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Title:

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

Authors:

Therese Fosholt Moe[a,†]

Anne Krag Brysting[a]

Tom Andersen[b]

Susanne Claudia Schneider[c]

Øyvind Kaste[c]

Dag O. Hessen[a]

† Corresponding author: e-mail: t.f.moe@bio.uio.no, telephone: (+47) 98 62 76 50.

a Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biology, University of Oslo, Pb 1066 Blindern, 0316 Oslo, Norway.

b Department of Biology, University of Oslo, Pb 1066 Blindern, 0316 Oslo, Norway.

c Norwegian Institute for Water Research (NIVA), Gaustadalléen 21, 0349 Oslo, Norway.

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SUMMARY

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).

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.

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^{o}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^{o}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 1º x 1º 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).

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).

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 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).

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.

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).

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 $\mu E\ m^{-2}\ s^{-1}$; (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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References

Aulio, K. (1987) Rapid decline of mass occurrences of *Juncus bulbosus* in a deacidified freshwater reservoir. *Memoranda Societatis pro Fauna et Flora Fennica*, **63,** 41-44.

Barko, J.W., Adams, M.S. & Clesceri, N.L. (1986) Environmental factors and their consideration in the management of submersed aquatic vegetation: A review. *Journal of Aquatic Plant Management*, **24,** 1-10.

Bellemakers, M.J.S., Maessen, M., Verheggen, G.M. & Roelofs, J.G.M. (1996) Effects of liming on shallow acidified moorland pools: A culture and a seed bank experiment. *Aquatic Botany*, **54,** 37-50.

Bergström, A.K., Blomqvist, P. & Jansson, M. (2005) Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. *Limnology and Oceanography*, **50,** 987-994.

Brandrud, T.E. (2002) Effects of liming on aquatic macrophytes, with emphasis on Scandinavia. *Aquatic Botany*, **73,** 395-404.

Brandrud, T.E. & Johansen, S.W. (1997) Tiltak mot krypsiv. Vegetasjonsfjerning i Sveindasområdet i Mandalsvassdraget 1996 [in Norwegian]. *NIVA report*, Norwegian Institute for Water Research, vol. 3759-97, p 26.

Dice, L.R. (1945) Measures of the amount of ecological association between species. *Ecology Brooklyn*, **26,** 297-302.

Ehrich, D. (2006) AFLPDAT: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes*, **6,** 603-604.

Ehrich, D., Gaudeul, M., Assefa, A., Koch, M.A., Mummenhoff, K., Nemomissa, S., *et al.* (2007) Genetic consequences of Pleistocene range shifts: contrast between the Arctic, the Alps and the East African mountains. *Molecular Ecology*, **16,** 2542-2559.

Elser, J.J., Andersen, T., Baron, J.S., Bergström, A.K., Jansson, M., Kyle, M. *Et al*. (2009) Shifts in Lake N:P Stoichiometry and Nutrient Limitation Driven by Atmospheric Nitrogen Deposition. *Science,* **326,** 835-837.

Falush, D., Stephens, M. & Pritchard, J.K. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes,* **7,** 574-578.

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. (2001) PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* **4,** Unpaginated.

Hessen, D.O., Andersen, T., Brettum, P. & Faafeng, B.A. (2003) Phytoplankton contribution to sestonic mass and elemental ratios in lakes: Implications for zooplankton nutrition. *Limnology and Oceanography,* **48,** 1289-1296.

Hindar, A., Johansen, S.W., Andersen, T. & Saloranta, T. (2003) Faktorer som påvirker problemvekst av krypsiv i Sør-Norge; datagjennomgang, analyser og forslag om videre studier [in Norwegian]. *NIVA report,* Norwegian Institute for Water Research, vol. OR-4688, p 34.

Holmes, R.M., Aminot, A., Kerouel, R., Hooker, B.A. & Peterson, B.J. (1999) A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences,* **56,** 1801-1808.

Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution,* **23,** 254-267.

Johansen, S.W. (1993) Krypsiv i Mandalsvassdraget. Status for utbredelse, vurdering av tilgroing og årsaker, samt forslag til tiltak [in Norwegian]. *NIVA report,* Norwegian Institute for Water Research, vol. 2954, p 56.

