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Early detection and the importance of environmental drivers for the invasive cyanobacterium *Raphidiopsis raciborskii*

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Cover: *Raphidiopsis raciborskii* filaments
(photo: Carlotta Meriggi)

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Abstract

Freshwater habitats often experience species loss, with the greatest pressures coming from land use alterations and the establishment of non-native species as they become invasive. One species of concern is the tropical invasive freshwater cyanobacterium *Raphidiopsis raciborskii*, currently expanding its geographic distribution towards northern regions of Europe. As a toxin-producing, bloom-forming species, population growths of *R. raciborskii* can negatively affect local biodiversity and ecosystem services. Hence, in this study, I wanted to evaluate and validate (i) the geographical extent of suitable habitats, (ii) its competitive capacities with a native phytoplankton assemblage and (iii) its adaptive capabilities when moved from ideal to colder temperatures. To identify suitable colonization areas, I used Species Distribution Models (SDMs), which revealed suitable habitats in areas where the invasive cyanobacterium has not been recorded, i.e. several parts of Europe including south-eastern Sweden. However, there were some inconsistencies between SDM results and the field studies used to validate the SDM prediction, where only a few of the European screened lakes matched with the SDM prediction. To better understand the competitive success of *R. raciborskii* we performed a laboratory study using different nutrient and temperature conditions and, as competitors, an assembly of phytoplankton species typically found in Scandinavian freshwaters. Results confirmed the importance of temperature on *R. raciborskii* growth and its ability to withstand suboptimal environments. The physiological changes and gene expressions of *R. raciborskii*, when transferred from optimal to suboptimal temperatures, were tested through a differential transcriptomic experiment. Results showed a difference in gene expression in different treatments, with a downregulation of genes in the suboptimal temperature. Although *R. raciborskii* has not been recorded in Sweden, it is a well-established invasive species in many European lakes. Combined results from this thesis show that *R. raciborskii* meets the climatic conditions matching with the south-eastern parts of Sweden. If able to surpass physical and ecological barriers, due to its ability to withstand a wide-range of environments, it might be able to survive in lakes with less favorable conditions and bloom when conditions become ideal. To be able to prevent and contain the expansion of this invasive species in northern European lakes, proactive monitoring programs with high temporal and spatial frequency are needed in combination with broad information campaigns to minimize further spread to other freshwater systems.

Keywords: *Raphidiopsis raciborskii*; invasive species; cyanobacteria; early detection; SDMs; metagenomics; competition experiment; transcriptomic experiment; DNA; eutrophication

Tidig upptäckt och betydelsen av miljöfaktorer för den invasiva cyanobakterien *Raphidiopsis raciborskii*

Sammanfattning

Sötvattenmiljöer upplever ofta artförluster, och det beror främst på förändringar i markanvändning och etablering av icke-inhemska arter som blir invasiva. En art att se upp med är den tropiska invasiva sötvattenscyanobakterien *Raphidiopsis raciborskii*, som för närvarande utökar sin geografiska spridning mot norra delen av Europa. Som en toxinproducerande, blombildande art kan populationstillväxten av *R. raciborskii* påverka den lokala biologiska mångfalden och ekosystemtjänsterna negativt. I denna studie ville jag därför utvärdera och validera (i) den geografiska omfattningen av lämpliga livsmiljöer, (ii) dess konkurrensförmåga med inhemska växtplankton och (iii) dess anpassningsförmåga när den flyttas från optimala till kallare temperaturer. För att identifiera lämpliga kolonisationsområden använde jag Species Distribution Models (SDMs), som avslöjade lämpliga habitat i områden där den invasiva cyanobakterien ännu inte har hittats, det vill säga stora delar av Europa inklusive sydöstra Sverige. Det fanns dock vissa inkonsekvenser mellan SDM-resultaten och de fältstudier som användes för att validera resultatet, där endast ett fåtal av de europeiska sjöarna från en stor DNA-databas matchade SDM-prediktionen. För att bättre förstå *R. raciborskiis* konkurrensframgång genomförde vi en laboratoriestudie med olika närings- och temperaturförhållanden och konkurrenter i form av en samling växtplanktonarter som vanligtvis finns i skandinaviska sötvatten. Resultaten bekräftade betydelsen av temperatur på *R. raciborskiis* tillväxt och dess förmåga att motstå suboptimala miljöer. De fysiologiska förändringarna och genuttrycket hos *R. raciborskii* när de överfördes från optimala till suboptimala temperaturer testades under ett endags experiment med transkriptomik. Resultaten visade en skillnad i genuttryck i de olika behandlingarna, med en generell nedreglering av gener i suboptimala temperatur. Även om *R. raciborskii* inte har registrerats i Sverige är den en väletablerad invasiv art i många europeiska sjöar. Sammantaget visar resultaten från avhandlingen att kraven *R. raciborskii* har för tillväxt uppfylls av klimatförhållanden som matchar de sydöstra delarna av Sverige. Om arten kan ta sig över fysiska och ekologiska barriärer, med hjälp av sin förmåga att klara olika miljöförhållanden, kan den kanske överleva i sjöar under mindre gynnsamma förhållanden för att sedan ta över när förhållandena någon varm sommar blir optimala. För att kunna förebygga och begränsa spridningen av denna invasiva art i nordeuropeiska sjöar behövs proaktiva övervakningsprogram, med hög tids- och rumsfrekvens i kombination med informationskampanjer för att hindra ytterligare spridning ifall den hittas.

Nyckelord: *Raphidiopsis raciborskii*; invasiv art; cyanobakterier; tidig upptäckt; SDMs; metagenomik; konkurrensförsök; transkriptomisk; DNA; eutrofiering

Dedication

A mamma e papà

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Meriggi, C.*, Drakare, S., Polaina Lacambra, E., Johnson, R. K., & Laugen, A. T. (2022). Species distribution models as a tool for early detection of the invasive *Raphidiopsis raciborskii* in European lakes. Harmful Algae, 113. https://doi.org/10.1016/j.hal.2022.102202.

II. Meriggi, C., Mehrshad, M., Johnson, R. K., Laugen, A. T., Drakare, S. Challenges in supplying empirical proof for predictions derived from Species Distribution Models (SDMs): the case of an invasive cyanobacterium (submitted to ISME communications).

III. Meriggi, C., Johnson, R. K., Laugen, A. T., Drakare, S. Competition dynamics of native mixed phytoplankton communities exposed to the invasive cyanobacterium *Raphidiopsis raciborskii* along gradients of N:P and temperature (manuscript).

IV. Mehrshad, M., Meriggi, C., Drakare, S. Adaptation strategies in response to lower temperature in the invasive cyanobacterium *Raphidiopsis raciborskii* revealed through comparative transcriptomics (manuscript).

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The contribution of Carlotta Meriggi to the papers included in this thesis was as follows:

I. Contributed to the design of the study, collected the data, performed the SDMs analysis and drafted the manuscript. All authors read and approved the final version of the manuscript.

II. Contributed to the design of the study, collected the samples, performed the laboratory work as well as bioinformatics analysis with the guidance of co-supervisor, and drafted the manuscript. All authors read and approved the final version of the manuscript.

III. Contributed to the design of the study, performed the experiment, collected and analyzed the samples in the FlowCam. Performed statistical analysis and drafted the manuscript. All authors read and approved the final version of the manuscript.

IV. Contributed to the design of the study and to running the experiment. Performed RNA extractions and measurements, but did not perform data analysis and interpretation of the results. Contributed to drafting the manuscript. All authors read and approved the final version of the manuscript.

1. Introduction

Habitat and climate change, overexploitation and pollution of resources as well as the introduction of non-native species are the main causes of biodiversity loss (Mazor et al., 2018; Wang et al., 2021). Freshwater habitats are particularly vulnerable to species loss, with the greatest pressures coming from land use changes and the establishment of non-native species as they become invasive (Havel et al., 2015; Ricciardi & Rasmussen, 1999; Sala et al., 2000). Non-native species are defined as organisms introduced outside their natural range of dispersal potential (Gren et al., 2022; Kernan, 2015), whilst invasive species are described as non-native species that, once introduced in a new environment, overcome the acclimatization period successfully, showing good capacity of settling and expanding in the new invaded environment (Leidenberger et al., 2015). Invasive species can cause ecological and economical damages, representing a global threat to native biodiversity, ecosystem services and economies (Lehtiniemi et al., 2015; Mannino & Balistreri, 2018). Additionally, they can alter native species assemblages causing changes in species composition and structure that ultimately may lead to drastic changes in ecosystem processes (Francis & Chadwick, 2012; Wang et al., 2021). Consequently, due to global environmental changes caused by the intensification of anthropogenic activities and a general lack of an active and proactive early detection surveillance and management, the risk posed by invasive species introduced to aquatic ecosystems all around the world is projected to increase (Litchman, 2010; Sukenik et al., 2015; Wilk-Woźniak et al., 2016). Detecting potential invasive species at an early stage is critical in order to mitigate the consequences of their potential expansion (Trebitz et al., 2017). However, early detection of aquatic invasive species, particularly microorganisms, is challenging and it is considered “early detection” only if the organisms are still few and spatially confined (Bolius et al., 2019; Litchman, 2010; Trebitz et al., 2017). Throughout this thesis, I will refer mainly to the term invasive, as the species in focus is a well-known invasive species in European freshwaters.

1.1 Invasive cyanobacteria

Cyanobacteria are a diverse group of prokaryotic autotrophs, reproducing asexually, by binary or multiple division in unicellular and colonial species or by fragmentation and spore formation in filamentous ones (Dehò & Galli, 2012). Favorable abiotic factors, such as temperature and nutrient availability, allow buoyant cyanobacteria with gas vacuoles to float upwards towards the water surface for better access to sunlight for photosynthesis, forming a dense mass called blooms (Dehò & Galli, 2012). The repeated incidence of cyanobacterial blooms is a worldwide concern of environmental health, posing severe threats to freshwater habitats (Gkelis & Zaoutsos, 2014; Kenne & Merwe, 2013). The most telling signs of cyanobacterial blooms are strong discoloration of water, accumulation of surface scums, strong smell and decrease in water quality by the release of off-flavour as well as cyanotoxins (Ibelings & Chorus, 2007; Moreira et al., 2013). The formation of planktonic cyanobacterial blooms is one of the main threats causing impaired water resources in shallow lakes (Bonilla et al., 2012). Furthermore, projected changes in the climate and anthropogenic stressors could potentially lead to shifts in species assemblage of cyanobacterial blooms, favouring the invasion of non-native microorganisms (Mehnert et al., 2010). Non-native microbes are quite deceitful as they can be present in an aquatic ecosystem while remaining undetected due to their small size and initially low biomass (Sukenik et al., 2012). Once environmental conditions are favorable, they may become abundant and widespread, causing ecological and economic impairments, thus becoming invasive (Sukenik et al., 2012). Additionally, the invasion of non-native cyanobacterial species in aquatic habitats could potentially affect the aquatic food web due to the production of secondary metabolites (cyanotoxins), functioning as allelochemicals, that inhibit the growth of other phytoplankton species and grazers (Paerl & Paul, 2012). Dispersal limitation is one of the main factors regulating the distribution and range expansion of a species (Ribeiro et al., 2018). Microorganisms encounter fewer dispersal barriers compared to macroorganisms, as dispersal limitation is often overcome by the presence of a resting stage (akinetes) that is easily transported by both physical and/or biological vectors (Incagnone et al., 2015; Litchman, 2010; Padisák, 1997). Specifically, cyanobacteria have been described as air-dispersed phytoplankton, as the action of wind helps with the dispersal of both microorganisms and resting stages (Incagnone et al., 2015). The invasion of microorganisms to aquatic habitats is difficult to detect mainly due to the lack of proactive and detailed water quality monitoring programs (Litchman, 2010; Sukenik et al., 2012).

1.2 The case of *Raphidiopsis raciborskii*

One species of concern is the invasive freshwater cyanobacterium *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*), a planktonic, filamentous cyanobacterium of the order Nostocales (Fig. 1).

Figure 1. A *Raphidiopsis raciborskii* filament with two akinetes (a resting stage cell type). Photo: Carlotta Meriggi, SLU.

R. raciborskii is an invasive species that has spread from the tropics to northern temperate regions and nowadays it is found in waterbodies in almost all continents and in various climate zones (Wilk-Woźniak et al., 2016). In Europe, *R. raciborskii* was first recorded 1938 in Lake Kastoria, Greece (Skuja, 1938; Wiedner et al., 2007) and, since its first sighting, it has been detected throughout southern and continental Europe. Within the last decades, *R. raciborskii* migrated towards the northern regions of Europe (Poland and Lithuania) and the most northern point recorded so far is from the Lake Nero, Russia (Babanazarova et al., 2015; Kokociński et al., 2017).

Its rapid and successful expansion and colonization is mainly due to the global climate changes affecting freshwaters and, due to its phenotypic plasticity, *R. raciborskii* is able to survive under an extensive range of temperature, nutrient and light conditions (Bonilla et al., 2012; Kokociński et al., 2017; Mehnert et al., 2010; Padisák & Istvánovics, 1997). Additionally, the presence of certain characteristics, e.g. production of heterocysts (used for N_2 fixation), akinetes (spore-like resting cells, resistant to unfavorable conditions) and toxic secondary metabolites (cyanotoxins), have been advantageous for its successful establishment in new invaded habitats, making *R. raciborskii* competitive against native phytoplankton species (Burford et al., 2016; Moisander et al., 2002; Rzymski et al., 2014; Soares et al., 2013). Strains of *R. raciborskii* differ in their ability of producing cyanotoxins, revealing a connection between geographic distribution of *R. raciborskii* strains and the produced toxins (Saker & Griffiths, 2000; Sinha et al., 2012; Zanchett & Oliveira-Filho, 2013). The Australian and New Zealand *R. raciborskii*'s strains produce cylindrospermopsin (CYN) (Hawkins et al., 1985; Wood & Stirling, 2003), a highly potent hepatotoxin, whilst the South American strains produce saxitoxins (STXs), a dangerous neurotoxin (Lagos et al., 1999). European strains, however, do not show the production of either CYN nor STXs, but certain *R. raciborskii* strains from Poland can produce toxic compounds similar to CYN and it is hypothesized to be involved in allelopathic relations (Rzymski et al., 2014; Sinha et al., 2012). These differences denote the high subspecies diversity of *R. raciborskii*, meaning that this species is a variegate mixture of multiple strains with different physiological responses to a wide range of environmental conditions (ecotypes) by differing in toxic cell quota (chemotypes) (Willis et al., 2016; Xiao et al., 2017; Yoshida et al., 2005). In Europe, *R. raciborskii* is usually found in shallow, turbid, warm-water eutrophic to hypereutrophic lakes and bloom events have been associated with a wide range of environmental factors such as high temperature, low light intensity, vertical water stratification, resistance to grazing and flexibility in response to nutrient acquisition (Kokociński et al., 2017; Kokociński & Soininen, 2012; O'Brien et al., 2009). As a toxin producing and bloom-forming species, its ongoing expansion throughout European freshwater habitats could negatively affect local biodiversity and ecosystem services, as already observed in Hungary and Serbia (Svirčev et al., 2016; Vehovszky et al., 2013). There is an urgent need to determine the geographic extent of potentially suitable habitats of *R. raciborskii* in order to understand and predict its possible invasive range. This is specifically important in lake-rich countries like Sweden, where the phytoplankton composition is monitored in only a small fraction of the over 100 000 lakes. Monitoring efforts targeted towards *R. raciborskii* and, in general, a framework for the early detection of invasive aquatic microbes is largely overlooked in monitoring programs, globally and in Sweden.

2. Overall aim of the thesis and specific objectives

The overall aim of this thesis was to predict and validate the potential expansion of the invasive cyanobacterium *R. raciborskii* in areas of unknown occurrence as well as to understand the environmental drivers underpinning its potential expansion and establishment.

