

## Habitat requirements of *Luronium natans* (L.) Raf. in northern Belgium

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### Highlights

- *Luronium natans* in northern Belgium has a broad environmental range, including eutrophic waters
- Competitive abilities of this species are limited, particularly regarding light
- As an early-successional species, *L. natans* has a ruderal advantage
- *L. natans* can withstand physical disturbance, showing strong stress tolerance
- The plasticity of *L. natans* in reproduction may be linked to nutrients and light availability

### Abstract

We investigated the environmental range of *Luronium natans* populations in lower Belgium (Flanders) with particular focus on key factors influencing its population size and the interplay of nutrient availability and physical disturbances in determining its abundance. Soil calcium content and total phosphorus in the water layer were the only physical-chemical variables related to *L. natans* abundance. Physical water body and vegetation characteristics influenced population dynamics and incidence of reproductive strategies. The species has a ruderal character, and occurs early in vegetation succession or depends on regular physical disturbance events for prolonged presence. Presenting limited competitive abilities, especially in terms of light availability, the species is hindered by taller-growing vegetation. Large populations are typically associated with well-lit conditions. Its flexibility to switch between vegetative and generative reproduction strategies may be associated with nutrient and light conditions. The need for long-term monitoring to consider population dynamics and evaluate the effectiveness of conservation efforts is emphasized. Overall, our study provides further insight into the relation between abiotic conditions, population dynamics and conservation management and extends the basis for sustainable management of the remaining *L. natans* populations in Flanders and similar regions of Atlantic Europe.

### 1. Introduction

Conservation management of endangered plant species has to take into account the realized niche, i.e. the actual abiotic and biotic environment the species is occurring. For soft-water plant communities and their characteristic isoetids and amphibious therophytes, limiting availability of carbon, phosphorus and nitrogen is considered imperative to enable them to fully exploit their specific physiological and morphological adaptations. At the same time it is essential to prevent competitive exclusion, e.g. by taller submerged plants and helophytes. Management plans for maintaining and restoring favorable conservation status of soft-water plant communities therefore usually aim at reducing acidification and eutrophication pressures and obtaining alkalinity and nutrient levels within the range where community composition or populations of constituent species are sustained (Roelofs, 1983; Kozłowski and Vallelian, 2009; Lucassen et al., 2016; Lansdown, 2022). Physical disturbance, either by natural phenomena such as wind erosion or seasonal water-level changes, management including temporary draw-down, vegetation removal or mowing and conservation actions such as pond clearing, may allow eutrophication-sensitive soft-water communities and

pioneer species to prosper longer and at higher nutrient levels than usual when such disturbances are lacking (Greulich et al., 2000a). Effects on soil conditions, i.e. removal of organic matter and enhanced oxygenation, and suppression of potential competitors are important mechanisms favoring non-competitive, early-successional species. Balancing nutrient status with appropriate levels of physical disturbance might therefore be an appropriate management strategy for conservation of certain soft-water plants as long as eutrophication pressures are not fully eliminated.

*Luronium natans* (Alismataceae), or floating water-plantain, is an exemplary target species in this respect. As an aquatic or amphibious pioneer, it disappears quickly when overgrown by other plants, yet it also tolerates a certain degree of physical disturbance (Greulich et al., 2000a). Endemic to west and central Europe, it occurs from Spain to the southern part of Sweden and Norway and from Ireland to the Czech Republic and Poland (<http://bd.eionet.europa.eu/article17>, accessed on 6 April 2023 and GBIF Secretariat, 2022). Its distribution shows four more or less disjunct main areas: Pomerania in Poland, the Atlantic part of the UK, Brittany and central France, and a central area including northern Belgium (Flanders) and the Netherlands. Scattered populations occur in between and in their periphery. *Luronium natans* declined markedly throughout Europe in recent decades (Willby and Eaton, 1993; IUCN, 2023; Makuch et al., 2023). Consequently, it is considered as 'vulnerable' to 'extinct' on Red Lists in the central area (LNV, 2004; Saintenoy-Simon et al., 2006; Van Landuyt et al., 2006; Metzging et al., 2018), as 'critically endangered' in Poland and the Czech Republic (Zarzycki and Szelag, 2006; Grulich, 2012) and as 'near threatened' to 'critically endangered' in Scandinavia (Moeslund et al., 2019; Artsdatabanken, 2021; Artfakta, 2023). Being listed as an Annex 2 and Annex 4 species of the Habitats Directive (European Commission, 1992), it requires protection and reporting on its population size, range, habitat quality and future prospects every 6 years by EU member states. In Flanders, *L. natans* occurs almost exclusively in the north-eastern coversand area. The 40 sites presently remaining here represent c. 8 % of all current occurrences (GBIF Secretariat, 2022), making Flanders and parts of the neighboring Netherlands a relative stronghold of the species on the continent.