Johansen, S.W., Brandrud, T.E. & Mjelde, M. (2000) Konsekvenser av reguleringsinngrep på vannvegetasjon i elver. Tilgroing av krypsiv. Kunnskapsstatus [in Norwegian]. *NIVA report*, Norwegian Institute for Water Research, vol. 4321, p 67.

Johnson, J.B. & Omland, K.S. (2004) Model selection in ecology and evolution. *Trends in Ecology & Evolution,* **19,** 101-108.

Jørgensen, M.H., Elven, R., Tribsch, A., Gabrielsen, T.M., Stedje, B. & Brochmann, C. (2006) Taxonomy and evolutionary relationships in the *Saxifraga rivularis* complex. *Systematic Botany,* **31,** 702-729.

Kaste, Ø., Henriksen, A. & Hindar, A. (1997) Retention of atmospherically-derived nitrogen in subcatchments of the Bjerkreim river in southwestern Norway. *Ambio,* **26,** 296-303.

Larsen, S., Andersen, T. & Hessen, D.O. (2011a) Climate change predicted to cause severe increase of organic carbon in lakes. *Global Change Biology,* **17,** 1186-1192.

Larsen, S., Andersen, T. & Hessen, D.O. (2011b) The pCO_2 in boreal lakes: Organic carbon as a universal predictor? *Global Biogeochemical Cycles,* **25**.

Lid, J. & Lid, D.T. (2005) *Norsk Flora [in Norwegian],* Det Norske Samlaget, Oslo.

Lucassen, E., Bobbink, R., Oonk, M.M.A., Brandrud, T.E. & Roelofs, J.G.M. (1999) The effects of liming and reacidification on the growth of *Juncus bulbosus*: a mesocosm experiment. *Aquatic Botany,* **64,** 95-103.

Maberly, S.C. & Madsen, T.V. (2002) Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. *Functional Plant Biology,* **29,** 393-405.

Murphy, K.J. (2002) Plant communities and plant diversity in softwater lakes of northern Europe. *Aquatic Botany,* **73,** 287-324.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L. *et al*. (2010). vegan: Community Ecology Package. R package version 1.17-5.

Pompanon, F., Bonin, A., Bellemain, E. & Taberlet, P. (2005) Genotyping errors: Causes, consequences and solutions. *Nature Reviews Genetics,* **6,** 847-859.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics,* **155,** 945-959.

Prockow, J. (2008a) *Juncus bulbosus* (Juncaceae), a species new to South America (Chile). *Acta Societatis Botanicorum Poloniae,* **77,** 225-227.

Prockow, J. (2008b) What is *Juncus bulbosus* subsp *kochii* (Juncaceae) and does it really exist? A taxonomic revision of bulbous rush subspecies. *Botanical Journal of the Linnean Society,* **156,** 501-512.

R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roelofs, J.G.M. (1983) Impact of acidification and eutrophication on macrophyte communities in soft waters in the Netherlands. 1. Field observations. *Aquatic Botany,* **17,** 139-155.

Roelofs, J.G.M., Brandrud, T.E. & Smolders, A.J.P. (1994) Massive expansion of *Juncus bulbosus* L. after liming of acidified SW Norwegian lakes. *Aquatic Botany,* **48,** 187-202.

Roelofs, J.G.M., Schuurkes, J.a.a.R. & Smits, A.J.M. (1984) Impact of acidification and eutrophication on macrophyte communities in soft waters. II. Experimental studies. *Aquatic Biology,* **18,** 389-411.

Roelofs, J.G.M., Smolders, A.J.P., Brandrud, T.E. & Bobbink, R. (1995) The effect of acidification, liming and reacidification on macrophyte development, water quality

and sediment characteristics of soft-water lakes. *Water Air and Soil Pollution,* **85,** 967-972.

Rørslett, B. (1987) Tilgroing i Otra nedstrøms Brokke. Problemanalyse og forslag om tiltak [in Norwegian]. *NIVA report*, Norwegian Institute for Water Research, vol. OR-1997, p 40.

Rørslett, B. (1990) Tilgroing i terskelbasseng i Otra ved Valle. Problemanalyse og forslag om tiltak [in Norwegian]. *NIVA report*, Norwegian Institute for Water Research, vol. 2442, p 117.

