

## Determination of habitat requirements of the glacial relict *Nuphar pumila* as basis for successful (re-)introductions

### Ermittlung der Habitatansprüche des Glazialrelikts *Nuphar pumila* als Basis für erfolgreiche (Wieder)ansiedlungen

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#### Abstract

*Nuphar pumila* is a glacial relict, which is nowadays rare throughout Europe and red-listed in most European regions. In Switzerland only three autochthonous populations and one population of the hybrid with *N. lutea* (*N. ×spenneriana*) have survived to date, one of them in the canton of Zurich. To protect this species regionally, the canton of Zurich has commissioned the *ex situ* propagation of specimens, which then had been introduced to 37 water bodies in protected areas, including one known former site. Since only about 10% of these introductions had been successful, there was a wish to identify causes of this lack of success. To this end, we compared the vegetation and physical-chemical parameters of the four natural sites in Switzerland with the successful and unsuccessful introduction sites. Additionally, for a subset of sites, we assessed diatom genus composition as a proxy of water quality. Moreover, we derived vegetation plots of *N. pumila* and *N. lutea* from across Europe from the European Vegetation Archive (EVA) to compare their site conditions, using mean ecological indicator values and bioclimatic variables of the localities. We found that inside Switzerland the main differences were between the natural sites and all introduction sites, while successful and unsuccessful introduction sites hardly differed in the determined parameters. Natural sites had cooler water with lower magnesium content, and according to mean ecological indicator values, also lower nutrient status. The diatom data, though limited in amount, point into the same direction. The EVA data demonstrate that stands of *N. pumila* are mainly more oligotrophic, but also cooler and more acidic than those of *N. lutea*. We could not find any factor that explains the success vs. lack of success of plantations of *N. pumila* in multiple sites in the canton of Zurich, but our results rather indicate that due to the relatively warm

climate, the high atmospheric nitrogen input and the predominantly base-rich bedrock, the sites in the canton are generally not particularly well suited for *N. pumila*. We consider it therefore more promising to protect and possibly (re-) introduce *N. pumila* in other cantons with higher elevation, base-poor bedrock and lower atmospheric nitrogen input.

**Keywords:** conservation, ecological indicator value (EIV), European Vegetation Archive (EVA), *ex situ* conservation, glacial relict, habitat requirement, *Nuphar lutea*, *Nuphar pumila*, re-introduction, Switzerland

**Erweiterte deutsche Zusammenfassung am Ende des Artikels**

## 1. Introduction

The least water lily (*Nuphar pumila* (Timm) DC.) is regarded as a very rare species in Central Europe (SEBALD et al. 1993), while globally it is classified as “least concern” (MAIZ-TOME 2016). In Switzerland, *N. pumila* is categorized as “endangered” (BORNAND et al. 2016), in Germany as “critically endangered” (METZING et al. 2018), in Estonia as “vulnerable”, in Denmark as “near threatened” and in the United Kingdom as “nationally scarce” (LANSDOWN 2011). The principal causes of threat are eutrophication, habitat destruction, isolation of populations, modification of the hydrological regime, as well as competition with other species and hybridization (KÄSERMANN & MOSER 1999, EGGENBERG & KEEL 2004, BÉTRISEY et al. 2020).

*Nuphar pumila* is mainly distributed in NE Europe and Northern Asia in the boreal zone as well as montane or alpine areas (HEGI 1974, KOZŁOWSKI & EGGENBERG 2005, LANSDOWN 2017). This distribution corresponds to a typical Central European glacial relict. In consequence of the last glaciation, the species was displaced in higher or more northern regions (BÉTRISEY 2018). *Nuphar pumila* grows in standing, slightly acidic, oligo- to mesotrophic, cool water bodies over peat mud soils up to a maximum depth of 300 cm (KÄSERMANN & MOSER 1999, OBERDORFER 2001, KOZŁOWSKI & EGGENBERG 2005). If the water depth exceeds 2 m, the plant individuals remain sterile (HEGI 1974).

If *N. pumila* occurs together with the widespread congener *N. lutea* (L.) Sm., the fertile hybrid *N. ×spenneriana* Gaudin (*N. ×intermedia* Ledeb.) is often found (SEBALD et al. 1993; EGGENBERG & KEEL 2004, KOZŁOWSKI & EGGENBERG 2005). *Nuphar pumila* is morphologically clearly distinct from *N. lutea* by the densely pubescent lower surface of the floating leaves and the deeply lobed stigma disk, which is reached by the rays (KOZŁOWSKI & EGGENBERG 2005, VAN DE WEYER et al. 2011, HESS et al. 2015). In contrast, the identification of *N. ×spenneriana* is problematic, since backcrossing and introgression between *N. pumila* and *N. lutea* occur (LANSDOWN 2017).

Various studies demonstrate that the site conditions in water bodies with *N. pumila* populations differ from those of other *Nymphaeaceae* species (KŁOSOWSKI & TOMASZEWICZ 1993, KŁOSOWSKI 2006, JABŁOŃSKA & KŁOSOWSKI 2012): Water bodies with *N. pumila* are weakly acidic and poor in calcium and magnesium, resulting in low total and carbonate hardness values. The concentrations of sulphate, dissolved silicate, sodium, potassium and chloride are lower in such water bodies, while those of total iron, phosphate and nitrate are higher. Vegetation surveys showed that *N. pumila* occurs together with only few other floating-leaved plants (KOZŁOWSKI & EGGENBERG 2005).

In Switzerland, four autochthonous populations of *N. pumila* survived until 2004 (KOZŁOWSKI & EGGENBERG 2005): Kämmoosweiher (canton of Zurich), Gräppelensee (canton of St. Gallen), Lac Lussy and Lac des Jones (both canton of Fribourg), but the plants

of Lac Lussy are meanwhile considered as a hybrid population (*N. ×spenneriana*) (BÉTRISEY et al. 2020). According to BÉTRISEY et al. (2020), there 13 more populations existed in Switzerland before 1970, among them three in the canton of Zurich (Egelsee, Lützelsee and a nameless pond near Wädenswil). The cantons of Zurich and Fribourg have a high responsibility for this rare species; thus, conservation plans have been developed (EGGENBERG & KEEL 2004, KOZLOWSKI s.a.). In the context of the conservation plan of the canton of Zurich, *ex situ* propagated *N. pumila* individuals were introduced in numerous water bodies. Among these, only the Egelsee is a reintroduction as *N. pumila* occurred there historically and got extinct (BÉTRISEY et al. 2020), while in all other sites the species was newly introduced. For the introductions, the plants were packed in gunny sacks and buried in the littoral in the years 1991–2017. The newly introduced populations developed with different degrees of success, but it was unclear why some thrived and others died.

Therefore, the aim of this study was to compare the site conditions of the successful and unsuccessful introductions in the canton of Zurich with one another and also with those of water bodies with natural *N. pumila* occurrences in Switzerland. To this end, vegetation surveys and water analyses were conducted. Complementary analyses of the diatom genus composition were carried out to provide information on the ecological status of the water bodies (see HILL et al. 2001). Since the data basis in Switzerland was limited, we additionally assessed the site conditions of *N. pumila* and compared it to *N. lutea* based on a large pan-European dataset (CHYTRÝ et al. 2016). All four sources of information should contribute to explaining the different levels of success of population introductions.