Specific objectives of the work described in **Paper I-IV** were to:

Paper I – Predict and visualize the potential distributional patterns of *R. raciborskii* across Europe to identify potential geographical areas, beyond its known distribution, by using Species Distribution Models (SDMs).

Paper II – Validate the SDM prediction (**paper I**) on the potential occurrence of *R. raciborskii* with field studies and highlight challenges in providing empirical confirmation.

Paper III – Test *R. raciborskii* capability to compete against a selection of phytoplankton species native to Scandinavian lakes under different environmental conditions.

Paper IV – Understand the impact of lower temperature on the gene expression profile of *R. raciborskii* and genes potentially involved in its adaptation to a new invaded habitat.

3. Materials and Methods

Below I give an overview of the main methods used in this thesis. Even though the papers are interrelated, the individual studies differ and therefore each section will outline the methods used. For more details on the methods described below, please see **Papers I-IV**.

3.1 Predicting and validating the potential distributional patterns of *R. raciborskii* beyond its known distribution

3.1.1 Species distribution models

A suitable tool to forecast where a species may find suitable areas to establish is the use of Species Distribution Models (SDMs), a statistical approach that links occurrence records of a species to environmental variables to estimate spatial distribution patterns using a correlative approach (Escobar et al., 2018; Leidenberger et al., 2015). Correlation between species occurrence and environmental variables might not imply direct effects, but it can be associated to broad-scale patterns of the species distribution and help to anticipate where a species is likely to occur (Real et al., 2017). When modelling invasive species, two central assumptions of SDMs do not hold (i) they are not in equilibrium with their environment and, therefore, (ii) niche quantification and transferability in space and time are limited (Gallien et al., 2012). The violation of the equilibrium assumption has some repercussions on the potential niche of the species, which could potentially underestimate geographical areas that the species can occupy (Barbet-Massin et al., 2018; Václavík & Meentemeyer, 2012). Even though SDMs cannot comprehensively predict the potential range of an invasive species, they are still valuable for invasive species management and can be a powerful tool to predict where invasive species are more likely to disperse and establish next (Barbet-Massin et al., 2018; Václavík & Meentemeyer, 2012; Warren & Seifert, 2011). Identifying areas where a species is more likely to occur can also be used to guide sampling protocols and prioritize areas of study (Guimarães et al., 2020). Hence, in **paper I**, we used SDMs to predict and visualize potential distributional patterns of *Raphidiopsis raciborskii* across Europe and to identify potential habitats, beyond its known distribution.

The first step in calibrating SDMs was to gather data on the occurrences of *Raphidiopsis raciborskii*. Presence records were retrieved mainly from published studies and the Global Biodiversity Information Facility (GBIF, 2019) database, confining the search within Europe. The compiled dataset of European lakes covered 209 presence records of *R. raciborskii* distributed across 17 countries. As second step, we collected environmental predictors for *R. raciborskii* by using publicly available, high spatial resolution bioclimatic variables related to temperature, precipitation, average altitude and yearly solar radiation (Fick & Hijmans, 2017). Only non-collinear environmental predictors were retained (Variance of Inflation Factor, VIF < 4) resulting in 7 of the 21 available environmental variables included in the final model: mean diurnal temperature range; temperature seasonality; precipitation of driest month; precipitation seasonality; precipitation of the warmest quarter; precipitation of coldest quarter and altitude. After collating data on species presence and environmental variables, a random set of pseudo-absences was included, as it was not possible to retrieve a set of "real" absence data. Thereafter it was possible to build an ensemble SDM prediction (Fig. 2) using the Biomod2 package for R version 3.6.1 (Thuiller et al., 2009; R Core Team, 2020). Four different algorithms were fit, Generalized Linear Model (GLM), Generalized Boosted Models (GBM), Maximum Entropy (Maxent; Phillips et al., 2004) and Generalized Additive Models (GAM). Model performance was evaluated with the true skill statistic (TSS) (Allouche et al., 2006; Ruete & Leynaud, 2015), defined as sensitivity+specificity-1 and a threshold of TSS ≥ 0.6 was used for the final ensemble model, to include accurate models only (Fig. 2).

Cyanobacterial blooms in Scandinavian lakes and elsewhere are correlated with elevated total phosphorus (TP) concentrations (Vuorio et al., 2020). Published data on the occurrence of *R. raciborskii* did not include suitable information of nutrient levels to be included as an environmental predictor variable in the SDM. Therefore, we included data from several nutrient-rich Swedish lakes to more accurately predict areas at high risk of *R. raciborskii* invasion as well as to compare with the SDM output. Accordingly, total phosphorus (TP) data for 4800 lakes retrieved from the Swedish environmental monitoring database (https://miljodata.slu.se/MVM/Search) were divided into three nutrient groups, representing TP concentrations that depict risk of low to high levels of cyanobacterial blooms.

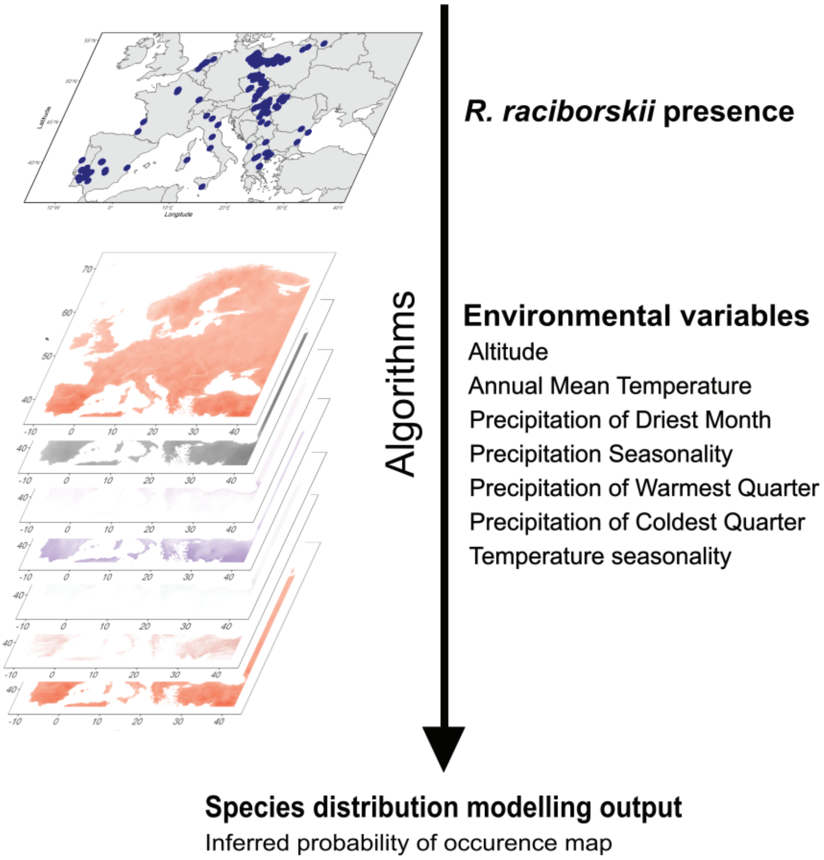

Figure 2. Inspired and adapted from Elith & Leathwick, 2009. SDM framework for detecting the possible areas of expansion for the invasive *Raphidiopsis raciborskii* in European lakes.

3.1.2 Validating SDM output through field and in-silico studies

Paper II builds on findings from **paper I**, with the intention of validating the SDM output (**paper I**) in order to provide empirical evidence of *R. raciborskii* presence/absence in lakes located in both Sweden and other parts of Europe (Fig. 3). During the summer of 2020, we performed a field study in Sweden by sampling water and surface sediment in 11 nutrient-rich lakes (Fig. 3), located in areas of both high (> 0.5) and low (< 0.5) probability of occurrence, in accordance with the SDM output from **paper I**. Replicates of water and sediment samples were processed for DNA extraction and the extracted DNA was used for Polymerase Chain Reaction (PCR) amplification of the chloroplast (rpoC1) gene with *R. raciborskii* specific primers (Fig. 4). In addition, to complement the field study, we performed an in-silico study, on a larger areal scale, by screening environmental DNA using publicly available metagenomes[1] from different European lakes (Fig. 3). The selected metagenomes were downloaded from the National Center for Biotechnology Information (NCBI), quality checked via the bbduck.sh tool, processed and the SSU-align tool (Nawrocki, 2009) was used to identify, align, mask as well as visualize bacterial 16S rRNA associated reads. The taxonomy of the extracted 16S rRNA reads was assigned using BLAST (Wheeler et al., 2007) against Silva SSU 138.1 (Quast et al., 2013), as reads assigned to invasive cyanobacterium *R. raciborskii* were detected, resulting in a map of confirmed presence/absence of the screened metagenomes (Fig. 4).

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[1] Metagenomes are the recovery and complete sequencing of genetic material extracted from environmental samples (Raza & Shahid, 2020), in our case water and sediment samples.

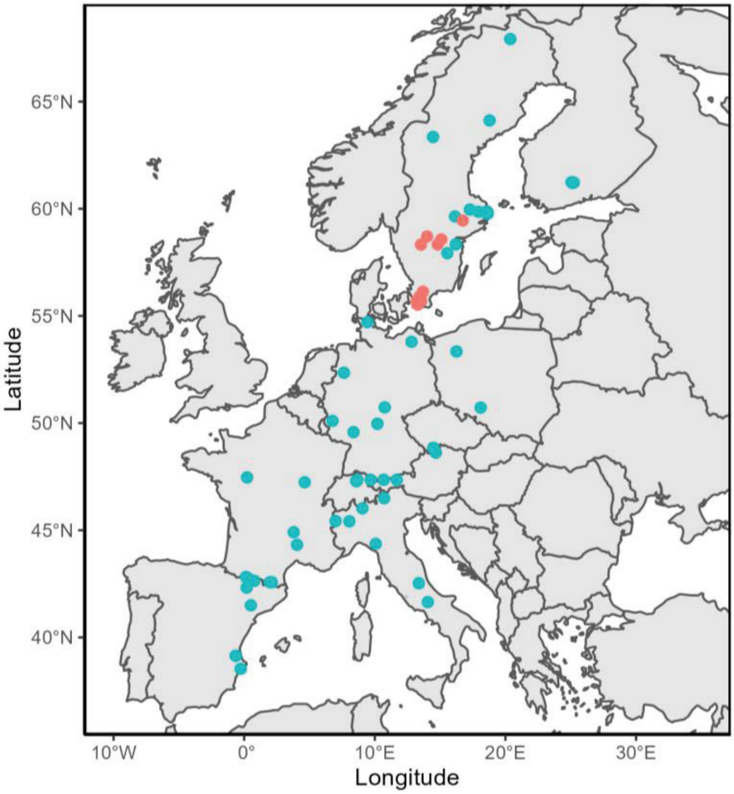

Figure 3. The map represents fieldwork in Sweden (red dots) and in-silico study through screened metagenomes (blue dots).

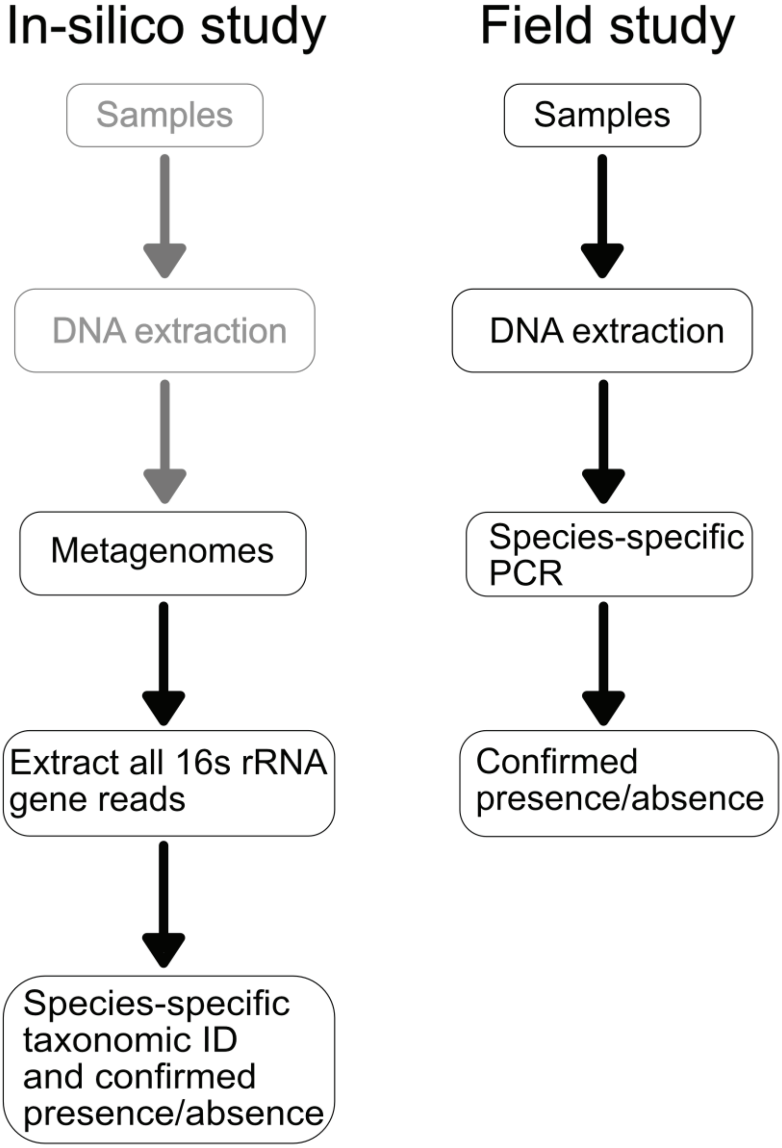

Figure 4. Methodology differences between field and in-silico studies described in **paper II**. In the **in-silico** study, publicly available metagenomes were downloaded from the National Center for Biotechnology Information (NCBI). Consequently, steps of the in-silico study that I did not perform are grayed out in the figure.

3.2 Test *R. raciborskii*'s competitive capacity and physiological adaptation under different stress conditions

Despite findings from the SDM and field-study approaches used in **paper I** and **paper II** it is still difficult to understand *R. raciborskii*'s competitive capabilities against native phytoplankton communities under varying conditions (**paper III**) as well as its physiological adaptation in a new invaded habitat (**paper IV**). This knowledge is essential for understanding the capabilities of *R. raciborskii* for potential expansion and settlement in areas of unknown occurrence, like Sweden.

3.2.1 Competition experiment

As mentioned in the introduction, the invasion success of *R. raciborskii* in freshwaters has been attributed to its phenotypic plasticity and ability to survive under an extensive range of temperature, nutrient and light conditions, giving *R. raciborskii* competitive advantages over other bloom-forming cyanobacteria (Zheng et al., 2023). Cyanobacterial blooms, in general, are favoured by the ratio of nitrogen to phosphorus (N:P ratio), dominating at low N:P ratio, usually below 16:1 (Huisman et al., 2018; Palus, 2015). It is still uncertain how the settlement of *R. raciborskii* in a newly invaded habitat could be affected by the presence of native species under varying temperature and nutrient conditions. In **paper III**, we address the potential risk of the invasive *R. raciborskii* to establish in Scandinavian freshwaters by conducting an experiment in which *R. raciborskii* competed against an assembled phytoplankton community. The community consisted of five strains from three major phytoplankton functional groups, native to many Scandinavian lakes and that are likely to be encountered by *R. raciborskii* following its invasion: chlorophytes (*Chlamydomonas reinhardtii*, *Scenedesmus* sp.), cyanobacteria (*Planktothrix agardhii*, *Microcystis aeruginosa*) and diatoms (*Asterionella formosa*) (Fig. 5).