Schartau, A.K., Fjellheim, A., Walseng, B., Skjelkvåle, B.L., Framstad, E., Halvorsen, G.A. *et al*. (2011) Monitoring long-range transboundary air pollution 2010. Summary report [in Norwegian, English summary]. *NIVA report*, Norwegian Institute for Water Research, vol. 6183-2011, p 86.

Schuurkes, J., Kok, C.J. & Denhartog, C. (1986) Ammonium and nitrate uptake by aquatic plants from poorly buffered and acidified waters. *Aquatic Botany,* **24,** 131-146.

Skjelkvåle, B.L., Borg, H., Hindar, A. & Wilander, A. (2007) Large scale patterns of chemical recovery in lakes in Norway and Sweden: Importance of seasalt episodes and changes in dissolved organic carbon. *Applied Geochemistry,* **22,** 1174-1180.

Smolders, A.J.P., Lucassen, E. & Roelofs, J.G.M. (2002) The isoetid environment: biogeochemistry and threats. *Aquatic Botany,* **73,** 325-350.

Snogerup, S. (2006) The annual species of *Juncus* in Norden [in Swedish]. *Svensk botanisk tidskrift,* **100,** 245-255.

Stoddard, J.L. (1994) Long-term changes in watershed retention of nitrogen - its causes and aquatic consequences. In: *Environmental Chemistry of Lakes and Reservoirs* (Ed: L.A. Baker), pp. 223-284. Advances in Chemistry Series. Amer Chemical Soc, Washington.

Súri, M., Huld, T.A. & Dunlop, E.W. (2005) PV-GIS: a web-based solar radiation database for the calculation of PV potential. *International Journal of Sustainable Energy,* **24,** 55-67.

Svedäng, M.U. (1990) The growth dynamics of *Juncus bulbosus* L. - a strategy to avoid competition? *Aquatic Botany,* **37,** 123-138.

Svedäng, M.U. (1992) Carbon dioxide as a factor regulating the growth dynamics of *Juncus bulbosus*. *Aquatic Botany,* **42,** 231-240.

Terbraak, C.J.F. & Prentice, I.C. (1988) A theory of gradient analysis. *Advances in Ecological Research,* **18,** 271-317.

Vanndirektivet, D. (2009) Klassifisering av miljøtilstand i vann [in Norwegian]. *Veileder 01:2009*, Direktoratsgruppa for gjennomføring av vanndirektivet. p. 184.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vandelee, T., Hornes, M. *et al*. (1995) AFLP - a new technique for DNA-fingerprinting. *Nucleic Acids Research,* **23,** 4407-4414.

Wetzel, R.G., Brammer, E.S. & Forsberg, C. (1984) Photosynthesis of submersed macrophytes in acidified lakes. 1. Carbon fluxes and recycling of CO_2 in *Juncus bulbosus* L. *Aquatic Botany,* **19,** 329-342.

Wetzel, R.G., Brammer, E.S., Lindström, K. & Forsberg, C. (1985) Photosynthesis of submersed macrophytes in acidified lakes. 2. Carbon limitation and utilization of benthic CO2 sources. *Aquatic Botany,* **22,** 107-120.

Whitlock, R., Hipperson, H., Mannarelli, M., Butlin, R.K. & Burke, T. (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology Resources,* **8,** 725-735.

Winkel, A. & Borum, J. (2009) Use of sediment CO_2 by submersed rooted plants. *Annals of Botany*, **103,** 1015-1023.

Økland, R.H. (1990) A phytoecological study of the mire Norhtern Kisselbergmosen, SE Norway. 2. Identification of gradients by detrended (canonical) correspondence-analysis. *Nordic Journal of Botany,* **10,** 79-108.

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

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		pH		Parameter 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*

Table 3 Basic information on the parameters included in the multiple linear models testing *Juncus bulbosus* growth in 105 Norwegian lakes 2007, sorted by R^2-values. R^2 and p-values reported are from single-predictor linear regression models of *J. 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.

(Dear reviewer: Table 3 is too big to be included here, please see separate file)

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

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).

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 (^oC) 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.

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.

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 44 lakes had identical position as at least one other lake, we included 2.5% jitter in both directions to show all 99 lakes.

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.