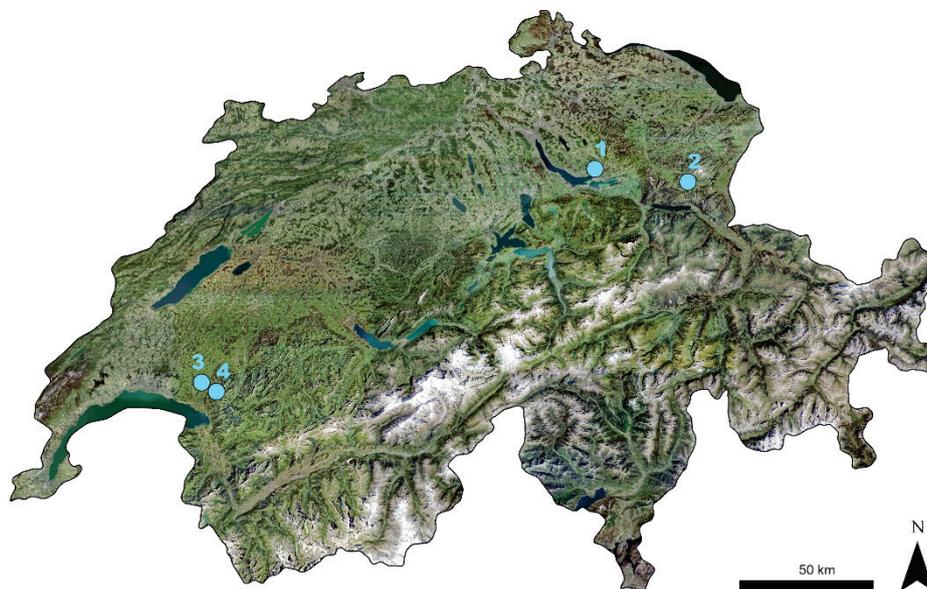
## 2. Methods

### 2.1 Study sites in Switzerland

*Nuphar pumila* (including the hybrid *N. ×spenneriana*) has four remaining natural populations in Switzerland (Fig. 1): Kämmoosweiher (Fig. 2), Gräppelensee, Lac des Jones and Lac Lussy. According to UNA, Bern (pers. comm.), *N. pumila* had been planted in 37 sites in 36 water bodies in the canton of Zurich, mainly in the “Zürcher Oberland”, except for two in Birmensdorf and Thalwil (Fig. 2, Supplement E1). The introduction attempts were made in natural or artificial water bodies and are situated in conservation areas.

Geologically (SCHWEIZERISCHE EIDGENOSSENSCHAFT 2018), the introduction sites in the Zürcher Oberland as well as the Kämmoosweiher belong to the geological units of the Upper Freshwater Molasse and moraines. In the canton of Fribourg the sites with autochthonous *N. pumila* are located on the Lower Freshwater Molasse (Lac Lussy), respectively on moraines (Lac des Jones). The Gräppelensee lies in the geological unit of the Lower Cretaceous. The mean annual temperature of the sites in the canton of Zurich are around 10 °C, while the natural sites in the canton of Fribourg (approx. 7 °C) and the Gräppelensee in the canton of St. Gallen (approx. 5 °C) have a cooler climate (METEOSCHWEIZ 2018).

The studied sites were divided into the groups “natural”, “successful” and “unsuccessful”. The term “natural” refers to the four sites with autochthonous *N. pumila* occurrence (including Lac Lussy, where, according to new analyses of BÉTRISEY et al. [2020], only an autochthonous population of *N. ×spenneriana* occurs). The terms “successful” and “unsuccessful” refer to the introduction sites, depending on the success of the introduction. An introduction was considered as successful when *N. pumila* was present in the vegetation surveys in 2018. Unfortunately, the introduction sites were not monitored regularly after introduction, so that we could not use survival time as dependent variable. In total, 41 sites were visited and analysed physically (4 natural, 4 successful, 33 unsuccessful; see Supplement E1). Fourteen of these (4 natural, 4 successful, 6 unsuccessful) were selected for vegetation



**Fig. 1.** Natural *Nuphar pumila* populations in Switzerland. 1: Kämmoosweiher (ZH), 2: Gräppelensee (SG), 3: Lac Lussy (FR), 4: Lac des Joncs (FR) (geodata kindly provided by swisstopo, DV084370).

**Abb. 1.** Natürliche *Nuphar pumila*-Populationen in der Schweiz. 1: Kämmoosweiher (ZH), 2: Gräppelensee (SG), 3: Lac Lussy (FR), 4: Lac des Joncs (FR) (Geodaten freundlicherweise zur Verfügung gestellt von swisstopo, DV084370).

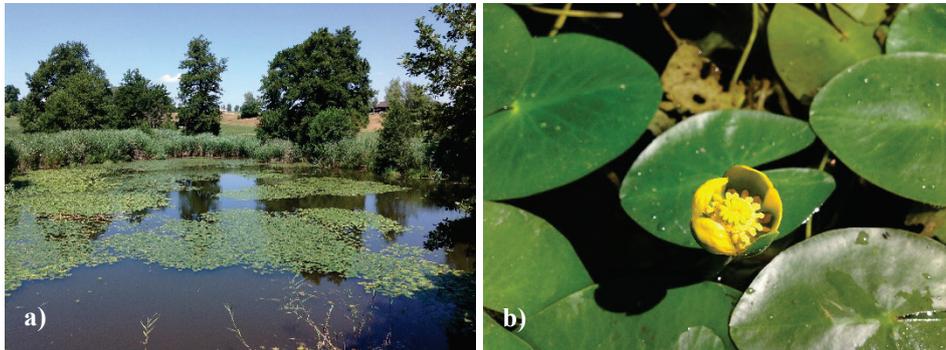
surveys, chemical water analyses and the analysis of diatoms. The six unsuccessful sites were selected to represent a wide ecological and geographical gradient. On the other hand, five introduction sites could not be subjected to standard sampling because they were not accessible or silted up in meantime, or the provided coordinates were erroneous.

## 2.2 Vegetation plots and their analysis

In July 2018, we recorded vegetation plots of 4 m<sup>2</sup> size in the selected sites, using a floating square frame made of polyethylene. This plot size is recommended for aquatic vegetation (CHYTRÝ & OTÝPKOVÁ 2003). Depending on the size of the site, we sampled two or three randomly located plots (Supplement E1), resulting in 32 plots in total. In the larger and deeper lakes Kämmoosweiher, Egelsee, Gräppelensee and Lac Lussy, a boat was used, while in the other sites, sampling was done from the shore. Plots were placed in representative stands, but not necessarily including *N. pumila*. We recorded the plot coordinates with a handheld GPS device (Garmin eTrex 20) (see Supplement E2).

In the vegetation plots, water depth was measured in three random points and averaged. All vascular plants were recorded and their cover estimated in percent. Moreover, total cover of emerged and submerged vegetation was noted. Species of the genera *Potamogeton*, *Utricularia* and *Callitriche* were collected and subsequently determined using a stereomicroscope.

