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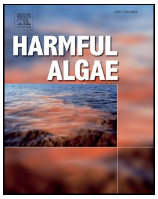

Causal relationships of *Raphidiopsis* (formerly *Cylindrospermopsis*) dynamics with water temperature and N:P-ratios: A meta-analysis across lakes with different climates based on inferential modelling

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ABSTRACT

Raphidiopsis raciborskii is a tropical toxic cyanobacterium that is rapidly expanding to diverse lake habitats in different climate zones by sophisticated adaptation mechanisms.

This meta-analysis investigated correlations of *R. raciborskii* with water temperature and N:P (nitrogen to phosphorus)-ratios across four lakes with different climates and trophic states by means of long-term time series and the hybrid evolutionary algorithm HEA. The results have shown that in the lakes with temperate and Mediterranean climate, *R. raciborskii* is strongly correlated with water temperature since germination and growth rely on rising water temperatures in spring. In contrast, there was a weaker correlation with water temperature in subtropical and tropical lakes where pelagic populations of *R. raciborskii* are overwintering, and are present all year round. However, the highest abundances of *R. raciborskii* coincided with highest water temperature for the Mediterranean, subtropical and tropical lakes, whilst in the temperate Langer See the highest abundances of *R. raciborskii* occurred at 24.1 °C, even though temperatures of up to 27 °C were recorded in 2013 and 2014. The correlation of *R. raciborskii* with N:P-ratios proved to be strongest for the meso- to eutrophic Lake Kinneret ($r^2 = 0.8$) and lowest for the eutrophic Lake Paranoa ($r^2 = 0.16$). However, the assumption has been confirmed that *R. raciborskii* is growing fastest when waters are N-limited regardless of trophic states. In terms of phenology, the temperate and Mediterranean lakes displayed "fastest growth" in spring and early summer. In contrast, the growing season in subtropical and tropical lakes lasted from spring to autumn most likely because of overwintering populations, and growing importance of direct and indirect biotic regulating factors such as competition, grazing, remineralisation of nutrients along warming climate. In order to carry out a meta-analysis of time series across four different lakes, HEA served as powerful tool resulting in inferential models with predictive capacity for population dynamics of *R. raciborskii* just driven by water temperature or N:P-ratios, whilst coefficients of determination r^2 served as criteria for hypotheses testing.

1. Introduction

The expanding global distribution of *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno (Aguilera et al., 2018) is attributed to a suite of sophisticated adaptation mechanisms characteristic of this bloom-forming nostocalean cyanobacterium. Akinetes that germinate only when water temperatures exceed 15 °C enable *R. raciborskii* to survive the cold months in temperate waters (Padisak, 1997; Rücker et al., 2009). In terms of nitrogen consumption, *R. raciborskii* shows a preference for dissolved inorganic (ammonium, nitrate) and organic (urea) forms of nitrogen (e.g. Saker and Neilan, 2001; Ammar et al., 2014; Burford et al., 2018), whilst heterocyte cells allow *R. raciborskii* to grow in N-limited waters by performing N_2-fixation (e.g. Plominsky et al., 2013).

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Table 1
Comparison of limnological properties of Langer See, Lake Kinneret, Lake Wivenhoe and Lake Paranoa.

	Langer See	Lake Kinneret	Lake Wivenhoe	Lake Paranoa
Circulation Type	Shallow Polymictic	Warm Monomictic	Warm Monomictic	Oligomictic
Trophic State	Hypertrophic	Meso- to Eutrophic	Meso- to Eutrophic	Eutrophic
WRT years	0.12	10	20	0.9
Depth m Mean/Max	2.1 / 3.5	25.6 / 41.7	11 / 44	15 / 40
WT °C Mean/Min/Max	13.5/0.2/27.3	22.7/14/31	22.7/15/30	23.8/19.5/28.5
DIN µg L^{-1} Mean/Min/Max	189.5/7.4/1291.7	146.6/11.8/523.5		546.5/10/2236.7
TN µg L^{-1} Mean/Min/Max	1123/346/2488	498/213/836	490.6/200/860	
DIP µg L^{-1} Mean/Min/Max	13/1.2/121.7	2.2/1/6.4		4.5/2/23
TP µg L^{-1} Mean/Min/Max	74.5/29/196	17.8/5.6/34.4	21.2/7/140	33.7/16/128
***R. raciborskii* cells mL^{-1} Mean/Min/Max**	4668.3/0/118321.1	19032.7/0/1387273	9557.4/3/173166	417280/58/5758421
Details of Data Sources	Mischke (2003)	Alster et al. (2010)	Orr et al. (2010)	Branco and Senna (1994)

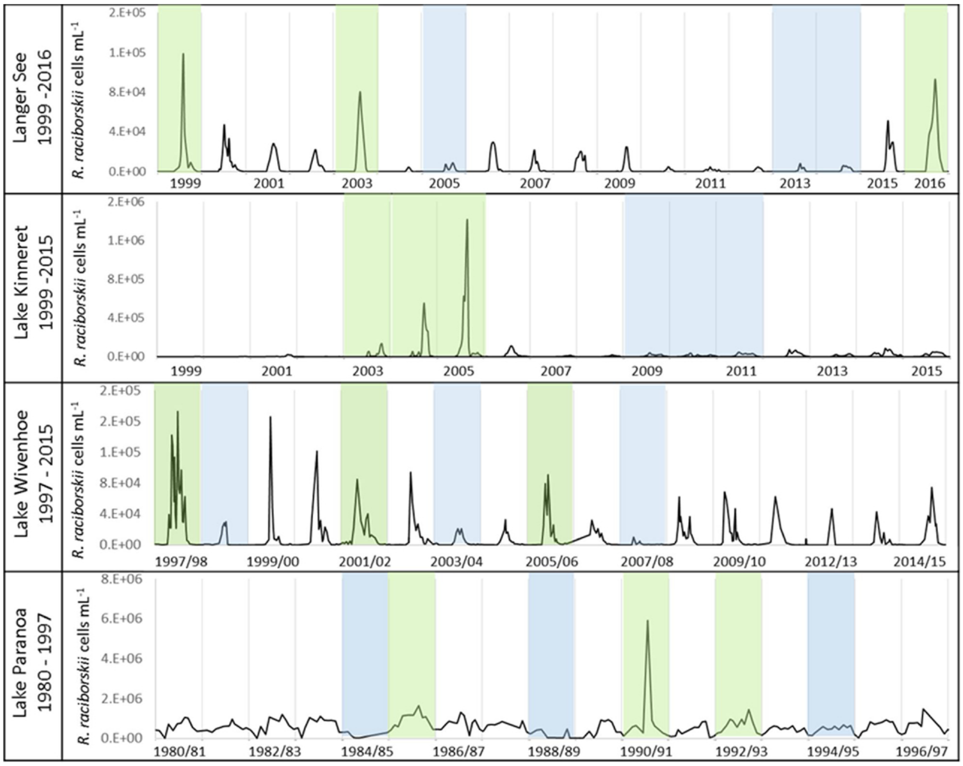

Fig. 1. Inter-annual time series of *R. raciborskii* cell concentrations in Lakes Langer See, Kinneret, Wivenhoe and Paranoá. Light green refers to years selected for 'high' abundances, and light blue refers to years selected for 'low' abundances (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

