Nuisance growth of *Juncus bulbosus* in lakes and rivers

- experimental and observational studies

Dissertation for the degree of *Philosophiae Doctor*

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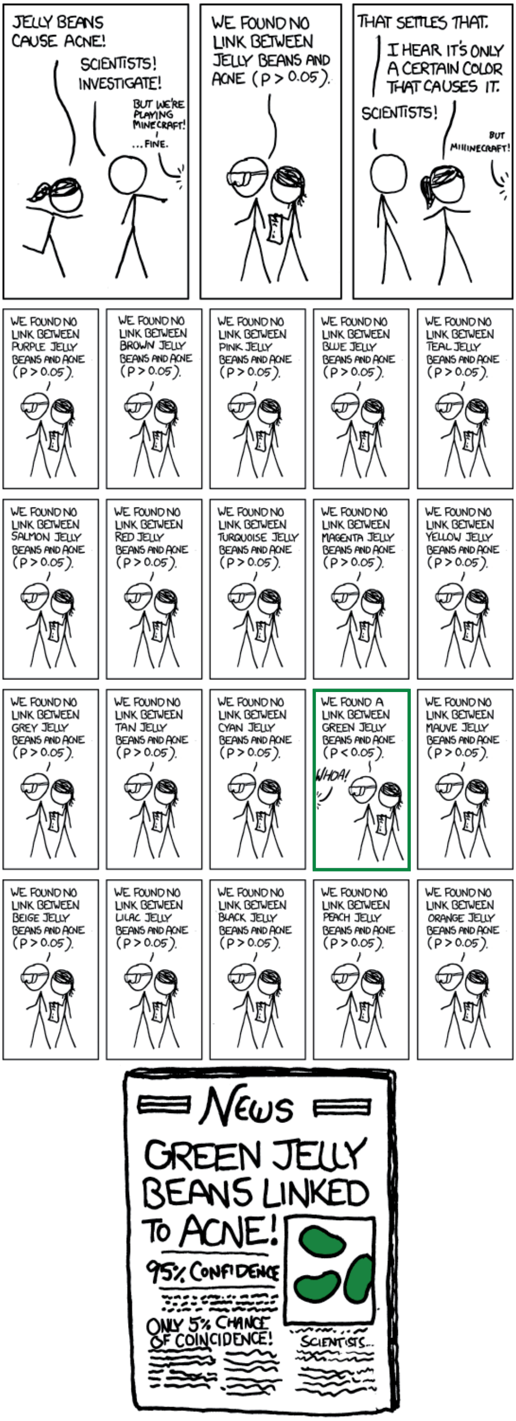
JELLY BEANS CAUSE ACNE!
SCIENTISTS! INVESTIGATE!
BUT WE'RE PLAYING MINECRAFT!
... FINE.
WE FOUND NO LINK BETWEEN JELLY BEANS AND ACNE (P > 0.05).
THAT SETTLES THAT.
I HEAR IT'S ONLY A CERTAIN COLOR THAT CAUSES IT.
SCIENTISTS!
BUT MIIIINECRAFT!
WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TURQUOISE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN MAGENTA JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN YELLOW JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN GREY JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN CYAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND A LINK BETWEEN GREEN JELLY BEANS AND ACNE (P < 0.05).
WHOA!
WE FOUND NO LINK BETWEEN MAUVE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BEIGE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN LILAC JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLACK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PEACH JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN ORANGE JELLY BEANS AND ACNE (P > 0.05).
NEWS
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...

(xkcd.com)

ACKNOWLEDGEMENTS

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Therese Fosholt Moe
Oslo 2012

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Submitted to Freshwater Biology Dec 2011.

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ABSTRACT

Nuisance growth of the aquatic macrophyte *Juncus bulbosus* has been observed since the 1980s in an increasing number of rivers and lakes in northern Europe. What causes such massive growth is not well understood, however, and our aim was to assess potential drivers behind the nuisance growth in both lakes and rivers. Our main hypothesis was based on the observation that there is a strong overlap between the areas receiving elevated nitrogen (N) deposition and those with the most severe growth of *J. bulbosus*. These areas generally have low levels of phosphorus (P) and we wanted to test whether the resulting elevated N:P ratios might promote *J. bulbosus* growth. To test this hypothesis, we collected data from 153 lakes and 28 river locations in Southern Norway (papers I, II and IV) and conducted a controlled growth experiment in which 100 *J. bulbosus* plants received different nutrient treatments (paper III). However, none of these approaches gave support for our hypothesis.

To search for other drivers for *J. bulbosus* nuisance growth, we collected a range of parameters from the lakes and rivers visited. These included catchment, lake water and sediment characteristics. Despite an extensive number of parameters tested, we were unable to detect any general drivers that could explain nuisance growth. Furthermore, a genetic screening (AFLP fingerprinting) showed no genetic differences between nuisance and non-nuisance plants. The upside of these “negative” conclusions however, is that we can now exclude several candidate parameters as the causes for nuisance growth. The most important being genetic differences and elevated N:P ratios.

Our data did show that the most problematic nuisance growth occurred in the most oligotrophic lakes (paper I). Analyses of plant elemental composition (paper IV) showed that *J. bulbosus* plants had high C:N and C:P ratios compared to other freshwater macrophytes (and even higher in roots compared to shoots). This allows *J. bulbosus* to build large biomasses on small amounts of nutrients, rendering it highly competitive in nutrient poor habitats. In the growth experiment (paper III), we found an increasing uptake of sediment NH_4 with increasing growth, pointing towards a special relevance of NH_4 for *J. bulbosus* growth. Furthermore, analyses of plant elemental composition revealed higher N contents of river plants compared to lake plants (paper IV). Based on these results, relevant literature and indications from the lake and river surveys (papers I and II), we found indications that different triggers might be responsible for nuisance growth in rivers (NH_4) compared to lakes (CO_2). More detailed analyses are needed before we can draw definite conclusions.

1. INTRODUCTION

1.1 *Juncus bulbosus*

Juncus bulbosus L. is a perennial plant native to Europe and North Africa (Prockow, 2008a), which can inhabit both terrestrial and aquatic habitats (Prockow, 2008b). The aquatic type is common in oligotrophic and ultra-oligotrophic lakes and rivers (Rørslett, 1987; Snogerup, 2006), where it starts out as a small rosette of 10-20 cm length. However, side branches can emerge, bearing new "budding" rosettes of up to 80 cm length (Fig. 1; Johansen, Brandrud & Mjelde, 2000). Multiple years of accumulating such new side branches (without winter dieback) can result in dense stands of *J. bulbosus*, with individual plants reaching a length of up to 2-3 m (Johansen *et al.*, 2000; Hindar, Johansen, Andersen *et al.*, 2003).

Fig. 1 *Juncus bulbosus* initially grows as a small rosette plant, but it can grow new budding rosettes on the original plant, resulting in a diversity of growth forms. Here are some examples from Norwegian lakes and rivers.

1.2 Nuisance growth and its consequences

Since the mid 1980's, massive expansion resulting in nuisance growth has occurred in an increasing number of European lakes and rivers (Roelofs, 1983; Aulio, 1987b; Svedäng, 1990; Brandrud, 2002), with *J. bulbosus* becoming the dominant macrophyte species in many of these ecosystems (Fig. 2). Among the consequences of such nuisance growth are reduced biodiversity, reduced suitability of the ecosystems for fish spawning, clogging of hydropower inlet screens and reduced suitability of the ecosystems for recreational use such as fishing, boating and bathing. Mechanical removal of the plants is not only laborious and costly, but it also only deals with the effects, not the cause of the nuisance growth and re-growth is always observed within few years (Brandrud & Johansen, 1997).

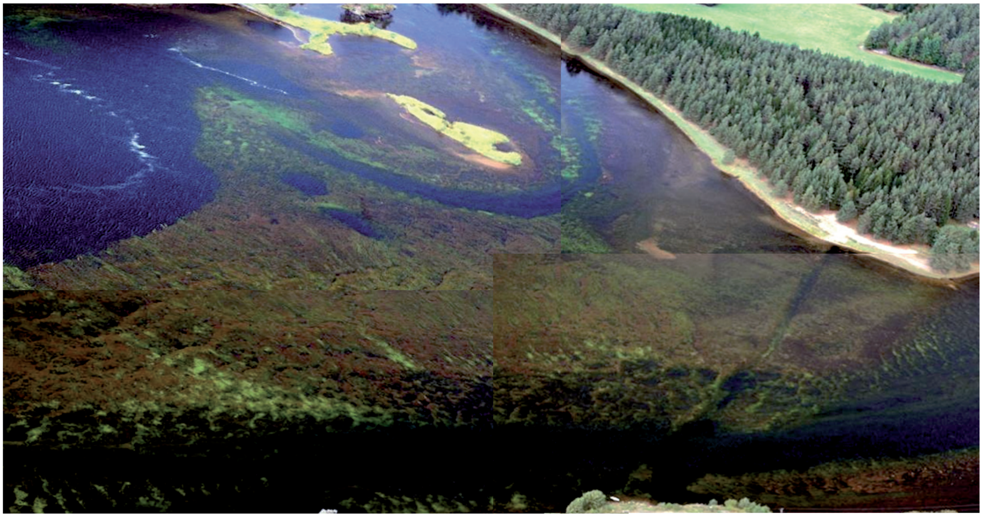

Fig. 2 Aerial photographs showing *Juncus bulbosus* nuisance growth in the Norwegian river Otra. Photo: Tor Kviljo.

1.3 Hypotheses concerning nuisance growth

Old descriptions of *J. bulbosus* indicate that the nuisance growth we now observe was uncommon in Norwegian lakes and rivers at the beginning of the 20th century (described by Buchenau, 1890; Ascherson & Graebner, 1902-04 and Braarud 1928; cited from Johansen *et al.*, 2000). Several hypotheses have been forwarded to explain the massive increase in *J. bulbosus* biomass. In lakes, the most common hypotheses concern acidification, liming and reacidification, coupled with an increase in CO_2, sediment ammonium and phosphorus (Aulio, 1987b; Svedäng, 1992; Roelofs, Brandrud & Smolders, 1994; Roelofs, Smolders, Brandrud *et* *al.*, 1995; Lucassen, Bobbink, Oonk *et al.*, 1999). In rivers, on the other hand, liming has not been considered a likely cause for nuisance growth (Johansen *et al.*, 2000). Instead, mild winters, leading to less ice erosion and thus to a higher survival (Johansen *et al.*, 2000; Hindar *et al.*, 2003), as well as hydropower development with resulting alterations in hydrology and ice cover (Johansen *et al.*, 2000; Hindar *et al.*, 2003) are the most common hypotheses.

However, in Norway we find massive *J. bulbosus* growth in both limed and unlimed lakes, and in rivers both with and without hydropower development, in both low lying (warm) and higher (cold) altitude regions, such that a consistent explanation for *J. bulbosus* nuisance growth is still lacking.

To address this issue, the current study is based on a different and new approach: Areas with elevated N deposition often show increased leaching of inorganic N to surface waters (Stoddard, 1994; Kaste, Henriksen & Hindar, 1997). Since other elements than N are primarily diluted by precipitation, one might expect ratios of inorganic N to C and P to increase in these areas, not only due to elevated N deposition, but also due to precipitation amount. One effect of such skewed inputs of N relative to P could be an intensified P limitation relative to N in surface waters (Hessen, Hindar & Holtan, 1997; Interlandi & Kilham, 1998), which has been shown for phytoplankton (Elser, Kyle, Steger *et al.*, 2009). Such alterations in surface water stoichiometry can have important implications for ecosystem diversity and functioning, and cause community shifts as well as proliferation of certain species. One example of the latter could be nuisance growth of *J. bulbosus*: In Southern Norway there is a remarkable similarity between the areas where massive *J. bulbosus* growth was first reported and the regional deposition pattern of atmospheric N (Fig. 3). Such excess N may have a double effect on *J. bulbosus*, firstly by promoting acidification of surface waters (Reuss & Johnson, 1986), and secondly by affecting the stoichiometry in lakes and rivers (Jassby, Goldman & Reuter, 1995; Kopacek, Prochazkova, Stuchlik *et al.*, 1995; Hessen *et al.*, 1997; Bergström, Blomqvist & Jansson, 2005). These areas are generally very low on phosphorus, and an elevated N:P ratio has been suggested as a reason for the massive *J. bulbosus* growth observed (Kaste, Johansen, Mjelde *et al.*, 2007). Furthermore, experiments with P fertilization in an acidified lake near Grimstad in Southern Norway resulted in a near 100% die back of *J. bulbosus* (Mjelde, 2004). Here, a change in the lake water N:P ratio was suggested as a possible explanation factor. How this affected *J. bulbosus* growth was not known, but as periphyton also increased considerably in this period, shading and/or competition from periphyton was suggested as an explanation. Such a relationship was also suggested by Svedäng (1990), who observed a decline in *J. bulbosus* in a Swedish lake at the same time as its epiphytic cover increased.

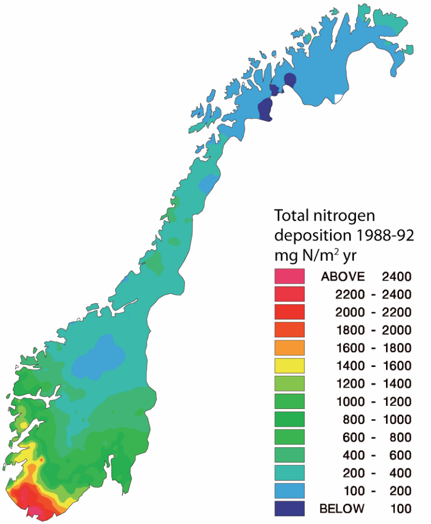

Fig. 3 Total nitrogen deposition (mg N/m^2 yr) over Norway 1988-1992 (based on Hole & Tørseth, 2002).

To test these hypotheses about skewed N:P inputs and periphyton abundance, this thesis combines three field seasons of observational studies, covering 153 lakes and 29 river locations (papers I and II). As the field work conducted was so extensive, we decided to also test a range of other factors that could potentially influence growth of *J. bulbosus* (see list of parameters in Table 1). Furthermore, the different growth forms of *J. bulbosus* could potentially be due to different genetics, and we used amplified fragment length polymorphisms (AFLP) to analyse plants and look for genetic structures (paper I). In addition, we have conducted a three month growth experiment specifically aimed at testing the N:P and periphyton abundance hypotheses (paper III). And finally, the elemental composition of *J. bulbosus* was analysed and tested for differences in N:P stoichiometry between plants from nuisance versus non-nuisance lakes (paper IV). The specific aims were:

1. To determine key factors explaining presence or absence of *J. bulbosus* in Norwegian lakes (paper I).
2. To determine key factors explaining the occurrence of different *J. bulbosus* growth forms and their abundances (nuisance growth or not) in Norwegian lakes, and especially see whether N deposition and/or sediment/water N concentrations were important factors (papers I and II).

3. To determine key factors explaining the occurrence of different *J. bulbosus* growth forms and their abundances (nuisance growth or not) in Norwegian rivers, and compare the findings between rivers and lakes (paper II).
4. To assess whether genetic differences in *J. bulbosus* can account for its different growth patterns (paper I).
5. To assess whether experimental additions of N and/or P affect periphyton abundance and *J. bulbosus* growth, and whether higher periphyton abundances reduce *J. bulbosus* growth (paper III).
6. Analyse the elemental composition of C, N and P in *J. bulbosus*, and look for differences in these elements between a) nuisance vs. non-nuisance lakes, b) roots vs. shoots and c) rivers compared to lakes.

2. MATERIALS & METHODS

2.1 Field work and analyses

Field work was conducted during three growing seasons (papers I, II and IV): In 2007 we visited 153 lakes (Fig. 4 A), and in both 2008 and 2010 we revisited 16 of these lakes along with 28 river sites from 15 different rivers (Fig. 4 B). At each lake and river site, *J. bulbosus* growth forms (rosette plants/small columns with annual shoots/large columns with annual shoots/surface mats; Fig. 5) and abundance (0 = not present; 1 = sparsely vegetated; 2 = covering large parts; 3 = dominating the site) of each growth form were estimated. Presence of other macrophyte species was also noted, as well as abundance of periphytic algae on *J. bulbosus*. At the site of most abundant *J. bulbosus* growth, sediment and water samples were collected, and a single *J. bulbosus* plant was randomly collected for genetic analyses (paper I) and analysis of elemental composition (papers II and IV).

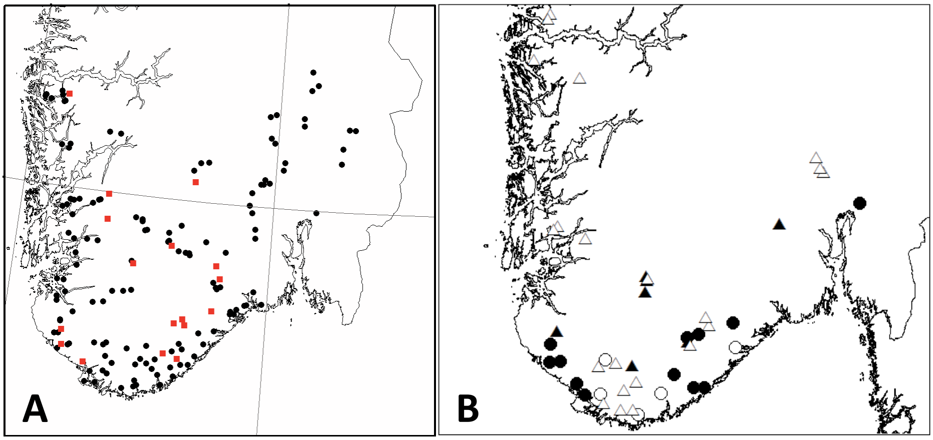

Fig. 4 Overview of the Norwegian lakes and rivers sampled for this thesis: A) 153 lakes sampled in 2007 (red squares represent *Juncus bulbosus* nuisance growth, black circles represent lakes without *J. bulbosus* or with *J. bulbosus* non-nuisance growth; paper I); B) the 17 lakes (circles) and 28 river sampling sites (triangles) visited in 2008 and 2010 (black symbols represent *Juncus bulbosus* nuisance growth, white symbols non-nuisance growth; paper II).

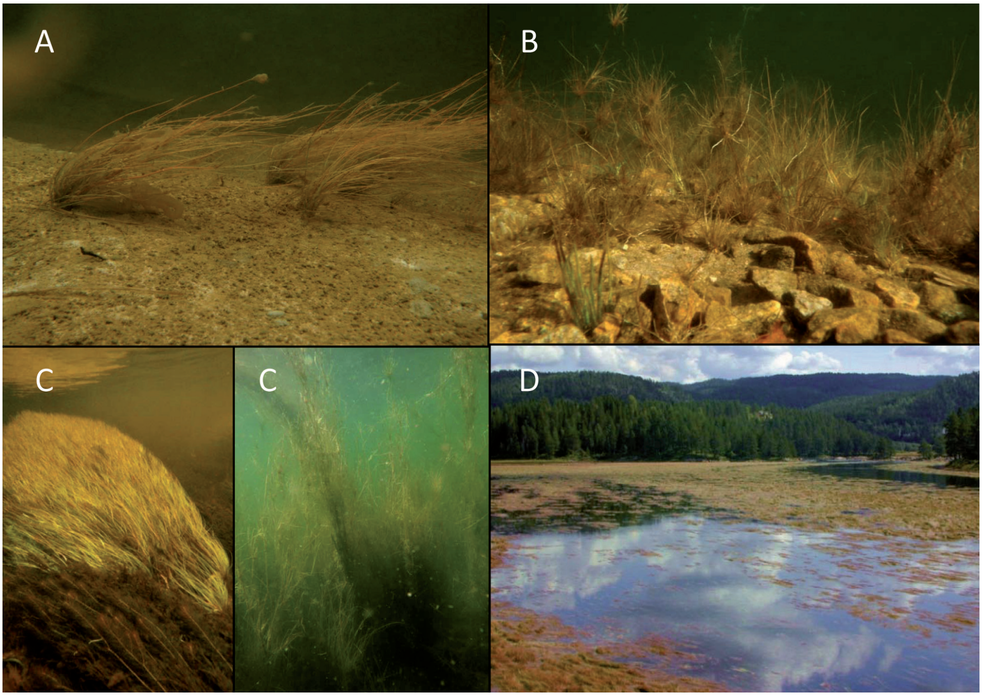

Fig. 5 Four categories of *Juncus bulbosus* growth forms: A) rosette plants; B) small columns with annual shoots; C) large columns with annual shoots; D) surface mats. Photos: T. F. Moe (A-C) and Edgar Vegge (D) (paper I).

Although our main hypotheses were related to N and P, we also wanted to test other water chemical parameters. The lake and river water was thus analysed for Ca, CO_2, DIC, TOC, TotN, NO_3, NH_4, TotP, PO_4, conductivity and pH (papers I and II). Water samples from the growth experiment (paper III) were only analysed for NO_3, NH_4 and PO_4. All sediments were analysed for organic content, water content and pore water NO_3, NH_4 and PO_4 (papers I, II and III).

Testing lake and river water nutrient concentrations by taking a single sample only gives a snapshot of the situation, and the resulting concentrations will be highly dependent on vegetation cover and phytoplankton abundances. Thus, to complement this picture, we applied a macrophyte trophic index (TIc) to all lakes in paper I. The TIc was calculated based on presence/absence of indicator macrophyte species with different degrees of eutrophication tolerance (paper I), and can thus give an indication of the more long term nutrient supply to each lake. The TIc ranges from -100 (eutrophic) to +100 (oligotrophic).

2.2 Catchment data

To assess the importance of N deposition on *J. bulbosus* growth we used geographical information systems (GIS) to define polygons on a digital map that corresponded with the catchments of each lake and river (papers I and II). In addition to atmospheric N deposition, we collected data on annual average temperature, precipitation, runoff, satellite derived normalized difference vegetation index (NDVI), terrain slope, area, altitude, solar irradiation, UVA and UVB irradiation. We also recorded the liming status of all rivers and lakes, and obtained information on hydropower development.