Depending merely on site conditions, floating water plantain is able to deploy different growth forms (Greulich et al., 2001; Lansdown and Wade, 2003; Lucassen et al., 2007). The isoetid growth form, consisting of a rosette of submerged linear leaves of up to 60 cm long, occurs in somewhat deeper parts of lakes or rivers. A nymphaeid growth form that combines rosettes of submerged linear leaves with elliptical, mostly floating leaves on long petioles. A terrestrial growth form with short-stalked elliptical leaves with pointed apex on temporarily drying soil, e.g. in marshland and on the banks of shallow ponds.

Both vegetative and generative reproduction are common for the species. Vegetative reproduction and propagation occur by means of stolons on which new shoots are formed. These can root and establish on the spot, or break off and spread in the water to settle elsewhere. Flowers produce seeds that remain viable for a long period (Lansdown and Wade, 2003; Janssen and Schaminée, 2004). Transport by water and persistent seed banks allow formation of metapopulations in space and time. Typically, population size varies substantially with germination conditions for sexually reproducing populations and remains more stable for vegetative populations.

Generally, *L. natans* is considered to occur mainly in weakly buffered, nutrient-poor conditions, in or along water with low mineral concentrations on a mineral-organic substrate (Bazydło, 2004; Szmeja and Bazydło, 2005). This is exemplified particularly by Polish populations, developing almost exclusively at low nitrogen and phosphorus concentrations (Table 1; Szankowski and Klosowski, 2001; Szmeja and Bazydło, 2005). Low phosphorus

availability is also claimed to explain why its occurrence is sometimes associated with iron-rich seepage (Lansdown and Wade, 2003; Lucassen et al., 2007). In Flanders, *L. natans* grows in stagnant waters, temporarily dry wetlands and a few slow-flowing lowland brooks, usually with weakly buffered and fairly nutrient-poor water. However, it also occurs in more nutrient-rich and alkaline conditions, as also reported from the Netherlands (Lucassen et al., 2007), British navigation canals (Willby and Eaton, 1993; Lansdown and Wade, 2003), drainage canals and rivers in Denmark (Nielsen et al., 2006) and cut-off river channels in France (Greulich et al., 2000b). Hence, the potential niche is quite broad, e.g. with considerable tolerance ranges for pH, alkalinity and calcium.

Genetic screening of populations from various habitats revealed 14 separate genetic clusters, a high degree of clonal reproduction and overall low genetic diversity in Flanders (Cox et al., 2014). However, identical clones occurred among geographically distant sites and populations exhibited high levels of differentiation. This suggests that several populations were established from one or a few migrants and that gene flow occurs among neighboring patches within a metapopulation, emphasizing the importance of connectivity for maintaining regional genetic diversity of the species.

The purpose of this paper is twofold. First of all, we investigate the realized niche and optimal growth conditions of *L. natans* in our study area, in comparison to abiotic conditions observed elsewhere. Special attention is given to the relative roles of nutrient conditions, the principal sources of physical disturbance and their potential interaction, as this carries important management implications and could also explain some of the observed regional habitat differences. If trophic tolerance is mediated by physical disturbances, the species would be restricted to low nutrient conditions in their absence, but this would be less so where the development of more competitive species is set back by intermittent disturbance events or physical processes. To this end, we examine whether: (A) the species is most abundant at low-nutrient conditions, as reflected by water and soil chemistry, and, (B) more optimal growth conditions depend on the presence or absence of physical disturbance. Secondly, plausible causes of population decline in the region are discussed. By doing so we intend to better inform sustainable management of the remaining populations, allow fine-tuning of restoration plans and furthermore support identification of potential growth sites in the study region, and by extension, similar parts of Atlantic Europe.

