-a and total phosphorus on calcification rates was found and including these variables with alkalinity in a multiple linear regression only decreased the strength of the model ($R^2=0.34$, $p<0.05$). However, in lakes, spontaneous calcification is unlikely to occur. Rather, it is mediated by picoplankton that promote the chemical environment necessary for calcification by increasing the pH and by offering ideal nucleation sites (Obst et al., 2009). The absence of any significant effect of chlorophyll-a and total phosphorus concentrations could be due to the fact that calcite saturation is more determined by alkalinity than by production of the system. The strength of primary production to promote calcification likely depends on the level of calcite saturation which is directly related to water alkalinity. A quantile regression using the 95% quantile suggests an alkalinity threshold for calcification at~ 1 meq L^{-1} (Figure 3.1a), below which calcification is unlikely to occur. This is consistent with the threshold of 1 meq L^{-1} reported in previous studies (Marcé et al., 2015; McConnaughey & Whelan, 1997). Considering that 57% of the global area occupied by lakes and reservoirs have alkalinity values above 1 meq L^{-1} (Marcé et al., 2015), pelagic calcite precipitation is likely to occur in most lakes and reservoirs around the globe. In addition, the 5% quantile regression indicates that virtually all lakes with alkalinity beyond 2.5 meq L^{-1} will show summer pelagic calcification (Figure 3.1a).

Calcite precipitation and dissolution reactions imply changes on both alkalinity and the relative abundance of DIC species (Ridgwell & Zeebe, 2005) (Equation 1.1, in general introduction), whereby calcite precipitation releases one mole of CO_2 per mole of calcite precipitated, increasing pCO_2. Because of the higher calcification rates found in lakes of higher alkalinity, one would expect the increase in pCO_2 caused by calcification to be stronger in such systems. However, the ratio between calcite precipitation and CO_2 release (commonly referred to as Ψ) depends on the capacity to buffer changes in pCO_2 (Michel Frankignoulle et al., 1994; S. V. Smith & Mackenzie, 2016). As a result, the buffering capacity of high alkalinity lakes mitigate pCO_2 increase preventing most of the released CO_2 to escape to the atmosphere (Figure. 3.1b). Therefore, the potential change in pCO_2 caused by calcite precipitation on a daily time scale is minimal (Figure 3.1b).

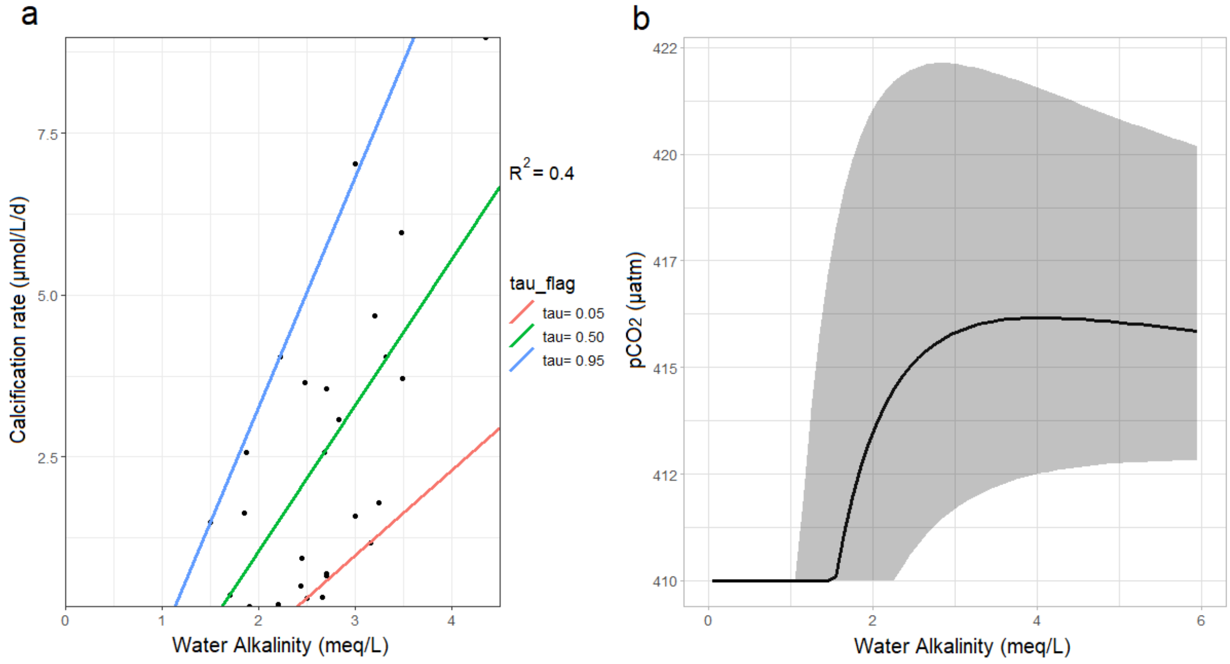

Figure 3.1. Effects of alkalinity on calcification rates. (**a**) relationship between alkalinity and calcification rates reported in the literature for global lakes. Lines corresponds to quantile regressions at the 95% quantile (blue), 50% (green), and 5% (red). (**b**) Daily effect of calcite precipitation on pCO_2 at different alkalinity levels. Initial pCO_2 value is at atmospheric equilibrium. The solid line represents the 50% quantile. The shaded area corresponds to the range of calcification found at a given alkalinity level using the 5% and 95% quantiles.

We used the alkalinity – calcification rates relationship together with the global distribution of lakes and reservoirs per alkalinity level (Marcé et al., 2015) for upscaling calcite precipitation rates to the entire globe. The resulting annual global rate of calcite precipitation of 0.03 Pg C yr^{-1} (range 0.01 – 0.07

Pg C yr^{-1}) is comparable in magnitude to global annual rates of OC burial of 0.15 Pg C yr^{-1} (range 0.06 – 0.25 Pg C yr^{-1}, (Mendonça et al., 2017), Figure 3.2a). Global calcite precipitation would thus be of the same order of magnitude as OC burial, making them both similarly relevant as main drivers of C cycling in lakes. As a comparison, the average areal calcification rates in lakes (13.8 g C m^{-2} yr^{-1}, range: 5.4 – 28, Supplementary Data File) correspond to 37% (14% - 75%) of areal rates of pelagic calcification in oceans (37 g C m^{-2} yr^{-1}) (S. V. Smith & Mackenzie, 2016).

The net role of pelagic calcification as a sedimentary inorganic carbon sink depends on calcite dissolution during sedimentation and in the sediments. In open oceans 80% of all carbonate that precipitates dissolves within the water column or in the sediments (Ridgwell & Zeebe, 2005). Although some studies have reported calcite dissolution in some lakes (Ohlendorf & Sturm, 2001; Ramisch et al., 1999), others report no dissolution during settling of calcite crystals (Stabel, 1986). A study on 13 lakes revealed that calcite dissolution in lakes and reservoirs is minor compared to calcite precipitation because rather than dissolving in the hypolimnion, calcite tends to accumulate in the sediments (Müller et al., 2016), thereby acting as a long-term C sink. These results are in agreement with another study on 5 lakes that reports calcite precipitation with no evidence of calcite dissolution (Lenz et al., 2018). Yet, high rates of calcite dissolution are reported in deeper stratified systems rich in OC where decomposition of organic matter promotes anoxic conditions and low pH in the hypolimnion (Ohlendorf & Sturm, 2001) and where calcite crystals may partly dissolve in the water column during sedimentation (Ramisch et al., 1999). The global calcification rate provided in this study is a gross estimate to be further built upon. The role of calcite dissolution needs to be assessed at a global scale to determine the net burial flux, which is beyond the scope of this study. Considering calcification alone, the total export of calcite to the sediments would represent between 10% and 70% of the total net oceanic sink for pelagic calcification (0.1 Pg C yr^{-1}) (Sabine & Tanhua, 2010). So long as calcite dissolution hasn't been estimated at a global scale, these values remain hypothetical. This is even more uncertain in the context of global change which is likely to increase the frequency of anoxic conditions in the hypolimnion of stratified systems (Jankowski et al., 2006; Schwefel et al., 2016) which could promote ideal conditions for calcite dissolution. Nevertheless, these values highlight the disproportionately important role of calcite precipitation in lakes and reservoirs as a potential carbon sink despite the relatively small area that these water bodies occupy on the globe.

The gross estimate of global CO_2 emissions caused by calcification is 0.0002 Pg C yr^{-1} (range: 0.00005 – 0.0007 Pg C yr^{-1}). This is an irrelevant fraction of the total CO_2 emissions from lakes and reservoirs (<0.5%, Figure 3.2b). Our results contrasts with those of saline lakes where calcite precipitation is expected to contribute significantly to CO_2 emissions (Duarte et al., 2008). The low contribution of calcification-derived CO_2 emissions is because higher rates of calcite precipitation are found in lakes of higher alkalinity (Figure 3.1) that have a stronger capacity to buffer changes in pCO_2. Recent studies found that inorganic processes including DIC loading from chemical weathering and calcite precipitation are important drivers of CO_2 emissions in lakes and reservoirs (Marcé et al., 2015; McDonald et al., 2013). Our results suggest that pelagic calcite precipitation represents a minor share of emissions caused by inorganic sources (Figure 3.2b).

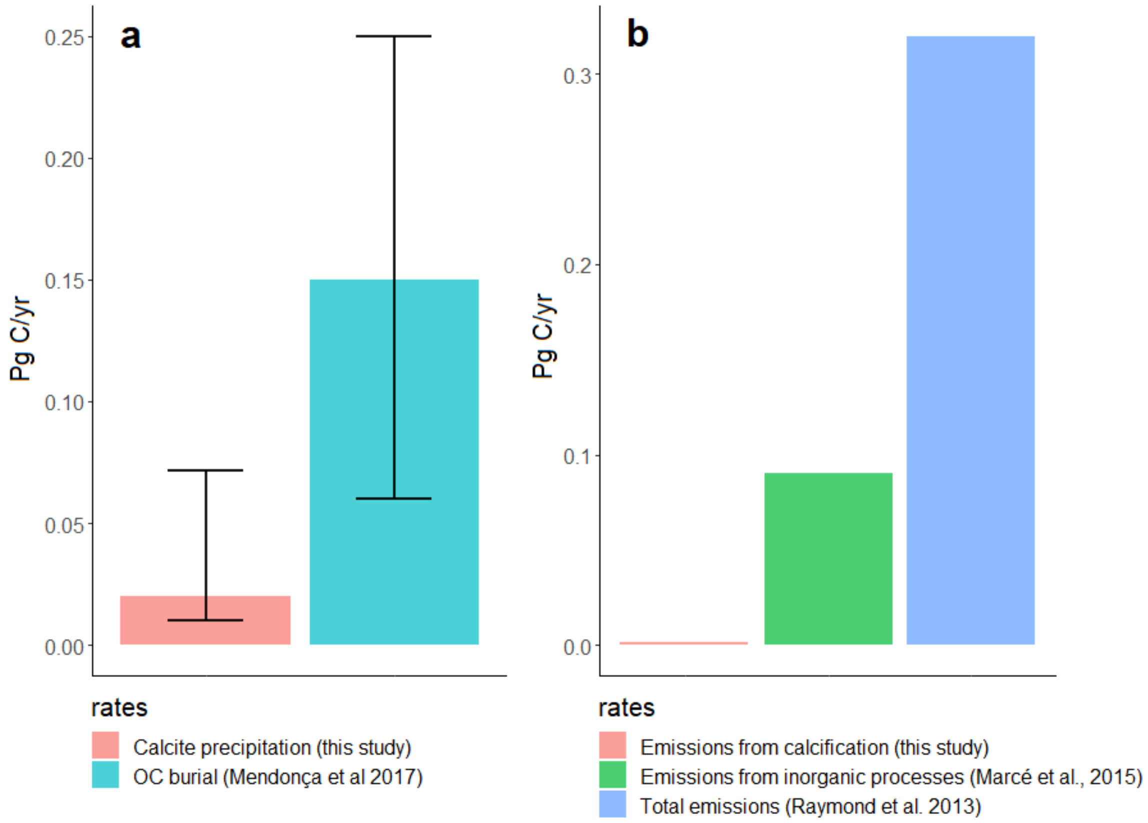

Figure 3.2. Global rates of calcification and CO_2 emissions. (**a**) Global annual rates of calcite precipitation (this study) and OC burial (Mendonça et al., 2017) in lakes and reservoirs. The error bars represent the range. (**b**) Global rates of CO_2 emissions from calcification (this study), inorganic processes (Marcé et al., 2015) and total emissions (Raymond et al., 2013a).

It is important to mention that pelagic calcification rates reported here are probably only a share of total calcification in lakes and reservoirs. Benthic primary production can account for a large fraction of the overall metabolism of lakes (Cremona et al., 2016), and benthic habitats dominated by calcifying macrophytes can promote daily losses of up to 0.57 meq L^{-1} of alkalinity due to intense calcification (M. R. Andersen et al., 2017). In the absence of a global estimate of lake benthic calcification we can only speculate about its relevance, but considering potentially high calcification rates from benthic macrophytes (McConnaughey & Whelan, 1997) and their prevalence in lakes, we posit benthic calcification could be even higher than pelagic. Furthermore, our global estimate only accounts for calcification during the summer season assuming that this represents the largest part within a year. While calcification usually occurs mainly in the summer season, it is variable in time and the possibility of its occurrence in other seasons cannot be excluded (Trapote et al., 2018). The seasonality of calcification is likely to be directly related to the seasonality of primary production since calcification in lakes is a biologically mediated process that is enhanced during peaks of primary production (Obst et al., 2009). Arriving at a mechanistic understanding of how primary production promotes calcification within the physicochemical context of a lake, including its alkalinity level, is a next step to predict calcification events in lakes and further refine its global estimation by integrating seasonality at a finer scale.

While CO_2 emissions caused by calcite precipitation is minor at a global scale, CO_2 release during summer calcification can have significant implications on C dynamics at a local scale, resulting in imbalances between metabolic rates and C concentrations (Khan et al., 2020). Such imbalances can reach a point where lakes are simultaneously in an autotrophic state, yet they emit CO_2 to the atmosphere, as is likely the case in approximately one third of lakes in the boreal region and in the contiguous United States (Bogard & Giorgio, 2016; McDonald et al., 2013). Accounting for the role of calcite precipitation on C dynamics at a local scale is necessary to improve the reliability and predictability of lake models that are currently unable to explain the reported imbalances between metabolism and C fluxes (Lu et al., 2018).

To conclude, the rates provided here highlight the role of pelagic calcite precipitation as an important component of the C cycle in lakes and reservoirs and the need to account for it in global C budgets (Marcé & Obrador, 2019). Research should aim at quantifying the ratios between production and calcification rates as well as between calcite precipitation and CO_2 release along alkalinity gradients in order to account for this process in models of inland water C processing. Temporal patterns of calcite precipitation and environmental conditions for its occurrence need to be investigated for predicting this process at short and longer temporal scales. Finally, calcite precipitation and dissolution in lakes and reservoirs need to be understood in the context of climate change predictions in order to assess their role as potential C sinks or C sources in a changing climate.

Chapter 4

4. Eutrophication and geochemistry drive pelagic calcite precipitation in lakes

Original publication (Appendix A):

Khan, H., Laas, A., Marcé, R., Sepp, M., Obrador, B. 2021. Eutrophication and Geochemistry Drive Pelagic Calcite Precipitation in Lakes. Water 13(5): 597. DOI: 10.3390/w13050597

4.1 Abstract

Pelagic calcification shapes the carbon budget of lakes and the sensitivity of dissolved inorganic carbon (DIC) responses to lake metabolism. This process, being tightly linked to primary production, needs to be understood within the context of summer eutrophication which is increasing due to human stressors and global change. Most lake carbon budget models do not account for calcification because the conditions necessary for its occurrence are not well constrained. This study aims at identifying ratios between calcification and primary production and the drivers that control these ratios in freshwater. Using in situ incubations in several European freshwater lakes, we identify a strong relationship between calcite saturation and the ratio between calcification and net ecosystem production (NEP) (p-value < 0.001, $R^2 = 0.95$). NEP-induced calcification is a short-term process that is potentiated by the increase in calcite saturation occurring at longer time scales, usually reaching the highest levels in summer. The resulting summer calcification event has effects on the DIC equilibria, causing deviations from the metabolic 1:1 stoichiometry between DIC and dissolved oxygen (DO). The strong dependency of the ratio between NEP and calcification on calcite saturation can be used to develop a suitable parameterization to account for calcification in lake carbon budgets.

4.2 Introduction

Considering their comparatively small global surface area, lakes and reservoirs play a disproportionate role in the continental carbon (C) cycle due to their large contributions of carbon dioxide (CO_2) and methane emissions to the atmosphere and burial of organic carbon (OC) in their sediments (Cole et al., 2007; Mendonça et al., 2017; Raymond et al., 2013a). Numerous observations from lakes with low water alkalinity and high dissolved organic carbon (DOC) concentrations have stressed the prevalence of heterotrophy and its paramount effect on lake CO_2 fluxes (Del Giorgio et al., 1999; Duarte & Prairie, 2005; Larsen et al., 2011). However, increasing evidence also stresses the importance of inorganic processes in driving C fluxes in lakes of higher alkalinity (i.e., >1 meq L^{-1}), comprising 57% of the world's lakes area (Marcé et al., 2015; McDonald et al., 2013). In such systems, pelagic calcite precipitation acts as an important alkalinity sink and CO_2 source (Müller et al., 2016). Identifying the drivers of this process is necessary to be able to estimate its contribution to carbon fluxes.

High rates of calcite precipitation are often found during the summer season when calcite saturation peaks due to enhanced primary production that raises the pH (Fuchs et al., 2016; Müller et al., 2016; Nõges et al., 2016; Trapote et al., 2018). The abundance of nucleation sites provided by autotrophic picoplankton, particularly picocyanobacteria, further facilitates calcite precipitation in the pelagic zones of lakes (Dittrich et al., 2004; Dittrich & Obst, 2004; Obst et al., 2009), which in some cases reach such intensities where lakes take on a milky appearance caused by the calcite crystals that can be seen from satellite imagery (Strong & Eadie, 1978). Calcification is therefore expected to be directly related to primary production, and hence to the trophic state of a lake. In recent decades, eutrophication has become a major issue as the concentrations of dissolved phosphorus and nitrogen in water bodies have increased due to human activities such as extensive agriculture or pollutant discharges from point sources such as sewage water treatment plants (Carpenter et al., 1998). The increasing nutrient supply increases the phytoplankton abundance (V. H. Smith, 2003) and can lead to cyanobacteria blooms (Conley et al., 2009). Such stressors are likely to promote calcification events in lakes by increasing the pH through increased primary production and by increasing the amount of nucleation sites through picocyanobacteria (Dittrich et al., 2004; Dittrich & Obst, 2004; Obst et al., 2009). Within this context of increasing eutrophication, quantifying and predicting the way primary production promotes calcification are necessary for estimating carbon budgets in lakes and reservoirs under present and future scenarios. This study is a first attempt at identifying a quantifiable relationship between primary production and calcite precipitation in freshwaters.

Calcification can have a significant effect on the C budget of a lake, simultaneously acting as a C sink to the sediments (Nõges et al., 2016) and increasing the water CO_2 partial pressure (pCO_2) due to the CO_2 release caused by the reaction of calcium carbonate formation (Perga et al., 2016; Stets et al., 2009). However, calcification is not accounted for in C budgets or in lake models for two reasons. Firstly, contrary to marine systems, pelagic calcification is assumed to play a minor role in carbon fluxes of freshwater systems, likely because most studies that link CO_2 supersaturation to net heterotrophy are conducted in lakes that have relatively low alkalinity and high dissolved organic carbon (DOC) values(McDonald et al., 2013). However, calcite precipitation has been presented as a potentially important component of global carbon budgets for saline lakes (Duarte et al., 2008) and recent findings suggest the same for freshwaters (Khan et al., n.d.). Secondly, there is a need to understand the mechanisms of calcification and the drivers that control it, so that calcification could be parameterized and included in lake models. Currently, the lack of a proper parameterization accounting for CO_2 derived from calcite precipitation results in discrepancies between theoretical models and empirical data (Lu et al., 2018). This study aims at solving this issue by identifying key variables that control calcite precipitation and that could be used for the parameterization of calcite precipitation.

Attempts to parameterize lake calcification rates have related them to net ecosystem production (NEP) by observing ratios between the two, although calcite precipitation to NEP molar ratios (α) have rarely been studied in freshwater systems. The few studies that have measured this ratio in freshwater systems constrain it in the range between 0.2 and 1 (McConnaughey, 1991; McConnaughey & Whelan, 1997; Megard, 1968). This range is similar for marine planktonic assemblages (Riebesell et al., 2000) but lower than the ratio of 1.3 found in marine benthic ecosystems (Jean-Pierre Gattuso et al., 1999). While the 1:1 ratio is often found in calcifying macrophytes, this ratio can increase at pH levels above 9 due to CO_3^{2-} competing with HCO_3^- for protonation (McConnaughey, 1991). The α has been assumed to be alkalinity-dependent (Khan et al., 2020; Marcé et al., 2015), yet the nature of this dependency is not fully understood nor has it been precisely quantified. The purpose of this study is to identify and quantify the effects of lake NEP on calcification rates under different environmental conditions, by observing variations in α along gradients of calcite saturation and alkalinity. A mechanistic understanding of the occurrence of calcification is necessary for including this process in carbon budgets and for parameterizing calcification effects on CO_2 in lake models under different eutrophication scenarios.

4.3 Materials and Methods

4.3.1 Study Sites

The study was conducted in 4 European freshwater lakes covering a wide range of water alkalinity (from 0.5 to 4 meq L^{-1}; Table A1). The lake with the lowest alkalinity (0.5 meq L^{-1}), Valguta Mustjärv, was included in this study to observe the absence of calcification expected from such low-alkalinity lakes. Three lakes in Estonia were sampled during summer 2019 and one reservoir in Spain was sampled during summer 2018. All four water bodies differ substantially in their water retention time as well as in their catchment characteristics (Table 4.1), especially in regard to land use. However, all of the studied lakes were highly productive, according to nutrient (nitrogen and phosphorus) concentrations and chlorophyll-a values (Supplementary Table A1 in Appendix A). Each of these lakes has been extensively studied and described in detail elsewhere (Cremona et al., 2016; Laas et al., 2016; Marcé et al., 2008; Rõõm et al., 2014).

Table 4.1. Characteristics of the studied lakes. Values represent long-term averages.

Lake	Trophic State	Mixing Regime	Area (Ha)	Mean Depth (m)	Maximum Depth (m)	Kd (m)	Watershed Size (km2)	Water Residence Time (y)
Valguta Mustjärv (EE)	hypertrophic	polymictic	20.4	<1	1	10.3	1.34	source lake
Erastvere (EE)	hypertrophic	dimictic	16.3	3.5	9.7	2.9	5.2	0.5
Võrtsjärv (EE)	eutrophic	polymictic	27000	2.8	6	2.7	3116	1
Sau Reservoir (ES)	eutrophic	monomictic	600	25.2	65	0.9	1522	0.3

4.3.2 Experimental Setup

Five experimental campaigns using in situ incubations were conducted in Sau Reservoir (Spain) from May to August 2018. The following year, the same experiments were performed twice (first in June, then in July 2019) in the three Estonian lakes; Valguta Mustjärv, Erastvere and Võrtsjärv. The sampling combined experimental determination of lake calcification and NEP from the incubations, with high-frequency monitoring (HFM) of lake water characteristics during extended time periods. These two combined approaches were used so that calcification, assessed through experimental samplings, could be understood within the context of seasonal variations in parameters that can affect the process of calcification, especially dissolved oxygen (DO) and temperature as indicators of primary production. The experiment consisted of in situ incubations of surface lake water for 5–6 hours, from where the initial and final values of temperature (T), pH, electrical conductivity (EC, µS cm^{-1}), dissolved oxygen (DO, mg L^{-1}), dissolved oxygen saturation (DOsat, %), calcium concentration ($[Ca^{2+}]$ mg L^{-1}) and total alkalinity (TA, meq L^{-1}) were measured. Surface water was collected in a large (>9 L) plastic container from the pelagic zone at the deepest area of each lake. The initial T, pH, EC and DO were determined in triplicate using a handheld sensor calibrated in situ immediately before the experiment (for campaigns in Estonia during June 2019: WTW 2FD47D Multi 3430 Multiparameter Meter with Sentix 940-3 for pH, Tetracon 925 for EC and FDO 925 for DO; for campaigns in Estonia in July: same as in June, except that for DO, we used a Dissolved Oxygen Meter MARVET JUNIOR; for campaigns in Spain: YSI ProODO Handheld for the DO and WTW 3320 for EC and pH). Triplicate water samples for the determination of initial TA (120 mL, stored at 4 ºC in Nalgene bottles) and $[Ca^{2+}]$ (10 mL filtered at 0.45 µm, acidified in situ with 100 µl of 60 % HNO_3 and stored at 4 °C in PP tubes) were taken for laboratory measurements.

The incubations were performed in 5 transparent Winkler-type glass bottles of 250 mL at 30 cm depth in the littoral zone of each lake to maintain the light conditions of the upper mixed layer. The incubation bottles were carefully filled with water from the container with a pipe to avoid air mixing using a 250 µm mesh fixed between the container and the pipe for filtering zooplankton and detritus. The absence of any small air bubbles inside the bottles was carefully checked before starting the incubations. After the incubation period, the final T, pH, EC and DO were determined immediately after opening each bottle, minimizing turbulence. Triplicate samples were taken from each bottle for later laboratory analysis to determine the final $[Ca^{2+}]$ and TA, following the same procedures as for the initial conditions. All alkalinity samples were stored in a fridge and were measured using H_2SO_4 0.02 N titration the following day. An automatic titrator (Metrohm 655 Dosimat (in Spain) and SI Analytics TitroLine® 6000 (in Estonia)) was used to determine TA in a large sample volume (60 mL) and slow injection settings. The titrators were configured to increase the precision of the alkalinity determinations to 20 µeq L^{-1} (determined by replicated titrations of $NaCO_3$ solutions). Samples for $[Ca^{2+}]$ were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). All ICP-MS analyses were performed in a Perkin Elmer Optima 8300 at the Scientific and Technological Centre (CCiT) of the University of Barcelona.

4.3.3 Open Water Dynamics

Surface water sampling and HFM were used simultaneously in all lakes. However, in Sau Reservoir, surface water sampling was performed 9 times at a time interval of 2–3 weeks from May until the end of September 2018, while only 2 surface water samplings were performed in each Estonian lake. The higher number of surface water samplings in Sau Reservoir allowed us to simultaneously observe, at a finer scale, the trends in [Ca^{2+}], TA and calcite saturation measured from water samples, and the trends in T and DO, from HFM, for the entire summer season.

Surface water samples were taken from the deepest point of the reservoir and analyzed following the same procedures as for the incubations. In Sau, the HFM station (Marcé et al., 2016) was located at the deepest point of the reservoir. DO and T measurements were automatically taken every 5 min using a multiparametric sonde (Yellow Springs Instruments 600XLM V2). The sensors were calibrated before deployment and were placed at a depth of 0.3 m at the deepest area of the reservoir. Sensors were regularly cleaned, and the oxygen sensor was equipped with an automated wiper. No drifts in the measured values were noticed between sampling campaigns, and the data were verified by comparison with the values provided by surface water monitoring using the handheld sensors.

All studied Estonian lakes were equipped with a high-frequency monitoring platform or small lake buoy (OMC-7012 data-buoy) for the whole experimental period. Continuous monitoring of DO and T was performed with Ponsel OPTOD sensors, and CO_2 concentrations were monitored with AMT Analysenmesstechnik GmbH sensors. In all lakes, sensors were placed between 0.3 and 0.5 m depth, close to the surface. In Lake Võrtsjärv, additional pH data were collected with a multiparametric sonde (Yellow Springs Instruments 6600 V2–4) at one meter depth. In Erastvere, the installed lake buoy stopped recording the measurements from the end of June until the beginning of July, due to technical issues. The time series for the measured parameters in the Estonian lakes are presented in the Appendix figures (Appendix Figures A2–A4). Measured water temperature and air pressure data were used to calculate the real CO_2 partial pressure (pCO_2) and dissolved CO_2 concentration from the signals captured by the sensors, according to the manufacturer manuals (Laas et al., 2016).

4.3.4 Measurements of Hydrochemical Parameters

All lakes were sampled for chlorophyll-*a* ($\mu g\ L^{-1}$), total phosphorus (TP, $mg\ L^{-1}$), total nitrogen (TN, $mg\ L^{-1}$) and dissolved organic carbon (DOC, $mg\ L^{-1}$) concentrations (Supplementary Table A1 in Appendix A). For chlorophyll-*a* analysis, water samples were concentrated on Whatman GF/F glass microfiber filters, extracted with 90% acetone and measured spectrophotometrically. Chlorophyll-*a* concentrations were calculated using equations of Jeffrey and Humphrey (Jeffrey & Humphrey, 1975). TP concentrations were determined using the molybdenum blue spectrophotometric method (Hansen & Koroleff, 1999) after oxidation to phosphates (PO_4^{3-}) with potassium persulfate ($K_2S_2O_8$). TN concentrations were determined with the ultraviolet spectrophotometric screening method ((APHA) et al., 1999) after oxidation to nitrates (NO_3^-) with $K_2S_2O_8$. For DOC analysis, water samples were filtered through Whatman GF/F glass microfiber filters. DOC concentrations were determined using a Shimadzu TOC-V_{CPH} analyzer, and this method conforms to the European standard (CEN European Commitee for Standardization, 1997).

4.3.5 Data Analysis

The dissolved inorganic carbon (DIC, mM) and carbonate ([CO_3^{2-}], mM) concentrations were determined using T, pH, EC and TA to solve the carbonate equilibrium as implemented in the CO_2sys application (Lewis et al., 1998), using the constants for freshwater in Millero (1979).

The calcite saturation index (SI) was calculated as the logarithm of the saturation state of calcite (Ω) at the *in situ* temperature and conductivity:

$$SI = \log (\Omega)$$

$$\Omega = ([Ca^{2+}][CO_3^{2-}])/Kcal,$$

where Kcal is the apparent solubility product for calcite at the observed salinity and temperature (Mucci, 1983).

The rates of change of DIC, DO and TA in the incubation bottles (dDIC/dt, dDO/dt, dTA/dt, respectively) were calculated by dividing the change between final and initial conditions by the time period of incubation in each bottle. Changes in TA (dTA/dt) were used as a proxy for calcification, following the chemical equation of calcite precipitation whereby 2 equivalents of alkalinity are lost per mole of calcite that precipitates. Therefore, negative changes in alkalinity were divided by −2 to obtain the corresponding calcification rates. The precision of Ca^{2+} analyses (~0.3 ppm) was not high enough to use Ca^{2+} concentrations directly as an indicator of calcification, which is why we used the changes in TA as a proxy instead.

The 5 incubation bottles were treated as replicates. Therefore, the values for each variable were expressed as the mean and standard deviation (s.d.) of the 5 replicates. For each campaign, extreme outliers within the 5 replicates were removed from the dataset (5 outliers for alkalinity and 4 outliers for DO). NEP in the bottles was measured from dDO/dt.

4.4 Results

All lakes had a positive calcite SI, so calcite precipitation was theoretically possible, except for Valguta Mustjärv, a brown-water bog lake with low alkalinity and pH (Figure 4.1a). As for the other lakes, calcification, detected as a decrease in alkalinity during incubations, was found in all campaigns except for Lake Erastvere on 18.6.19 and Sau Reservoir on 16.5.18 (Figure 4.1b). Hereinafter, the analyzed dataset will be restricted to the seven campaigns with detectable calcification, i.e., those which showed a positive calcite SI as well as a decrease in TA.

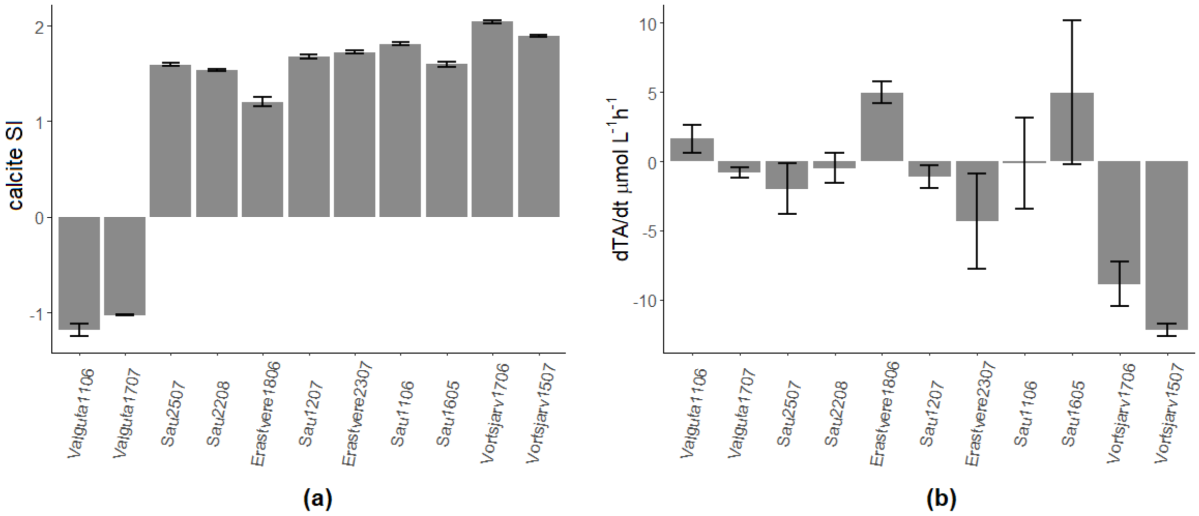

Figure 4.1. (a) Calcite saturation index (SI) in the 11 experimental campaigns. (b) Mean (± sd) rate of change of alkalinity. Numbers after the name of lakes represent the day and month of sampling; all lakes were sampled in 2019 except for Sau Reservoir in 2018.

In the seven campaigns that showed calcification, no significant direct effect of NEP on calcification was found (Figures 4.2a and Supplementary Figure A1a in Appendix A), nor was there a strong direct relationship between calcite saturation and calcification (Figure 4.2b). However, the ratio between calcification and NEP showed a strong power relationship with the calcite SI (Figures 4.3 and Supplementary Figure A1c in Appendix A).

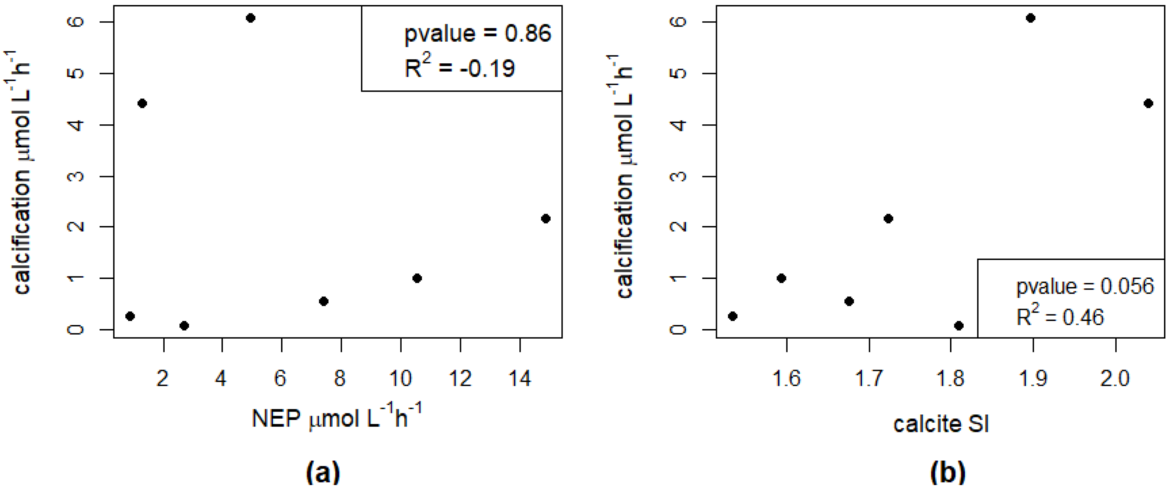

Figure 4.2. (a) Relationship between net ecosystem production (NEP) and calcification rates. (b) Relationship between calcite SI and calcification rates.