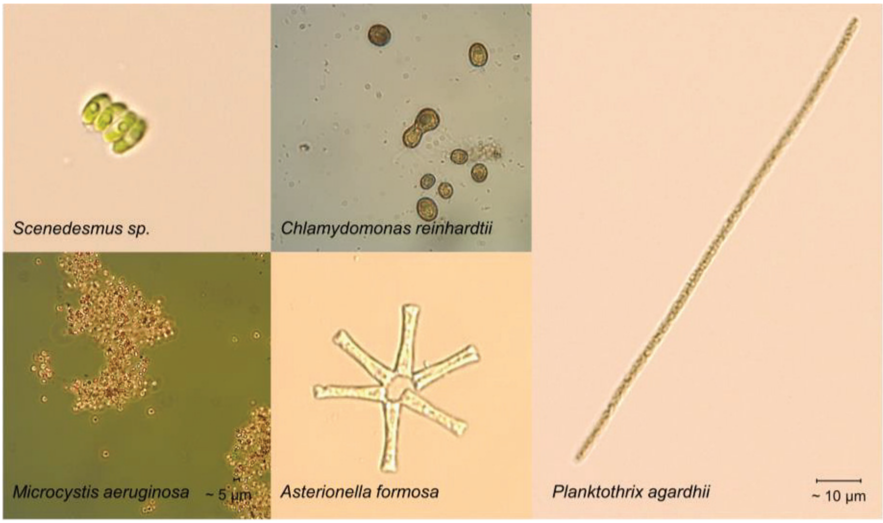

Figure 5. The experimental phytoplankton assemblage used in **paper III** together with *R. raciborskii* (Fig. 1). Photos: Carlotta Meriggi, SLU.

A 3x3 factorial experiment was performed and the mixed community was exposed to three temperatures (17°C; 22°C; 26°C) and three different N:P atomic ratio concentrations (8:1; 16:1; 32:1) (Fig. 6) to produce N-limiting, optimal and P-limiting conditions (Vrede et al., 2009), achieved by modifying the growth media. Phytoplankton's response to gradients of temperature and N:P might be species-specific and could impact species composition in the water body, especially in the first phase of invasion. The growth media was replaced daily to achieve a semi-continuous system. Every third day 10 ml of culture was removed during the media exchange dilution and preserved in Lugol's solution for later analysis of the phytoplankton species composition and biovolume. Every tenth day the volume removed during the daily media exchange was analyzed to monitor nutrient conditions throughout the experiment. The experiment was performed using irradiance levels between 70 and 90 µE m^{-2} s^{-1} and a 16:8 light/dark cycle. The experiment was run for 31 days. The samples were analyzed by a flow cytometer and microscope, better known as FlowCam (VS1, Fluid Imaging Technologies Inc., Scarborough., ME, USA), which is a flow imaging microscope that combines imaging and laser light to detect particles from a fluid sample (Vorobjev & Barteneva, 2016). The FlowCam, coupled with the VisualSpreadsheet software (Version 4.15.1), gave the possibility to analyze, classify and count each species separately in the experimental samples.

Biovolume estimates (µm^3/mL) of each species were calculated based on formula for their geometric shapes. A two-way ANOVA was performed to analyze effects of temperature and nutrient conditions and their interactions on biovolume of the five strains. The statistical analysis was performed on day 16 of the experiment when nutrient ratios showed clear differences in nutrient limitation using the R version 4.2.1 (R Core Team, 2022). Since *A. formosa* was not able to grow in any of the experimental treatments it was excluded from the analysis.

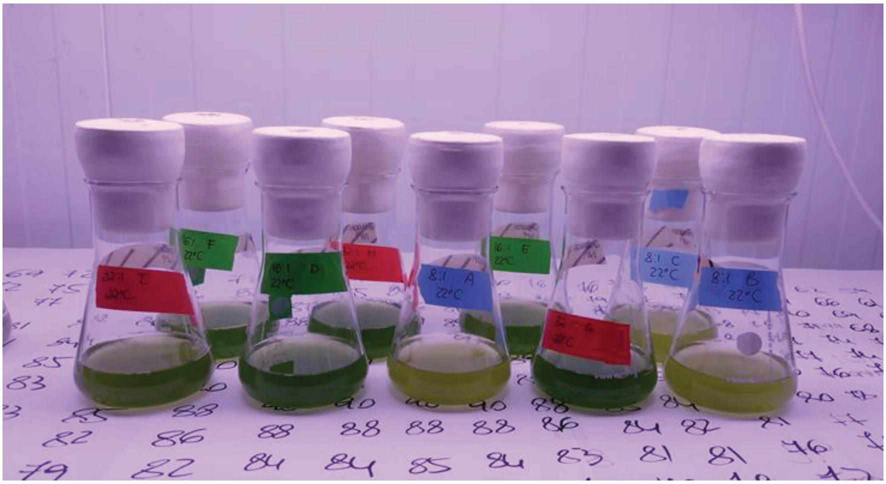

Figure 6. Experimental set-up, exemplified with a photo from the 22°C controlled temperature room (same set-up in the 17°C and 26°C). The different label colors refer to the different concentration of nutrients and the numbers on the table cover show irradiance levels.

3.2.2 Transcriptomic experiment to track *R. raciborskii*'s gene expression

Based on results from **paper III**, we decided to perform a differential transcriptomic[2] experiment to study the impact of temperature on the gene expression profile of *R. raciborskii* (Fig. 7). In **paper IV**, we aimed to determine whether differences in temperature could regulate the gene expression profile of *R. raciborskii*. Additionally, via this experiment we studied the expressed metabolic pathways and certain key genes that were differentially regulated for *R. raciborskii*'s adaptation from its optimal growth temperature (26°C) to suboptimal and colder one (17°C), relevant for its potential colonization in Sweden. Six *R. raciborskii* cultures were acclimatized and grown at *R. raciborskii*'s optimal growth temperature (26°C). These cultures were sampled at time point 0; afterwards, three out of the six cultures were transferred from the 26°C to the 17°C temperature treatment. After 4 and 12 hours, from time point 0, samples were collected at both 26°C and 17°C treatments (time point 1 and 2, respectively) (Fig. 7). For each sampling round, a total of 3 mL sample was collected from each culture. The biomass was collected on 0.22 µm filters and filters were frozen immediately to stop metabolic processes. The experiment was performed using irradiance levels between 70 and 90 µE m^{-2} s^{-1} and a 16:8 light/dark cycle. Total cellular RNA was extracted for each treatment and used for sequencing library preparation. Generated libraries were sequenced using NovaSeq 6000 SP flowcell (PE150). The first step to perform the gene-resolved transcriptome analysis was to download *R. raciborskii*'s reference genome from GenBank. Gene prediction on this genome was performed using Prodigal (Hyatt et al., 2010) and predicted genes were preliminarily annotated using Prokka (Seemann, 2014). Annotations of tRNA and rRNA sequences were done using tRNAscan (Chan et al., 2021) and barrnap, respectively. The sequenced metatranscriptomes were quality checked and the resulting good quality reads were mapped against the *R. raciborskii* reference genome. The number of mapped reads to each gene were counted by using an in-house script. The read count per gene values were normalized and processed using DESeq2 (Love et al., 2014) via the iDEP pipeline. Differentially expressed genes were then analyzed using DESeq2. Genes

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[2] Transcriptomic is the study of the transcriptome, which is the complete set of all the ribonucleic acid (RNA) molecules produced by the genome under specific circumstances or in a specific cell (Milward et al., 2016). When comparing transcriptomes, it is possible to identify differences in genes expression in response to different treatments as presented in **Paper IV**.

were annotated using BlastKoala, Phmmer, NCBI conserved domain and gene annotations were manually checked via the results of NCBI conserved domain annotations. Differentially expressed genes in 17°C versus 26°C, at time point 1 and 2 (4 and 12 h after the transfer, respectively), were categorized in different modules based on their annotation. Functional annotation of these genes and their expression level were used to disentangle the adaptation strategy of *R. raciborskii* to the lower temperature.

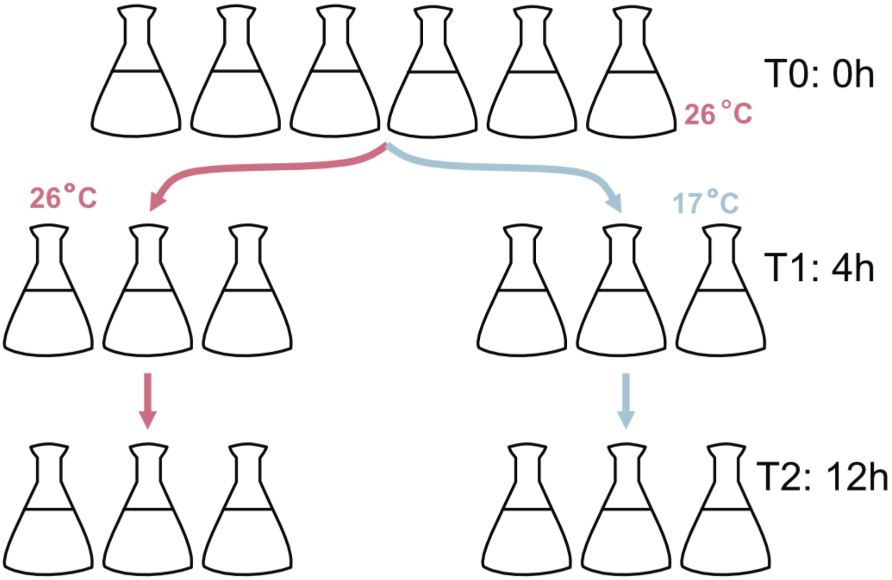

Figure 7. Experimental setup from transcriptomic experiment, adapted from **Paper IV**.

4. Results and discussion

In this section, I briefly discuss the results from **papers I-IV**. For more details, refer to the individual papers.

4.1 *Raphidiopsis raciborskii* predicted and validated potential expansion

In **paper I**, we predicted and identified geographical areas that have suitable environmental conditions for *R. raciborskii* potential invasions. SDM predicted approximately 85% of the presences in Europe (sensitivity) and 89% of the pseudo-absences (specificity) for *R. raciborskii* with an ensemble TSS value of **0.747**. Low temperature seasonality (less variation between seasons, resulting in a longer growth season) and low altitude (under 500 m a.s.l.) were the main factors determining the habitat suitability for *R. raciborskii* in Europe. The ensemble probability of occurrence map showed suitable habitats (> 0.5 probability) for the occurrence of *R. raciborskii* in areas where the cyanobacterium has been already recorded, particularly in central Europe. Somewhat disconcerting, the southern and central regions of Sweden correspond to areas with high probabilities of occurrence (> 0.5, Fig. 8). *R. raciborskii* has not yet been recorded in Sweden, however, the SDM output indicated locations where *R. raciborskii* might be already present or where future invasions may occur. Some of the areas of suitable habitat for *R. raciborskii,* located in the south-eastern parts of Sweden correspond to regions with lakes having moderate to high nutrient concentrations, conditions that favor cyanobacterial blooms.

Field-studies output from **Paper II** showed negative results in which none of the 11 sampled lakes in Sweden resulted in the amplification of the target region, indicating the absence of *R. raciborskii*. The in-silico study had reads corresponding to the *R. raciborskii* 16S rRNA sequence in only five of the 153-screened metagenomes in Europe and none in Sweden. Thus, the low number of reads made it difficult to interpret the SDM prediction.

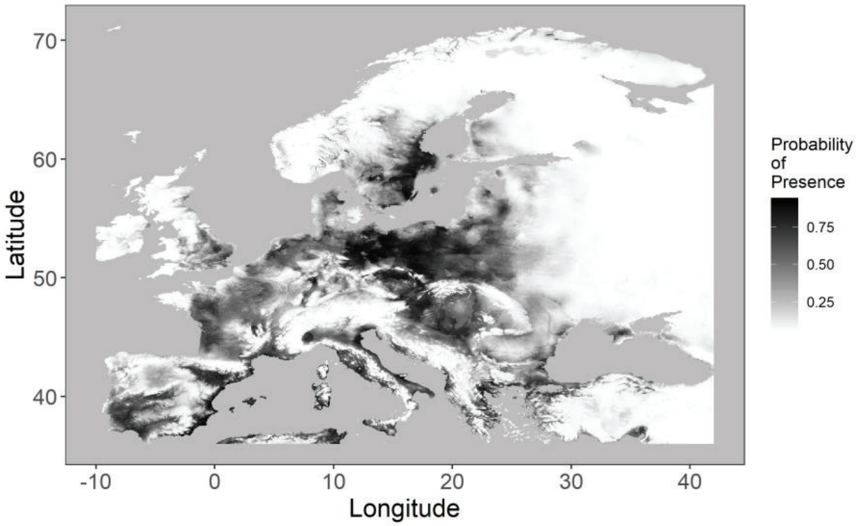

Figure 8. *Raphidiopsis raciborskii* SDM ensemble probability of occurrence map for Europe. Black color shows high probability of occurrence if standing water is present, while lighter shades of black show intermediate probability of occurrence, and white color shows a probability of occurrence close to zero (**paper I**).

4.2 Constraints

One of the main constraints encountered when building SDMs for predicting the potential expansion of *R. raciborskii* was the lack of important environmental variables. In **paper I**, it was not possible to include information regarding, nutrient concentrations, water temperature and other abiotic factors relevant for *R. raciborskii* settlement in freshwaters, as reports were not supplemented by detailed environmental metadata. Therefore, we used easily accessible bioclimatic predictors that have been used for predicting cyanobacterial blooms (Guimarães et al., 2020) and that are also relevant for *R. raciborskii*. The inclusion of more specific predictor variables would have likely increased the accuracy of the SDM in predicting the potential blooms of *R. raciborskii* in freshwaters, rather than climatically feasible areas only, highlighting lakes in regions at higher risk of invasion. Additionally, the implementation of true absences, instead of pseudo-absences, in the modelling approach could have increased model accuracy (Pineda & Lobo, 2009). On the other hand, I decided to use pseudo-absences as it was difficult to know if the absences of *R. raciborskii* was the result of inadequate surveying, seasonality, biotic interactions or dispersal limitations. Furthermore, areas where the invasive species is not found could become suitable in the near future; for this reason absences should not be included if the purpose is to estimate the complete areas at risk of invasion (Jiménez-Valverde et al., 2011; Lobo et al., 2010).

Some of these constraints are reflected in **paper II,** as there are inconsistencies between detected presences of the invasive *R. raciborskii* and habitat suitability based on the bioclimatic factors used across many parts of Europe, making the validation/interpretation of the SDM equivocal. The results underline the importance to report findings regarding additional biological and environmental factors connected to the presence of the target species in already invaded areas and to implement detailed monitoring programs focused on the early detection of invasive microbes. This data could then be used to predict the likelihood of invasion into new aquatic habitats. In addition, dispersal limitation, competition and predation are essential components for the potential spreading, settlement and establishment of an invasive species in a new habitat. However, these factors are seldom included in SDMs, as biological information is usually lacking and the addition of these factors would entail building mechanistic models that are conceptually and computationally intensive and therefore usually not feasible. The power of SDMs by using open access environmental predictors was to have a manageable method to limit and select high-risk sites for regular monitoring, as blindly monitoring for "door-knocker" species, such as *R. raciborskii* in Sweden, entails a relatively large economic investment as well as tedious and labor-intensive monitoring. In addition, the empirical validation of SDM prediction could have also be limited due to the lower abundance of the target species at an early stage of invasion, inconsistent spatial and temporal frequency of monitoring or due to an underrepresentation of cyanobacteria in the screened metagenomics datasets. Hence, SDMs are valuable tools for predicting areas of potential invasion for the invasive *R. raciborskii* and these results should mainly be considered as a guide for further monitoring/management efforts rather than evidence of presence/invasion.