## **2. Material and methods**

### **2.1 Study area**

The distribution of *Luronium natans* in Flanders was documented by Ronse et al. (2015). Its core distribution area lies within the Kempen ecoregion, which covers north-eastern Belgium. This low-lying region (3 – 200 m above mean sea level) is mainly characterized by a thick cover of carbonate-poor aeolian sandy soils, which slopes gently towards the north. Along intersecting lowland rivers, somewhat richer soils and fen peat may occur locally and at places, less permeable lenses of clay or loam are present in the subsurface. The relief is mainly flat with some inland dunes and erosional questas. The climate is maritime temperate with relatively cool summers (average 19 °C), mild winters (4 °C) and significant precipitation in all seasons, ranging from 47 to 87 mm per month. Before agricultural and industrial development, land cover in this area consisted mainly of sandy heath, moors and wetlands. Actually, it is mainly agricultural (52 %), followed by built area and infrastructure (33 %) and 10 % woodland, predominantly pine plantations. Most populations of *L. natans* are in shallow ponds, either natural moorland ponds, old man-made fish-farming ponds or excavations for sand or loam. Only a few are in slow-flowing brooks or canals.

## 2.2 Vegetation surveys

Data included in this study are mainly from vegetation surveys in 2008 and 2009 and population monitoring for EU reporting since 2015 (data included until 2021). Together, they cover all 100 sites in the Flemish region where the occurrence of *L. natans* has been reported since 2000 (Figure 1). Data collection also included 36 sites where the species proved to be absent or disappeared during the study period, allowing us to examine possible causes for its disappearance.

Population monitoring for Article 17 reporting of the Habitats Directive followed a standard protocol (Leyssen, 2022) with a vegetation survey during the growing season (June-September). This includes searching for rosettes of *L. natans*, by systematically wading through transects of 3 to 4 meters wide. Depending on the site, deeper parts are screened with a telescopic rake, or spot-checked with a dredge rake from a boat. In the latter case, transects and sampling points were at ca. 10-20 m from each other. In watercourses, wading was against the current to avoid visibility due to upturned substrate along a zig-zag pattern from one bank to the other. Stands of *L. natans* were located with GPS, their surface area recorded and the number of rosettes estimated in each one. Reproduction strategy was noted by recording the presence of flowers, seeds and stolons. The percentage of the water volume occupied by submerged vegetation (PVI; Plant Volume Infested or percent plant volume inhabited; Canfield et al., 1984) and the percentage of vegetation higher than *L. natans* (tall vegetation) were recorded at population level. Depending on the dominant growth form of *L. natans*, the latter includes helophytes, but it can also include submerged vegetation, nymphaeid or duckweed cover.

For each site the abundances of *L. natans* were aggregated in three classes: absence, low abundance (< 100 rosettes and < 5 m<sup>2</sup>) and high abundance (> 100 rosettes or > 5 m<sup>2</sup>; based on Huck and Michl, 2006). A more detailed estimation was considered less useful, given the expected amplitude of temporal inter-annual variation (Willby et al., 2003). Reproductive status was classified as vegetative (no flowers or seeds) or generative (seeds or flowers present). Additional site and vegetation characteristics recorded during field work are given in Appendix A, partim water body and vegetation characteristics.

## 2.3 Soil and water sampling

Soil samples were collected in May and June 2010 with a plexi tube corer (diameter of 4 cm). Four systematically positioned sub-samples were taken per 1 m<sup>2</sup> plot where most *L. natans* plants occurred, retrieving the top 10 cm (excluding liquid mud), corresponding roughly to the principal root zone of the species. All sub-samples were stored in a plastic bag and homogenized prior to analysis (Appendix A). Extraction with barium chloride (BaCl<sub>2</sub>) was used to determine CEC and cations (Al<sup>3+</sup>, Fe<sup>2+</sup>, Mn<sup>2+</sup>) were analyzed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES; Optima 8300 Perkin Elmer). Loss on ignition was determined after drying in a muffle furnace at 550°C. Sediment sampling occurred one to two years after the vegetation survey to avoid interference. We assumed relative stability of the measured characteristics over this period.