2.3 AFLP analyses

J. bulbosus shows great diversity of growth forms, and we hypothesise that this is due to great plasticity, meaning it can change growth form depending on water flow, nutrient supply, water depth etc. However, it is possible that the difference between massive stands of *J. bulbosus* and the populations consisting of small rosette plants is related to genetic differences. To address this question, we collected 69 specimens of *J. bulbosus* from 14 lakes and 27 river localities (from 15 different rivers) in 2008 and 2010. The fresh plant material was dried on silica gel and was later analysed using amplified fragment length polymorphisms (AFLPs) (paper I). The AFLP markers in the range of 60-500 base pairs were scored, and the resulting presence/absence matrix was analysed using three different approaches in order to detect possible genetic structures of the 69 *J. bulbosus* samples: 1) principle coordinate (PCO) analysis; 2) neighbour networks; and 3) Bayesian clustering.

2.4 Experimental setup

To test the N deposition and periphyton hypotheses, we designed a growth experiment with four different treatment groups: N additions, P additions, N+P additions and a control group (paper III). We used a total of 100 *J. bulbosus* plants (25 in each treatment group), and growth was assessed by measuring biomass and length (and other parameters, see paper III) both at the beginning and end of the experiment. At the end we also measured periphyton abundance, as well as sediment and water nutrients.

All plants were put in separate pots filled with partly organic sediments (from the same lake as the plants were collected). All pots were then submerged in 3 L glass beakers with water (Fig. 6 A), and all beakers were partly submerged in water in one of two large tanks (Fig. 6 B). Light in the range of 24-53 μmol PAR s^{-1} m^{-2} per μA was on for 12 hours each day, and the room temperature was kept constant at about 18^{o}C. Air was bubbled into all beakers through air stones, and nutrients were added weekly to the beakers according to treatment group: 1) 49.4 μg N (from NH_4NO_3) was added to the N group; 2) 6.8 μg P (from KH_2PO_4) was added to the P group; 3) the N+P group received the same treatment as both groups N and P added together; and 4) the Control group received no additions. The experiment was terminated after three months.

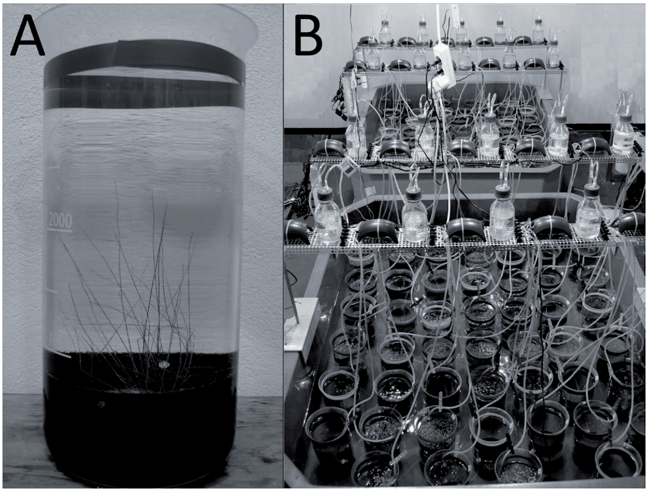

Fig. 6 Experimental setup of (B) 100 *Juncus bulbosus* plants grown in lake sediment in (A) separate three litre beakers (paper III).

2.5 Statistics

We were able to find *J. bulbosus* in 118 of the 153 lakes examined and at all river sites. For various reasons (see paper I), we excluded some of the lakes from the analyses, leaving a total of 139 lakes, of which 105 had *J. bulbosus* growth. In paper I, we first wanted to find which of the lake and/or catchment parameters could best explain absence or presence (regardless of abundance) of *J. bulbosus*. For this purpose, we used multiple logistic regression. In a next step, we focused on the 105 lakes where we had observed *J. bulbosus*, this time trying to find what parameters could best explain nuisance vs. non-nuisance growth. However, as there is no obvious way of categorising *J. bulbosus* (nuisance) growth, we tested several different growth categorizations to parameterize our response variable (Table 1, paper I). The starting point for all these approaches was the division of *J. bulbosus* into the observed growth forms (0-3; see section 2.1) and their abundances (0-3). In papers I, II and IV, we used several different ways of categorising nuisance growth into two (nuisance vs. non-nuisance growth) or three (nuisance, partly nuisance or non-nuisance) categories. In addition, in paper I, we also chose to use a linear approach; a “DCA1-score”. This score was calculated through a Detrended Correspondence Analysis (DCA) (Ter Braak & Prentice, 1988), which takes into consideration all the different growth forms and their abundances in each lake. The result is a number for each lake, denoting its “level of nuisance growth”. Low numbers represented lakes with mainly small rosette plants, and higher numbers indicated mass abundances of *J. bulbosus* (nuisance growth). These DCA-scores were then used as the response variable for single-predictor linear regression models as well as a multiple linear model selection based on the explanatory variables listed in Table 1.

In paper II, we tested for differences between sites with nuisance compared to non-nuisance growth and between rivers and lakes using Mann-Whitney U tests. In paper III, we used Kruskal-Wallis tests to measure the effects of nutrient treatment and periphyton abundance, and regression models to measure the effects of sediment and water nutrients on *J. bulbosus* growth. Mann-Whitney U tests were also used to test for differences in *J. bulbosus* C, N and P content and element ratios between nuisance and non-nuisance lakes, roots and shoots and river compared to lake plants (paper IV).

To avoid type II errors, we corrected the significance levels of all tests where we did not use multivariate approaches using Bonferroni corrections ($\alpha = 0.05/n$, where n = number of tests), with or without the refinement of Holmes (Stahel, 1995; Bärlocher, 1999).

3. MAIN FINDINGS

3.1 *Juncus bulbosus* in lakes (paper I)

J. bulbosus is known to prefer acidic, nutrient poor waters (Rørslett, 1987; Lid & Lid, 2005; Snogerup, 2006) and our logistic model describing presence/absence of *J. bulbosus* confirmed this picture: *J. bulbosus* appeared most frequently in slightly acidic lakes with low phosphate concentrations and high N:P ratios (Fig. 4 A-C in paper I). Furthermore, *J. bulbosus* was generally absent from habitats with the lowest minimum temperatures (Fig. 4 D in paper I). This probably reflects that *J. bulbosus* is not very frost tolerant (Svedäng, 1990), yet it may also be linked to the minimum length of the growing season or the amount of ice cover during the winter (which can cause mechanical stress on the plants and uprooting during ice break).

When we focused our studies on the 105 lakes where *J. bulbosus* was present, however, we found no single parameters (Table 1) or multiple models that could significantly explain differences in nuisance versus non-nuisance growth. This was true both when we tried to categorise *J. bulbosus* growth into two or three nuisance growth categories, and when we used the linear approach based on DCA1-scores. Thus we did not find any support for our N deposition or periphyton hypotheses, but nor did we find support for any of the other factors most often put forward to explain *J. bulbosus* nuisance growth (e.g. liming, NH_4, or CO_2, see introduction). There are many possible reasons for this lack of significant results, and this will be discussed thoroughly in section 4. We do know, however, that the differences in *J. bulbosus* growth are definitely not due to genetic differences (Fig. 7 in paper I).

As an addition to the measurements of sediment and water nutrient concentrations, we also assigned a TIc to all the lakes (see section 2.1). The TIc was based on presence/absence of (perennial) macrophytes tolerant to eutrophication, and can therefore give us an indication of the recent nutrient history of each lake. Although not being significantly related to *J. bulbosus* nuisance growth (as DCA-1 scores; $p = 0.0068$; paper I), plotting the TIc against *J. bulbosus* growth forms suggests that the most troublesome growth forms occurred in the most oligotrophic lakes (Fig. 7). This can probably be explained by the increased competition from other macrophyte species with increasing nutrient and DIC availability (see section 4.4).

Table 1 Basic information on the parameters included in the multiple linear models testing *Juncus bulbosus* growth in 105 Norwegian lakes 2007, sorted by R2-values. R^2 and p-values reported are from single-predictor linear regression models of *Juncus bulbosus* growth (as DCA1-scores, see text). Significance level with Bonferroni correction is 0.05/34 = 0.0015, thus no single-predictor models were significant. Effect indicates whether a parameter has a positive or negative relation to *J. bulbosus* growth. Non-parametric parameters were ln-transformed before model selection. Group indicates whether the data are collected on the basis of the lake or the lakes catchment area.

Parameter	Unit	Median	Mean	Range	R^2	p-value	Effect	n	Transformation	Group
Dissolved Inorganic Carbon (DIC)	mg/L	1.00	1.23	0.36-4.60	0.060	0.024	-	105	Ln	Lake
Phosphate (PO_4)	µg P/L	0.50	0.89	0.5-3.0	0.058	0.026	-	105	Ln	Lake
Total Organic Carbon (TOC)	mg/L	5.20	5.39	0.53-16.40	0.057	0.028	-	105	Ln	Lake
Sediment NO_3	µg N/L sediment	14.4	32.4	3.7-1345.3	0.038	0.073	-	85	Ln	Lake
Solar irradiation	W/m^2	868	865	805-904	0.033	0.095	+	105	None	Catchment
DIN:TotalP	Element ratio	17.7	43.0	0.4-554.7	0.030	0.11	+	105	Ln	Lake
Yearly average of global horizontal UVA irradiation	10 kJ/m^2	18162	18062	16369-19641	0.027	0.013	+	105	None	Catchment
Total Phosphorus	µg P/L	5	6	1-17	0.019	0.20	-	105	Ln	Lake
Calcium (Ca)	mg/L	1.19	1.55	0.15-5.47	0.018	0.22	-	105	Ln	Lake
CO_2	mg C/L	0.70	0.78	0.05-2.00	0.018	0.22	-	105	Ln	Lake
pH	-	6.12	6.06	4.82-7.02	0.014	0.29	-	105	None	Lake
Dissolved Inorganic Nitrogen (DIN = NH_4 + NO_3)	µg N/L	46.0	80.8	1.5-629.0	0.014	0.28	+	105	Ln	Lake
Yearly average of global horizontal UVB irradiation	kJ/m^2	15369	15628	13433-18366	0.014	0.28	+	105	None	Catchment
Liming	Yes, Indirectly, Previously, No	-	-	-	0.014	0.77	-	105	None	Lake
Total Nitrogen	µg N/L	305	313	86-785	0.013	0.31	-	105	Ln	Lake
Conductivity	mS/m	2.25	2.72	0.55-7.01	0.010	0.35	-	105	Ln	Lake
Sediment NH_4	µg N/L sediment	764	1275	25-5464	0.0067	0.46	+	85	Ln	Lake
Nitrate (NO_3)	µg N/L	37.00	72.78	0.5-620.0	0.0059	0.49	+	105	Ln	Lake
Nitrogen deposition	mg/m^2/year	0.85	0.82	0.37-1.09	0.0056	0.49	+	105	None	Catchment
Area	km^2	7	34	0.5-1233.6	0.0054	0.50	+	105	Ln	Catchment
Runoff	mm/year	1089	1430	230-4467	0.0046	0.54	+	105	Ln	Catchment
Normalized Difference Vegetation Index (NDVI)	-	0.38	0.35	0.02-0.49	0.0044	0.55	-	105	None	Catchment
Mean temperature of the coldest month	^oC	-4.9	-5.8	-13.4- -0.7	0.0044	0.55	+	105	None	Catchment
Altitude	mas	361	408	41-1280	0.00091	0.78	+	105	None	Catchment
Sediment organic content	%	8	17.2	0.3-78.3	0.00089	0.79	-	85	None	Lake
Regulation	Yes/No	-	-	-	0.00084	0.79	+	105	None	Catchment
Precipitation	mm/year	1411	1465	635-2799	0.00048	0.84	-	105	None	Catchment
Mean temperature of the warmest month	^oC	16.4	16.6	11.7-20.2	0.00041	0.85	-	105	None	Catchment
Slope inclination	^{o}	6.65	7.69	1.32-23.17	0.00034	0.87	+	105	None	Catchment
Algae on the *Juncus bulbosus*	None, Periphyton, "Clouds"	-	-	-	0.00014	0.92	-	105	None	Lake
Sediment PO_4	µg P/L sediment	7.57	11.01	0.01-53.43	0.000087	0.93	-	85	Ln	Lake
Yearly mean temperature	^oC	4.8	4.5	-1.6-7.2	0.000025	0.96	-	105	None	Catchment
Sediment water content	%	58	56	16-96	0.0000083	0.98	+	85	None	Lake
Ammonium (NH_4)	µg N/L	6	8	1-629	0.0000031	0.99	+	105	Ln	Lake

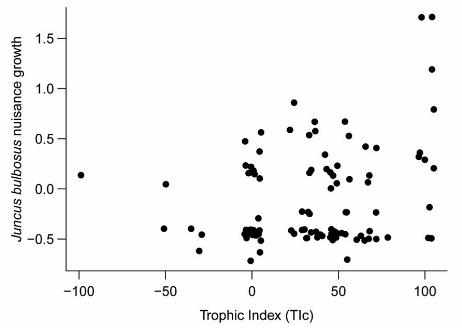

Fig. 7 *Juncus bulbosus* nuisance growth (here represented as DCA1 site scores, higher values indicating more nuisance growth) plotted against the macrophyte trophic index (TIc, high values indicate oligotrophic lakes, low values indicate eutrophic lakes) of 99 S Norwegian lakes 2007. As several lakes had overlapping positions, we included 2.5% jitter in both directions to show all 99 lakes (paper I).

3.2 *Juncus bulbosus* in rivers – compared to lakes (paper II)

This study aimed towards finding possible causes for *J. bulbosus* nuisance growth in Norwegian rivers, and to compare these findings to similar analyses in lakes. To do this, we used data from 16 lakes and 28 river locations visited in 2008 and 2010. All sample locations were defined as either nuisance or non-nuisance lakes/rivers based on *J. bulbosus* growth forms and abundances.

In the search for factors that could explain nuisance growth, we tested for differences in water and sediment chemistry, catchment characteristics and elemental composition between plants from nuisance compared to non-nuisance stands. This was done separately for rivers and lakes, but resulted in no significant differences (Tables 1 and 2 in paper II).

As a second approach, we tested whether nuisance sites in rivers shared the same characteristics as nuisance sites in lakes, and found that the N content of river nuisance plants was significantly higher than that in lake nuisance plants ($p = 0.0003$; Table 3 in paper II). We also tested for differences between non-nuisance sites in rivers and lakes, and these analyses revealed significantly lower NH_4 ($p = 0.0013$; Fig. 8) and TotN ($p = 0.0009$; Table 3 in paper II) concentrations in non-nuisance rivers compared to non-nuisance lakes. The generally higher N content in river plants, despite an equal or even lower N concentration in rivers compared to lakes, might reflect differences in nutrient supply. In rivers, plants are in receipt of a continuous supply of nutrients, whereas a slow diffusion of ions occurs through nutrient depleted zones around the shoots of plants in standing waters (described by Ruttner, 1940; cited from Elster, 1962).

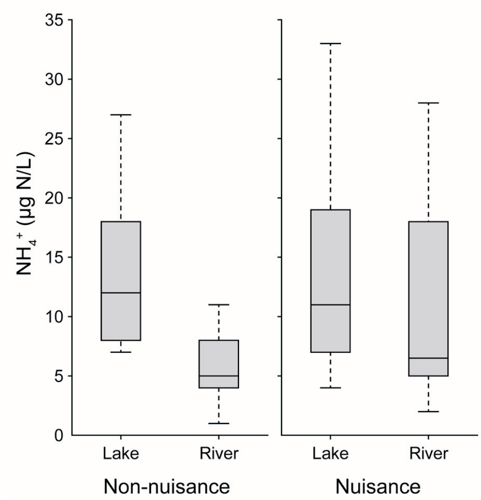

Fig. 8 Water NH_4^+-N concentrations at rivers and lakes with and without *J. bulbosus* nuisance growth in Southern Norway, using averages of samplings from 2008 and 2010. The difference between lakes and rivers is significant for non-nuisance sites ($p = 0.0013$), but not for nuisance sites ($p = 0.34$). Boxes indicate 1st and 3rd quantiles, horizontal lines indicate medians and dotted lines indicate minimum and maximum values (paper II).

Despite testing a range of different parameters in both rivers and lakes, few results became significant. Thus, we still lack clear indications with respect to possible triggers of nuisance growth of *J. bulbosus*. We have, however, isolated some parameters which are more likely than others to play a role in *J. bulbosus* nuisance growth in rivers: The predominant difference observed between nuisance and non-nuisance sites is that no difference in NH_4 concentration occurs between nuisance lakes and rivers, whereas NH_4 concentrations are lower in non-nuisance rivers than in non-nuisance lakes (Fig. 8). NH_4 is usually rapidly removed in streams (Peterson, Wollheim, Mulholland *et al.*, 2001), such that lower NH_4 concentrations in non-nuisance rivers compared to lakes are not surprising. We therefore suggest that the relatively high NH_4 concentrations measured in rivers with nuisance growth are probably enhanced compared to background conditions. In paper II, we demonstrate how enhanced NH_4 concentrations could potentially trigger *J. bulbosus* nuisance growth in rivers.

3.3 *Juncus bulbosus* growth experiment (paper III)

The growth experiment was designed to test the effects of N and P on growth of both periphyton and *J. bulbosus*, as well as the effect of periphyton abundance on *J. bulbosus* growth. And as hypothesised, we found significantly more periphyton in the P and N+P treatment groups than in the control and N only groups (Fig. 9 here and Fig. 2 in paper III).

This indicates that the periphyton in our experiment was P-limited., which is assumed to be the case also in most South Norwegian lakes and rivers (Elser *et al*., 2009). In contrast to the observations of Mjelde (2004), however, this increase in periphyton biomass did not lead to reduced *J. bulbosus* growth (Fig 4 in paper III).

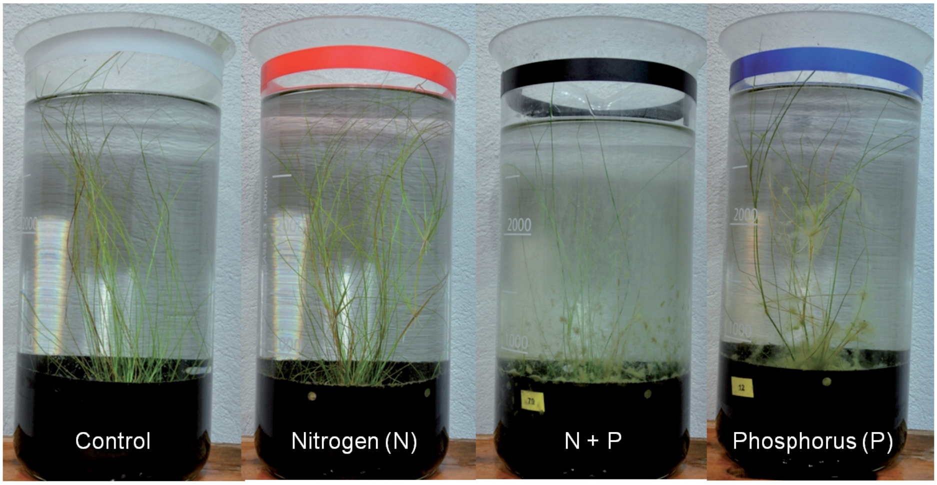

Fig. 9 Beakers representing the four treatment groups at the end of the growth experiment. The most obvious finding is the significantly (data not shown) higher periphyton abundance in the two P-treatment groups (paper III).

From personal experience, the amount of periphyton observed in our experiment corresponded relatively well with the amounts we regularly observe in the field, although we did not achieve the most extreme amounts. Thus, we cannot exclude that very high periphyton biomass still might impact *J. bulbosus* growth. Furthermore, there is uncertainty about the mechanisms by which periphyton might influence macrophyte growth: With our experimental setup we have only tested for *J. bulbosus* growth inhibition through competition for nutrients and/or light. In lakes, on the other hand, periphyton might also compete for CO_2 (Sand-Jensen & Borum, 1984), an effect our experimental setup did not allow. This potential CO_2 competition could be somewhat counterbalanced by *J. bulbosus*' ability to take up CO_2 through the roots (Roelofs, Schuurkes & Smits, 1984; Wetzel, Brammer, Lindström *et al.*, 1985), but such an effect on *J. bulbosus* growth might be more evident in the field than what we were able to simulate in our experiment; especially in waters with a short supply of CO_2 and massive periphyton growth.

We found no significant differences in *J. bulbosus* growth between the four treatment groups (Fig. 10), thus the experiment gave no support for elevated N deposition levels as a primary trigger for *J. bulbosus* nuisance growth. Unfortunately, while the treatment groups' N concentrations were significantly different (approximately 65 vs. 80 µg N/L), the rather high background levels of NO_3 from the source water made it impossible to achieve really low N concentrations as a contrast to the elevated N treatments. We thus conclude that a difference of 65 vs. 80 µg N/L did not induce significant differences in *J. bulbosus* growth, but we cannot, however, exclude that a larger difference would have induced growth differences.

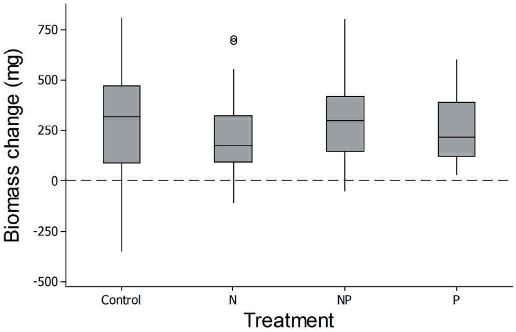

Fig. 10 Change in *Juncus bulbosus* biomass (n = 93) in response to different nutrient treatments during a three month growth experiment. Boxes show 1st and 3rd quantiles, horizontal lines mark the median and hollow circles mark outliers. Dashed line indicates zero biomass change (paper III).

In addition to measuring periphyton and *J. bulbosus* growth, we also measured sediment and water NO_3, NH_4 and PO_4 concentrations at the end of the experiment. From this we found that sediment NH_4 concentrations decreased significantly with increasing *J. bulbosus* growth (Fig. 11). We believe it is more likely that the lower sediment NH_4 concentrations in the larger plants were a result of growth-proportional uptake, rather than NH_4 having a negative effect on *J. bulbosus* growth. As we could not relate the growth differences to any of the other measured factors, it is possible they are the result of some factor we have not accounted for. However, these differences could also simply be a result of minor inequalities in starting conditions (genetic relationships, nutrient storage, root size etc), which is always a source of variation when working with natural systems.