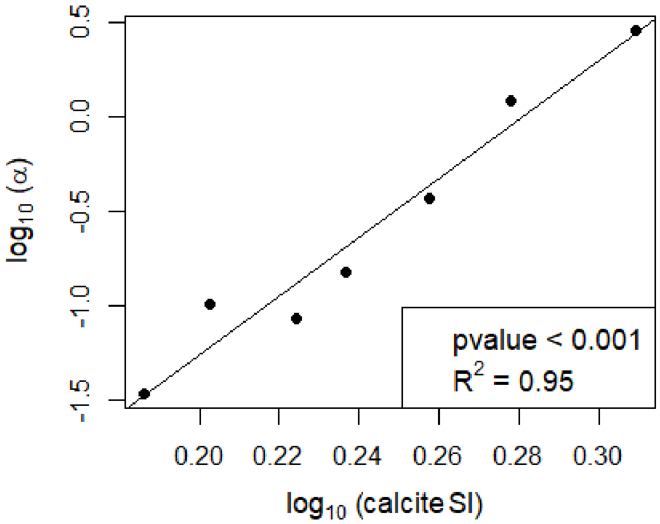

Figure 4.3. Relationship between the calcification to NEP ratio (α) and the calcite SI (note the log–log scale).

The calcification/NEP ratio (α) had a strong effect on the response of DIC to metabolic changes in oxygen, as shown by the relationship between α and the ratio between dDIC/dt and dDO/dt (dDIC/dDO, Figure 4.4). At α close to 0, DIC changes followed the metabolic signal with a dDIC/dDO ratio close to the expected value of −1 when metabolism dominates the DIC variability. At larger α (i.e., in conditions of higher calcification than expected by metabolism), the DIC change is greatly amplified, with a dDIC/dDO ratio up to ~−8.

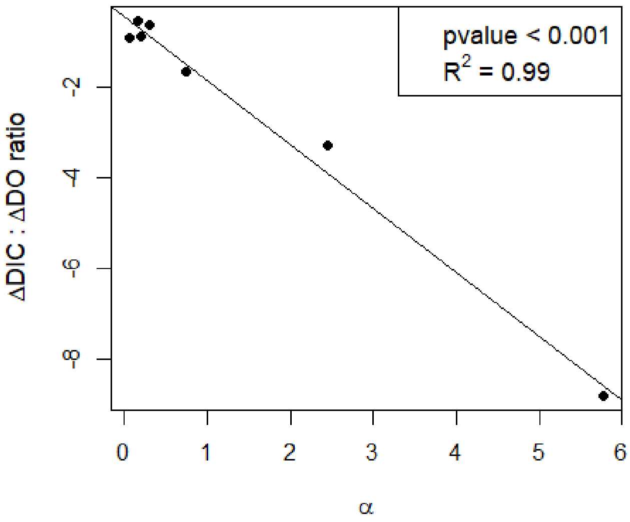

Figure 4.4. Relationship between the calcification/NEP ratio (α) and the response of dissolved inorganic carbon (DIC) to metabolism.

Surface water samples in Sau Reservoir revealed a decrease in calcium concentrations, likely caused by calcification, during the second half of the month of June and the first half of the month of July 2018 (Figure 4.5). During this period, the calcite saturation was at its highest value. The HFM measurements and surface samples for DO revealed a sudden increase in DO during the second half of June and then a second wave of increase during the first half of July, coinciding with the period of calcium decrease (Figure 4.5). This increase in DO resulted from peaks in primary production caused by the increase in temperature during this period (Figure 4.5), and this was also confirmed by increasing chlorophyll-a values (Supplementary Table A1 in Appendix A). From the number of calcium samples, it is unclear whether calcium decreased continuously during this period or followed the two peaks in DO (Figure 4.5). After this calcification period, no significant decrease in calcium concentrations was found despite the water being highly saturated in oxygen.

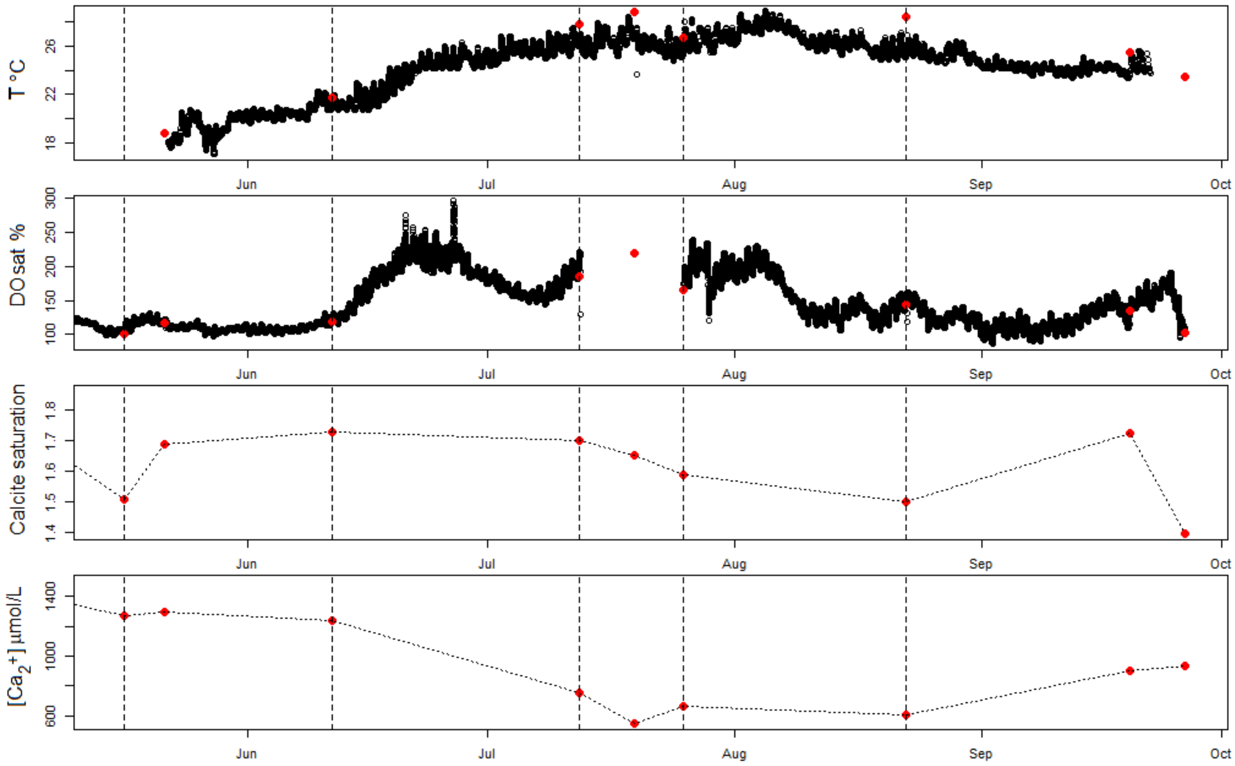

Figure 4.5. Seasonal evolution of surface water variables in Sau Reservoir. The high-frequency measurements of temperature (T) (in degrees Celsius) and dissolved oxygen saturation (DOsat) (dark points) are shown together with the spot surface water measurements (red points) of T, DOsat, calcite SI and [Ca2+] at the time of the campaigns.

4.5 Discussion

Alkalinity and NEP have been described as the two main parameters controlling calcification (Hamilton et al., 2009; Khan et al., 2020; Müller et al., 2016; Obst et al., 2009), yet they have mostly been studied independently. NEP increases pH, shifting the DIC equilibrium to saturation of carbonates, offering an ideal environment for calcite formation around nucleation points in the form of picocyanobacteria (Obst et al., 2009). Alkalinity implies availability of DIC and Ca^{2+}, enabling higher rates of calcification, with a threshold of 1 meq L^{-1}, below which calcification is unlikely to occur (Khan et al., 2020; Marcé et al., 2015; Müller et al., 2016).

Our results suggest that the effect of NEP on calcification is to be understood within the larger context of a lake's physicochemical properties, especially in relation to its equilibrium with calcite. At short-term scales, NEP alone is not sufficient to explain the observed calcification (Supplementary Figure 2A in Appendix A). However, it is the ratio between calcification and NEP (α) which is predictable from the calcite SI (Figure 4.3). Similarly, the relationship between calcite saturation alone and calcification is not significant (Figure 4.2B) and is much weaker than when considering its effect on α (Figure 4.3). Therefore, the short-term effect of NEP on calcification can be comprehended within the context of calcite saturation which depends on processes that act at a longer time scale. In the incubation bottles, we observe the changes in NEP at an hourly scale, and the short-term effect of primary production within the bottles is therefore evident. However, the calcite saturation in these bottles corresponds to a legacy of biogeochemical processes that occur in the lakes at a larger temporal scale than the time frame of the incubations. Changes in calcite saturation in lakes are reported to be gradual and follow a seasonal pattern with higher saturation from spring to fall with the peak during summer, coinciding with the rise in temperature and primary production (Trapote et al., 2018).

The higher calcite saturation during summer, together with the summer peak in NEP, is likely the main cause for the seasonal variation in calcification. In Sau Reservoir, calcification seems to have occurred during the peak of primary production and during the higher calcite saturation found in June–July that came along with rising temperatures (Figure 4.5). During this period, the calcium concentration decreased, likely as a result of calcification (Figure 4.5). The potentiating role of calcite saturation in the effect of NEP on calcification can result in intense calcification events extending usually from a few days to a few weeks, which are sometimes visible in the form of whitening events which have a big impact on the carbon budget of a lake (Strong & Eadie, 1978). Our results suggest that such events are triggered by peaks of production and that the intensity of such events is likely caused by a gradual departure from the calcite equilibrium until it reaches a state that strongly potentiates the calcification-inducing factor of NEP. Within the context of global change, these results suggest that calcification is likely to be further promoted by anthropogenic stressors that affect the trophic state of a lake. The increase in dissolved phosphorus and nitrogen in water bodies, caused by human activities such as agriculture or urban activities including industry or sewage water treatment plants (Carpenter et al., 1998), is causing an increase in eutrophication and in the proliferation of cyanobacterial blooms (Conley et al., 2009). Such conditions can trigger intense calcification, especially in systems that are highly saturated in calcite. In turn, such events can improve the trophic state of eutrophic lakes due to co-precipitation with phosphates, acting as a self-remediation mechanism (Walsh et al., 2019). Furthermore, eutrophication can cause dramatic changes in the phytoplankton community due to shifts in the trophic state (Hsieh et al., 2010). Hence, the resulting change in planktonic size spectra may affect the availability of nucleation sites that trigger calcification (Dittrich et al., 2004; Dittrich & Obst, 2004; Obst et al., 2009). This is an added complexity which is beyond the scope of this study. Disentangling the effect of nucleation, metabolism and calcite saturation should be addressed in further research. Besides increasing eutrophication, global change may promote calcification through its effect on water alkalinity. An increase in the export of alkalinity due to global change has been observed in large Russian Arctic rivers, as well as in North America’s largest river, the Mississippi (Drake et al., 2018; Raymond & Cole, 2003). A rise in water alkalinity would increase the calcite SI, which is directly dependent on alkalinity, among other variables. This would potentiate the calcification-inducing effect of NEP, allowing for higher rates of calcite precipitation. In this view, global change could possibly enhance calcite precipitation events, both in frequency and intensity, due to the increase in eutrophication and water alkalinity. However, more research is needed to confirm this hypothesis.

Most campaigns showed potential calcification, except both campaigns at Valguta Mustjärv, Lake Erastvere on 18.6.19 and Sau Reservoir on 16.5.18 (Figure 4.1). Valguta Mustjärv is a humic (DOC 41 mg L^{-1}), brown-water bog lake with low alkalinity (Supplementary Table A1 in Appendix A) and low pH (6.8) and was therefore undersaturated in calcite. The main reason for including this lake in this study was to observe the absence of calcification expected from such low-alkalinity lakes. The HFM time series of DO saturation for this lake shows a peak of production shortly before the first incubation campaign (Supplementary Figure A2 in Appendix A). However, despite this peak of production, calcification did not occur because of the undersaturation in calcite. Lake Erastvere on 18.6.19 had the lowest calcite SI among the campaigns with positive calcite saturation (Figure 4.1a), combined with relatively low values in chlorophyll-*a* and NEP (Supplementary Table A1 and Figure A1a in Appendix A), which is likely the reason for the absence of calcification. Looking at the HFM time series of DO and

T, it appears that the timing of the two campaigns did not coincide with the peaks of production in this lake (Supplementary Figure A3 in Appendix A), which could be another explanation for the lack of calcification during this campaign. As for the medium-alkalinity Sau Reservoir on 16.5.18, the relatively low calcite SI (Figures 4.1a and 4.5) and NEP (Supplementary Figure A1a in Appendix A) could be potential reasons for the absence of measurable calcification. The HFM time series of DO indicate that this reservoir was the least productive during this campaign because the timing (Spring) was too early to capture the strong summer rise in production that started in the month of June (Figure 4.5). In this reservoir, two campaigns show the lowest rates of calcification (11.6.2018 and 22.8.2018, Figure 4.1b). In the first case (11.6.2018), the campaign occurred during the initial stage of the summer rise in production and therefore DO saturation was still relatively low compared to the following period (Figure 4.5). The second case (22.8.2018) corresponds to a period where calcite SI strongly decreased, likely because of the calcification that occurred previously (Figure 4.5). As for the two campaigns (12.7.2018 and 25.7.2018) that were held between these two cases during the month of July, the clear occurrence of calcification is justified by the high values in DO and in calcite SI during this period. This further supports the explanation that calcification depends simultaneously on two parameters, namely, NEP and calcite saturation. The two highest rates of calcification were both observed in Võrtsjärv, which had the highest calcite SI among all the campaigns (Figure 4.1). The lake was saturated in DO during both campaigns, suggesting high production (Supplementary Figure A4 in Appendix A). Here, again, the high rates of calcification are explained by the high values of NEP and calcite SI observed in this lake.

The α ratio has been described as a function of alkalinity (Khan et al., 2020; McConnaughey & Whelan, 1997). Our results suggest that it is rather a function of the calcite SI. While calcite saturation is dependent on alkalinity, it is also dependent on other variables such as T, EC and pH and is therefore a better predictor for calcification than alkalinity alone. In marine studies, α has been reported at a value of 1.67 in coral reefs (J.-P. Gattuso et al., 1999; Suzuki, 1998). Calcite saturation in the pelagic ocean is much more constant than in lakes, with an average value of 1.5 for the Central Pacific Ocean water column (Lerman & Mackenzie, 2018). In contrast, lakes exhibit large variations in calcite SI and can have much higher values. In Lake Constance, variations in calcite SI between 1 and 9 have been reported (Stabel, 1986). Our results suggest that at the higher calcite SI commonly found in lakes, α can reach much more extreme values than the ones reported in marine studies, and therefore pelagic calcification rates in lakes can largely exceed those found in the ocean. The α ratio in lakes has only rarely been reported and appears to range between 0 and 1 (McConnaughey & Whelan, 1997). However, its power relationship with calcite saturation results in ratios of up to nearly 3:1 for Lake Võrtsjärv on 15.7.2019 (Figure 4.4). Therefore, the effect of NEP on calcification is strongly potentiated at higher calcite saturation.

This potentially strong effect of NEP on calcification has important implications for DIC dynamics of lakes and the way we relate calcification to metabolic processes. Besides acting as a C sink in the sediments (Khan et al., n.d.), calcification releases CO_2, thereby promoting CO_2 supersaturation and CO_2 emissions to the atmosphere (Kalokora et al., 2020; Ridgwell & Zeebe, 2005). Due to its association with NEP, calcification is reported to be a cause of the deviations from the 1:1 metabolic stoichiometry between DO and DIC that is often reported in lakes (R. M. Andersen et al., 2019; Khan et al., 2020; Obrador & Pretus, 2013; Vachon et al., 2020). It is not uncommon to find lakes that are simultaneously emitting CO_2 while being in an autotrophic state [8,20,30]. Our results suggest that these deviations are correlated to α (Figure 4.4), and we show that this is not only relevant in hard water lakes as commonly assumed, but also in lakes of alkalinity above 1 meq L^{-1}, as suggested by Marcé et al. (2015) (Marcé et al., 2015). When α is close to 0, DIC dynamics follows the classical 1:1 metabolic stoichiometry with DO. At higher α, the overall stoichiometry changes, with larger differences in DIC per mole of oxygen produced. This is clearly visible in Lake Võrtsjärv on 15.07.2019, where the DIC/DO ratio reaches nearly −8 (Figure 4.3). Such deviations caused by calcification are not accounted for in lake models, potentially causing discrepancies between theoretical models and empirical data and biasing our understanding of lake ecosystems through modeling (Finlay et al., 2019; Lu et al., 2018). Understanding and quantifying the interplay between calcification, NEP and calcite saturation may provide a more realistic parameterization of lake carbon budget models under different eutrophication scenarios.

4.6 Conclusions

Calcification is driven simultaneously by the long-term effects of calcite saturation and the short-term effects of NEP, whereby calcite saturation strongly potentiates the NEP-induced calcification effect. This would explain the seasonal and episodic nature of pelagic calcification in lakes, whereby calcification occurs when the level of calcite saturation is high enough to trigger this process usually during the summer season, allowing high rates of calcite precipitation to be induced by NEP. Considering human stressors that increase lake eutrophication, calcification is likely to be intensified with the increase in summer production rates. Our results also suggest that the strong effect found between calcite saturation and α provides a mechanistic relationship that could be used to parameterize this process in lake models. This way, the calcite SI is an indicator of the NEP-induced calcification effect and the resulting deviations from the metabolic 1:1 stoichiometry between DO and DIC.

Chapter 5

5. Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes

Original publication (Appendix A):

Khan, H., Laas, A., Marcé, R., Obrador, B. 2020. Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes. Ecosystems 23: 1566-1580. DOI: 10.1007/s10021-020-00488-6

5.1 Abstract

Several findings suggest that CO_2 emissions in lakes are not always directly linked to changes in metabolism but can be associated to interactions with the dissolved inorganic carbon equilibrium. Alkalinity has been described as a determining factor in regulating the relative contributions of biological and inorganic processes to carbon dynamics in lakes. Here we analyzed the relationship between metabolic changes in dissolved oxygen (DO) and dissolved inorganic carbon (DIC) at different time scales in eight lakes covering a wide range in alkalinity. We used high frequency data from automatic monitoring stations to explore the sensitivity of DIC to metabolic changes inferred from oxygen. To overcome the problem of noisy data, commonly found in high frequency measurements datasets, we used Singular Spectrum Analysis to enhance the diel signal to noise ratio. Our results suggest that in most of the studied lakes, a large part of the measured variability in DO and DIC reflects non-metabolic processes. Furthermore, at low alkalinity, DIC dynamics appear to be mostly driven by aquatic metabolism, but this relationship weakens with increasing alkalinity. The observed deviations from the metabolic 1:1 stoichiometry between DO and DIC were strongly correlated to the deviations expected to occur from calcite precipitation, with a stronger correlation when accounting also for the benthic contribution of calcite precipitation. This highlights the role of calcite precipitation as an important driver of CO_2 supersaturation in lakes with alkalinity above 1 meq L^{-1}, which represents 57% of the global area of lakes and reservoirs around the world.

5.2 Introduction

Considering that lakes occupy a relatively small area in the global landscape, they are disproportionally active sites for carbon cycling due to their important roles in carbon transport, transformation and storage (Battin et al., 2009; Cole et al., 2007; Tranvik et al., 2009). Globally, most lakes are supersaturated in CO_2 and act as carbon sources to the atmosphere (Sobek et al., 2005). The carbon emitted by these ecosystems is relevant and comparable in magnitude to global terrestrial net ecosystem production (Tranvik et al., 2009). The CO_2 emissions from lakes have widely been attributed to their heterotrophic activity that oxidizes terrestrially produced carbon (Del Giorgio et al., 1999; Duarte & Prairie, 2005; Jonsson et al., 2003; Larsen et al., 2011; Roehm et al., 2009). Many studies that directly link CO_2 supersaturation to net heterotrophy are conducted in lakes that have relatively low alkalinity and high dissolved organic carbon (DOC) values. While this paradigm is often valid for such systems, it has recently been rejected for lakes and reservoirs of higher alkalinity. A study that relates CO_2 emissions to lake metabolism in nineteen lakes of higher alkalinity shows that despite a positive relationship between CO_2 emissions and respiration of organic matter, CO_2 emissions often exceed the rates expected from lake metabolism (Sand-jensen & Staehr, 2009). Several findings suggest that in such systems, major drivers of carbon dynamics include direct exogenous dissolved inorganic carbon (DIC) inputs from soil respiration and mineral weathering of the catchment, interactions with the DIC equilibrium, and calcite precipitation (Maberly et al., 2012; Marcé et al., 2015; McDonald et al., 2013; Weyhenmeyer et al., 2015; Wilkinson et al., 2016).

The chemical equilibrium between photosynthesis and respiration implies a balance between inorganic carbon variability and net ecosystem production (NEP), whereby one mole of O_2 respired produces one mole of DIC, and inversely, one mole of DIC is photosynthesized into one mole of O_2 (Stumm & Morgan, 1996). Imbalances between inorganic carbon variability and metabolic rates resulting in deviations from the one to one stoichiometry between dissolved oxygen (DO) and DIC in lakes are widely reported in the literature (López et al., 2011; McDonald et al., 2013; Peeters et al., 2016; Perga et al., 2016; Stets et al., 2009)(Peeters et al., 2016)(Peeters et al., 2016)(Peeters et al., 2016). Such imbalances have in some cases reached a point where lakes are simultaneously in an autotrophic state, yet they emit CO_2 to the atmosphere (Marcé et al., 2015; Peeters et al., 2016; Perga et al., 2016; Stets et al., 2009). According to a recent model, this could be the case for approximately 35% of the lakes and reservoirs in the contiguous United States (McDonald et al., 2013). Hydrologic inputs and CO_2 released by calcite precipitation are likely to be the main factors driving CO_2 emissions in such lakes (Marcé et al., 2015; McDonald et al., 2013; Perga et al., 2016; Stets et al., 2009). A recent study shows that the sensitivity of CO_2 supersaturation to DO disequilibrium weakens with increasing alkalinity and that above an alkalinity threshold of 1 meq L^{-1} it is possible to find lakes that are simultaneously supersaturated in CO_2 and DO (Marcé et al., 2015). This implies that above this alkalinity threshold CO_2 supersaturation in lakes is directly regulated by DIC inputs from carbonate weathering and calcite precipitation, and that this is a widespread phenomenon since 57% and 34% of the global area occupied by lakes and reservoirs have alkalinity values above 1 and 2 meq L^{-1} respectively (Marcé et al., 2015).

In such lakes, summer DIC depletion due to photosynthesis comes largely at the expense of alkalinity, with only minimal changes in pCO_2 and pH (Mcconnaughey et al., 1994). An explanation lies in the biogenic process of calcite precipitation that is enhanced during summer stratification, when algal blooms increase pH through photosynthesis and offer abundant nucleation sites around the surface of micro-algae (Müller et al., 2016). Summer calcification reduces alkalinity and calcium levels, generating an equal molar quantity of CO_2, thereby compensating photosynthetic CO_2 depletion and maintaining CO_2 partial pressure (pCO_2) values within bounds that sustain primary production (R. M. Andersen et al., 2019; Mcconnaughey et al., 1994). This mechanism is less relevant in lakes of low alkalinity where calcite precipitation is unlikely to occur at high rates and therefore primary production is potentially carbon limited because of the lack of inorganically produced CO_2 (Kragh & Sand-Jensen, 2018). The mass balance equation of calcite precipitation implies the release of one mole of CO_2, yet it results in a net loss of 1 mole of DIC and two equivalents of total alkalinity (TA) (Equation 1.1 in general introduction).

A recent study on a lake of relatively high alkalinity (4.5 meq L^{-1}) shows that about 40-70% of sediment organic carbon originates from alkalinity export from the catchment, suggesting that primary producers actively take up DIC of terrestrial origin, promoting calcite precipitation and turning such lakes into "alkalinity sinks" during summer (Nõges et al., 2016). Despite acting as an alkalinity sink, it is widely acknowledged that calcification promotes CO_2 supersaturation of surface waters by releasing CO_2 (Michel Frankignoulle et al., 1994, 1995; J.-P. Gattuso et al., 1993, 1999; Jean-Pierre Gattuso et al., 1996; Opdyke & Walker, 1992; Suzuki, 1998). Therefore, summer calcification could be an explanation for the deviation from the 1:1 stoichiometry between DO and DIC variability commonly found in lakes of high alkalinity. However, other processes could result in such deviations, such as anaerobic respiration (Stumm & Morgan, 1996; Torgersen & Branco, 2007; Wissel et al., 2008), physical processes such as gas exchanges with the atmosphere (Wissel et al., 2008) or horizontal and vertical gas exchanges with nearshore or deeper waters (Antenucci et al., 2013), as well as direct DIC inputs caused by storm events (Vachon & del Giorgio, 2014). While the importance of calcite precipitation in CO_2 levels and emissions is well documented in marine systems, it is yet to be recognized as an important component of the carbon cycle in freshwater systems.

The objective of this research is to assess the sensitivity of DIC variability to metabolic changes in DO along a gradient of alkalinity. For this we use high frequency data of dissolved oxygen, pCO_2, pH and temperature from lakes covering a wide range in alkalinity. A theoretical model that accounts for the effect of calcite precipitation on the DO – DIC relationship is used as a comparison to the empirical data to highlight the importance of this process as a driver of carbon dynamics in lakes. The overarching aim of this study is thus to determine the main drivers of DIC dynamics along an alkalinity gradient. Our hypothesis is that lake metabolism is the main driver of carbon dynamics in lakes of low alkalinity but that with increasing alkalinity the importance of this driver becomes relatively low compared to other processes such as calcite precipitation.

5.3 Methods

5.3.1 Study sites

The data for this study was collected from eight Estonian lakes during the summer period from July to September in 2014 and is available in open access (Laas & Khan, 2019). The studied lakes include Lake Peipsi, Võrtsjärv, Saadjärv, Lake Ülemiste, Mullutu Suurlaht, Lake Erastvere, Äntu Sinijärv, and Valguta Mustjärv (Table 5.1). Based on their area, conductivity, thermal stratification, alkalinity, chloride content and color, each of these lakes belong to different lake types (Ministry of the Environment 2009) according to the European Water Framework Directive (European Union, 2000). Total alkalinity ranges from 0.5 to 4.8 meq L^{-1}. The lakes also cover a variety of trophic states, ranging from mesotrophic to hypertrophic states according to the OECD trophic state classification (Caspers, 1984). In addition, they differ substantially in their water retention time as well as in their catchment characteristics, especially in regard to land use. Each of these lakes have been described in detail in previous studies (Cremona et al., 2016; Laas et al., 2016; Rõõm et al., 2014).

Table 5.1. Characteristics of the studied lakes. Average values from the measurements and earlier data.

lakes	Trophic state	Mixing regime	Area (ha)	Mean depth (m)	Max depth (m)	Chl-*a** (µg L^{-1})	Alkalinity* (meq L^{-1})	DOC* (mg L^{-1})	Kd** (m)	Watershed size (km^2)	Water residence time (y^{-1})
Valguta Mustjärv	hypertrophic	polymictic	20.4	<1	1	23.19	0.500	35.2	10.34	1.34	source lake
Erastvere	hypertrophic	dimictic	16.3	3.5	9.7	125.64	1.632	12.3	2.96	5.2	0.5
Mullutu Suurlaht	eutrophic	polymictic	412.7	<1	1.7	9.04	1.800	18.1	0.58	238	0.2
Saadjärv	mesotrophic	dimictic	724.5	8	25	5.62	2.466	9.2	0.42	28.4	0.13
Peipsi	eutrophic	polymictic	261100	8.3	12.9	13.4	2.799	12	1.6	47800	2
Ülemiste	eutrophic	polymictic	944	2.5	4.2	24.7	3.299	13.7	3.5	98.8	0.33
Võrtsjärv	eutrophic	polymictic	27000	2.8	6	35.71	3.614	11.8	2.76	3116	1
Äntu Sinijärv	alkalitrophic	polymictic	2.1	6	8	1	4.800	4.72	0.25	1.37	source lake

* depth-integrated averages

** vertical light attenuation coefficient

5.3.2 Monitoring stations and measured parameters

All lakes were continuously monitored between 6 to 12 full days using a high frequency measurement platform or a small lake buoy (OMC-7012 data-buoy). In most lakes, the automated stations were installed near the deepest point, except in lake Peipsi, where it was placed in Mustvee bay, approximately 1 km from the western shore because of security reasons. A multiparametric sonde (Yellow Springs Instruments 6600 V2-4) was used for continuous measurements of DO concentration, pH and water temperature at 0.5 or 1-meter depth in all lakes. The multiparametric sonde was equipped with an in-built cleaning system for optical DO sensor, whereas other sensors were manually cleaned on a weekly basis. Dissolved CO_2 was measured at 0.5 or 1-meter depth using membrane covered optical CO_2 sensors (AMT Analysenmesstechnik GmbH) with measuring ranges of 30 mg L^{-1} and 80 mg L^{-1}. Measured water temperature and air pressure data was used to calculate the real pCO_2 and dissolved CO_2 concentration from the signals captured from the sensor, according to manufacturer manuals (Laas et al., 2016). A calibration of all sensors was performed before and after the deployment. For data collection and storage, all platforms were equipped with OMC-045-II GPRS data loggers. Sensors were automatically measuring at a time interval of 10 or 30 minutes depending on power availability.

5.3.3 Data analysis

For each lake, an alkalinity value for each time step was calculated using pCO_2 and pH as input parameters for the CO_2sys program (Lewis et al., 1998) following Millero (1979). These were then averaged to obtain the mean alkalinity value for each lake and sampling period. Dissolved inorganic carbon (DIC) was calculated for each time step using pH and the average alkalinity as input parameters in CO_2sys. Alkalinity had to be averaged to avoid unrealistic DIC values due to the large errors in alkalinity propagated by the limited precision of the field pH sensors (typically ~0.2 pH units). Using average alkalinity and pH as inputs resulted in cleaner and more realistic DIC values compared to other alternatives of calculating DIC (e.g., using CO_2 and pH as inputs).

After obtaining the DIC time-series, the next step was to identify the presence of a 24-hour metabolic signal for DO and DIC. This was done by performing a continuous wavelet-based analysis using the R (R Development Core Team, 2015) “biwavelet” package (Gouhier et al., 2015). Wavelets can be used to identify time-dependent structures in time-series, in this case the diel metabolic pattern known to occur for DO and DIC concentrations (Hanson et al., 2006) (see supplementary material). As a support to the wavelet analysis, periodograms for DO and DIC concentrations were made using the time series analysis “TSA” package in R (Chan & Ripley, 2018). Periodograms offer a more general view of the main time dependent structures within an entire time series.

Then we assessed how DIC variability relates to DO variability by testing the sensitivity of DIC to changes in DO. Bivariate wavelets were used to identify common time periods at which DO and DIC oscillate, and how these common periods evolve during the entire length of the time series. Beside indicating common oscillations between two time-series, cross-wavelets also examine if there’s a consistent phase relationship between the series, which is suggestive of causality between the two time series (Grinsted et al., 2004) (see supplementary material). In case of common oscillations between DO and DIC, one would expect an anti-phase relationship because the metabolic reaction implies that an increase in one leads to a decrease of the other, and vice-versa. Because of the large amount of noise and random signals that are not related to the metabolic diel signal, we decided to enhance the signal to noise ratio by isolating the 24h-frequency oscillations of DO and DIC from all other signals. To do so, we used the Singular Spectrum Analysis “SSA” R package (Zhao, 2016). SSA is a tool that allows identifying and extracting fundamental oscillatory components from an original time series (see supplementary material). The R code that was used in this analysis is published and freely available (Khan, 2019). Once the 24-hours diel cycle components were identified, they were grouped and reconstructed into a new time series following Golyandina & Korobeynikov (2014). This method efficiently isolates the diel seasonal signal from a time series, even if it is weak or if the time series is noisy (see supplementary Figure S5 and Figure S6 for comparison of a diel signal extracted from a clean versus noisy time series). For lake Äntu Sinijärv, the diel signal in DIC was very weak and had to be extracted by performing a Multivariate Singular Spectrum Analysis (MSSA) using the isolated diel signal of DO and the original time series of DIC as inputs. DIC and DO rates of change (dDO/dt and dDIC/dt) were calculated form the signal enhanced series and expressed in $\mu mol\ L^{-1}\ min^{-1}$. Therefore, corrections for air-water gas exchanges are not necessary because the rates are calculated from isolated metabolic signals that have been removed from the effect of any non-metabolic process, including air-water gas exchanges.

Linear regressions were used to test the effect of dDO/dt on dDIC/dt in all lakes. While all these relationships were highly significant, the main parameter of interest was the slope of the relationship. A theoretical slope of ~-1 is expected from the stoichiometry of aerobic respiration and photosynthesis (Stumm & Morgan, 1996; Torgersen & Branco, 2007). The slope of -1 is an estimation that can be applied only in aerobic conditions without anaerobic respiration or other processes that can affect DIC concentrations such as calcite precipitation or direct DIC inputs following a storm event (Stumm & Morgan, 1996; Vachon & del Giorgio, 2014). All measurements were taken from surface waters where the effect of anaerobic respiration on DIC concentrations is not relevant according to the depth distributions of temperature, DO and CO_2 reported for each lake during the same period of measurements in a previous study (Laas et al., 2016). However, calcite precipitation could influence DIC concentrations in these lakes. Therefore, deviations from the theoretical -1 metabolic slope were interpreted with a focus on calcite precipitation.

The final step was to assess how alkalinity affects the sensitivity of DIC to metabolism. To do so, the slopes of the linear regression models were plotted against the alkalinity for all studied lakes. We compared the empirical slopes with the theoretical slopes under the presence of reactions with calcite. For each lake we calculated a theoretical time series of dDIC/dt that accounts for the effect of precipitation and dissolution of carbonates on dDIC/dt following a simple model:

Equation 5.1: $$dDIC/dt_{theoretical} = -dDO/dt - \alpha\, dDO/dt$$

Where α is the molar ratio between precipitation and dissolution of carbonates and NEP (McConnaughey & Whelan, 1997). The values of α were calculated according to three different hypotheses (Table 5.2). The first hypothesis assumes that α=0 which corresponds to a system with no calcite precipitation, i.e. where all changes in DIC are explained by aerobic metabolism. The second hypothesis assumes an alkalinity-dependent value for α, using the same values as suggested in Marcé and others (2015), whereby α = 0 at alkalinity below 1 meq L^{-1} (McConnaughey & Whelan, 1997), α = 0.2 at alkalinity between 1 and 2.23 meq L^{-1}, and α = 0.6 at alkalinity above 2.23 meq L^{-1}. The third hypothesis assumes these same alkalinity-dependent values for α with an additional correction for the benthic fraction (BF) of total primary production in the lakes. By doing this correction, the α parameter is able to account for pelagic calcite precipitation as well as benthic calcification by calcifying macrophytes. To do this, we used the percentage of benthic primary production of each lake obtained from a previous study on these same lakes that was conducted during the same period of study as the data used here (Cremona et al., 2016). We assumed benthic production to be composed of calcifying macrophytes, based on previous studies of these eight lakes (Cremona et al., 2016; Laas et al., 2016). An approximate 1:1 calcification to photosynthesis ratio in such organisms have been reported (McConnaughey, 1991; Mcconnaughey et al., 1994; McConnaughey & Whelan, 1997). Therefore, we attributed an α value of 1 to the benthic production and corrected the α values from Marcé and others (2015) according to the ratio between pelagic primary production and benthic primary production.

Table 5.2. α values and α corrections for benthic fraction (BF) used to build the expected slopes of the dDO/dt – dDIC/dt relationships.

Lake	Alkalinity (meq L^{-1})	BF (%) *	Hypothesis 1 α value	Hypothesis 2 α value **	Hypothesis 3 α value	Hypothesis 1 theoretical slope	Hypothesis 2 theoretical slope	Hypothesis 3 theoretical slope
Valguta Mustjärv	0.500	NA	0	0.0	0.00	-1.0	-0.86	-0.86
Erastvere	1.632	10	0	0.2	0.21	-1.0	-1.31	-1.33
Mullutu Suurlaht	1.800	46	0	0.2	0.57	-1.0	-0.85	-1.59
Saadjärv	2.466	26	0	0.6	0.70	-1.0	-1.55	-1.75
Peipsi	2.799	10	0	0.6	0.64	-1.0	-1.47	-1.55
Ulemiste	3.299	10	0	0.6	0.64	-1.0	-1.43	-1.51
Võrtsjärv	4.425	4	0	0.6	0.62	-1.0	-1.77	-1.81
Äntu Sinijärv	4.800	94	0	0.6	0.98	-1.0	-1.09	-1.85

* (Cremona et al., 2016)
** (Marcé et al., 2015)

At this point one must remember that the observed dDIC/dt values come from DIC values that were calculated using a constant average alkalinity. While the diurnal changes in alkalinity are too small to be accurately estimated by using CO_2 and pH coming from field probes, assuming a constant alkalinity value tends to underestimate the changes in DIC, as it omits changes in the chemical equilibrium of the carbonate system. To overcome this limitation, we calculated a corrected observed dDIC/dt that accounts for calcite reactions:

$$\text{Equation 5.2:}\quad dDIC/dt_{corrected} = dDIC/dt - \alpha\, 2\, dDIC/dt$$

where α is the calcification parameter in Equation 5.1. This procedure is thus generating a corrected observed dDIC/dt dataset (and thus an observed slope between dDIC/dt and dDO/dt) which is dependent on the three hypotheses used for assigning values to α. Note that for Hypothesis 1 α equals 0 and $dDIC/dt_{corrected}$ equals dDIC/dt. The slopes $dDIC/dt_{corrected}$ vs. dDO/dt and $dDIC/dt_{theoretical}$ vs. dDO/dt were calculated using linear regression and compared by plotting them along the alkalinity gradient. The rationale of the analysis is that the theoretical slopes and the observed slopes (using $dDIC/dt_{corrected}$) will agree only in case of using the appropriate hypothesis for the calcite reactions (α).