Water samples were taken bimonthly during the growing season of 2009 (April-September). A number of sites was resampled monthly in the same period between 2014 and 2021, in the course of ongoing water chemistry monitoring. Temperature, conductivity, dissolved oxygen and pH were measured in the field with a digital precision meter (WTW Multi 3430 with WTW IDS TetraCon 925, WTW IDS FDO925 and WTW IDS Sentix 940 electrodes). Three 2 liter sub-samples were collected at least 1 m apart from each other and ca. 30 cm below the water surface and mixed in plastic containers (Scheers, 2021). For measurement of metals,

0.30 ml of nitric acid (1 %) was added to 30 ml of filtered sample (0.20 µm). Samples were cooled during transport, stored at 1-5 °C and analyzed within 24 h. Analyses were carried out in an ISO-17025-certified laboratory by means of spectrophotometry (SPECORD 200 PLUS), ICP-AES (Optima 8300 Perkin Elmer), ion chromatography (Metrohm 930 Compact IC Flex) and high temperature catalytic combustion (FormacsHT analyzer, Skalar) according to ISO, Belgian (NBN) or Flemish standards (CMA, 2023; Appendix A). Values below detection thresholds were set at 50 % of the threshold for calculations. For each water variable and site, the mean value was used for analyses. Not all water samples were collected in the same year as the vegetation survey, but only those within a time frame of less than 2 years were retained. To our knowledge, no important changes in water quality (e.g. due to pollution spills or flooding) occurred between the time of the water sampling and the vegetation survey. We were unable to collect representative interstitial pore water samples (e.g., Roelofs, 1983) in the course of this study.

## 2.4 GIS and additional data

Geospatial water body characteristics were calculated in ArcGIS (ArcGIS Desktop 10.8.1; version 10.8.1.14362). Minimum Bounding Geometry was applied to identify morphological metrics (rectangularity, circularity and shore development). The linear distance to the perimeter of the nearest site with *L. natans* was calculated. Water body age and eventual restoration date were derived from historical maps, time-series of orthophotographs, literature and information retained from site owners and managers. As the exact dates were not available for all water bodies, data were grouped into classes (>2015; 2010-2015; 2000-2010; 1971-2000; 1939-1971; <1939). The time elapsed since creation or restoration was categorized within year intervals (0-2; >2 -5; >5 -10; >10 - 100; >100; time since last disturbance). Bank slope, mud thickness and management regime were recorded during field visits (see Appendix A for a detailed description).

## 2.5 Data analysis

Data analysis was performed in R (R version 4.3.2; R Core Team, 2023). To avoid sampling time bias, an exploratory analysis checked for differences in the ranges of soil and water chemistry variables between data preceding, coinciding with or following the year of the respective vegetation survey. The correlation between potential explanatory variables was calculated as the Spearman rank-correlation on pairwise complete observations (see Appendix C).

Conditions at sites where *L. natans* disappeared were compared to sites with low abundance and high abundance by means of boxplots and bar charts to identify potential discriminating characteristics. These were complemented with ecologically relevant variables mentioned in the literature (Arts et al., 1990; Greulich and Bornette, 1999; Greulich et al., 2000b; Lansdown and Wade, 2003; Willby et al., 2003; Bazydło, 2004; Bazydło and Szmeja, 2004; Szmeja and Bazydło, 2005; Nielsen et al., 2006) and for highly correlated variables, only one was kept. Because the entire dataset was high-dimensional and to fully exploit differently sized observation frames, four subdatasets were distinguished: soil, water column, water body and vegetation characteristics. These were analyzed separately and results used for joint treatment. To test hypothesis (A), models are built based on soil and water column characteristics. If (A) is true, we expect optimal conditions (high abundance of *L. natans* and flowering) to coincide with low soil and water nutrients and low physical-chemical disturbance indicating variables. To test hypothesis (B), models are built based on water body and vegetation characteristics. If (B) is valid, we expect optimal conditions to coincide with low presence of other species (i.e. low PVI, low percentage of shading, low percentage of tall vegetation) and presence of physical disturbance. Concentrations, distance to nearest