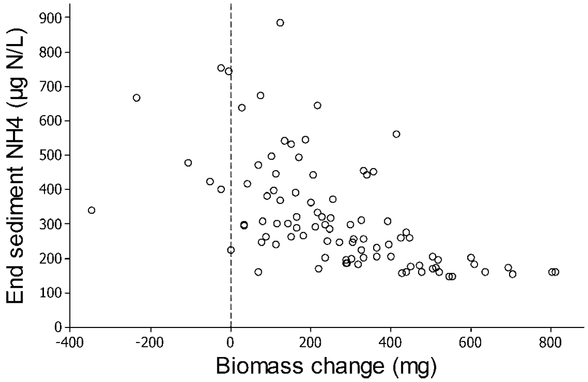

Fig. 11 Sediment ammonium concentration (in µg N/L) in response to *Juncus bulbosus* change in biomass (in mg; n = 93) after a three month growth experiment. Dashed line indicates zero biomass change (paper III).

From mass balance calculations (see paper III), we found that the initial NH_4 concentrations in the sediments most likely were far too low for the plants to have achieved the biomass increases observed. We conclude that a substantial amount of the N taken up by the plants must have been NO_3 from the water column. Thus, although *J. bulbosus* has been shown to prefer NH_4 over NO_3 (Schuurkes, Kok & Denhartog, 1986), more NO_3 than NH_4 was assimilated in our experiment. We believe this to be a result of NO_3 being more readily available due to its higher concentration as well as the accessibility of surface water nutrients in comparison to sediments nutrients: The air bubbled into the beakers caused a continuous mixing of the water, reducing any nutrient depleted layer around the leaf surface to a minimum. As a consequence, both NO_3 and NH_4 concentrations in the water were reduced to virtually zero between the weekly nutrient additions. In contrast, the sediment was left undisturbed during the course of the experiment, and the low diffusion rate in water most probably led to nutrient depleted zones around the root surfaces. However, water nutrient concentrations obviously were not enough to support the observed *J. bulbosus* growth, since there was a negative correlation between sediment NH_4 concentrations at the end of the experiment and *J. bulbosus* growth. Thus we believe that in oligotrophic waters, growth could be reliant on a readily available pool of sediment NH_4.

3.4 *Juncus bulbosus* nutrient stoichiometry (paper IV)

Different plant species have different allocation rules for nutrients, and also widely different elemental ratios. *J. bulbosus* is adapted to very nutrient poor conditions and has shown massive nuisance growth in ultra-oligotrophic lakes (Fig. 7). We thus hypothesised that its nutrient content would be very low, and our survey, covering a large number of observations, confirmed this: we found that *J. bulbosus* was high in C and low in N and P. Indeed, comparing with a wide range of other macrophytes and plant groups (Table 2), *J. bulbosus* had the lowest N content of all plant groups included in this comparative survey, and far lower than the average of macrophytes (freshwater angiosperms). The mean C content and C:N ratio of *J. bulbosus* were somewhere in between those of terrestrial plants and macrophytes, probably reflecting the terrestrial origin and physiological properties of this species. Macrophytes had on average nearly twice the tissue-specific concentrations of P compared to *J. bulbosus*, and the average C:P ratio of *J. bulbosus* was exceptionally high (1067 by atoms), more than three times the average of macrophytes in general (339) and higher than all the other plant groups in Table 2.

Table 2 Mean carbon, nitrogen and phosphorus content (as % of dry weight) and element ratios in *Juncus bulbosus* from 112 Norwegian lakes in comparison to other studies and plant groups (paper IV).

Plant Type	C (%)	N (%)	P (%)	C:P	C:N	N:P	Reference
Phytoplankton[a]	34.1	5.5	1.14	77	7	11	Duarte 1992
Redfield ratio	-	-	-	106	7	16	Redfield 1958
Freshwater angiosperms[a]	38.0	2.4	0.29	339	18	18	Duarte 1992
Seagrass[a]	33.5	1.9	0.24	361	21	18	Duarte 1992
Macroalgae[a]	24.8	1.9	0.10	641	15	42	Duarte 1992
Terrestrial plants[b]	50.7	1.8	0.25	740	44	18	Mysterud *et al.*, 2011
J. bulbosus 112 lakes	45.7	1.7	0.16	1067	33	31	Our findings
J. bulbosus 1 lake[a]	-	2.2	0.02	-	-	255	Aulio 1987a
J. bulbosus 6 limed lakes[a]	-	1.5	0.10	-	-	33	Roelofs *et al.*, 1994
J. bulbosus 4 unlimed lakes[a]	-	1.6	0.30	-	-	12	Roelofs *et al.*, 1994

[a] Element ratios are calculated based on mean dry weight C, N and P content.

[b] Based on raw data of averages from 233-266 plants of each species.

The high C to N and P ratios of *J. bulbosus* allows it to build large biomasses on small amounts of nutrients. This could give it a competitive advantage under nutrient poor conditions, and it might help explain the success of this species in oligotrophic softwater lakes. In addition, herbivores generally prefer plants with relatively low C:N and C:P ratios (e.g. Moran & Hamilton, 1980; Moran & Bjorndal, 2007; Dorenbosch & Bakker, 2011), such that *J. bulbosus* presumably is also “low quality food” for grazers.

Autotrophs generally are not very homeostatic, i.e. their elemental content should to a large extent reflect ambient, available nutrient concentrations and ratios. Hence if N concentrations and N:P ratios affect growth form, one might expect a higher N content in plants from areas with high N deposition and nuisance stands compared to plants from non-nuisance areas, and correspondingly also elevated N:P-ratios. However, we found no such differences (or any other differences), and concluded that, to the extent that elevated N deposition has caused increased *J. bulbosus* growth in Southern Norway, it was not reflected in plant stoichiometry. In contrast to our hypothesis, Roelofs *et al.* (1984) concluded that CO_2 was the most important factor governing growth of *J. bulbosus*, yet we did neither find significantly higher C contents in the nuisance plants compared to the non-nuisance plants. One should keep in mind, however, that while growth may initially be stimulated by either nutrients or inorganic C, this may not be reflected in the final elemental content of the massive stands (due to e.g. growth by dilution).

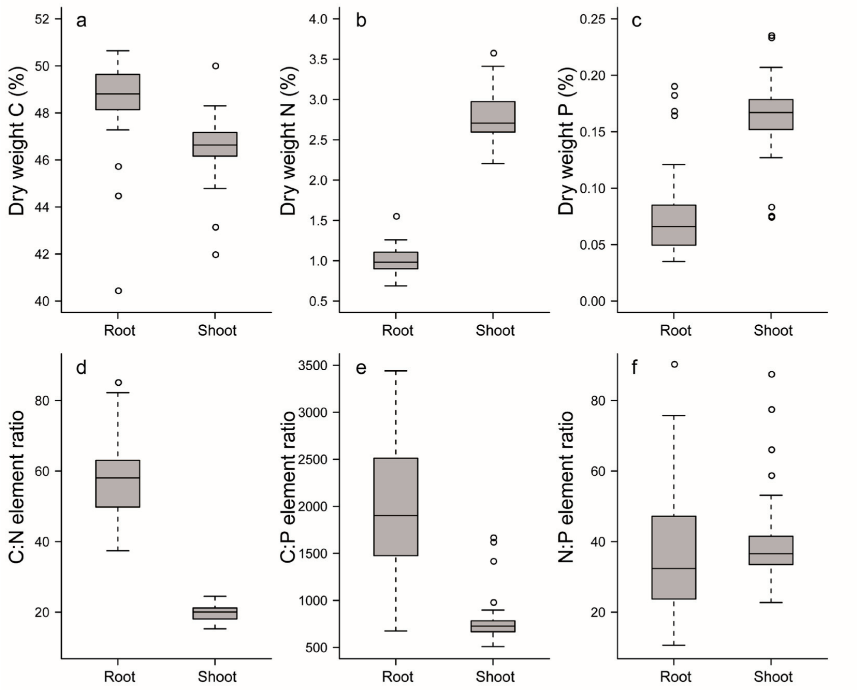

Fig. 12 Differences in *Juncus bulbosus* root compared to shoot a) carbon (C), b) nitrogen (N) and c) phosphorus (P) content (% of dry weight) as well as d) C:N, e) C:P and f) N:P element ratios of 62 plants from Lake Breisjøen, Oslo, Norway 2010. All differences are tested with Mann Whitney U-tests and are significant at $p = 6.9 \times 10^{-9}$ or lower (except N:P element ratio; $p = 0.11$). Lower and upper box boundaries and internal lines indicate 1st and 3rd quantiles and median, respectively. Dotted lines mark min and max values, extreme observations are marked with circles (paper IV).

As hypothesised, *J. bulbosus* roots exhibited a higher C content than shoots, reflected also in higher C:N and C:P ratios (Fig. 12). Roots usually serve as a storage tissue, and in *J. bulbosus*, the higher root C content might in addition reflect its ability to form bulbs in the root section (assumed to be C-rich storage material). Furthermore, we found that the relative nutrient content in the shoots was higher than in roots. This probably reflects that N and P are important components of photosynthesis. The pronounced differences between root and shoot nutrient contents and element ratios observed in this study show the importance of analysing these plant parts separately. Especially when comparing small and large plants, there will be a bias due to the relatively more important root section of the smaller plants. This would result in too high estimates of C:N and C:P ratios in smaller plants compared to larger plants, and could as such mask potential differences between these two groups.

There were no significant differences between river and lake plants with respect to P and C content, but we found more N (and a lower C:N ratio) in the river plants compared to the lake plants (Fig. 2 in paper IV). This was also found in paper II, and is discussed in more detail in section 3.2.

4. DISCUSSION

4.1 N deposition and periphyton abundance

What is most striking from the collection of papers in this study is probably the lack of significant results. Based on observational studies in 153 lakes and 28 river locations, as well as a growth experiment with 100 plants and studies of elemental compositions, we find no support for our initial hypotheses: There are no significant differences in N deposition, plant N content or any of the water or sediment N parameters measured in nuisance compared to non-nuisance lakes or rivers (papers I, II and IV). Also, the growth experiment showed no significant differences in growth between the N and non-N treatment groups (paper III). Thus, the overall picture is that increased N deposition, with resulting alterations in N:P stoichiometry is probably not the reason for the massive *J. bulbosus* growth we observe today.

A secondary hypothesis of this study was that increased periphyton abundances would lead to a decline in *J. bulbosus* nuisance growth. This was tested experimentally in the growth experiment, as well as in rivers and lakes studies (papers I, II and III). In contrast to earlier hypotheses (Mjelde, 2004), however, shading or competition for nutrients or CO_2 by periphyton did not seem to hamper *J. bulbosus* nuisance growth. Svedäng (1990) suggested that *J. bulbosus* avoid competition from periphyton by starting the growing season very early in spring, when the CO_2 levels are usually high (Kelly, Fee, Ramlal *et al.*, 2001) and periphyton are not yet present in large amounts. But although we cannot exclude that very high periphyton biomasses might impact *J. bulbosus* growth, our growth experiment showed that *J. bulbosus* can sustain relatively high periphyton abundances without reducing growth (paper III).

We found no support for any of our main hypotheses, but we tested a range of other parameters as well, both in rivers and lakes (e.g. Table 1). As with the N deposition hypothesis, we tested for differences in these parameters between nuisance and non-nuisance sites. But even with an extensive list of parameters to test, covering catchment characteristics, water and sediment chemistry and plant elemental composition, we failed to come up with any models or single parameters that could significantly explain *J. bulbosus* nuisance growth. We did, however, isolate some parameters which we believe are more likely than others to play a role in *J. bulbosus* nuisance growth.

4.2 Inorganic carbon - CO_2

From the lake and river studies (papers I and II), we could not detect any effects of CO_2 or DIC concentrations in ambient water on growth of *J. bulbosus*. In Southern Norway, intense *J. bulbosus* growth is generally observed in soft water lakes with low buffer capacities, and most of these lakes became acidified during the past decades (Schartau, Fjellheim, Walseng *et al.*, 2011). To counteract the acidification process, many of these lakes have been limed and some are still being limed today. As the lime dissolves, lake pH and decomposition rates increase, and this again leads to a temporary increase in CO_2 levels. Like isoetids, *J. bulbosus* cannot use bicarbonate, but is reliant on CO_2 for photosynthesis (Roelofs *et al.*, 1984; Maberly & Madsen, 2002). Macrophyte production in softwater lakes is often limited by carbon (Maberly & Madsen, 2002), and increased CO_2 supply has previously been suggested as the most likely primary trigger for *J. bulbosus* nuisance growth (Roelofs *et al.*, 1984). In our study, however, we found no direct support for increasing CO_2 levels (or liming or acidification) to be important for nuisance growth (papers I and II). Indeed, if anything, there was a negative relationship between *J. bulbosus* growth and CO_2 in the lake study (Table 1).

A general problem when comparing plant mass with potentially limiting elements is of course that positive correlations could indicate a causative relationship, but so could a negative correlation – if the nutrients have already been incorporated into plant biomass.

Hence a lacking or even slightly negative correlation with CO_2 could simply reflect that more CO_2 is fixed by photosynthesis in these high plant biomass areas ("ghost of uptake past").

Furthermore, CO_2 could to a large extent be obtained from the sediments, a parameter we did not analyse in our study. Sediment CO_2 is the most important C-source for most isoetids (Smolders, Lucassen & Roelofs, 2002), but Winkel & Borum (2009) showed that also many non-isoetid macrophytes relied heavily upon sediment CO_2 for C-uptake. Given ample supplies, Roelofs *et al.* (1984) found that *J. bulbosus* CO_2 uptake was mainly through the leaves. However, given more moderate supplies, a substantial amount of the CO_2 fixed by *J. bulbosus* can come from root uptake (Wetzel *et al.*, 1985). When it comes to nutrients and macrophytes, root uptake is most important in oligotrophic waters, whereas shoot uptake proliferates in eutrophic waters (Rattray, Howard-Williams & Brown, 1991). Such supply dependent uptake could very well also apply to CO_2 uptake, and as CO_2 concentrations in oligotrophic softwater lakes usually are up to 100-fold higher in sediments compared to the overlying water (Smolders *et al.*, 2002), sediment CO_2 could potentially be an important factor influencing growth of *J. bulbosus*.

From paper II we also had some indications that C could be important for nuisance growth in lakes, and the overall picture when combining our findings with the relevant literature is that CO_2 is the most likely primary trigger for *J. bulbosus* nuisance growth in lakes. In earlier works, however, acidification and lake liming were assumed to be the reasons for the increased CO_2 supplies to nuisance lakes (e.g. Roelofs *et al.*, 1994; Brandrud, 2002). As stated in the introduction, however, these explanations are not sufficient today.

One possible explanation for enhanced CO_2 supply to *J. bulbosus* in nuisance lakes might be an increased mineralization of organic matter. Both the present recovery from acidification (Skjelkvåle, Borg, Hindar *et al.*, 2007) and the observed increases in dissolved organic carbon (DOC) concentrations (Monteith, Stoddard, Evans *et al.*, 2007) in southern Norway could account for such a process (paper II). If this is the case, we might witness a general shift in the macrophyte vegetation of ultra-oligotrophic softwater lakes, from slow growing isoetids (which hardly increase growth rates with increasing CO_2 levels; Spierenburg, Lucassen, Lotter *et al.*, 2009) towards a dominance of the faster growing *J. bulbosus*.

In addition, annual variations in lake CO_2 concentrations should also be considered. Svedäng (1990) suggested that *J. bulbosus* has the capacity to effectively utilize the rich CO_2 supply which is often observed in lakes in early spring (Kelly *et al.*, 2001). Consequently, high spring CO_2 concentrations in nuisance lakes, possibly resulting from increased mineralization of organic matter, might have stimulated *J. bulbosus* growth.

4.3 Phosphorus and nitrogen

J. bulbosus nuisance growth could potentially be a stage in a general succession of oligotrophic lakes or rivers turning eutrophic. However, we found no signs of *J. bulbosus* nuisance growth being more abundant in nutrient rich compared to nutrient poor sites (papers I and II), and the growth experiment showed no differences in growth between the P and non-P treatment groups (paper III). Furthermore, we found no differences in P content between nuisance and non-nuisance sites (paper IV), and these results are supported by the findings of Roelofs *et al.* (1984), who reported that phosphate and/or ammonium enrichments did not lead to increased *J. bulbosus* growth in their experiments. Indeed, our data on the macrophyte trophic index (TIc) suggested that the most troublesome growth forms occurred in the most oligotrophic lakes (Fig. 7). The implications of this will be discussed further in section 4.4.

In general, however, all the surveyed lakes were nutrient poor (Table 1), and due to the remarkably high C:P and C:N ratios in *J. bulbosus* (paper IV); this species is capable of building large biomasses on low concentrations of P and N. This point towards the conclusion that elevated nutrient supply is not the primary reason behind the large *J. bulbosus* biomasses we now observe, or that that the increase in nutrient supplies is too small to be detected by our snapshot survey.

However, the growth experiment revealed a significant negative relationship between *J. bulbosus* growth and sediment NH_4, indicating that the plants with the highest biomass increase also absorbed the highest amounts of sediment NH_4. NH_4 is the preferred form of N for *J. bulbosus* (Schuurkes *et al.*, 1986), and Roelofs *et al.* (1995), Lucassen *et al.* (1999) and Brandrud (2002) have all indicated that NH_4 is an important factor for *J. bulbosus* nuisance growth. That we did not find any such relationships in our lake survey (paper I) could be explained by either “the ghost of uptake past” (see section 4.2) or by NH_4 not being the primary limiting factor for growth in Norwegian lakes. From paper II we concluded that the latter could possibly be the case: When comparing rivers and lakes, we found indications of NH_4 being important for nuisance growth in rivers, whereas CO_2, as concluded in section 4.1, seems to be a more plausible factor when explaining nuisance growth in lakes.

Such a potential difference in the triggers of nuisance growth between rivers and lakes could be explained as follows: CO_2 diffuses very slowly in water, and lake plants can easily become C-limited due to boundary layer depletion zones around the shoots (described by Ruttner, 1940; cited from Elster, 1962). In rivers, on the other hand, the water is in constant motion, mixing the upper, air saturated water with the rest of the water body, such that CO_2 limitation in river plants seems less plausible. An indication of what could be the primary limiting factor in rivers was found from the growth experiment (paper III): as we bubbled air down into all the beakers, none of the plants should have experienced CO_2 limitation. This is analogous to the conditions in rivers. And from the growth experiment, we found that the only factor that could be related to *J. bulbosus* growth was NH_4. Furthermore, we found a higher N content (and lower C:N ratio) in river plants compared to lake plants (Fig. 2 in paper IV), which could also indicate an importance of nitrogen-nutrition for *J. bulbosus* growth in rivers. Finally, sediment (and to a lesser extent water) NH_4 concentrations tended to be higher in nuisance river sites than in non-nuisance river sites (albeit not significant, Table 1 in paper II).

In summary, our results fit published literature on *J. bulbosus*, and indicate that increased biomasses of *J. bulbosus* may be a result of enhanced NH_4 supplies (in rivers). In contrast to earlier works, however, which directly assumed increased N deposition to be responsible for increased water NH_4 concentrations and *J. bulbosus* nuisance growth (Schuurkes, Elbers, Gudden *et al.*, 1987), we did not find any direct influence from N deposition. Instead, increased river NH_4 concentrations could be due to more direct causes, e.g. treated wastewater in rural areas (scattered settlement in rural areas, with concomitant small-scale wastewater treatment is a common phenomenon in Norway, see e.g. Paruch, Maehlum, Obarska-Pempkowiak *et al.* (2011)), or runoff from cattle grazed areas (we did in fact observe cattle grazing in the immediate surrounding of at least some of the river nuisance sites). Increased NH_4 supply might also be a result of reduced flow velocities in weir basins, leading to enhanced sedimentation of nutrient-rich material and a concomitant increase in supply of sediment NH_4 (paper II). Irrespective of NH_4 origin, however; the initially enhanced *J. bulbosus* biomass will likely start a positive feed-back mechanism: as dense stands are known to trap more fine sediment, this will lead to an even better supply of nutrients to the plants.

4.4 Macrophyte competition

Analyses of plant elemental composition revealed that *J. bulbosus* had a high C and low N and P content. Comparing with a wide range of other macrophytes and plant groups (Table 2), *J. bulbosus* had the lowest N content and by far the highest average C:P ratio

(1067, more than three times the amount of C per P compared to the average for macrophytes). The high C to N and P ratios of *J. bulbosus* allows it to build large biomasses on small amounts of nutrients. This could give it a competitive advantage under nutrient poor conditions, and indeed, we observed the most extensive nuisance growth in the most oligotrophic lakes (Fig. 7). This can probably be explained by a lack of competition from other macrophytes in these lakes, as macrophyte vegetation (apart from *J. bulbosus*) in the most nutrient poor softwater lakes is usually dominated by slow growing isoetids. Both isoetids and *J. bulbosus* are adapted to very low nutrient availabilities, and they both use CO_2 as their only carbon source (Roelofs *et al.*, 1984; Maberly & Madsen, 2002; Smolders *et al.*, 2002). However, *J. bulbosus* has a higher intrinsic growth rate and a higher affinity for CO_2 than isoetids (Roelofs *et al.*, 1984; Madsen, Olesen & Bagger, 2002), such that a slightly increased CO_2 supply will likely be more advantageous to *J. bulbosus*. If the nutrient and DIC availability increases, however, there will be increased competition from other macrophyte species such as *Potamogeton* sp., *Elodea* sp., *Callitriche hamulata* or *Nuphar lutea* (Murphy, 2002; Spierenburg *et al.*, 2009). These species can inhibit *J. bulbosus* growth in all but the very most oligotrophic, low DIC lakes.