5.4 Results

5.4.1 Presence and strength of metabolic signals in DO and DIC dynamics

The presence of a metabolic signal was assessed using periodograms which estimate the spectral density of a time series and shows what are the dominating frequencies of oscillation composing it. The dominating spikes at 24-hour frequency indicate a clear diel metabolic signal (24-hours pattern) for DO and DIC in most lakes, except in Äntu Sinijärv, Saadjärv and lake Ülemiste where the diel signals are weak resulting in a shorter spike at the 24-hour frequency mark (Figure 5.1, Figure 5.2). The periodograms showed an important number of signals at other frequencies that didn't correspond to the metabolic diel pattern. While the spectral density of these non-metabolic signals was relatively low, their relevance was amplified when using rates of change (dDO/dt and dDIC/dt) rather than raw concentrations, especially the noisy signals at short frequencies (see Supplementary Figure S1, Figure S2 in Appendix A).

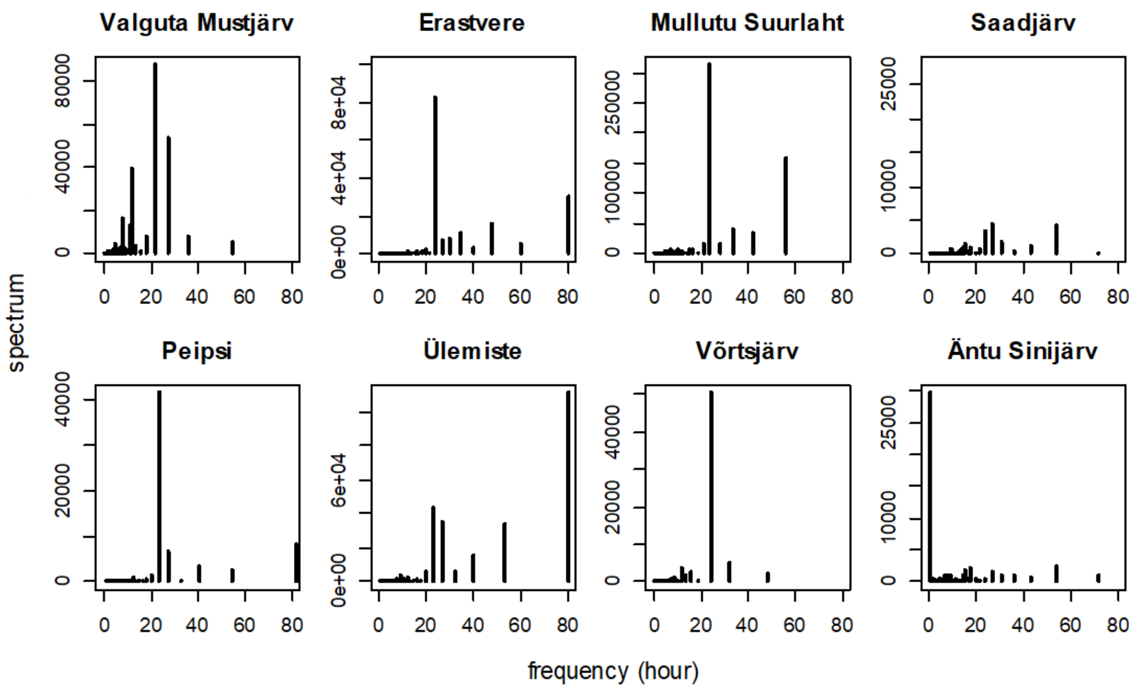

Figure 5.1. Periodograms for dissolved oxygen (DO) in the studied lakes. The x-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated. The y-axis corresponds to the spectral density or power spectrum, with higher values indicating important features of the signal at a given frequency. Notice the dominating spikes at 24-hour frequency

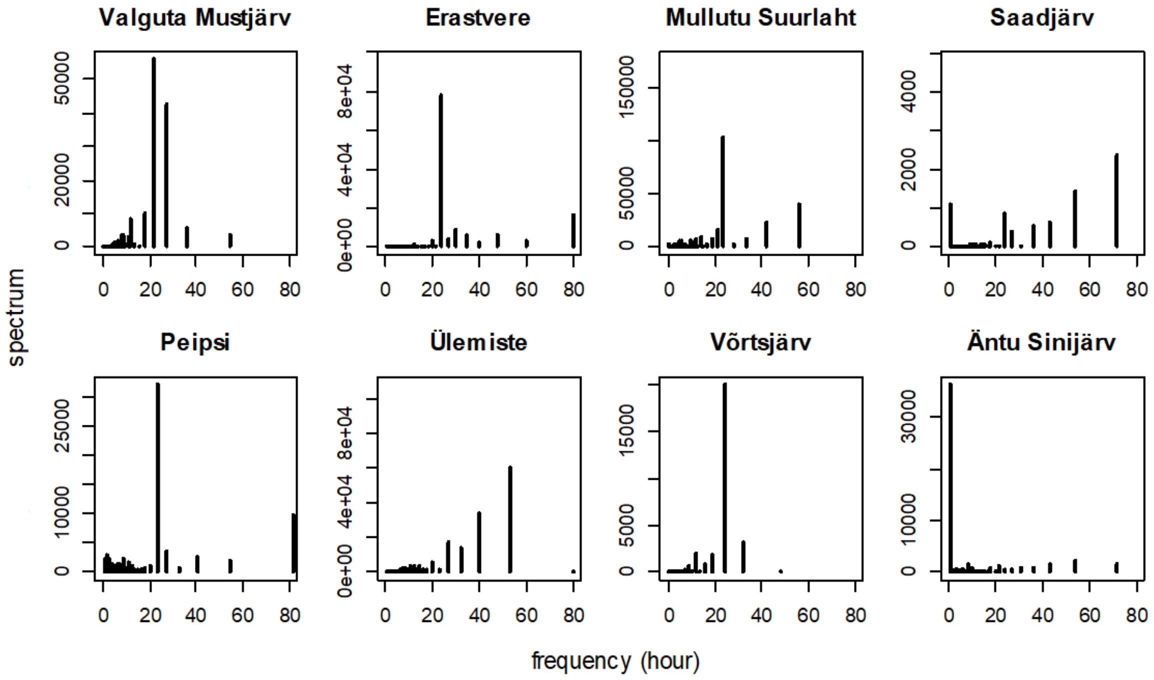

Figure 5.2. Periodograms for dissolved inorganic carbon (DIC) in the studied lakes. The x-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated (y-axis). The y-axis corresponds to the spectral density or power spectrum, with higher values indicating important features of the signal at a given frequency. Notice the dominating spikes at 24-hour frequency

5.4.2 Relationship between DIC and DO variability

Cross-wavelets were used to identify at what time-period synchronized oscillations can be found between DO and DIC and the phase of these synchronizations. Due to the metabolic stoichiometry between DO and DIC, an anti-phase synchronization between both variables is to be expected at the 24-hour frequency, whereby an increase in DO results in a decrease in DIC, and vice versa. This was apparent for most of the lakes in the cross-wavelets, where a “cloud” of arrows pointing to the left indicate a relatively strong anti-phase synchronization around the 24-hour period (Figure 5.3). While this diel synchronization in oscillation was relatively strong in some lakes (Valguta Mustjärv, Erastvere, Mullutu Suurlaht, Peipsi and Võrtsjärv), it was weak in others (Saadjärv, Ülemiste and Äntu Sinijärv), causing disruptions in the synchronicity between DO and DIC along the time-series (Figure 5.3). The lack of coupling was most obvious in lake Äntu Sinijärv. All lakes showed a relatively important amount of significant coupling in oscillation at time periods shorter than 24 hours (Figure 5.3). These common oscillations ranged from time-periods of less than one hour up to several hours and could be caused by water movements caused by convection, minor mixing events or seiches, as well as noise resulting from the sensitivity thresholds of the sensors.

The presence of non-metabolic signals in DO and DIC that were oscillating at other frequencies than the metabolic 24-hour frequency resulted in poor relationships between dDO/dt and dDIC/dt (Supplementary Figure S3 in Appendix A). However, using the isolated metabolic diel signals to calculate dDO/dt and dDIC/dt (Supplementary Figure S4 in Appendix) resulted in much stronger relationships between the two (Figure 5.4). These relationships were significant for all lakes, with substantial differences in slope between lakes.

In most of the lakes, the slopes of these relationships were close to the ones found by performing linear regression models on dDO/dt and dDIC/dt obtained from the raw data, yet the R^2 values dramatically increased (Table 5.3). However, the slope for this relationship in Saadjärv turned from positive to negative. Considering the low R^2 value (0.05) of the relationship using the raw data and its high increase (R^2=0.91) when using the diel data, we concluded that on a 24-hourly scale, the slope was indeed negative, which goes in line with the anti-phase relationship between DO and DIC expected from ecosystem metabolism.

Table 5.3. Results of linear regression models testing the effect of dDO/dt on dDIC/dt using the raw data (left) and the isolated 24-hour signal data (right).

linear models using raw data					linear models using isolated diel signal data		
Lake	alkalinity	slope	p-value slope (**<0.001)	R^2	slope	p-value slope (**<0.001)	R^2
Valguta Mustjärv	0.50	-1.00	**	0.25	-0.89	**	0.97
Erastvere	1.63	-0.91	**	0.92	-0.91	**	1.00
Mullutu Suurlaht	1.80	-0.12	**	0.03	-0.46	**	0.74
Saadjärv	2.47	0.46	**	0.05	-0.35	**	0.91
Peipsi	2.80	-0.22	**	0.19	-0.28	**	0.97
Ulemiste	3.30	-0.58	**	0.46	-0.23	**	0.95
Võrtsjärv	3.61	-0.47	**	0.25	-0.57	**	0.95
Äntu Sinijärv	4.80	0.74	**	0.53	0.11	**	0.37

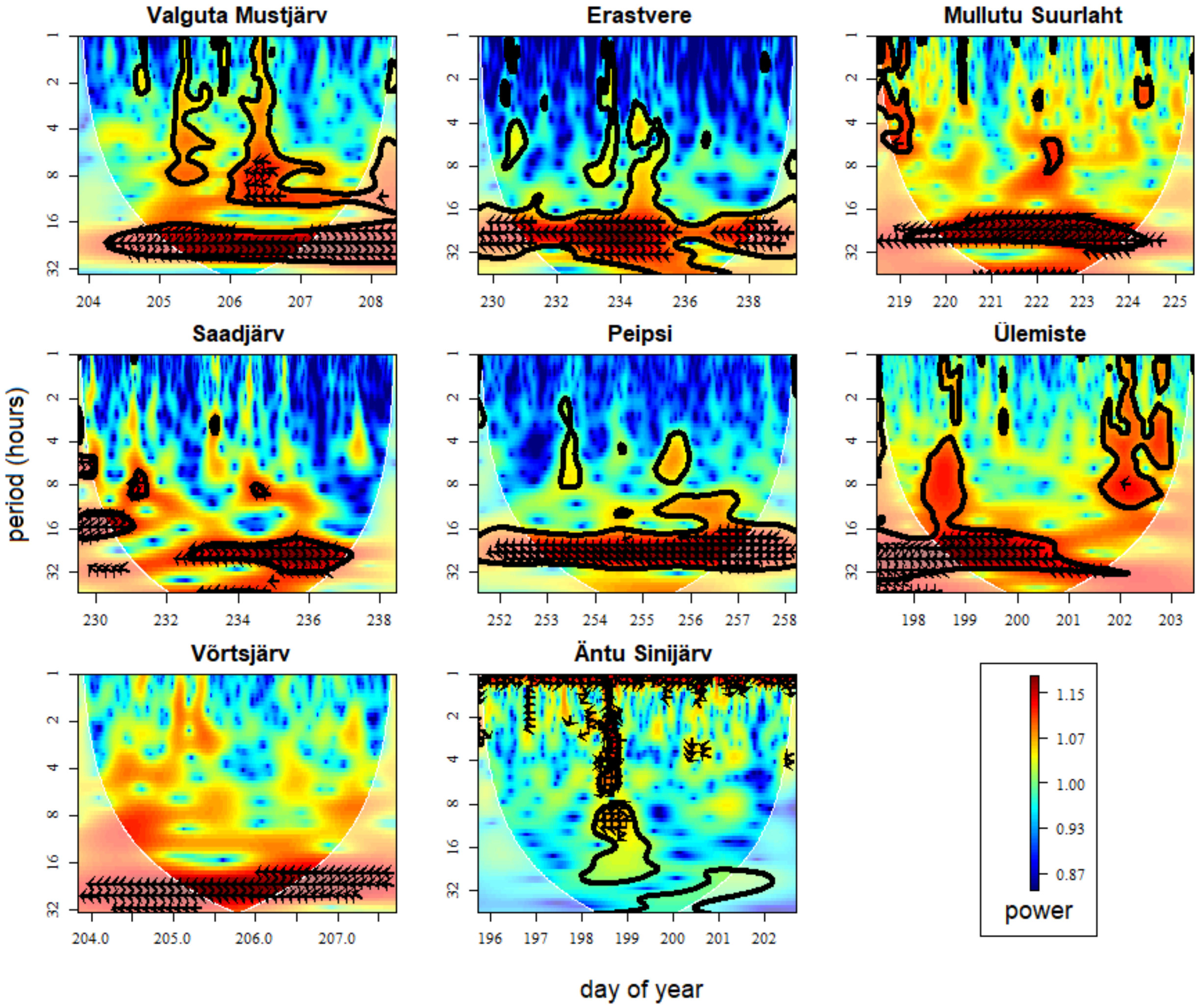

Figure 5.3. Cross-wavelets between DO and DIC for each lake, showing regions in the time-frequency space with common power. The power bar represents the amplitude-squared of signals, with higher values corresponding to important features of the signal. The x-axis represents the time (expressed as day of the year) and allows to see the evolution of common oscillatory patterns between DO and DIC in time. The y-axis represents the period of oscillation (in hours). Significant synchronizations in oscillations between DO and DIC at a certain period are delimited by the black contours. Arrows pointing to the left symbolize an anti-phase relationship. Notice the dominance of a synchronized anti-phase pattern around 24-hour frequency in most lakes.

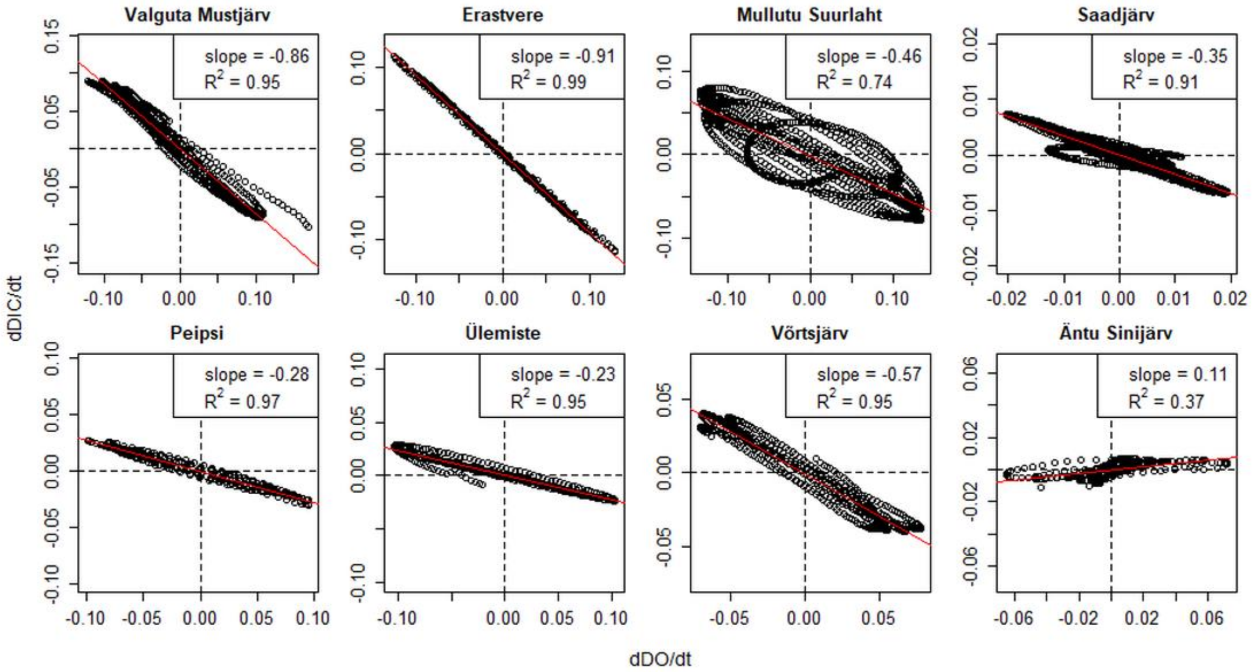

Figure 5.4. Relationship between rates of change in DO (dDO/dt) calculated from the isolated diel signal of DO, and rates of change in DIC (dDIC/dt) calculated from the isolated diel signal in DIC. All relationships are significant ($p<0.001$), with the slope and R^2 values indicated in the top-right window of each plot. The lakes are ordered from low to high alkalinity (top left to bottom right).

5.4.3 Effect of alkalinity on the sensitivity of DIC to metabolism

Finally, we wanted to know how alkalinity affects the sensitivity of DIC to metabolic changes in DO. For this, we tested the three hypotheses using $dDIC/dt_{theoretical}$ (Equation 5.1) to see if metabolism is the only driver for changes in dDIC/dt (Figure 5.5A), if pelagic calcite precipitation plays a role (Figure 5.5B) or if pelagic and benthic calcite precipitation both affect the sensitivity of DIC to metabolism (Figure 5.5C). The observed slopes did not fit the expected slope under the first hypothesis, equal to -1 in all lakes (Figure 5.5A). For the two last scenarios (Figure 5.5B, Figure 5.5C), the expected slopes of the dDO/dt vs. dDIC/dt relationships were very close to the observed ones, especially when using the correction for benthic metabolism (Hypothesis 3, Figure 5.5C). The relationship between observed and expected slopes had the highest R^2 value for the third hypothesis (Figure 5.5C).

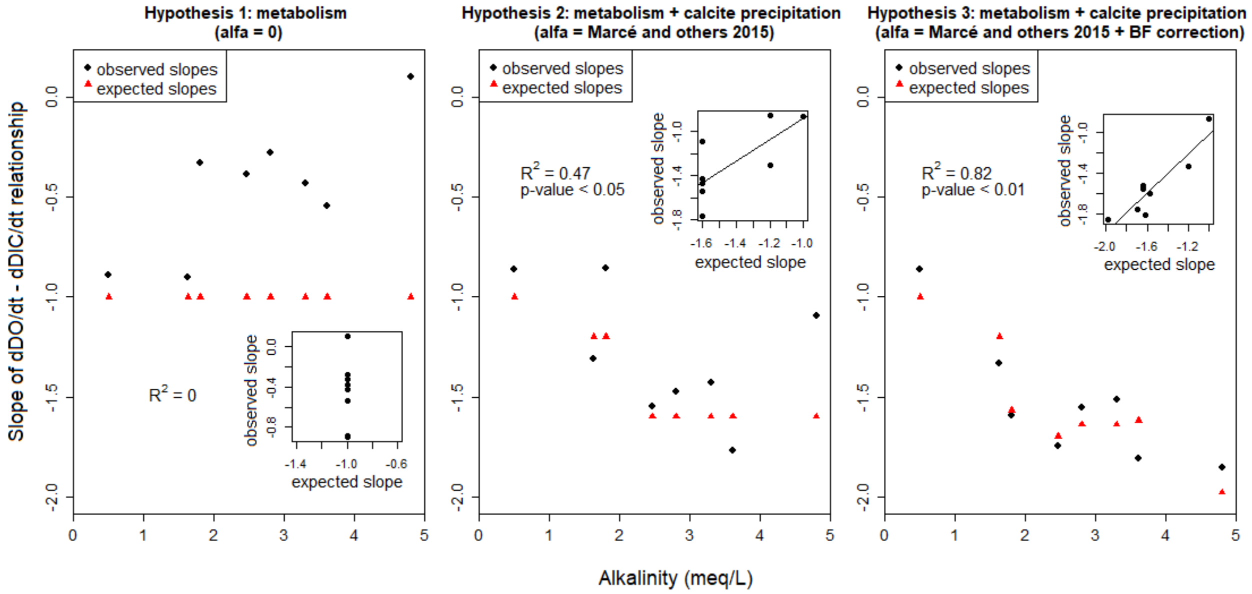

Figure 5.5. Effect of alkalinity on the sensitivity of dDIC/dt to changes in dDO/dt for each lake. Comparing the observed slopes (corrected using Equation 5.2) of the dDO/dt – dDIC/dt relationship with expected slopes (calculated using Equation 5.1) for each lake based on three different hypotheses: A) metabolism is the only factor controlling carbon dynamics (α=0), B) the α parameter (as defined in Marcé and others 2015) is added to the metabolism equation to account for effects of pelagic calcite precipitation on carbon dynamics, C) the value of α is corrected for each lake for its benthic fraction to account for the effect of pelagic calcite precipitation and benthic calcifying macrophytes.

5.5 Discussion

5.5.1 Effects of calcite precipitation on the relationship between DO and DIC

Comparing dDO/dt with dDIC/dt calculated from the isolated 24-hour signals is a way to assess the relative importance of metabolism in controlling carbon dynamics. The stoichiometry of photosynthesis and respiration implies a 1:1 relationship between changes in DIC and DO whereby one mole of DO respired produces one mole of DIC, and inversely, one mole of DIC is photosynthesized into one mole of DO (Stumm & Morgan, 1996). Due to this stoichiometry, it is expected that a slope close to -1 is found in the linear relationship between dDO/dt with dDIC/dt if aerobic metabolism is the main driver of carbon dynamics (Stumm & Morgan, 1996). Calcite precipitation/dissolution processes can involve deviations from the metabolic slope of -1 due to its release/consumption of CO_2 (Barrón et al., 2006; Gattuso et al., 1999; Obrador & Pretus, 2013). Such deviations can also be caused by anaerobic processes (Torgersen & Branco, 2007) or can be correlated to major gradients of ecosystem level, substrate level, and bacterial community level characteristics (Berggren et al., 2011).

Among the eight studied lakes, only two lakes, Erastvere and Valguta Mustjärv, showed a slope near -1 for the dDO/dt – dDIC/dt relationship (Figure 5.4). This implies that changes in dDIC/dt are strongly negatively correlated, at an almost equal molar ratio, to metabolic changes in dDO/dt. These two lakes have the lowest alkalinity values (1.6 and 0.5 meq L^{-1} respectively). In all other lakes of higher alkalinity, the slopes between dDO/dt and dDIC/dt decreases and gradually approaches a value of 0 at higher alkalinity (Figure 5.5A), which involves a deviation from the metabolic 1:1 stoichiometry between changes in DO and DIC. This means that with increasing alkalinity, the importance of metabolism in driving changes in DIC diminishes, as highlighted by Marcé and others (2015). DIC dynamics in lakes of higher alkalinity can be also affected by chemically enhanced uptake of atmospheric CO_2 (Kragh &

Sand-Jensen, 2018). However, our method of isolating the metabolic diel signal eliminates any physical effects such as gas exchanges or water movements and therefore chemically enhanced uptake of atmospheric CO_2 is unlikely to be the cause of the observed deviations from the metabolic 1:1 stoichiometry.

Because of the chemical stoichiometry of respiration and photosynthesis, our studied lakes had an anti-phase coupling between the diel signals of DO and DIC, but with deviations from the 1:1 stoichiometry at increasing alkalinity, suggesting that although the anti-phase is tightly linked to photosynthetic or respiratory processes, deviations from this is influenced by the carbonate equilibrium system. The most plausible explanation of this mechanism is calcite precipitation, as it is tightly linked to alkalinity and photosynthetic activity. High alkalinity lakes are likely to precipitate larger quantities of calcite due to the high content of bicarbonates and carbonates. Photosynthesis raises the pH, influencing the carbonic acid equilibrium and shifting the balance towards oversaturation of carbonates (Dittrich & Obst, 2004; Minder-Zürich, 1922). Therefore, calcite precipitation has been reported to peak with high summer rates of primary production (Müller et al., 2016). Additionally, picoplankton and cyanobacteria can act as nucleation sites for calcite precipitation (Dittrich & Obst, 2004; Obst et al., 2009; Stabel, 1986). The relationship between primary production and calcite precipitation has rarely been quantified in freshwater systems. The few studies that do so have suggested that depending on the lake alkalinity, one mole of DIC consumed to produce one mole of O_2 through photosynthesis induces between 0.2 and 1 mole of calcite precipitation (Mcconnaughey et al., 1994; McConnaughey & Whelan, 1997; Megard, 1968) and that below an alkalinity threshold of 1 meq L^{-1}, calcification is unlikely to occur (Marcé et al., 2015). This range is consistent with the α values used in this study. A similar range is reported in marine planktonic assemblages (Riebesell et al., 2000).

The chemical equation describing calcite precipitation implies that for each mole of calcite that precipitates, there is a loss of one mole of DIC and 2 moles of TA (Equation 1.1 in general introduction). The combined effect of metabolism and calcite precipitation results in a net loss of -1 to -2 moles of DIC per mole of DO produced. However, the observed slopes of the dDO/dt – dDIC/dt relationships in the 8 studied lakes ranged between -1 and 0.1, which is inconsistent with the theoretical slope of -1 to -2. This inconsistency can easily be explained by the methods used to calculate DIC. The high variability in alkalinity caused by the limited sensitivity of field pH sensors made it impossible to use diurnal changes in alkalinity. Instead, the alkalinity values were averaged and used in combination with pH to calculate DIC. Correcting dDIC/dt for theoretical changes in alkalinity (Equation 5.2) results in slopes that are in the correct range between -1 and -2. This highlights the importance of considering diurnal changes in alkalinity for correct estimations of DIC, something still challenging with current sensor technologies.

Here we show that the slopes clearly deviated from the metabolic slope of -1, especially at higher alkalinity (Figure 5.5A). Accounting for pelagic calcite precipitation showed a positive relationship ($R^2 = 0.47$) between the observed slopes and the expected slopes (Figure 5.5B). However, in case of two lakes, Mullutu Suurlaht and Äntu Sinijärv, the difference between observed and expected slope was still relatively high compared to the other lakes (Figure 5.5B, alkalinity 1.8 and 4.8 meq L^{-1} respectively). These two lakes share the common characteristics of being shallow and having a thick layer of calcifying macrophytes of the genus *Chara* at their bottom floor. An approximate 1:1 calcification to photosynthesis ratio ($\alpha = 1$) in such organisms has been reported (McConnaughey, 1991; Mcconnaughey et al., 1994; McConnaughey & Whelan, 1997). This is due to their ability to use HCO_3- instead of CO_2 for photosynthesis and due to an efficient coupling between carbon uptake at the acidic part of the cell surface and $CaCO_3$ precipitation at the alkaline surface of the cell

(Mcconnaughey et al., 1994). The benthic fraction of primary production for these two lakes is 46% for Mullutu Suurlaht and 94% for Äntu Sinijärv (Table 5.2) (Cremona et al., 2016). In these two lakes, correcting the α values for the BF of primary production increased the fit between observed and expected slopes (Figure 5.5C). This suggests that benthic calcification by macrophytes can be equally or more important than pelagic calcite precipitation in driving changes in dDIC/dt in lakes that have a high fraction of primary production of benthic origin, and highlights the need to be spatially inclusive and include all metabolic components (benthic and pelagic) when addressing ecosystem-scale DIC dynamics (Obrador & Pretus, 2013). This is especially relevant in shallow oligotrophic systems where benthic charophytes can reach similar production rates as pelagic phytoplankton communities under highly eutrophic conditions (Christensen et al., 2013).

Besides calcite precipitation, other factors may lead to deviations from the metabolic slope of -1, such as physical processes or anaerobic respiration. However, any physical effects such as gas exchanges, water movements, or external inputs of DIC due to storm events can be discarded because the metabolic diel signals were isolated from any non-metabolic signal. As for anaerobic respiration, the vertical distributions of temperature, DO and CO_2 presented in Laas and others (2016) for each lake during the same measurement period as this study, suggest that anaerobic processes have no major effect on surface waters at the measuring depths that were used for this study, especially considering the absence or very weak presence of gradient in CO_2 concentrations in the surface water layer. Two strong evidences suggest that calcite precipitation is the main mechanism causing these deviations: firstly, the observed slopes are strongly correlated to the expected slopes under calcite precipitation conditions and secondly, the degree of deviation from the metabolic slope is positively correlated to lake alkalinity. Several other studies in other aquatic systems have suggested that calcite precipitation/dissolution is responsible for such deviations due to the CO_2 release/consumption (Barrón et al., 2006; J.-P. Gattuso et al., 1999; Obrador & Pretus, 2013).

Recent evidence suggests that in lakes of high alkalinity, large pools of DIC support higher rates of primary production, whereas in lakes of low alkalinity, primary production can be strongly DIC-limited (Kragh & Sand-Jensen, 2018). Considering the coupling between primary production and calcite precipitation, it is likely that high production in lakes of high alkalinity during summer production peaks results in high rates of calcite precipitation that compensates photosynthetic DIC depletion by releasing CO_2. However, this mechanism is likely irrelevant in low alkalinity lakes, where DIC limitation minimizes primary production and calcite precipitation.

This study focuses on inorganic carbon dynamics within the aquatic systems without directly addressing questions related to CO_2 emissions to the atmosphere. An interesting addition to this work would be to relate the observed and expected sensitivity of DIC to metabolic changes in DO, to CO_2 emissions. Even though there is a net loss of 1 mole of DIC per mole of calcite that precipitates, the process of calcification is acknowledged to increase CO_2 supersaturation in surface waters because it also releases 1 mole of CO_2, thereby acting as a carbon source to the atmosphere (Michel Frankignoulle et al., 1995; Opdyke & Walker, 1992; Suzuki, 1998). Considering that DIC dynamics are largely driven by calcite precipitation in lakes of high alkalinity, this process is likely to be an important driver of CO_2 supersaturation in these lakes. Marcé and others (2015) suggested a threshold of 1 meq L^{-1} above which metabolism alone isn't enough in explaining DIC dynamics. This threshold seems consistent with our results (Figure 5.5), but only one lake at alkalinity lower than 1 meq L^{-1} is represented in this study and therefore we cannot clearly identify and confirm this threshold. Marcé and others (2015) further suggest that 57% and 34% of the global area occupied by lakes have alkalinity values above 1 meq L^{-1} and 2 meq L^{-1} respectively which implies an important global contribution of calcite precipitation to CO_2 supersaturation in lakes. Our results agree well with this statement, as all of the lakes above 1 meq L^{-1} (7 out of 8 lakes) show clear deviations from the -1 metabolic slope, with a tendency of stronger deviations at higher alkalinity (Figures 5.5B and 5.5C). Therefore, calcite precipitation may have a major contribution in global atmospheric CO_2 emissions from surface waters.

5.5.2 *Numerical considerations*

The periodograms for DO and DIC showed a large number of signals with oscillations at shorter time periods than the 24-hour metabolic diel signal (Figure 5.1, Figure 5.2), but the spectrum was so weak that it seemed of minor importance. However, when calculating rates of change from the raw DO and DIC data, these signals at shorter period were amplified to the extent where the diel signal became hidden in shorter oscillations of high amplitude (Supplementary Figure S1, Figure S2 in Appendix A). These short time period signals cannot be explained by the metabolic balance between photosynthesis and respiration and are caused by non-metabolic processes. All the studied lakes have a large amount of noise in their DO and DIC data resulting from the high frequency in measurements that capture noisy random signals, and imprecisions in the pH sensors that create substantial errors when calculating DIC values due to the exponential scale of pH. Other physical processes may also cause the random short time period signals observed in these lakes, such as atmospheric or internal gas exchanges (Antenucci et al., 2013; Wissel et al., 2008), as well as water movements caused by convection, turbulence, minor mixing events or seiches. The cross-wavelets for DO and DIC reveal that significant oscillatory signals are commonly found between 1 to 8-hours frequency. This cannot be classified as noise and needs to be linked to internal processes of the lakes. One explanation could be found in the seasonal pattern of internal seiches which corresponds well to the 1 to 8-hour frequency in oscillation (Bernhardt & Kirillin, 2013). Despite the amount of noise and non-metabolic signals, the cross wavelets revealed a clear anti-phase coupling between DO and DIC around the 24-hour period in most of the lakes, which is to be expected if aerobic metabolism is the main driver of both DO and DIC dynamics (Figure 5.3). In some lakes, this coupling was weak or interrupted, especially in Äntu Sinijärv where it was almost totally absent due to the particularities of this lake (DIC dynamics are mostly driven by groundwater DIC-rich inputs).

Despite the amount of noise and sometimes weak diel signals, Singular Spectrum Analysis is an efficient tool that allowed us to enhance the metabolic diel signal to noise ratio, by isolating and extracting the diel signals for DO and DIC. In contrast to a smoothing function which would smooth out the noisy signals without getting rid of them, SSA identifies the fundamental oscillations within a time series. Therefore, the diel signals that are isolated using this tool are not affected by unwanted signals such as noise or non-metabolic processes.

5.6 Conclusions

- Calcite precipitation is an important process in driving carbon dynamics in lakes of high alkalinity, which represents more than half of the global area occupied by lakes and reservoirs. Acknowledged to act as a carbon source to the atmosphere, it is likely that calcite precipitation is an important driver of CO_2 fluxes in lakes globally.
- The importance of calcification in driving carbon dynamics can be assessed using an alkalinity-dependent parameter (α). In lakes that have a high benthic fraction of primary production, α must be corrected to account for calcification by macrophytes.
- Considering diurnal changes in alkalinity is mandatory for precise estimations of DIC concentrations and dynamics.

The problem of noisy high frequency measurements datasets can be effectively overcome using Singular Spectrum Analysis to enhance the signal to noise ratio by extracting the signal of interest, such as diel signals.

6. General discussion

6.1 The relevance of calcification in lakes: addressing the assumptions

The important role of calcification is well established in marine studies where it is acknowledged as a major sedimentary carbon sink and a carbon source that regulates atmospheric CO_2 concentrations at geological time scales (Frankignoulle et al., 1994, 1995; Gattuso et al., 1999, 1993, 1996; Opdyke & Walker, 1992; Ridgwell & Zeebe, 2005; Suzuki, 1998). However, in freshwater systems, calcification is assumed to play a negligible role and is therefore not accounted for in any global carbon budgets of inland waters (Aufdenkampe et al., 2011; Battin et al., 2009; Cole et al., 2007; Mendonça et al., 2017; Raymond et al., 2013a; Tranvik et al., 2009). This is likely due to three main reasons that shall now be addressed.

Firstly, pelagic calcification in open marine ecosystems is usually triggered by planktonic organisms that produce calcareous shells, such as foraminifera and coccolitophorales species, that generate a continuous rain of calcite to the deep oceans, thereby producing an important fraction of the world's carbonate (Henehan et al., 2017; Monteiro et al., 2016). In comparison, calcifying planktonic species are rare in freshwater systems (Kelts & Hsü, 1978), which has likely led to the assumption that pelagic calcification is a rare process in freshwaters. This is misleading because the underlying mechanisms responsible for calcification in freshwaters are different than those found in marine systems and do not require calcifying organisms' calcification (Dittrich & Obst, 2004; Obst et al., 2009). This is confirmed by the results in the fourth chapter of this thesis which show that the two main drivers of calcification in lakes are primary production and calcite saturation, whereby the calcifying inducing effect of the first is potentiated by the latter.

Secondly, most studies related to the carbon cycle in inland waters have been done in low alkalinity systems. Studies that have been related with CO_2 fluxes through organic processes have been conducted in lakes of low alkalinity and high DOC, where DOC is the main driver of the observed fluxes and where DIC dynamics are mostly dependent on metabolic processes such as mineralization of DOC (McDonald et al., 2013). Therefore, inorganic processes such as in-lake calcite precipitation or carbonate weathering in the catchment, that would otherwise weaken the relationship between DOC and CO_2 fluxes, were treated as negligible or even absent processes in such systems (Del Giorgio et al., 1999; Duarte & Prairie, 2005; Larsen et al., 2011). This understanding has been generalized to most lakes, giving precedence to organic processes over inorganic ones in the studies of DIC dynamics and carbon fluxes in lakes. The results discussed in chapter four of this thesis showed that lake metabolism is indeed the main driver of DIC dynamics in lakes of low alkalinity, but in lakes of higher alkalinity it must be considered along with calcification for correctly understanding DIC dynamics. This finding is supported by previous studies that challenge the notion that net heterotrophy is the main cause of carbon fluxes in lakes (Marcé et al., 2015; McDonald et al., 2013; Müller et al., 2016; Weyhenmeyer et al., 2015).