4.3 *R. raciborskii* competition dynamic and transcriptomic experimental outputs

In the competition experiment (**paper III**), the increase in phytoplankton cell densities were reflected in declines in nutrient concentrations throughout the experiment. *R. raciborskii* did not dominate in any of the treatments. Nevertheless, it was able to grow in all tested conditions, and after *C. reinhardtii* and *M. aeruginosa* it was the dominant species in all treatments. *Scenedesmus* sp. and *P. agardhii* were the less dominant taxa in all treatments and, in particular, *P. agardhii* was negatively affected by the low temperature. Temperature, in general, is one of the most important factors influencing phytoplankton growth, nutrient uptake, spatial-temporal distribution in freshwater systems and competition is one of the major factor shaping phytoplankton structure and succession in freshwaters (Lei et al., 2020). In this experiment, temperature was the most significant factor affecting the growth and structure of the phytoplankton assemblage. In high temperature treatment there was an increase in species' growth, except for *Scenedesmus* sp. that showed the opposite behavior. Nutrient response was strain-specific and was only significant for *C. reinhardtii*, being the species with the highest growth. *C. reinhardtii* was probably controlling nutrient availability for the other taxa that became severely nutrient limited. For *R. raciborskii* temperature was the significant variable affecting its growth. *R. raciborskii*'s growth was highest in the high temperature treatments (22°C and 26°C), but it was able to survive and maintain its biomass even in the less favorable temperature (17°C). These results corroborated those from other studies in which *R. raciborskii* was able to withstand temperatures as low as 12°C, showing good growth capabilities from 17°C and reaching its optimum temperature between 22-30°C (Soares et al., 2013; **paper III**). This is a key result considering typical summer temperatures in Swedish lakes, being at around 15°C only (average temperature for the months of July and August, year 2021 and 2022, according to the Swedish environmental monitoring database, https://miljodata.slu.se/MVM/Search). If *R. raciborskii* could be able to start the growing season at low temperature and potentially develop blooms early in the season, it may replace other native filamentous cyanobacteria with a decrease in diversity (Sukenik et al., 2015). In addition, low grazing pressure, as many zooplankton prefer to feed on high quality phytoplankton like *C. reinhardtii*, could promote the invasion success of *R. raciborskii*, as in general cyanobacteria lack important fatty acids and can produce potentially toxic compounds (Hong et al., 2013). In the case of *R. raciborskii*, if able to surpass ecological as well as natural barriers and disperse to Swedish freshwaters, it would most likely be found in shallow, warm, eutrophic lakes first. Based on the experimental findings and considering its wide acclimation capabilities as well as its ecological plasticity, *R. raciborskii* could be able to survive and maintain a consistent biomass in relative low summer temperatures, overcome unfavorable winter conditions, due to the development of akinetes, and form cyanobacterial blooms when environmental conditions become ideal (e.g. during occasional warm summers).

However, extrapolating experimental findings to field conditions is difficult. In this experiment we did not consider the presence of genetic differences within species, variation in environmental conditions and the presence of top-down control, which are considered as important factors influencing invasion outcomes in specific lakes (Ryan et al., 2017). Nevertheless, experiments can be used to unravel mechanisms and help to understand the factors underpinning dispersal and establishment of invasive species in new potential habitats.

The effect of temperature on *R. raciborskii*'s gene expression and growth was studied in detail in **paper IV**. Via this experiment, we were able to unravel *R. raciborskii*'s gene expression profiles when transferred from optimal temperature for growth (26°C) to a less favourable one (17°C). At the start point of the experiment, when all samples were growing at 26°C, all six cultures represented a similar transcription profile, as expected. However, already after 4 hours, transcription profiles diverged in the two temperatures and the relative difference between the treatments remained similar after 12 hours as visualized by Principal Component Analysis (PCA) (see **paper IV**). The divergences were related mainly to *R. raciborskii* growth over time. However, when comparing the gene expression profiles of the 17°C treatment versus the 26°C treatment, at each time point, a higher number of genes were downregulated compared to those upregulated. Functional distribution of annotated up- and downregulated genes differ at each time point, suggesting that cells were fine-tuning their metabolism, at different growth stages, in order to respond to the colder temperature, adapt and grow in the suboptimal treatment. The results from **paper IV** are in line with the results in **paper III**, in which *R. raciborskii* was able to maintain its biomass also at 17°C by slowing down the cell metabolism, as higher number of genes are downregulated in modules related to energy, carbohydrate, amino acid and lipid metabolism. Additionally, genes related to stress response such as toxin-antitoxin systems, endonucleases, and chaperones and folding catalysts were upregulated in the 17°C treatment, which is potentially suggesting a role for these genes in responding and adapting to this suboptimal growth temperature by halting the growth and redirecting the metabolism. These results emphasize *R. raciborskii*'s adaptability in response to less favorable conditions and its capability to survive in a potentially newly invaded cold-climate freshwater.

The environmental variables tested in the experimental studies (**paper III** and **IV**) are only a small fraction of the processes, compared to natural conditions, for understanding the mechanisms controlling the dispersal potential of invasive microbes in a new habitat. Nonetheless, experiments are valuable to disentangle mechanisms that control microbial communities in aquatic systems, leading to a better understanding of the factors underpinning the establishment of invasive microbes and ultimately to better predictive capabilities (Havel et al., 2015; Ryan et al., 2017). For example, experimental information could be used for developing SDMs to predict future *R. raciborskii* blooms in areas where the species has not been observed (**Paper I**).

4.4 The necessity of monitoring potential invasive microbes

As discussed, the dispersal of invasive cyanobacteria into new freshwaters has several important consequences for native aquatic food web and ecosystem services (Havel et al., 2015) as numerous cyanobacterial strains produce cyanotoxins, inhibiting the growth of native phytoplankton species and grazers, affecting the entire food web (Paerl & Paul, 2012). This can cause a shift in native species abundance and diversity to a more homogeneous assemblage, with a predominance of more robust and more competitive invasive species, adapted to environmental shifts caused by the ongoing global changes. Therefore, preventing the establishment and spread of invasive species is a more cost-effective management strategy than eradication, containment and control that may be required when an invasive species has fully established (Trebitz et al., 2017). Eradication of an invasive species when already establish is quite challenging and, in the case of invasive microbes, likely impossible. This is mainly due to the global changes that would have to be implemented to cause an abrupt change in societal behavior, which, on a global scale, is frustratingly hard. On a local scale, some attention could be applied to avoid the spread of invasive species from one freshwater system to another by engaging local communities to be more responsible users of freshwaters. Nevertheless, it is necessary to implement a proactive monitoring program with high temporal and spatial frequency to be able to prevent and contain the expansion of *R. raciborskii* in northern European lakes.

5. Conclusion and future outlook

The overall aim of this thesis was to predict and validate the potential expansion of the invasive cyanobacterium *R. raciborskii* in areas of unknown occurrence as well as to understand the environmental drivers underpinning its potential expansion and establishment. This study concludes that:

- Although currently there is no record of *R. raciborskii* in Sweden, SDM output indicates favorable conditions in the south-eastern regions of the country. Lakes with moderate and high TP concentrations are recommended to be targeted for increased monitoring as well as implementation of management interventions for early detection of this invasive species.

- By validating the SDM output through field studies, we were able to identify constraints linked to the lack of important environmental variables for constructing the SDMs, underlining the importance of making publicly available metadata regarding *R. raciborskii*'s geographic distribution as well as other environmental factors related to its presence.

- The results from the competition experiment confirmed the importance of temperatures for the growth of *R. raciborskii* and its ability to withstand unfavorable conditions when competing with native species likely to be encountered during invasion.

- Through the transcriptomic study, we were able to better understand *R. raciborskii*'s physiological adaptation via stress response and reduced cell metabolism and its specific gene expression when transferred from an optimal to suboptimal and colder temperature.

Future work

In the presented SDMs, we used publicly available environmental predictors linked to the occurrence of *R. raciborskii* in European lakes. As mentioned, in this approach, species interactions (e.g. competition for resource, space etc.) was not taken into account. This kind of information is rarely included/available when modelling the potential expansion of an invasive species over broad geographic areas, such as Europe, where there is a lack of metadata connected to the presence of the target species, especially when the species in focus is a microorganism. However, when taking into account well-sampled lakes, in which an invasive microbe has already invaded, with publicly available and well developed metadata, the use of more complex models, i.e. Joint Species Distribution Models (JSDMs), could then be used to predict the interactions between the invasive organisms and other species as a function of the environment. This information could help to understand niche overlap between native vs invasive species and predict possible ways to prevent potential expansion to other freshwaters.

In the experimental design presented in **Paper III**, an additional level of complexity could have been met by adding a grazer from the next trophic level, disentangling mechanisms underpinning the establishment and settlement of the invasive cyanobacterium in freshwater habitat. *C. reinhardtii* was the dominant species in all treatments, but the addition of grazers may have changed the outturn. Additionally, a gene expression analysis, similar to the one performed in **paper IV**, could have helped to understand gene regulation in all the experimental taxa to have a better overview of the physiological changes occurring under the tested conditions. This kind of analysis could be used as well to evaluate which genes connected to the production of toxins were expressed and in which taxa. The type of cyanotoxins and their concentrations could also be analyzed in different treatments, to check for correlations between treatments and toxin production using High-Performance Liquid Chromatography analysis.

Additionally, as mentioned in many studies, invasive nitrogen-fixing cyanobacteria could alter the N budgets in invaded freshwaters, making it an interesting research topic to study the impact of invasive microbes on major biogeochemical cycles.

When an invasive species establish, experts should focus on how to control population growths in the invaded habitat, mitigate effects and minimize dispersal to the nearby freshwaters. In the case of an undesirable microorganism like *R. raciborskii*, experts may need to implement information campaigns before its invasion, to minimize the spread to other freshwater systems via swimwear, diving equipment, fishing gear, boats and monitoring gears. Finally, there should be a general control of cyanobacterial bloom intensity through land management use and nutrient loading as this would reduce the possibility of *R. raciborskii* to develop blooms.

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Popular science summary

Freshwater habitats have been undergoing major biodiversity loss due to alterations caused by anthropogenic activities and the introduction of non-native species. Non-native species, once introduced into a new habitat can eventually become invasive, disrupting ecosystem processes. A species of global concern is the cyanobacterium *Raphidiopsis raciborskii*, considered an invasive phytoplankton species with tropical origins that was isolated for the first time in 1912 from a lake in Indonesia. The first record in Europe dates to 1938 from a lake in Greece, and since then it has been recorded in several lakes throughout Europe. *R. raciborskii*'s occurrence in freshwaters is often associated with major habitat disruptions caused by its blooms, ranging from environmental asphyxiation due to excessive consumption of oxygen (causing the deaths of fish and benthic invertebrates, among others), to problems in leisure areas, when blooms form a colourful and often malodourous scum on the water surface. It is also known to produce toxic secondary metabolites, called cyanotoxins, causing human and animal poisoning. Although found in many European freshwaters *R. raciborskii* has not been recorded in Scandinavia. There is an urgent need to evaluate its potential range of expansion in Nordic countries and its potential adaptability to establish and survive in cold-climate freshwaters.

During my PhD project, I used a modelling approach with selected climatic variables to predict the probability of occurrence and possible expansion of *R. raciborskii* in European freshwaters. I found that *R. raciborskii* could potentially thrive in the south-eastern parts of Sweden, particularly in lakes with relatively high concentrations of nutrients. Several lakes in the south of Sweden, located in areas with both high and low risk of *R. raciborskii* occurrence, were sampled but analyses failed to detect its presence. The modelling approach used to assess which areas are suitable for *R. raciborskii* was not comprehensive as many potential predictor variables were not included. For example, when an invasive species invades a new habitat, it is faced with numerous challenges dictated by the new environment. Specifically, an invasive species must interact and compete with the native phytoplankton community. To understand these mechanisms, I performed a laboratory experiment in which *R. raciborskii* competed against an assembled phytoplankton community typically found in Scandinavian lakes. Additionally, a second level of stress was added by exposing the phytoplankton communities to different temperatures and nutrients, ranging from optimal to suboptimal. The invasive cyanobacterium was not able to outcompete the native phytoplankton community but was able to survive even in the least favorable conditions, underlining its high adaptability. These capabilities were further tested in a second experiment, in which cultures of *R. raciborskii* were grown at an optimal temperature (26°C) and then half of the cultures were moved to a less optimal one (17°C). When a species colonizes a new habitat, it undergoes changes that can be detected only on a molecular level. Examining the mechanisms behind the over- or underexpression of certain genes fundamental for the survival of a species in the newly invaded environment is one approach used for assessing adaptability. With this experiment, I showed that *R. raciborskii*, when exposed to a less optimal temperature, changed its metabolism as a response to the lower temperature, indicating its ability to quickly adapt and grow in suboptimal temperatures. These results emphasize its ability to colonize less favourable conditions, and help us to understand how an invasive species might become more successful relative to other species. As stated, *R. raciborskii* has tropical origins and therefore thrives in freshwaters with high temperatures and nutrient concentrations. Taken together the experimental and modelling results suggest that *R. raciborskii* should be able to establish viable populations in Swedish freshwaters, compete with native phytoplankton and grow during summers even with relatively low water temperatures. Although *R. raciborskii* has not been recorded in Sweden, it is prudent to consider a proactive monitoring program as well as information campaigns to reduce the risk of unintentionally transferring the species between lakes. These precautionary measures are important to minimize its establishment in northern European lakes and, in general, to prevent the expansion of other aquatic invasive species.

Populärvetenskaplig sammanfattning

Sötvatten har genomgått stora förluster av biologisk mångfald framförallt på grund av habitatförändringar i dem orsakade av antropogena aktiviteter och introduktion av främmande arter. Icke-inhemska arter, när de väl introducerats i en ny livsmiljö, kan så småningom bli invasiva och orsaka störningar i ekosystemen. En sådan främmande art är cyanobakterien *Raphidiopsis raciborskii*, som anses vara en invasiv växtplanktonart av tropiskt ursprung, isolerad för första gången från en sjö i Java i Indonesien 1912. I Europa hittades den för första gången 1938 i sjön Kastoria, Grekland och den har sedan dess hittats i sjöar i stora delar av Europa. *R. raciborskiis* förknippas med stor påverkan på ekosystemet den invaderar genom att dess stora biomassa ansamlas på vattenytan (cyanobakterieblomning), som sedan leder till syrgasbrist när den ska brytas ner. Syrgasbristen kan då orsaka dödsfall för bland annat fiskar och ryggradslösa bottendjur. För oss människor orsakar det problem för bad och friluftsliv, när blomningen bildar ett färgat och ofta illaluktande skum på vattenytan. *R. raciborskiis* är också känd för att bilda giftiga ämnen, kallade cyanotoxiner, som kan förgifta av människor och djur. Denna art finns i många europeiska sötvatten men har ännu inte hittats i Skandinavien. Det finns ett akut behov av att utvärdera artens möjlighet att sprida sig till Skandinavien samt anpassningsförmågan att etablera sig och överleva i länder med kallt klimat.