Additionally, herbivores generally prefer plants with relatively low C:N and C:P ratios (e.g. Moran & Hamilton, 1980; Moran & Bjorndal, 2007; Dorenbosch & Bakker, 2011). *J. bulbosus*, with its low “stoichiometric value”, should thus generally be avoided by selective grazers, especially if other more nutrient rich macrophytes are present. To which being nutrient poor and carbon rich should be seen as a strategy of this plant, or simply reflects the fact that it is a terrestrially adapted plant that has rather recently entered the aquatic realm, may however be open to discussion.

4.5 Negative results

Despite a range of parameters and models tested, we failed to come up with a model that could offer a satisfactory explanation to the differences in *J. bulbosus* growth forms. Neither did the AFLP-screening reveal genetic differences consistent with the different growth forms or abundances. There were small scale geographical patterns in the *J. bulbosus* plant material, but no correlation between *J. bulbosus* nuisance growth and AFLP phenotype. We cannot rule out that there could be key ambient drivers that were not included in our survey (e.g. sediment CO_2). But still, this lack of consistent trends even with the long list of parameters at hand is striking, and reflects a general problem of multivariate ecosystem based analysis in ecology; it is often hard to arrive at strong conclusions with regard to key forcing parameters. This again raises intriguing questions about apparently stochastic responses, hidden interactions between variables or simply matters of response times and resolution. By and large, nutrient concentrations usually reflect the general productivity of a system (Hessen, Andersen, Brettum *et al.*, 2003). However, for instance with regard to nutrients and CO_2, a snapshot study such as ours cannot account for the "ghost of uptake past". These nutrient concentrations represent the chemical situation of a particular lake at a particular moment in time, but fail to say anything about nutrient dynamics/supply, and to what extent nutrients are allocated into plant and animal biomass. This could also be illustrated by the fact that there were no correlations higher than $r = 0.3$ between the elemental compositions C, N and P in the *J. bulbosus* plants and water or sediment chemistry at the different sampling sites (data not shown), indicating that potential differences in supply are masked due to rapid incorporation into biomass. Furthermore, we may have performed sampling in the midst of an ongoing expansion of the species within this region, so that small stands simply may reflect early successions after recent colonization. If so, the full response or potential of the plant within a given locality will only be realized after some years.

5. CONCLUSIONS

This study shows no support for the hypothesis of elevated N deposition and resulting skewed N:P ratios promoting nuisance growth of *J. bulbosus*. The same is true for our hypothesis about increased periphyton abundance. We have however isolated some parameters which we believe are more likely than others to play a role in *J. bulbosus* nuisance growth. Collectively, our results are consistent with earlier studies which conclude that CO_2 is the most likely primary trigger for *J. bulbosus* nuisance growth in lakes. On the other hand, we found indications that NH_4 might be the primary limiting factor in rivers. Also, we believe that the elemental allocation and stoichiometry of this plant may explain some of its success when it comes to competition, building large biomasses in nutrient poor systems and at the same time being unattractive to grazers.

Despite mainly "negative" conclusions, we can now exclude a range of candidate parameters for *J. bulbosus* nuisance growth. We have shown that variations in *J. bulbosus* growth is not due to genetic differences, and it is probably also not a direct result of N deposition or due to large differences in climate, light or nutrients. Since we measured concentrations rather than supply, we cannot exclude the possibility that small variations in nutrient supply and/or (especially sediment) CO_2 might be important. These issues can only be settled through controlled experiments and long term monitoring (throughout the whole vegetation period) of preferably oligotrophic, isoetid and/or *J. bulbosus* dominated lakes, including separate analyses of water and sediments. Together, this should put us in a better position to answer what influences *J. bulbosus* growth over time, and what management strategies should be applied to resolve the present problems of *J. bulbosus* nuisance growth.

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Nuisance growth of *Juncus bulbosus* related to catchment characteristics, lake water and sediment chemistry

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Key words:

Macrophyte, bulbous rush, AFLP, nutrient, catchment

Abstract

Nuisance growth of the freshwater macrophyte *Juncus bulbosus* has become a large-scale problem in many lakes and rivers in northern Europe, strongly affecting biodiversity and human use, not the least hydroelectric power plants. The causes of the proliferation of these massive stands of *J. bulbosus* are not finally settled, however. In this study, a wide range of catchment, lake and sediment parameters were collected from 153 lakes in Southern Norway, with the aim to explain presence or absence of *J. bulbosus* and to assess potential drivers behind the nuisance growth. However, despite the extensive number of parameters from a wide range of lakes across environmental gradients, we were unable to detect any general drivers that could explain nuisance growth. Neither did the strong gradient of N-deposition, climate, light nor nutrients generate consistent patterns in growth forms or abundances. Furthermore, a genetic screening (AFLP fingerprinting) showed no genetic differences between the various growth forms. Based on a macrophyte index, however, we found that the most problematic nuisance growth occurred in the most oligotrophic lakes. The lack of consistent patterns may either reflect drivers that were not covered by our survey, or reflect that the current extension of stands represents a cumulative response over time, not traced by our snapshot survey. The upside of these “negative” conclusions from our survey, however, is that we can now exclude several candidate parameters as the causes for nuisance growth.

Introduction

Juncus bulbosus L. is a perennial plant native to Europe and North Africa (Prockow, 2008a), which can inhabit both terrestrial and aquatic habitats (Prockow, 2008b). The aquatic form of *J. bulbosus* initially grows as a small rosette of 10-20 cm length, but under certain conditions side branches emerge, bearing new “budding” rosettes of 5-80 cm length (Johansen, Brandrud & Mjelde, 2000). Multiple years of accumulating such new side branches (without winter dieback) can result in dense stands of *J. bulbosus*, with individual plants reaching a length of up to 2-3 m (Hindar, Johansen, Andersen *et al.*, 2003; Johansen *et al.*, 2000).

Since the late 1980’s, nuisance growth resulting in massive stands has occurred in an increasing number of European lakes and rivers (Aulio, 1987; Brandrud, 2002; Roelofs, 1983; Svedäng, 1990), with *J. bulbosus* becoming the dominating macrophyte species in many of these ecosystems (Fig. 1). Among the consequences of such nuisance growth are reduced biodiversity, reduced suitability of the ecosystems for fish spawning, clogging of hydropower inlet screens and reduced suitability of the ecosystems for recreational use such as fishing, boating and bathing. Mechanical removal of the plants is not only laborious and costly, but it also only deals with the effects, not the cause of the nuisance growth, and re-growth is always observed within few years (Brandrud & Johansen, 1997).

Fig. 1 *Juncus bulbosus* nuisance growth in Norwegian lakes and rivers. Photos: Edgar Vegge, Tor Kviljo and Liv-Bente Scancke.

Several hypotheses have been forwarded to explain the massive increase in *J. bulbosus* biomass in rivers and lakes, the most common being hydropower development with resulting alterations in hydrology and ice cover (Rørslett, 1987; Rørslett, 1990; Johansen, 1993; Hindar *et al.*, 2003; Johansen *et al.*, 2000), increased water temperatures (Johansen, 1993; Rørslett, 1987; Hindar *et al.*, 2003), and acidification, liming and reacidification coupled with an increase in CO_2 and sediment ammonium and phosphorus (Roelofs, Brandrud & Smolders, 1994; Roelofs, Smolders, Brandrud *et al.*, 1995; Aulio, 1987; Lucassen, Bobbink, Oonk *et al.*, 1999; Svedäng, 1992). However, in Norway we find massive *J. bulbosus* growth in waters both with and without hydropower development, in both low lying and higher altitude regions, and in both limed and unlimed lakes and rivers, such that a consistent explanation for *J. bulbosus* nuisance growth in both rivers and lakes is still lacking.

In this study, we focus on lakes, and the main objectives were threefold; 1) to determine key factors explaining presence or absence of *J. bulbosus* in Norwegian lakes; 2) to explain the occurrence of different *J. bulbosus* growth forms and their abundances in these lakes; and 3) to assess whether genetic differences in *J. bulbosus* can account for its different growth patterns. To address these issues, we conducted a survey of 153 lakes, covering major geographical and water quality gradients in Southern Norway. In the surveyed lakes, we collected data on *J. bulbosus* growth forms, macrophyte vegetation, catchment characteristics, periphyton coverage, lake water chemistry as well as sediment characteristics and chemistry. Additionally, we collected plant material, which was later screened for genetic affinities by use of amplified fragment length polymorphism (AFLP), to explore whether the differences in *J. bulbosus* growth forms could be due to genetic differences.

Methods

Field work

This study is based on a synoptic survey of 153 lakes in Southern Norway during autumn 2007 (Fig. 2). In each lake, *J. bulbosus* growth forms (rosette plants/small columns with annual shoots/large columns with annual shoots/surface mats; Fig. 3) and abundances (0 = not present; 1 = sparsely vegetated; 2 = covering large parts; 3 = dominating the lake) were estimated from a boat using an aqua-scope. Abundance of periphytic algae on *J. bulbosus* was estimated as 0 = no macroscopic algae visible, 1 = macroscopic algae clearly visible, and 2 =

J. bulbosus plants were covered with large amounts of filamentous algae. Presence of other macrophyte species was also noted. A sediment core of approximately 7 cm length was taken at the site of most prolific stands in each lake where the plant was present. The sediment samples were frozen on dry ice immediately after sampling and kept frozen until the analysis. Water samples were collected at approximately 10 cm depth within the area of highest abundance of *J. bulbosus* (if present). Water for CO_2 and dissolved inorganic carbon (DIC) analyses were collected in 125 mL gas-tight serum vials which were stored in lake water (in separate plastic containers) until analysed. 1 mL $HgCl_2$ was used as fixative for CO_2 vials to block biotic uptake and respiration. The remaining analyses were conducted on water sampled in 0.5 L acid-washed plastic bottles. The plastic bottles were stored cold until analysed; the glass bottles were stored at room temperature.

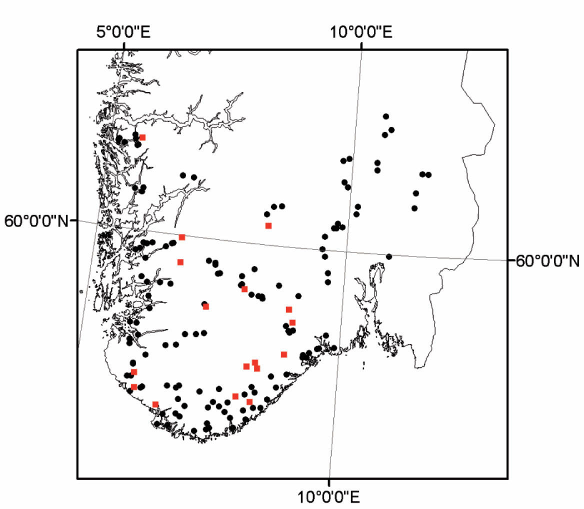

Fig. 2 The 153 lakes of Southern Norway sampled during summer/autumn 2007. Red squares indicate *Juncus bulbosus* nuisance growth as described in materials and methods (AFLP section). Black circles indicate lakes without *J. bulbosus* or with *J. bulbosus* non-nuisance growth.

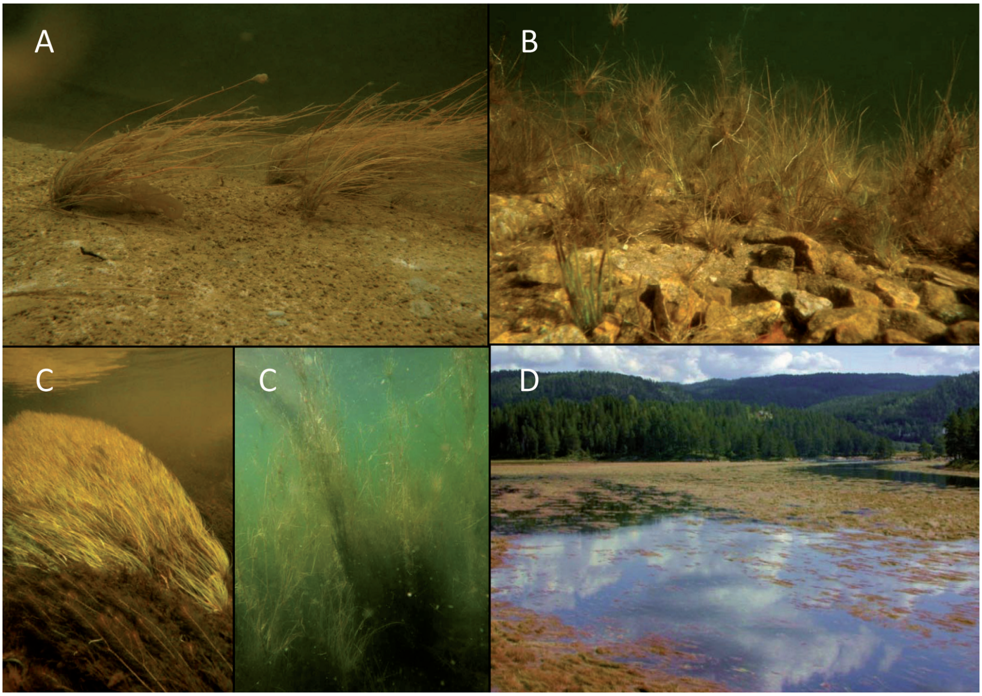

Fig. 3 Four categories of *Juncus bulbosus* growth forms: A) rosette plants; B) small columns with annual shoots; C) large columns with annual shoots; D) surface mats. Photos: T. F. Moe (A-C) and Edgar Vegge (D).

Water and sediment analyses

Lake water chemical parameters were analysed at the Norwegian Institute for Water Research (NIVA): pH was analysed on a Metrohm titrator model 799 GPT Titrino (Metrohm AG, Herisau, Switzerland) using the Norwegian Standard (NS) 4720. Conductivity was measured on a Metrohm Conductivity Meter (Metrohm AG, Herisau, Switzerland) (NS-ISO 7888). Calcium (Ca), nitrate (NO_3) and ammonium (NH_4) were analysed through ion chromatography on a Dionex DX320 with IonPac CS16/CG16 for cations and AS15/AG15 for anions (Dionex Corporation, Sunnyvale, California, US) (NS-EN ISO 10304-1 and NS-EN-ISO 14911). Concentrations below the detection limits were given the value of ½ the detection limit (< 1 µg N/L = 0.5 for NO_3 and < 2 µg N/L = 1 for NH_4). Total organic carbon (TOC), dissolved inorganic carbon (DIC) and carbon dioxide (CO_2) were analysed on a Dohrmann Phoenix 8000 TOC-TC analyser (Teledyne Tekmar, Mason, Ohio, US) according to NS-ISO 8245 for TOC, NS-EN 1484 for DIC and Standard Methods 4500-CO2, 4-12-4-18 for CO_2. Total nitrogen (TotN), total phosphorus (TotP) and phosphate (PO_4) were analysed on a Skalar San Plus autoanalyser (Skalar Analytical B.V., Breda, The Netherlands) according to NS 4743, NS 4725 and NS 4724, respectively. PO_4 concentrations below the detection limit (< 1 µg P/L) were given the value of 0.5. Dissolved Inorganic Nitrogen (DIN) was calculated as the sum of NH_4 and NO_3.

Sediment pore water was extracted from the thawed sediments in the lab through centrifugation and analysed for PO_4, NO_3, NH_4, water content and organic content. Pore water NH_4 was analysed using protocol B from Holmes, Aminot, Kerouel *et al.* (1999). Pore water NO_3 and PO_4 were analysed in an auto analyser with applications G-297-03 for PO_4 and G-172-96 for NO_3 (Auto analyser 3, SEAL Analytical/BRAN LUEBBE, Norderstedt, Germany). To account for sediment water content, we calculated sediment nutrient concentrations as pore water nutrients per volume sediment. We also tested pore water nutrients itself, but with similar results as sediment nutrients, so we have only reported the latter. Sediment water content was calculated as wet weight minus dry weight divided by wet weight. Dry weight was measured after drying the sediments at 105°C for 24 hours. Organic content was measured as dry free ash weight minus dry weight; dry free ash weight being measured after burning the dried sediment sample in a muffle furnace for 2h at 450°C and cooling the sample to room temperature in a desiccator.

Catchment data

To assess the roles of catchment properties and thus catchment related export to the lakes, the catchment boundaries for each investigated lake were delineated according to the procedures described in Larsen, Andersen & Hessen (2011a), and data on annual average temperature, precipitation, runoff and satellite derived normalized difference vegetation index (NDVI, an index describing vegetation cover) as well as data on terrain slope, area types and altitude were obtained according to Larsen, Andersen & Hessen (2011b). Atmospheric nitrogen deposition was averaged for each catchment from a digital map of yearly, accumulated total atmospheric nitrogen deposition (including dry deposition) for 1995. The nitrogen deposition map was constructed by spatial interpolation (kriging with a spherical semivariogram model) on 1° x 1° gridded output data from the Unified EMEP MSC-W modelling system (http://www.emep.int/). Data on solar, UVA and UVB irradiation (based on yearly averages of global horizontal irradiation for the period 1981-1990) were obtained from the Photovoltaic Geographic Information System (PVGIS) of the European Commission Joint Research Centre (JRC) (http://re.jrc.ec.europa.eu/pvgis/) (Súri, Huld & Dunlop, 2005). County governors assisted with information on liming status of all the lakes. Information on hydropower development was obtained from the Norwegian Water Resources and Energy Directorate (NVE).

AFLP analyses

Plant material was collected from all *J. bulbosus* lakes in 2007, and 14 lakes were revisited together with 27 river localities (from 15 different rivers) in 2008 and 2010. During the latter two sampling years, a total of 69 specimens of *J. bulbosus* were collected, fresh plant material being dried on silica gel to ensure high quality, non-degraded DNA. The 2007 material was not dried on silica gel, and preliminary analyses showed bad reproducibility of replicates. This material was not included in the final analyses, where the 69 silica dried specimens were analysed using amplified fragment length polymorphisms (AFLPs).

Each location from where we collected plant material was assigned to one of three *J. bulbosus* nuisance growth categories based on growth form abundances: All locations with surface mats/large columns abundance 3 were assigned to the “nuisance growth”-category (n = 15). Locations with surface mats/large columns abundance 2 and/or small columns/rosette plants abundance 3 were assigned to the “partly nuisance growth” category (n = 13). The remaining locations were assigned to the “no nuisance growth”-category (n = 41). Several other categorizations/quantifications were also tested, all with similar results (data not shown).

Silica-dried leaf tissue was crushed in 2 mL tubes with two tungsten carbide beads for 2 x 1 min at 20 Hz on a mixer mill (MM301, Retsch GmbH & Co., Haan, Germany), and DNA was extracted using the E.Z.N.A. Plant DNA Mini Kit (Omega Bio-tek, Norcross, Georgia, USA) according to the manufacturer’s manual. We performed the elution (50 µL buffer) twice in the same tube and used the first eluate in the second elution step to ensure high concentrate DNA. DNA concentration was measured with a spectrophotometer (NanoDrop ND-1000, Thermo Fisher Scientific, Wilmington, Delaware, USA), and diluted with MilliQ (MQ) water to approximately 50 ng/µL. Some samples had initial concentrations lower than 50 ng/µL and were used undiluted; in the few cases where the concentration was lower than 10 ng/µL, the samples were replicated through the whole AFLP procedure to check for reproducibility. Altogether, 31 samples were replicated to enable the estimation of an error rate.

The AFLP procedure followed Vos, Hogers, Bleeker *et al.* (1995) with several of the modifications implemented by Jørgensen, Elven, Tribsch *et al.* (2006). For adapter and primer sequences, see Vos *et al.* (1995). After a screening of selective primers, four primer combinations with two or three selective nucleotides were selected for the final analyses: 6FAM-*Eco*RI-ACC/*Mse*I-CA; NED-*Eco*RI-ACA/*Mse*I-CA; PET-*Eco*RI-AGA/*Mse*I-CAA; VIC-*Eco*RI-AGC/*Mse*I-CG). The 6FAM primer and all non-labelled primers and adapters were ordered from MWG (Ebersberg, Germany), the other labelled primers from Applied Biosystems (Carlsbad, California, USA).

Restriction-ligation (RL) of genomic DNA was done in one step, starting with digestion of genomic DNA by two restriction endonucleases, *Eco*RI and *Mse*I, followed by ligation of double-stranded *Eco*RI and *Mse*I adapters. The reaction mix (final volume 11 µL) contained 2 µL genomic DNA, 1.1 µL 10 x T4 DNA ligase buffer (Roche, Basel, Switzerland), 1.1 µL 0.5 M NaCl, 0.55 µL 1 mg/mL BSA (bovine serum albumin; New England Biolabs, Ipswich, Massachusetts, USA), 1 U MseI (New England Biolabs), 5 U *Eco*RI (Roche), 1 U T4 DNA ligase (Roche), 1 µL 10 µM *Mse*I-adapters, and 1 µL 10 µM *Eco*RI-adapters. The RL-mix was incubated for 3h at 37ºC in a Mastercycler epgradient (Eppendorf AG, Hamburg, Germany), and afterwards diluted 10-fold with MQ water.

The preselective amplification reaction mix (final volume 12.5 µL) contained 1.25 µL 10 x PCR buffer II (Applied Biosystems, Carlsbad, California, USA), 0.075 µL AmpliTaq (Applied Biosystems), 0.75 µL 25 mM $MgCl_2$, 1 µL 10 mM dNTPs (Applied Biosystems), 0.25 µL of each of the two preselective primers (10 µM; *Eco*RI-A, *Mse*I-C) and 1.5 µL diluted RL product. The fragments were amplified under the following PCR conditions: 2 min at 72ºC, 30 cycles each consisting of 30 sec at 94ºC, 30 sec at 56ºC, and 2 min at 72ºC, and one last hold of 10 min at 72ºC. The resulting PCR products were diluted 10-fold with MQ water.