Thirdly, calcification has been assumed to occur only in lakes of high alkalinity, or shallow oligotrophic lakes, or saline lakes that represent the minority of global lacustrine environments (Cole et al., 2007; Duarte et al., 2008; Tranvik et al., 2009). Shallow oligotrophic lakes are often densely populated with bottom-dwelling calcifying macrophytes (Andersen et al., 2017; Sand-Jensen et al., 2018), hence the occurrence of calcification is an acknowledged process in such systems but restricted to the benthic environments (Andersen et al., 2019; Mcconnaughey et al., 1994). As for lakes of high alkalinity, these systems usually have relatively high levels of calcite saturation which might suggest that spontaneous precipitation of calcite could possibly occur as a result of calcite oversaturation (Dittrich & Obst, 2004; Ridgwell & Zeebe, 2005). Yet, spontaneous calcification has not been observed neither in lakes, nor in oceans because the initial step of crystal nucleation is kinetically unfavorable and requires very high levels of calcite oversaturation which are beyond the levels found in lakes and oceans (Dittrich & Obst, 2004; Ridgwell & Zeebe, 2005). Rather, it is a biologically mediated process (Dittrich & Obst, 2004; Hartley et al., 1995; Obst et al., 2009), as was shown in the fourth chapter of this thesis that exposes the strong relationship between calcification and primary production. The assumption that calcification occurs mostly in lakes of high alkalinity is partially true. The results in chapters three, four, and five of this thesis confirm that calcification is likely to be more intense in lakes of higher alkalinity. However, chapter three suggests that the alkalinity threshold for pelagic calcification to occur is at 1 meq L^{-1} in agreement with previous approaches (Marcé et al., 2015). This level of alkalinity is rather low considering that 57% of the surface area of lakes and reservoirs is above this threshold (Marcé et al., 2015). The results presented in chapters four and five further point to this threshold, because the lakes above 1 meq L^{-1} show signs of calcification, whereas the one below it (ie. the case study lake in Estonia, Valguta Mustjärv) does not.

Due to this threshold, calcification appears to be a widespread process in lakes and reservoirs at a global scale. This contradicts the assumption that its role is negligible in lakes or that it is only relevant in lakes of high alkalinity. In view of the extensive nature of this process, it is necessary to estimate its contribution within the global carbon budget of lakes and reservoirs, and to understand its drivers and implications for lake carbon dynamics and for modelling lake carbon fluxes.

6.2 Pelagic calcification in the global lake carbon budget: its role as a sedimentary carbon sink and atmospheric carbon source

To date, calcification in freshwaters has never been quantified at a global scale and is overlooked in all the global carbon budget estimations for lakes and reservoirs (Aufdenkampe et al., 2011; Battin et al., 2009; Cole et al., 2007; Mendonça et al., 2017; Raymond et al., 2013a; Tranvik et al., 2009). These estimations are largely based on organic processes, emphasizing the role of organic carbon burial over inorganic carbon deposition, and the role of DOC and heterotrophy over inorganic carbon dynamics in regulating carbon emissions (Battin et al., 2009; Cole et al., 2007; Mendonça et al., 2017; Raymond et al., 2013a).

The results that are discussed in the third chapter of this thesis offer a first global gross estimate of pelagic calcification from lakes. The results show that pelagic calcification contributes significantly to the global carbon budget of lakes and reservoirs. While organic processes represent the main drivers of carbon fluxes, gross global pelagic calcification rates (0.03 Pg C yr^{-1}, range: 0.01 – 0.07 Pg C yr^{-1}) are lower, yet comparable in magnitude to those of OC burial (0.15 Pg C yr^{-1}, range: 0.06 – 0.25 Pg C yr^{-1}) (Mendonça et al., 2017). These results have important implications on shaping our understanding of carbon cycling in lakes and how we approach future research. Most studies in lake carbon cycling consider organic processes without accounting for inorganic processes such as calcite precipitation. Neglecting pelagic calcification in carbon budgets is misleading considering its relevance at a global scale and its similar order of magnitude in comparison to the burial of organic carbon. Furthermore, the inorganic carbon sink is not prone to the degradation processes that characterize the organic carbon sink. Organic carbon in the sediments is either mineralized to CO_2 or CH_4 by heterotrophic microbial communities or buried and stored over geological timescales (Sobek et al., 2009). A study over several lakes showed an average OC burial efficiency (ratio between buried OC and deposited OC) of 48% (Sobek et al., 2009). This efficiency can be greater in reservoirs due to anoxic conditions often found in these systems, which promote efficient burial of organic carbon (Isidorova et al., 2019). In this sense, the inorganic carbon sink is more stable than its organic counterpart because it is not affected by mineralization processes. Hence, the inorganic carbon sink is a relevant additional component of the carbon cycle that stores carbon at geological timescales. However, inorganic carbon in lake sediments is essentially composed of calcite and aragonite which are subject to redissolution that can be enhanced by low pH at anoxic conditions. This mostly occurs in the hypolimnion and sediment pore waters (Bluszcz et al., 2009; Kufel et al., 2020; Roeser et al., 2016). In oceans, redissolution of carbonates can be pressure induced at depths beyond the lysocline (Pälike et al., 2012) which is why 80% of all carbonates that precipitate in the ocean redissolve within the water column (Ridgwell & Zeebe, 2005). However, calcite in freshwaters cannot be redissolved for this reason because such depths are not found in lakes. Arriving at a global estimation of calcite dissolution in lakes is still needed in order to quantify the net long-term inorganic carbon sink of lakes.

In contrast, the contribution of pelagic calcification to CO_2 emissions appears to be minor at a global scale (0.0002 Pg C yr^{-1}, range: 0.00005 – 0.0007 Pg C yr^{-1}) due to the ability of lakes of higher alkalinity to buffer the CO_2 released by calcification, thereby limiting changes in pCO_2, as shown in the third chapter. However, these interactions with the DIC equilibria can have considerable effects on the DIC dynamics of a lake at a local scale. This is addressed in chapter five of this thesis that shows that calcification can cause large deviations from the typical balance expected between DIC dynamics and metabolic rates. Therefore, it is necessary for studies in lake carbon cycling to account for calcification not only at global scales in carbon budgets, but also at local scales, due to its effect on DIC dynamics. This is especially true when relating lake metabolism with inorganic carbon fluxes, as will be further discussed.

The confusing double role of calcite precipitation as a carbon sink in the sediments and a carbon source to the atmosphere (Ridgwell & Zeebe, 2005) has given rise to a debate in marine studies on whether calcification acts as an overall carbon sink or carbon source in coral reefs (J.-P. Gattuso et al., 1999). The general consensus has been that the CO_2 emitted from calcification in such systems is greater than the sedimentary deposition of carbonates, hence its primary role as a carbon source (J.-P. Gattuso et al., 1999; Ware et al., 1992). The current understanding of the role of calcification as an overall carbon sink or carbon source in lakes is much less clear. Several studies have observed that CO_2 emissions can be largely driven by inorganic processes such as calcification, chemical weathering or inorganic carbon loading, although we lack information on the relative contributions of each of these processes (Marcé et al., 2015; McDonald et al., 2013; Stets et al., 2009). The contribution of inorganic processes to CO_2 emissions in lakes have been estimated at 0.09 Pg C yr^{-1} (range: 0.05 – 0.17 Pg C yr^{-1}) (Marcé et al., 2015), representing 28% of the total CO_2 emissions from lakes (0.32 Pg C yr^{-1}, range: 0.06 – 0.84 Pg C yr^{-1}) (Raymond et al., 2013a). This suggests that inorganic processes are relevant drivers of CO_2 emissions that must be accounted for together with organic processes. However, our results in chapter three suggest that the contributions of calcification to CO_2 emissions are negligible in comparison to the total CO_2 emissions of lakes and represent only a minor fraction of the CO_2 emitted from inorganic processes. This is surprising considering the relatively high value of global gross calcification rates that was estimated in chapter three. The reason for the disparity observed between global calcification rates and its resulting CO_2 emissions lies in the buffering capacity of lakes. Higher calcification rates are expected to occur in lakes of higher alkalinity which have a greater buffering capacity thereby limiting the changes in pCO_2 that would be expected from the CO_2 released from calcification.

Given the relatively high value for global gross calcification rates in comparison to the CO_2 emissions caused by global calcification, pelagic calcite precipitation appears to act mainly as a carbon sink in lakes if calcite dissolution plays a minor role. A global estimation of calcite dissolution would be necessary to confirm this statement. While the direct contribution of calcification to CO_2 emissions is minor, it may nevertheless have important indirect effects on CO_2 emissions by offsetting photosynthetic uptake of CO_2. The CO_2 released during calcification can directly sustain photosynthesis, thereby compensating CO_2 depletion (Andersen et al., 2019; Mcconnaughey et al., 1994). This was shown in lake Annecy where calcite precipitation supported nearly 100% of summer primary production (Perga et al., 2016). Furthermore, calcification decreases the pH resulting in higher concentrations of CO_2 (Stumm & Morgan, 1996), which may further offset CO_2 consumption of primary producers. Therefore, calcification supports primary production, which is itself a key driver of calcification, as discussed in the fifth chapter of this thesis. Yet, our estimations of global gross calcification rates and its resulting CO_2 emissions are based on the relationship between alkalinity and calcification rates without accounting for the effect of primary production. This is because a mechanistic understanding of the effects of primary production on calcification is still missing. This issue was addressed in the fourth chapter of this thesis by identifying a mechanistic relationship that can be used to account for calcification in earth system models and carbon budgets. This will now be further discussed by addressing the drivers of calcification.

6.3 Understanding the role of primary production and geochemistry as drivers of calcification

Primary production has been identified as a key driver of calcification (Dittrich & Obst, 2004; Hartley et al., 1995; McConnaughey & Whelan, 1997). Its occurrence has usually been observed during summer when high primary production rates promote calcification events (Dittrich & Obst, 2004; Trapote et al., 2018). The occurrence of pelagic calcification is variable in its duration in different lakes and can range from a single week (Rodrigo et al., 1993) to one month (Vanderploeg et al., 1987) to half a year (Reynolds, 1978) or can also be a continuous process throughout the year (Dobolyi & Herodek, 1980). Yet, primary production alone is insufficient to fully explain the periodic nature of this process. Therefore, it is necessary to understand what controls the calcification-inducing effect of primary production. Our results presented in chapter four suggest that the effect of primary production on calcification needs to be assessed with NEP, which accounts for the net balance between primary production and respiration. Hence it is the net production at an ecosystem level which promotes calcification. This relationship between calcification and NEP depends on calcite saturation. More precisely, it is the ratio between calcification and NEP (α) which is predictable from the calcite saturation index (SI). This relationship can be used to assess the intensity of calcification. Furthermore, it explains the periodic nature of its occurrence. The effect of NEP on calcification acts at a short temporal scale whereby an increase in NEP can induce calcification. The efficiency of this process is dependent on calcite SI which varies at longer seasonal temporal scales, usually reaching its summit during summers (Figure 6.1). Hence, the short-term efficiency of NEP to induce calcification can be exponentially potentiated during summer when calcite saturation is highest. During the calcification event, calcite SI decreases along with water alkalinity due to the precipitation of carbonates, resulting in a decrease in calcification rates (Figure 6.1). This might explain why summer calcification is frequently a periodic process that is limited in time.

Although the global estimation of calcification rates in chapter three are based on the relationship between lake alkalinity and calcification, our results in chapter four suggest that calcite SI is a better predictor of calcification. But because calcite SI is strongly dependent on water alkalinity, the use of lake alkalinity is still suitable for our global estimation, especially since calcite SI values for lakes are less commonly provided in the literature. Furthermore, the global estimation in chapter three is based on literature data that is linked to reference alkalinity values of lakes. This approach is subject to large variability, particularly at a temporal scale. At such precision, it is likely that using alkalinity or calcite saturation as predictors would not make much of a difference. However, in chapter four, the analysis is done at a smaller scale whereby incubation bottles were used to identify the mechanistic relationship between NEP and calcification. The use of calcite SI to describe this relationship is therefore needed at this scale of analysis.

Our results discussed in chapter four show that the α ratio increases exponentially with increasing calcite SI due to the potentiating effect of calcite SI on the calcification inducing effect of NEP. Therefore, it is more likely to observe intense calcification such as whiting events in systems with high calcite SI, usually consisting of lakes of high alkalinity due to the strong dependency of alkalinity on calcite SI. This is relevant considering that 57% and 34% of the global area occupied by lakes and reservoirs have alkalinity values above 1 and and 2 meq L^{-1} respectively (Marcé et al., 2015). The 1 meq L^{-1} threshold marks the minimum condition required for calcification to occur (chapter 3), whereas at 2 meq L^{-1} the intensity in calcification is expected to have acute effects on CO_2 supersaturation in lakes (Marcé et al., 2015). In fact, at latitudes between 30°-50°N, and 10°-20°S, more than half of the surface area of lakes and reservoirs have alkalinity values beyond the 2 meq L^{-1} threshold (Marcé et al., 2015). Furthermore, an increase in the export of alkalinity has been observed in several parts of the world due to global change (Drake et al., 2018; Raymond & Cole, 2003). The resulting rise in alkalinity would potentiate the calcification inducing effect of NEP (Figure 6.1). Interestingly, recent studies show that lakes of higher alkalinity can support higher productivity than low-alkalinity lakes at equal light and nutrient levels due to an efficient use of DIC for photosynthesis as well as chemically enhanced atmospheric CO_2 uptake (Hammer et al., 2019; Kragh & Sand-Jensen, 2018). For this reason, the most productive lakes are nutrient-rich hard water lakes (Kragh & Sand-Jensen, 2018). This may be an added effect that promotes intense calcification rates by enabling high NEP in combination with the already high calcite SI usually found in such systems. The release of CO_2 coupled to calcification supports primary production and prevents CO_2 depletion in such systems (Kragh & Sand-Jensen, 2018). Primary production is also enhanced by nutrient increase and its resulting lake eutrophication (Hamilton et al., 2009). Human activities related to agriculture or urban activities such as sewage and industrial discharges are increasing eutrophication and the proliferation of cyanobacterial blooms (Carpenter et al., 1998; Conley et al., 2009; Lund et al., 1972). Eutrophication can promote calcification through its potentiating effect on primary production which usually results in an increase in NEP (Figure 6.1). However, inorganic phosphorus which causes eutrophication, also inhibits the growth of calcite crystals (House, 1990). Hence, when eutrophication of a lake increases along with the concentrations of soluble reactive phosphate, calcite precipitation is progressively inhibited (House, 1990) (Figure 6.1).

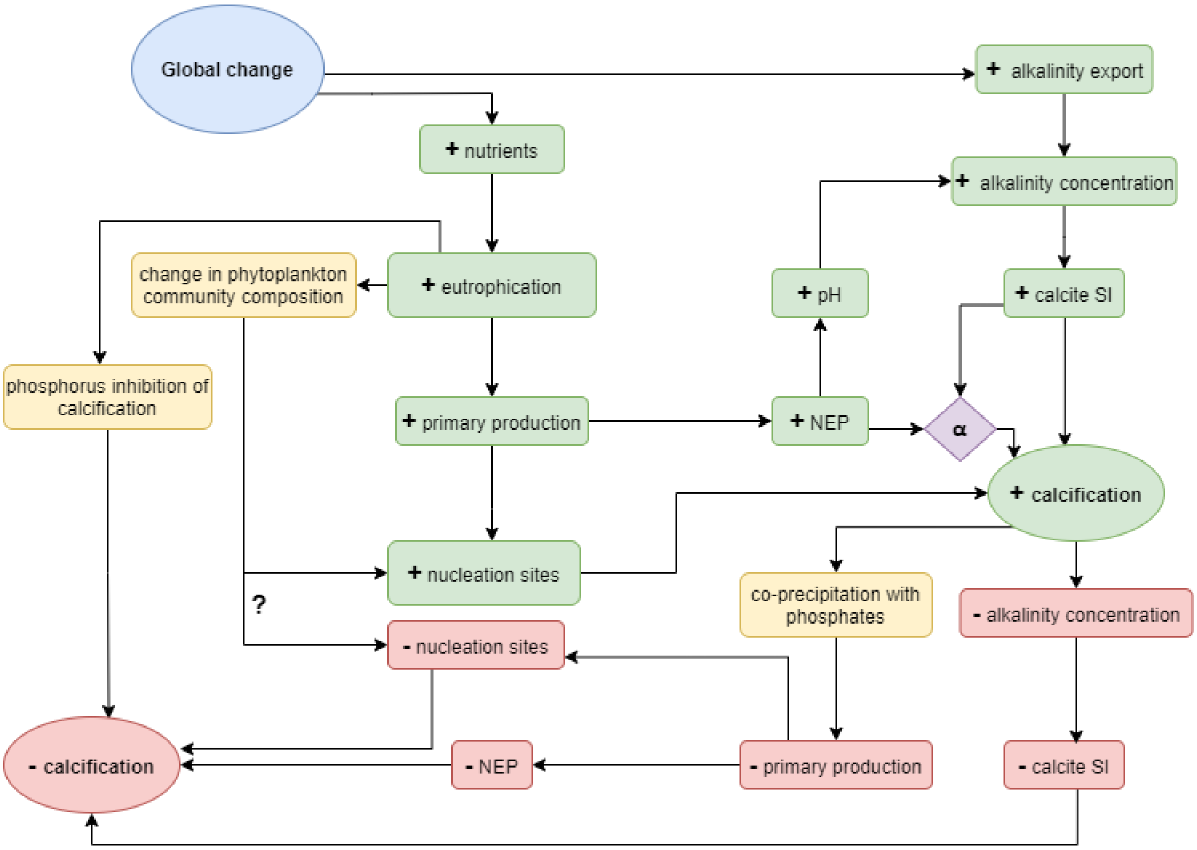

Figure 6.1. Conceptual model of the parameters and processes that increase or decrease lake calcification, including the effect of global change through its effect on increasing nutrient concentrations and alkalinity export to inland waters. Green color indicates an increase, red color indicates a decrease, orange color is neutral.

6.4 Calcification and modelling of lake carbon cycling

Beyond confirming previous findings that relate calcification to primary production (Dittrich et al., 2004; Hartley et al., 1995; McConnaughey & Whelan, 1997; Obst et al., 2009), the results of this thesis provide the first freshwater parametrization for calcification, which may facilitate the incorporation of pelagic calcification in current models of carbon cycling in lakes and their inclusion in Earth System Models. As discussed above, most carbon models for lakes rely on lake metabolism to explain carbon fluxes, using DOC as the main predictor of pCO_2 and assuming a negligible role of inorganic processes (Finlay et al., 2019). Our results in chapter five suggest that this approach is suitable for lakes of low alkalinity (<1 meq L^{-1}), where DIC dynamics are mostly driven by lake metabolism. However, above an alkalinity of 1 meq L^{-1}, we observed large deviations from the metabolic 1:1 stoichiometry that characterizes the diel incursions between dissolved oxygen (DO) and DIC, implying that metabolism alone cannot fully explain DIC dynamics in such systems. The observed deviations were strongly correlated with the deviations expected from calcification, with an even stronger correlation when considering both pelagic and benthic calcification in those lakes shallow enough to be influenced by benthic processes. This highlights the necessity to account for pelagic calcification in carbon models in lakes above 1 meq L^{-1}, which, as said, are more than half of the world's lakes (Marcé et al 2015). It also suggests that accounting for benthic calcification is also necessary in shallow lakes where the total primary production of the lake is considerably determined by benthic metabolism.

The deviations from the metabolic 1:1 stoichiometry between DO and DIC that are caused by calcification, could be the reason for the large imbalances reported in marine and brackish waters between inorganic carbon variability and metabolic rates (Barrón et al., 2006; Gattuso et al., 1999;

Obrador & Pretus, 2013). Many lakes are reported to have reached a strong imbalance whereby they emit CO_2 while being in an autotrophic state (McDonald et al., 2013; Perga et al., 2016; Stets et al., 2009). This could be due to the CO_2 released during calcification. Although calcification releases CO_2, it acts as a DIC sink, whereby two moles of DIC are consumed per mole of calcite that precipitates, and one mole of CO_2 is released, resulting in a net loss of one mole of DIC. This loss, when added to the effect of DIC consumption from photosynthesis, affects the 1:1 metabolic stoichiometry between DO and DIC. The results exposed in chapter four show that these deviations are themselves related to α. Hence, the parameterization of calcification described in chapter four can be used to, firstly, describe the occurrence and intensity of calcification rates, and secondly, for estimating the imbalances between DIC variability and lake metabolism. Ultimately, these results can be incorporated into integrative lake functioning models aiming to cover the global variability in lake geochemical properties.

6.5 Research limits and future research agenda

This thesis is a first attempt at estimating global calcification rates and its resulting CO_2 emissions and offers a first parameterization that can be potentially used to account for freshwater calcification in carbon cycling models. In this sense, the results presented in this thesis should be considered as a stepping stone to be further built upon. In this section, the limits of this research will be discussed, as well as the knowledge gaps that still need to be addressed to complement the results presented in this thesis.

The global estimate of calcification presented in chapter three is a first gross estimate of pelagic calcification which does not account for the contribution of calcite dissolution, nor does it account for benthic calcification by macrophytes. To fully understand the role of calcification in the global carbon budget of lakes, a net estimation of this process is needed, hence the need for a global estimation of calcite dissolution. Yet, several studies involving multiple lakes suggest that calcite dissolution in lakes and reservoirs is minor compared to calcite precipitation (Lenz et al., 2018; Müller et al., 2016; Stabel, 1986), although this statement is likely less applicable for deep stratified lakes that present anoxic and low pH conditions in the hypolimnion (Ohlendorf & Sturm, 2001; Ramisch et al., 1999). Following the provisional assumption that calcite dissolution is negligible, net global calcification rates would be similar in magnitude to organic carbon burial (Mendonça et al., 2017). This would present a major shift in the current understanding of lake carbon cycling, as explained above, which further stresses the need to account for calcite dissolution to assess the net contribution of global calcification rates in the global carbon budget. Similarly, the total global contribution of lake calcification can only be accurate when accounting for both pelagic and benthic calcification. Therefore, the results in chapter three are likely underestimating the total global calcification rates because the benthic fraction of primary production can sometimes be greater than the pelagic fraction, especially in shallow lakes (Cremona et al., 2016) which represent a large percentage of the world’s lakes (Beklioğlu et al., 2016), and in littoral zones which can cover extensive areas in deeper lakes (Vadeboncoeur et al., 2008). Since the results discussed in chapter four highlight the strong dependence of calcification on primary production, systems with a large ratio of benthic primary production could contribute to a large amount of benthic calcification. This assumption is supported by the high calcification rates that have been observed in calcifying macrophytes (McConnaughey & Whelan, 1997), suggesting that benthic calcification may have a large contribution to the total global calcification rates. Further research is therefore needed to refine the global estimate of calcification by accounting for its benthic contribution.

Furthermore, the global estimate of calcification provided in chapter three is based on summer calcification rates, assuming that this process occurs exclusively during the summer season, which again implies our estimate to be conservative. Although calcification occurs mostly during summer, it is variable in time and can occur throughout the year (Rodrigo et al., 1993; Trapote et al., 2018). The results in the fourth chapter of this thesis showed that the temporal variation in calcification depends on the interaction between calcification, NEP and calcite SI that controls both the temporal occurrence of calcification and its intensity. Integrating these drivers within the global estimate and accounting for seasonal variations in calcification throughout the year would result in a more accurate global estimation.

The mechanistic relationship between the calcification to NEP ratio and calcite SI described and discussed in chapter four offers a first suitable parameterization of calcification. Further research should aim at calibrating lake metabolism models using this parameter with empirical data to see how the strength of calcification varies across different lake types. Such efforts should assess the suitability of this parameter to describe both the temporal variability of the occurrence of calcification, and its intensity. Furthermore, such models should compare the expected deviations from the 1:1 metabolic stoichiometry between DIC and DO using this parameter with observed deviations in a similar way as presented in chapter five of this thesis. This would enable an assessment of the strength of this parameter to describe DIC dynamics in lakes, which undoubtedly go beyond the simplistic view that relates it solely to lake metabolism. Besides highlighting these deviations in lakes of higher alkalinity, chapter five offers innovative methods that can be used for identifying such deviations by isolating diel signals from time series that often present a lot of noisy or random signals. Once the ability of this parameter to describe calcification and its effect on DIC dynamics has successfully been tested along various lake types, environmental gradients, and seasons, scientific efforts should be made to readapt lake carbon cycle research by accounting for this process along with the more widely acknowledged processes such as lake metabolism. This would minimize the discrepancies between theoretical models and observed data (Lu et al., 2018) and would likely be a solution for explaining the commonly observed imbalances between carbon fluxes and metabolic states (McDonald et al., 2013; Perga et al., 2016; Stets et al., 2009).

While the results in chapter four identify the general effect of NEP on calcification, further research should aim at disentangling the effect of nucleation sites from the effect of NEP, both of which are directly related to primary production. Even though eutrophication likely increases calcification by increasing NEP, research has shown that calcification acts as a self-remediation process against lake eutrophication due to the co-precipitation with phosphates which improves the trophic state of eutrophic lakes (Walsh et al., 2019) (Figure 6.1). However, this mechanism can be prevented if inorganic phosphorus concentrations reach high levels that inhibit calcification (House, 1990) (Figure 6.1). It is still unclear how phytoplankton community composition and their size distribution affect calcification rates. The calcifying planktonic freshwater chlorophyte *Phacotus lenticularis* has shown remarkable contributions to calcification in hard water lakes (Krienitz et al., 1993; Lenz et al., 2018). However, the relative contribution of non-calcifying species that induce calcification by changing the chemical microenvironment of the water layer adjacent to the cell is not well understood. These are added complexities which are beyond the scope of this study. Further research is needed to understand calcite precipitation within the context of lake eutrophication and global change.

Finally, further research should aim at understanding the role of calcification within the context of global change. Chapter four of this thesis identifies NEP and calcite SI as the main drivers of calcification. Both drivers are affected by global change whereby primary production is intensified by lake eutrophication (Carpenter et al., 1998; Conley et al., 2009; Hamilton et al., 2009) and calcite SI is likely to raise with the increase in alkalinity export observed in several parts of the world (Drake et al., 2018; Raymond & Cole, 2003) (Figure 6.1). The consequences of global change effects on lake calcification are not clear yet. Considering the relevance of this process on a global scale, it is necessary to understand its response to a changing environment.

7. General conclusions

Overall, this thesis contributes to a better understanding of pelagic calcification in lakes. It addresses its role as a sedimentary carbon sink and an atmospheric carbon source at a global scale by presenting a first estimation of its contribution in the global carbon budget of lakes. Furthermore, this thesis offers a deeper understanding of the main drivers of lake calcification. The role of planktonic metabolism in driving pelagic calcification is explained within the larger context of water geochemistry, allowing to identify a parameter for this process. At a local scale, this parameter describes the temporal pattern and intensity of calcification, as well as the response of DIC variability to lake metabolism. The main conclusions of this thesis can be summarized as follows:

- Pelagic calcification potentially occurs in lakes that have water alkalinity above 1 meq L^{-1}. This represents more than half of the global surface area covered by lakes and reservoirs. In this regard, pelagic calcification in lakes deserves scientific attention.
- The sink of inorganic carbon related to pelagic calcification is an important component of the carbon budget in lakes, similar in magnitude to the burial of organic carbon. However, a global estimation of calcite dissolution is needed for refining the estimate of the net carbon sink related to pelagic calcification.
- CO_2 emissions due to pelagic calcification are negligible at a global scale. This is explained by the greater buffering capacity of high alkalinity lakes, whereby the CO_2 released by calcification interacts with the DIC equilibria, limiting the evasion of CO_2 to the atmosphere.
- Primary production is a main driver of calcification at a short time scale. Its efficiency in driving calcification depends on calcite saturation that changes at a seasonal scale. This defines the α parameter that describes the relationship whereby the ratio between calcification and NEP depends on calcite saturation. This explains the temporal patterns and intensities of calcification events and offers a parameter that could potentially be used in carbon cycling models.
- Deviations from the 1:1 metabolic stoichiometry between the diel variations in DO and in DIC are dependent on the molar ratio between calcification and NEP.
- In low alkalinity lakes, DIC dynamics can be assesed from metabolic processes. At higher alkalinity, DIC dynamics are controlled simultaneously by metabolic processes and calcification.

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Appendices

Limnetica, 41(1): 000-000 (2022). DOI: 10.23818/limn.41.02
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The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs

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Received: 29/09/20 Accepted: 04/12/20

ABSTRACT

The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs

Calcite precipitation acts as a carbon sink in the sediments and a short-term source of carbon dioxide (CO_2) to the atmosphere, as widely acknowledged in marine studies. However, pelagic calcite precipitation has received limited attention in lakes. Here we use the relationship between lake water alkalinity and reported calcification rates to provide the first global estimate of pelagic calcification in lakes. Global gross calcification rates amount to 0.03 Pg C yr^{-1} (0.01 – 0.07) comparable to rates of organic carbon burial, whereas its related CO_2 release is largely buffered by the carbonate equilibria. Calcification occurs at water alkalinity above 1 meq/L corresponding to 57 % of global lake and reservoir surface area. Pelagic calcification therefore is a prevalent process in lakes and reservoirs at the global scale, with a potentially relevant role as a sedimentary inorganic carbon sink, comparable in magnitude to the total calcite accumulation rates in ocean sediments.

Key words: calcite precipitation, pelagic calcification, carbon cycle, lake carbon budget, carbon fluxes, carbon sink

RESUMEN

La relevancia de la calcificación pelágica en el balance global de carbono de lagos y embalses

La precipitación de calcita actúa como un sumidero de carbono en sedimentos y una fuente a corto plazo de dióxido de carbono (CO_2) a la atmósfera, como es ampliamente reconocido en sistemas marinos. Sin embargo, la precipitación pelágica de calcita ha recibido una atención limitada en lagos. Aquí utilizamos la relación entre la alcalinidad del agua y tasas reportadas de calcificación para proporcionar una primera estima global de calcificación pelágica en lagos. Las tasas de calcificación bruta global ascienden a 0.03 Pg C año^{-1} (0.01 - 0.07) comparables a las tasas de enterramiento de carbono orgánico, mientras que la consecuente liberación de CO_2 está en gran medida amortiguada por los equilibrios de sistema de carbono inorgánico disuelto. La calcificación se produce a alcalinidad superior a 1 meq/L, que corresponde al 57 % de la superficie global de lagos y embalses. Por lo tanto, la calcificación pelágica es un proceso frecuente en lagos y embalses a escala global, con un rol potencialmente relevante como sumidero de carbono inorgánico sedimentario, comparable en magnitud a las tasas de acumulación total de calcita en los sedimentos oceánicos.

Palabras clave: *precipitación de calcita, calcificación pelágica, ciclo del carbono, balances de carbono en lagos, flujos de carbono, sumidero de carbono*

INTRODUCTION

Lakes and reservoirs are important components of the continental carbon (C) cycle due to large emissions of methane and CO_2 to the atmosphere and burial of organic carbon (OC) in their sediments (Cole *et al.*, 2007; Mendonça *et al.*, 2017; Raymond *et al.*, 2013). OC production, decomposition and burial have been described as the main drivers of C fluxes (Duarte & Prairie, 2005). Most studies relating C fluxes to OC metabolism in lakes have been conducted in systems with low water alkalinity (i.e. less than 1 meq/L) and high dissolved OC concentrations (McDonald *et al.*, 2013). However, increasing evidence suggests that inorganic carbon (IC) may be equally relevant in lakes of higher alkalinity (Marcé & Obrador, 2019), comprising 57 % of world's lakes area (Marcé *et al.*, 2015; McDonald *et al.*, 2013). While the role of benthic calcification is acknowledged in such systems, calcification in the pelagic zone is assumed to be negligible due to the rarity of planktonic organisms that produce calcareous shells in freshwaters (Kelts & Hsü, 1978). However, recent evidences stress the important role of pelagic calcite precipitation in lakes as an alkalinity sink and CO_2 source (Müller *et al.*, 2016). This double-edged role is widely acknowledged and quantified in marine studies (Frankignoulle *et al.*, 1994; Ridgwell & Zeebe, 2005), but its relevance in lakes has been assumed to apply only to saline systems. Evidence suggests that calcification contributes to the large CO_2 emissions found in saline lakes, yet those systems represent only a fifth of the Earth's lake surface (Duarte *et al.*, 2008). The biogeochemistry of carbon in saline lakes usually differs from those of freshwater lakes, because of the large carbon pools supporting high CO_2 exchange rates and chemical enhancement processes that are usually less relevant in freshwater systems (Duarte *et al.*, 2008). It is therefore necessary to study pelagic calcification in freshwater lakes separately to assess the relevance of this process in these systems representing the vast majority of the Earth's lake surface. Moreover, the majority of freshwater lakes are above the alkalinity threshold of 1 meq/L where calcification is likely to occur (Khan *et al.*, 2020; Marcé *et al.*, 2015; McConnaughey & Whelan, 1997). While the occurrence of pelagic calcification in freshwater lakes has often been reported, it has never been quantified at a global scale, nor has its derived CO_2 emissions.

High rates of calcite precipitation are often found during the summer season of lakes when calcite saturation peaks due to enhanced primary production that rises the pH by depleting CO_2 concentrations, thereby shifting the carbonic acid equilibrium towards oversaturation of carbonates (Müller *et al.*, 2016). Yet, spontaneous calcification is unlikely to occur, rather it is biologically mediated by picocyanobacteria that provide ideal nucleation sites that further facilitate calcite precipitation in the pelagic zones of lakes (Obst *et al.*, 2009). Calcite precipitation in lakes has been presented as a natural process that can reduce summer eutrophication (Rodrigo *et al.*, 1993). Because of this seasonal association and its occurrence during high primary production, most studies that have quantified calcite precipitation in lakes have focused on the summer season. Using summer pelagic calcification rates from the literature (n = 26, Supplementary information, available at http://www.limnetica.net/en/limnetica), here we identify a clear association with water alkalinity. Using water alkalinity as the driver variable with obvious mechanistic links with calcification, we provide a first gross global estimate of calcification rates and its derived CO_2 emissions. We intentionally excluded rates measured in other seasons than summer or averaged over the year, rates for benthic calcification caused by calcifying macrophytes, rates in brackish systems and saline lakes.

METHODS

Data on pelagic calcite precipitation was obtained from published literature reporting areal or volumetric rates from either sediment traps, sediment cores, C or Ca^{2+} mass balances, incubations, or modelling (Supplementary information, available at http://www.limnetica.net/en/limnetica). Most rates found in the literature are average rates covering only the summer season, therefore we excluded mean annual rates from this analysis. When reported per unit area, areal rates were divided by the mixed layer depth (Zmix) to obtain rates per unit volume, assuming that most pelagic calcification occurs in the epilimnion (Hartley *et al.*, 1995; Obst *et al.*, 2009). If Zmix of a specific lake was not specified in the literature, it was calculated using FLAKE Global model (Kirillin *et al.*, 2011). The strong relationship found between alkalinity and calcification rates was used in the upscaling to global rates, using the 5 %, 50 % and 95 % quantiles to obtain a range of uncertainty. The volumetric rates obtained from this relationship were converted to areal rates by multiplying the volumetric rates with an average Zmix value of 6.72. The average Zmix was calculated from a dataset of 628 lakes (Woolway & Merchant, 2019), using a hyperbolic function between Zmix and lake fetch (Lewis, 2011). The areal rates by alkalinity level were multiplied by the global surface area of lakes corresponding to each level of alkalinity (alkalinity intervals of 0.05 meq/L) (Marcé *et al.*, 2015). The sum of these rates was multiplied by the average number of days of measurements (94 days) from which each reported daily rate in the literature was averaged. These calculations are presented in the supplementary Data file.