Under mitt doktorandprojekt identifierade jag möjliga etableringsområden för denna invasiva cyanobakterie i sötvatten över hela Europa genom att använda en modell som kopplar ihop redan kända förekomster av en art till utvalda klimatvariabler för dessa områden. Genom att använda denna metod fann jag att *R. raciborskii* potentiellt skulle kunna trivas i de sydöstra delarna av Sverige, särskilt i sjöar som rapporterats ha höga koncentrationer av näringsämnen. Flera sjöar i södra delen av Sverige belägna i områden med både hög och låg risk för *R. raciborskii* enligt modellen provtogs och analyserades, men jag kunde inte påvisa dess förekomst i någon av de studerade sjöarna. Detta tyder på att *R. raciborskii* kanske inte är spridits till Sverige ännu, men baserat på modellresultatet bör sjöar i de sydöstra delarna av Sverige vara i fokus för riktade övervakningsprogram. Modellen skulle kunna förbättras genom att ta hänsyn till fler viktiga faktorer. Till exempel, när en invasiv art invaderar en ny livsmiljö, måste den hantera många utmaningar som dikteras av den nya miljön. Specifikt måste en invasiv art interagera med och konkurrera med det inhemska växtplanktonsamhället för att kunna etablera sig. För att förstå dessa mekanismer gjorde jag ett laboratorieexperiment där *R. raciborskii* utsattes för ett växtplanktonsamhälle med fem andra arter som är vanliga i Skandinaviska sjöar och som *R. raciborskii* borde stöta på. Dessutom testade jag två andra parametrar som är viktiga, olika temperatur och mängder av näringsämnen, från optimala till icke-optimala. Den invasiva cyanobakterien kunde inte ta över det inhemska växtplanktonsamhället men kunde överleva som den tredje mest förekommande arten, även under de minst gynnsamma förhållandena som svalt vatten (17°C) och lite näringsämnen, vilket understryker dess höga anpassningsförmåga. I ännu mer detalj studerades effekten av nedkylning. I ett andra experiment, odlades *R. raciborskii* i flaskor en optimal temperatur (26°C) och sedan flyttades hälften av flaskorna till en svalare temperatur (17°C). När en art sprider sig till en ny livsmiljö genomgår den förändringar som bara kan upptäckas på molekylär nivå. Det innebär att man studerar vilka gener som används eller inte används vid olika förhållanden. Med detta experiment kunde jag se att *R. raciborskii*, när den exponerades för sval temperatur, ändrade sin ämnesomsättning snabbt, dvs. skillnad kunde ses mellan start, 4 och 12 timmar, vilket visar anpassningsförmågan till att kunna växa i den nya temperaturen. Dessa resultat är mycket viktiga eftersom de betonar artens höga anpassningsförmåga till mindre gynnsamma förhållanden och hjälper till att förstå hur en invasiv art kan bli så framgångsrik i förhållande till andra arter. Som tidigare nämnts är *R. raciborskii* en art av tropiskt ursprung och dess idealiska förhållanden för att frodas uppfylls i sötvatten med höga temperaturer och höga koncentrationer av näringsämnen. Att jämföra de experimentella resultaten med modelleringsresultatet tyder på att *R. raciborskii* skulle kunna bosätta sig i svenska sötvatten, konkurrera med inhemska växtplankton och växa till även under somrar med relativt svala vattentemperaturer. Även om *R. raciborskii* ännu inte hittats i Sverige är det viktigt att genomföra ett proaktivt övervakningsprogram med hög tids- och rumsfrekvens i kombination med breda informationskampanjer för att minska risken att oavsiktligt sprida arten mellan sjöar. Dessa förebyggande åtgärder är väsentliga för att kunna minimera risken att sprida *R. raciborskii* till nordeuropeiska sjöar och för att förhindra spridning även av andra invasiva vattenlevande arter generellt.

Riassunto scientifico semplificato

Gli habitat di acqua dolce stanno subendo una grave perdita di biodiversità a causa di alterazioni causate dalle attività antropiche e dall'introduzione di specie non-indigene. Le specie non-indigene (dette anche aliene, esotiche o alloctone), una volta introdotte in un nuovo habitat, potrebbero diventare invasive, provocando gravi cambiamenti agli ecosistemi autoctoni. Una specie che desta preoccupazione a livello globale è il cianobatterio *Raphidiopsis raciborskii*, specie fitoplanctonica invasiva di origine tropicale che fu isolata per la prima volta nel 1912 in un lago Indonesiano e successivamente nel 1938 in Grecia. Da allora è stato registrato in numerosi laghi in tutto il territorio europeo.

La presenza di *R. raciborskii* nelle acque dolci è solitamente associata a gravi alterazioni dell'habitat autoctono causate dalle sue fioriture (blooms), che vanno dall’asfissia ambientale, dovuta all'eccessivo consumo di ossigeno (causando la morte di pesci ed invertebrati bentonici) a problemi nelle aree di balneazione dovuti alla formazione di uno strato colorato e maleodorante sulla superficie dell'acqua. Tale cianobatterio è anche noto per la produzione di metaboliti secondari tossici, chiamati cianotossine, che possono causare avvelenamento umano e animale. Sebbene già presente in molti laghi europei, *R. raciborskii* non è stato ancora rilevato in Scandinavia.

Durante il mio progetto di dottorato, ho utilizzato un approccio di modellazione, selezionando specifiche variabili climatiche al fine di prevedere le potenziali aree di espansione di *R. raciborskii* nei bacini di acqua dolce distribuiti sul territorio europeo. Attraverso l’utilizzo di questa metodologia, è emerso che *R. raciborskii* potrebbe potenzialmente stabilirsi nelle parti sud-orientali della Svezia, in particolare nei laghi con concentrazioni relativamente elevate di nutrienti. Sono stati campionati ed analizzati diversi laghi nel sud della Svezia, ma le analisi eseguite non hanno rilevato la presenza di questa specie, suggerendo quindi che *R. raciborskii* potrebbe non essersi ancora stabilito in Svezia.

L'approccio di modellazione utilizzato per valutare quali aree potrebbero essere potenzialmente idonee affinché *R. raciborskii* possa stabilirsi risulta limitato, in quanto non è stato possibile includere nel modello altre variabili importanti. Ad esempio, quando una specie invasiva si stabilisce in un nuovo habitat, deve affrontare numerose sfide dettate dal nuovo ambiente, come l'interazione e la competizione con la comunità fitoplanctonica nativa. Per comprendere questi meccanismi, ho eseguito un esperimento di laboratorio in cui *R. raciborskii* è stato esposto ad una comunità fitoplanctonica tipica di laghi scandinavi, esponendoli a diversi livelli di temperatura e nutrienti, da ottimale a subottimale. Al termine dell'esperimento, *R. raciborskii* non è riuscito a competere con successo con la comunità fitoplanctonica autoctona, ma è riuscito a crescere e sopravvivere anche nelle condizioni meno favorevoli, sottolineando la sua elevata adattabilità. Queste sue capacità sono state testate in un secondo esperimento, in cui ho esposto inizialmente colture di *R. raciborskii* ad una temperatura ottimale (26°C) e spostando successivamente metà delle colture ad una temperatura meno ottimale (17°C). I cambiamenti che una specie subisce nel momento in cui questa colonizza un nuovo habitat sono rilevabili solamente a livello molecolare. Tali cambiamenti possono essere verificati tramite i meccanismi alla base della sovra o sottoespressione di alcuni geni fondamentali per la sopravvivenza delle specie invasiva nell'ambiente appena invaso, un approccio ultilizzato per valutare la sua adattabilità. Con questo esperimento ho dimostrato che *R. raciborskii*, se esposto ad una temperatura meno ottimale, modifica il suo metabolismo in risposta alle temperature più basse, evidenziando la sua capacità di adattarsi rapidamente a condizioni meno favorevoli. Questo comportamento dimostra come una specie invasiva potrebbe avere più successo rispetto ad altre specie.

Nonostante *R. raciborskii* sia una specie di origini tropicali che tende quindi a preferire laghi caratterizzati da temperature più miti e con alte concentrazioni di nutrienti, si potrebbe ipotizzare che, grazie a questa sua alta capacità di adattamento, sia in grado di stabilirsi nei laghi svedesi, competendo con il fitoplancton nativo e crescendo durante i periodi estivi anche con temperature dell'acqua relativamente basse. Sebbene non ci siano ancora evidenze che *R. raciborskii* sia presente in Svezia, risulta importante l'attuazione di un programma di monitoraggio in combinazione con campagne informative per ridurne il rischio di trasferimento involontario tra i bacini di acqua dolce. Queste misure precauzionali sono fondamentali per ridurre al minimo il suo potenziale insediamento nei laghi nordeuropei e, in generale, per prevenire l'espansione di altre specie acquatiche invasive.

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Species distribution models as a tool for early detection of the invasive *Raphidiopsis raciborskii* in European lakes

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ABSTRACT

In freshwater habitats, invasive species and the increase of cyanobacterial blooms have been identified as a major cause of biodiversity loss. The invasive cyanobacteria *Raphidiopsis raciborskii* a toxin-producing and bloom-forming species affecting local biodiversity and ecosystem services is currently expanding its range across Europe. We used species distribution models (SDMs) and regional bioclimatic environmental variables, such as temperature and precipitation, to identify suitable areas for the colonization and survival of *R. raciborskii*, with special focus on the geographic extent of potential habitats in Northern Europe. SDMs predictions uncovered areas of high occurrence probability of *R. raciborskii* in locations where it has not been recorded yet, e.g. some areas in Central and Northern Europe. In the southeastern part of Sweden, areas of suitable climate for *R. raciborskii* corresponded with lakes of high concentrations of total phosphorus, increasing the risk of the species to thrive. To our knowledge, this is the first attempt to predict areas at high risk of *R. raciborskii* colonization in Europe. The results from this study suggest several areas across Europe that would need monitoring programs to determine if the species is present or not, to be able to prevent its potential colonization and population growth. Regarding an undesirable microorganism like *R. raciborskii*, authorities may need to start information campaigns to avoid or minimize the spread.

1. Introduction

Climate change is a catalyst for the global expansion of harmful invasive bloom-forming algae in aquatic environments (Paerl and Huisman, 2009). Outside their native range, invasive species pose a threat to biodiversity, ecosystem functioning, economy and human health (Litchman, 2010). In recent decades, the number of species that have spread into new freshwater habitats has increased, mainly due to dispersal through natural pathways such as active or passive movement through connected waterways, wind and, migrating birds as well as human-mediated mechanisms such as commercial and recreational activities (Incagnone et al., 2015). In freshwaters, bloom-forming cyanobacteria can be problematic, resulting in impaired water quality and security (Richardson et al., 2019). The impacts of cyanobacterial blooms are extensive, ranging from environmental asphyxiation due to excessive consumption of oxygen (causing the deaths of fish and benthic invertebrates, among others) to problems in leisure areas, when blooms forms colorful and often smelly scum on the water surface (Benayache et al., 2019). Additionally, some species of cyanobacteria produce secondary metabolites (cyanotoxins) leading to neurological, hepatic and digestive diseases, which cause water quality problems for fisheries, aquaculture, farming, and sanitary threats to human and animal health (Benayache et al., 2019).

Detecting an invasive species at an early stage is crucial to ameliorate or mitigate potentially harmful effects in a cost-effective way (Morisette et al., 2020; Reaser et al., 2020). However, the early detection of aquatic invasive species, particularly microorganisms, is challenging (Bolius et al., 2019; Litchman, 2010). For example, if their biomass is low, many cyanobacteria are able to survive undetected in freshwater habitats, allowing them to continue dispersing unobserved (Sukenik et al., 2012),

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hence the term “invisible invader” (Litchman, 2010). This might create a significant risk to freshwater habitats and native species that will be exposed to the microorganism’s harmful effects (Figueredo and Giani, 2009). For example, one of the main threats of non-native cyanobacteria is the production of secondary metabolites, functioning as allelochemicals that could inhibit the growth of other phytoplankton species and grazers, affecting the whole food web (Ger et al., 2014; Paerl and Paul, 2012).

A suitable tool to forecast where a species may find suitable conditions to establish are species distribution models (SDMs), statistical procedures that link occurrence records of a species to environmental variables to estimate spatial distribution patterns using a correlative approach (Escobar et al., 2018; Leidenberger et al., 2015). In invasion biology, SDMs are increasingly used to forecast invasion risk and to improve approaches to control their expansion (Barbet-Massin et al., 2018; Bradley et al., 2010; Tingley et al., 2018). However, two central assumptions of SDMs do not hold when modeling invasive species, (i) they are not in equilibrium with their environment and, therefore, (ii) niche quantification and transferability in space and time are limited (Gallien et al., 2012). The violation of the climatic equilibrium assumption has some repercussions on the potential climatic niche of the species, which could potentially underestimate the geographical area the species can occupy (Barbet-Massin et al., 2018; Václavík and Meentemeyer, 2012). Even though SDMs cannot predict the complete potential invasion range of an invasive species that has recently established, they are still valuable for invasive species management and can be a powerful tool to predict where invasive species are more likely to disperse and establish next (Barbet-Massin et al., 2018; Václavík and Meentemeyer, 2012; Warren and Seifert, 2011). Identifying areas where a species is more likely to occur can also be used to guide sampling protocols and prioritize areas of study (Guimarães et al., 2020).

The invasive cyanobacteria *Raphidiopsis raciborskii* (former name *Cylindrospermopsis raciborskii*), a toxin-producing and bloom-forming species known to impact local biodiversity (Svirčev et al., 2016; Svirčev et al., 2014) and ecosystem services (Hawkins et al., 1985), is currently expanding its range across Europe (Wilk-Woźniak et al., 2016). As a cosmopolitan species of tropical origin, *R. raciborskii* thrives in reservoirs, lakes, and rivers worldwide (Sinha et al., 2012; Yang et al., 2018); in Europe this species has been shown to proliferate in shallow, turbid, and eutrophic/hyper-eutrophic lakes (Kokociński et al., 2009; Kokociński and Soininen, 2012). Dispersing from the tropics to northern temperate regions, *R. raciborskii* is now found on almost all continents in many climatic zones (Wilk-Woźniak et al., 2016). In the last few decades, *R. raciborskii* has migrated towards the northern regions of Europe, reaching lakes in countries such as Poland and Lithuania (Kokociński et al., 2017), with the most northern point recorded being Lake Nero, in Russia (57°09′26.0″N, 39°25′35.5″E; Babanazarova et al., 2015). Prediction of the geographic areas that meet the climatic conditions required by *R. raciborskii* can be used to estimate areas where *R. raciborskii* is present but currently undetected. This is especially important in lake rich countries like Sweden, where the phytoplankton composition is known in only a small proportion (1500 lakes in the National monitoring database, https://miljodata.slu.se/MVM/Search) of the over 100 000 lakes, to focus monitoring efforts of this species.

In this paper, we used SDMs to visualize and predict the potential distributional patterns of *R. raciborskii* across Europe to 1) identify potential habitats for *R. raciborskii*; 2) identify important climatic variables underpinning establishment; and 3) ultimately predict lakes in northern parts of Europe that are at risk of invasion. We based the SDMs on published observations of *R. raciborskii* and environmental predictors obtained from climatic models. The resulting probability of occurrence map can be used in risk assessments by authorities and to design monitoring programs and information campaigns to protect the health of both citizens, domestic animals and the aquatic environment.

2. Materials and methods

2.1. Occurrence data

Raphidiopsis raciborskii records were retrieved from published studies and reports found in Scopus, Google scholar and Google using the search terms “*Raphidiopsis raciborskii*”, “*Cylindrospermopsis raciborskii*” or “*Amoeba raciborskii*” (i.e. including former names of the species), and delimiting the search to European countries (Table S1, Supplementary materials). When an exact location (latitude and longitude) was not included, but there was a map showing the sampling sites, Google Earth was used to extract the points to match with the locations shown on the maps. When information regarding a country was not retrievable via literature due to scarcity of complete datasets or ambiguity, the Global Biodiversity Information Facility (GBIF) database (https://www.gbif.org/) or direct contact with the author of the paper were used. All records with positional uncertainty and duplicates with same coordinates were removed to increase accuracy and reduce risk of overfitting. Occurrences were grouped to obtain one observation per 2.5 arc-minute grid cell (~4.5 km^2) to match the Worldclim (Fick and Hijmans, 2017) environmental predictors’ resolution. These steps resulted in a European dataset of lakes comprising 209 unique data points, with records of *R. raciborskii* (Fig. 1) distributed across 17 countries (Austria, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Lithuania, Montenegro, the Netherlands, Poland, Portugal, Serbia, Slovenia and Spain; Table S1).

2.2. Environmental variables

High spatial resolution climatic data were used in the model: bioclimatic variables related to temperature, precipitation, average altitude, and yearly solar radiation (Table S2, from WorldClim 2.1, http://worldclim.org/version2; Fick and Hijmans, 2017). These 21 environmental variables have previously been used to predict cyanobacterial blooms as well as the invasive potential of selected phytoplankton, phytobenthic and aquatic plants species (Guimarães et al., 2020; Meichtry de Zaburlín et al., 2016; Montecino et al., 2014; Wittmann et al., 2017). Air-temperature was used as a proxy for water-temperature as they are highly correlated (Montecino et al., 2014) and temperature is known to be a strong predictor of cyanobacteria growth and metabolism (Guimarães et al., 2020). Precipitation is considered as one of the most important carriers of nutrients to freshwater habitats due to runoff (Guimarães et al., 2020). Solar radiation is the basic energy input for autotrophic organisms like cyanobacteria (Khanipour Roshan et al., 2015). Altitude, besides being related to temperature, often indicates exposure to human activities and eutrophication (De Oliveira et al., 2019; Guimarães et al., 2020; Teittinen et al., 2016). Only non-collinear predictors were used in the final model (Feng et al., 2019).