Subsequently, weighted indicator values for humidity, pH, nutrients, light, temperature and continentality were calculated for each vegetation plot (LANDOLT et al. 2010). *Nuphar pumila* and *×spenneriana* as well as plants determined only at the genus level (e.g. ornamental varieties of the genus *Nymphaea*) were excluded from the calculations.



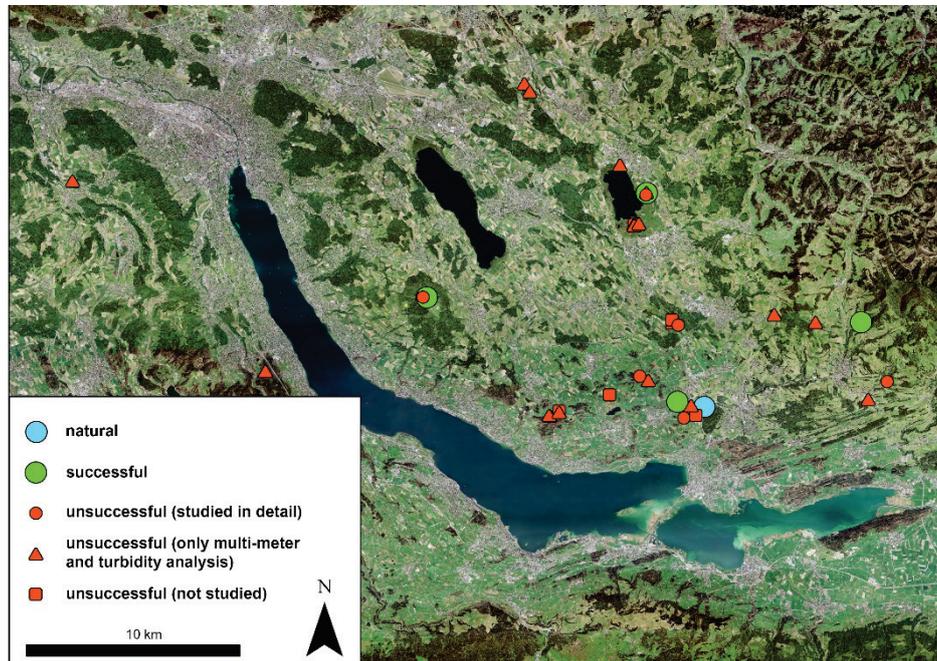
**Fig. 2. a)** Kämmoosweiher with the sole remaining autochthonous populations of *Nuphar pumila* in the canton of Zurich; **b)** detail of *N. pumila* from there (Photos: M. Babbi, July 2018).

**Abb. 2. a)** Der Kämmoosweiher mit den einzigen verbleibenden autochthonen Populationen von *Nuphar pumila* im Kanton Zürich; **b)** Detail von *N. pumila* von dort (Fotos: M. Babbi, Juli 2018).

### 2.3 Measurement and analysis of chemical-physical parameters

From May to July 2018, pH value, conductivity ( $\mu\text{S}$ ), temperature ( $^{\circ}\text{C}$ ), oxygen content ( $\text{mg/l}$ ), turbidity (FNU = Formazin Nephelometric Units) and oxygen saturation (%) were recorded once in 36 measured sites at a water depth of about 40 cm with a multi-meter (HQ40d, Hach Lange GmbH) and the mobile turbidity meter (2100Q, Hach Lange GmbH). These parameters were measured a second time in the fourteen waters in which vegetation was recorded. In the sites Bachtelweiher, Hanfländer 1 and Hanfländer 3, Oberhöfler Riet, Strickelweiher, Waldweiher, Wilderspitz, Kämmoosweiher, Lac des Joncs and Lac Lussy two spots were sampled; in the Gräppelensee four. To reduce the effects of measurement errors, these measurements were repeated per spot in three points at a distance of 1 m. Subsequently, the mean was calculated and outliers were removed (KENT 2012, NOLLET & DE GELDER 2014). This was done when a value was more than 1.5 times the mean of the two closer values (this affected 3% of all measured data). In the 14 selected sites, the multi-meter and turbidity measurements were repeated at a later visit. Acknowledging that our measurements incompletely account for the diurnal and seasonal changes in these parameters, they still provide a picture of prevailing conditions in the water (WEINER 2013, NOLLET & DE GELDER 2014), complementing the results of vegetation surveys, chemical water analyses and diatom studies.

In the 14 selected sites, water samples were taken and examined for the parameters water hardness (measuring range 1–20  $^{\circ}\text{dH}$ ), calcium (Ca in  $\text{mg/l}$ ), magnesium (Mg in  $\text{mg/l}$ ), nitrate ( $\text{NO}_3\text{-N}$ ; measuring range 0.23–13.5  $\text{mg/l}$ ) and phosphate ( $\text{PO}_4\text{-P}$ ; measuring range 0.05–1.5  $\text{mg/l}$ ). The water samples were taken in the same places as the multi-meter and turbidity measurements. In July and August 2018, a total of three samples per site were taken at intervals of two to three weeks. An unfiltered sample of 500 ml each and a filtered sample (mesh size 45  $\mu\text{m}$ ) of 100 ml each was taken and immediately cooled at about 10  $^{\circ}\text{C}$  to reduce the decomposition and remodelling processes of the substances in the water (WETZEL 1983, NOLLET & DE GELDER 2014). The chemical analyses in the laboratory were done the day after sampling. If this was not possible, the water samples were frozen. The water hardness as well as the content of calcium and magnesium were determined with cuvette tests (Hach Lange, LCK 327) in the unfiltered water samples. To determine the nitrate and phosphate concentrations in the filtered samples, the tests LCK 339 and LCK 349 by Hach Lange were used with a spectrophotometer (DR3800, Hach Lange). If the concentration of nitrate and phosphate was below the detection limit, the mean between 0 and the detection limit was used, i.e. 0.115  $\text{mg/l}$  for nitrate and 0.025  $\text{mg/l}$  for phosphate. For calcium and magnesium 0 was used if negative values occurred.



**Fig. 3.** Sites with *Nuphar pumila* introductions and the Kämmoosweiher with a natural population of the species in the canton of Zurich. We treated those introduction sites as successful where the species still existed in the year 2018 (geodata kindly provided by swisstopo, DV084370).

**Abb. 3.** Gewässer mit *Nuphar pumila*-Ansiedlungen und der Kämmoosweiher mit einer natürlichen Population der Art im Kanton Zürich. Wir haben diejenigen Ansiedlungsgewässer als erfolgreich bewertet, in denen die Art im Jahr 2018 noch existierte (Geodaten freundlicherweise zur Verfügung gestellt von swisstopo, DV084370).