High uptake affinity (Wu et al., 2009) and storage capacity for phosphorus (Istvánovics et al., 2000) make *R. raciborskii* competitive in lakes with seasonally pulsing P-concentrations (Amaral et al., 2014). Under P-limiting conditions it increases its alkaline phosphatase (APA) activity (Wu et al., 2011) and utilises organic phosphorus (Bai et al., 2014). In addition, buoyancy by gas vesicles allows *R. raciborskii* to access horizons with optimum light and nutrient conditions within the water column (Fabbro and Duivenvoorden, 1996; Istvánovics et al., 2000; Pierangelini et al., 2015). It is assumed that increasing water temperatures enhance growth of *R. raciborskii,* as evidenced by the increasing frequency of bloom events worldwide with global climate change (e.g. Briand et al., 2004; Wiedner et al., 2007; Paerl et al., 2011; Sinha et al., 2012; Sukenik et al., 2012). However, with regards to combined effects of global climate change on *R. raciborskii*, manifold adjustments within the plankton community reflected by altered competition and grazing have also to be taken into account (Moustaka-Gouni et al., 2006; Gaedke et al., 2010; Hong et al., 2014).

Since synergetics between water temperature, nutrient conditions, local and climate–specific habitat conditions determine strongly the appearance of *R. raciborskii,* this meta-analysis focuses on the strength of relationships of *R. raciborskii* with water temperature (WT), nitrogen to phosphorus (N:P)-ratios as universal stoichiometric reference for nutrient limitation of planktonic production (Redfield, 1958), and dissolved inorganic phosphorus (DIP) concentration in four lakes

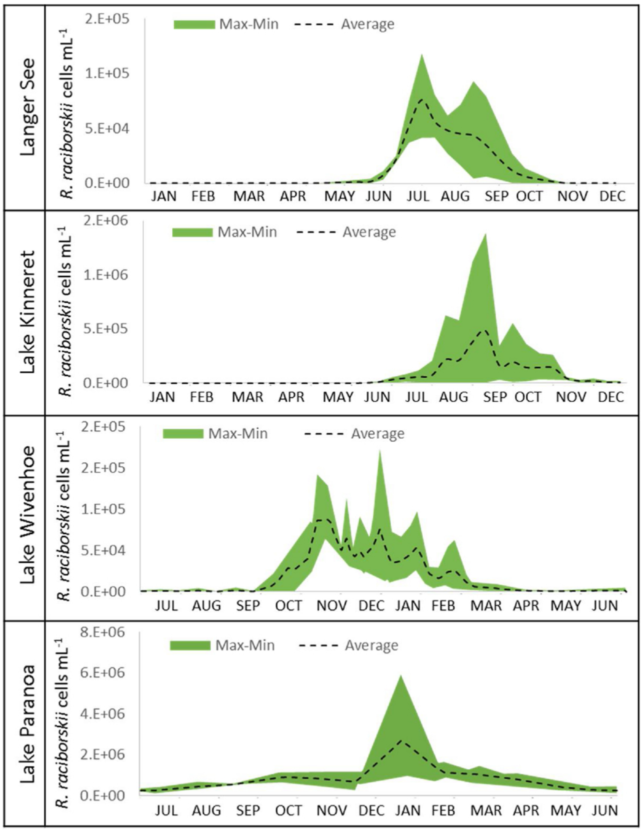

Fig. 2. Averaged annual cell concentrations and Max-Min envelopes of *R. raciborskii* of three selected 'high' abundance years from Lakes Langer See (1999, 2003, 2016), Kinneret (2003, 2004, 2005), Wivenhoe (1997/98, 2001/02, 2005/06) and Paranoá (1985/86, 1990/91, 1992,93) as highlighted by light green in Fig. 1 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

characterized by different climate and trophic states. As suggested by Osenberg et al. (1999), meta-analysis becomes more conclusive for ecological applications if time-series data and quantitative models can be included. Hence, the hybrid evolutionary algorithm HEA (Cao et al., 2014) has been applied to model time series of three years with "high" and three years with "low" abundances of *R. raciborskii* that have been selected from the temperate Langer See (Germany), the Mediterranean Lake Kinneret (Israel), the sub-tropical Lake Wivenhoe (Australia) and the tropical Lake Paranoá (Brazil). Results reveal coefficients of determination r^2 by inferential models of *R. raciborskii* solely driven by WT and N:P-ratios, and identify lake specific thresholds of WT and N:P-ratios that indicate exceeding abundances of *R. raciborskii*. Based on the acquired information we tested the following hypotheses:

(1) There is a similar high correlation between *R. raciborskii* and WT across lakes with different climates, and *R. raciborskii* reaches highest abundances during episodes of warmest WT (e.g. Briand et al., 2004).
(2) There is a similar high correlation between *R. raciborskii* and N:P-ratios across lakes with different trophic states, and *R. raciborskii* is growing fastest during episodes of N-limitation by performing N_2-fixation (e.g. Plominsky et al., 2013).
(3) *R. raciborskii* maintains a distinct phenology across lakes regardless of differences in climate and trophic state.

2. Materials and methods

2.1. Data

Langer See is shallow-polymictic with a maximum depth of 3.8 m, and is one of the northernmost lakes in Europe where populations of *R. raciborskii* have been detected. The other three lakes are warm-monomictic with maximum depths between 40 and 44 m. *R. raciborskii* was first recorded in Langer See in 1993 (Mischke, 2003) where their occurrence was attributed to an earlier rise in WT associated with climate change that enables the akinetes to germinate earlier (Wiedner et al., 2007; Rücker et al., 2009). No explanation has been found yet for the distinct inter-annual variability in *R. raciborskii* population size that is typical for Langer See and other lakes as well. *R. raciborskii* was first recorded in Lake Kinneret in 1998, and reached a maximum biomass (65 g m^{-2}) in August 2005, when this species accounted for 82% of the total phytoplankton biomass (Zohary and Shlichter, 2009; Alster et al., 2010). Lake Wivenhoe has experienced recurrent blooms of *R. raciborskii* for more than two decades, and it is the dominant toxic cyanobacterial species in that lake. Lake Paranoá was created adjacent to the Barazillian capital of Brasilia in the early 1960′s. *R. raciborskii* was first recorded there during the 1980′s (Branco and Senna, 1994), and it now dominates the phytoplankton community.