2.3. Species distribution models (SDMs)

We built ensemble SDMs prediction using the Biomod2 package for R version 3.6.1 (Thuiller et al., 2009; R Core Team, 2020). Together with *R. raciborskii* presence data and the environmental variables, we included a random set of pseudo-absences at a minimum distance of 0 km to presence records (1000 pseudo-absences) as recommended for reliable models (Barbet-Massin et al., 2012). We used four different algorithms, Generalized Linear Model (GLM), Generalized Boosted Models (GBM), Maximum Entropy (Maxent; Phillips et al., 2004) and Generalized Additive Models (GAM). As independent data were not available, 70% of the data were used for model calibration, whilst the remaining 30% of the data were used for model validation. Each model algorithm was run four times. Model performance was evaluated with the true skill statistic (TSS; Allouche et al., 2006; Ruete & Leynaud, 2015), defined as sensitivity + specificity -1. For the final ensemble model, we used a

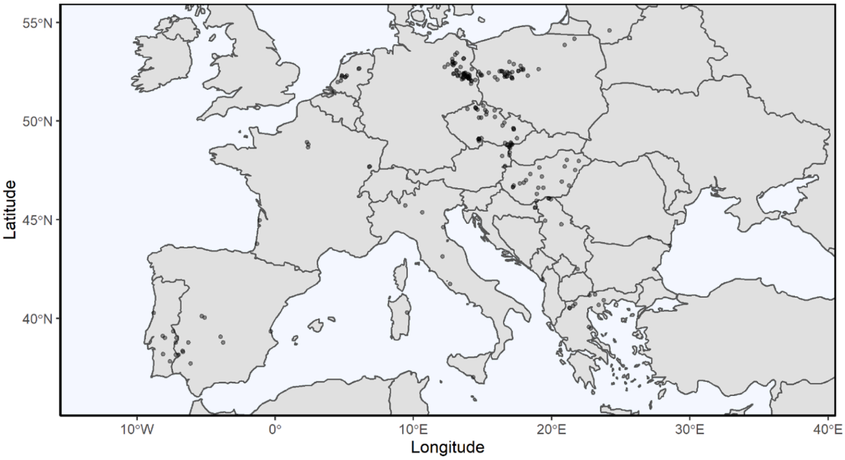

Fig. 1. *Raphidiopsis raciborskii* occurrences in European lakes based on published data in the scientific literature, GBIF or direct contact with the author of the paper.

threshold of TSS ≥ 0.6 to guarantee that only accurate models were included.

2.4. *Targeting results to eutrophic lakes*

Cyanobacteria blooms in Scandinavian lakes and elsewhere are correlated with high total phosphorus (TP) concentrations (Vuorio et al., 2020). However, published data on the occurrence of *R. raciborskii* did not include adequate information of nutrient levels to be included as an environmental predictor variable in the SDMs. We included data from a number of nutrient-rich Swedish lakes to more accurately predict areas at risk of *R. raciborskii* invasion, to compare with the probability of occurrence map. Total phosphorus data for 4800 lakes, sampled between mid-September and mid-November (once during a six-year reporting cycle) and analyzed by a certified lab (Fölster et al., 2014), were retrieved from the Swedish environmental monitoring database (https://miljodata.slu.se/MVM/Search). Three nutrient groups were used: 0 - 20 μg/L, 20 - 50 μg/L and > 50 μg/L, representing TP concentrations that characterize risk of low to high levels of cyanobacterial blooms (Vuorio et al., 2020).

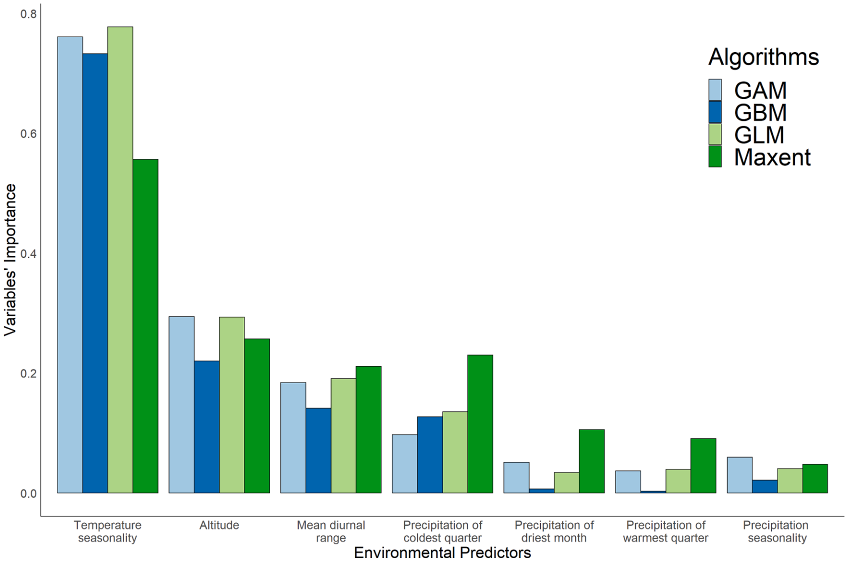

Fig. 2. Variables Importance, for each algorithm, influencing the probability of occurrence of the cyanobacterium *Raphidiopsis raciborskii* in Europe.

3. Results

In model calibration, only non-collinear predictors were retained (Variance of Inflation Factor, VIF < 4) resulting in 7 of the 21 available environmental variables included in the final model: mean diurnal temperature range; temperature seasonality; precipitation of driest month; precipitation seasonality; precipitation of the warmest quarter; precipitation of coldest quarter; and altitude.

SDMs accurately predicted approximately 85% of *R. raciborskii* presences in Europe (sensitivity) and 89% of the pseudo-absences (specificity) with an ensemble TSS value of 0.747 (Table S3). Variable importance estimates showed that temperature seasonality and altitude were the two most relevant environmental predictors in all four algorithms (Fig. 2; Table S4). Temperature seasonality between 4–8 °C resulted in a high probability of occurrence of *R. raciborskii*, whereas at higher values probabilities decreased (Fig. S1). For altitude, the probability of occurrence was lower at altitudes > 500 m a.s.l., suggesting a preference for lowland lakes (Fig. S2).

The ensemble probability of occurrence map shows suitable habitats for the occurrence of *R. raciborskii* in many areas of Europe (Fig. 3). The map also highlighted areas with zero or low occurrence probability (<0.5), e.g. in the mountainous regions of continental Europe and the Nordic countries (Norway, Finland, and northern Sweden). Areas with occurrence probabilities > 0.5 were concentrated in areas where *R. raciborskii* has been recorded, particularly in central Europe. Interestingly, the southern and central regions of Sweden showed areas with high occurrence probability (> 0.5), indicating areas where *R. raciborskii* may have already colonized or where future invasions may occur (Fig. 3). In addition, the probability of occurrence map identified several areas of high suitability in the southern and central regions of Europe, where occurrences of *R. raciborskii* have not been recorded. Thus, the results from this study suggest several areas across Europe that need further study to determine if this problematic species is present or not.

The area of suitable climate (Fig. 3) for *R. raciborskii* corresponded to areas with lakes of medium and high concentrations of TP in the southeast of Sweden (Fig. 4). Accordingly, lakes in this area with moderate to high TP concentrations are recommended to be targeted for increased monitoring for early detection and to possibly implement management interventions.

4. Discussion

4.1. Climatic conditions influencing potential occurrences of Raphidiopsis raciborskii

This study showed that low temperature seasonality was the main factor determining the high probability of occurrence of *R. raciborskii* in Europe. Based on results, we interpreted that with low temperature seasonality the number of days with warmer weather are prolonged, as there is less variation between seasons, resulting in a longer growth season for *R. raciborskii* to thrive. This conjecture is supported by a recent study of eutrophic lakes in Poland. Lenard et al. (2019) showed that concentrations of chlorophyll-a, total biomass of phytoplankton and cyanobacteria were considerably higher after milder winters. Hence, as the seasons become milder and there is less extreme or pronounced seasonal variability, the presence and duration of cyanobacterial bloom events might increase. Although *R. raciborskii* has not yet been recorded in Sweden, our modeling indicates that favorable conditions exist in the southern and central regions. Consequently, there is a high probability that the species may increase its geographical distribution into this area in the near future. Altitude was the second-best predictor determining the probability of occurrence of *R. raciborskii*. Our model results show that above 500 m a.s.l. the probability of *R. raciborskii* decreases markedly. This finding is not surprising as lowland areas generally have milder climates. Furthermore, lowland lakes are often situated in catchments that are affected by land use such as agricultural and urbanization, resulting in elevated nutrients that favor cyanobacterial blooms (Cordeiro et al., 2020). By complementing climatic variables with information on the nutrient status of Swedish lakes, we were able to identify areas of high risk for invasion success. These findings indicate that if/when dispersal occurs it is highly probable that *R. raciborskii* will become established and possibly result in problematic cyanobacterial

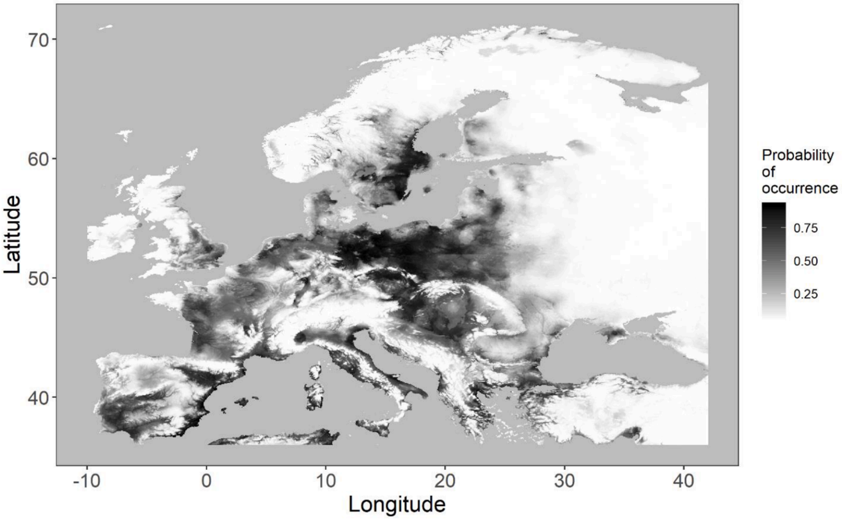

Fig. 3. *Raphidiopsis raciborskii* (Cyanobacteria) SDMs ensemble probability of occurence map for Europe based on presence data from 209 sites and climatic grid based variables. Black color show high probability of occurrence if standing water is present, while lighter shades of black show intermediate probability of occurrence, and white color shows a probability of occurrence close to zero.

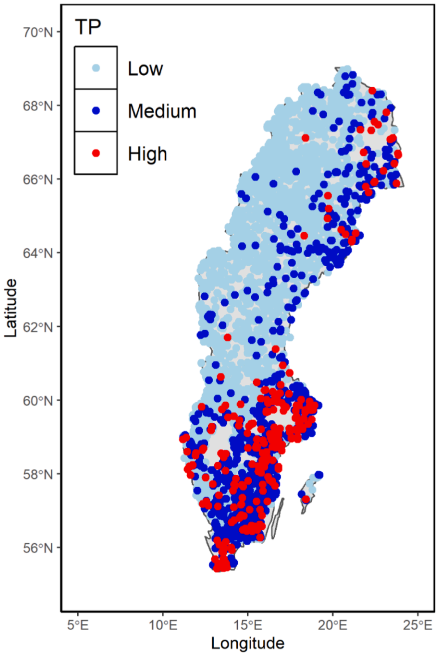

Fig. 4. TP concentrations of 4800 Swedish lakes. Light blue dots, represent lakes with low concentrations of TP (< 20 μg/l), blue dots represents lakes with moderate concentrations of TP (between 20 and 50 μg/l) and red dots represent high concentrations of TP (> 50 μg/l). Eutrophic and hypereutrophic lakes are mainly located in central/south of Sweden, where the large cities and agricultural areas are situated.

blooms. This result narrow down the number of lakes for specific monitoring efforts in search for this invasive cyanobacterium.

4.2. Non-climatic factors helping with microorganism's dispersal

Dispersal limitation is one of the main factors regulating the distribution and range expansion of a species. For an invasive microorganism like *R. raciborskii*, dispersal limitation is often overcome by the presence of a resting stage (e.g. akinetes), as these are easily transported by both physical and/or biological vectors (Padisák, 1997). Migratory water birds are considered as an important long-distance vector for the dispersal of resting stages between freshwater habitats (Bauer and Hoye, 2014; Incagnone et al., 2015). Indeed this is likely a very important vector in Europe as birds migrate between southern and northern areas for nesting and overwintering (Incagnone et al., 2015). Moreover, there is a high probability that resting stages of certain cyanobacteria are continuously being transported into new areas, but viable populations are unable to establish until favorable environmental conditions are reached (Cellamare et al., 2010; Kaštovský et al., 2010). On smaller spatial scales, humans may facilitate dispersal by using lakes for recreation. Although it is unknown if *R. raciborskii* is currently occupying new freshwater habitats predicted by our models, it is important to closely monitor its ongoing range of expansion in Europe and, in particular, in Northern Europe.

4.3. How to improve the model

Species distribution models are useful to predict habitats suitable for colonization by an invasive species outside the endemic range, in particular when knowledge of a species geographic distribution is incomplete (Guimarães et al., 2020). This information is critical to evaluate if colonization of a region is climatically feasible, to predict potential dispersal pathways, and for selecting high-risk sites for regular monitoring (Guimarães et al., 2020; Marcelino and Verbruggen, 2015). The invasive cyanobacterium *R. raciborskii* has been expanding its range relatively unobserved for the past few decades, although interest in understanding the factors underpinning its dispersal, colonization and population growths has recently increased (Falfushynska et al., 2019; Svirčev et al., 2016; Supplementary material S1). In our study, we predicted geographic areas that meet the environmental conditions of *R. raciborskii*, and accordingly we have identified areas that are suitable for successful invasions.

When a species occupies a new environment, it will have to cope with not only environmental factors but also potential biotic resistance in the ecosystem, in form of e.g. interspecific interactions, that can act as a species-sorting filter reducing the probability of occurrence (Bombi et al., 2009; Engström-Öst et al., 2015). Biotic interactions may be implemented through mechanistic models and can have large effects on species distributions. However, biotic interactions are difficult to include in the modeling processes as they are less well known; this is particularly true for invasive microorganisms. Our database comprised published scientific data, reports and occurrences recorded in GBIF. The addition of true absences and biotic interactions in the modeling approach would probably increase model accuracy of the actual distribution of the species (Pineda and Lobo, 2009).

The development of cyanobacterial blooms is usually associated with high nutrient levels and weather conditions such as high temperature and precipitation (Deng et al., 2014; Guimarães et al., 2020; Vuorio et al., 2020). Phosphorus is considered the most important nutrient for the development of cyanobacterial blooms in freshwater habitats (Guimarães et al., 2020; Vuorio et al., 2020). However, as discussed, in our study information on lake nutrient levels was not included in the SDMs, as information from the literature was incomplete. Inclusion of total phosphorus as a predictor variable would have likely increased the accuracy of our models in predicting potential blooms of *R. raciborskii* and not simply occurrences in freshwater environments. At a continental level, climate can be considered the dominant factor affecting species distributions, while at local scales factors like substrate (e.g. nutrients), biotic interactions and anthropogenic impacts typically become more important (Marcelino and Verbruggen, 2015). When information in areas with high probability of occurrence is available, local drivers affecting the distribution of *R. raciborskii* can be used to develop a mechanistic model to better understand and ultimately manage range expansions of undesirable organisms.