## 2.4 Analysis of diatoms

From the 14 selected sites, one submerged piece of a randomly selected aquatic plant was sampled during the vegetation survey. In the lab, an aliquot of the homogenized sample was applied to a microscopic slide. Due to financial constraints, standard diatom sample preparation and identification (cleaning with acid, 500 valves to species level) were not possible. Accordingly, the quality of the slides was relatively poor, and diatoms were only identified to genus level, using HOFMANN et al. (2011). However, in a comparison of water assessments inferred from diatoms identified at species and genus level, HILL et al. (2001) concluded that genus level identification, while not as detailed as species level information, still provides useful evidence about the ecological water quality. Per sample, 100 periphytic diatom valves were identified to genus level from a burn mount slide at 1000× magnification, using Nomarski optics. The relative abundances of the genera were plotted according to their weighted average along a gradient of occurrence of *N. pumila*.

## 2.5 European plot data

On 10 May 2018, we retrieved from the European Vegetation Archive (EVA; <http://euroveg.org/eva-database>; CHYTRÝ et al. 2016) all vegetation plots of *N. pumila* and *N. lutea* that were available in free or semi-restricted access. We included *N. lutea* in the request to allow comparisons of *N. pumila* with the widespread and well-known species. In total, we got 7,920 plots (97 with *N. pumila*, 7,799 with *N. lutea*, 24 with both species), of which 6,324 were georeferenced (see Supplement E3). We

assigned the EVA taxon names to their equivalent in LANDOLT et al. (2010), provided the taxon occurs also in Switzerland. That way, 573 of 1399 (43%) occurring taxa could be assigned to indicator values, corresponding to 86% of constancies and 88% of cover values across the retrieved European plots.

To compare the ecological conditions of the stands of the two *Nuphar* species, we used the ecological indicator values of LANDOLT et al. (2010; scaled from 1 to 5) to be comparable with the analyses within Switzerland. We calculated weighted mean indicator values for moisture, reaction, nutrients, light, temperature and continentality, while excluding the two *Nuphar* species to avoid circular reasoning. Additionally, we characterised the temperature niche of both species via bioclimatic variables derived via the plot coordinates from WorldClim (FICK & HIJMANS 2017). We restricted ourselves to the temperature variables BIO4 (temperature seasonality [standard deviation]), BIO7 (temperature annual range), BIO10 (mean temperature of warmest quarter) and BIO11 (mean temperature of coldest quarter) as precipitation variables should be largely irrelevant for water plants.

## 2.6 Statistical analyses

The statistical tests and visualizations were computed with the statistical environment R 3.5.1 (R CORE TEAM 2018), using the packages ‘car’ and ‘multcompView’. The significance level of all tests was set at  $\alpha = 0.05$ . Since visual inspection of boxplots (in case of group comparisons) and residual plots in case of regressions did not show severe violations of the assumptions of linear models (QUINN & KEOUGH 2002), we applied standard linear models.

We used analyses of variance (ANOVAs) to test for differences in physical-chemical parameters between the three site groups (successful, unsuccessful, natural). If an ANOVA showed a significant pattern, Tukey’s post hoc test implemented in the R package ‘multcompView’ was used to determine homogenous groups. To relate the performance (expressed as % cover) of *N. pumila* to chemical parameters, the results of the three samples taken in July and August 2018 were averaged. Using only the plots with successful introductions of *N. pumila*, we then applied bivariate linear regressions for each parameter (command ‘lm’ in R).

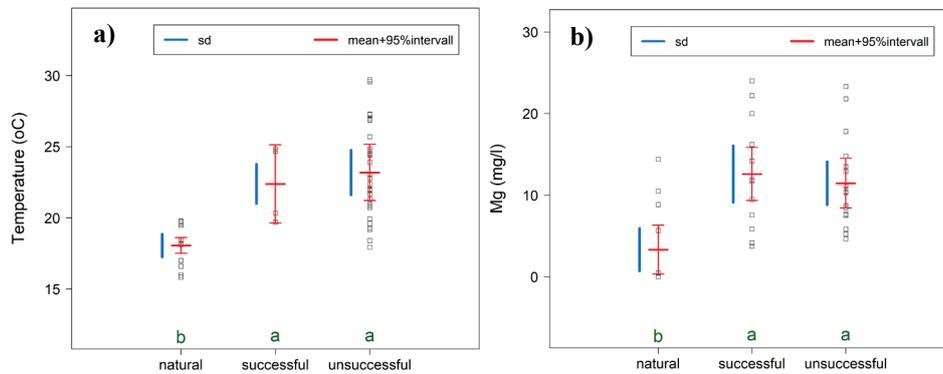
The mean indicator values and bioclimatic variables of the stands with the two species in Europe were compared with *t*-tests for unequal variances (default setting in R). We further tested for differences in the variances of indicator values (which would indicate different niche widths) by means of Levene tests.

## 3. Results

### 3.1 Swiss data

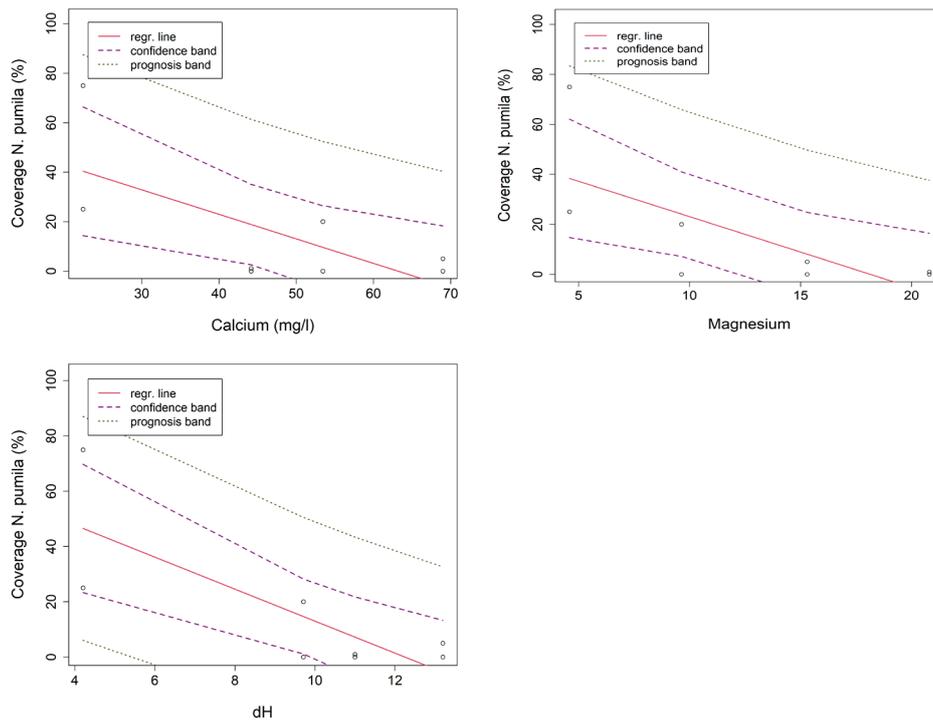
#### 3.1.1 Chemical-physical parameters

Water temperatures varied widely between the studied sites, from 15.8 °C in the natural site Lac des Jones to 29.7 °C in the unsuccessful introduction pond of Robenhuserriet\_TP2. They were significantly lower in the sites with natural occurrences than in those where introductions had been attempted, while successful and unsuccessful introduction sites did not differ (Fig. 4a, Supplement E2). Magnesium content showed a similar pattern with lower values in the natural sites, higher values in the introduction sites, but no difference between successful and unsuccessful (Fig. 4b, Supplement E2). All other tested chemical and physical parameters showed no significant pattern among the three site groups (Supplement E2). The concentrations of nitrate and phosphate were almost exclusively below the measurable range. Among the sites with successful introductions, we found a significant decrease of *Nuphar pumila* cover with increasing calcium and magnesium contents as well as hardness (Fig. 5, Supplement E5).