Table 1 displays the broad range of limnological properties recorded by 16 to 17 years of monitoring, and shows that Lake Paranoá is the most eutrophic lake with average DIP-concentrations up to 7-times higher than Lake Kinneret which is the least eutrophic. Cell counts of *R. raciborskii* were made from weekly, bi-weekly or monthly phytoplankton samples, collected and depth-integrated from central lake stations, and preserved with Lugol's solution according to the standard methods (Utermöhl, 1958). For more details on data sources of the four lakes please see references in Table 1.

The inter-annual trajectories of *R. raciborskii* abundance of the 4 lakes represented in Fig. 1 reveal that Langer See and Lake Wivenhoe experienced numerous years with high abundances and several years with low to very low abundances of *R. raciborskii.* However, the history of *R. raciborskii* in the Lakes Kinneret and Paranoá has been dominated by major bloom events in 2005 and 1991/92, respectively.

In order to have a baseline for the meta-analysis, we selected from each lake three years with high abundances (highlighted in light green in Fig. 1) and three years with low abundances (highlighted in light blue). The averaged time-series with Max-Min-envelopes of the three selected 'high' abundance years for each of the four lakes are shown in Fig. 2.

Fig. 2 shows that *R. raciborskii* in the two tropical/sub-tropical lakes (Wivenhoe and Paranoá) is present throughout the year with cell concentrations increasing from early spring of the southern hemisphere (September and October) and resulting summer peaks in November followed by oscillating high abundances towards autumn with the major peak in early January in Lake Wivenhoe. Lake Paranoá maintains steady abundances between October and April which peak in January. *R. raciborskii* in Langer See and Lake Kinneret is almost absent during winter of the northern hemisphere. Cell concentrations increase in early summer of the northern hemisphere between May and June in both lakes, and peak in a mid-summer bloom in July followed by a late-summer peak in early September in Langer See while Lake Kinneret tends to have its peak in September. Cell concentrations decrease in both lakes through to June the following year.

2.2. Inferential modelling by HEA

The hybrid evolutionary algorithm HEA (Cao et al., 2014) has been

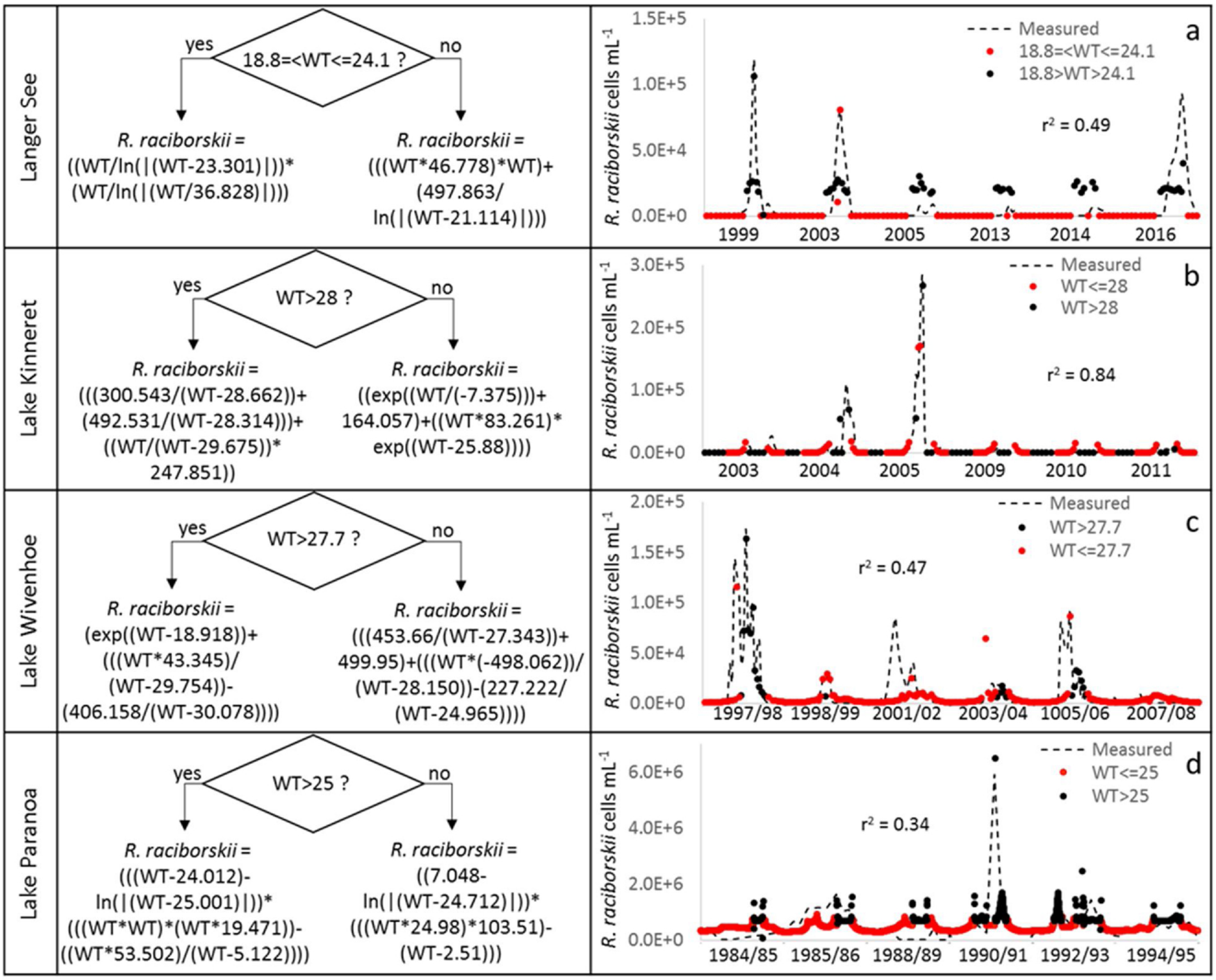

Fig. 3. IF-THEN-ELSE-rules and validation results of inferential models of *R. raciborskii* for Lakes Langer See, Kinneret, Wivenhoe and Paranoá predicted solely from water temperatures. For more detailed validation of the models and water temperature thresholds of the four lakes see Figs. A1 and A2.

designed to evolve predictive models represented by IF-THEN-ELSE-rules from complex time-series data. It applies genetic programming (Koza, 1992) to evolve the optimum structure, and differential evolution (Storn and Price, 1997) to optimise the parameters of the "fittest" models. IF-conditions of the rules display population, community or habitat thresholds discovered by HEA. HEA has been successfully applied for threshold analysis of plankton communities of Lake Kinneret (Israel), Lake Taihu (China), Lake Müggelsee (Germany), Lakes Lajes and Descoberto (Brazil) and River Nakdong (Recknagel et al., 2016, 2014; Recknagel et al., 2013, 2017; Rocha et al., 2019) and of macroinvertebrate communities in South Australian catchments (Sultana et al., 2019) as well as for early warning of cyanobacteria blooms in Lake Wivenhoe (Australia) and Vaal Reservoir (South Africa) (Recknagel et al., 2017a, 2017b). In this study the C^{++} version of HEA has been used running by cloud computing in a cyclic boot-strap scheme that creates 80 generations of models by randomly selecting 75% of all data points for training and 25% of all data points for testing. After 100 boot-strap runs it determines the overall "fittest model" of all generations by cross-validation of six years of data sets from each lake. The models with the lowest root-mean-square error (RMSE) and the highest coefficient of determination r^2 will be selected for evaluation.