In addition, to further predict *R. raciborskii* blooms in new invaded areas, we need an understanding of genetic diversity and potential for local adaptation. In fact, the worldwide expansion of *R. raciborskii* could also be explained by the presence of several ecotypes with differences in their physiology, which might explain its tolerance and success in a wide range of different environmental conditions (Piccini et al., 2011). This intraspecific variation is fundamental for *R. raciborskii* success by providing an adaptable population to different environmental pressure and this plasticity, in reaction to different environmental conditions, can increase its realized niche (Baxter et al., 2020; Burford et al., 2016). An explanation for the occurrence of *R. raciborskii* in single lakes both in Lithuania and Russia may be explained by phenotypic plasticity or local adaptation to lower temperatures (Kokociński et al., 2017). However, why the species has not yet expanded to neighboring lakes remains unknown (Kokociński et al., 2017). According to our results, there is a medium-high probability of occurrence of *R. raciborskii* in Lithuanian lakes. This means that based on environmental conditions alone, *R. raciborskii* might be able to establish at those relatively high latitudes. Intraspecific variation should be taken into consideration in experimental designs for predicting and understanding potential success of invasive cyanobacteria. This information could then be used for developing SDMs to predict future *R. raciborskii* blooms in areas where the species has not been observed.

4.4. Management implications

Due to the high cost of monitoring and surveillance programs designed for early detection of invasive species in aquatic habitats, predictive models are often a cost-effective management tool (Barbet-Massin et al., 2018). The benefits of estimating and projecting invasion risk can be very important to assist decision makers and implement sampling effort in areas at high risk of invasion (Srivastava et al., 2019). If the invasive species is already established, managers need to focus more on how to control population growths in the invaded ecosystem to mitigate effects as well as to minimize dispersal and colonization to other ecosystems (Prior et al., 2018). For example, regarding an undesirable microorganism like *R. raciborskii*, authorities may need to start information campaigns to minimize the spread to other lakes via swimwear, diving equipment, fishing gear and boats. In addition, managers may try to control the severity of cyanobacteria blooms through land management and nutrient loading (Stroom and Kardinaal, 2016).

5. Conclusions

The need to model the potential occurrence of this species has been recently highlighted because of its adverse effects in aquatic habitats, human health, and economical loss. To our knowledge, this is the first attempt to predict the suitability of European lakes for the establishment of *R. raciborskii*. Our model predictions showed that the southeastern part of Sweden is an area of concern for potential invasion. Furthermore, our results suggest many suitable habitats across many parts of Europe, signaling that regionally monitoring programs should specifically focus on this invasive and harmful species. In lakes, where *R. raciborskii* is recorded, monitoring programs should include measures of nutrient status to be able to refine predictive models and be able to understand the variables underpinning dispersal and establishment of this unwanted species, as it is the first line of defense.

Declaration of Competing Interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2022.102202.

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Supplementary material

Species distribution models as a tool for early detection of the invasive *Raphidiopsis raciborskii* in European lakes

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Table S1 Raphidiopsis raciborskii occurrences were retrieved from published studies and reports found in Scopus, Google scholar and Google using the search topic = "Raphidiopsis raciborskii", "Cylindrospermopsis raciborskii" or "Amoeba raciborskii" (i.e. including former names of the species), and delimiting the search to European countries.

N	**Literature**	**Countries**	**Lakes**	**Longitude**	**Latitude**
1	Christophoridis et al., 2018; Kormas et al., 2010; Vardaka et al., 2005	Greece	Lake Doirani (Dojran)	22.750000	41.18333
2	Kormas et al., 2010	Greece	Lake Kastoria	21.300000	40.51667
3	Moustaka-Gouni et al., 2007	Greece	Lake Kastoria	21.279639	40.52944
4	Vardaka et al., 2005	Greece	Lake Zazari	21.546548	40.62145
5	Christophoridis et al., 2018; Gkelis & Zaoutsos, 2014; Vardaka et al., 2005	Greece	Lake Volvi	23.416667	40.68333
6	Christophoridis et al., 2018; Gkelis & Zaoutsos, 2014	Greece	Lake Kerkini	23.131216	41.21032
7	Christophoridis et al., 2018	Greece	Lake Petron	21.697459	40.72919
8	Gkelis et al., 2017	Greece	Lake Karla	22.828601	39.48985
9	Gkelis et al., 2017	Greece	Kalamaki reservoir	22.707481	39.55546
10	Padisák, 1997	Greece	River Struma	23.778335	40.92602
11	Messineo et al., 2009	Italy	Lake Albano	12.658997	41.75000
12	Manti & Mattei, 2005	Italy	Lake Trasimeno	12.100000	43.13333
13	Manganelli, 2016	Italy	Sartirana Lake	9.425350	45.71712
14	Barone et al., 2010	Italy	Il biviere di Gela	14.342508	37.02101
15	Manganelli, 2016	Italy	Lago di Castellaro	10.637381	45.37138
16	Ghion et al., 2005	Italy	Valle di Comacchio	12.155256	44.61232
17	Manti & Mattei, 2005	Italy	Lake Cedrino	9.537147	40.30340
18	GBIF (Dutch Foundation for Applied Water Research)	NL	De Poel, Amstelveense	4.838930	52.29861

19	GBIF (Dutch Foundation for Applied Water Research)	NL	Kleine Poel, Amstelveense	4.827072	52.29258
20	GBIF(Dutch Foundation for Applied Water Research)	NL	Sluipwyk, Plas sgravenkoop	4.764536	52.04048
21	GBIF(Dutch Foundation for Applied Water Research)	NL	Binnenschelde, Bergen op Zoom	4.262688	51.48858
22	GBIF(Dutch Foundation for Applied Water Research)	NL	Kortenhoefse Plassen, Kortenhoef	5.098393	52.24480
23	GBIF(Dutch Foundation for Applied Water Research)	NL	Naarden	5.166790	52.29415
24	GBIF(Dutch Foundation for Applied Water Research)	NL	Schutsloterwijde	6.084957	52.66766
25	GBIF(Dutch Foundation for Applied Water Research)	NL	Bergschenhoek	4.514851	51.96657
26	GBIF(Dutch Foundation for Applied Water Research)	NL	Wijde Gat, Kortenhoef	5.080234	52.23452
27	GBIF(Dutch Foundation for Applied Water Research)	NL	Canal, Uithoorn	4.812238	52.23595
28	GBIF(Dutch Foundation for Applied Water Research)	NL	Agricultural Canal, Sint Jansklooster	6.044864	52.65575
29	GBIF(Botanic Garden and Botanical Museum Berlin)	Germany	Eichenteich	13.307819	52.45254
30	GBIF(Botanic Garden and Botanical Museum Berlin)	Germany	Falkenhagener See	13.120802	52.57482
31	GBIF(Botanic Garden and Botanical Museum Berlin)	Germany	Neuer See	13.125551	52.56828
32	GBIF(Botanic Garden and Botanical Museum Berlin)	Germany	Kleiner Schwielochsee	14.188629	52.02087
33	GBIF(Botanic Garden and Botanical Museum Berlin)	Germany	Schwielochsee	14.207392	52.04831
34	Fastner et al., 2003; Mischke, 2003	Germany	Langer See	13.618423	52.40960
35	Fastner et al., 2003; Mischke, 2003	Germany	Melangsee	13.989210	52.16191
36	Wiedner et al., 2007	Germany	Melang See	13.173078	52.33346
37	Stüken et al., 2006	Germany	Bützsee	12.903286	52.82722
38	Stüken et al., 2006	Germany	Ruppiner See	12.803638	52.89572
39	Stüken et al., 2006	Germany	Molchowsee	12.829677	52.96937
40	Stüken et al., 2006	Germany	Zermützelsee	12.832645	53.01274
41	Stüken et al., 2006	Germany	Buckwitzer See	12.490665	52.87060
42	Stüken et al., 2006	Germany	Vielitzsee	13.018435	52.93666
43	Stüken et al., 2006	Germany	Werbellinsee	12.947187	52.92309
44	Stüken et al., 2006	Germany	Braminsee	12.809126	53.13593
45	Stüken et al., 2006	Germany	Großer Zernsee	12.933414	52.40224
46	Stüken et al., 2006	Germany	Rahmer See	13.410485	52.74908
47	Stüken et al., 2006	Germany	Fauler See	13.657150	53.17932
48	Stüken et al., 2006	Germany	Lieps	13.158333	53.45194
49	Stüken et al., 2006	Germany	Petersdorfer See	14.072405	52.31409

50	Stüken et al., 2006	Germany	Scharmützelsee	14.056298	52.25099
51	Stüken et al., 2006	Germany	Großer Glubigsee	14.001849	52.19408
52	Stüken et al., 2006	Germany	Springsee	13.992314	52.17650
53	Stüken et al., 2006	Germany	Großer Kossenblatter See	14.101793	52.13480
54	Stüken et al., 2006	Germany	Lebbiner See	13.939257	52.27937
55	Stüken et al., 2006	Germany	Großer Storkower See	13.969021	52.24361
56	Stüken et al., 2006	Germany	Schaplowsee	13.926285	52.23694
57	Stüken et al., 2006	Germany	Großer Schauener See	13.896874	52.23395
58	Stüken et al., 2006	Germany	Wolziger See	13.820608	52.25333
59	Stüken et al., 2006	Germany	Kutzingsee	13.833071	52.23754
60	Stüken et al., 2006	Germany	Zemmin-See	13.635310	52.16135
61	Rücker & Wiedner, 2007; Stüken et al., 2006	Germany	Pätzer Vordersee	13.652048	52.23488
62	Stüken et al., 2006	Germany	Motzener See	13.569831	52.21457
63	Stüken et al., 2006	Germany	Rangsdorfer See	13.406839	52.28985
64	Stüken et al., 2006	Germany	Croisensee	13.681105	52.36532
65	Stüken et al., 2006	Germany	Zeuthener See	13.640465	52.35384
66	Stüken et al., 2006	Germany	Großer Zeschsee	13.515172	52.10972
67	Stüken et al., 2006	Germany	Siethener See	13.203181	52.28503
68	Stüken et al., 2006	Germany	Flakensee	13.759802	52.44212
69	Stüken et al., 2006	Germany	Großer Krampe	13.662071	52.40457
70	Stüken et al., 2006	Germany	Kietzersee	14.219097	52.62206
71	Stüken et al., 2006	Germany	Oder-Spree	13.997810	52.36726
72	Stüken et al., 2006	Germany	Spree	13.739126	52.40702
73	Stüken et al., 2006	Germany	Byhleguhrer See	14.165833	51.89667
74	Stüken et al., 2006	Germany	Gülper See	12.256389	52.74139
75	Stüken et al., 2006	Germany	Wirchensee	14.481944	52.07556
76	Stüken et al., 2006	Germany	Petznicksee	13.615000	53.15556
77	Wiedner et al., 2007	Germany	Großer Plessower See	12.900000	52.38361
78	Mehnert et al., 2010	Germany	Zierker See	13.028278	53.36369
79	Briand et al., 2002 Coute et al., 1997	France	Francs Pêcheurs	2.389538	48.67097
80	Cellamare et al., 2010	France	Lacanau	-1.125278	44.97583
81	Cellamare et al., 2010	France	Soustons	-1.315278	43.77667
82	Druart & Briand, 2002	France	River Seine a Ivry	2.405044	48.81784
83	Cellamare et al., 2010	France	Malsaucy ponds	6.801551	47.68608
84	Cellamare et al., 2010	France	Étang Léchir	6.852876	47.69483
85	Cellamare et al., 2010	France	Lake ChanteraŒnes	2.312627	48.93587
86	Caetano, 2015	Portugal	Alqueva Reservoir	-7.179722	38.77460
87	Saker et al., 2003	Portugal	Ardila River	-7.195749	38.15562
88	Saker et al., 2003	Portugal	Odivelas Reservoir	-8.096866	38.19364
89	Saker et al., 2003	Portugal	Maranhão Reservoir	-7.975355	39.01507
90	Saker et al., 2003	Portugal	Caia Reservoir	-7.148519	39.01553
91	Caetano, 2015	Portugal	Montargil Reservoir	-8.135166	39.09788
92	Caetano, 2015	Portugal	Velas Lagoon	-8.791377	40.26954

93	Caetano, 2015	Portugal	Mertola Reservoir	-7.603093	37.81943
94	Caetano, 2015	Portugal	Riberiro do Murtega or Bufo reservoir	-6.985337	38.15154
95	Valério et al., 2005	Portugal	Portalegre (Albufeira da Barragem da Apartadura)	-7.381928	39.34607
96	Cirés et al., 2014	Spain	Vega del Jabalón	-3.774584	38.77450
97	Cirés et al., 2014	Spain	Vicario	-3.953582	39.06412
98	Romo & Miracle, 1994	Spain	Albufeira de Valencia	-0.323561	39.33802
99	de Hoyos et al., 2004	Spain	Embalse de Valuengo	-6.668323	38.30515
100	de Hoyos et al., 2004	Spain	Embalse de Brovales	-6.694274	38.35589
101	Wörmer et al., 2011	Spain	Embalse de Cala (Lagos del Serrano)	-6.104909	37.71152
102	Wörmer et al., 2011	Spain	Embalse de Rosarito	-5.308750	40.10302
103	Wörmer et al., 2011	Spain	Embalse de Alange	-6.265514	38.77869
104	Wörmer et al., 2011	Spain	Embalse de Navalcán	-5.104890	40.04429
105	Svirčev et al., 2016	Serbia	Aleksandrovac Lake	21.899651	42.48955
106	Predojević et al., 2015	Serbia	Zasavica River	19.527106	44.95726
107	Karadžić et al., 2013	Serbia	Ponjavica River	20.717254	44.75925
108	Ćirić et al., 2015	Serbia	Kapetanski Rit fish farm	19.940245	46.03812
109	Trbojevic, 2015	Serbia	Palic	19.757503	46.08637
110	Tokodi et al., 2018	Serbia	Ludaš Lake	19.830144	46.08652
111	Mihaljević & Stević, 2011	Croazia	Sakadaš Lake	18.801331	45.60888
112	Mihaljević & Stević, 2011	Croazia	Kopački Rit	18.790269	45.60824
113	Kokociński et al., 2017	Lithuania	Jiezno ež	24.176930	54.59381
114	Kokociński & Soininen, 2012	Poland	Kursko	15.457261	52.45256
115	Kokociński & Soininen, 2012	Poland	Biskupieckie	20.950114	53.86530
116	Kokociński & Soininen, 2012	Poland	Strykowskie	16.606186	52.25203
117	Kokociński & Soininen, 2012	Poland	Kierskie Male	16.787463	52.48651
118	Kokociński & Soininen, 2012	Poland	Żabiniec	14.725987	52.50496
119	Kokociński & Soininen, 2012	Poland	Busko	14.849819	52.38433
120	Kokociński & Soininen, 2012	Poland	Pniewskie	21.651297	54.16407
121	Kokociński & Soininen, 2012	Poland	Chodzieskie	16.933728	52.99308
122	Kokociński & Soininen, 2012	Poland	Kowalskie	17.157262	52.47722
123	Kokociński & Soininen, 2012	Poland	Berzyńskie	16.090485	52.09481
124	Kokociński & Soininen, 2012	Poland	Szydłowskie	17.934744	52.58695
125	Kokociński & Soininen, 2012	Poland	Buszewskie	16.379199	52.54448
126	Kokociński & Soininen, 2012	Poland	Niepruszewskie	16.615510	52.37893
127	Kokociński & Soininen, 2012	Poland	Tomickie	16.637794	52.31786
128	Kokociński & Soininen, 2012	Poland	Jelonek	17.587990	52.53488
129	Kokociński & Soininen, 2012	Poland	Mogileńskie	17.951995	52.65087
130	Kokociński & Soininen, 2012	Poland	Witobelskie	16.727116	52.26525