For upscaling CO_2 emissions, the 5 %, 50 % and 95 % quantiles of the relationship between alkalinity and calcification was used to build a model that simulates the resulting range of lake CO_2 partial pressure (pCO_2) increase at different daily calcification rates that are dependent on the initial alkalinity value. The model is based on the chemical dissolved inorganic carbon (DIC) equilibria whereby 1 mole of calcite that precipitates results in a loss of 1 mole of DIC (because 2 moles of HCO_3^{2-} are lost in the reaction and 1 mole of CO_2 is released) and a loss of 2 equiva-

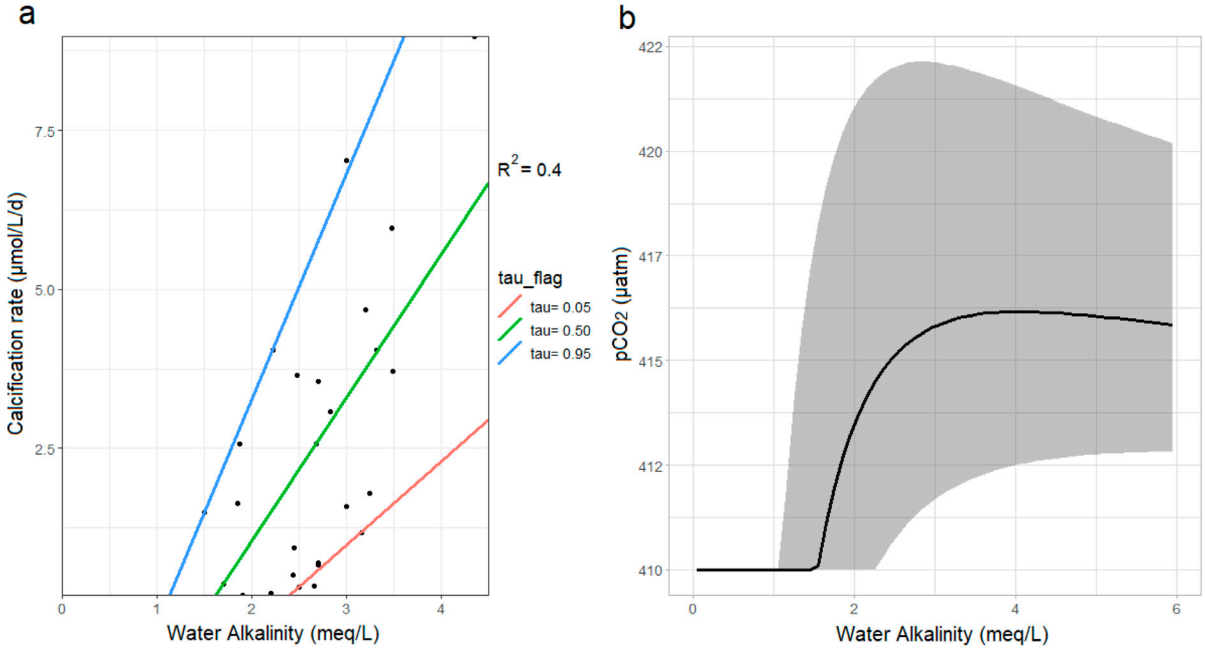

Figure 1. Effects of alkalinity on calcification rates. (a) relationship between alkalinity and calcification rates reported in the literature for global lakes. Lines corresponds to quantile regressions at the 95 % quantile (blue), 50 % (green), and 5 % (red). (b) Daily effect of calcite precipitation on pCO_2 at different alkalinity levels. Initial pCO_2 value is at atmospheric equilibrium. The solid line represents the 50 % quantile. The shaded area corresponds to the range of calcification found at a given alkalinity level using the 5% and 95% quantiles. *Efectos de la alcalinidad sobre las tasas de calcificación. (a) relación entre alcalinidad y tasas de calcificación reportadas en la bibliografía para lagos. Las líneas corresponden a regresiones de cuantiles al cuantil 95 % (azul), 50 % (verde) y 5 % (rojo). (b) Efecto diario de la precipitación de calcita sobre la* pCO_2 *a diferentes niveles de alcalinidad. El valor inicial de* pCO_2 *está en equilibrio atmosférico. La línea sólida representa el cuantil 50 %. El área sombreada corresponde al rango de calcificación encontrado a un nivel dado de alcalinidad usando los cuantiles del 5 % y 95 %.*

lents of alkalinity. The fate of the CO_2 released by calcification and its interaction with the DIC equilibria is therefore taken into account in this model. The initial pCO_2 value was set at the atmospheric equilibrium value of 410 ppm. The model is freely available online in the following link (http://hdl.handle.net/2445/172489). The global upscaling was done similarly to the upscaling of calcification rates, using existing data of the global surface area of lakes per alkalinity levels (Marcé *et al.*, 2015). CO_2 flux was calculated using Fick's Law of gas diffusion (Millero, 1979) using the global average Piston velocity (k_{600}) value of 1 m/d with an uncertainty range between 0.74 and 1.33 (Raymond *et al.*, 2013). The sum of the fluxes correspond to the global annual CO_2 emissions caused by planktonic calcification. It is a first estimate of the CO_2 emissions related to calcification and should therefore be understood as an approximate estimation that can be further refined by research, rather than an ultimate value. All calculations are available in the Supplementary Data File.

RESULTS AND DISCUSSION

A significant positive linear relationship ($R^2 = 0.4$, n = 26, $p < 0.001$) was found between water alkalinity and calcite precipitation rates, suggesting that the importance of this process is significantly higher with increasing alkalinity (Fig. 1a). No significant effect of chlorophyll-a and total phosphorus on calcification rates was found and including these variables with alkalinity in a multiple linear regression only decreased the strength of the model ($R^2 = 0.34$, $p < 0.05$). However, in lakes, spontaneous calcification is unlikely to occur. Rather, it is mediated by picoplankton that promote the chemical environment necessary for calcification by increasing the pH and by offering ideal nucleation sites (Obst *et al.*, 2009). The absence of any significant effect of chlorophyll-*a* and total phosphorus concentrations could be due to the fact that calcite saturation is more determined by alkalinity than by production of the system. The strength of primary production to promote calcification likely depends on the level of calcite saturation which is directly related to water alkalinity. A quantile regression using the 95 % quantile suggests an alkalinity threshold for calcification at ~ 1 meq/L (Fig. 1a), below which calcification is unlikely to occur. This is consistent with the threshold of 1 meq/L reported in previous studies (Marcé *et al.*, 2015; McConnaughey & Whelan, 1997). Considering that 57 % of the global area occupied by lakes and reservoirs have alkalinity values above 1 meq/L (Marcé *et al.*, 2015), pelagic calcite precipitation is likely to occur in most lakes and reservoirs around the globe. In addition, the 5 % quantile regression indicates that virtually all lakes with alkalinity beyond 2.5 meq/L will show summer pelagic calcification (Fig. 1a).

Calcite precipitation and dissolution reactions imply changes on both alkalinity and the relative abundance of (DIC) species (Ridgwell & Zeebe, 2005) (1).

$$Ca^{2+} + 2HCO^{3-} \leftrightarrow CaCO_3 + CO_2 + H_2O$$
equation (1)

Whereby calcite precipitation releases one mole of CO_2 per mole of calcite precipitated, increasing pCO_2. Because of the higher calcification rates found in lakes of higher alkalinity, one would expect the increase in pCO_2 caused by calcification to be stronger in such systems. However, the ratio between calcite precipitation and CO_2 release (commonly referred to as Ψ) depends on the capacity to buffer changes in pCO_2 (Frankignoulle *et al.*, 1994; Smith & Mackenzie, 2016). As a result, the buffering capacity of high alkalinity lakes mitigate pCO_2 increase preventing most of the released CO_2 to escape to the atmosphere (Fig. 1b). Therefore, the potential change in pCO_2 caused by calcite precipitation on a daily time scale is minimal (Fig. 1b).

We used the alkalinity – calcification rates relationship together with the global distribution of lakes and reservoirs per alkalinity level (Marcé *et al.*, 2015) for upscaling calcite precipitation rates to the entire globe. The resulting annual global rate of calcite precipitation of 0.03 Pg C yr^{-1} (range 0.01 – 0.07 Pg C yr^{-1}) is comparable in magnitude to global annual rates of OC burial of 0.15 Pg C yr^{-1} (range 0.06 – 0.25 Pg C yr^{-1}, (Mendonça *et al.*, 2017), Fig. 2a). Global calcite precipitation would thus be of the same order of magnitude as OC burial, making them both similarly relevant as main drivers of C cycling in lakes. As a comparison, the average areal calcification rates in lakes (13.8 g C m^{-2} yr^{-1}, range: 5.4 – 28, Supplementary Data File) correspond to 37 % (14 % - 75 %) of areal rates of pelagic calcification in oceans (37 g C m^{-2} yr^{-1}) (Smith & Mackenzie, 2016).

The net role of pelagic calcification as a sedimentary inorganic carbon sink depends on calcite dissolution during sedimentation and in the sediments. In open oceans 80 % of all carbonate that precipitates dissolves within the water column or in the sediments (Ridgwell & Zeebe, 2005). Although some studies have reported calcite dissolution in some lakes (Ohlendorf & Sturm, 2001; Ramisch *et al.*, 1999), others report no dissolution during settling of calcite crystals (Stabel, 1986). A study on 13 lakes revealed that calcite dissolution in lakes and reservoirs is minor compared to calcite precipitation because rather than dissolving in the hypolimnion, calcite tends to accumulate in the sediments (Müller *et al.*, 2016), thereby acting as a long-term C sink. These results are in agreement with another study on 5 lakes that reports calcite precipitation with no evidence of calcite dissolution (Lenz *et al.*, 2018). Yet, high rates of calcite dissolution are reported in deeper stratified systems rich in OC where decomposition of organic matter promotes anoxic conditions and low pH in the hypolimnion (Ohlendorf & Sturm, 2001) and where calcite crystals may partly dissolve in the water column during sedimentation (Ramisch *et al.*, 1999). The global calcification rate provided in this study is a gross estimate to be further built upon. The role of calcite dissolution needs to be assessed at a global scale to determine the net burial flux, which is beyond the scope of this study. Considering calcification alone, the total export of calcite to the sediments would represent between 10 % and 70 % of the total net oceanic sink for pelagic calcification (0.1 Pg C yr^{-1}) (Sabine & Tanhua, 2010). So long as calcite dissolution hasn't been estimated at a global scale, these values remain hypothetical. This is even more uncertain in the context of global change which is likely to increase the frequency of anoxic conditions in the hypolimnion of stratified systems (Jankowski *et al.*, 2006; Schwefel *et al.*, 2016) which could promote ideal conditions for calcite dissolution. Nevertheless, these values highlight the disproportionately important role of calcite precipitation in lakes and reservoirs as a potential carbon sink despite the relatively small area that these water bodies occupy on the globe.

The gross estimate of global CO_2 emissions caused by calcification is 0.0002 Pg C yr^{-1} (range: 0.00005 – 0.0007 Pg C yr^{-1}). This is an irrelevant fraction of the total CO_2 emissions from lakes and reservoirs (< 0.5 %, Fig. 2b). Our results contrasts with those of saline lakes where calcite precipitation is expected to contribute significantly to CO_2 emissions (Duarte *et al.*, 2008). The low contribution of calcification-derived CO_2 emissions is because higher rates of calcite precipitation are found in lakes of higher alkalinity (Fig. 1) that have a stronger capacity to buffer changes in pCO_2. Recent studies found that inorganic processes including DIC loading from chemical weathering and calcite precipitation are important drivers of CO_2 emissions in lakes and reservoirs (Marcé *et al.*, 2015; McDonald *et al.*, 2013). Our results suggest that pelagic calcite precipitation represents a minor share of emissions caused by inorganic sources (Fig. 2b).

It is important to mention that pelagic calcification rates reported here are probably only a share of total calcification in lakes and reservoirs. Benthic primary production can account for a large fraction of the overall metabolism of lakes (Cremona *et al.*, 2016), and benthic habitats dominated by calcifying macrophytes can promote daily losses of up to 0.57 meq/L of alkalinity due to intense calcification (Andersen *et al.*, 2017). In the absence of a global estimate of lake benthic calcification we can only speculate about its relevance, but considering potentially high calcification rates from benthic macrophytes (McConnaughey & Whelan, 1997) and their prevalence in lakes, we posit benthic calcification could be even higher than pelagic. Furthermore, our global estimate only accounts for calcification during the summer season assuming that this represents the largest part within a year. While calcification usually occurs mainly in the summer season, it is variable in time and the possibility of its occurrence in other seasons cannot be excluded (Trapote

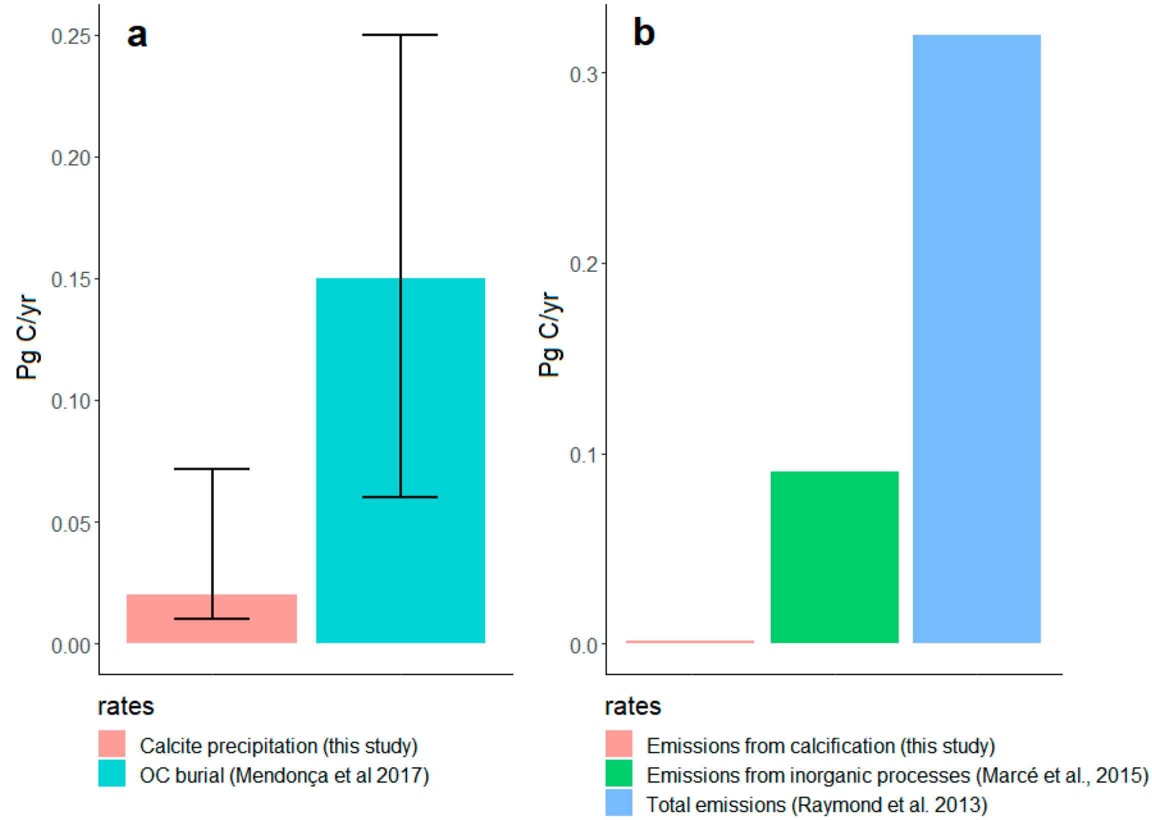

Figure 2. Global rates of calcification and CO_2 emissions. (a) Global annual rates of calcite precipitation (this study) and OC burial (Mendonça *et al.*, 2017) in lakes and reservoirs. The error bars represent the range. (b) Global rates of CO_2 emissions from calcification (this study), inorganic processes (Marcé *et al.*, 2015) and total emissions (Raymond *et al.*, 2013). *Tasas globales de calcificación y emisiones asociadas de* CO_2. *(a) Tasas globales anuales de precipitación de calcita y enterramiento de carbono orgánico en lagos y embalses. Las barras de error representan el rango. (b) Tasas globales de emisiones de* CO_2 *en lagos asociadas a la calcificación (este estudio), procesos inorgánicos (Marcé* et al.*, 2015) y emisiones totales (Raymond* et al.*, 2013).*

et al., 2018). The seasonality of calcification is likely to be directly related to the seasonality of primary production since calcification in lakes is a biologically mediated process that is enhanced during peaks of primary production (Obst *et al.*, 2009). Arriving at a mechanistic understanding of how primary production promotes calcification within the physicochemical context of a lake, including its alkalinity level, is a next step to predict calcification events in lakes and further refine its global estimation by integrating seasonality at a finer scale.

While CO_2 emissions caused by calcite precipitation is minor at a global scale, CO_2 release during summer calcification can have significant implications on C dynamics at a local scale, resulting in imbalances between metabolic rates and C concentrations (Khan *et al.*, 2020). Such imbalances can reach a point where lakes are simultaneously in an autotrophic state, yet they emit CO_2 to the atmosphere, as is likely the case in approximately one third of lakes in the boreal region and in the contiguous United States (Bogard & Giorgio, 2016; McDonald *et al.*, 2013). Accounting for the role of calcite precipitation on C dynamics at a local scale is necessary to improve the reliability and predictability of lake models that are currently unable to explain the reported imbalances between metabolism and C fluxes (Lu *et al.*, 2018).

To conclude, the rates provided here highlight the role of pelagic calcite precipitation as an important component of the C cycle in lakes and reservoirs and the need to account for it in global C budgets (Marcé & Obrador, 2019). Research should aim at quantifying the ratios between production and calcification rates as well as between calcite precipitation and CO_2 release along alkalinity gradients in order to account for this process in models of inland water C processing. Temporal patterns of calcite precipitation and environmental conditions for its occurrence need to be investigated for predicting this process at short and longer temporal scales. Finally, calcite precipitation and dissolution in lakes and reservoirs need to be understood in the context of climate change predictions in order to assess their role as potential C sinks or C sources in a changing climate.

ACKNOWLEDGMENTS

This study was funded by MANTEL ITN (Management of climatic extreme events in lakes and reservoirs for the protection of ecosystem services) through European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 722518, by the Estonian Research Council grant (PUT PSG32), and by the project C-HYDROCHANGE (CGL2017-86788-C3-3-P and CGL2017-86788-C3-2-P) funded by the Spanish Ministry of Science, Innovation and Universities.

DATA AVAILABILITY STATEMENT

All calcification rates collected from the literature are compiled in the Supplementary Data Excel file, available at http://www.limnetica.net/en/limnetica. The calculations for the global upscaling of calcification rates and its associated CO_2 emissions are presented in this same data Excel file. The code that was used for calculating the effect of calcification on CO_2 partial pressure is available online (http://hdl.handle.net/2445/172489).

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Eutrophication and Geochemistry Drive Pelagic Calcite Precipitation in Lakes

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Citation: Khan, H.; Laas, A.; Marcé, R.; Sepp, M.; Obrador, B. Eutrophication and Geochemistry Drive Pelagic Calcite Precipitation in Lakes. *Water* **2021**, *13*, 597. https://doi.org/10.3390/w13050597

Academic Editor: Soren Brothers

Received: 18 January 2021
Accepted: 19 February 2021
Published: 25 February 2021

Abstract: Pelagic calcification shapes the carbon budget of lakes and the sensitivity of dissolved inorganic carbon (DIC) responses to lake metabolism. This process, being tightly linked to primary production, needs to be understood within the context of summer eutrophication which is increasing due to human stressors and global change. Most lake carbon budget models do not account for calcification because the conditions necessary for its occurrence are not well constrained. This study aims at identifying ratios between calcification and primary production and the drivers that control these ratios in freshwater. Using in situ incubations in several European freshwater lakes, we identify a strong relationship between calcite saturation and the ratio between calcification and net ecosystem production (NEP) (p-value < 0.001, $R^2 = 0.95$). NEP-induced calcification is a short-term process that is potentiated by the increase in calcite saturation occurring at longer time scales, usually reaching the highest levels in summer. The resulting summer calcification event has effects on the DIC equilibria, causing deviations from the metabolic 1:1 stoichiometry between DIC and dissolved oxygen (DO). The strong dependency of the ratio between NEP and calcification on calcite saturation can be used to develop a suitable parameterization to account for calcification in lake carbon budgets.

Keywords: calcite precipitation; eutrophication; metabolism; geochemistry; lake carbon budget; primary production; alkalinity; calcite saturation

1. Introduction

Considering their comparatively small global surface area, lakes and reservoirs play a disproportionate role in the continental carbon (C) cycle due to their large contributions of carbon dioxide (CO_2) and methane emissions to the atmosphere and burial of organic carbon (OC) in their sediments [1–3]. Numerous observations from lakes with low water alkalinity and high dissolved organic carbon (DOC) concentrations have stressed the prevalence of heterotrophy and its paramount effect on lake CO_2 fluxes [4–6]. However, increasing evidence also stresses the importance of inorganic processes in driving C fluxes in lakes of higher alkalinity (i.e., >1 meq/L), comprising 57% of the world's lakes area [7,8]. In such systems, pelagic calcite precipitation acts as an important alkalinity sink and CO_2 source [9]. Identifying the drivers of this process is necessary to be able to estimate its contribution to carbon fluxes.

High rates of calcite precipitation are often found during the summer season when calcite saturation peaks due to enhanced primary production that raises the pH [9–12]. The abundance of nucleation sites provided by autotrophic picoplankton, particularly picocyanobacteria, further facilitates calcite precipitation in the pelagic zones of lakes [13–

15], which in some cases reach such intensities where lakes take on a milky appearance caused by the calcite crystals that can be seen from satellite imagery [16]. Calcification is therefore expected to be directly related to primary production, and hence to the trophic state of a lake. In recent decades, eutrophication has become a major issue as the concentrations of dissolved phosphorus and nitrogen in water bodies have increased due to human activities such as extensive agriculture or pollutant discharges from point sources such as sewage water treatment plants [17]. The increasing nutrient supply increases the phytoplankton abundance [18] and can lead to cyanobacteria blooms [19]. Such stressors are likely to promote calcification events in lakes by increasing the pH through increased primary production and by increasing the amount of nucleation sites through picocyanobacteria [13–15]. Within this context of increasing eutrophication, quantifying and predicting the way primary production promotes calcification are necessary for estimating carbon budgets in lakes and reservoirs under present and future scenarios. This study is a first attempt at identifying a quantifiable relationship between primary production and calcite precipitation in freshwaters.

Calcification can have a significant effect on the C budget of a lake, simultaneously acting as a C sink to the sediments [10] and increasing the water CO_2 partial pressure (pCO_2) due to the CO_2 release caused by the reaction of calcium carbonate formation [20,21]. However, calcification is not accounted for in C budgets or in lake models for two reasons. Firstly, contrary to marine systems, pelagic calcification is assumed to play a minor role in carbon fluxes of freshwater systems, likely because most studies that link CO_2 supersaturation to net heterotrophy are conducted in lakes that have relatively low alkalinity and high dissolved organic carbon (DOC) values[8]. However, calcite precipitation has been presented as a potentially important component of global carbon budgets for saline lakes [22] and recent findings suggest the same for freshwaters [23]. Secondly, there is a need to understand the mechanisms of calcification and the drivers that control it, so that calcification could be parameterized and included in lake models. Currently, the lack of a proper parameterization accounting for CO_2 derived from calcite precipitation results in discrepancies between theoretical models and empirical data [24]. This study aims at solving this issue by identifying key variables that control calcite precipitation and that could be used for the parameterization of calcite precipitation.

Attempts to parameterize lake calcification rates have related them to net ecosystem production (NEP) by observing ratios between the two, although calcite precipitation to NEP molar ratios (α) have rarely been studied in freshwater systems. The few studies that have measured this ratio in freshwater systems constrain it in the range between 0.2 and 1 [25–27]. This range is similar for marine planktonic assemblages [28] but lower than the ratio of 1.3 found in marine benthic ecosystems [29]. While the 1:1 ratio is often found in calcifying macrophytes, this ratio can increase at pH levels above 9 due to CO_3^{2-} competing with HCO_3^- for protonation [26]. The α has been assumed to be alkalinity-dependent [7,30], yet the nature of this dependency is not fully understood nor has it been precisely quantified. The purpose of this study is to identify and quantify the effects of lake NEP on calcification rates under different environmental conditions, by observing variations in α along gradients of calcite saturation and alkalinity. A mechanistic understanding of the occurrence of calcification is necessary for including this process in carbon budgets and for parameterizing calcification effects on CO_2 in lake models under different eutrophication scenarios.

2. Materials and Methods

2.1. Study Sites

The study was conducted in 4 European freshwater lakes covering a wide range of water alkalinity (from 0.5 to 4 meq/L; Table A1). The lake with the lowest alkalinity (0.5 meq/L), Valguta Mustjärv, was included in this study to observe the absence of calcification expected from such low-alkalinity lakes. Three lakes in Estonia were sampled during summer 2019 and one reservoir in Spain was sampled during summer 2018. All four water bodies differ substantially in their water retention time as well as in their catchment characteristics (Table 1), especially in regard to land use. However, all of the studied lakes were highly productive, according to nutrient (nitrogen and phosphorus) concentrations and chlorophyll-a values (Table A1). Each of these lakes has been extensively studied and described in detail elsewhere [31–34].

Table 1. Characteristics of the studied lakes. Values represent long-term averages.

Lake	Trophic State	Mixing Regime	Area (Ha)	Mean Depth (m)	Maximum Depth (m)	Kd (m)	Watershed Size (km2)	Water Residence Time (y)
Valguta Mustjärv (EE)	hypertrophic	polymictic	20.4	<1	1	10.3	1.34	source lake
Erastvere (EE)	hypertrophic	dimictic	16.3	3.5	9.7	2.9	5.2	0.5
Võrtsjärv (EE)	eutrophic	polymictic	27000	2.8	6	2.7	3116	1
Sau Reservoir (ES)	eutrophic	monomictic	600	25.2	65	0.9	1522	0.3

2.2. Experimental Setup

Five experimental campaigns using in situ incubations were conducted in Sau Reservoir (Spain) from May to August 2018. The following year, the same experiments were performed twice (first in June, then in July 2019) in the three Estonian lakes; Valguta Mustjärv, Erastvere and Võrtsjärv. The sampling combined experimental determination of lake calcification and NEP from the incubations, with high-frequency monitoring (HFM) of lake water characteristics during extended time periods. These two combined approaches were used so that calcification, assessed through experimental samplings, could be understood within the context of seasonal variations in parameters that can affect the process of calcification, especially dissolved oxygen (DO) and temperature as indicators of primary production. The experiment consisted of in situ incubations of surface lake water for 5–6 hours, from where the initial and final values of temperature (T), pH, electrical conductivity (EC, $\mu S\ cm^{-1}$), dissolved oxygen (DO, $mg\ L^{-1}$), dissolved oxygen saturation (DOsat, %), calcium concentration ($[Ca^{2+}]$ $mg\ L^{-1}$) and total alkalinity (TA, $meq\ L^{-1}$) were measured. Surface water was collected in a large (>9 L) plastic container from the pelagic zone at the deepest area of each lake. The initial T, pH, EC and DO were determined in triplicate using a handheld sensor calibrated in situ immediately before the experiment (for campaigns in Estonia during June 2019: WTW 2FD47D Multi 3430 Multiparameter Meter with Sentix 940-3 for pH, Tetracon 925 for EC and FDO 925 for DO; for campaigns in Estonia in July: same as in June, except that for DO, we used a Dissolved Oxygen Meter MARVET JUNIOR; for campaigns in Spain: YSI ProODO Handheld for the DO and WTW 3320 for EC and pH). Triplicate water samples for the determination of initial TA (120 mL, stored at 4 ºC in Nalgene bottles) and $[Ca^{2+}]$ (10 mL filtered at 0.45 µm, acidified in situ with 100 µl of 60 % HNO_3 and stored at 4 °C in PP tubes) were taken for laboratory measurements.

The incubations were performed in 5 transparent Winkler-type glass bottles of 250 mL at 30 cm depth in the littoral zone of each lake to maintain the light conditions of the upper mixed layer. The incubation bottles were carefully filled with water from the container with a pipe to avoid air mixing using a 250 µm mesh fixed between the container and the pipe for filtering zooplankton and detritus. The absence of any small air bubbles inside the bottles was carefully checked before starting the incubations. After the incubation period, the final T, pH, EC and DO were determined immediately after opening each bottle, minimizing turbulence. Triplicate samples were taken from each bottle for later laboratory analysis to determine the final $[Ca^{2+}]$ and TA, following the same procedures as for the initial conditions. All alkalinity samples were stored in a fridge and were measured using H_2SO_4 0.02 N titration the following day. An automatic titrator (Metrohm 655 Dosimat (in Spain) and SI Analytics TitroLine® 6000 (in Estonia)) was used to determine TA in a large sample volume (60 mL) and slow injection settings. The titrators were configured to increase the precision of the alkalinity determinations to 20 μeq L^{-1} (determined by replicated titrations of $NaCO_3$ solutions). Samples for $[Ca^{2+}]$ were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). All ICP-MS analyses were performed in a Perkin Elmer Optima 8300 at the Scientific and Technological Centre (CCiT) of the University of Barcelona.

2.3. Open Water Dynamics

Surface water sampling and HFM were used simultaneously in all lakes. However, in Sau Reservoir, surface water sampling was performed 9 times at a time interval of 2–3 weeks from May until the end of September 2018, while only 2 surface water samplings were performed in each Estonian lake. The higher number of surface water samplings in Sau Reservoir allowed us to simultaneously observe, at a finer scale, the trends in $[Ca^{2+}]$, TA and calcite saturation measured from water samples, and the trends in T and DO, from HFM, for the entire summer season.

Surface water samples were taken from the deepest point of the reservoir and analyzed following the same procedures as for the incubations. In Sau, the HFM station [35] was located at the deepest point of the reservoir. DO and T measurements were automatically taken every 5 min using a multiparametric sonde (Yellow Springs Instruments 600XLM V2). The sensors were calibrated before deployment and were placed at a depth of 0.3 m at the deepest area of the reservoir. Sensors were regularly cleaned, and the oxygen sensor was equipped with an automated wiper. No drifts in the measured values were noticed between sampling campaigns, and the data were verified by comparison with the values provided by surface water monitoring using the handheld sensors.

All studied Estonian lakes were equipped with a high-frequency monitoring platform or small lake buoy (OMC-7012 data-buoy) for the whole experimental period. Continuous monitoring of DO and T was performed with Ponsel OPTOD sensors, and CO_2 concentrations were monitored with AMT Analysenmesstechnik GmbH sensors. In all lakes, sensors were placed between 0.3 and 0.5 m depth, close to the surface. In Lake Võrtsjärv, additional pH data were collected with a multiparametric sonde (Yellow Springs Instruments 6600 V2–4) at one meter depth. In Erastvere, the installed lake buoy stopped recording the measurements from the end of June until the beginning of July, due to technical issues. The time series for the measured parameters in the Estonian lakes are presented in the Appendix figures (Appendix Figures A2–A4). Measured water temperature and air pressure data were used to calculate the real CO_2 partial pressure (pCO_2) and dissolved CO_2 concentration from the signals captured by the sensors, according to the manufacturer manuals [31].

2.4. Measurements of Hydrochemical Parameters

All lakes were sampled for chlorophyll-*a* (μg L^{-1}), total phosphorus (TP, mg L^{-1}), total nitrogen (TN, mg L^{-1}) and dissolved organic carbon (DOC, mg L^{-1}) concentrations (Table A1). For chlorophyll-*a* analysis, water samples were concentrated on Whatman GF/F glass microfiber filters, extracted with 90% acetone and measured spectrophotometrically. Chlorophyll-*a* concentrations were calculated using equations of Jeffrey and Humphrey [36]. TP concentrations were determined using the molybdenum blue spectrophotometric method [37] after oxidation to phosphates (PO_4^{3-}) with potassium persulfate ($K_2S_2O_8$). TN concentrations were determined with the ultraviolet spectrophotometric screening method [38] after oxidation to nitrates (NO_3^-) with $K_2S_2O_8$. For DOC analysis, water samples were filtered through Whatman GF/F glass microfiber filters. DOC concentrations were determined using a Shimadzu TOC-V_{CPH} analyzer, and this method conforms to the European standard [39].

2.5. Data Analysis

The dissolved inorganic carbon (DIC, mM) and carbonate ([CO_3^{2-}], mM) concentrations were determined using T, pH, EC and TA to solve the carbonate equilibrium as implemented in the CO$_2$sys application [40], using the constants for freshwater in Millero (1979) [41].

The calcite saturation index (SI) was calculated as the logarithm of the saturation state of calcite (Ω) at the in situ temperature and conductivity:

$$SI = \log(\Omega)$$

$$\Omega = ([Ca^{2+}][CO_3^{2-}])/Kcal,$$

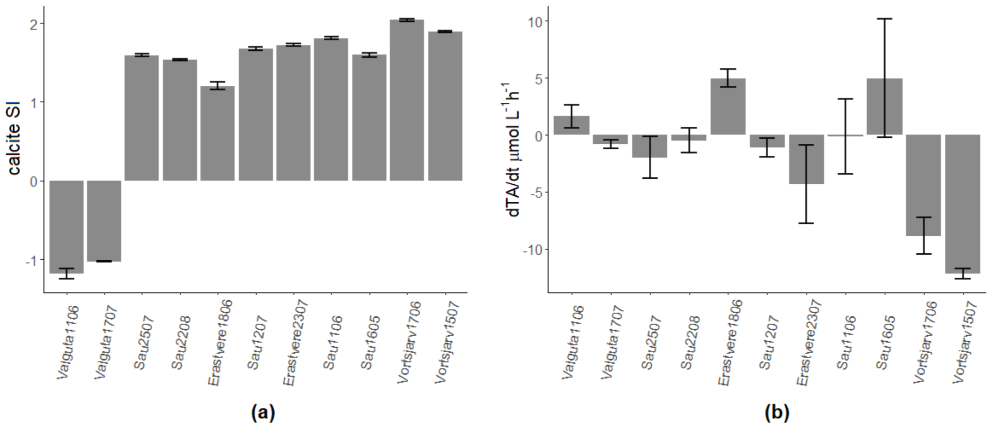
where Kcal is the apparent solubility product for calcite at the observed salinity and temperature [42].

The rates of change of DIC, DO and TA in the incubation bottles (dDIC/dt, dDO/dt, dTA/dt, respectively) were calculated by dividing the change between final and initial conditions by the time period of incubation in each bottle. Changes in TA (dTA/dt) were used as a proxy for calcification, following the chemical equation of calcite precipitation whereby 2 equivalents of alkalinity are lost per mole of calcite that precipitates. Therefore, negative changes in alkalinity were divided by −2 to obtain the corresponding calcification rates. The precision of Ca^{2+} analyses (~0.3 ppm) was not high enough to use Ca^{2+} concentrations directly as an indicator of calcification, which is why we used the changes in TA as a proxy instead.

The 5 incubation bottles were treated as replicates. Therefore, the values for each variable were expressed as the mean and standard deviation (s.d.) of the 5 replicates. For each campaign, extreme outliers within the 5 replicates were removed from the dataset (5 outliers for alkalinity and 4 outliers for DO). NEP in the bottles was measured from dDO/dt.

3. Results

All lakes had a positive calcite SI, so calcite precipitation was theoretically possible, except for Valguta Mustjärv, a brown-water bog lake with low alkalinity and pH (Figure 1a). As for the other lakes, calcification, detected as a decrease in alkalinity during incubations, was found in all campaigns except for Lake Erastvere on 18.6.19 and Sau Reservoir on 16.5.18 (Figure 1b). Hereinafter, the analyzed dataset will be restricted to the seven campaigns with detectable calcification, i.e., those which showed a positive calcite SI as well as a decrease in TA.



(a)

(b)

Figure 1. (**a**) Calcite saturation index (SI) in the 11 experimental campaigns. (**b**) Mean (± sd) rate of change of alkalinity. Numbers after the name of lakes represent the day and month of sampling; all lakes were sampled in 2019 except for Sau Reservoir in 2018.

In the seven campaigns that showed calcification, no significant direct effect of NEP on calcification was found (Figures 2a and A1a), nor was there a strong direct relationship between calcite saturation and calcification (Figure 2b). However, the ratio between calcification and NEP showed a strong power relationship with the calcite SI (Figures 3 and A1c).