The selective amplification reaction mix (final volume 10 µL) contained 1.25 µL 10 x PCR gold buffer (Applied Biosystems), 0.1 µL AmpliTaq Gold (Applied Biosystems), 1.25 µL 25 mM $MgCl_2$, 0.10 µL 10 mM BSA, 1 µL 10 mM dNTPs, 0.10 µL 10 µM *Eco*RI selective primer, 0.25 µL 10 µM *Mse*I selective primer, and 2.5 µL diluted preselective product. The PCR profile consisted of 10 min at 95ºC, 13 cycles each consisting of 30 sec at 94ºC, 1 min at 65-56ºC (the temperature decreasing 0.7ºC after each cycle), and 1 min at 72ºC, 23 cycles each consisting of 30 sec at 94ºC, 1 min at 56ºC, and 1 min at 72ºC, and a final10 min hold at 72ºC.

Of each selective PCR product, 2 µL were mixed with 11.7 µL HiDi formamide (Applied Biosystems) and 0.3 µL GeneScan Liz 500 size standard (Applied Biosystems), denatured at 95ºC for 5 min and cooled on ice. Electrophoresis of PCR fragments was performed on an ABI PRISM 3100 genetic analyser (Applied Biosystems).

Scoring of AFLP markers in the range of 60-500 base pair was performed using GeneMapper v. 3.7 (Applied Biosystems) and the semi-automated procedure described in Whitlock, Hipperson, Mannarelli *et al.* (2008), using the interactive R script "AFLPScore" version 1.4 in the statistical package R version 2.13.1 (R Development Core Team 2010). The method uses thresholds of peak height created by GeneMapper to exclude AFLP loci that are likely to contribute to high error rates, and determine the AFLP phenotype (fragment absence or presence) at the retained loci. The data were filtered to remove putative noise peaks by applying the phenotype-calling threshold prior to locus selection. Error rate analysis (mismatch error rate; (Pompanon, Bonin, Bellemain *et al.*, 2005)) is an integral part of this process. Markers that were present in or absent from only one sample (possibly owing to PCR errors) were removed.

The resulting presence/absence matrix was analysed using three different approaches in order to detect possible genetic structures of the 69 *J. bulbosus* samples: (1) principle coordinate (PCO) analysis, (2) neighbour networks, and (3) Bayesian clustering. PCO analysis was run in PAST v. 1.9.3 (Hammer, Harper & Ryan, 2001) using Dice similarity coefficient (Dice, 1945). NeighborNet analysis, also using Dice similarity coefficient, was performed in SplitsTree4 (Huson & Bryant, 2006). Bayesian clustering was performed in Structure 2.3.3 with the approach developed for dominant AFLP markers (Falush, Stephens & Pritchard, 2007; Pritchard, Stephens & Donnelly, 2000). We applied the admixture model with the recessive model with uncorrelated allele frequencies and did 10 replicate runs for each K from K = 1 to K = 18 on the freely available Bioportal, University of Oslo (http://www.bioportal.uio.no), using a burn-in of 1 x 10^5 iterations followed by 1 x 10^6 additional Monte Carlo Markov Chain iterations. The Structure outputs were summarized using the R-script Structure-sum v. 2011 (Ehrich, 2006; Ehrich, Gaudeul, Assefa *et al.*, 2007) and calculations of the log probability of data (LnP(D)). The similarity coefficient between different runs, and delta K were used to choose K. Altogether, 146 polymorphic AFLP loci were retained that had a mean mismatch error rate of 1.2 %.

Statistics

We observed *J. bulbosus* in 118 of the 153 lakes examined. In nine of these lakes, only a few small rosette plants were observed. In several of these cases, we observed only one single plant, some of these being observed in places like private docks and man-made beaches, where it is likely that the plants were accidentally introduced. To avoid misleading data we chose to exclude these nine lakes from all analyses. Also, four lakes were excluded because of lack of catchment data (we observed *J. bulbosus* in three of these lakes). Finally, one lake was excluded because the water samples were confounded during sampling. Thus in total, 139 lakes were finally included in the statistical analyses, 105 of which had *J. bulbosus* growth.

Statistical analyses were performed with R version 2.12.0 (R Development Core Team 2009), extended with the "vegan" package 1.17-5 (Oksanen, Blanchet, Kindt *et al*. 2010). We first computed a logistic regression model of presence/absence of *J. bulbosus*, with explanatory variables selected through forward selection with Bayesian Information Criterion (BIC) (Johnson & Omland, 2004). All catchment and water chemical parameters were included in this logistic model selection.

For the remaining analyses, we focused on differences in *J. bulbosus* growth forms observed in the 105 lakes where the species was present. Due to problems during sampling (sediments too rocky/organic/coarse/deep), the sample size of sediment characteristics was only 85, thus n = 85 for analyses including these parameters. As there is no obvious way of categorising *J. bulbosus* (nuisance) growth, we tested several different growth categorizations to parameterize our response variable (Table 1). The starting point for all of these approaches was the division of *J. bulbosus* into the observed growth forms (0-3; see field work section) and their abundances (0-3). But as all categorizations showed similar results, we have chosen to report only the results from using the "DCA 1-scores" as the response variable. Furthermore, we visually inspected graphic plots of all explanatory variables in relation to growth forms to look for non-linear relationships. As no obvious non-linear relationships were observed, all response variables were tested with linear and multiple linear regressions for significance (or logistic regression where the response variable was binary).

Table 1 Different ways of classifying *Juncus bulbosus* (nuisance) growth.

Response variable	Description	Regression
Nuisance vs. not nuisance growth	Nuisance = presence of surface mats/large columns	Logistic
Nuisance vs. not nuisance growth	Nuisance = presence of surface mats/large columns > 1	Logistic
Nuisance growth categories	Nuisance, partly nuisance, no nuisance	For genetics
Maximum growth forms	"Maximum" observed growth form (1-4)	Linear
Total abundances	Sum of abundances for all growth forms (1-7)	Linear
Total weighted abundances	Sum of weighted abundances for all growth forms, larger growth forms rated higher (1-21)	Linear
DCA1-scores	"Average" growth form abundances from DCA	Linear

The "DCA1-score" response variable was calculated through a Detrended Correspondence Analysis (DCA) (TerBraak & Prentice, 1988) on the abundances of the different growth forms of *J. bulbosus*. We used the DCA axis 1 site scores of each lake as the response variable for the multiple linear regressions (see results). As several lakes had the same growth form distributions/abundances, global nonmetric multidimensional scaling (GNMDS) was not applicable to our data set. We considered DCA to be the best alternative since we will not meet the prospective problem of a tongue effect (Økland, 1990) when we are only using DCA axis 1. Multiple linear regression model selection was conducted through backward selection with Bayesian Information Criterion (BIC) using the "step" function in R. We also tested single parameter models, and to avoid type II errors due to many tests we used Bonferroni correction with $\alpha = 0.05/n$ as our significance level (n = number of explanatory variables; in this case $\alpha = 0.05/34 = 0.0015$).

The models resulting from the multiple linear regression model selections all had very low explanatory power (max $R^2 = 0.21$, see results). To explore this further, we wanted to see how much of the variation in growth forms could be explained by random combinations of our explanatory variables. As a trade off between good explanatory power and making a too complicated model, we chose to combine four explanatory variables per model. To do this, we computed a loop that selected four of our variables randomly (n = 32 as we excluded liming and regulation), and this process was repeated 10.000 times, each time reporting the R^2-value of the model.

Our way of testing the nutrient content of the lake water gives a snapshot of the situation, and the resulting concentrations will be highly dependent on vegetation cover and phytoplankton abundance. Thus, to complement this picture, we wanted to make a parameter that could tell us something about the nutrient history of each lake. We did this by using the macrophyte index commonly applied in Norwegian lakes; TIc. The TIc was calculated based on presence/absence of indicator macrophyte species according to Vanndirektivet (2009), and it ranges from -100 (eutrophic) to +100 (oligotrophic). We excluded *J. bulbosus* as an indicator species, and the TIc was assigned to a total of 99 lakes (there were no other indicator species in the remaining six of the *J. bulbosus* lakes). We did not include this variable in the linear regression model as this would have reduced the number of observations from 105 to 99, but we ran a separate DCA on this subset of 99 lakes and tested the DCA axis 1 site scores against TIc according to the methods described for the multiple linear models above.

Results

Juncus bulbosus presence/absence

Of the 139 lakes analysed, *J. bulbosus* was found in 105 (rosette plants in 83, small columns in 103, large columns in 30 and surface mats in 10 lakes). Multiple logistic regression model selection of *J. bulbosus* presence/absence revealed increasing odds of finding *J. bulbosus* with decreasing pH and phosphate levels, and increasing DIN:TotP element ratio (R^2 = 0.29; Fig. 4 A-C). We also tried to include interactions between these three parameters to the model, but they were not significant (data not shown). PO_4 was weakly correlated to pH and DIN:TotP, whereas pH and DIN:TotP were not correlated (Table 2). We also tested all initial parameters separately, and the three parameters chosen in the multiple model were among the top four most significant single parameter models. The second most significant among the single parameter models was minimum temperature (Table 2), with higher minimum temperature being positively associated with *J. bulbosus* presence (Fig. 4 D). Minimum temperature was negatively correlated with pH and positively correlated to DIN:TotP (Table 2).

Table 2 Single parameter logistic models and correlation between the top four most significant parameters related to presence/absence of *Juncus bulbosus* in 139 Norwegian lakes 2007. Significant correlations are marked with asterisk (*).

	Single parameter logistic regression		Parameter pH		PO_4		DIN:TotP	
Parameter	r^2	p-value	r	p-value	r	p-value	r	p-value
pH	0.13	5.8E-05	-	-	-	-	-	-
PO_4	0.13	3.1E-05	0.23	0.0075*	-	-	-	-
DIN:TotP	0.12	8.9E-05	-0.14	0.098*	-	-	-	-
Min temperature	0.12	5.1E-05	-0.41	4.9E-07*	-0.16	0.058	0.44	4.1E-08*

Juncus bulbosus growth forms

A DCA of *J. bulbosus* growth forms and abundances arranged the four growth forms in an increasing order of "nuisance" along DCA axis 1 (left to right in Fig. 5 A). This means that for each lake, we can extract a site score of the DCA axis 1 (the x-coordinate for each lake in Fig. 5 B), giving us a number that can be used to denote the "level of nuisance growth" in that lake. The DCA1 site scores for each lake ranged from -0.67 to 1.68, with the lowest numbers denoting lakes with mainly small rosette plants, and higher numbers indicating mass abundances of *J. bulbosus* with mats and extensive coverage. These DCA-numbers were then

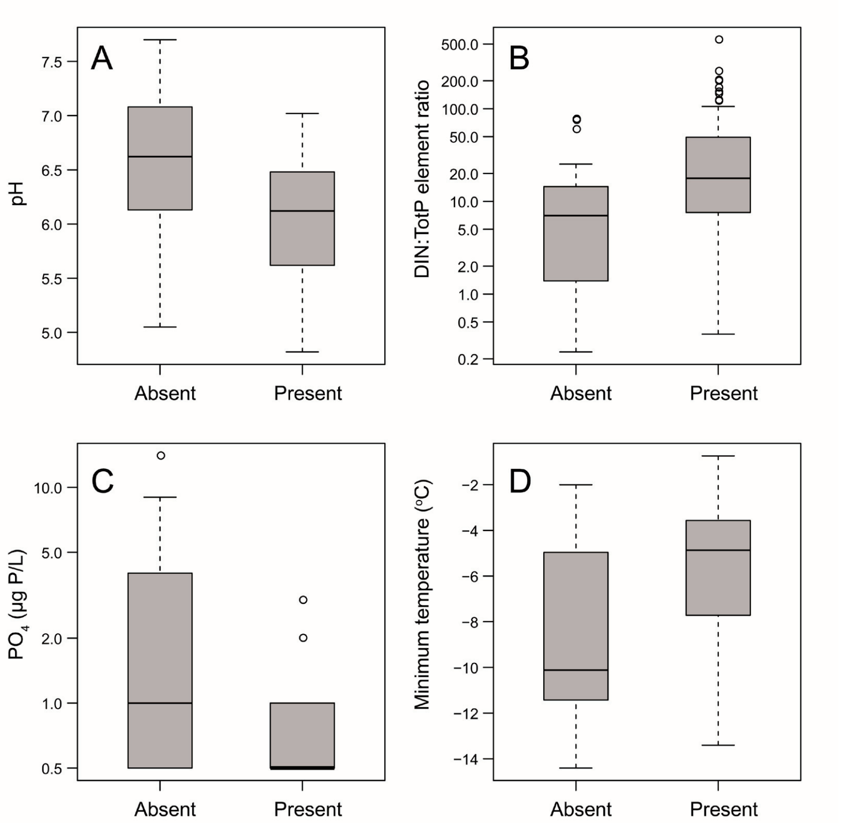

Fig. 4 Box plots showing significant differences in A) pH, B) DIN:TotP element ratio, C) PO_4 concentration (µg P/L) and D) minimum temperature (°C) in S Norwegian lakes in 2007 where *Juncus bulbosus* is absent (n = 34) compared to where it is present (n = 105). Boxes indicate 25 and 75 percentiles, with medians represented by a solid line, dotted lines indicating min and max values and outliers marked with open circles.

used as the response variable for a multiple linear model selection with the same initial explanatory variables as was the starting point for the logistic model, this time also including sediment characteristics and periphyton abundance (Table 3). However, no single parameter could account for the different growth forms observed (Table 3), and neither did a multivariate approach with backward selection provide robust predictions (robust in the sense that they do not change when making small alterations in initial parameters or observations included) for growth forms either (data not shown).

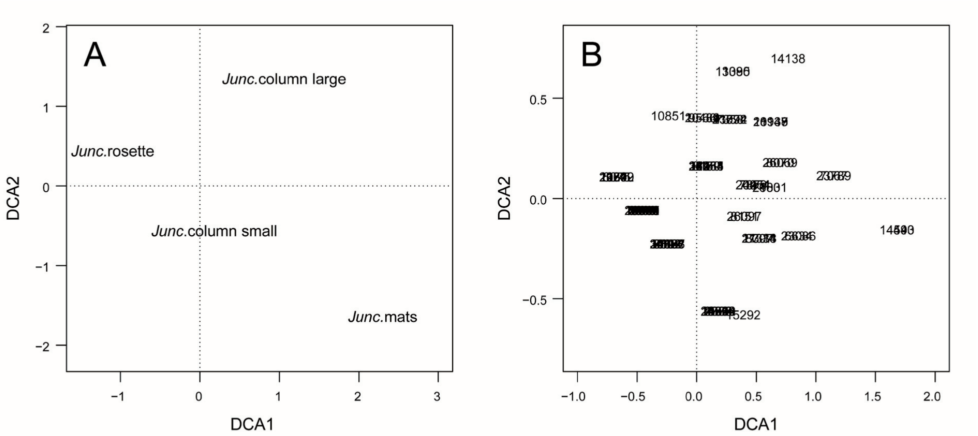

Fig. 5 DCA ordination of A) different growth forms of *Juncus bulbosus* and B) site scores for each lake (each number indicates a lake) from 105 lakes in S Norway 2007.

We also tested how much of the observed variation in growth forms that could be explained by *any* arbitrary combination of four of the explanatory variables from Table 3. The maximum R^2-value obtained from 10.000 random combinations of four of these explanatory variables was 0.21, a result not substantially better than a similar test with completely random, normally distributed numbers (max $R^2 = 0.15$). This strongly suggests that there is no obvious linear relationship between our measured environmental variables and *J. bulbosus* growth form abundances, and that changing the order in which the parameters entered the model would not have affected this result.

Finally, testing the macrophyte trophic index (TIc) on the subset of 99 lakes showed a positive, but not significant (at $\alpha = 0.0015$), relationship with *J. bulbosus* growth forms ($r^2 = 0.07$; $p = 0.0068$). Furthermore, we plotted the TIc against the growth forms (as DCA1-scores; Fig. 6), and while this plot was quite scattered, it suggests that the most troublesome growth forms occurred in the most oligotrophic lakes, with minor problems in the more eutrophic lakes.

Table 3 Basic information on the parameters included in the multiple linear models testing *Juncus bulbosus* growth in 105 Norwegian lakes 2007, sorted by R2-values. R^2 and p-values reported are from single-predictor linear regression models of *Juncus bulbosus* growth (as DCA1-scores, see text). Significance level with Bonferroni correction is 0.05/34 = 0.0015, thus no single-predictor models were significant. Effect indicates whether a parameter has a positive or negative relation to *J. bulbosus* growth. Non-parametric parameters were ln-transformed before model selection. Group indicates whether the data are collected on the basis of the lake or the lakes catchment area.

Parameter	Unit	Median	Mean	Range	R^2	p-value	Effect	n	Transformation	Group
Dissolved Inorganic Carbon (DIC)	mg/L	1.00	1.23	0.36-4.60	0.060	0.024	-	105	Ln	Lake
Phosphate (PO_4)	µg P/L	0.50	0.89	0.5-3.0	0.058	0.026	-	105	Ln	Lake
Total Organic Carbon (TOC)	mg/L	5.20	5.39	0.53-16.40	0.057	0.028	-	105	Ln	Lake
Sediment NO_3	µg N/L sediment	14.4	32.4	3.7-1345.3	0.038	0.073	-	85	Ln	Lake
Solar irradiation	W/m^2	868	865	805-904	0.033	0.095	+	105	None	Catchment
DIN:TotalP	Element ratio	17.7	43.0	0.4-554.7	0.030	0.11	+	105	Ln	Lake
Yearly average of global horizontal UVA irradiation	10 kJ/m^2	18162	18062	16369-19641	0.027	0.013	+	105	None	Catchment
Total Phosphorus	µg P/L	5	6	1-17	0.019	0.20	-	105	Ln	Lake
Calcium (Ca)	mg/L	1.19	1.55	0.15-5.47	0.018	0.22	-	105	Ln	Lake
CO_2	mg C/L	0.70	0.78	0.05-2.00	0.018	0.22	-	105	Ln	Lake
pH	-	6.12	6.06	4.82-7.02	0.014	0.29	-	105	None	Lake
Dissolved Inorganic Nitrogen (DIN = NH_4 + NO_3)	µg N/L	46.0	80.8	1.5-629.0	0.014	0.28	+	105	Ln	Lake
Yearly average of global horizontal UVB irradiation	kJ/m^2	15369	15628	13433-18366	0.014	0.28	+	105	None	Catchment
Liming	Yes, Indirectly, Previously, No	-	-	-	0.014	0.77	-	105	None	Lake
Total Nitrogen	µg N/L	305	313	86-785	0.013	0.31	-	105	Ln	Lake
Conductivity	mS/m	2.25	2.72	0.55-7.01	0.010	0.35	-	105	Ln	Lake
Sediment NH_4	µg N/L sediment	764	1275	25-5464	0.0067	0.46	+	85	Ln	Lake
Nitrate (NO_3)	µg N/L	37.00	72.78	0.5-620.0	0.0059	0.49	+	105	Ln	Lake
Nitrogen deposition	mg/m^2/year	0.85	0.82	0.37-1.09	0.0056	0.49	+	105	None	Catchment
Area	km^2	7	34	0.5-1233.6	0.0054	0.50	+	105	Ln	Catchment
Runoff	mm/year	1089	1430	230-4467	0.0046	0.54	+	105	Ln	Catchment
Normalized Difference Vegetation Index (NDVI)	-	0.38	0.35	0.02-0.49	0.0044	0.55	-	105	None	Catchment
Mean temperature of the coldest month	^oC	-4.9	-5.8	-13.4- -0.7	0.0044	0.55	+	105	None	Catchment
Altitude	mas	361	408	41-1280	0.00091	0.78	+	105	None	Catchment
Sediment organic content	%	8	17.2	0.3-78.3	0.00089	0.79	-	85	None	Lake
Regulation	Yes/No	-	-	-	0.00084	0.79	+	105	None	Catchment
Precipitation	mm/year	1411	1465	635-2799	0.00048	0.84	-	105	None	Catchment
Mean temperature of the warmest month	^oC	16.4	16.6	11.7-20.2	0.00041	0.85	-	105	None	Catchment
Slope inclination	°	6.65	7.69	1.32-23.17	0.00034	0.87	+	105	None	Catchment
Algae on the *Juncus bulbosus*	None, Periphyton, "Clouds"	-	-	-	0.00014	0.92	-	105	None	Lake
Sediment PO_4	µg P/L sediment	7.57	11.01	0.01-53.43	0.000087	0.93	-	85	Ln	Lake
Yearly mean temperature	^oC	4.8	4.5	-1.6-7.2	0.000025	0.96	-	105	None	Catchment
Sediment water content	%	58	56	16-96	0.0000083	0.98	+	85	None	Lake
Ammonium (NH_4)	µg N/L	6	8	1-629	0.0000031	0.99	+	105	Ln	Lake

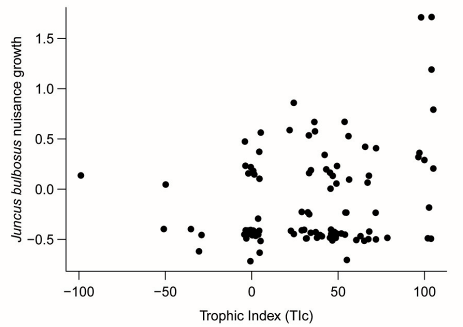

Fig. 6 *Juncus bulbosus* nuisance growth (here represented as DCA1 site scores, higher values indicating more nuisance growth) plotted against the macrophyte trophic index (TIc, high values indicate oligotrophic lakes, low values indicate eutrophic lakes) of 99 S Norwegian lakes 2007. As several lakes had overlapping positions, we included 2.5% jitter in both directions to show all 99 lakes.

Genetic analyses

The possibility remained that the different morphs and growth forms simply reflected underlying genetic differences. However, the genetic screening of different populations representing different growth forms (69 samples in total) revealed no clear-cut genetic structure by neither of the three approaches (PCO, NeighborNet and Structure analyses), as seen by no clear groupings in the PCO plot (Fig. 7) and neither any major splits in the NeighborNet (data not shown). In the Structure analysis, K = 2 was chosen as the most appropriate number of groups based on an overall evaluation of LnP(D), the similarity coefficient between different runs, and delta K (data not shown). Most samples were assigned to group 1, whereas only eight samples were assigned with more than 50 % to group 2, and additional 13 samples with more than 10 %. Group 2 (defined as samples with > 50 % assignment) could be identified in the PCO plot as the samples located at the upper end of PCO axis 1 (Fig. 7), and in the NeighborNet, where the eight samples constituted a cluster of their own. There seemed to be no geographical explanation for this cluster, however, and the grouping did not match any of the phenotypic or ecological characteristics of the samples (data not shown).