131	Kokociński & Soininen, 2012	Poland	Rzepinko	14.889835	52.32066
132	Kokociński et al., 2009; Kokociński & Soininen, 2012	Poland	Bnińskie	17.109364	52.21094
133	Kokociński et al., 2009; Kokociński & Soininen, 2012	Poland	Bytyńskie	16.509294	52.50404
134	Kokociński et al., 2009	Poland	Lubosińskie	16.387426	52.52839
135	Kokociński et al., 2013	Poland	Pniewskie	16.240833	52.51139
136	Kokociński et al., 2013	Poland	Grylewskie	17.251111	52.88528
137	Kokociński et al., 2013	Poland	Świętokrzyskie	17.595278	52.54417
138	Kokociński et al., 2013	Poland	Boczowskie	14.946389	52.31944
139	Kokociński et al., 2013	Poland	Bnińskie	17.116389	52.20056
140	Kokociński et al., 2013	Poland	Biskupińskie	17.748889	52.79472
141	Kokociński et al., 2013	Poland	Zbąszyńskie	15.903611	52.23472
142	Kokociński et al., 2013	Poland	Kruchowskie	17.806111	52.60361
143	Budzyńska & Gołdyn, 2017	Poland	Lake Rusałka	16.877998	52.42675
144	Wilk-Woźniak & Najberek, 2013	Poland	Pątnowskie Lake	18.289356	52.30302
145	Kobos et al., 2013	Poland	Jezioro Góreckie	16.782846	52.26760
146	Kobos et al., 2013	Poland	Jezioro Łękno	17.145230	52.15592
147	Kobos et al., 2013	Poland	Lake Malta	16.974722	52.40232
148	Kobos et al., 2013	Poland	Jezioro Borusa	19.060833	52.63444
149	Bláhová et al., 2009	C. Republic	Dubice reservoir	14.510613	50.67134
150	Bláhová et al., 2009	C. Republic	Svět	14.746063	48.99117
151	Bláhová et al., 2009	C. Republic	Máchovo jezero	14.649331	50.58095
152	Bláhová et al., 2009	C. Republic	Hamrys (Slavonin Lake)	17.249644	49.56784
153	Bláhová et al., 2009	C. Republic	Oborský rybnik	15.306563	50.47280
154	Dvorak & Hasler, 2007	C. Republic	Morava river (close to Chomoutov, Olomouc)	17.236694	49.64193
155	Dvorak & Hasler, 2007	C. Republic	Zámecký	15.274840	50.33230
156	Jan Kaštovský personal communication	C. Republic	Nové Mlýny	16.642511	48.89648
157	Jan Kaštovský personal communication	C. Republic	Babice u Uherského Hradiště	17.491181	49.12136
158	Jan Kaštovský personal communication	C. Republic	Meadow poll south of Lány	16.929601	48.68655
159	Jan Kaštovský personal communication	C. Republic	Zámecký rybník	16.812380	48.80986
160	Jan Kaštovský personal communication	C. Republic	Dubice u České Lípy	14.497851	50.68172
161	Jan Kaštovský personal communication	C. Republic	Malhostický rybník u Rtyně nad Bílinou	13.922964	50.60883
162	Jan Kaštovský personal communication	C. Republic	Novozámecký rybník	14.540326	50.62844
163	Jan Kaštovský personal communication	C. Republic	Dvorský rybník	17.072348	48.85495
164	Jan Kaštovský personal communication	C. Republic	Morava, Olomouc (Mlýnský potok)	17.259235	49.59929

165	Jan Kaštovský personal communication	C. Republic	Mlýnský rybník	16.822180	48.78674
166	Jan Kaštovský personal communication	C. Republic	Rybník Láska	14.749138	49.11066
167	Jan Kaštovský personal communication	C. Republic	Dobrá vůle	14.755794	49.10567
168	Jan Kaštovský personal communication	C. Republic	Koclířov	14.694212	49.06724
169	Jan Kaštovský personal communication	C. Republic	Rožmberk	14.763303	49.04798
170	Jan Kaštovský personal communication	C. Republic	Srálkovský rybník	17.055791	48.90471
171	Jan Kaštovský personal communication	C. Republic	Očko (Káraný)	14.745087	50.16861
172	Jan Kaštovský personal communication	C. Republic	VN Harcov	15.075928	50.77089
173	Jan Kaštovský personal communication	C. Republic	Jezero Ostrá	14.899289	50.17631
174	Jan Kaštovský personal communication	C. Republic	Koupaliště Sedmihorky	15.191440	50.55842
175	Jan Kaštovský personal communication	C. Republic	Březina	14.928208	49.13551
176	Jan Kaštovský personal communication	C. Republic	Rybník Rosnička	16.451388	49.77142
177	Jan Kaštovský personal communication	C. Republic	Stříbrný rybník	15.891985	50.20464
178	Jan Kaštovský personal communication	C. Republic	Koupaliště Trutnov - Dolce park	15.869746	50.54520
179	Jan Kaštovský personal communication	C. Republic	Rybník Dlouhý u Lanškrouna	16.589646	49.91934
180	Horecká & Komárek, 1979	Slovakia	Kopčany	17.088889	48.77739
181	Marsâlek et al., 2000	Slovakia	Janíčkov dvor, gravel pit lake	17.000018	48.63695
182	Marsâlek et al., 2000	Slovakia	Gajary, village lake	16.962046	48.41075
183	Hindáková & Hindák, 2012	Slovakia	Devínske	16.950639	48.27511
184	Hindák & Hindáková, 2013	Slovakia	Castle-moat water in Holíč	17.157680	48.80863
185	Rakočević, 2018	Montenegro	Šasko Lake	19.345018	41.97424
186	Stoyneva, 2003	Bulgaria	Srebarna Lake	27.071367	44.10651
187	Dimitrova et al., 2014; Stoyneva, 2016	Bulgaria	Lake Burgas (Vaya)	27.406792	42.50127
188	Stoyneva, 2016	Bulgaria	Shabla (Durankulashko ezero Durankulak)	28.558399	43.68902
189	Dokulil, 2016; Dokulil & Mayer, 1996)	Austria	Alte Donau	16.427802	48.23584
190	Vehovszky et al., 2013	Hungary	Fancsika I. víztároló	21.715580	47.51245
191	Présing et al., 2008	Hungary	Lake Balaton (Easter Basin)	18.135692	47.00071

192	Présing et al., 2008	Hungary	Lake Balaton (Wester Basin)	17.254668	46.73487
193	Padisák, 1997	Hungary	Lake Balaton	17.706351	46.83564
194	Padisák, 1997	Hungary	Danube	18.923895	46.31085
195	Padisák, 1997	Hungary	Dunakeszi / Dead arm of the Danube	19.115911	47.64078
196	Padisák, 1997	Hungary	Levelek/fish pond	21.962722	47.97309
197	Padisák, 1997	Hungary	Zala river	17.210632	46.65992
198	Horecká & Komárek, 1979	Hungary	Dabas stream	19.305074	47.18503
199	Padisák, 1997	Hungary	Lake Szelid	19.041548	46.62385
200	Padisák, 1997	Hungary	Tisza river	20.934827	47.69820
201	Padisák, 1997	Hungary	Tomalom	16.624734	47.71838
202	Padisák, 1997	Hungary	Vadkerti-to	19.392778	46.61335
203	Padisák, 1997	Hungary	Zámolyi-víztározó	18.452384	47.31998
204	Vasas et al., 2010	Hungary	Kis-Balaton	17.197961	46.64627
205	Vasas et al., 2010	Hungary	Zamardi	17.982684	46.86926
206	Vasas et al., 2010	Hungary	Doboz, fish pond	21.270795	46.72616
207	Vasas et al., 2010	Hungary	Endrőd- Középső- Holt-Körös	20.732924	46.92184
208	Kidolgozása, 2004	Hungary	Tiszadobi Holt-Tisza	21.112044	48.02831
209	Kidolgozása, 2004	Hungary	Klágya-Duna	18.894654	45.96422

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Table S2 Environmental predictors used to build SDMs for R. raciborskii (Fick & Hijmans, 2017; https://www.worldclim.org/data/worldclim21.html). Variables in bold were used in the final models.

Environmental predictors	**Description**
Bio1	Annual mean temperature
Bio2	**Mean diurnal range (mean of monthly max temp-min temp)**
Bio3	Isothermality (Bio2/bio7)*100
Bio4	**Temperature Seasonality (standard deviation*100)**
Bio5	Max Temperature of Warmest Month
Bio6	Min Temperature of Coldest Month
Bio7	Temperature Annual Range (BIO5-BIO6)
Bio8	Mean Temperature of Wettest Quarter
Bio9	Mean Temperature of Driest Quarter
Bio10	Mean Temperature of Warmest Quarter
Bio11	Mean Temperature of Coldest Quarter
Bio12	Annual Precipitation
Bio13	Precipitation of Wettest Month
Bio14	**Precipitation of Driest Month**
Bio15	**Precipitation Seasonality (Coefficient of Variation)**
Bio16	Precipitation of Wettest Quarter
Bio17	Precipitation of Driest Quarter
Bio18	**Precipitation of Warmest Quarter**
Bio19	**Precipitation of Coldest Quarter**

Elevation	**Derived from the SRTM elevation data.**
Solar radiation	Monthly solar radiation

Table S3 Results on the predictive ability of different models fitted with R. raciborskii datasets. TSS is the true skill statistic. Sensitivity is the proportion of presences correctly predicted. Specificity is the proportion of absences correctly predicted. Cut-off binary shows the value of suitability (0–1) that maximized the sum of sensitivity and specificity.

	TSS	Sensitivity (%)	Specificity (%)	Cut-off
R. r iborskii	0.747	84.8	89.8	0.596

Table S4 Variables importance, for each algorithm, influencing the probability of presence of the cyanobacterium Raphidiopsis raciborskii in Europe.

	GLM	GAM	Maxent	GBM
Temperature seasonality	0.777333	0.760917	0.556333	0.732417
Altitude	0.293417	0.294583	0.256833	0.220083
Mean diurnal range	0.190917	0.18425	0.21125	0.141417
Precipitation of coldest quarter	0.135667	0.097333	0.230083	0.127333
Precipitation of warmest quarter	0.034	0.05125	0.105917	0.006583
Precipitation of driest month	0.040583	0.059833	0.047917	0.021417
Precipitation seasonality	0.039167	0.037083	0.091	0.002917

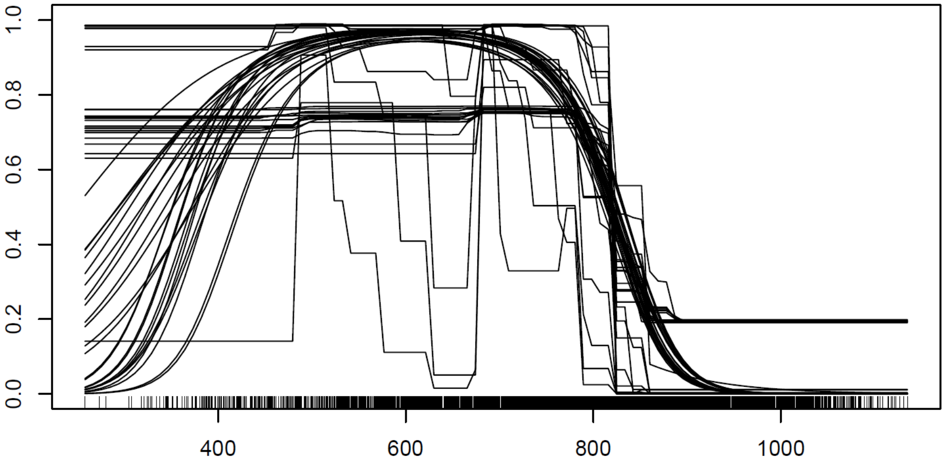

Figure S1 Response of occurrence of R. raciborskii for variable seasonal temperature range including results from all algorithms. X-axis showing standard deviation in temperature (oC) x 100, i.e. 400 corresponds to 4 oC deviation. The probability (y-axis) of finding R. raciborskii is according to the model very low when the seasonal range in temperature is slightly over 8 oC.

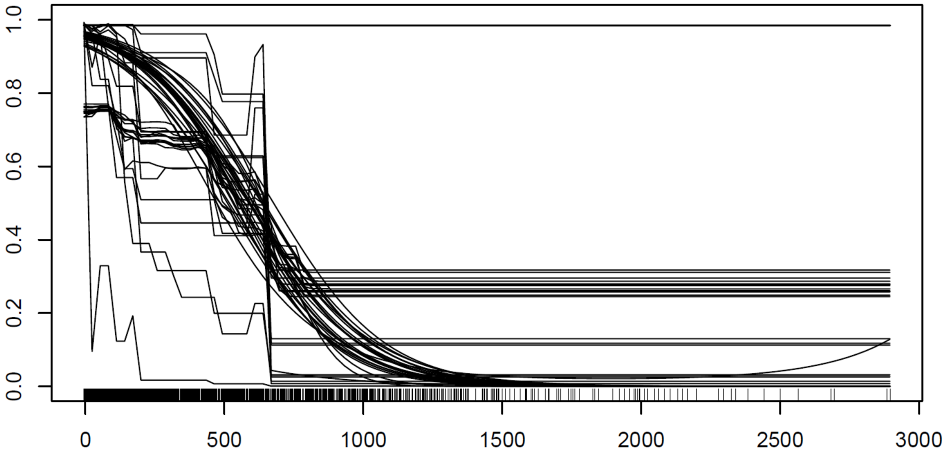

Figure S2 Response plot elevation all algorithms. Response of occurrence of R. raciborskii for altitude including results from all algorithms. X-axis showing the elevation gradient (m). The probability (y-axis) of finding R. raciborskii decreases after 500 m of altitude.

S.1. Raphidiopsis raciborskii toxicity increasing need of risk assessment

In Serbia, there was a mass fish mortality in Alexander Lake correlated with a mass bloom of *R. raciborskii*, linked with the production of secondary toxic metabolites different from saxitoxins SXT and cylindrospermopsin CYN (Svirčev et al., 2016). In Hungary, an anatoxin-*a* like neurotoxic toxin linked with *R. raciborskii* blooms was associated with mass fish mortality (Vehovszky et al., 2015; Svirčev et al., 2016). Additionally, *R. raciborskii* extracted from Polish strains produced alkaline phosphatase, similar to that observed for cylindrospermopsin and it may be involved in allelopathic relations (Rzymski et al., 2014). Recent studies are exploring the unknown toxicity of European strains and the potential effect on freshwater ecosystems. (Falfushynska et al., 2019) study assessed the in vitro toxicity of *R. raciborskii* strains isolated from Poland and Ukraine in common carp (*Cyprinus carpio*), being the first to testimony that some *R. raciborskii* strains can produce second metabolites that can potentially affect freshwater fish. A study from (Wejnerowski et al., 2020) revealed that there was no clear or common pattern regarding the degree of cyanobacterial bloom toxicity with different cyanobacteria species (*R. raciborskii* included), at a given stage of development. This means that young cyanobacteria bloom can be as equally toxic to aquatic vertebrates and ecosystem functioning as the stable or old ones that have tendency to collapse. These outcomes are significant for ecological risk assessment if *R. raciborskii* is expected and considered to increase its range of expansion throughout Europe (Rzymski et al., 2014) and an early monitoring should be implemented in the areas where the species has potential for expansions.

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Freshwater habitats are experiencing unprecedented species loss where the greatest pressures are related to land use alterations and the establishment of non-native species as they become invasive. The tropical invasive freshwater cyanobacterium *Raphidiopsis raciborskii* is currently expanding its geographic distribution towards the northern regions of Europe. Within this thesis work, theoretical models, field surveys and controlled laboratory experiments were used to identify probable habitats beyond *R. raciborskii's* current distribution and to understand the environmental drivers underpinning its potential expansion and establishment in Scandinavian lakes.

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