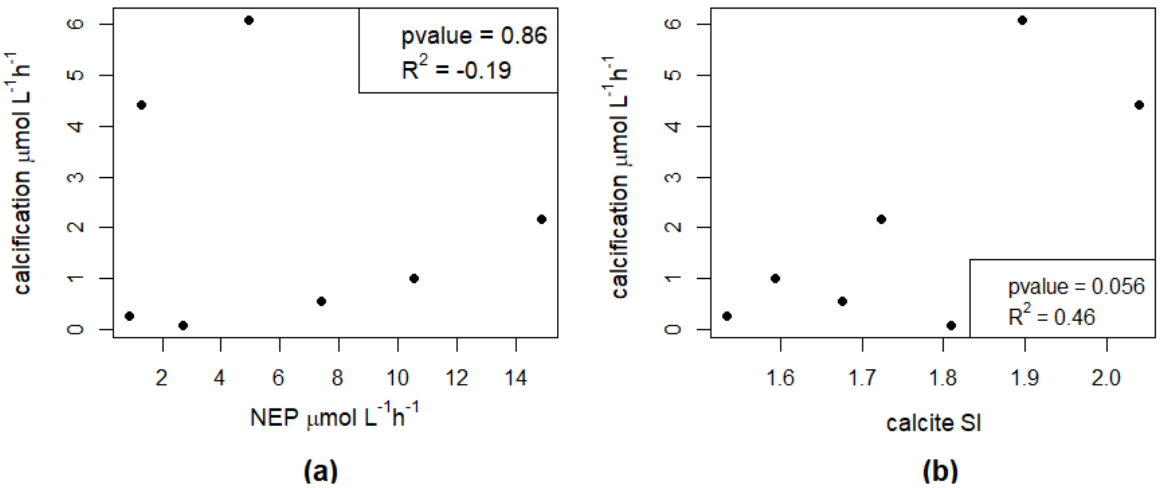


Figure 2. (**a**) Relationship between net ecosystem production (NEP) and calcification rates. (**b**) Relationship between calcite SI and calcification rates.

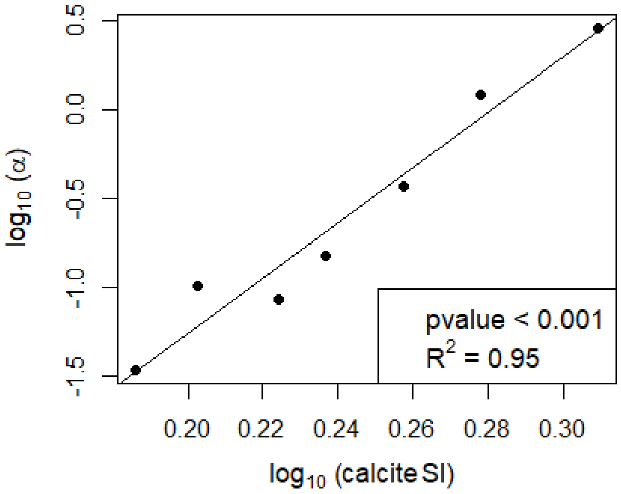


Figure 3. Relationship between the calcification to NEP ratio (α) and the calcite SI (note the log–log scale).

The calcification/NEP ratio (α) had a strong effect on the response of DIC to metabolic changes in oxygen, as shown by the relationship between α and the ratio between dDIC/dt and dDO/dt (dDIC/dDO, Figure 4). At α close to 0, DIC changes followed the metabolic signal with a dDIC/dDO ratio close to the expected value of −1 when metabolism dominates the DIC variability. At larger α (i.e., in conditions of higher calcification than expected by metabolism), the DIC change is greatly amplified, with a dDIC/dDO ratio up to ~−8.

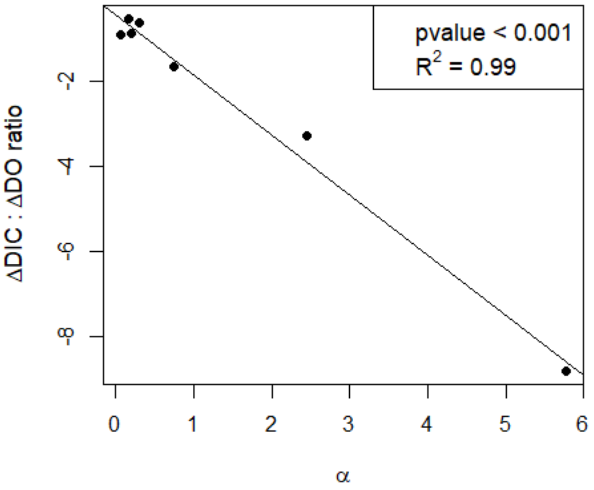


Figure 4. Relationship between the calcification/NEP ratio (α) and the response of dissolved inorganic carbon (DIC) to metabolism.

Surface water samples in Sau Reservoir revealed a decrease in calcium concentrations, likely caused by calcification, during the second half of the month of June and the first half of the month of July 2018 (Figure 5). During this period, the calcite saturation was at its highest value. The HFM measurements and surface samples for DO revealed a sudden increase in DO during the second half of June and then a second wave of increase during the first half of July, coinciding with the period of calcium decrease (Figure 5). This increase in DO resulted from peaks in primary production caused by the increase in temperature during this period (Figure 5), and this was also confirmed by increasing chlorophyll-a values (Table A1). From the number of calcium samples, it is unclear whether calcium decreased continuously during this period or followed the two peaks in DO (Figure 5). After this calcification period, no significant decrease in calcium concentrations was found despite the water being highly saturated in oxygen.

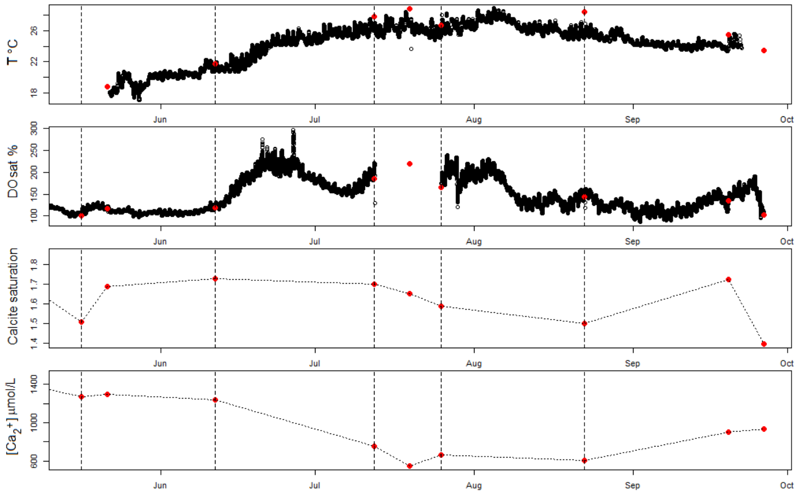


Figure 5. Seasonal evolution of surface water variables in Sau Reservoir. The high-frequency measurements of temperature (T) (in degrees Celsius) and dissolved oxygen saturation (DOsat) (dark points) are shown together with the spot surface water measurements (red points) of T, DOsat, calcite SI and [Ca2+] at the time of the campaigns.

4. Discussion

Alkalinity and NEP have been described as the two main parameters controlling calcification [9,13,30,43], yet they have mostly been studied independently. NEP increases pH, shifting the DIC equilibrium to saturation of carbonates, offering an ideal environment for calcite formation around nucleation points in the form of picocyanobacteria [13]. Alkalinity implies availability of DIC and Ca^{2+}, enabling higher rates of calcification, with a threshold of 1 meq L^{-1}, below which calcification is unlikely to occur [7,9,44].

Our results suggest that the effect of NEP on calcification is to be understood within the larger context of a lake's physicochemical properties, especially in relation to its equilibrium with calcite. At short-term scales, NEP alone is not sufficient to explain the observed calcification (Figure 2A). However, it is the ratio between calcification and NEP (α) which is predictable from the calcite SI (Figure 3). Similarly, the relationship between calcite saturation alone and calcification is not significant (Figure 2B) and is much weaker than when considering its effect on α (Figure 3). Therefore, the short-term effect of NEP on calcification can be comprehended within the context of calcite saturation which depends on processes that act at a longer time scale. In the incubation bottles, we observe the changes in NEP at an hourly scale, and the short-term effect of primary production within the bottles is therefore evident. However, the calcite saturation in these bottles corresponds to a legacy of biogeochemical processes that occur in the lakes at a larger temporal scale than the time frame of the incubations. Changes in calcite saturation in lakes are reported to be gradual and follow a seasonal pattern with higher saturation from spring to fall with the peak during summer, coinciding with the rise in temperature and primary production [12].

The higher calcite saturation during summer, together with the summer peak in NEP, is likely the main cause for the seasonal variation in calcification. In Sau Reservoir, calcification seems to have occurred during the peak of primary production and during the higher calcite saturation found in June–July that came along with rising temperatures (Figure 5). During this period, the calcium concentration decreased, likely as a result of calcification (Figure 5). The potentiating role of calcite saturation in the effect of NEP on calcification can result in intense calcification events extending usually from a few days to a few weeks, which are sometimes visible in the form of whitening events which have a big impact on the carbon budget of a lake [16]. Our results suggest that such events are triggered by peaks of production and that the intensity of such events is likely caused by a gradual departure from the calcite equilibrium until it reaches a state that strongly potentiates the calcification-inducing factor of NEP. Within the context of global change, these results suggest that calcification is likely to be further promoted by anthropogenic stressors that affect the trophic state of a lake. The increase in dissolved phosphorus and nitrogen in water bodies, caused by human activities such as agriculture or urban activities including industry or sewage water treatment plants [17], is causing an increase in eutrophication and in the proliferation of cyanobacterial blooms [19]. Such conditions can trigger intense calcification, especially in systems that are highly saturated in calcite. In turn, such events can improve the trophic state of eutrophic lakes due to co-precipitation with phosphates, acting as a self-remediation mechanism [45]. Furthermore, eutrophication can cause dramatic changes in the phytoplankton community due to shifts in the trophic state [46]. Hence, the resulting change in planktonic size spectra may affect the availability of nucleation sites that trigger calcification [13–15]. This is an added complexity which is beyond the scope of this study. Disentangling the effect of nucleation, metabolism and calcite saturation should be addressed in further research. Besides increasing eutrophication, global change may promote calcification through its effect on water alkalinity. An increase in the export of alkalinity due to global change has been observed in large Russian Arctic rivers, as well as in North America's largest river, the Mississippi [47,48]. A rise in water alkalinity would increase the calcite SI, which is directly dependent on alkalinity, among other variables. This would potentiate the calcification-inducing effect of NEP, allowing for higher rates of calcite precipitation. In this view, global change could possibly enhance calcite precipitation events, both in frequency and intensity, due to the increase in eutrophication and water alkalinity. However, more research is needed to confirm this hypothesis.

Most campaigns showed potential calcification, except both campaigns at Valguta Mustjärv, Lake Erastvere on 18.6.19 and Sau Reservoir on 16.5.18 (Figure 1). Valguta Mustjärv is a humic (DOC 41 mg L^{-1}), brown-water bog lake with low alkalinity (Appendix Table A1) and low pH (6.8) and was therefore undersaturated in calcite. The main reason for including this lake in this study was to observe the absence of calcification expected from such low-alkalinity lakes. The HFM time series of DO saturation for this lake shows a peak of production shortly before the first incubation campaign (Appendix Figure A2). However, despite this peak of production, calcification did not occur because of the undersaturation in calcite. Lake Erastvere on 18.6.19 had the lowest calcite SI among the campaigns with positive calcite saturation (Figure 1a), combined with relatively low values in chlorophyll-*a* and NEP (Appendix Table A1 and Figure A1a), which is likely the reason for the absence of calcification. Looking at the HFM time series of DO and T, it appears that the timing of the two campaigns did not coincide with the peaks of production in this lake (Appendix Figure A3), which could be another explanation for the lack of calcification during this campaign. As for the medium-alkalinity Sau Reservoir on 16.5.18, the relatively low calcite SI (Figures 1a and 5) and NEP (Appendix Figure A1a) could be potential reasons for the absence of measurable calcification. The HFM time series of DO indicate that this reservoir was the least productive during this campaign because the timing (Spring) was too early to capture the strong summer rise in production that started in the month of June (Figure 5). In this reservoir, two campaigns show the lowest rates of calcification (11.6.2018 and 22.8.2018, Figure 1b). In the first case (11.6.2018), the campaign occurred during the initial stage of the summer rise in production and therefore DO saturation was still relatively low compared to the following period (Figure 5). The second case (22.8.2018) corresponds to a period where calcite SI strongly decreased, likely because of the calcification that occurred previously (Figure 5). As for the two campaigns (12.7.2018 and 25.7.2018) that were held between these two cases during the month of July, the clear occurrence of calcification is justified by the high values in DO and in calcite SI during this period. This further supports the explanation that calcification depends simultaneously on two parameters, namely, NEP and calcite saturation. The two highest rates of calcification were both observed in Võrtsjärv, which had the highest calcite SI among all the campaigns (Figure 1). The lake was saturated in DO during both campaigns, suggesting high production (Appendix Figure A4). Here, again, the high rates of calcification are explained by the high values of NEP and calcite SI observed in this lake.

The α ratio has been described as a function of alkalinity [27,30]. Our results suggest that it is rather a function of the calcite SI. While calcite saturation is dependent on alkalinity, it is also dependent on other variables such as T, EC and pH and is therefore a better predictor for calcification than alkalinity alone. In marine studies, α has been reported at a value of 1.67 in coral reefs [49,50]. Calcite saturation in the pelagic ocean is much more constant than in lakes, with an average value of 1.5 for the Central Pacific Ocean water column [51]. In contrast, lakes exhibit large variations in calcite SI and can have much higher values. In Lake Constance, variations in calcite SI between 1 and 9 have been reported [52]. Our results suggest that at the higher calcite SI commonly found in lakes, α can reach much more extreme values than the ones reported in marine studies, and therefore pelagic calcification rates in lakes can largely exceed those found in the ocean. The α ratio in lakes has only rarely been reported and appears to range between 0 and 1 [27]. However, its power relationship with calcite saturation results in ratios of up to nearly 3:1 for Lake Võrtsjärv on 15.7.2019 (Figure 4). Therefore, the effect of NEP on calcification is strongly potentiated at higher calcite saturation.

This potentially strong effect of NEP on calcification has important implications for DIC dynamics of lakes and the way we relate calcification to metabolic processes. Besides acting as a C sink in the sediments [23], calcification releases CO_2, thereby promoting CO_2 supersaturation and CO_2 emissions to the atmosphere [53,54]. Due to its association with NEP, calcification is reported to be a cause of the deviations from the 1:1 metabolic stoichiometry between DO and DIC that is often reported in lakes [30,55–57]. It is not uncommon to find lakes that are simultaneously emitting CO_2 while being in an autotrophic state [8,20,30]. Our results suggest that these deviations are correlated to α (Figure 4), and we show that this is not only relevant in hardwater lakes as commonly assumed, but also in lakes of alkalinity above 1 meq L^{-1}, as suggested by Marcé et al. (2015) [7]. When α is close to 0, DIC dynamics follows the classical 1:1 metabolic stoichiometry with DO. At higher α, the overall stoichiometry changes, with larger differences in DIC per mole of oxygen produced. This is clearly visible in Lake Võrtsjärv on 15.07.2019, where the DIC/DO ratio reaches nearly −8 (Figure 3). Such deviations caused by calcification are not accounted for in lake models, potentially causing discrepancies between theoretical models and empirical data and biasing our understanding of lake ecosystems through modeling [24,58]. Understanding and quantifying the interplay between calcification, NEP and calcite saturation may provide a more realistic parameterization of lake carbon budget models under different eutrophication scenarios.

5. Conclusions

Calcification is driven simultaneously by the long-term effects of calcite saturation and the short-term effects of NEP, whereby calcite saturation strongly potentiates the NEP-induced calcification effect. This would explain the seasonal and episodic nature of pelagic calcification in lakes, whereby calcification occurs when the level of calcite saturation is high enough to trigger this process usually during the summer season, allowing high rates of calcite precipitation to be induced by NEP. Considering human stressors that increase lake eutrophication, calcification is likely to be intensified with the increase in summer production rates. Our results also suggest that the strong effect found between calcite saturation and α provides a mechanistic relationship that could be used to parameterize this process in lake models. This way, the calcite SI is an indicator of the NEP-induced calcification effect and the resulting deviations from the metabolic 1:1 stoichiometry between DO and DIC.

Author Contributions: Conceptualization, B.O. and R.M.; methodology, B.O., R.M., A.L., H.K. and M.S.; validation, B.O. and A.L.; formal analysis, H.K., B.O., A.L. and M.S.; resources, B.O., and A.L.; writing—original draft preparation, H.K.; writing—review and editing, H.K., B.O., A.L., R.M. and M.S.; supervision, B.O. and A.L.; funding acquisition, B.O. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by MANTEL ITN (Management of climatic extreme events in lakes and reservoirs for the protection of ecosystem services) through the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 722518, by the Estonian Research Council grants (PSG32 and PUTJD954) and by the project C-HYDROCHANGE (CGL2017-86788-C3-3-P and CGL2017-86788-C3-2-P) funded by the Spanish Ministry of Science, Innovation and Universities.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: Data available on request.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Ancillary data of the studied lakes.

Lake	Date	Alkalinity (meq/L)	Chlorophyll-a (µg/L)	TP (µg/L)	TN (mg/L)	DOC (mg/L)	DIC (µmol/L)	Ca2+ (µmol/L)
Valguta Mustjärv (EE)	11/6/2019	0.52	14.0	641	2.0	42.1	696.6	234.7
Valguta Mustjärv (EE)	17/7/2019	0.67	NA	572	1.7	39.3	868.1	245.8
Erastvere (EE)	18/6/2019	1.48	10.5	43	0.9	12.6	1469.9	487.6
Erastvere (EE)	23/7/2019	1.79	NA	56	1.6	11.8	1568.4	491.3
Võrtsjärv (EE)	17/6/2019	3.23	26.1	23	1.1	12.0	3054.6	1066.3
Võrtsjärv (EE)	15/7/2019	4.08	22.3	36	1.4	12.4	3949.2	1091.9
Sau Reservoir (ES)	16/5/2018	2.57	NA	NA	NA	20.5	2540.1	1312.1
Sau Reservoir (ES)	11/6/2018	2.45	20.1	59	6.2	NA	2393.8	1260.3
Sau Reservoir (ES)	12/7/2018	1.52	4.9	36	4.3	NA	1412.1	711.1
Sau Reservoir (ES)	25/7/2018	1.19	28.6	73	3.7	NA	1047.3	602.8
Sau Reservoir (ES)	22/8/2018	1.19	32.0	39	3.0	NA	1099.6	607.7

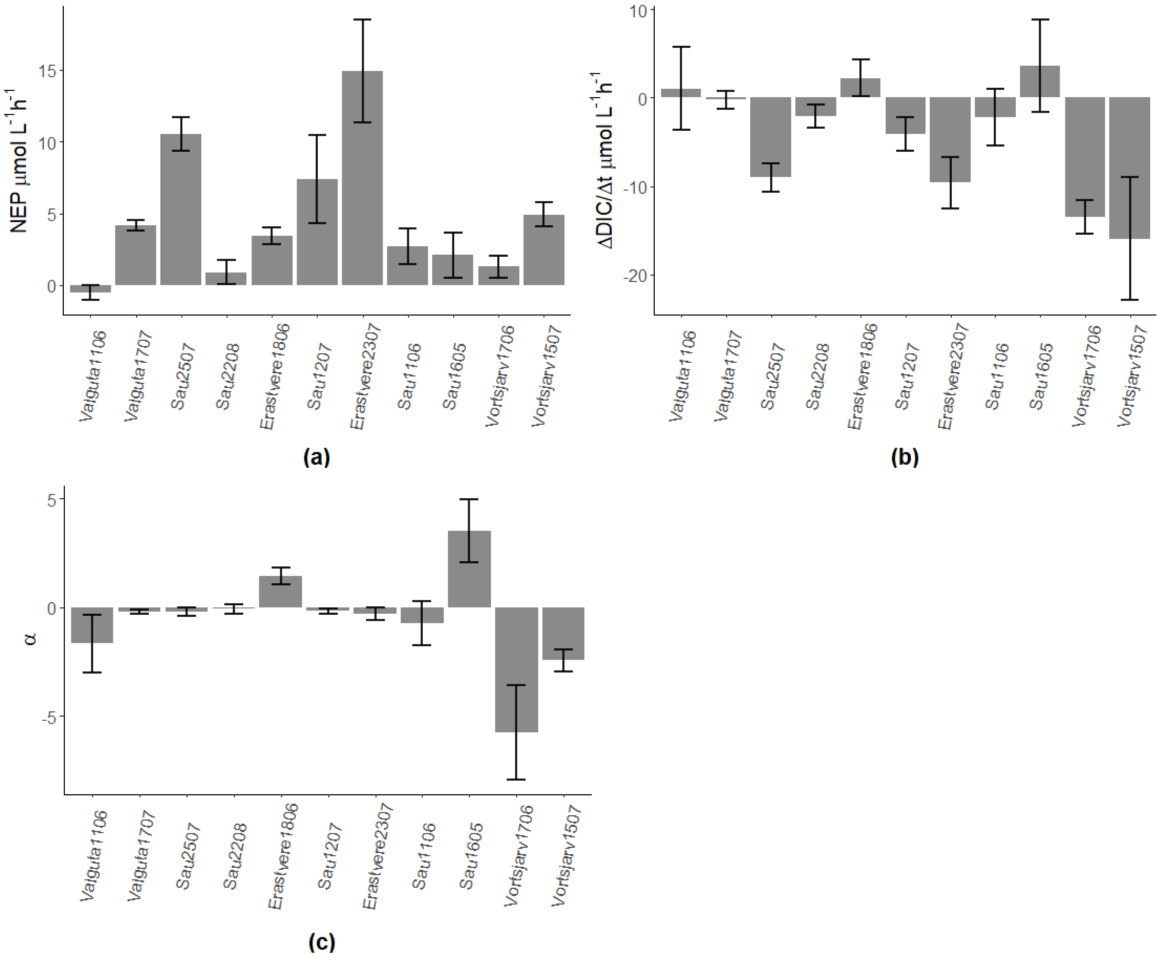


Figure A1. (**a**) NEP rates obtained from rates of change of oxygen (dDO/dt), (**b**) rates of change of DIC and (**c**) ratios between calcification and NEP (α). Numbers after the name of lakes represent the day and month of sampling; all lakes were sampled in 2019 except for Sau Reservoir in 2018.

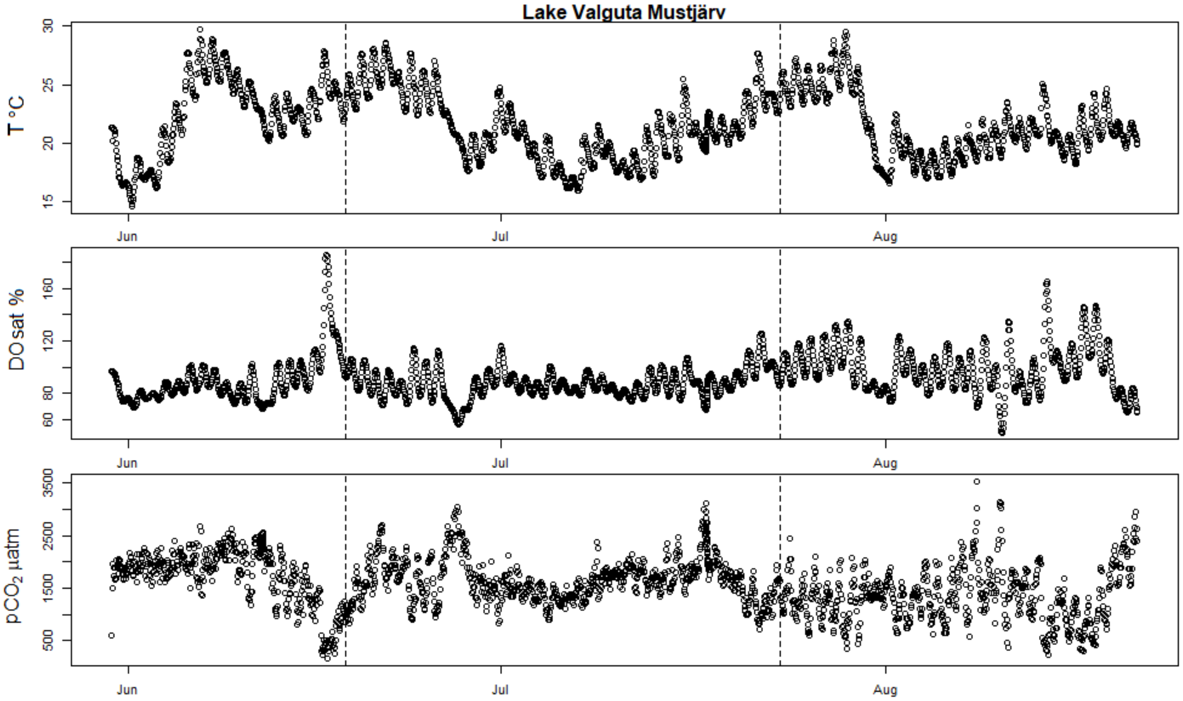


Figure A2. High-frequency measurements showing seasonal evolution of surface water T (in degrees Celsius), DOsat and pCO$_2$ partial pressure in Valguta Mustjärv. The two vertical dotted bars mark the date of the two incubation campaigns held in this lake (11.6.2019 and 17.7.2019).

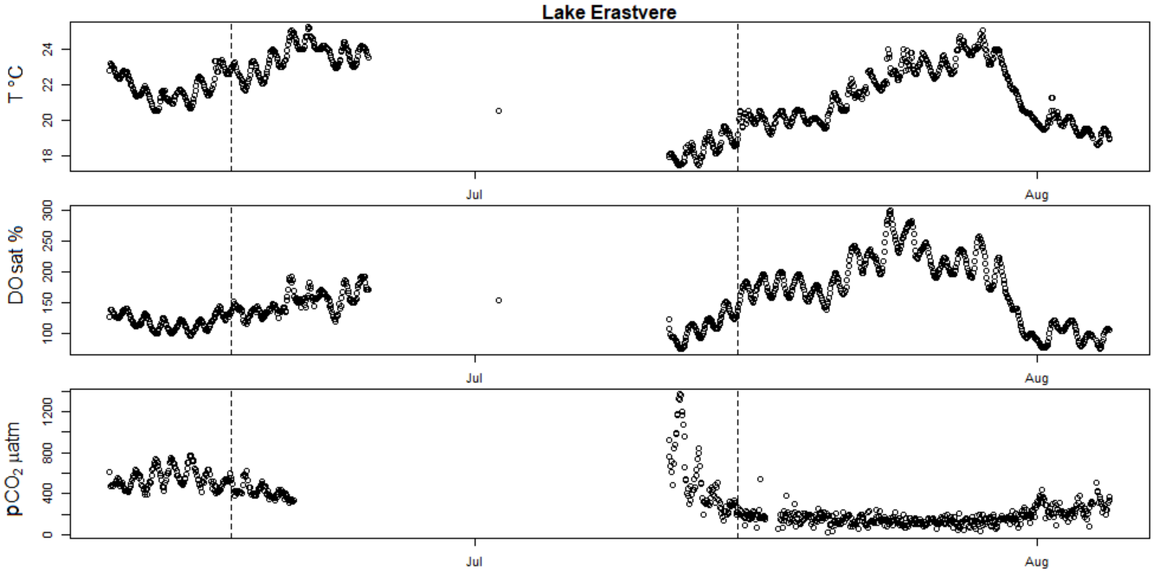


Figure A3. High-frequency measurements showing seasonal evolution of surface water T (in degrees Celsius), DOsat and pCO$_2$ partial pressure in Lake Erastvere. The two vertical dotted bars mark the date of the two incubation campaigns held in this lake (18.6.2019 and 23.7.2019).

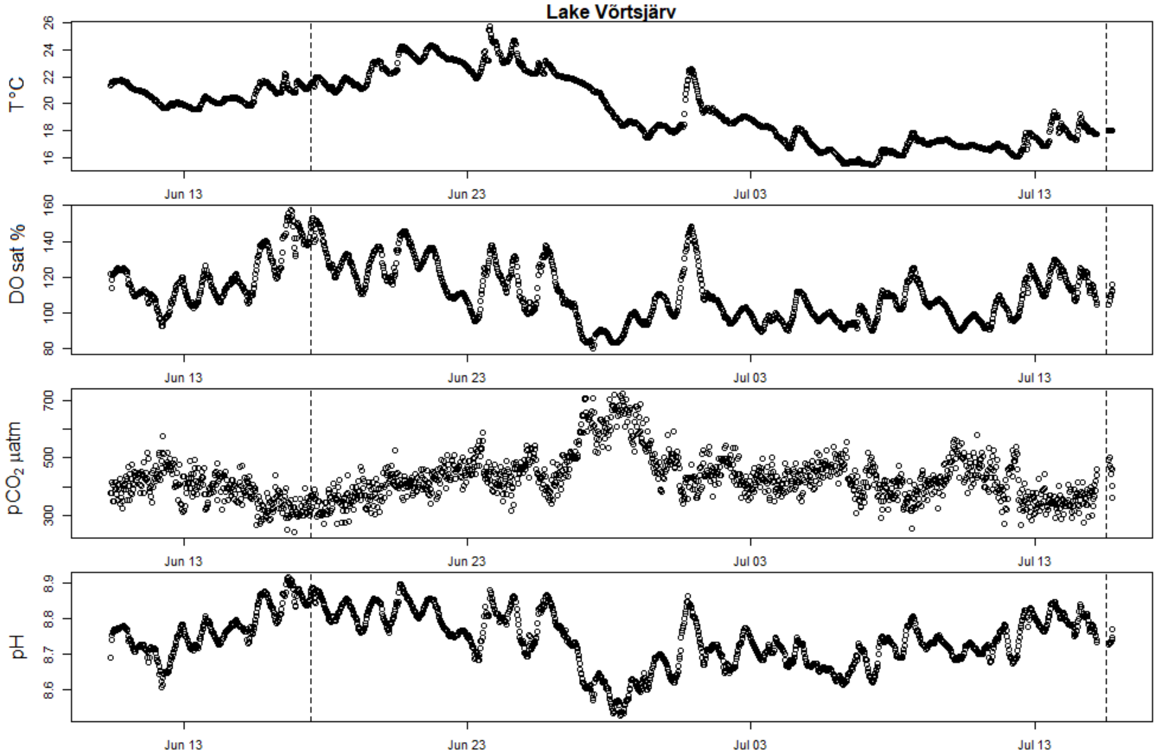


Figure A4. High-frequency bouy measurements showing seasonal evolution of surface water T (in degrees Celsius), DOsat, pCO$_2$ partial pressure and pH in Võrtsjärv. The two vertical dotted bars mark the date of the two incubation campaigns (17.6.2019 and 15.7.2019).

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Ecosystems
https://doi.org/10.1007/s10021-020-00488-6

ECOSYSTEMS

Major Effects of Alkalinity on the Relationship Between Metabolism and Dissolved Inorganic Carbon Dynamics in Lakes

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ABSTRACT

Several findings suggest that CO_2 emissions in lakes are not always directly linked to changes in metabolism but can be associated with interactions with the dissolved inorganic carbon equilibrium. Alkalinity has been described as a determining factor in regulating the relative contributions of biological and inorganic processes to carbon dynamics in lakes. Here we analyzed the relationship between metabolic changes in dissolved oxygen (DO) and dissolved inorganic carbon (DIC) at different timescales in eight lakes covering a wide range in alkalinity. We used high-frequency data from automatic monitoring stations to explore the sensitivity of DIC to metabolic changes inferred from oxygen. To overcome the problem of noisy data, commonly found in high-frequency measurements datasets, we used Singular Spectrum Analysis to enhance the diel signal-to-noise ratio. Our results suggest that in most of the studied lakes, a large part of the measured variability in DO and DIC reflects non-metabolic processes. Furthermore, at low alkalinity, DIC dynamics appear to be mostly driven by aquatic metabolism, but this relationship weakens with increasing alkalinity. The observed deviations from the metabolic 1:1 stoichiometry between DO and DIC were strongly correlated with the deviations expected to occur from calcite precipitation, with a stronger correlation when accounting also for the benthic contribution of calcite precipitation. This highlights the role of calcite precipitation as an important driver of CO_2 supersaturation in lakes with alkalinity above 1 meq L^{-1}, which represents 57% of the global area of lakes and reservoirs around the world.

Key words: Carbon dynamics; Calcite precipitation; Metabolism; Alkalinity; Dissolved inorganic carbon; High-frequency measurements; Singular spectrum analysis; Cross-wavelets analysis.

HIGHLIGHTS

- Calcite precipitation is a major driver of carbon dynamics in lakes with alkalinity above 1 meq L^{-1}.
- At low alkalinity, metabolism is the main driver of carbon dynamics.

Received 10 April 2019; accepted 22 January 2020

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10021-020-00488-6) contains supplementary material, which is available to authorized users.

Author Contributions BO, RM and AL conceived this study, AL collected the data, HK analyzed the data with support of all authors and wrote the first versions of the manuscript with inputs and edits by all others.

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INTRODUCTION

Considering that lakes occupy a relatively small area in the global landscape, they are disproportionally active sites for carbon cycling due to their important roles in carbon transport, transformation and storage (Cole and others 2007; Battin and others 2009; Tranvik and others 2009). Globally, most lakes are supersaturated in CO_2 and act as carbon sources to the atmosphere (Sobek and others 2005). The carbon emitted by these ecosystems is relevant and comparable in magnitude to global terrestrial net ecosystem production (Tranvik and others 2009). The CO_2 emissions from lakes have widely been attributed to their heterotrophic activity that oxidizes terrestrially produced carbon (Del Giorgio and others 1999; Jonsson and others 2003; Duarte and Prairie 2005; Roehm and others 2009; Larsen and others 2011). Many studies that directly link CO_2 supersaturation to net heterotrophy are conducted in lakes that have relatively low alkalinity and high dissolved organic carbon (DOC) values. Although this paradigm is often valid for such systems, it has recently been rejected for lakes and reservoirs of higher alkalinity. A study that relates CO_2 emissions to lake metabolism in nineteen lakes of higher alkalinity shows that despite a positive relationship between CO_2 emissions and respiration of organic matter, CO_2 emissions often exceed the rates expected from lake metabolism (Sand-jensen and Staehr 2009). Several findings suggest that in such systems, major drivers of carbon dynamics include direct exogenous dissolved inorganic carbon (DIC) inputs from soil respiration and mineral weathering of the catchment, interactions with the DIC equilibrium, and calcite precipitation (Maberly and others 2012; McDonald and others 2013; Marcé and others 2015; Weyhenmeyer and others 2015; Wilkinson and others 2016).

The chemical equilibrium between photosynthesis and respiration implies a balance between inorganic carbon variability and net ecosystem production (NEP), whereby 1 mol of O_2 respired produces 1 mol of DIC, and inversely, 1 mol of DIC is photosynthesized into 1 mol of O_2 (Stumm and Morgan 1996). Imbalances between inorganic carbon variability and metabolic rates resulting in deviations from the one to one stoichiometry between dissolved oxygen (DO) and DIC in lakes are widely reported in the literature (Stets and others 2009; López and others 2011; McDonald and others 2013; Peeters and others 2016; Perga and others 2016). Such imbalances have in some cases reached a point where lakes are simultaneously in an autotrophic state, yet they emit CO_2 to the atmosphere (Stets and others 2009; Marcé and others 2015; Peeters and others 2016; Perga and others 2016). According to a recent model, this could be the case for approximately 35% of the lakes and reservoirs in the contiguous United States (McDonald and others 2013). Hydrologic inputs and CO_2 released by calcite precipitation are likely to be the main factors driving CO_2 emissions in such lakes (Stets and others 2009; McDonald and others 2013; Marcé and others 2015; Perga and others 2016). A recent study shows that the sensitivity of CO_2 supersaturation to DO disequilibrium weakens with increasing alkalinity and that above an alkalinity threshold of 1 meq L^{-1} it is possible to find lakes that are simultaneously supersaturated in CO_2 and DO (Marcé and others 2015). This implies that above this alkalinity threshold CO_2 supersaturation in lakes is directly regulated by DIC inputs from carbonate weathering and calcite precipitation and that this is a widespread phenomenon since 57% and 34% of the global area occupied by lakes and reservoirs have alkalinity values above 1 and 2 meq L^{-1}, respectively (Marcé and others 2015).