2.3. Meta-analysis

Meta-analysis as powerful tool for quantitative synthesis, analysis and summary of a collection of studies (Hedges and Olkin 1985) will be more relevant and conclusive for ecological applications by a more empirical approach utilizing time-series and dynamic models for testing hypotheses across same type ecosystems (Osenberg et al., 1999). In this study, we applied meta-analysis firstly, by developing separate models for relationships of WT and N:P with *R. raciborskii* over six years across the four lakes with different climate and trophic state, secondly, by identifying the coefficients of determination r^2 for the WT- and N:P-driven models evolved by HEA with lowest RMSE and p-values $< =$ 0.05 as indicators of the strength of correlations, and thirdly, by testing the hypotheses (1) and (2) by analysing trends in the r^2-values of models across the lakes. Since meta-analysis requires a standardised approach, we selected from each lake three years with 'high' abundances (highlighted in light green in Fig. 1) and three years with 'low' abundances (highlighted in light blue) as baseline. To compare 3 "high"

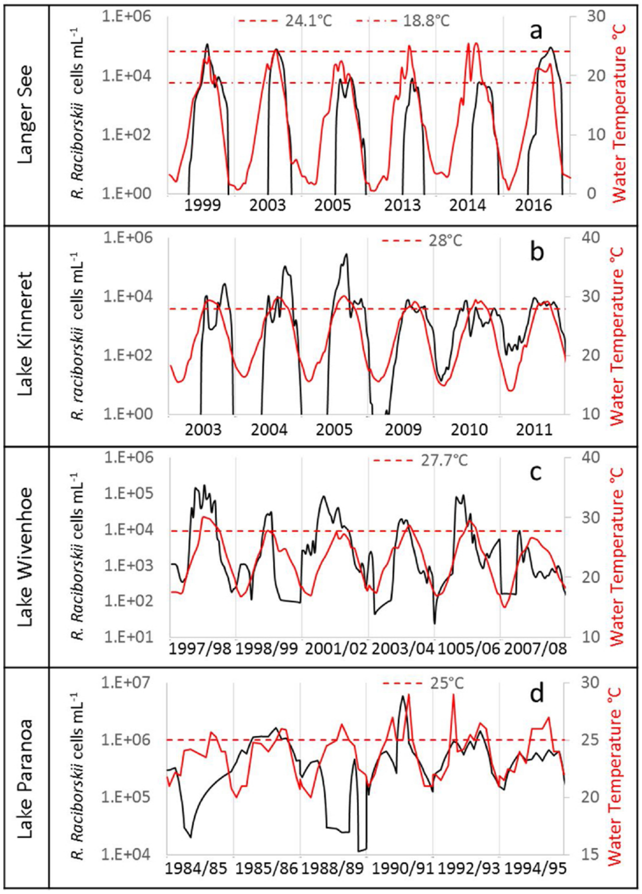

Fig. 4. Water temperature thresholds of the inferential models (Fig. 3) illustrated in conjunction with time series of *R. raciborskii* (cell concentrations in log scale) and water temperatures for each of the six selected years of Lakes Langer See, Kinneret, Wivenhoe and Paranoá.

abundance and 3 “low” abundance years of each lake allowed us to study similarities and differences of the 4 lakes in terms of abundance extremes.

3. Results

After modelling population dynamics of *R. raciborskii* over the six selected years for each of the four lakes solely dependent on WT, results for Lake Kinneret showed the highest coefficient of determination $r^2 = 0.84$ suggesting that fastest population growth takes place at WT greater than 28 °C (see Fig. 3b). Such temperature conditions occurred during the two major bloom events of *R. raciborskii* in Lake Kinneret in 2004 and 2005. As illustrated in Fig. 4b, the highest abundances of *R. raciborskii* in the years 2003, 2004 and 2005 coincided with WT exceeding 28 °C. The model for Langer See achieved a $r^2 = 0.49$ suggesting an optimal WT range between 18.8 and 24.1 °C that would favour fastest population growth, which is common for early summer of most years. However, the model underestimated the peak abundance observed in summer 2016 (Fig. 3a). Whilst the model correctly predicted the peak abundance of *R. raciborskii* in 2003 at WT greater than 24.1 °C, cell numbers remained relatively low in 2013 and 2014 at these temperatures (Fig. 4a). This suggests that WT above 24.1 °C does not necessarily trigger bloom events of *R. raciborskii* in Langer See. In contrast, the highest abundances of *R. raciborskii* were predicted at WT greater than 27.7 °C in Lake Wivenhoe (Figs. 3c and 4 c) and at WT greater than 25 °C in Lake Paranoá (Figs. 3d and 4 d).

The models of population dynamics of *R. raciborskii* solely driven by N:P-ratios for Lakes Langer See, Kinneret and Paranoa considered DIN/DIP–ratios as drivers. Since no measurements of DIN and DIP concentrations were available for Lake Wivenhoe, TN:TP-ratios were used as drivers.

The *R. raciborskii* model with the highest coefficient of determination $r^2 = 0.8$ has been developed for Lake Kinneret (Fig. 5b). It suggests that fastest population growth takes place at DIN:DIP-ratios smaller than 6.4 indicative for N-deficient conditions, according to the atomic ratio 16 N : 1 P of phytoplankton that corresponds with the mass ratio 7.2 N : 1 P (Redfield, 1958). The Fig. 6b illustrates the seasonally inverse oscillations of DIN:DIP-ratio and *R. raciborskii* during the six selected years for Lake Kinneret. The N:P-driven model for Langer See achieved an $r^2 = 0.62$ and suggested DIN:DIP-ratios smaller than 6.8 (also indicative of N-limitation) as being typical for increased abundances of *R. raciborskii.* As Figs. 5a and 6 a show, this condition was met for the years 1999 and 2003 but was not met for the bloom event in 2016. The Fig. 7 displays the averaged time-series of *R. raciborskii* and DIN:DIP-ratios in Langer See from April to September separately for ‘high’ and ‘low’ abundance years. It reveals that steady high growth and peak abundances of *R. raciborskii* in Langer See from May to July of 1999, 2003 and 2016 (Fig. 7a) coincide strongly with N-limited periods indicated by DIN:DIP-ratios below 7.2 (Redfield, 1958). By contrast, DIN:DIP-ratios of 2005, 2013 and 2014 appeared to be well above 7.2 indicating P-limitation that may have caused low abundances during summer months (see Fig. 7b).