Nevertheless, a geographical component was clearly present in the dataset as samples collected from the same location in most cases grouped together both in the PCO plot and in the NeighborNet. When categories of nuisance growth were marked in the PCO plot, NeighborNet, or Structure groups, no correspondence was seen between AFLP phenotype and nuisance growth (Fig. 7).

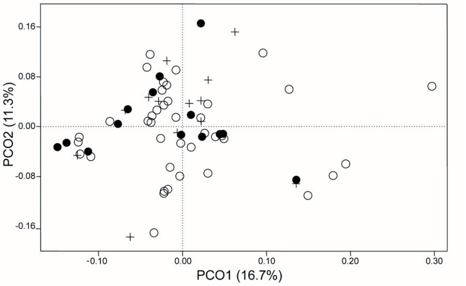

Fig. 7 Principle coordinate (PCO) analysis of 69 *Juncus bulbosus* samples and 146 AFLP loci. Samples are labeled with regard to nuisance growth: open circle – no nuisance growth, filled circle – nuisance growth, cross – partly nuisance growth. PCO axis 3 explained 10.2% of the total variation in the dataset but did not correspond with further structure.

Discussion

Juncus bulbosus presence/absence

J. bulbosus is a macrophyte with very high C:P and C:N ratios (Moe & Hessen, submitted manuscript), and thus presumably low nutrient demands. It is known to prefer acidic, nutrient poor waters (Snogerup, 2006; Rørslett, 1987; Lid and Lid, 2005), and our logistic model describing presence/absence of *J. bulbosus* confirmed this picture: *J. bulbosus* appeared most frequently in slightly acidic lakes with low phosphate concentrations and high N:P ratios. These lakes are generally soft water lakes with low buffer capacities and historically high loads of acid rain, and *J. bulbosus*, with its low nutrient demands and high affinity for CO_2 rather than HCO_3 (Roelofs, Schuurkes & Smits, 1984), seems very well adapted to this environment.

From the single parameter logistic models we also found that *J. bulbosus* generally is absent from habitats with the lowest minimum temperatures. This probably reflects that *J. bulbosus* is not very frost tolerant (Svedäng, 1990), yet it may also be linked to the minimum length of the growing season or the amount of ice cover during the winter (which can cause mechanical stress on the plants and uprooting during ice break).

Juncus bulbosus growth forms

Given suitable temperatures, the most common inorganic parameters limiting macrophyte growth are the availability of light, nutrients and inorganic carbon (Barko, Adams & Clesceri, 1986). Increased submerged macrophyte growth is consequently often related to increased availability of one of these parameters. In our study, however, none of them gave a clear response.

The lack of relationships between *J. bulbosus* growth forms and any of the measured light parameters can be explained by *J. bulbosus* preferably growing in oligotrophic lakes. These lakes are generally highly transparent, and *J. bulbosus* has a very low light compensation point (1.5 – 6 μE m^{-2} s^{-1}; (Wetzel, Brammer & Forsberg, 1984)) such that increased light is unlikely to have caused *J. bulbosus* nuisance growth (though light might influence depth distribution).

Our results did not show an impact of N or P sediment or water concentrations either. Firstly, *J. bulbosus* nuisance growth could potentially be a stage in a general succession of oligotrophic lakes turning eutrophic. However, we found no signs of *J. bulbosus* nuisance growth being more abundant in nutrient rich compared to nutrient poor lakes. Secondly, the areas from where nuisance growth was originally reported correspond very well with the areas that have received the highest amounts of nitrogen deposition and precipitation since the 1970's, thus promoting acidification, elevated NO_3 (and to some extent NH_4) concentrations as well as elevated N:P ratios in recipient waters (Kaste, Henriksen & Hindar, 1997; Stoddard, 1994; Bergström, Blomqvist & Jansson, 2005). In a recent study, Elser, Andersen, Baron *et al.* (2009) found that phytoplankton in lakes in high N-deposition areas had shifted from primarily N-limitation to P-limitation. However, despite a strong N-deposition over the surveyed regions, we failed to detect any effects of neither N-deposition nor N concentrations in water or sediment. Thirdly, all of the surveyed lakes were nutrient poor, TotP ranging from 1 to 17 μg P/L (median 5 μg P/L), and NH_4 ranging from 1 μg N/L to 629 μg N/L (median 6 μg N/L; Table 3). Due to its remarkably high C:P and C:N ratios compared to other macrophytes (median C:P = 792:1, median C:N = 32:1; T.F. Moe, unpubl. data), *J. bulbosus* plants are capable of building large biomasses on low concentrations of P and N. Thus, we interpret the lack of explanatory power of P and N as a signal that elevated nutrient supply either is not the reason behind the large *J. bulbosus* biomasses we now observe, or that the increase in nutrient supply is too small to be detected by our snapshot survey. Indeed, our data on TIc suggested that the most troublesome growth forms occurred in the most oligotrophic lakes (Fig. 6). This can probably be explained by the increased competition from other macrophyte species (e. g. *Potamogeton* sp., *Elodea* sp. or *Nuphar lutea* L.) with increasing nutrient and DIC availability (Murphy, 2002), and these species can inhibit *J. bulbosus* growth in all but the very most oligotrophic lakes. The macrophyte vegetation (apart from *J. bulbosus*) in the most oligotrophic lakes, on the other hand, is generally dominated by slow growing isoetids. Both isoetids and *J. bulbosus* are adapted to very low nutrient availabilities, and they both use CO_2 as their only carbon source (Maberly & Madsen, 2002; Roelofs *et al.*, 1984; Smolders, Lucassen & Roelofs, 2002). But in contrast to the isoetids, *J. bulbosus* is capable of fast growth and tall stands, thus it has the potential to completely dominate the macrophyte vegetation in these lakes.

We did not detect any effects of CO_2 or DIC concentrations in ambient water on growth of *J. bulbosus*. In Southern Norway, intense *J. bulbosus* growth is generally observed in soft water lakes with low buffer capacities, and most of these lakes became acidified during the past decades (Schartau, Fjellheim, Walseng *et al.*, 2011). Acidification shifts the inorganic carbon balance towards CO_2, and this can potentially reduce the competition from the faster growing elodeids, most of which otherwise have the advantage of using both CO_2 and HCO_3 (Maberly & Madsen, 2002). Furthermore, to counteract the acidification process, many of these lakes have been limed and some are still being limed today. As the lime dissolves, lake pH increases and so do the decomposition rates. This again leads to a temporary increase in CO_2 levels, which, as stated previously, is the preferred C-form of *J. bulbosus* (Roelofs *et al.*, 1984; Maberly & Madsen, 2002). In addition, increased pH due to liming promotes the formation and release of phosphorus and ammonium from the sediments (Bellemakers, Maessen, Verheggen *et al.*, 1996; Roelofs *et al.*, 1995), the latter being the preferred N-species of *J. bulbosus* (Schuurkes, Kok & Denhartog, 1986). Acidification, liming and reacidification have previously been assumed to be responsible for *J. bulbosus* nuisance growth (Roelofs *et al.*, 1984; Roelofs *et al.*, 1995; Lucassen *et al.*, 1999). However, although pH is currently rising due to reduced atmospheric deposition of sulphur compounds (Skjelkvåle, Borg, Hindar *et al.*, 2007), we expected the underlying factors with respect to CO_2 and NH_4 to be related to mass growth of *J. bulbosus* (Roelofs *et al.*, 1995). But we find no direct support for any of these factors in our lakes. Indeed, if anything, there was a negative relationship between *J. bulbosus* growth and CO_2 (Table 3). However, a general problem when comparing plant mass with potentially limiting elements is of course that positive correlations could indicate a causative relationship, but so could also a negative correlation – if the nutrients have already been incorporated into plant biomass. Hence a lacking or even slight negative correlation with CO_2 could simply reflect that more CO_2 is fixed by photosynthesis in these high plant biomass areas. Furthermore, the large stands of *J. bulbosus* we observe today could be a reminiscence of previous elevations in e.g. CO_2 concentrations, which we would not be able to detect today. Maybe more probable, however, is the possibility that CO_2 could to a large extent be obtained from the sediments, a parameter we did not analyse. Sediment CO_2 is the most important C-source for most isoetids (Smolders *et al.*, 2002), but Winkel & Borum (2009) showed that also non-isoetid macrophytes like *Lilaeopsis macloviana* (Gand.) A.W. Hill relied heavily upon sediment CO_2 for C-uptake (>75%). (Wetzel, Brammer, Lindström *et al.*, 1985) reported that an average of 34 % of the CO_2 fixed by *J. bulbosus* came from root uptake. As CO_2 concentrations in oligotrophic softwater lakes are usually up to 100-fold higher in sediments compared to the overlying (Smolders *et al.*, 2002), sediment CO_2 could potentially be an important factor influencing growth of *J. bulbosus*.

Despite a range of parameters and models tested, we failed to come up with a model that could offer a satisfactory explanation to the differences in *J. bulbosus* growth forms. Neither did the AFLP-screening reveal genetic differences consistent with the different growth forms or abundance. There were small scale geographical patterns in the *J. bulbosus* plant material, but no correlation between *J. bulbosus* nuisance growth and AFLP phenotype. We cannot rule out that there are key ambient drivers that were not included in our survey (e.g. sediment CO_2). But still, this lack of consistent trends even with the long list of parameters at hand is striking, and reflects a general problem of multivariate ecosystem based analysis in ecology; it is often hard to arrive at strong conclusions with regard to key forcing parameters. This again raises intriguing questions about apparently stochastic responses, hidden interactions between variables or simply matters of response times and resolution. By and large, nutrient concentrations usually reflect the general productivity of a system (Hessen, Andersen, Brettum *et al.*, 2003). However, for instance with regard to nutrients and CO_2, a snapshot study such as ours cannot account for the "ghost of uptake past". These nutrient concentrations represent the chemical situation of a particular lake at a particular moment in time, but fail to say anything about nutrient dynamics/supply and to what extent nutrients are allocated into plant and animal biomass. Furthermore, we may have performed sampling in the midst of an ongoing expansion of the species within this region, so that small stands simply may reflect early successions after recent colonization. If so, the full response or potential of the plant within a given locality will only be realized after some years.

Although this survey has resulted in mainly "negative" conclusions, we can now exclude a range of candidate parameters for *J. bulbosus* nuisance growth. We have shown that variations in *J. bulbosus* growth is not due to genetic differences, and it is probably also not a direct result of N-deposition or due to large differences in climate, light or nutrients. However, since we measured concentrations rather than supply, we cannot exclude the possibility that small variations in nutrient supply and/or (especially sediment) CO_2 might be important. These issues can only be settled through controlled experiments and long term monitoring of preferably oligotrophic, isoetid/*J. bulbosus* dominated lakes, including separate analyses of water and sediments. Together, this should put us in a better position to answer what influences *J. bulbosus* growth over time, and what management strategies should be applied to resolve the present problems of *J. bulbosus* nuisance growth.

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II

Juncus bulbosus nuisance growth in freshwater ecosystems: different triggers for the same phenomenon in rivers and lakes?

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Macrophyte, bulbous rush, nitrogen, carbon dioxide, ammonium

Abstract

Since the 1980s nuisance growth of the aquatic macrophyte *Juncus bulbosus* has been observed in an increasing number of rivers and lakes in Europe. Acidification processes and liming measurements, as well as the impact of hydropower plants and climate-related parameters have previously been assumed to have caused *J. bulbosus* mass development, but cannot be the sole reason for today's phenomenon. To find drivers for *J. bulbosus* nuisance growth and to assess whether or not different patterns emerge in rivers and lakes, we analyzed macrophyte vegetation, periphyton coverage, water and sediment chemistry, catchment characteristics, and *J. bulbosus* elemental composition in 17 lakes and 28 river sites in southern Norway. Despite a large number of measured parameters, we still lack clear indications with respect to possible triggers of nuisance growth. However, our results with respect to changes in water CO_2 and plant % C composition are consistent with literature suggesting CO_2 as the most likely trigger for *J. bulbosus* nuisance growth in lakes. While *J. bulbosus* seems to be C-limited in not-nuisance lakes, we have no such indications in nuisance lakes. For rivers, our study indicates that the most likely primary trigger for *J. bulbosus* nuisance growth is a slightly enhanced NH_4^+ supply. We suggest that different triggers are likely responsible for *J. bulbosus* nuisance growth in rivers and lakes.

1. Introduction

Juncus bulbosus L. is a perennial macrophyte native to Europe and North Africa and can inhabit both aquatic and terrestrial habitats (Casper & Krausch, 1980). The species is described as preferring nutrient and calcium poor, acidic water (Casper & Krausch, 1980; Johansen et al., 2000). Its ecological amplitude, however, extends to calcareous, eutrophic and even brackish waters (Olsen, 1950 and literature cited therein).

J. bulbosus exhibits an extreme variation in morphology (Proćków, 2008). The terrestrial form (f. *terrestris*) grows as short green or reddish meadows, plants of the submerged form (f. *submersus*) grow up to 2 m long and can have up to 60 cm long leaves, and the floating form (f. *fluitans*) grows in floating mats, with richly branched stems and plant tips growing above the water surface (Casper & Krausch, 1980). Aquatic plants of *J. bulbosus* initially grow as a small rosette10-20 cm in length. Stems can, however, emerge from the rootstalk, and the plant often develops new rosettes and roots on each node of the stem (Casper & Krausch, 1980; Johansen et al., 2000).

These different growth forms of *J. bulbosus* have been recognized historically (Glück, 1936), but only since the 1980s nuisance growth of the submerged and floating forms, have been observed in a number of rivers and lakes in Europe (Roelofs et al., 1994; Johansen et al., 2000; Brandrud, 2002). In lakes, the enhanced growth of *J. bulbosus*, often together with *Sphagnum* sp. and at the expense of other macrophyte species, has been related to acid deposition with sulfate and ammonium as major components (Melzer, 1984; Schuurkes et al., 1987). The resulting increase in NH_4^+ and CO_2 concentrations have been suggested to favor *J. bulbosus* compared to other macrophyte species (Roelofs et al., 1995). In addition, lake liming combined with re-acidification of the water has been shown to promote *J. bulbosus* nuisance growth, since liming leads to temporary increased levels of CO_2 and NH_4^+ (Roelofs et al., 1995, Lucassen et al., 1999). Roelofs et al. (1984) concluded that water CO_2 concentration was the primary trigger for *J. bulbosus* nuisance growth in lakes, since phosphate and/or NH_4^+ enrichment without concomitant CO_2 enrichment did not lead to an increase in *J. bulbosus* biomass.

Fewer data are available from rivers than from lakes. Increased *J. bulbosus* growth in South Norwegian rivers is reported to have begun around the 1960s to 1980s (Johansen et al., 2000), and several hypotheses have been advocated to explain these changes: mild winters leading to less ice erosion and thus to a higher survival of *J. bulbosus* biomass (Johansen et al., 2000;

Hindar et al., 2003), hydropower plants with resulting alterations in flow patterns, flow velocity and ice cover (Johansen et al., 2000; Hindar et al., 2003), as well as increased NH_4^+ concentrations (Hindar et al., 2003). In contrast to lakes, liming has not been considered a likely cause for nuisance growth in rivers (Johansen et al., 2000).

Lakes in Norway exhibit clear signs of recovery from acidification since the 1990s (Skjelkvåle et al., 1998; 2007), and currently massive growth of *J. bulbosus* occurs also in non-limed Norwegian lakes. Thus, acidification and liming, which previously have been assumed to cause *J. bulbosus* nuisance growth in lakes (Schuurkes et al., 1987; Roelofs et al., 1995; Lucassen et al., 1999), can clearly not be the sole reason for the mass occurrence. In addition, data on *J. bulbosus* nuisance growth in rivers are generally scarce. We analyzed macrophyte vegetation, periphyton coverage, water and sediment chemistry, catchment characteristics and *J. bulbosus* elemental composition in 17 lakes and 28 river sites in Southern Norway. Sampling was conducted in both 2008 and 2010 at the same sampling sites. The main objectives of this study were i) to detect differences in development of *J. bulbosus* growth forms between nuisance and not-nuisance sites, ii) to find drivers for *J. bulbosus* nuisance growth, and iii) to assess whether or not different patterns emerge in rivers and lakes.

2. Materials and Methods

2.1 Field work

This study is based on a survey of 17 lakes and 28 sites from 15 different rivers in South Norway (Fig. 1). Each river site was visited twice, in July 2008 and August 2010 and all but one lake site were visited three times, in June 2008, September 2008 and July 2010. The remaining lake site was visited in June 2008 only. At each river site, macrophyte vegetation was registered along a stretch of approximately 50 m, while at lake sites, a transect of approximately 20 m breadth was investigated to the depth where we could no longer see the bottom. Each lake site was situated at the site of most abundant *J. bulbosus* growth in the respective lake, based on information from previous investigations. At each lake and river site *J. bulbosus* growth forms (rosette plants/small columns with shoots/large columns with shoots/fresh surface mats/old surface mats) were registered according to a 3-point scale: 1 = sparsely; 2 = covering large parts; 3 = dominating the site. For data analyses, fresh and old mats were later combined. At lake sites, the abundance of other macrophyte species was recorded according to a 5-point scale (1 = very rare, 2 = infrequent, 3 = common, 4 = frequent, 5 = abundant, predominant), and the sum of isoetids was calculated as the total sum of abundance of *Isoetes*, *Lobelia* and *Littorella* species. We also noted presence or absence of small but clearly visible periphytic algae on *J. bulbosus* leaves and of large amounts of filamentous algae around *J. bulbosus* plants. At river sites, flow velocity was estimated as 1=low, 2=medium, 3=high, and at lake sites Secchi depth was recorded. At each site, a sediment core of 5.5 cm in diameter and approximately 10 cm length was taken at the site of most prolific *J. bulbosus* growth. Due to coarse sediment, we were unable to sample sediment at 6 river sites (5 not-nuisance and 1 nuisance site). The sediment samples were frozen in dry ice immediately after sampling and kept frozen until analysis. Water samples were collected at approximately 10 cm depth at each site, and $HgCl_2$ was used to preserve samples for CO_2 analysis in 125 ml serum vials with gas-tight stoppers. In addition, a *J. bulbosus* plant was collected at each site from the most abundant stand, dried and later analysed for carbon (C), nitrogen (N) and phosphorus (P).

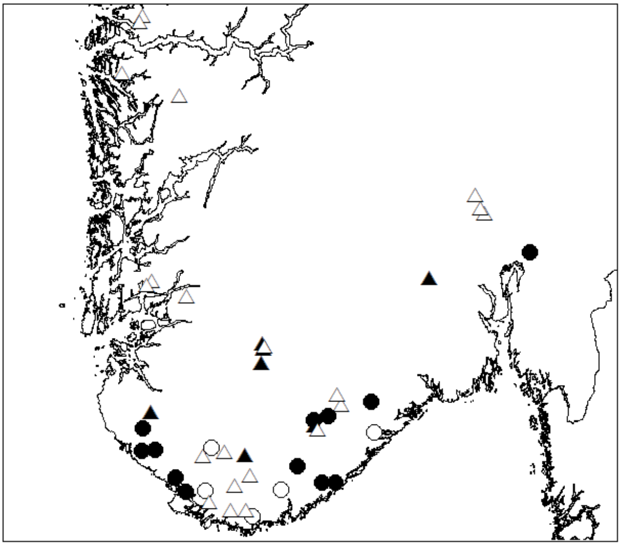

Fig 1 Sampling sites in Southern Norway 2008 and 2010; circles represent lakes (n = 17), triangles river sites (n = 28). Black symbols represent *Juncus bulbosus* nuisance growth, white symbols not-nuisance growth.

2.2 Water and sediment analyses

Water chemical parameters were analysed at the Norwegian Institute for Water Research (NIVA) according to standard methods (pH: NS 4720; conductivity: NS-ISO 7888; Calcium (Ca), nitrate (NO_3^-), and ammonium (NH_4^+): ion chromatography according to NS-EN ISO 10304-1 and NS-EN-ISO 14911; total organic carbon (TOC): NS-ISO 8245; dissolved inorganic carbon (DIC): NS-EN 1484; CO_2: Standard Methods 4500-CO2, 4-12-4-18; Total nitrogen (TotN): NS 4743; Total phosphorus (TotP): NS 4725; phosphate (PO_4^{3-}): NS 4724). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3^- and NH_4^+.

Sediment porewater was extracted from the thawed sediments through centrifugation and analysed for PO_4^{3-}, NO_3^-, and NH_4^+. Porewater NH_4^+ was analysed using protocol B from Holmes et al. (1999). Porewater NO_3^- and PO_4^{3-} were analysed using an auto-analyser with applications G-297-03 for PO_4^{3-} and G-172-96 for NO_3^- (Auto-analyser 3, SEAL Analytical/BRAN LUEBBE, Norderstedt, Germany). To account for sediment water content, we calculated sediment nutrient concentrations as pore-water nutrients per volume sediment. Sediment water content was calculated as (wet weight minus dry weight)/wet weight. Dry weight was measured after drying the sediments at 105°C for 24 hours. Organic content was measured as (dry weight minus ash free dry weight)/dry weight. Ash free dry weight was measured after burning the dried sediment sample in a muffle furnace for 2h at 450°C and cooling the sample to room temperature in a desiccator.

2.3 Plant elemental composition

For the 2008 samples, the entire plant was analysed for carbon (C), nitrogen (N), and phosphorus (P), whereas the plants sampled in 2010 were analysed separately at both root and shoot (the transition zone between the root and the shoot was not analysed). All plants were manually cleaned of detritus and periphyton in the field, dried and stored until elemental analyses. The dried plants were grinded 45 sec at 6500 rpm on a Precellys 24 (Bertin Technologies, Montigny, France), and C and N was analysed on an element analyser (Flash EA 1112 NC Analyser, ThermoFisher Scientific, MA, US). P was analysed in an autoanalyzer with application G-297-03 (Autoanalyzer 3, SEAL Analytical/BRAN LUEBBE, Norderstedt, Germany). All results are reported as % of dry weight.