In such lakes, summer DIC depletion due to photosynthesis comes largely at the expense of alkalinity, with only minimal changes in pCO_2 and pH (Mcconnaughey and others 1994). An explanation lies in the biogenic process of calcite precipitation that is enhanced during summer stratification, when algal blooms increase pH through photosynthesis and offer abundant nucleation sites around the surface of micro-algae (Müller and others 2016). Summer calcification reduces alkalinity and calcium levels, generating an equal molar quantity of CO_2, thereby compensating photosynthetic CO_2 depletion and maintaining CO_2 partial pressure (pCO_2) values within bounds that sustain primary production (Mcconnaughey and others 1994; Andersen and others 2019). This mechanism is less relevant in lakes of low alkalinity where calcite precipitation is unlikely to occur at high rates and therefore primary production is potentially carbon limited because of the lack of inorganically produced CO_2 (Kragh and Sand-Jensen 2018). The mass balance equation of calcite precipitation implies the release of 1 mol of CO_2, yet it results in a net loss of 1 mol of DIC and two equivalents of total alkalinity (TA):

$$\begin{aligned} Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 \\ + H_2O\ (\Delta TA = -2\ \text{eq}) \end{aligned} \tag{1}$$

A recent study on a lake of relatively high alkalinity (4.5 meq L^{-1}) shows that about 40–70% of sediment organic carbon originates from alkalinity export from the catchment, suggesting that primary producers actively take up DIC of terrestrial origin, promoting calcite precipitation and turning such lakes into ''alkalinity sinks'' during summer (Nõges and others 2016). Despite acting as an alkalinity sink, it is widely acknowledged that calcification promotes CO_2 supersaturation of surface waters by releasing CO_2 (Opdyke and Walker 1992; Gattuso and others 1993, 1996, 1999; Frankignoulle and others 1994, 1995; Suzuki 1998). Therefore, summer calcification could be an explanation for the deviation from the 1:1 stoichiometry between DO and DIC variability commonly found in lakes of high alkalinity. However, other processes could result in such deviations, such as anaerobic respiration (Stumm and Morgan 1996; Torgersen and Branco 2007; Wissel and others 2008), physical processes such as gas exchanges with the atmosphere (Wissel and others 2008) or horizontal and vertical gas exchanges with nearshore or deeper waters (Antenucci and others 2013), as well as direct DIC inputs caused by storm events (Vachon and del Giorgio 2014). While the importance of calcite precipitation in CO_2 levels and emissions is well documented in marine systems, it is yet to be recognized as an important component of the carbon cycle in freshwater systems.

The objective of this research is to assess the sensitivity of DIC variability to metabolic changes in DO along a gradient of alkalinity. For this, we use high-frequency data of dissolved oxygen, pCO_2, pH and temperature from lakes covering a wide range in alkalinity. A theoretical model that accounts for the effect of calcite precipitation on the DO–DIC relationship is used as a comparison to the empirical data to highlight the importance of this process as a driver of carbon dynamics in lakes. The overarching aim of this study is thus to determine the main drivers of DIC dynamics along an alkalinity gradient. Our hypothesis is that lake metabolism is the main driver of carbon dynamics in lakes of low alkalinity but that with increasing alkalinity the importance of this driver becomes relatively low compared to other processes such as calcite precipitation.

METHODS

Study Sites

The data for this study were collected from eight Estonian lakes during the summer period from July to September in 2014 and are available in open access (Laas and Khan 2019). The studied lakes include Lake Peipsi, Võrtsjärv, Saadjärv, Lake Ülemiste, Mullutu Suurlaht, Lake Erastvere, Äntu Sinijärv and Valguta Mustjärv (Table 1). Based on their area, conductivity, thermal stratification, alkalinity, chloride content and color, each of these lakes belongs to different lake types (Ministry of the Environment 2009) according to the European Water Framework Directive (European Union 2000). Total alkalinity ranges from 0.5 to 4.8 meq L^{-1}. The lakes also cover a variety of trophic states, ranging from mesotrophic to hypertrophic states according to the OECD trophic state classification (Caspers 1984). In addition, they differ substantially in their water retention time as well as in their catchment characteristics, especially in regard to land use. Each of these lakes has been described in detail in previous studies (Rõõm and others 2014; Cremona and others 2016; Laas and others 2016).

Monitoring Stations and Measured Parameters

All lakes were continuously monitored between 6 and 12 full days using a high-frequency measurement platform or a small lake buoy (OMC-7012 data-buoy). In most lakes, the automated stations were installed near the deepest point, except in lake Peipsi, where it was placed in Mustvee bay, approximately 1 km from the western shore because of security reasons. A multi-parametric sonde (Yellow Springs Instruments 6600 V2-4) was used for continuous measurements of DO concentration, pH and water temperature at 0.5 or 1-m depth in all lakes. The multi-parametric sonde was equipped with an in-built cleaning system for optical DO sensor, whereas other sensors were manually cleaned on a weekly basis. Dissolved CO_2 was measured at 0.5 or 1-m depth using membrane-covered optical CO_2 sensors (AMT Analysenmesstechnik GmbH) with measuring ranges of 30 mg L^{-1} and 80 mg L^{-1}. Measured water temperature and air pressure data were used to calculate the real pCO_2 and dissolved CO_2 concentration from the signals captured from the sensor, according to manufacturer manuals (Laas and others 2016). A calibration of all sensors was performed before and after the deployment. For data collection and storage, all platforms were equipped with OMC-045-II GPRS data loggers. Sensors were automatically measuring at a time interval of 10 or 30 min depending on power availability.

Table 1. Characteristics of the Studied Lakes

Lakes	Trophic state	Mixing regime	Area (ha)	Mean depth (m)	Max depth (m)	Chl-*a** (μg L^{-1})	Alkalinity* (meq L^{-1})	DOC* (mg L^{-1})	K_d** (m)	Watershed size (km^2)	Water residence time ($year^{-1}$)
Valguta Mustjärv	Hypertrophic	Polymictic	20.4	< 1	1	23.19	0.500	35.2	10.34	1.34	Source lake
Erastvere	Hypertrophic	Dimictic	16.3	3.5	9.7	125.64	1.632	12.3	2.96	5.2	0.5
Mullutu Suurlaht	Eutrophic	Polymictic	412.7	< 1	1.7	9.04	1.800	18.1	0.58	238	0.2
Saadjärv	Mesotrophic	Dimictic	724.5	8	25	5.62	2.466	9.2	0.42	28.4	0.13
Peipsi	Eutrophic	Polymictic	261,100	8.3	12.9	13.4	2.799	12	1.6	47,800	2
Ülemiste	Eutrophic	Polymictic	944	2.5	4.2	24.7	3.299	13.7	3.5	98.8	0.33
Võrtsjärv	Eutrophic	Polymictic	27,000	2.8	6	35.71	3.614	11.8	2.76	3116	1
Äntu Sinijärv	Alkalitrophic	Polymictic	2.1	6	8	1	4.800	4.72	0.25	1.37	Source lake

*Depth-integrated averages.
**Vertical light attenuation coefficient.
Average values from the measurements and earlier data.

Data Analysis

For each lake, an alkalinity value for each time step was calculated using pCO_2 and pH as input parameters for the CO_2sys program (Lewis and others 1998) following Millero (1979). These were then averaged to obtain the mean alkalinity value for each lake and sampling period. Dissolved inorganic carbon (DIC) was calculated for each time step using pH and the average alkalinity as input parameters in CO_2sys. Alkalinity had to be averaged to avoid unrealistic DIC values due to the large errors in alkalinity propagated by the limited precision of the field pH sensors (typically ~ 0.2 pH units). Using average alkalinity and pH as inputs resulted in cleaner and more realistic DIC values compared to other alternatives of calculating DIC (for example, using CO_2 and pH as inputs).

After obtaining the DIC time series, the next step was to identify the presence of a 24-h metabolic signal for DO and DIC. This was done by performing a continuous wavelet-based analysis using the R (R Development Core Team 2015) ''biwavelet'' package (Gouhier and others 2015). Wavelets can be used to identify time-dependent structures in time series, in this case the diel metabolic pattern known to occur for DO and DIC concentrations (Hanson and others 2006) (see supplementary material). As a support to the wavelet analysis, periodograms for DO and DIC concentrations were made using the time series analysis ''TSA'' package in R (Chan and Ripley 2018). Periodograms offer a more general view of the main time-dependent structures within an entire time series.

Then we assessed how DIC variability relates to DO variability by testing the sensitivity of DIC to changes in DO. Bivariate wavelets were used to identify common time periods at which DO and DIC oscillate, and how these common periods evolve during the entire length of the time series. Besides indicating common oscillations between two time series, cross-wavelets also examine whether there is a consistent phase relationship between the series, which is suggestive of causality between the two time series (Grinsted and others 2004) (see supplementary material). In the case of common oscillations between DO and DIC, one would expect an anti-phase relationship because the metabolic reaction implies that an increase in one leads to a decrease in the other, and vice versa. Because of the large amount of noise and random signals that are not related to the metabolic diel signal, we decided to enhance the signal-to-noise ratio by isolating the 24-h-frequency oscillations of DO and DIC from all other signals. To do so, we used the Singular Spectrum Analysis "SSA" R package (Zhao 2016). SSA is a tool that allows identifying and extracting fundamental oscillatory components from an original time series (see supplementary material). The R code that was used in this analysis is published and freely available (Khan 2019). Once the 24-h diel cycle components were identified, they were grouped and reconstructed into a new time series following Golyandina and Korobeynikov (2014). This method efficiently isolates the diel seasonal signal from a time series, even if it is weak or if the time series is noisy (see supplementary Figures S5 and S6 for comparison of a diel signal extracted from a clean versus noisy time series). For lake Äntu Sinijärv, the diel signal in DIC was very weak and had to be extracted by performing a Multivariate Singular Spectrum Analysis (MSSA) using the isolated diel signal of DO and the original time series of DIC as inputs. DIC and DO rates of change (dDO/dt and dDIC/dt) were calculated from the signal enhanced series and expressed in μmol L^{-1} min^{-1}. Therefore, corrections for air–water gas exchanges are not necessary because the rates are calculated from isolated metabolic signals that have been removed from the effect of any non-metabolic process, including air–water gas exchanges.

Linear regressions were used to test the effect of dDO/dt on dDIC/dt in all lakes. Although all these relationships were highly significant, the main parameter of interest was the slope of the relationship. A theoretical slope of about − 1 is expected from the stoichiometry of aerobic respiration and photosynthesis (Stumm and Morgan 1996; Torgersen and Branco 2007). The slope of − 1 is an estimation that can be applied only in aerobic conditions without anaerobic respiration or other processes that can affect DIC concentrations such as calcite precipitation or direct DIC inputs following a storm event (Stumm and Morgan 1996; Vachon and del Giorgio 2014). All measurements were taken from surface waters where the effect of anaerobic respiration on DIC concentrations is not relevant according to the depth distributions of temperature, DO and CO_2 reported for each lake during the same period of measurements in a previous study (Laas and others 2016). However, calcite precipitation could influence DIC concentrations in these lakes. Therefore, deviations from the theoretical − 1 metabolic slope were interpreted with a focus on calcite precipitation.

The final step was to assess how alkalinity affects the sensitivity of DIC to metabolism. To do so, the slopes of the linear regression models were plotted against the alkalinity for all studied lakes. We compared the empirical slopes with the theoretical slopes under the presence of reactions with calcite. For each lake, we calculated a theoretical time series of dDIC/dt that accounts for the effect of precipitation and dissolution of carbonates on dDIC/dt following a simple model:

$$\mathrm{dDIC/dt}_{\mathrm{theoretical}} = -\mathrm{dDO/dt} - \alpha\,\mathrm{dDO/dt} \qquad (2)$$

where α is the molar ratio between precipitation and dissolution of carbonates and NEP (McConnaughey and Whelan 1997). The values of α were calculated according to three different hypotheses (Table 2). The first hypothesis assumes that $\alpha = 0$ which corresponds to a system with no calcite precipitation, that is, where all changes in DIC are explained by aerobic metabolism. The second hypothesis assumes an alkalinity-dependent value for α, using the same values as suggested in Marcé and others (2015), whereby $\alpha = 0$ at alkalinity below 1 meq L^{-1} (McConnaughey and Whelan 1997), $\alpha = 0.2$ at alkalinity between 1 and 2.23 meq L^{-1}, and $\alpha = 0.6$ at alkalinity above 2.23 meq L^{-1}. The third hypothesis assumes these same alkalinity-dependent values for α with an additional correction for the benthic fraction (BF) of total primary production in the lakes. By doing this correction, the α parameter is able to account for pelagic calcite precipitation as well as benthic calcification by calcifying macrophytes. To do this, we used the percentage of benthic primary production of each lake obtained from a previous study on these same lakes that was conducted during the same period of study as the data used here (Cremona and others 2016). We assumed benthic production to be composed of calcifying macrophytes, based on previous studies of these eight lakes (Cremona and others 2016; Laas and others 2016). An approximate 1:1 calcification to photosynthesis ratio in such organisms has been reported (McConnaughey 1991; Mcconnaughey and others 1994; McConnaughey and Whelan 1997). Therefore, we attributed an α value of 1 to the benthic production and corrected the α values from Marcé and others (2015) according to the ratio between pelagic primary production and benthic primary production.

At this point, one must remember that the observed dDIC/dt values come from DIC values that were calculated using a constant average alkalinity. Although the diurnal changes in alkalinity are too small to be accurately estimated by using CO_2 and pH coming from field probes, assuming a constant alkalinity value tends to underestimate the changes in DIC, as it omits changes in the chemical equi-

Table 2. α Values and Corrections for Benthic Fraction (BF) Used to Build the Expected Slopes of the dDO/dt–dDIC/dt Relationships

Lake	Alkalinity (meq L^{-1})	BF (%) *	Hypothesis 1 α value	Hypothesis 2 α value**	Hypothesis 3 α value	Hypothesis 1 Theoretical slope	Hypothesis 2 Theoretical slope	Hypothesis 3 Theoretical slope
Valguta Mustjärv	0.500	NA	0	0.0	0.00	− 1.0	− 0.86	− 0.86
Erastvere	1.632	10	0	0.2	0.21	− 1.0	− 1.31	− 1.33
Mullutu Suurlaht	1.800	46	0	0.2	0.57	− 1.0	− 0.85	− 1.59
Saadjärv	2.466	26	0	0.6	0.70	− 1.0	− 1.55	− 1.75
Peipsi	2.799	10	0	0.6	0.64	− 1.0	− 1.47	− 1.55
Ülemiste	3.299	10	0	0.6	0.64	− 1.0	− 1.43	− 1.51
Võrtsjärv	4.425	4	0	0.6	0.62	− 1.0	− 1.77	− 1.81
Äntu Sinijärv	4.800	94	0	0.6	0.98	− 1.0	− 1.09	− 1.85

*Cremona and others (2016).
**Marcé and others (2015).

librium of the carbonate system. To overcome this limitation, we calculated a corrected observed dDIC/dt that accounts for calcite reactions:

$$dDIC/dt_{corrected} = dDIC/dt - \alpha 2\ dDIC/dt \qquad (3)$$

where α is the calcification parameter in equation 2. This procedure is thus generating a corrected observed dDIC/dt dataset (and thus an observed slope between dDIC/dt and dDO/dt) which is dependent on the three hypotheses used for assigning values to α. Note that for Hypothesis 1 α equals 0 and $dDIC/dt_{corrected}$ equals dDIC/dt. The slopes $dDIC/dt_{corrected}$ versus dDO/dt and $dDIC/dt_{theoretical}$ versus dDO/dt were calculated using linear regression and compared by plotting them along the alkalinity gradient. The rationale of the analysis is that the theoretical slopes and the observed slopes (using $dDIC/dt_{corrected}$) will agree only in the case of using the appropriate hypothesis for the calcite reactions (α).

RESULTS

Presence and Strength of Metabolic Signals in DO and DIC Dynamics

The presence of a metabolic signal was assessed using periodograms which estimate the spectral density of a time series and shows what are the dominating frequencies of oscillation composing it. The dominating spikes at 24-h frequency indicate a clear diel metabolic signal (24-h pattern) for DO and DIC in most lakes, except in Äntu Sinijärv, Saadjärv and lake Ülemiste where the diel signals are weak resulting in a shorter spike at the 24-h-frequency mark (Figures 1 and 2). The periodograms showed an important number of signals at other frequencies that did not correspond to the metabolic diel pattern. While the spectral density of these non-metabolic signals was relatively low, their relevance was amplified when using rates of change (dDO/dt and dDIC/dt) rather than raw concentrations, especially the noisy signals at short frequencies (see Supplementary Figures S1 and S2).

Relationship Between DIC and DO Variability

Cross-wavelets were used to identify at what time period synchronized oscillations can be found between DO and DIC and the phase of these synchronizations. Due to the metabolic stoichiometry between DO and DIC, an anti-phase synchronization between both variables is to be expected at the

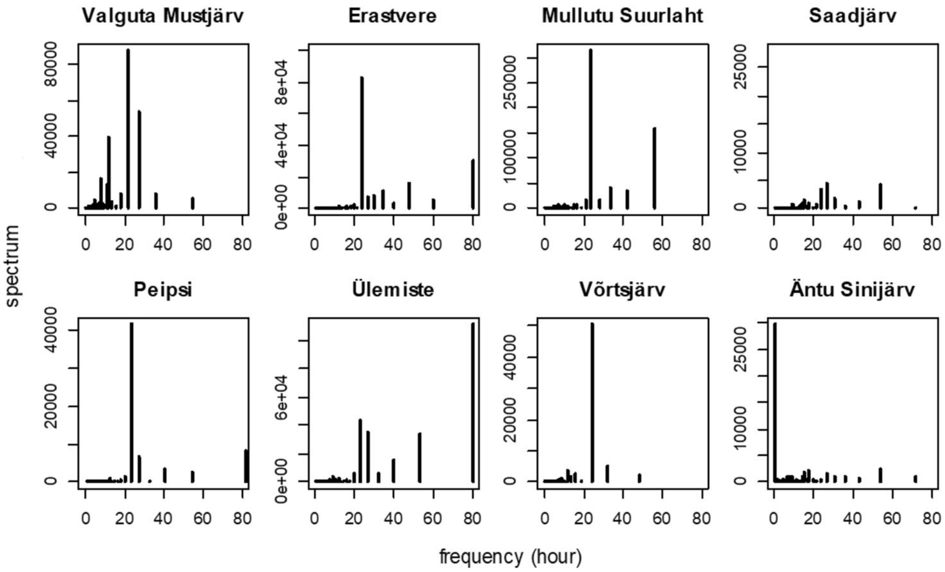

Figure 1. Periodograms for dissolved oxygen (DO) in the studied lakes. The *x*-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated. The *y*-axis corresponds to the spectral density or power spectrum, with higher values indicating important features of the signal at a given frequency. Notice the dominating spikes at 24-h frequency.

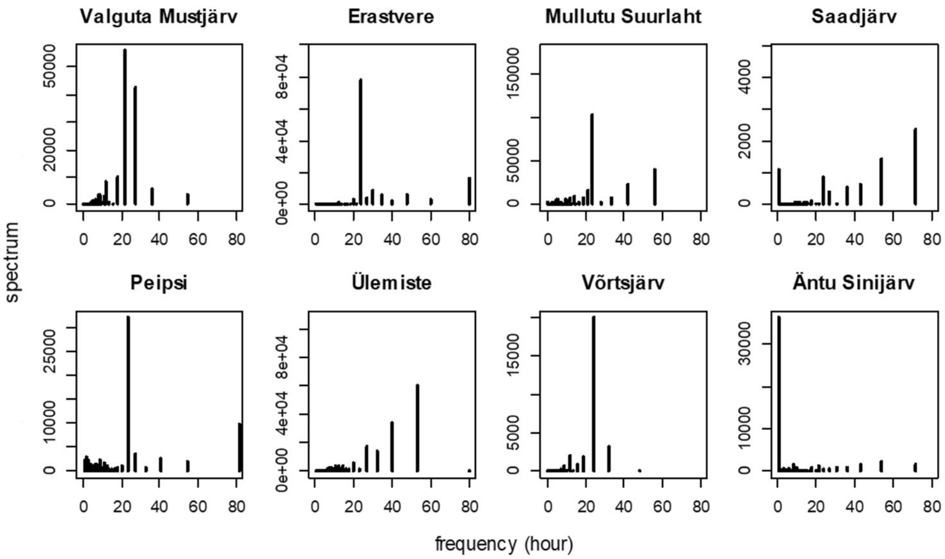

Figure 2. Periodograms for dissolved inorganic carbon (DIC) in the studied lakes. The *x*-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated (*y*-axis). The *y*-axis corresponds to the spectral density or power spectrum, with higher values indicating important features of the signal at a given frequency. Notice the dominating spikes at 24-h frequency.

24-h frequency, whereby an increase in DO results in a decrease in DIC, and vice versa. This was apparent for most of the lakes in the cross-wavelets, where a ‘‘cloud’’ of arrows pointing to the left indicate a relatively strong anti-phase synchronization around the 24-h period (Figure 3). Although this diel synchronization in oscillation was relatively strong in some lakes (Valguta Mustjärv, Erastvere, Mullutu Suurlaht, Peipsi and Võrtsjärv), it was weak in others (Saadjärv, Ülemiste and Äntu Sinijärv), causing disruptions in the synchronicity between DO and DIC along the time series (Figure 3). The lack of coupling was most obvious in lake Äntu Sinijärv. All lakes showed a relatively important amount of significant coupling in oscillation at time periods shorter than 24 h (Figure 3).

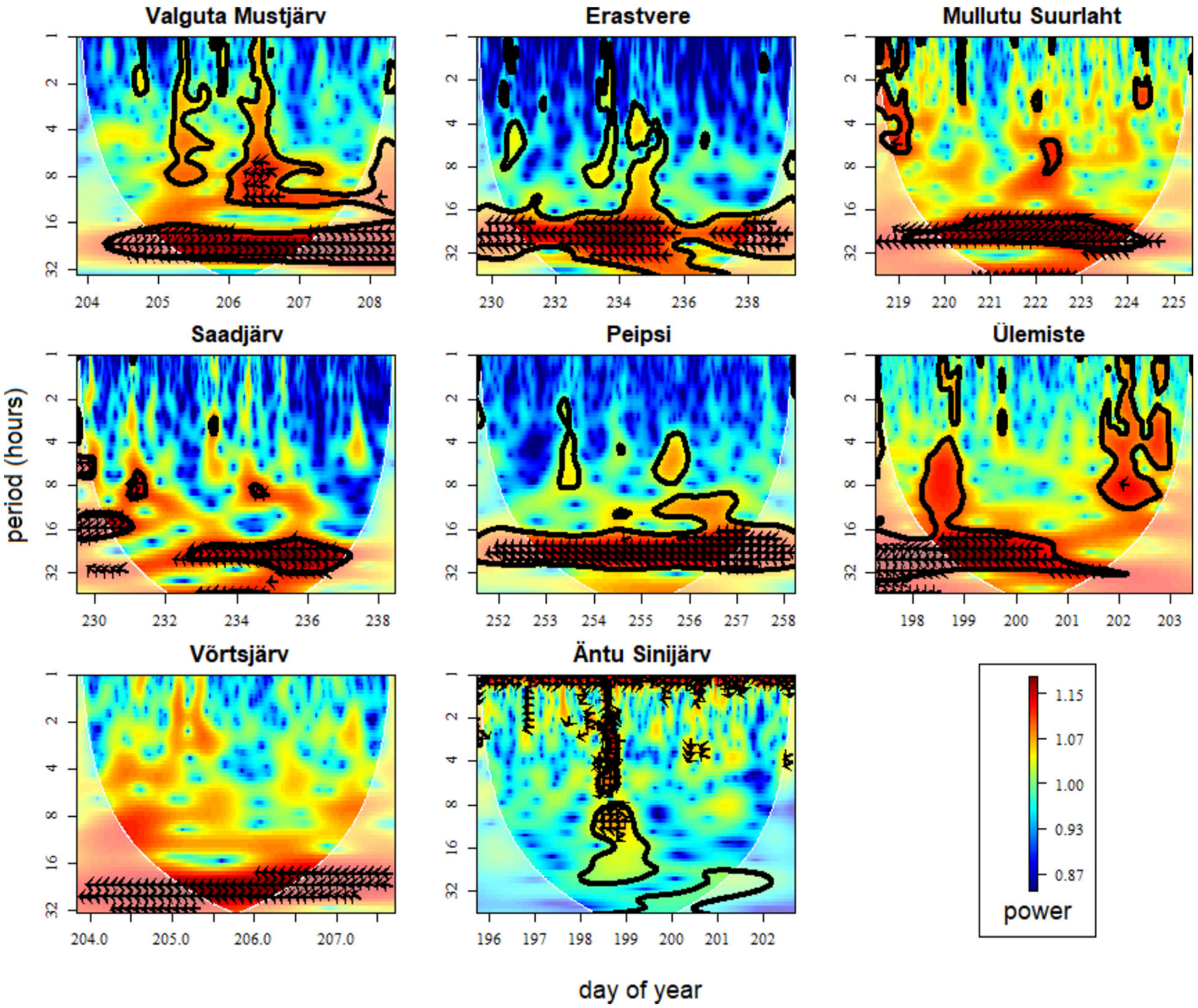

Figure 3. Cross-wavelets between DO and DIC for each lake, showing regions in the time–frequency space with common power. The power bar represents the amplitude-squared of signals, with higher values corresponding to important features of the signal. The *x*-axis represents the time (expressed as day of the year) and allows to see the evolution of common oscillatory patterns between DO and DIC in time. The *y*-axis represents the period of oscillation (in hours). Significant synchronizations in oscillations between DO and DIC at a certain period are delimited by the black contours. Arrows pointing to the left symbolize an anti-phase relationship. Notice the dominance of a synchronized anti-phase pattern around 24-h frequency in most lakes.

These common oscillations ranged from time periods of less than 1 h up to several hours and could be caused by water movements caused by convection, minor mixing events or seiches, as well as noise resulting from the sensitivity thresholds of the sensors.

The presence of non-metabolic signals in DO and DIC that were oscillating at other frequencies than the metabolic 24-h frequency resulted in poor relationships between dDO/dt and dDIC/dt (Supplementary Figure S3). However, using the isolated metabolic diel signals to calculate dDO/dt and dDIC/dt (Supplementary Figure S4) resulted in much stronger relationships between the two (Figure 4). These relationships were significant for all lakes, with substantial differences in slope between lakes.

In most of the lakes, the slopes of these relationships were close to the ones found by performing linear regression models on dDO/dt and dDIC/dt obtained from the raw data, yet the R^2 values dramatically increased (Table 3). However, the slope for this relationship in Saadjärv turned from positive to negative. Considering the low R^2 value (0.05) of the relationship using the raw data and its high increase ($R^2 = 0.91$) when using the

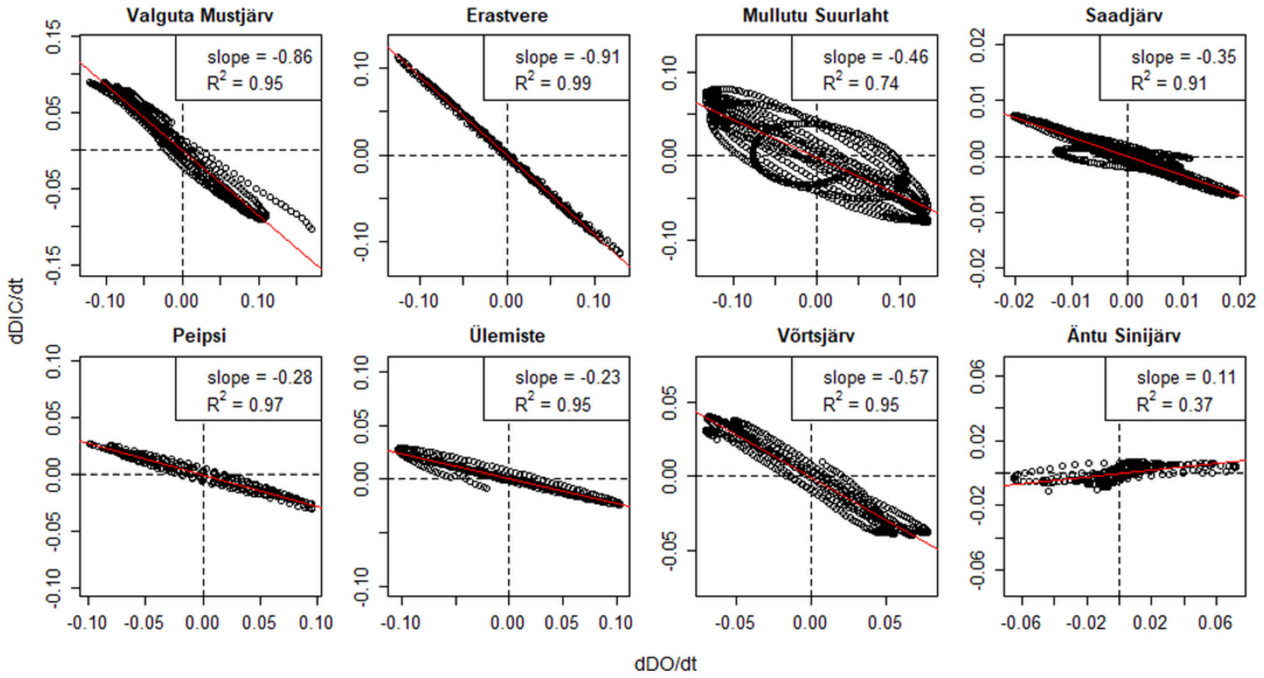

Figure 4. Relationship between rates of change in DO (dDO/dt) calculated from the isolated diel signal of DO, and rates of change in DIC (dDIC/dt) calculated from the isolated diel signal in DIC. All relationships are significant ($p < 0.001$), with the slope and R^2 values indicated in the top-right window of each plot. The lakes are ordered from low to high alkalinity (top left to bottom right).

Table 3. Results of Linear Regression Models Testing the Effect of dDO/dt on dDIC/dt Using the Raw Data (left) and the Isolated 24-h Signal Data (right)

Linear models using raw data					**Linear models using isolated diel signal data**		
Lake	**Alkalinity**	**Slope**	***p* value slope (** < 0.001)**	**R^2**	**Slope**	***p* value slope (** < 0.001)**	**R^2**
Valguta Mustjärv	0.50	− 1.00	**	0.25	− 0.89	**	0.97
Erastvere	1.63	− 0.91	**	0.92	− 0.91	**	1.00
Mullutu Suurlaht	1.80	− 0.12	**	0.03	− 0.46	**	0.74
Saadjärv	2.47	0.46	**	0.05	− 0.35	**	0.91
Peipsi	2.80	− 0.22	**	0.19	− 0.28	**	0.97
Ülemiste	3.30	− 0.58	**	0.46	− 0.23	**	0.95
Võrtsjärv	3.61	− 0.47	**	0.25	− 0.57	**	0.95
Äntu Sinijärv	4.80	0.74	**	0.53	0.11	**	0.37

diel data, we concluded that on a 24-hourly scale, the slope was indeed negative, which goes in line with the anti-phase relationship between DO and DIC expected from ecosystem metabolism.

Effect of Alkalinity on the Sensitivity of DIC to Metabolism

Finally, we wanted to know how alkalinity affects the sensitivity of DIC to metabolic changes in DO. For this, we tested the three hypotheses using dDIC/dt$_{\text{theoretical}}$ (equation 2) to see whether metabolism is the only driver for changes in dDIC/dt (Figure 5A), whether pelagic calcite precipitation plays a role (Figure 5B) or whether pelagic and benthic calcite precipitation both affect the sensitivity of DIC to metabolism (Figure 5C). The observed slopes did not fit the expected slope under the first hypothesis, equal to − 1 in all lakes (Figure 5A). For the two last scenarios (Figure 5B and

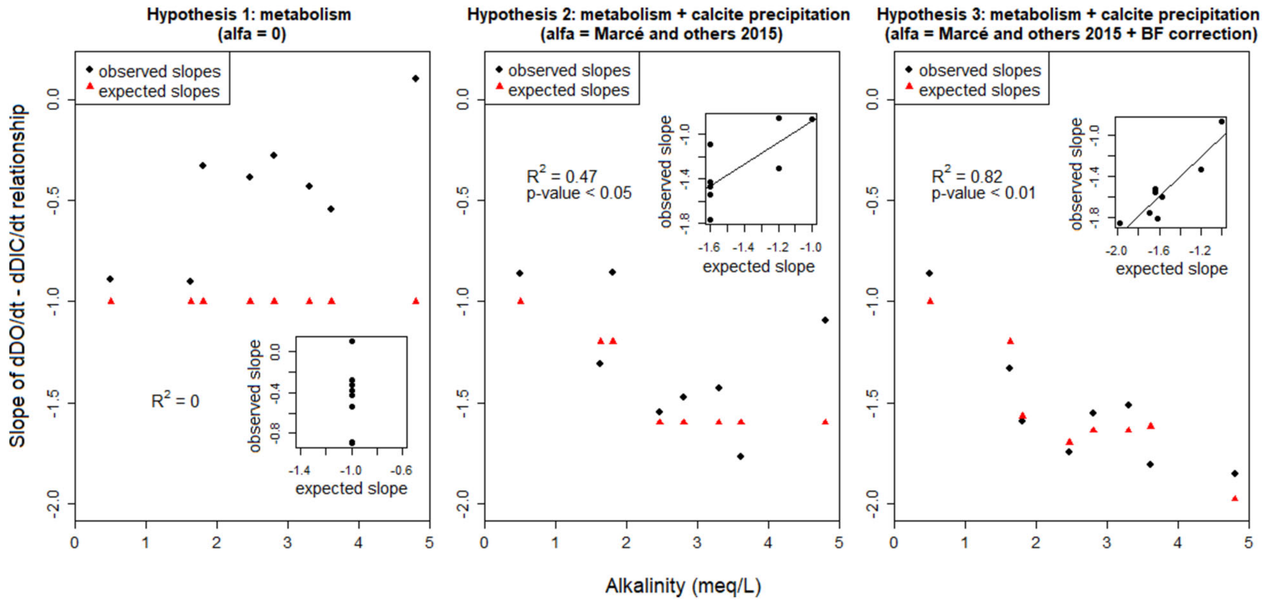

Figure 5. Effect of alkalinity on the sensitivity of dDIC/dt to changes in dDO/dt for each lake. Comparing the observed slopes (corrected using Eq. 3) of the dDO/dt–dDIC/dt relationship with expected slopes (calculated using Eq. 2) for each lake based on three different hypotheses: **A** metabolism is the only factor controlling carbon dynamics ($\alpha = 0$), **B** the α parameter (as defined in Marcé and others 2015) is added to the metabolism equation to account for effects of pelagic calcite precipitation on carbon dynamics, **C** the value of α is corrected for each lake for its benthic fraction to account for the effect of pelagic calcite precipitation and benthic calcifying macrophytes.

C), the expected slopes of the dDO/dt versus dDIC/dt relationships were very close to the observed ones, especially when using the correction for benthic metabolism (Hypothesis 3, Figure 5C). The relationship between observed and expected slopes had the highest R^2 value for the third hypothesis (Figure 5C).

DISCUSSION

Effects of Calcite Precipitation on the Relationship Between DO and DIC

Comparing dDO/dt with dDIC/dt calculated from the isolated 24-h signals is a way to assess the relative importance of metabolism in controlling carbon dynamics. The stoichiometry of photosynthesis and respiration implies a 1:1 relationship between changes in DIC and DO whereby 1 mol of DO respired produces 1 mol of DIC, and inversely, 1 mol of DIC is photosynthesized into 1 mol of DO (Stumm and Morgan 1996). Due to this stoichiometry, it is expected that a slope close to − 1 is found in the linear relationship between dDO/dt with dDIC/dt if aerobic metabolism is the main driver of carbon dynamics (Stumm and Morgan 1996). Calcite precipitation/dissolution processes can involve deviations from the metabolic slope of − 1 due to its release/consumption of CO_2 (Barrón and others 2006; Gattuso and others 1999; Obrador and Pretus 2013). Such deviations can also be caused by anaerobic processes (Torgersen and Branco 2007) or can be correlated with major gradients of ecosystem level, substrate level, and bacterial community level characteristics (Berggren and others 2011).

Among the eight studied lakes, only two lakes, Erastvere and Valguta Mustjärv, showed a slope near − 1 for the dDO/dt–dDIC/dt relationship (Figure 4). This implies that changes in dDIC/dt are strongly negatively correlated, at an almost equal molar ratio, with metabolic changes in dDO/dt. These two lakes have the lowest alkalinity values (1.6 and 0.5 meq L^{-1}, respectively). In all other lakes of higher alkalinity, the slopes between dDO/dt and dDIC/dt decrease and gradually approach a value of 0 at higher alkalinity (Figure 5A), which involves a deviation from the metabolic 1:1 stoichiometry between changes in DO and DIC. This means that with increasing alkalinity, the importance of metabolism in driving changes in DIC diminishes, as highlighted by Marcé and others (2015). DIC dynamics in lakes of higher alkalinity can be also affected by chemically enhanced uptake of atmospheric CO_2 (Kragh and Sand-Jensen 2018). However, our methods of isolating the metabolic diel signal eliminates any physical effects such as gas exchanges or water movements and therefore chemically enhanced uptake of atmospheric CO_2 is unlikely to be the cause of the observed deviations from the metabolic 1:1 stoichiometry.