The model for Lake Wivenhoe identified a TN:TP-ratio of between 37.5 and 43.7 indicative of N-limitation (Sterner, 2008) as condition for the extreme bloom event in summer 1997/98 (Fig. 5c).

The model for Lake Paranoá identified also N-deficiency by a DIN:DIP-ratio smaller than 7.6 as condition for elevated abundances of *R. raciborskii* throughout the six selected years (Figs. 5d and 6 d).

In order to better understand timing and intensity of changing abundance of *R. raciborskii* in conjunction with changing P-concentrations and water temperature along the three ‘high’ abundance years of the four lakes, we calculated the positive change rates day^{-1} of these variables between consecutive days throughout the three years of each lake (e.g. (WT(day 2)–WT(day 1))/WT (day2) > 0). The Fig. 8 displays the resulting positive daily rates of cell numbers of *R. raciborskii* in conjunction with positive daily rates of WT and P-concentrations of the four lakes. Four of the six events with fast positive rates of *R. raciborskii* in Langer See (Fig. 8a) coincided with relative high DIP rates, only one with high WT rates (19/4/1999) and one with both, high WT and DIP rates (6/5/2016). The majority of events with fast positive rates of *R. raciborskii* in Lake Kinneret (Fig. 8b) was associated with moderate positive WT and DIP rates, whilst only the event on 2/9/2004 corresponded solely with DIP rates, whereby those on 7/7/2003, 6/6/2004 and 10/4/2005 corresponded solely with WT rates. Lake Wivenhoe demonstrated high positive rates of *R. raciborskii* with coincidental moderate WT and DIP rates in 1997/98 and 2001/02 whilst some events across all years were only linked to positive WT rates (Fig. 8c). The Fig. 8d revealed only one event of high positive *R. raciborskii* rates corresponding with very low WT and DIP rates in Lake Paranoá (22/7/1985), and some events associated with very small WT gradients for all years (Fig. 8d). Whilst positive rates of WT and DIP ranged between 0.01 and 0.1 day^{-1} in Langer See, these rates were declining from Lake Kinneret towards Lake Paranoá up to 0.001 day^{-1}.

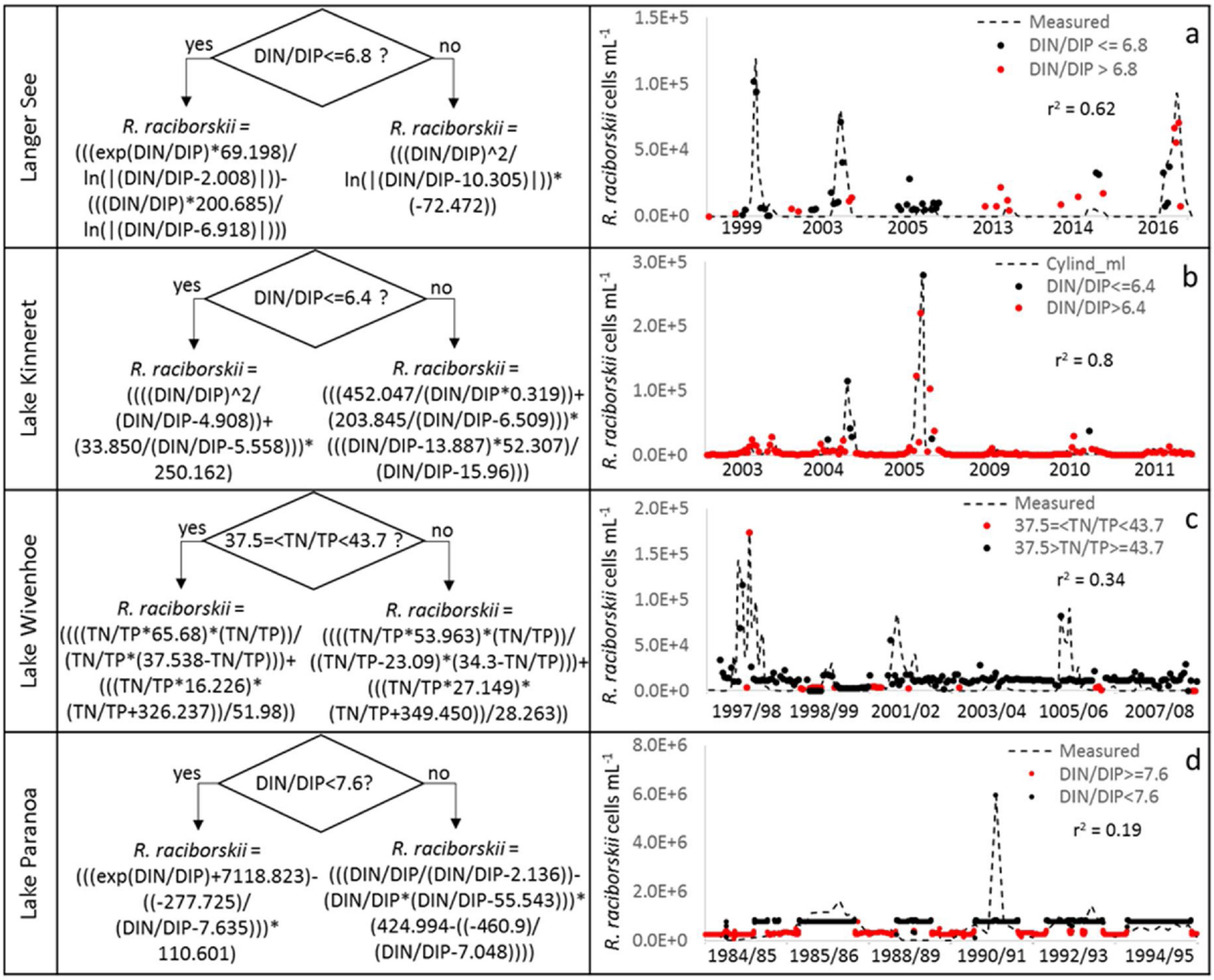

Fig. 5. IF-THEN-ELSE-rules and validation results of inferential models of *R. raciborskii* for Lakes Langer See, Kinneret, Wivenhoe and Paranoá solely predicted from N:P-ratios. For more detailed validation of the models and N:P-thresholds of the four lakes see Figs. A1 and A2.

4. Discussion

Physiological plasticity enables *R. raciborskii* to evolve strains that are adapted to local environmental conditions, and also differ in toxicity (e.g. Orr et al., 2010). This meta-analysis applied strains of four lakes to examine their response to different climate and eutrophication conditions.

Whilst water temperature data was investigated as indicator and driver for warming and climate-related changes of *R. raciborskii,* the N:P-ratios were used to reflect nutrient limitation-related changes of *R. raciborskii.* There are alternative concepts for assessing nutrient limitation of planktonic production discussed in the literature (e.g. Dolman and Wiedner, 2014), however, the stoichiometric reference N:P-ratios for nutrient limitation (Redfield, 1958) served well as indicator in terms of the present meta-analysis.