2.4 Catchment data

Catchment boundaries for each investigated lake and river site were delineated according to the procedures described in Larsen et al. (2011a), and data on annual average temperature, precipitation, runoff and satellite derived normalized difference vegetation index (NDVI) as well as data on terrain slope, area and altitude was obtained according to Larsen et al. (2011b). Atmospheric nitrogen deposition was averaged for each catchment from a digital map of yearly, accumulated total atmospheric nitrogen deposition (including dry deposition) for 1995. The nitrogen deposition map was constructed by spatial interpolation (kriging with a spherical semivariogram model) on 1° x 1° gridded output data from the Unified EMEP MSC-W modelling system (http://www.emep.int/). Data on solar, UVA and UVB irradiation (based on yearly averages of global horizontal irradiation for the period 1981-1990) was obtained from the Photovoltaic Geographic Information System (PVGIS) of the European Commission Joint Research Centre (JRC) (http://re.jrc.ec.europa.eu/pvgis/) (Súri et al. 2005). County governors assisted with information on liming status. Information on hydropower development was obtained from the Norwegian Water Resources and Energy Directorate (NVE), in collaboration with local hydropower companies and county governors.

2.5 Data handling

A site was defined as having nuisance growth when either large columns or surface mats dominated the site (i.e. abundance was noted as 3), or large columns and mats together dominated the site (i.e. the sum of abundances of large columns, old mats and fresh mats was 5 or more). This definition matches the visual impression in the field, that a site is "overgrown" with *J. bulbosus*. When a site was categorized as "nuisance" during one sampling event but not the other, the site was generally categorized as "nuisance". Using these categories, we had a dataset of 21 river sites and 5 lakes where *J. bulbosus* was present but not showing nuisance growth, and 7 river sites and 12 lakes with nuisance growth (Fig. 1).

Since most of the measured variables were not normally distributed and normal distribution for some of these parameters was not achieved by transformation, non-parametric Mann-Whitney U tests were used when testing for differences between nuisance growth and not-nuisance growth, and between river and lake locations. For these tests, average values per site were used for the two (rivers) or three (lakes) sampling events. To correct for multiple testing, a Bonferroni correction with the refinement of Holmes was applied (Stahel 1995, Bärlocher 1999). All analyses were performed using STATISTICA 10.

3. Results

3.1 Characterization of nuisance versus not-nuisance sites

The main growth forms in not-nuisance lakes and rivers were rosette plants and small columns, while nuisance lakes and rivers were dominated by small and large columns (data not shown). Not surprisingly, nuisance lakes and rivers were generally characterized by a higher abundance of surface mats and large and small columns than not-nuisance lakes and rivers. In contrast, we observed no such difference in the abundance of rosette plants between nuisance and not-nuisance lakes and rivers (Fig. 2).

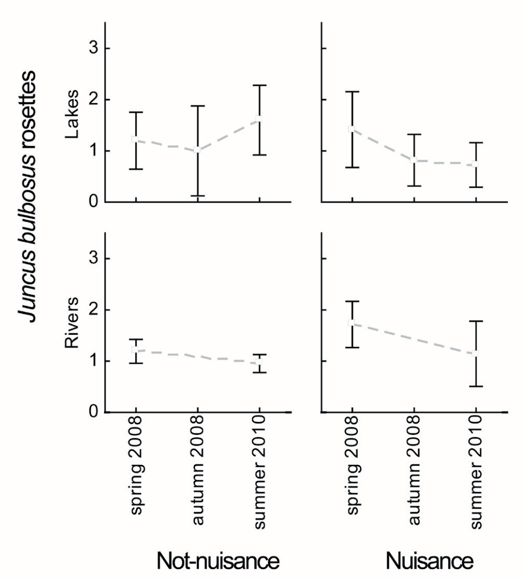

Fig 2 Mean abundance ± 0.95 confidence interval of *Juncus bulbosus* rosette plants at 21 not-nuisance and 7 nuisance river sites, as well as 5 not-nuisance and 12 nuisance lake sites at each sampling event.

The abundance of mats and large columns increased slightly from 2008 to 2010 in both nuisance lakes and rivers, whereas the abundance of small columns and rosette plants decreased slightly. No clear patterns were observed in not-nuisance lakes and rivers (data not shown).

There are generally very few differences between river sites with and without *J. bulbosus* nuisance growth and none of the observed differences are significant after Bonferroni correction (Table 1). In general, however, *J. bulbosus* nuisance sites in our dataset are less limed than sites without nuisance growth and have a higher sediment NH_4^+ content but lower sediment NO_3^- concentration than not-nuisance sites (p-values < 0.05, see Table 1).

Like river sites, there were only minor differences between lakes with and without *J. bulbosus* nuisance growth and none of the observed differences are significant after Bonferroni correction (Table 2). In general, however, lakes with nuisance growth were generally characterized by a steep catchment morphology.

3.2 Differences in sediment and water chemistry between sampling events

As a next step, we calculated the differences in all measured water and sediment chemical variables and the plant CNP composition between sampling events, and tested if the observed changes were different between nuisance and not-nuisance sites. Again, there were no consistent differences after Bonferroni correction, yet in four instances, p-values of <0.05 between nuisance and not-nuisance sites were observed, two in both river and lake locations. At river sites with *J. bulbosus* nuisance growth, sediment NH_4^+ increased from 2008 to 2010 by 502 ± 514 µg N/dm^3, compared to a decrease of 109 ± 324 µg N/dm^3 at not-nuisance sites (p=0.046). Water pH also increased by 0.38 ± 0.12 at nuisance sites, compared to an increase of only 0.15 ± 0.19 at not-nuisance sites (p=0.004).

In lakes with *J. bulbosus* nuisance growth, water CO_2 concentration did not change from spring 2008 to summer 2010 (we calculated an average decrease of 0.04 ± 0.11 mg C/l), whereas it decreased by 0.31 ± 0.12 mg C/l in not-nuisance lakes during the same period (p=0.005). The %C in *J. bulbosus* plants slightly increased from spring to autumn 2008 (0.73 ± 2.12 %C) in nuisance lakes, but decreased by 1.83 ± 2.11 in not-nuisance lakes (p=0.031).

Table 1 River water and sediment chemistry, catchment characteristics, presence of epiphytic algae, and plant CNP composition in Norway (21 river sites without and 7 river sites with nuisance growth). Data are averaged from one measurement in 2008 and one in 2010; p-values < 0.05 are marked in bold, even though they are not significant after Bonferroni correction.

a) estimated as 1=low, 2=medium, 3=high velocity; b) estimated as 0=absent, 1=present; c) 0=not regulated, 0.5=min water flow, 1=full flow; d) 0=not limed, 0.5=indirect liming, 1=direct liming.

		not nuisance growth						p-value for difference between groups	nuisance growth					
	unit	**N**	**Mean**	**Median**	**Min**	**Max**	**Std.Dev.**		**N**	**Mean**	**Median**	**Min**	**Max**	**Std.Dev.**
pH	-	21	6.46	6.48	5.82	6.86	0.29	0.254	7	6.35	6.37	5.89	6.73	0.25
Conductivity	mS/m	21	1.97	1.68	1.01	4.20	0.85	0.075	7	1.40	1.24	0.94	2.58	0.57
Ca	mg/l	21	1.50	1.28	0.63	2.52	0.63	0.075	7	1.09	0.91	0.58	2.29	0.56
DIC	mg/l C	21	0.96	0.89	0.48	1.65	0.37	1.000	7	0.98	0.82	0.58	1.60	0.41
CO2	mg/l C	21	0.44	0.37	0.16	0.81	0.19	0.202	7	0.54	0.59	0.25	0.82	0.22
TOC	mg/l C	21	2.84	3.00	0.70	5.60	1.18	0.193	7	2.41	1.95	1.20	4.65	1.19
TOTN	µg/l N	21	237.1	257.5	134.5	400.0	64.0	0.265	7	202.6	195.0	123.0	292.5	63.2
NO3-N	µg/l N	21	85.60	92.50	0.50	205.00	58.86	0.232	7	60.43	36.00	0.50	225.00	77.77
NH4-N	µg/l N	21	5.98	5.50	2.00	12.50	2.79	0.202	7	15.21	6.50	3.50	46.50	15.85
DIN	µg/l N	21	91.57	94.50	5.50	214.50	59.22	0.559	7	75.64	47.00	20.50	228.50	74.05
TOTP	µg/l P	21	4.10	4.00	1.51	6.50	1.56	0.377	7	3.50	2.50	2.00	7.00	1.73
PO4-P	µg/l P	21	0.75	0.50	0.50	1.25	0.34	0.142	7	0.54	0.50	0.50	0.75	0.09
Velocity	a)	21	1.62	1.50	1.00	3.00	0.59	0.345	7	1.36	1.50	1.00	2.00	0.38
filamentous algae	b)	21	0.10	0.00	0.00	1.00	0.30	0.843	7	0.07	0.00	0.00	0.50	0.19
small epiphytic algae	b)	21	0.40	0.50	0.00	1.00	0.44	0.113	7	0.71	1.00	0.00	1.00	0.39
Sediment water content	% wet weight/100	16	0.38	0.34	0.22	0.72	0.15	0.060	6	0.50	0.48	0.30	0.73	0.14
Sediment organic cont.	% dry weight	16	4.01	2.36	0.66	21.34	4.94	0.060	6	6.75	6.28	1.56	14.32	4.16
Pore water NH4-N	µg/L N	16	1274	949	204	2990	823	0.113	6	2084	1447	959	4111	1350
Sediment NH4-N	µg/dm^3 N	16	513	355	115	2161	510	**0.030**	6	1064	912	332	1963	640
Pore water PO4-P	µg/l P	16	26.36	15.88	7.01	98.72	26.56	0.083	6	10.21	9.37	5.00	18.56	4.60
Sediment PO4-P	µg/dm^3 P	16	8.65	5.42	1.71	33.91	8.34	0.285	6	5.06	3.88	2.46	8.87	2.71
Pore water NO3-N	µg/L N	16	51.14	35.75	4.60	164.04	41.28	**0.014**	6	18.52	18.46	9.54	30.35	7.15
Sediment NO3-N	µg/dm^3 N	16	16.60	12.37	3.24	46.95	11.80	0.113	6	8.39	7.85	4.70	13.32	3.28
Altitude	m	21	565	617	210	1031	226	0.106	7	797	972	394	1023	269
Area	km^2	21	558	398	15	1705	529	0.095	7	1439	1705	58	4058	1398
Runoff	mm/year	21	1803	1520	620	3570	960	0.474	7	1415	1504	610	2395	542
N deposition	mg/m^2/year	21	0.71	0.71	0.49	0.95	0.14	0.853	7	0.74	0.71	0.64	1.00	0.11
NDVI	index	21	131.1	131.9	110.3	144.3	8.8	0.106	7	124.9	126.6	116.5	135.8	8.2
Slope	degree	21	8.66	9.13	3.38	14.01	3.23	0.811	7	8.24	9.13	6.49	9.94	1.42
UVB	kJ/m^2	21	8312	8518	7300	8931	561	0.254	7	8615	8653	8083	8907	253
T.mean	°C	21	3.62	3.62	0.66	6.10	1.76	0.060	7	2.06	0.78	0.03	4.95	1.99
T.max	°C	21	15.44	15.36	12.80	18.27	1.63	0.095	7	14.21	14.34	12.90	15.96	1.30
T.min	°C	21	-6.39	-5.55	-12.45	-1.82	3.36	0.145	7	-8.31	-9.56	-12.41	-4.74	2.85
Precipitation	mm/year	21	1659	1584	747	2745	612	0.577	7	1482	1580	955	1912	306
regulation code	c)	21	0.50	0.50	0.00	1.00	0.47	0.353	7	0.71	1.00	0.00	1.00	0.39
weir basin	yes/no	21	0.10	0.00	0.00	1.00	0.30	0.474	7	0.29	0.00	0.00	1.00	0.49
liming code	d)	21	0.69	1.00	0.00	1.00	0.37	**0.021**	7	0.21	0.00	0.00	1.00	0.39
% P root	% dry weight	14	0.09	0.08	0.03	0.18	0.05	0.746	5	0.08	0.08	0.04	0.15	0.04
% N root	% dry weight	18	1.34	1.22	0.82	1.98	0.36	0.301	6	1.51	1.42	1.06	2.01	0.37
% C root	% dry weight	18	43.94	44.38	36.80	50.20	3.98	0.868	6	43.78	44.44	38.19	47.92	3.52
% P shoot	% dry weight	20	0.17	0.16	0.06	0.37	0.08	0.194	7	0.13	0.11	0.08	0.23	0.05
% N shoot	% dry weight	21	2.39	2.30	1.35	3.31	0.55	0.750	7	2.43	2.36	2.04	3.18	0.36
% C shoot	% dry weight	21	42.80	42.58	38.22	51.06	2.50	1.000	7	42.47	42.58	40.01	44.01	1.33
%P average total plant	% dry weight	21	0.17	0.16	0.07	0.43	0.08	0.124	7	0.12	0.11	0.07	0.19	0.05
%N average total plant	% dry weight	21	1.76	1.69	1.15	2.61	0.41	0.367	7	1.55	1.50	1.15	2.04	0.29
%C average total plant	% dry weight	21	46.96	46.94	44.41	49.03	1.14	0.340	7	46.52	45.76	44.74	48.84	1.64

Table 2 Lake water and sediment chemistry, catchment characteristics, presence of epiphytic algae, and plant CNP composition in Norway (5 lakes without and 12 lakes with *Juncus* bulbosus nuisance growth). Data are averaged from two measurements in 2008 and one in 2010; p-values < 0.05 are marked in bold, even though they are not significant after Bonferroni correction.

b) estimated as 0=absent, 1=present; d) 0=not limed, 0.5=indirect liming, 1=direct liming.

		not nuisance growth						**p-value for difference between groups**	**nuisance growth**					
	unit	**N**	**Mean**	**Median**	**Min**	**Max**	**Std.Dev.**		**N**	**Mean**	**Median**	**Min**	**Max**	**Std.Dev.**
pH	-	5	6.01	6.19	4.94	6.65	0.70	0.635	12	5.96	5.93	5.43	6.92	0.43
Conductivity	mS/m	5	4.34	3.32	2.53	7.73	2.23	0.429	12	3.09	3.17	1.17	5.01	1.33
Ca	mg/l	5	1.62	1.18	0.27	3.50	1.27	0.317	12	1.05	0.83	0.53	2.43	0.57
DIC	mg/l C	5	0.97	0.94	0.41	1.57	0.41	0.712	12	0.88	0.76	0.42	1.70	0.39
CO2	mg/l C	5	0.71	0.66	0.34	1.05	0.29	0.712	12	0.64	0.63	0.35	1.03	0.20
TOC	mg/l C	5	4.57	5.60	1.43	6.23	2.06	0.493	12	3.69	2.62	1.24	8.73	2.49
TOTN	µg/l N	5	360.3	380.0	313.3	403.3	39.6	0.126	12	299.6	300.0	193.3	465.0	74.8
NO3-N	µg/l N	5	115.93	116.00	6.00	235.00	107.75	0.752	12	88.88	71.83	2.33	268.33	81.83
NH4-N	µg/l N	5	14.00	15.67	8.33	18.67	5.01	1.000	12	13.94	13.33	7.67	21.67	5.89
DIN	µg/l N	5	129.9	131.7	14.3	253.3	108.3	0.958	12	102.8	84.0	22.0	276.7	78.2
TOTP	µg/l P	5	8.67	5.67	2.67	18.00	7.07	0.597	12	5.44	5.17	2.33	10.67	2.76
PO4-P	µg/l P	5	1.60	1.00	0.50	4.17	1.53	0.170	12	0.63	0.50	0.50	1.00	0.18
Secchi depth	m	5	4.92	3.10	2.25	10.00	3.23	0.955	11	5.77	5.00	0.00	17.50	4.77
filamentous algae	b)	5	0.20	0.00	0.00	0.67	0.30	0.952	12	0.19	0.00	0.00	0.67	0.26
small epiphytic algae	b)	5	0.60	0.67	0.00	1.00	0.43	1.000	12	0.61	0.67	0.00	1.00	0.31
max. vegetation depth	m	5	3.45	4.00	1.75	4.00	0.97	0.748	12	3.63	3.75	2.00	6.00	1.41
total abundance isoetids	sum of abund.	5	6.27	6.00	4.67	8.00	1.52	0.313	12	4.94	5.50	1.33	8.50	2.29
Sediment water content	% wet weight/100	5	0.68	0.73	0.30	0.85	0.22	0.712	12	0.72	0.81	0.25	0.93	0.20
Sediment organic cont.	% dry weight	5	17.74	17.42	3.34	34.17	11.57	0.562	12	27.90	17.23	1.13	76.94	23.43
Pore water NH4-N	µg/L N	5	1290	577	195	3023	1216	0.792	12	2173	877	224	12493	3404
Sediment NH4-N	µg/dm^3 N	5	919	494	161	2104	902	0.562	12	1629	590	169	9566	2640
Pore water PO4-P	µg/l P	5	13.75	18.35	2.40	21.38	9.12	0.792	12	16.61	7.53	2.23	60.04	20.04
Sediment PO4-P	µg/dm^3 P	5	9.93	12.87	2.00	17.73	7.42	0.874	12	12.44	5.41	1.83	42.88	14.92
Pore water NO3-N	µg/L N	5	34.44	29.66	14.27	62.15	19.97	0.792	12	88.36	28.33	10.06	619.11	173.30
Sediment NO3-N	µg/dm^3 N	5	20.55	14.62	10.39	39.55	11.96	0.874	12	74.77	18.76	5.00	558.17	157.52
Altitude	m	5	223.0	247.7	132.1	349.8	92.2	0.317	12	278.0	285.2	86.1	507.4	127.9
Area	km^2	5	6.7	3.1	0.8	16.4	6.8	0.958	12	17.8	4.1	1.2	160.9	45.3
Runoff	mm/year	5	1113.7	1072.1	599.9	1537.3	397.0	0.792	12	1230.4	1089.1	706.9	1998.6	423.3
N deposition	mg/m^2/year	5	0.96	0.96	0.88	1.03	0.05	0.073	12	0.90	0.91	0.71	0.97	0.07
NDVI	index	5	142.1	145.9	134.3	146.7	6.0	0.317	12	138.7	138.0	130.2	146.5	4.3
Slope	degree	5	2.88	3.24	1.32	3.84	1.01	**0.018**	12	5.99	5.83	1.54	12.30	2.86
Solar irradiation	W/mm^2	5	885	888	867	900	13	0.188	12	877	880	856	889	12
UVA	10 kJ/m^2	5	18584	18526	17655	19380	667	0.712	12	18519	18494	17557	19641	611
UVB	kJ/m^2	5	16738	17034	14690	18363	1501	0.370	12	15962	16156	13433	18036	1299
T.mean	°C	5	5.92	5.96	4.97	6.73	0.68	0.370	12	5.55	5.61	4.25	6.83	0.85
T.max	°C	5	16.91	16.90	15.60	18.96	1.32	0.958	12	17.02	16.87	15.49	19.79	1.28
T.min	°C	5	-3.90	-3.80	-4.64	-2.78	0.76	0.958	12	-4.60	-3.75	-8.90	-2.67	1.88
Precipitation	mm/year	5	1424	1397	908	1738	330	0.874	12	1369	1296	813	1794	354
liming code	d)	5	0.10	0.00	0	0.5	0.22	1.000	12	0.13	0.00	0	0.5	0.23
% P root	% dry weight	3	0.07	0.06	0.04	0.12	0.04	0.699	6	0.11	0.09	0.04	0.26	0.08
% N root	% dry weight	5	1.22	0.99	0.80	1.91	0.47	0.626	7	1.02	0.96	0.86	1.34	0.17
% C root	% dry weight	5	42.03	43.45	34.92	46.76	4.52	0.516	7	43.49	45.09	37.06	46.51	3.37
% P shoot	% dry weight	4	0.13	0.11	0.08	0.24	0.07	0.744	11	0.12	0.12	0.06	0.20	0.05
% N shoot	% dry weight	5	1.87	1.59	1.46	2.96	0.62	0.821	11	1.71	1.75	1.01	2.23	0.35
% C shoot	% dry weight	5	42.81	42.75	41.59	43.69	0.89	0.113	11	43.64	43.79	41.38	45.05	1.19
%P average total plant	% dry weight	5	0.17	0.15	0.08	0.27	0.07	0.429	12	0.14	0.13	0.07	0.24	0.05
%N average total plant	% dry weight	5	1.70	1.64	1.56	1.88	0.13	0.429	12	1.63	1.61	1.41	1.92	0.14
%C average total plant	% dry weight	5	45.96	45.18	44.73	47.68	1.31	0.268	12	46.87	46.84	45.07	48.33	0.96

3.3 Difference between rivers and lakes

Shoots of river plants from nuisance sites had a significantly higher N-content than shoots from nuisance lake plants ($p = 0.0003$; Table 3). Not-nuisance rivers had a significantly lower NH_4^+ concentration than not-nuisance lakes ($p = 0.0013$; Fig. 3). No other parameters differed significantly between rivers and lakes after Bonferroni correction, but we have listed parameters with $p < 0.05$ in Table 3.