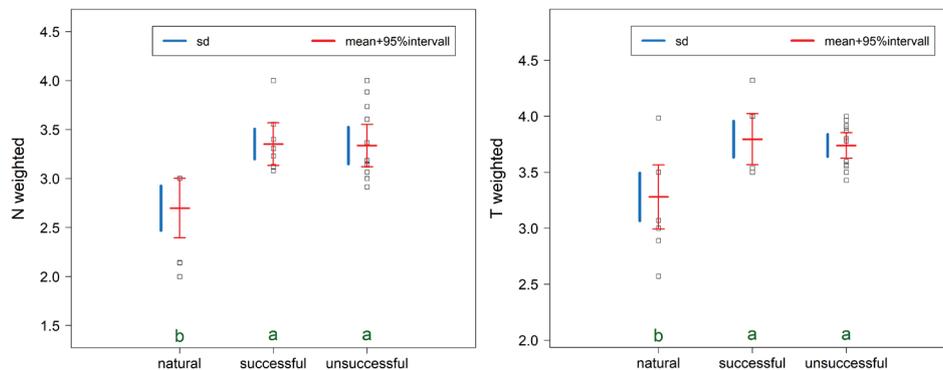
**Fig. 4.** Comparison of **a)** water temperature and **b)** magnesium content between natural, successful and unsuccessful sites. The lower-case letters indicate homogeneous groups according to Tukey's post-hoc test following a significant ANOVA.

**Abb. 4.** Vergleich von **a)** Wassertemperatur und **b)** Magnesiumgehalt zwischen natürlichen, erfolgreichen und erfolglosen Standorten. Die Kleinbuchstaben geben homogene Gruppen gemäß Tukeys Post-hoc-Test nach einer signifikanten Varianzanalyse an.



**Fig. 5.** Regression between the cover of *Nuphar pumila* (%) in successful sites and the calcium concentration ( $p = 0.006$ ), magnesium concentration ( $p = 0.048$ ) and German hardness ( $p = 0.002$ ).

**Abb. 5.** Regression zwischen der Deckung von *Nuphar pumila* (%) an erfolgreichen Stellen und der Calciumkonzentration ( $p = 0,006$ ), der Magnesiumkonzentration ( $p = 0,048$ ) und der deutschen Härte ( $p = 0,002$ ).



**Fig. 6.** Comparison of the mean ecological indicator values (LANDOLT et al. 2010) of nutrients (N) and temperature (T) between natural, successful and unsuccessful sites. The lower-case letters indicate homogeneous groups according to Tukey's post-hoc test following a significant ANOVA.

**Abb. 6.** Vergleich der mittleren ökologischen Ziegerwerte (LANDOLT et al. 2010) von Nährstoffen (N) und Temperatur (T) zwischen natürlichen, erfolgreichen und erfolglosen Standorten. Die Kleinbuchstaben zeigen homogene Gruppen gemäß Tukeys Post-hoc-Test nach einer signifikanten ANOVA an.

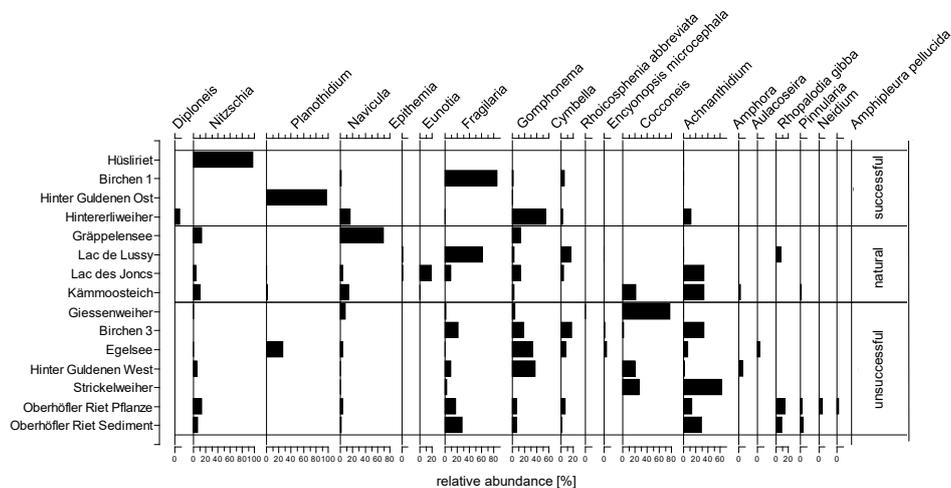
### 3.1.2 Vegetation data and mean indicator values

In the 32 vegetation plots, 31 species were recorded (Supplement E6). In the plots with *N. pumila* ( $n = 12$ ), one to four other species of aquatic macrophytes occurred, e.g. *Potamogeton praelongus*, *P. berchtoldii*, *Equisetum fluviatile* and *Elodea canadensis*. Four plots contained *N. lutea* but only a single plot, Kämmoosweiher 2, together with *N. pumila*. The hybrid *N. ×spenneriana* was found in all plots from the Kämmoosweiher where it co-occurred with *N. pumila*, as well as in Lac Lussy 1 (there without *N. pumila*). The lowest *N. pumila* coverage of 0.5% was in Hüsliriet 1, the highest in Gräppelensee 1 with 93%.

Among the six tested mean indicator values, those for nutrients and temperature showed a significant pattern (Supplement E4). The natural sites in both cases differed significantly from the introduction sites, whether successful or not (Fig. 6). Natural sites had lower indicator values for nutrients and temperature than introduction sites. The indicator values for soil moisture, reaction, light and continentality did not differ significantly among the site groups (Supplement E4).

### 3.1.3 Diatoms

The main genera found in the periphytic samples from 14 sites were *Achnanthydium*, *Gomphonema*, *Fragilaria*, *Navicula*, *Nitzschia* and *Planothidium* (Fig. 7). *Achnanthydium* and *Gomphonema* were found in eight sites with relative abundances between 10 and 60%. In two water bodies low occurrences (5%) of *Eunotia* and in one pond high occurrence (20%) of *Epithemia* were found. These sites had a natural presence of *N. pumila*. *Cocconeis* was found mainly in the ponds with unsuccessful introduction of *N. pumila*, while *Fragilaria* had highest occurrence (60–80%) in ponds with presence of *N. pumila*, including both natural occurrence as well as successful introductions. *Cocconeis* was rare in the sites with natural *N. pumila* occurrence and absent in the sites with successful introduction (Fig. 7).