With respect to hypothesis (1), there is evidence that *R. raciborskii* germinates and grows in spring in response to increasing water temperatures between April and May in Langer See and Lake Kinneret. In contrast, *R. raciborskii* has overwintering pelagic populations in the two subtropical/tropical lakes all year round. The highest abundances of *R. raciborskii* coincided with highest water temperature for Lakes Kinneret, Wivenhoe and Paranoá. However, in Langer See the highest abundances of *R. raciborskii* occurred at 24.1 °C even though temperatures of up to 27 °C were recorded in 2013 and 2014. This suggests that strains of *R. raciborskii* that typically occur in Langer See may not be adapted to the higher temperatures and/or solar radiation found in the other three lakes, or populations may be more susceptible to other factors including photoinhibition, competition or grazing (Wiedner et al., 2007; Rücker et al., 2009).

The weakest correlation with the lowest r^2 for the WT-driven models was found for Lake Paranoá and the highest for Lakes Kinneret and Langer See, possibly reflecting the fact that the annual initial stages of the *R. raciborskii* growth in Lakes Langer See and Kinneret correlate with increasing water temperature. The relatively lower importance of water temperature for the development of *R. raciborskii* in tropical/subtropical lakes because of overwintering populations may also been demonstrated during years of high positive abundance rates in Lake Paranoá such as in 1990/91 in the absence of positive water temperature rates.

With regards to hypothesis 2, there is strong evidence that the

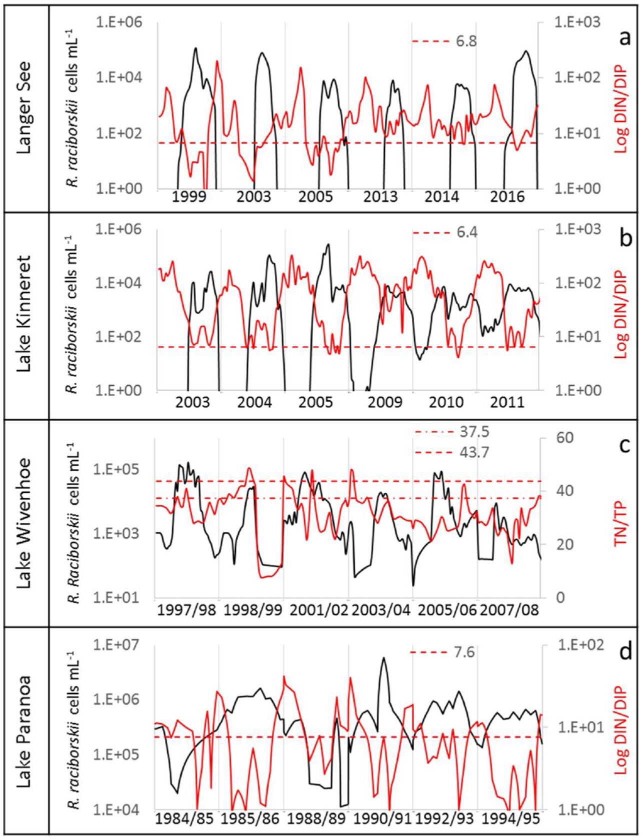

Fig. 6. N:P-thresholds of the inferential models (Fig. 5) illustrated in conjunction with time-series of *R. raciborskii* (cell concentrations in log scale) and N:P-ratios for each of the six selected years of Lakes Langer See, Kinneret, Wivenhoe and Paranoá.

population growth of *R. raciborskii* in all four lakes is highest during periods of N-deficiency characterised by pulses of positive rates of DIP and TP, respectively. Steady high growth and peak abundances of *R. raciborskii* in the polymictic Langer See from May to July of 1999, 2003 and 2016 suggested that a combination of N-limited periods and pulsing DIP loads from anaerobic sediments in summer may have favoured bloom events. As suggested by Amaral et al. (2014), *R. raciborskii* may grow faster by pulses rather than constant concentrations of DIP.

Results for Lake Kinneret also show that summer episodes of high abundances of *R. raciborskii* in the years 2003, 2004 and 2005 occurred when DIN:DIP-ratios fall below 6.4 and N becomes limiting.

During episodes of high *R. raciborskii* abundances in Lake Wivenhoe in the years 1997/98, 2001/02 and 2005/06 there were also declining TN:TP-ratios corresponding with findings by Sterner (2008) suggesting N-limitation at TN:TP-ratios smaller than 22. For Lake Paranoá, the three years with the highest *R. raciborskii* abundances during summer (1985/86, 1990/91 and 1992/93) also correlated with N-limiting conditions with DIN:DIP-ratios below 7.2.

However, the strength of correlation with N:P-ratios is declining from temperate and Mediteranean Lakes Langer See and Kinneret with $r^2 > 0.62$ towards the tropical Lake Paranoá with $r^2 = 0.19$ also reflected by only one episode of fast *R. raciborskii* growth in Lake Paranoá coinciding with a positive DIP rate on 20/7/1985.

Hypothesis (3) must be rejected since *R. raciborskii* grows fastest in spring and early summer in the temperate and Mediterranean lakes, and becomes extinct in winter. On the other hand, data from the subtropical/tropical lakes demonstrates a lasting ‘fast growing’ season from spring through until autumn most likely because of overwintering populations. However, as the visualisation of daily positive rates of *R. raciborskii* in conjunction with daily positive rates of WT and DIP for the ‘high’ abundance years has revealed, there is also strong evidence that despite ‘optimal’ water temperatures, *R. raciborskii* still requires conditions where P is not limiting.

This study was characterised by limiting of factors that influence population dynamics of *R. raciborskii* to water temperature and N:P-ratios. Other abiotic factors that have been shown to affect the physiology and phenology of *R. raciborskii* populations include light and nutrients other than N and P such as dissolved inorganic carbon (Pierangelini et al., 2015).

Future work aims to investigate inter-annual variability of cyanobacteria and interrelationships between changing community structures of zooplankton and phytoplankton over decades across lakes with different climate.