Because of the chemical stoichiometry of respiration and photosynthesis, our studied lakes had an anti-phase coupling between the diel signals of DO and DIC, but with deviations from the 1:1 stoichiometry at increasing alkalinity, suggesting that although the anti-phase is tightly linked to photosynthetic or respiratory processes, deviations from this are influenced by the carbonate equilibrium system. The most plausible explanation of this mechanism is calcite precipitation, as it is tightly linked to alkalinity and photosynthetic activity. High alkalinity lakes are likely to precipitate larger quantities of calcite due to the high content of bicarbonates and carbonates. Photosynthesis raises the pH, influencing the carbonic acid equilibrium and shifting the balance toward oversaturation of carbonates (Minder-Zürich 1922; Dittrich and Obst 2004). Therefore, calcite precipitation has been reported to peak with high summer rates of primary production (Müller and others 2016). Additionally, picoplankton and cyanobacteria can act as nucleation sites for calcite precipitation (Stabel 1986; Dittrich and Obst 2004; Obst and others 2009). The relationship between primary production and calcite precipitation has rarely been quantified in freshwater systems. The few studies that do so have suggested that depending on the lake alkalinity, 1 mol of DIC consumed to produce 1 mol of O_2 through photosynthesis induces between 0.2 and 1 mol of calcite precipitation (Megard 1968; Mcconnaughey and others 1994; McConnaughey and Whelan 1997) and that below an alkalinity threshold of 1 meq L^{-1}, calcification is unlikely to occur (Marcé and others 2015). This range is consistent with the α values used in this study. A similar range is reported in marine planktonic assemblages (Riebesell and others 2000).

The chemical equation describing calcite precipitation implies that for each mole of calcite that precipitates, there is a loss of 1 mol of DIC and 2 mol of TA (equation 1). The combined effect of metabolism and calcite precipitation results in a net loss of -1 to -2 mol of DIC per mole of DO produced. However, the observed slopes of the dDO/dt–dDIC/dt relationships in the 8 studied lakes ranged between -1 and 0.1, which is inconsistent with the theoretical slope of -1 to -2. This inconsistency can easily be explained by the methods used to calculate DIC. The high variability in alkalinity caused by the limited sensitivity of field pH sensors made it impossible to use diurnal changes in alkalinity. Instead, the alkalinity values were averaged and used in combination with pH to calculate DIC. Correcting dDIC/dt for theoretical changes in alkalinity (equation 3) results in slopes that are in the correct range between -1 and -2. This highlights the importance of considering diurnal changes in alkalinity for correct estimations of DIC, something still challenging with current sensor technologies.

Here, we show that the slopes clearly deviated from the metabolic slope of -1, especially at higher alkalinity (Figure 5A). Accounting for pelagic calcite precipitation showed a positive relationship ($R^2 = 0.47$) between the observed slopes and the expected slopes (Figure 5B). However, in the case of two lakes, Mullutu Suurlaht and Äntu Sinijärv, the difference between observed and expected slope was still relatively high compared to the other lakes (Figure 5B, alkalinity 1.8 and 4.8 meq L^{-1}, respectively). These two lakes share the common characteristics of being shallow and having a thick layer of calcifying macrophytes of the genus *Chara* at their bottom floor. An approximate 1:1 calcification to photosynthesis ratio ($\alpha = 1$) in such organisms has been reported (McConnaughey 1991; Mcconnaughey and others 1994; McConnaughey and Whelan 1997). This is due to their ability to use HCO_3- instead of CO_2 for photosynthesis and due to an efficient coupling between carbon uptake at the acidic part of the cell surface and $CaCO_3$ precipitation at the alkaline surface of the cell (Mcconnaughey and others 1994). The benthic fraction of primary production for these two lakes is 46% for Mullutu Suurlaht and 94% for Äntu Sinijärv (Table 2) (Cremona and others 2016). In these two lakes, correcting the α values for the BF of primary production increased the fit between observed and expected slopes (Figure 5C). This suggests that benthic calcification by macrophytes can be equally or more important than pelagic calcite precipitation in driving changes in dDIC/dt in lakes that have a high fraction of primary production of benthic origin, and highlights the need to be spatially inclusive and include all metabolic components (benthic and pelagic) when addressing ecosystem-scale DIC dynamics (Obrador and Pretus 2013). This is especially relevant in shallow oligotrophic systems where benthic charophytes can reach similar production rates as pelagic phytoplankton communities under highly eutrophic conditions (Christensen and others 2013).

Besides calcite precipitation, other factors may lead to deviations from the metabolic slope of − 1, such as physical processes or anaerobic respiration. However, any physical effects such as gas exchanges, water movements, or external inputs of DIC due to storm events can be discarded because the metabolic diel signals were isolated from any non-metabolic signal. As for anaerobic respiration, the vertical distributions of temperature, DO and CO_2 presented in Laas and others (2016) for each lake during the same measurement period as this study, suggest that anaerobic processes have no major effect on surface waters at the measuring depths that were used for this study, especially considering the absence or very weak presence of gradient in CO_2 concentrations in the surface water layer. Two strong evidences suggest that calcite precipitation is the main mechanism causing these deviations: firstly, the observed slopes are strongly correlated with the expected slopes under calcite precipitation conditions, and secondly, the degree of deviation from the metabolic slope is positively correlated with lake alkalinity. Several other studies in other aquatic systems have suggested that calcite precipitation/dissolution is responsible for such deviations due to the CO_2 release/consumption (Gattuso and others 1999; Barrón and others 2006; Obrador and Pretus 2013).

Recent evidence suggests that in lakes of high alkalinity, large pools of DIC support higher rates of primary production, whereas in lakes of low alkalinity, primary production can be strongly DIC-limited (Kragh and Sand-Jensen 2018). Considering the coupling between primary production and calcite precipitation, it is likely that high production in lakes of high alkalinity during summer production peaks results in high rates of calcite precipitation that compensates photosynthetic DIC depletion by releasing CO_2. However, this mechanism is likely irrelevant in low alkalinity lakes, where DIC limitation minimizes primary production and calcite precipitation.

This study focuses on inorganic carbon dynamics within the aquatic systems without directly addressing questions related to CO_2 emissions to the atmosphere. An interesting addition to this work would be to relate the observed and expected sensitivity of DIC to metabolic changes in DO, to CO_2 emissions. Even though there is a net loss of 1 mol of DIC per mole of calcite that precipitates, the process of calcification is acknowledged to increase CO_2 supersaturation in surface waters because it also releases 1 mol of CO_2, thereby acting as a carbon source to the atmosphere (Opdyke and Walker 1992; Frankignoulle and others 1995; Suzuki 1998). Considering that DIC dynamics are largely driven by calcite precipitation in lakes of high alkalinity, this process is likely to be an important driver of CO_2 supersaturation in these lakes. Marcé and others (2015) suggested a threshold of 1 meq L^{-1} above which metabolism alone is not enough in explaining DIC dynamics. This threshold seems consistent with our results (Figure 5), but only one lake at alkalinity lower than 1 meq L^{-1} is represented in this study and therefore we cannot clearly identify and confirm this threshold. Marcé and others (2015) further suggested that 57% and 34% of the global area occupied by lakes have alkalinity values above 1 meq L^{-1} and 2 meq L^{-1}, respectively, which implies an important global contribution of calcite precipitation to CO_2 supersaturation in lakes. Our results agree well with this statement, as all of the lakes above 1 meq L^{-1} (7 out of 8 lakes) show clear deviations from the − 1 metabolic slope, with a tendency of stronger deviations at higher alkalinity (Figure 5B and C). Therefore, calcite precipitation may have a major contribution in global atmospheric CO_2 emissions from surface waters.

Numerical Considerations

The periodograms for DO and DIC showed a large number of signals with oscillations at shorter time periods than the 24-h metabolic diel signal (Figures 1 and 2), but the spectrum was so weak that it seemed of minor importance. However, when calculating rates of change from the raw DO and DIC data, these signals at shorter period were amplified to the extent where the diel signal became hidden in shorter oscillations of high amplitude (Supplementary Figures S1 and S2). These short time period signals cannot be explained by the metabolic balance between photosynthesis and respiration and are caused by non-metabolic processes. All the studied lakes have a large amount of noise in their DO and DIC data resulting from the high frequency in measurements that capture noisy random signals, and imprecisions in the pH sensors that create substantial errors when calculating DIC values due to the exponential scale of pH. Other physical processes may also cause the random short time period signals observed in these lakes, such as atmospheric or internal gas exchanges (Wissel and others 2008; Antenucci and others 2013), as well as water movements caused by convection, turbulence, minor mixing events or seiches. The cross-wavelets for DO and DIC reveal that significant oscillatory signals are commonly found between 1- and 8-h frequency. This cannot be classified as noise and needs to be linked to internal processes of the lakes. One explanation could be found in the seasonal pattern of internal seiches which corresponds well to the 1- to 8-h frequency in oscillation (Bernhardt and Kirillin 2013). Despite the amount of noise and non-metabolic signals, the cross-wavelets revealed a clear anti-phase coupling between DO and DIC around the 24-h period in most of the lakes, which is to be expected if aerobic metabolism is the main driver of both DO and DIC dynamics (Figure 3). In some lakes, this coupling was weak or interrupted, especially in Äntu Sinijärv where it was almost totally absent due to the particularities of this lake (DIC dynamics are mostly driven by groundwater DIC-rich inputs).

Despite the amount of noise and sometimes weak diel signals, Singular Spectrum Analysis is an efficient tool that allowed us to enhance the metabolic diel signal-to-noise ratio, by isolating and extracting the diel signals for DO and DIC. In contrast to a smoothing function which would smooth out the noisy signals without getting rid of them, SSA identifies the fundamental oscillations within a time series. Therefore, the diel signals that are isolated using this tool are not affected by unwanted signals such as noise or non-metabolic processes.

CONCLUSIONS

- Calcite precipitation is an important process in driving carbon dynamics in lakes of high alkalinity, which represents more than half of the global area occupied by lakes and reservoirs. Acknowledged to act as a carbon source to the atmosphere, it is likely that calcite precipitation is an important driver of CO_2 fluxes in lakes globally.
- The importance of calcification in driving carbon dynamics can be assessed using an alkalinity-dependent parameter (α). In lakes that have a high benthic fraction of primary production, α must be corrected to account for calcification by macrophytes.
- Considering diurnal changes in alkalinity is mandatory for precise estimations of DIC concentrations and dynamics.
- The problem of noisy high-frequency measurements datasets can be effectively overcome using Singular Spectrum Analysis to enhance the signal-to-noise ratio by extracting the signal of interest, such as diel signals.

ACKNOWLEDGEMENTS

This study was funded by MANTEL ITN (Management of climatic extreme events in lakes and reservoirs for the protection of ecosystem services) through European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie Grant Agreement No 722518, by Estonian Ministry of Education and Research (IUT 21-02), by the Estonian Research Council grant (PUT PSG32) and by the project C-HYDROCHANGE (CGL2017-86788-C3-3-P and CGL2017-86788-C3-2-P) funded by the Spanish Ministry of Science, Innovation and Universities.

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DATA AVAILABILITY

Data are available at: https://doi.org/10.6073/pasta/421c3435f2a735fe472511fda4ed5e5f.

Code information is available at: https://doi.org/10.5281/zenodo.3465312.

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Appendices/Supplemental material

Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes

Hares Khan, Alo Laas, Rafael Marcé, Biel Obrador

Supplementary figures

The supporting information presented here provides more comprehensive details that complement the analysis presented in the main text, allowing a more complete assessment of our findings.

Periodograms and wavelets were used to identify the presence of a metabolic diel signal for DO and DIC, while cross-wavelets were used for assessing the presence and strength of a metabolic coupling between DO and DIC. A time-series is usually composed of several oscillating patterns occurring simultaneously at different frequencies of oscillation. Most traditional mathematical methods for analyzing the frequency and periodicity of these patterns, such as Fourrier analysis, assume that the underlying processes are stationary in time. Wavelets have the advantage of projecting a time series into the frequency space, allowing to identify localized intermittent periodicities (Grinsted and others 2004). Similarly to wavelets, cross-wavelets use the time-frequency space to visualize common oscillatory patterns which allows to identify localized intermittent periodicities that are common between two time series.

At first, rates of change in dissolved oxygen (dDO/dt) and dissolved inorganic carbon (dDIC/dt) were calculated from the raw DO and DIC data. However, the noisy non-metabolic signals that were only weakly present in the raw DO and DIC data were much more present and amplified in the rates of change (Fig. S1 for dDO/dt, Fig. S2 for dDIC/dt). This is why instead, we first isolated the diel signal in DO and DIC using Singular Spectrum Analysis (SSA), and then calculated the rates of change from these isolated signals.

Linear regression models testing for the effect of dDO/dt on dDIC/dt showed poor relationships in most of the lakes because the diel signal was covered in noise (Fig. S3). This was why we had to calculate rates of change from the isolated diel signals.

As an alternative, SSA was used to identify and isolate the diel signal in DO and DIC. Rates of change were then calculated from these diel signals, and the relationship between dDO/dt and dDIC/dt obtained from the diel signals were tested using linear regressions (Fig. S4). SSA is a tool that allows identifying and extracting fundamental oscillatory components, also known as Eigenvectors, from an original time series. The SSA package in R is able to decompose a time series into 50 Eigenvectors, some of which are trends, seasonal patterns, noise and random signals. The 24-hours diel cycle components were identified visually as well as by viewing scatterplots of paired Eigenvectors, and by viewing the correlations between the Eigenvectors using the specific commands in the SSA R package, following the recommendations in Golyandina & Korobeynikov (2014). Once the 24-hours diel cycle components were identified, they were grouped and reconstructed into a new time series following Golyandina & Korobeynikov (2014). The R code is published online and freely available (Khan 2019). This method efficiently isolates the diel seasonal signal from a time series, even if it is weak or if the time series is noisy. In such cases, the isolated diel signal will look very different to the original time series because it is devoid of any signal that is not related to the metabolic 24-hour signal (Figure S5 and Figure S6 for comparison of a diel signal extracted from a clean versus noisy time series). The multivariate SSA (MSSA) method used for lake Äntu Sinijärv works similarly to the SSA method but here both variables are decomposed into Eigenvectors that share a common oscillatory pattern.

Supplementary Figure Legends

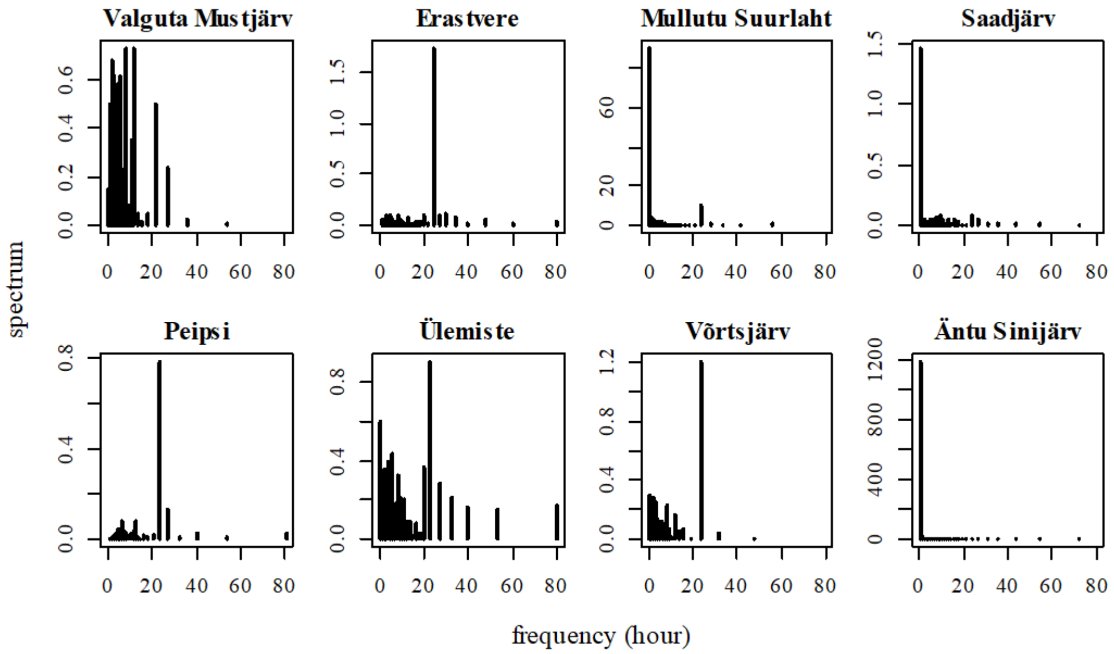

Figure S1. periodograms for rates of change of DO (dDO/dt). The x-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated (y-axis). Notice the dominating spikes at 24-hour frequency.

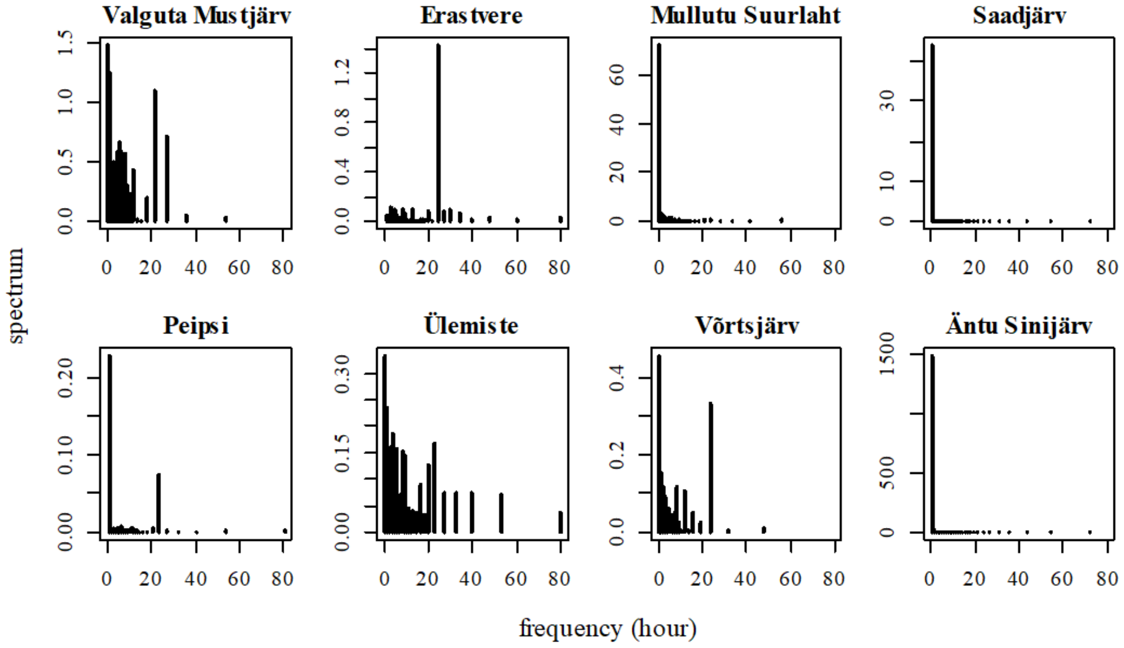

Figure S2. periodograms for rates of change of DIC (dDIC/dt). The x-axis corresponds to the frequency of oscillation (hours) at which the spectral density is estimated (y-axis). Notice the dominating spikes at 24-hour frequency.

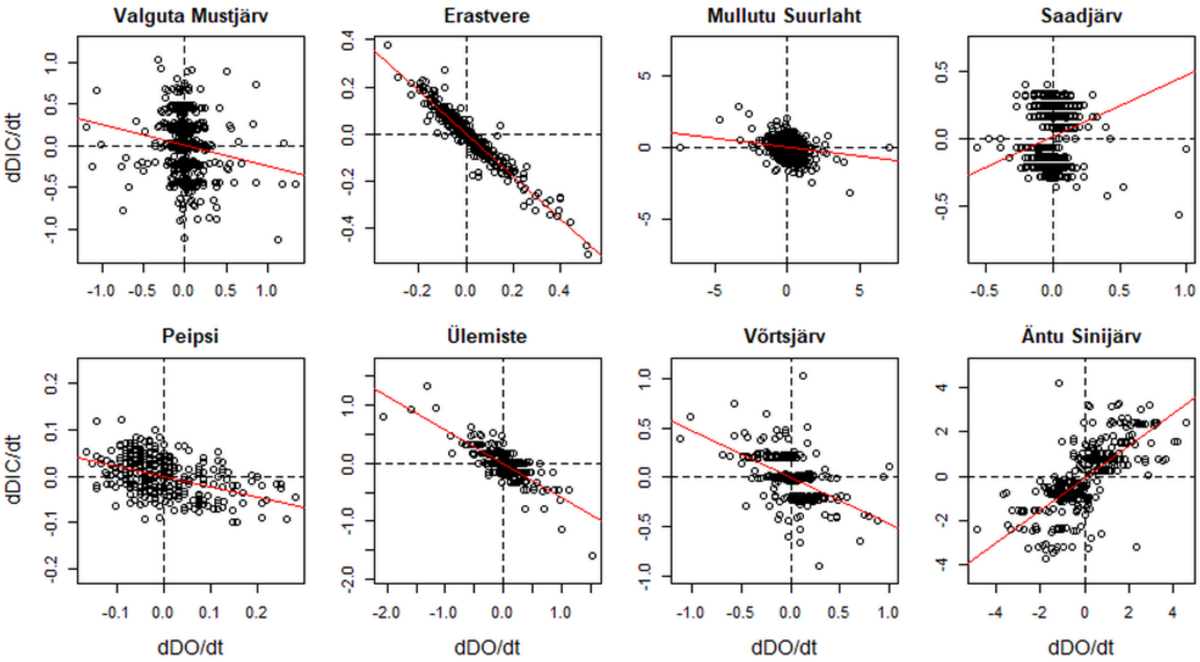

Figure S3. Relationship between rates of change in DO (dDO/dt) calculated from the raw DO data, and rates of change in DIC (dDIC/dt) calculated from the raw DIC data.

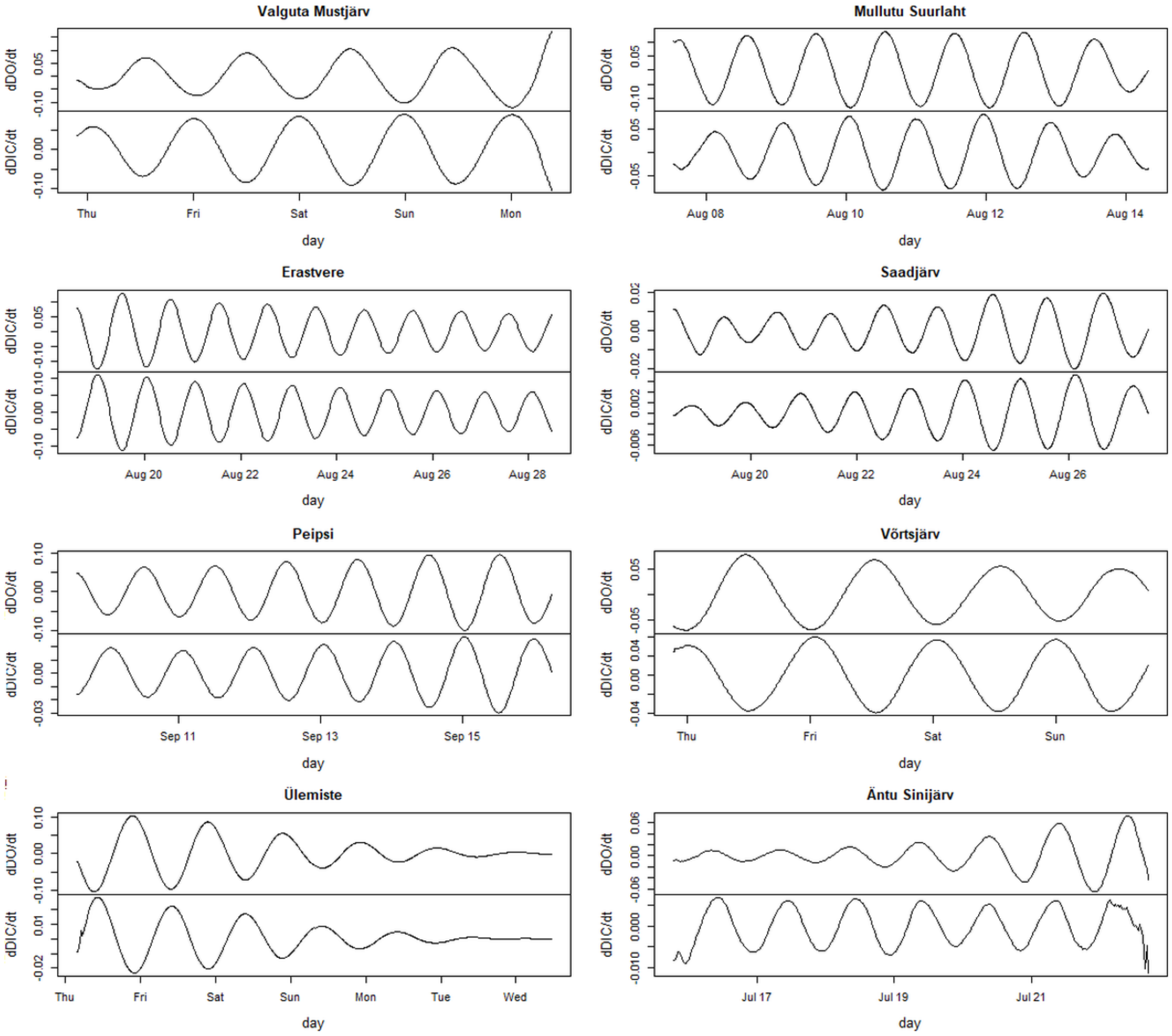

Figure S4. Times series of rates of change of DO and DIC (dDO/dt and dDIC), calculated from the isolated diel signals of DO and DIC that were extracted using SSA.

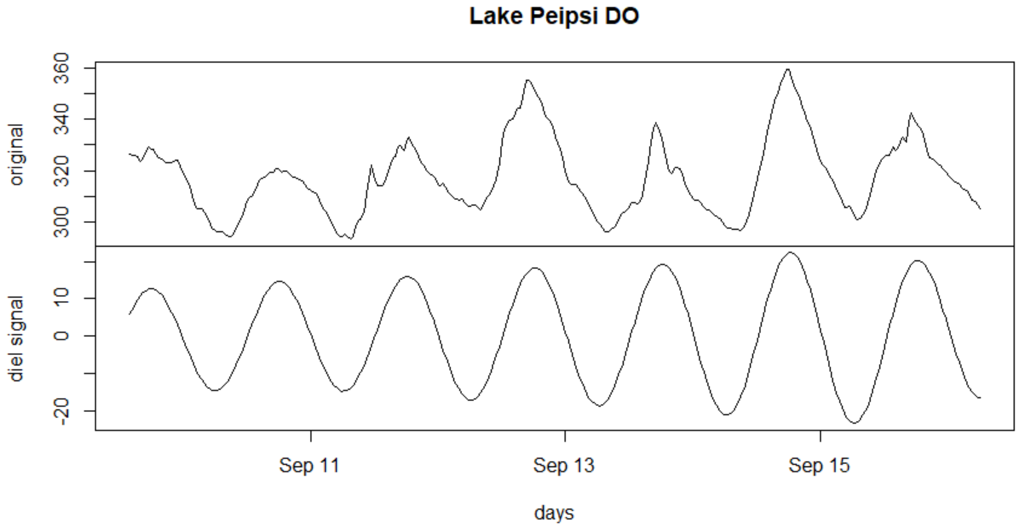

Figure S5. Example of a diel metabolic signal of DO extracted from a time series with less noisy and random signals.

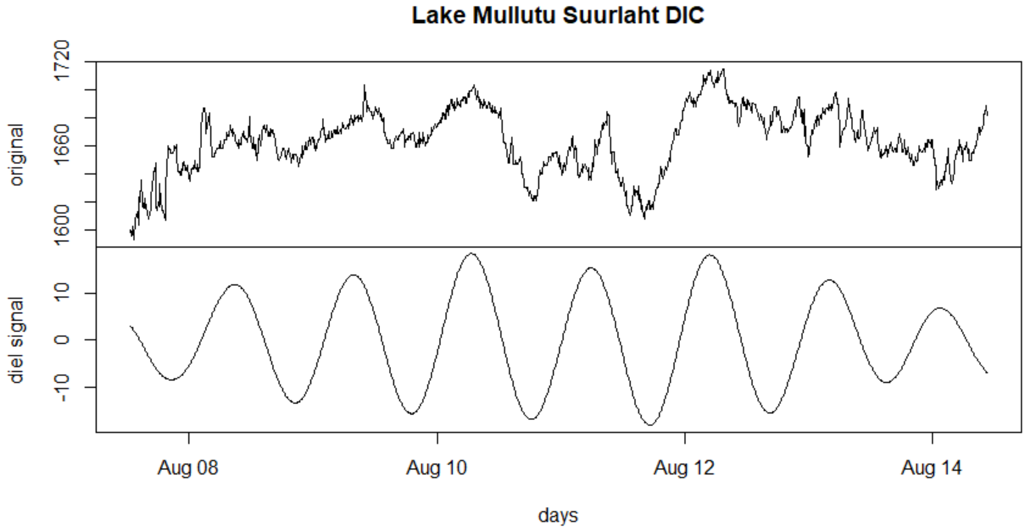

Figure S6. Example of a diel metabolic signal of DIC extracted from a noisy time series.

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Education

Ongoing PhD in limnology on the relationships between aquatic metabolism and carbon emissions in lakes and reservoirs and the effects of extreme climatic events on lake carbon cycle 2017 – 2020
Within the MANTEL ITN project (Management of climatic extreme events in lakes and reservoirs for the protection of ecosystem services) – A Marie Sklodowska-Curie European Joint Doctorate Innovative Training Network. More information on the project's website: www.mantel-itn.org
Joint doctorate between University of Barcelona (Spain) – Department of Evolutionary Biology, Ecology and Environmental Sciences, and University of Life Sciences (Estonia) – Institute of Agricultural and Environmental Sciences, Centre for limnology

The main objective is to understand the relative contributions of organic processes (i.e. lake metabolism) and inorganic processes (i.e. calcite precipitation) in driving carbon dynamics in different types of lakes across Europe, using High Frequency Measurements data, in situ incubation experiments and sediment traps.

In the framework of this PhD program, I have attended several intensive training workshops:

- Introduction to Mantel, extreme climatic events and high frequency monitoring, 2017, Estonia
- Understanding methods and tools used for high frequency data analysis, 2018, Germany
- Highland Statistics: Data Exploration, Regression, GLM & GAM with introduction to R, 2018, Sweden
- Ecosystem Services Workshop, 2019, The Netherlands
- Lake Modelling Workshop, 2019, Estonia

Complementary certificate in geomatics 2016 – 2017
University of Geneva – Faculty of sciences, Fac. of social sciences

Interdisciplinary Master's Degree in environmental sciences (MUSE) – Specialisation in Water sciences 2013 – 2015
University of Geneva – Faculty of sciences, Fac. of social sciences, Fac. of economy and management

Bachelor in ethnology, geography and biology 2010 – 2013
University of Neuchâtel – main subjects: ethnology and geography, secondary subject: biology

Matura 2005 – 2009
High school Jean-Piaget in Neuchâtel

Language and computing

French and English: mother tongue, excellent speaking and writing skills

Swiss German: spoken fluently

German: spoken fluently

Spanish: spoken fluently

IT: MS-Office, MS-Excel, MS-Access, MS-PowerPoint, R-studio, ArcGIS, Operations Dashboard for ArcGIS, Windows Movie Maker

Professional experiences

PhD – Maintenance, deployment and data analysis of high frequency measurement (HFM) stations 2017 – 2020
In the frame of my PhD research work I am currently working with automated HFM stations where I am assisting in the deployment and maintenance of several measuring stations across Estonia and Spain. I am also working with HFM data from several lakes across Europe to explore the effects of biological and inorganic processes as well as seasonal variations and episodic events on carbon dynamics.

PhD – Experimental setup, field work and laboratory analysis

In conjunction to HFM data analysis, I am performing in situ incubation experiments, sampling of surface water and sediment traps in several lakes across Estonia and Spain. The samples are then processed and analysed in laboratory for measuring a set of parameters including alkalinity, calcium content, nutrients, dissolved organic carbon and chlorophyll.

PhD – Numerical modelling and time-series analysis
For analysis of HFM data, I am using different approaches that involve numerical modelling such as lake models and regressions, or time-series analysis such as wavelet analysis or singular spectrum analysis. Using such tools, I have acquired programming skills using the R programming language.

Internship in geomatics at the State of Geneva May – July 2016
DGAN (Direction Générale de l'Agriculture et de la Nature) in the Geomatics section
Tasks: using ArcGIS' Operations Dashboard, created common view "Dashboards" for monitoring, tracking, and reporting events with the environmental data of DGAN. Creating several maps using ArcGIS, and collaborating with the expert network "GE21" in developing a tool for mapping ecosystem services

Research work in a laboratory for the Master's thesis 2015
University of Geneva, Forel Institute

Title of my Master's thesis: *Influence of green algae diversity (taxonomic, morphological and phylogenetic) on the uptake of nitrate from water.* The work was marked with the best note (6 / 6).

In this "Biodiversity on Ecosystem Functioning" (BEF) research work, we experimentally explored the impact of the functional diversity of phytoplankton communities on their capacity to use nitrogen as a resource. Using a set of microcosm experiments, we manipulated the functional diversity of phytoplankton diversity and measured nitrogen uptake and biomass production, functions that are directly related to water quality and nutrient pollution. Statistical modelling was used to identify trends between phytoplankton diversity, nitrate uptake and biomass production.

French and English teacher, animator Summers 2012 – 2015
SLC – Swiss Language Club in Leysin, Swiss Education Academy
Tasks: teaching English and French for beginners, intermediate and advanced levels. Co-developer of teaching methodologies. Responsible for several daily activities, such as, animator during day and evening activities, driving students from the airport to the campus in Leysin

Assistance in old age homes 2010 – 2016
Several missions for Civil Protection

Journal publications

Khan, H., Marcé, R., Laas, A. Obrador, B. 2021. The relevance of pelagic calcification in the global carbon budget of lakes and reservoirs. Limnetica *in press* DOI: 10.23818/limn.41.02

Khan, H., Laas, A., Marcé, R., Sepp, M., Obrador, B. 2021. Eutrophication and Geochemistry Drive Pelagic Calcite Precipitation in Lakes. Water 13(5): 597. DOI: 10.3390/w13050597

Khan, H., Laas, A., Marcé, R., Obrador, B. 2020. Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes. Ecosystems 23: 1566-1580. DOI: 10.1007/s10021-020-00488-6

Oral presentations at international scientific conferences

Khan H., Laas A., Marcé R., Obrador B., 2019. "Calcite precipitation events, understanding its drivers and implications". Book of abstracts, MANTEL Symposium online conference.

Khan H., Laas A., Marcé R., Obrador B., 2019. "Calcite precipitation, a major driver of carbon fluxes in lakes and reservoirs". Book of abstracts, SEFS conference (Symposium of European Freshwater Sciences), Zagreb, Croatia

Khan H., Marcé R., Laas A., Obrador B., 2019. "Major effects of alkalinity on the relationship between metabolism and dissolved inorganic carbon dynamics in lakes". Book of abstracts, SIBECOL conference, Barcelona.

Khan H., Marcé R., Laas A., Obrador B., 2018. "Effects of alkalinity and trophic state on the relationship between metabolism and carbon dynamics in several lakes". Book of abstracts, AIL conference, Coimbra.

Poster presentations at international scientific conferences

Khan H., Marcé R., Laas A., Obrador B., 2019. Limnology meets oceanography: Calcite precipitation as a relevant player on CO2 emissions from lakes. GLEON-21 meeting ("Global Lake Ecological Observatory Network") in Huntsville, Canada

Khan H., Laas A., Marcé R., Obrador B., 2018. "Effects of alkalinity on the relationship between metabolism and carbon dynamics in eight Estonian lakes". GLEON-20, Rottnest Island.

Published data

Laas A, Khan H. 2019. "Short-term high-frequency water dissolved carbon dioxide, temperature, dissolved oxygen, salinity and pH data from 8 Estonian lakes in year 2014". Environmental Data Initiative. https://doi.org/10.6073/pasta/421c3435f2a735fe472511fda4ed5e5f

Published codes

Khan H. 2019. Singular Spectrum Analysis using rSSA package. https://doi.org/10.5281/zenodo.3465312

Khan H. 2020. Calcite precipitation and pCO2 model. http://doi.org/10.5281/ZENODO.3727551

Other experiences

Active observer and volunteer collaborator in the development of innovative water treatment solutions, products development and scaling up with PakoSwiss Technologies Ltd – a company in Pakistan that works in research and development of innovative water treatment solutions to meet the needs of the Bottom of the Pyramid population (2013 – 2017)

Driving license

Category: B

Date of issue: 07.06.2011

Associations

GLEON – The Global Lake Ecological Observatory Network conducts innovative science by sharing and interpreting high-resolution sensor data to understand, predict and communicate the role and response of lakes in a changing global environment. www.gleon.org 2018

AIL – The Iberian Association of Limnology brings together all those interested in advancing knowledge on fundamental processes and relations between the different components of inland water ecosystems. www.limnetica.com 2018

Reference contacts

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Dr. Alo Laas – Supervisor of my PhD, Senior Researcher at Estonian University of Life Sciences – Institute of Agricultural and Environmental Sciences, Centre for limnology
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