**Fig. 7.** Relative frequency of the diatom genera. X-axis above: Diatom taxon. X-axis below: Relative frequency (%). Y-axis left: Sampled water bodies. Y-axis right: Status.

**Abb. 7.** Relative Häufigkeit der Diatomeengattungen. X-Achse oben: Diatomeentaxon. X-Achse unten: Relative Frequenz (%). Y-Achse links: entnommene Gewässerproben. Y-Achse rechts: Status.

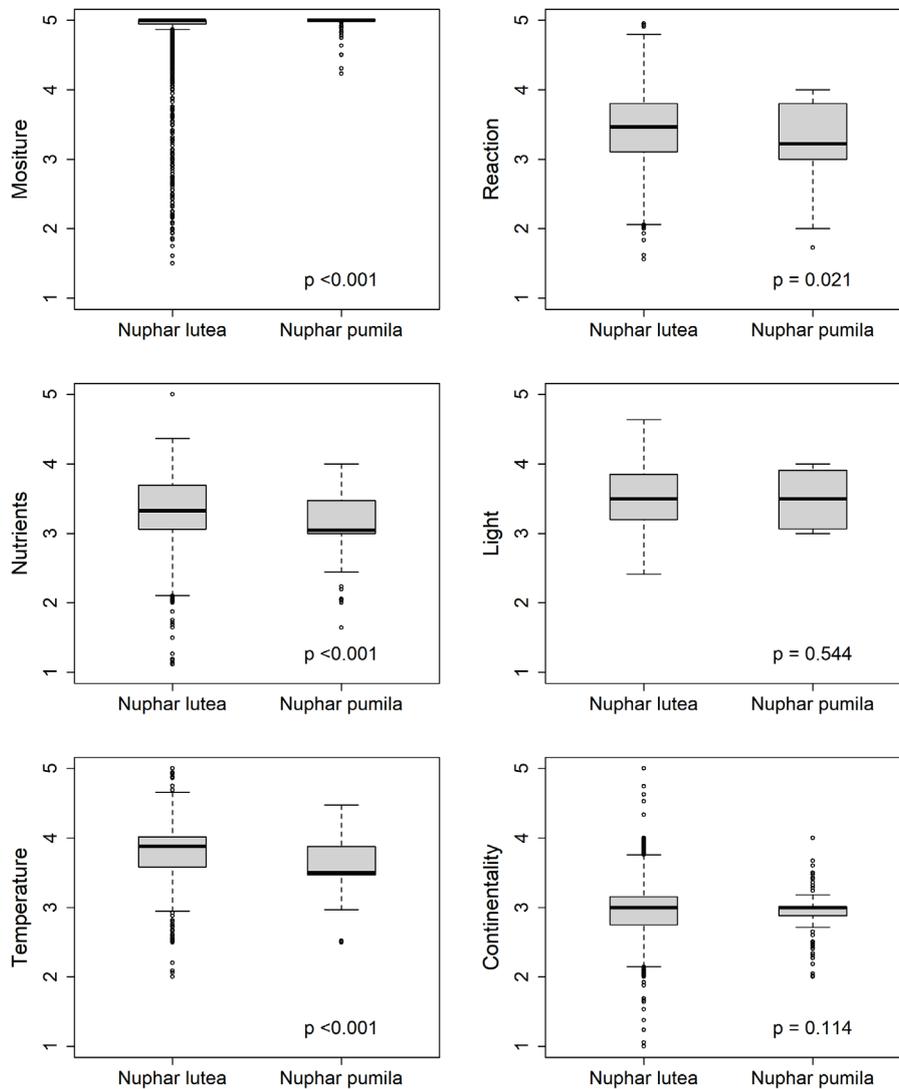
### 3.2 European data

Based on the mean ecological indicator values, the two *Nuphar* species differed most in their habitat requirements for nutrients (lower in *N. pumila*), followed by temperature (lower in *N. pumila*), moisture (higher for *N. pumila*) and soil reaction (lower pH for *N. pumila*), whereas differences for continentality and light were insignificant (Fig. 8, Supplement E5). Habitat conditions were less variable for *N. pumila* in the case of moisture and continentality, but more variable in the case of soil reaction and light (Fig. 8, Supplement E7). According to the bioclimatic variables, *N. pumila* on average inhabits regions with lower mean temperatures and higher thermal continentality, with the mean temperature of the coldest quarter showing the highest difference (-0.2 vs. 2.5 °C) (Supplement E8). The analysis of EVA data indicates that in Europe *Lemna minor*, *Sparganium emersum* and *Potamogeton natans* are the most frequent species occurring together with *N. pumila*.

## 4. Discussion

### 4.1 Predictors of re-introduction success

The significantly lower water temperatures in water bodies with natural occurrence of *Nuphar pumila* correspond to the ecological preferences of the species given in floras (SEBALD et al. 1993, OBERDORFER 2001). It can be explained by the fact that all these locations are large lakes, compared to rather small ponds, in which the introductions took place. The lack of significant differences between successful and unsuccessful introduction sites might be related to their similar elevation and possibly also by the statistical power of our test with only few measurements in relation to the known seasonal and diurnal fluctuations of water temperature (GLANDT 2006).



**Fig. 8.** Boxplots of the mean ecological indicator values (LANDOLT et al. 2010) of the stands of the two *Nuphar* species in Europe. Their means were compared by *t*-tests (for details, see Supplement E5).

**Abb. 8.** Boxplots der mittleren ökologischen Zeigerwerte (LANDOLT et al. 2010) der Bestände der beiden *Nuphar*-Arten in Europa. Ihre Mittelwerte wurden durch *t*-Tests verglichen (Einzelheiten siehe Anhang E5).

Lower concentrations of magnesium in the Swiss sites with natural *N. pumila* populations are in accordance with findings from Poland that highlight that low magnesium (and calcium) concentrations are characteristic for natural sites of *N. pumila* (KŁOSOWSKI & TOMASZEWICZ 1993, KŁOSOWSKI 2006). Therefore, the higher magnesium content in the re-introduction sites could be one reason for the failure of the introduction attempts. This assumption is reinforced by the fact that the regression analyses show a significant negative influence of the water hardness, magnesium and calcium concentrations on the coverage of

*N. pumila*. However, the fact that all these parameters did not differ significantly between successful and unsuccessful introduction sites, indicates that additional factors influence introduction success. Moreover, three of the currently successful sites have been established only in 2017; thus, it remains to be seen whether the species will survive there in the medium term.

Although nitrate and phosphate levels were almost always below the measurable range for natural, successful and unsuccessful site, those with natural *N. pumila* occurrence showed significantly lower mean nutrient indicator values compared to the introduction sites. These results are in agreement with the relative abundances of diatoms (compare Section 4.2). Nutrient poverty could favour the population development of *N. pumila*, which is weak in competition (ROWECK & REINÖHL 1986, BETRISEY et al. 2020). If the water bodies are richer in nutrients, it would be displaced by species that are able to translate the higher nutrient availability into faster growth. Since we measured nutrients only in the water, not in the sediment, we might have underestimated the actual nutrient availability in the introduction sites, while the mean indicator values provide a more realistic picture. The fact that the introduction sites are located in lower elevation, thus experiencing higher mean annual temperatures, might lead to a faster nutrient cycling. Moreover, the fact that the “Zürcher Oberland” is among the regions in Switzerland with the highest atmospheric nitrogen deposition with typically 20–30 kg N ha<sup>-1</sup> yr<sup>-1</sup>, but locally above 40 kg N ha<sup>-1</sup> yr<sup>-1</sup> (RIHM & ACHERMANN 2016) might play a role. It is well known and recently has been demonstrated for Switzerland that the higher the N deposition, the stronger the loss of plant species diversity across habitats (ROTH et al. 2015).