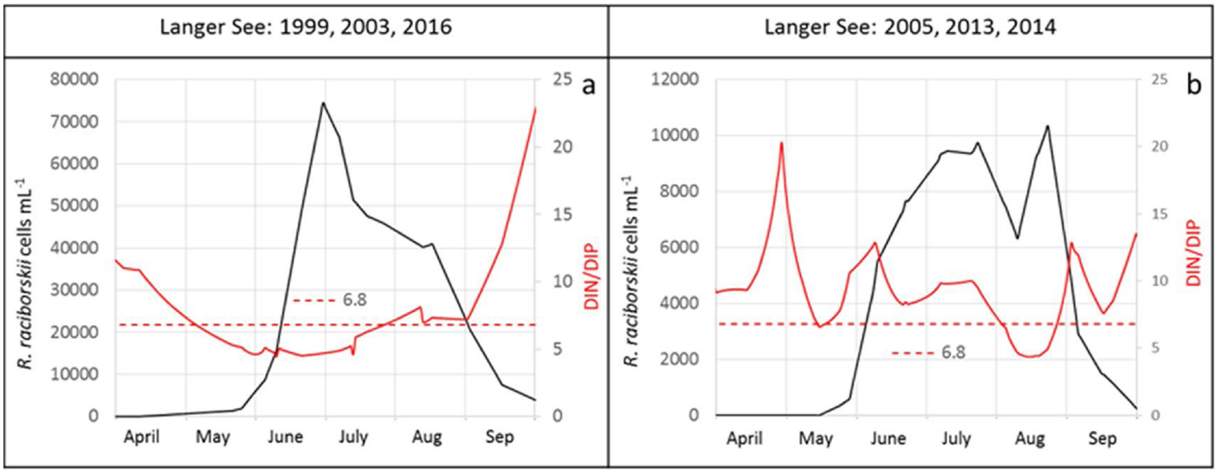

Fig. 7. Relationship between averaged time-series of *R. raciborskii* and DIN:DIP-ratios in Langer See from April to September in ‘high’ abundance years (a), and in ‘low’ abundance years (b). The dotted lines indicate the DIN:DIP-threshold suggested by the model in Fig. 5a.

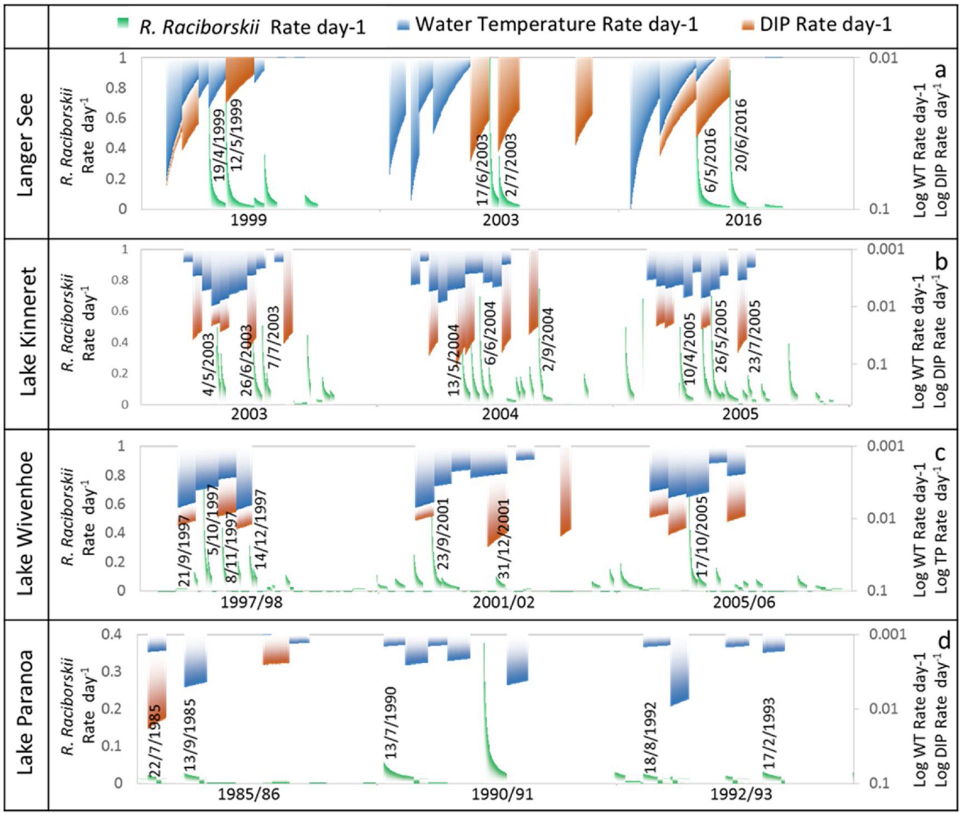

Fig. 8. Correspondence between positive daily rates in the abundance of *R. raciborskii* 🟩 and positive daily rates in water temperature 🟦 and DIP (TP) concentrations 🟧 in the three selected ‘high’ abundance years of Lakes Langer See, Kinneret, Wivenhoe and Paranoá. The dates indicate the first day when positive rates of *R. raciborskii,* water temperature and/or DIN (TP) coincided.

5. Conclusions

(1) Our results have shown that seasonal population dynamics of *R. raciborskii* are strongly correlated with water temperature in the temperate and Mediterranean lakes but weakly correlated in the tropical Lake Paranoá. This may suggest that warmer water temperatures elevate biotic control mechanisms of *R. raciborskii* such as competition by coexisting phytoplankton populations and grazing by zooplankton from seasonal to annual regulating factors of the physiology of *R. raciborskii.*

(2) Testing assumption on relationships between *R. raciborskii* and episodic nutrient limitations by inferential modelling, the nitrogen to phosphorus (N:P)-ratios served well as stoichiometric reference for nutrient limitation of planktonic production (Redfield, 1958). When applied to the four lakes, results have shown that fast growth of *R. raciborskii* coincided with episodes of N-limited waters regardless of trophic states. There was also an indication that pulsing DIP-concentrations in summer that are particularly typical for the polymictic Langer See further stimulate growth of *R. raciborskii* as suggested by Amaral et al. (2014).

(3) Distinct seasonal features of the phenology of *R. raciborskii* appeared to become compromised by growing importance of biotic regulating factors along warming climates.

(4) Applications of HEA as tool for meta-analysis resulted in inferential models just driven by water temperature or N:P-ratios that demonstrated high predictive capacity for population dynamics of *R. raciborskii.* Quantified thresholds and coefficients of determination r^2 of the models with p-values $< = 0.05$ allowed testing of hypotheses.