Table 3 p-values for differences between rivers and lakes (averaged from 2008 and 2010 measurements), divided into *Juncus bulbosus* nuisance and not-nuisance sites. All water and sediment chemical variables given in Tables 1 and 2, as well as plant elemental compositions were tested; here we only show variables where p-values are < 0.05 at either nuisance or not-nuisance growth (marked in bold); significant differences after Bonferroni correction are marked with *.

	not nuisance growth					nuisance growth				
	p-value for difference between rivers and lakes	median value lakes	median value rivers	N lakes	N rivers	p-value for difference between rivers and lakes	median value lakes	median value rivers	N lakes	N rivers
pH	0.2000	6.19	6.48	5	21	**0.0449**	5.93	6.37	12	7
conductivity	**0.0025**	3.32	1.68	5	21	**0.0026**	3.17	1.24	12	7
TOTN	**0.0009***	380.0	257.5	5	21	**0.0171**	300.0	195.0	12	7
NH4-N	**0.0013***	15.67	5.50	5	21	0.3402	13.33	6.50	12	7
Sediment water content	**0.0194**	0.73	0.34	5	16	**0.0320**	0.81	0.48	12	6
Sediment organic content	**0.0041**	17.42	2.36	5	16	**0.0415**	17.23	6.28	12	6
% N shoot	**0.0342**	1.59	2.30	5	21	**0.0003***	1.75	2.36	11	7

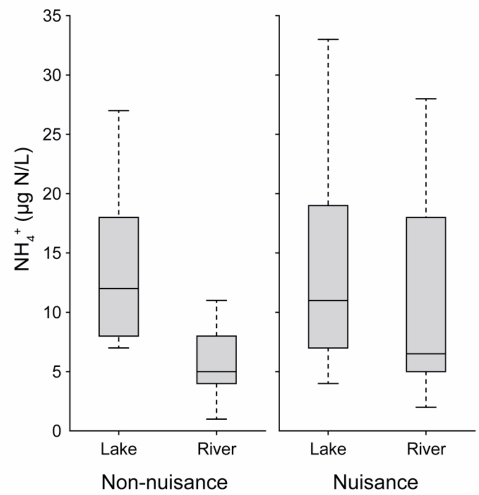

Fig 3 Water NH_4^+-N concentrations at rivers and lakes with and without *J. bulbosus* nuisance growth in Southern Norway, using averages of samplings from 2008 and 2010. The difference between lakes and rivers is significant for not-nuisance sites ($p = 0.0013$), but not for nuisance sites ($p = 0.34$). Boxes indicate 1st and 3rd quantiles, squares indicate median and black bars indicate minimum and maximum values.

4. Discussion

4.1 *Juncus bulbosus* development between sampling events

The development of plant nuisance biomasses could principally be related to increased plant recruitment from e.g. seeds or propagules, or to length growth of existing plants. Aquatic plants of *J. bulbosus* initially grow as rosettes, before stems emerge from the rootstalk (Johansen et al., 2000). In our data, we did not find evidence for differences in the abundance of rosette plants between nuisance and not-nuisance rivers and lakes (Fig 2), thus, we conclude that *J. bulbosus* nuisance growth is likely a consequence of increased plant growth or reduced dieback, rather than increased young plant recruitment. *J. bulbosus* nuisance populations are mainly composed of large columns in both lakes and rivers, while mats and small columns play a relatively minor role (data not shown), however both contribute towards nuisance populations.

4.2 Differences between rivers and lakes

At nuisance sites, *J. bulbosus* shoots had significantly higher nitrogen contents in rivers than in lakes (Table 3). A similar tendency was also observed in not-nuisance sites, but this was not significant after Bonferroni correction ($p=0.03$; Table 3). The generally higher nitrogen content observed in river plants, despite an equal or actually lower nitrogen concentration in rivers when compared to lakes (Tables 1, 2), might reflect differences in nutrient supply. In rivers, plants are in receipt of a continuous supply of nutrients, whereas a slow diffusion of ions occurs through nutrient depleted zones around the shoots of plants in standing waters (described by Ruttner (1940), cited from Elster (1962)).

The predominant difference observed between nuisance and not-nuisance sites is that no difference in NH_4^+ concentration occurs between nuisance lakes and rivers, whereas NH_4^+ concentration is lower in not-nuisance rivers than in not-nuisance lakes (Fig. 3). NH_4^+ is usually rapidly removed in streams, often within a few tens to hundreds of meters (Peterson et al., 2001), such that lower NH_4^+ concentrations in not-nuisance rivers compared to lakes are not surprising. We therefore suggest that the relatively high NH_4^+ concentrations measured in rivers with nuisance growth are probably enhanced compared to background conditions. We will demonstrate below that enhanced NH_4^+ concentrations may actually trigger *J. bulbosus* nuisance growth in rivers.

4.3 Juncus bulbosus nuisance growth in rivers

Our study was aimed at finding possible causes for *J. bulbosus* nuisance growth in Norwegian lakes and rivers, yet only few results became significant. Thus, we still lack clear indications with respect to possible triggers of nuisance growth. We have however, isolated some parameters which are more likely than others to play a role in *J. bulbosus* nuisance growth and which require analysis in greater detail.

In contrast to lakes, liming is unlikely to trigger *J. bulbosus* nuisance growth in rivers. This was already suspected by Johansen et al. (2000) and Brandrud (2002). On the contrary, in our dataset, five of seven nuisance sites were not limed, as opposed to only three of 21 not-nuisance sites (see also Table 1, liming code). In limed lakes, the trigger for *J. bulbosus* nuisance growth was suggested to be the sedimentation of carbonate rich material, together with re-acidification of the water and the concomitant increase in sediment and water CO_2 concentrations (Roelofs et al., 1995). In contrast, river flow should prevent a major sedimentation of carbonate rich material (with the possible exception of areas immediately downstream of the lime dosers) and pH in limed rivers is kept constant by continous dosing, such that re-acidification only occurs during doser malfunctioning. Our dataset is too small to draw any conclusions on whether liming could actually prevent *J. bulbosus* nuisance growth in rivers. A possible mechanism could be an increased competition by other fast growing macrophytes, as e.g. *Myriophyllum alterniflorum*, *Sparganium angustifolium*, or *Potamogeton* species. In lakes, an increase of these species after liming has been described by Brandrud (2002). It is important to note however, that such an effect if occurring would likely only prevent the massive growth of a single species, in this instance *J. bulbosus*, at the expense of an increased growth of other macrophyte species.

The different proportion of limed sites, with most nuisance sites being not limed and most not-nuisance sites being limed, likely explains the observed variability in pH; while pH increased by 0.38 ± 0.12 from July 2008 to August 2010 at nuisance sites, it only increased by 0.15 ± 0.19 at not-nuisance sites (see chapter 3.2). Calcium-poor rivers in southern Norway usually exhibit annual variations in pH with generally lowest values in spring and highest values in late summer/early autumn (DN, 2011). In contrast, pH in limed rivers varies less

(DN, 2011), thus explaining the relatively small difference between July 2008 and August 2010.

Although not significant, river nuisance sites were generally characterized by sediments having higher NH_4^+ and lower NO_3^- concentrations than not-nuisance sites (Tab. 1). In addition, sediment NH_4^+ concentrations at nuisance sites tended to increase from 2008 to 2010, compared to a rather decreasing trend at not-nuisance sites (chapter 3.2). Furthermore, there were significantly lower NH_4^+ concentrations in the water of not-nuisance rivers compared to lakes, but no difference at nuisance sites (Fig. 3). All of this points towards NH_4^+ as an important factor for *J. bulbosus* nuisance growth in rivers. *J. bulbosus* has been shown to prefer NH_4^+ over NO_3^- as its N-source and it has also been shown that NH_4^+ can be taken up through both leaves and roots (Schuurkes et al., 1986). The relative quantity taken up through leaves and roots is suggested to depend on the relative concentrations in water and sediment (Rattray et al., 1991), such that both an enrichment of water and sediment NH_4^+ can lead to increased plant growth. The importance of NH_4^+ for *J. bulbosus* nuisance growth was already assumed by Roelofs et al. (1995), Lucassen et al. (1999), and Brandrud (2002) and our own unpublished findings of a controlled growth experiment support the importance of NH_4^+ for *J. bulbosus* growth. In the present study, shoots of *J. bulbosus* river plants generally had a higher N-content than lake plants (Table 3). This could indicate an importance of nitrogen-nutrition for *J. bulbosus* growth in rivers. The fact that we were unable to measure a significant difference in water NH_4^+ concentrations between nuisance and not-nuisance rivers, is likely explained by the typically rapid removal of NH_4^+ in streams, which often occurs within a few tens to hundreds of meters (Peterson et al., 2001). *J. bulbosus* could very likely play an important role in that removal.

River macrophytes are, however, well known to modify their own sediment by trapping particles from the water column (Kleeberg et al., 2010). Consequently, NH_4^+-rich material can be accumulated in dense *J. bulbosus* stands, such that enhanced sediment NH_4^+ concentrations would be an effect of, rather than a cause for, *J. bulbosus* nuisance growth. While this is true for sediments, it is unlikely that enhanced water NH_4^+ concentrations can actually be caused by nuisance *J. bulbosus* stands in rivers. Although there is a theoretical possibility of sediment porewater diffusing into the water column and thereby causing enhanced water NH_4^+ concentrations, the flow velocity and discharge of rivers in Norway, together with the known rapid removal of NH_4^+ in streams (Peterson et al., 2001), renders this possibility unlikely.

Weir basins were earlier suspected to cause *J. bulbosus* nuisance growth in rivers (Johansen et al. 2000; Hindar et al., 2003). Since we find *J. bulbosus* nuisance as well as not-nuisance stands in weir basins as well as outside such areas (Table 1), they probably are not the primary trigger for *J. bulbosus* nuisance growth. It is, however, likely that they add to the problem. Reduced flow velocity, as is observed in weir basins, usually leads to increased sedimentation of small and often nutrient rich particles (Rother & Kohler, 2005). In river stretches where sedimentation prevails, increased NH_4^+ concentration in the upper 10 cm of sediment characteristically occurs (Thouvenot et al., 2007). From there it can be readily taken up by *J. bulbosus* and therefore lead to increased biomass production.

In summary, our results fit with published literature on *J. bulbosus* and are indicative of the following scenario; that an increase in biomass of *J. bulbosus* may be a result of an enhanced supply of NH_4^+. In contrast to earlier works, who directly assumed increased ammoniumsulfate deposition to be responsible for increased water NH_4^+- concentrations and *J. bulbosus* nuisance growth (Schuurkes et al., 1987), we did not find any direct influence from N-deposition (Table 1). This is probably due to the influence of climate and vegetation, as there is often no direct correlation between N-deposition and stream-N in Norway (de Wit et al., 2008). Increased river NH_4^+-concentrations might instead be due to direct causes, e.g. treated wastewater in rural areas (scattered settlement in rural areas, with concomitant small-scale wastewater treatment is a common phenomenon in Norway, see e.g. Paruch et al., 2011), or runoff from cattle grazed areas (we in fact observed cattle grazing in the immediate surrounding of at least some of the river nuisance sites). Increased NH_4^+ supply might also be a result of reduced flow velocities in weir basins, leading to enhanced sedimentation of nutrient-rich material and a concomitant increase in supply of sediment NH_4^+. Irrespective of NH_4^+-origin will the initially enhanced *J. bulbosus* biomass likely start a positive feed-back mechanism: Dense stands will trap more fine sediment, thus leading to an even better supply of nutrients to the plants. It is interesting to note, that despite the nutrient rich sediments likely accumulated in *J. bulbosus* nuisance stands, sediment PO_4^{3-} concentrations did not increase from 2008 to 2010, whereas sediment NH_4^+ concentrations appeared to do so. This would indicate that more NH_4^+ is accumulated than taken up. Such a scenario would be consistent with a shift from N- to P-limitation in *J. bulbosus* nuisance stands. For phytoplankton in Norwegian lakes , a shift from N- to P-limitation due to increased N-input was shown by Elser et al. (2009).

4.4 Juncus bulbosus nuisance growth in lakes

Smolders et al. (2002) and literature cited therein, described a “spectacular growth“ of *J. bulbosus* within “a very short time“ in acidified and limed lakes during the 1980s and 90s. In contrast to these earlier observations, we have observed a more gradual increase in *J. bulbosus* biomass over time. These gradual changes are probably also triggered by gradual processes, such that we today might expect the differences between nuisance and not-nuisance lakes to be less pronounced than in earlier investigations. In addition, we face the problem that measuring concentrations indeed describes the chemical status at a particular site at a particular time, but fails to say anything about nutrient supply. In short, if an additional nutrient supply should be fully incorporated into plant biomass, this will not be reflected in increased water nutrient concentrations. As a consequence we are unable to provide strong conclusions with respect to triggers and drivers of *J. bulbosus* nuisance growth in lakes. However, as in rivers, we isolated some parameters which seem more likely than others to play a role in *J. bulbosus* nuisance growth. These should be addressed with further, more detailed analysis.

There was no significant difference in the abundance of epiphytic algae on *J. bulbosus* between nuisance and not-nuisance lakes and plants with and without epiphytes were found in both nuisance and not-nuisance lakes (Table 2). Thus, in contrast to earlier hypotheses (Mjelde, 2004), shading or competition for nutrients by epiphytes seems not to hamper *J. bulbosus* nuisance growth. Our results are consistent with the results of Svedäng (1990), who suggested that *J. bulbosus* can avoid competition for CO_2 with epiphytic algae by using the relatively high CO_2 concentrations in early spring, when epiphytes are not yet present in large amounts.

We found no significant differences in the measured water and sediment C, N and P concentrations between nuisance and not-nuisance lakes (Table 2). Nevertheless, relatively more C, N, and P is stored in the *J. bulbosus* biomass of nuisance lakes, because *J. bulbosus* is obviously more abundant in nuisance lakes than in not-nuisance lakes and plant % C, N and P is the same in both nuisance and not-nuisance lakes (Table 2). As the macrophyte vegetation of the not-nuisance lakes largely consists of slow growing isoetids, who generally do not reach high biomasses (Madsen et al., 2002), other macrophytes are unlikely to store large amounts of C, N and P in not-nuisance lakes. As a consequence, the clue to answering *J. bulbosus* nuisance growth probably lies in nutrient supply rather than concentrations. We suggest that, to enable the production of large *J. bulbosus* biomasses in nuisance lakes without lowering nutrient concentrations compared to not-nuisance lakes, the nutrient supply to nuisance lakes likely was higher than to not-nuisance lakes. This needs not be a large difference, since *J. bulbosus* is a perennial species and biomass is built over several years.

We observed that in not-nuisance lakes, carbon content in *J. bulbosus* tended to decrease from spring to autumn 2008, while in nuisance plants, it remained constant. This could indicate an autumn C-limitation in not-nuisance lakes, while we observed no such indications in nuisance lakes. Likewise, water CO_2 concentration tended to decrease from spring 2008 to summer 2010 in not nuisance lakes, while it remained constant in nuisance lakes. This would be consistent with a summer CO_2 decline in not-nuisance lakes but not in nuisance lakes. Although none of these differences were significant after Bonferroni correction, they both point towards a C-limitation of *J. bulbosus* in not-nuisance lakes, while no such signs were observed in nuisance lakes.

Availability of CO_2 as the most likely primary trigger for *J. bulbosus* nuisance growth in lakes has already been suggested by Roelofs et al. (1984). In earlier works, however, acidification and lake liming were found to be the reasons for increased CO_2 supply in nuisance lakes (e.g. Roelofs et al., 1994; Brandrud, 2002). As we have shown above, these explanations are not sufficient today, such that we are obliged to look into possible carbon-sources for *J. bulbosus*. The species cannot take up bicarbonate (Svedäng, 1992; Maberly & Madsen, 2002), but Roelofs et al. (1984) and Wetzel et al. (1985) have shown that *J. bulbosus* can take up CO_2 through both roots and shoots. Thus, several different explanations might account for an enhanced CO_2 supply to *J. bulbosus*.

J. bulbosus surface mats were present in all investigated nuisance lakes, while not-nuisance lakes only rarely had surface mats. CO_2 might therefore be transported from the aerial leaves of surface mats to other plant parts via the internal lacunal airspace. Sand-Jensen & Frost-Christensen (1999) showed that photosynthesis of several species of amphibious macrophytes was 2-3 times higher in air than in stream water, even when stream water was supersaturated with CO_2. Wetzel et al. (1985) showed that CO_2 can diffuse via the internal lacunal airspace in *J. bulbosus*, therefore transport of CO_2 from aerial leaves to other plants parts theoretically is possible. Therefore, the uptake of aerial CO_2 and transport to other plant parts may lead to increased *J. bulbosus* growth. It is interesting to note that such a mechanism would imply a self-stabilizing state of *J. bulbosus* nuisance growth; the more surface mats, the more CO_2 could diffuse from the aerial leaves and thus support further growth.

Another explanation for enhanced CO_2 supply to *J. bulbosus* in nuisance lakes might be an increased mineralization of organic matter. Both the recovery from acidification (Skjelkvåle et al., 2007) and the observed increase in dissolved organic carbon (DOC) concentrations (Monteith et al., 2007) in southern Norway could account for such a process. All investigated lakes are located in an area which was formerly acidified and lakes in this region have been displaying signs of acidification recovery since the 1990s (Skjelkvåle et al., 1998; 2007). During severe acidification, decomposition of organic matter is inhibited (Kelly et al., 1984), such that organic matter can be accumulated. Due to oxygen release from roots, sediments colonized by isoetids and *J. bulbosus* are usually oxidized (Chabbi, 1999; Smolders et al., 2002). Therefore the stimulated decomposition of organic material in formerly acidified lakes (van Kleef et al., 2010), in recovery processes probably leads to increased sediment CO_2 concentrations. Likewise, dissolved organic carbon (DOC) concentrations in surface waters in Norway have increased in the last decades (Monteith et al., 2007) and mineralization of DOC generally leads to enhanced production of CO_2 (Larsen et al., 2011b). In addition, annual variations in lake CO_2 concentrations should also be considered. Svedäng (1990) suggested that *J. bulbosus* has the capacity to effectively utilize the rich CO_2 supply which may occur in lakes in early spring. Consequently, high spring CO_2 concentrations in nuisance lakes, possibly resulting from increased mineralization of organic matter (see above), might have stimulated *J. bulbosus* growth. Future analyses should therefore include CO_2 measurements in early spring. Since the majority of boreal lakes are CO_2 supersaturated and thus vent CO_2 to the atmosphere (Larsen et al., 2011b, and literature cited therein), the increase in atmospheric CO_2 concentrations observed since the industrial revolution is likely not directly responsible for an increased CO_2 supply to *J. bulbosus*.

J. bulbosus is expected to profit more from increased CO_2-supply than isoetids because it has a higher intrinsic growth rate and a higher affinity for CO_2 than isoetids (Roelofs et al., 1984; Madsen et al., 2002). *J. bulbosus* is, possibly together with *Myriophyllum alterniflorum,* the only species capable of fast growth in extremely softwater lakes but *Myriophyllum alterniflorum* prefers a slightly higher alkalinity than *J. bulbosus* (Brandrud, 2002) and lacks the adaptations to a carbon-poor environment typical for *J. bulbosus* and isoetids (Chabbi, 1999; Smolders et al., 2002). Thus, a slightly increased supply of CO_2 will likely be advantageous to *J. bulbosus* over other macrophyte species typical for extremely soft water lakes.

Collectively, our results are consistent with earlier studies which conclude that CO_2 is the most likely primary trigger for *J. bulbosus* nuisance growth in lakes (Roelofs et al.; 1984). The probable cause for not observing enhanced CO_2 concentrations in nuisance compared to not-nuisance lakes is that, the increase in CO_2 supply is small and that CO_2 had been taken up into plant biomass. Moreover, *J. bulbosus* has been shown to be an effective user of sediment CO_2 (Wetzel et al., 1985), a parameter which we have not measured. Future efforts should therefore be directed towards identifying possible reasons for an increased availability of CO_2 and should include carbon flux measurement throughout the whole vegetation period in addition to sediment CO_2 measurement.

The impact of catchment slope on *J. bulbosus* nuisance growth (Table 2), though not significant after Bonferroni correction (p=0.018), might also be connected to lake carbon metabolism. It is reasonable to assume that catchment topography reflects bathymetric properties of lakes. This will affect the ratio of sediment surface to lake volume, as well as lake area to lake volume, which in turn influence the fate of dissolved organic carbon and CO_2 export (Kelly et al., 2001; Flanagan & McCauley, 2008). Since we lack information about lake depth, volume or sediment area, we cannot explicitly link catchment slope to these lake properties. *J. bulbosus* is, however, assumed to enhance C-recycling in the sediment (Chabbi, 1999), such that a lower rate of C-recycling in the water column, as described for deep lakes with a low ratio of sediment surface to lake volume (Kelly et al., 2001; Flanagan & McCauley, 2008), could give a competitive advantage to *J. bulbosus*.

5. Conclusions

We conclude that the most likely primary trigger for *J. bulbosus* nuisance growth in rivers is a slightly enhanced NH_4^+ supply. Weir basins or other measures reducing flow velocity aggravate the problem by leading to increased sedimentation, in turn leading to increased NH_4^+ supply to the plants. Since *J. bulbosus* nuisance stands in rivers have the ability to trap nutrient rich sediment, instigating a positive feed-back mechanism leading to an even greater supply of nutrients for plant growth, measures against nuisance growth need to include the removal of *J. bulbosus* from nuisance sites. This will only be a temporary solution as long as NH_4^+ supply continues to be too high, as *J. bulbosus* will return, thus repeating the cycle. Consequently, sustainable measures to reduce nuisance growth should include reducing NH_4^+ input to these rivers.

In lakes, we suggest that the most likely trigger for *J. bulbosus* nuisance growth is CO_2. This has already been shown by Roelofs et al. (1984), such that we only need to re-assess the reasons behind an increased CO_2 supply and not the principal mechanisms behind *J. bulbosus* nuisance growth. If a general trend like the increasing DOC concentration in surface waters should be responsible for *J. bulbosus* nuisance growth, we might witness a general shift in the macrophyte vegetation of ultraoligotrophic softwater lakes, from slow growing isoetids towards a dominance of the faster growing *J. bulbosus*.

Our results indicate that different primary triggers could be responsible for *J. bulbosus* nuisance growth in rivers compared to lakes. Mass et al. (2010) investigated the influence of flow to marine benthic autotrophs and found that photosynthesis in flowing conditions is enhanced. They showed that this augmentation of photosynthesis is due to flow-driven enhancement of oxygen efflux from the organism to the water, which increases the affinity of the RuBisCO enzyme to CO_2. Such a mechanism can physiologically explain a shift from C-limitation in lakes towards another nutrient in rivers. If such a shift occurs, then enhanced lake N- or P-concentrations should have less consequence in the lake itself than in its outlet, because only the latter is N or P limited. Indeed, during field work we often observed high *J. bulbosus* biomasses in lake outlets, which in most cases not yet have reached nuisance status.

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