#### 4.2 Diatoms

The genus *Cocconeis*, which was rare in the sites with natural occurrence of *N. pumila* and absent those with successful introductions, is known to indicate eutrophic conditions (HILL et al. 2001), thus supporting the known preference of *N. pumila* for nutrient poor water (LANDOLT et al. 2010, BETRISEY et al. 2020). The high abundances of the genus *Fragilaria* in those sites with natural or introduced occurrence of *N. pumila* is worth mentioning as this genus might indicate high light availability, which indirectly would hint to low algae production, thus low nutrient levels. It is striking that the genera *Eunotia* and *Epithemia*, which indicate low alkalinity (CAMBURN & CHARLES 2000), were only found in Lac des Jones and Lac Lussy with natural *N. pumila* occurrence. It appears that the ponds where the introduction was not successful had the highest richness of diatom genera, but this should be considered as preliminary result as it was based on only 100 valves per sample. A more profound study including the diatoms archived in a sediment core (e.g. HAUSMANN & KIENAST 2006) would be promising to compare historic occurrence of *N. pumila* with changing water quality.

#### 4.3 Habitat requirements derived from EVA

The findings from the plot data across Europe for mean indicator values and bioclimatic variables point into the same direction as the analyses of the Swiss data (environmental parameters, mean indicator values, diatoms). The sites of *N. pumila* are mainly poorer in nutrients, more acidic and cooler than those of *N. lutea* as a reference, whereby the differences are most pronounced for nutrients (difference in mean Landolt values of 0.6, Supplement E7). The European patterns for the temperature niche were consistent whether based on mean indicator values or bioclimatic variables (mean temperatures for the coldest and warmest quarter).

This highlights nutrient status, pH and temperature as key factors that decide on introduction success of newly established populations or chance of survival of remaining autochthonous populations, which is in agreement with the general knowledge on the ecology of *N. pumila* (e.g. KŁOSOWSKI, S. & TOMASZEWICZ 1993, SEBALD et al. 1993, BETRISEY et al. 2020). The most important parameter seems to be eutrophication, but likely higher nutrient levels do not affect *N. pumila* directly, but rather they give *N. lutea* and other hydrophytes a strong competitive advantage (see also BETRISEY et al. 2020). While our inference from the EVA data was only indirect, they would offer the chance for a more direct assessment of drivers of *N. pumila* decline across Europe. One could resurvey known old localities of the species and link the survival to changes in mean indicator values of the communities and/or measured environmental parameters. Such resurvey studies are generally considered as one of the most promising tools to reconstruct vegetation change over the past decades and its attribution to drivers (KAPFER et al. 2017, VERHEYEN et al. 2017).

#### 4.4 Conclusions for nature conservation

The underlying hypothesis of this study that there are clear environmental factors that are causal for whether introductions of *N. pumila* in various water bodies of the canton of Zürich are successful or not, was not supported. Rather all the introduction sites systematically differed from the natural sites in Switzerland by warmer and less acidic water, and based on mean ecological indicator values, by higher nutrient availability. While we tested the main factors discussed in the literature, it cannot be categorically excluded that other, unmeasured factors were responsible for the unequal and overall very low success rate of introductions (4 out of 37). We conclude that the available water bodies in the canton of Zurich are generally not very promising for introductions of the species, and it was rather a matter of chance than of specific differences that *N. pumila* survived in a few ponds in which the species was planted but not in most others. However, since these introductions are rather recent and given the ecological situation of the water bodies, it remains questionable whether the species will survive there in the medium run.

The “problem” of the sites that are available for introductions in the canton of Zürich is that their elevation is generally too low, i.e. the air and consequently the water temperatures are too high (which is amplified in very small water bodies), their pH/hardness is too high and, most importantly, they are too rich in nutrients. While one could also more fundamentally question whether introducing species in sites where they never had occurred historically, is a proper conservation measure, it is even more questionable if the environmental conditions do not adequately meet the requirements of the species. According to LAUTERBACH (2013), the combination of limited habitat suitability with the fact that, except in one case, the introduction sites did not have historical occurrences, are clear counter-indications against introduction of *ex situ* propagated specimens of rare species.

*Nuphar pumila* is a glacial relict that generally suffers from the natural developments after the last glaciation, which across Europe led to higher temperatures (LANG 1994) and, via natural succession, to higher nutrient status of water bodies (POTT & REMY 2000). These natural processes have been amplified by anthropogenic global warming and nitrogen and phosphorous eutrophication (WORLD RESOURCES INSTITUTE 2005). We therefore recommend that *N. pumila* should primarily be protected (and potentially re-introduced) in regions with lower temperatures, lower eutrophication rates and preferentially acidic bedrock as there the chances of success are much higher. To ensure the survival of native populations in Switzerland, i.e. to reduce the risk of extinction due to a local event, it might thus be more

sensible to attempt reintroductions in former sites at higher elevation and/or with lower atmospheric nitrogen input. If the former sites identified by BETRISEY et al. (2000) in other cantons also should not be suitable, one could consider – for risk minimisation – new introductions in water bodies in the surroundings of the surviving populations in Gräppelensee (canton of St. Gallen) and Lac des Jones (canton of Fribourg), provided they are free of local nitrogen input and are not inhabited by *N. lutea* (to avoid the hybridisation risk). For the canton of Zurich, we believe that the most sensible conclusions for conservation are, (a) to protect the remaining autochthonous population of Kämmoosweiher from any avoidable threats (like local nutrient input), (b) conserve the genotype from there *ex situ* and (c) stop further *N. pumila* plantations in the wild and spend the freed money for more promising conservation measures for other species and habitats.

### Erweiterte deutsche Zusammenfassung

**Einleitung** – Die Kleine Teichrose (*Nuphar pumila*) ist ein Glazialrelikt, das heutzutage in ganz Europa selten ist und daher in den meisten europäischen Ländern auf der Roten Liste steht. In der Schweiz gibt es nur noch drei autochthone Populationen und eine Population der Hybride mit *N. lutea* (*N. ×spenneriana*) (KOZLOWSKI & EGGENBERG 2005, BÉTRISEY et al. 2020; Abb. 1). Eine der drei autochthonen Populationen kommt im Kämmoosweiher im Kanton Zürich vor (Abb. 2). Wegen der Seltenheit auf kantonaler Ebene hat die Naturschutzverwaltung des Kantons eine *ex situ*-Vermehrung und Auswilderungen der so gewonnenen Pflanzen in Auftrag gegeben. Diese wurden bislang in 37 verschiedenen Gewässern in Naturschutzgebieten des Kantons ausgebracht, darunter in einem bekannten ehemaligen Fundort. Da nur 10 % dieser Ansiedlungen erfolgreich waren, war es das Ziel weiterer Untersuchungen, die Ursachen für den Misserfolg herauszufinden.