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Appendix

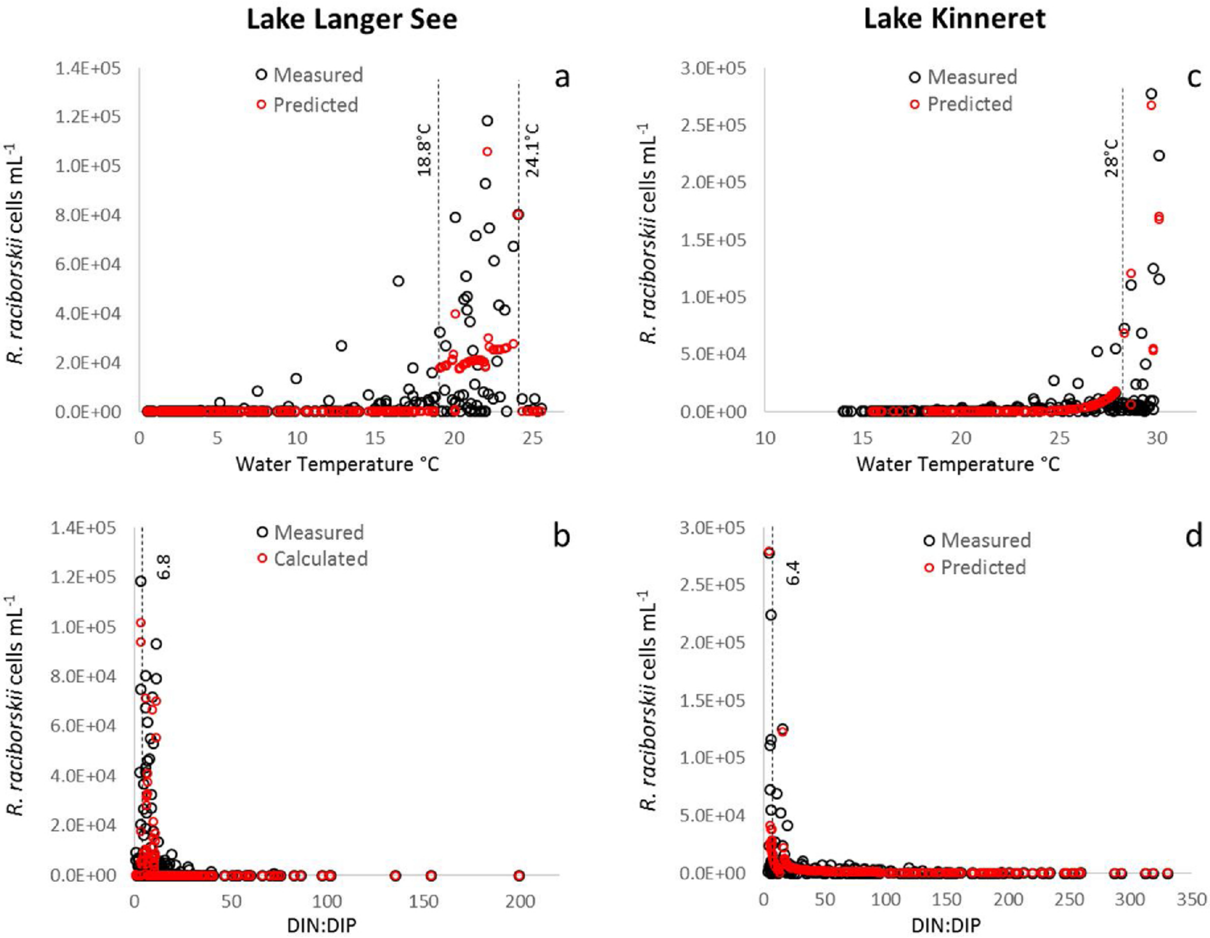

Fig. A1. Validation of the inferential models and thresholds discovered for water temperature and DIN:DIP-ratios of *R. raciborskii* in Lake Langer See (a, b) and Lake Kinneret (c, d).

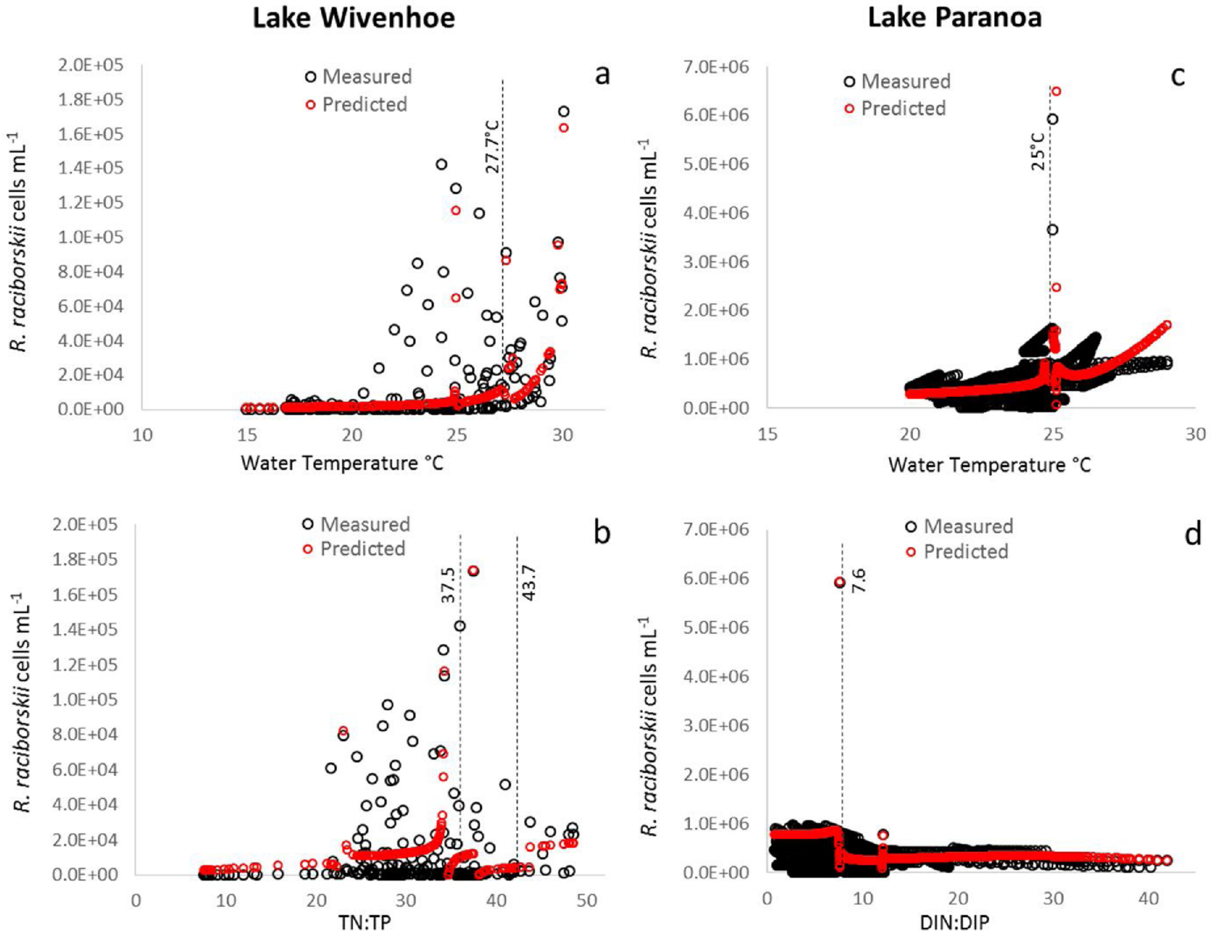

Fig. A2. Validation of the inferential models and thresholds discovered for water temperature and DIN:DIP (TN:TP)-ratios of *R. raciborskii* in Lake Wivenhoe (a, b) and Lake Paranoa (c, d).

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