**Methoden** – Wir haben die Vegetation (Vegetationsaufnahmen von 4 m<sup>2</sup>) und chemisch-physikalische Parameter der autochthonen Populationen (einschließlich der Hybridpopulation, *n* = 4) mit den Parametern erfolgreicher (*n* = 4) und erfolgloser (*n* = 33) Ansiedlungsgewässer verglichen (Abb. 1 und 3, Anhänge E1, E2 und E6). Für 14 Gewässer haben wir zudem die Zusammensetzung der Kieselalgenflora auf Gattungsebene analysiert, um so integrale Aussagen über die Gewässerqualität zu bekommen. Schließlich haben wir aus dem *European Vegetation Archive* (EVA; CHYTRÝ et al. 2016) alle verfügbaren Vegetationsaufnahmen mit *Nuphar pumila* (*n* = 121) und *N. lutea* (*n* = 7823) bezogen (vgl. Karte in Anhang E3), um die Standortansprüche der beiden Arten basierend auf mittleren ökologischen Zeigerwerten (LANDOLT et al. 2010) und thermischen Klimadaten (FICK & HIJMANS 2017) zu vergleichen.

**Ergebnisse** – In der Schweiz unterschieden sich die natürlichen Standorte in verschiedenen betrachteten Parametern von den Ansiedlungsgewässern, während es keine signifikanten Unterschiede zwischen erfolgreichen und erfolglosen Ansiedlungsgewässern gab. Die Gewässer mit autochthonen Vorkommen hatten eine tiefere Wassertemperatur und einen niedrigeren Magnesiumgehalt (Abb. 4 und Anhang E4). Auch nach den mittleren ökologischen Zeigerwerten waren sie kälter und zudem nährstoffärmer (Abb. 6 und Anhang E4). Innerhalb der erfolgreichen Ansiedlungsgewässer nahm die Deckung von *N. pumila* mit zunehmendem Calcium- und Magnesiumgehalt sowie höherer deutscher Härte ab (Abb. 5 und Anhang E5). Unter den Kieselalgen kam die Gattung *Cocconeis* hauptsächlich in erfolglosen Ansiedlungsgewässern und die Gattung *Fragilaria* hauptsächlich in Gewässern mit aktuellem *N. pumila*-Vorkommen vor (Abb. 7). Nach den EVA-Daten unterscheiden sich die beiden *Nuphar*-Arten am stärksten in ihren Habitatansprüchen für Nährstoffe (niedriger bei *N. pumila*), gefolgt von Temperatur (niedriger bei *N. pumila*), Feuchte (höher bei *N. pumila*) und pH-Wert (niedriger bei *N. pumila*) (Abb. 8 und Anhang E7). Basierend auf den Plotkoordinaten sind die Standorte von

*N. pumila* im Mittel kühl und weisen eine größere thermische Kontinentalität auf (Anhang E8). Die häufigsten Begleitarten von *N. pumila* europaweit waren *Lemna minor*, *Sparganium emersum* und *Potamogeton natans*.

**Diskussion** – Insgesamt konnten wir keine signifikanten Unterschiede zwischen den erfolgreichen und den erfolglosen Ansiedlungsgewässern im Kanton Zürich feststellen. Beide Typen von Gewässern scheinen im Großen und Ganzen heutzutage ungeeignet für dauerhafte Vorkommen von *N. pumila*, da sie klimatisch zu warm sind und überwiegend über basenreichem Untergrund liegen. Dazu kommt noch der sehr hohe atmogene Stickstoffeintrag in großen Teilen des Kantons (RIHM & ACHERMANN 2016), der für die konkurrenzschwache, nährstoffliebende Art problematisch ist. Es scheint also eher Zufall oder eine Frage der Zeit zu sein, dass sich *N. pumila* bislang (noch) in vier Ansiedlungsgewässern halten konnte. Wir schließen daher, dass Wiederansiedlungsversuche der Art in Gewässern des Kantons keine effiziente Verwendung von Naturschutzgeldern darstellen. Statt dessen wäre es besser, das eine noch bestehende Vorkommen im Kanton konsequent zu schützen (u. a. vor Nährstoffeinträgen und Hybridisierung), während für (Wieder-) Ansiedlungen Gewässer höherer Lagen über silikatischem Untergrund und mit geringer Eutrophierung ausgewählt werden sollten, wie sie in anderen Kantonen, aber nicht im Kanton Zürich vorkommen.

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## Author contribution statement

S.K. conducted the research underlying this paper as a Bachelor thesis under the supervision of J.D. and M.B. She carried out the field sampling, with help of M.B. and J.G., performed the statistical analyses of the Swiss data. S.K. and J.D. planned the paper and led the writing. S.H. identified the diatoms, visualized and interpreted the results, C.B., F.L. and K.Š. contributed a major part of the European vegetation data and helped with their interpretation, while P.S., S.W. and M.B. analysed and visualised them. All authors contributed to the writing and/or revising of the manuscript and approved its content

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## Supplements

**Additional supporting information may be found in the online version of this article.**

**Zusätzliche unterstützende Information ist in der Online-Version dieses Artikels zu finden.**

**Supplement E1.** Overview of all studied sites and applied methods.

**Anhang E1.** Übersicht aller beprobten Wasserkörper und der verwendeten Methoden.

**Supplement E2.** Coordinates and dates of the Swiss relevés.

**Anhang E2.** Koordinaten und Daten der Schweizer Vegetationsaufnahmen.

**Supplement E3.** Spatial distribution of the vegetation-plots from EVA.

**Anhang E3.** Räumliche Verteilung der Vegetationsaufnahmen aus EVA.

**Supplement E4.** Results of ANOVAs for differences in site conditions between natural, successful and unsuccessful sites.

**Anhang E4.** Ergebnisse der Varianzanalysen für Unterschiede in den Standortbedingungen zwischen natürlichen, erfolgreichen und erfolglosen Standorten.

**Supplement E5.** Results of regression analyses of *Nuphar pumila* cover against various predictors.

**Anhang E5.** Ergebnisse von Regressionsanalysen der *Nuphar pumila*-Deckungen gegen verschiedene Prädiktoren.

**Supplement E6.** Vegetation table of the Swiss relevés. The coverage is indicated as percentage (%).

**Anhang E6.** Vegetationstabelle der Schweizer Vegetationsaufnahmen. Der Deckungsgrad ist in Prozent (%) angegeben.

**Supplement E7.** Detailed statistical results for comparison of stands of the two *Nuphar* species in Europe.

**Anhang E7.** Detaillierte statistische Ergebnisse für den Vergleich der Bestände mit den beiden *Nuphar*-Arten in Europa.

**Supplement E8.** Bioclimatic comparison of stands of the two *Nuphar* species in Europe.

**Anhang E8.** Bioklimatischer Vergleich der Bestände mit den beiden *Nuphar*-Arten in Europa.

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