population and surface area of the water body were log-transformed and water depth was square-root transformed for modelling. Generalized linear models using the 'ocat' family for ordered categorical data (Wood et al., 2017b) were built to model the ordinal response for the three abundance classes: absence, low abundance and high abundance. Similarly, logistic regression (generalized linear model; binomial family) was used to model the incidence of generative reproduction as a function of covariates. Model comparison and selection was performed by using the function 'dredge' of the R-package 'MuMIn' (Bartoń, 2023). This function performs an automated model selection, based on a full model containing all putatively important covariates. All possible reduced models (dropping terms from the full model) are fitted one by one through repeated evaluation. Models are compared and ranked based on Akaike Information Criterion for small sample sizes (AICc). The highest-ranked models were visualized by plotting the conditional predictions using the R-package 'marginaleffects' (Arel-Bundock, 2024). These plots show the predictions of the models on the y-axis against values of predictors on the x-axis and using a different color for the different abundance classes. These conditional predictions hold all covariates, whose values are not specified explicitly, at their mean.

### 3. Results

Water body and vegetation characteristics were the best predictors of *Luronium* abundance. Temporary drying, time since last disturbance, water-level fluctuations, water-level regulation and wind action were all relevant predictors (Figure 2; Figure 3; Appendix E). High abundance was positively related to water-level fluctuations and water-level regulation, wind action, absence of temporary drying and 2 – 5 years since last disturbance event. Absence of *L. natans* was related to absence of water fluctuations and occurrence of temporary drying. Considering vegetation characteristics, high abundance concurred with a low percentage cover of tall vegetation and eutrophication indicators, but a high percentage of acidification indicators and invasive species (Figure 2; Figure 4; Appendix E). Absence was more likely in water bodies with a high percentage of tall vegetation, shading or eutrophication indicators.

*Luronium* abundance is explained to a lesser extent by the abiotic variables assessed in this survey. High Ca-soil levels appear to restrict the presence of *L. natans*; although the effect was not significant (Figure 2; Figure 5.A; Appendix E). From the water column variables, TP was identified as a significant explanatory variable and O<sub>2</sub>-saturation as marginally relevant (Figure 2; Figure 5.B-C; Appendix E). Low TP was associated with high abundance and high TP and high O<sub>2</sub>-saturation with absence.

To assess the relative importance and potential interaction effects of all relevant variables in the aforementioned models jointly, a combined model was evaluated. The final model included TP and the presence or absence of any form of physical disturbance (management of aquatic vegetation, water-level fluctuations, water-level regulation or wind action). Physical disturbances were more decisive than TP for *L. natans* abundance. Notably, only the main effects turned out to be significant and no interaction between TP and disturbance occurred (Appendix E).

The models predicting generative reproductive were also mainly explained by water body and vegetation characteristics. Flowering was related to low water transparency, deeper water, lower mud thickness and low circularity (irregular water body shape); although all predictors were marginally significant (Figure 6; Appendix E). Of the vegetation characteristics, PVI and percentage of eutrophication-indicating species were significant for reproduction type (Figure 6; Appendix E): more generative reproduction was observed with high PVI, whereas a high percentage of eutrophication indicators was associated with more

vegetative reproduction. Soil nor water-chemistry variables influenced generative reproduction (Appendix E).

Figure 2 shows the different ranges of the three abundance classes for the variables included in the above mentioned models. It illustrates the variance among the surveyed sites, distinguishing between sites where *L. natans* disappeared (absent), where it occurred with low abundance and where it attained high abundance. All other variables show broad ranges for abundant *L. natans* vegetation (Appendix D) and, at their best, only result in marginal differences with other two abundance classes. Tables 1 and 2 shows the mean values and ranges obtained in this study for the selected variables and compares them to those reported in literature. Appendix B includes the four basic datasets used for this study.

We found no reason to suspect biased results due to the time lapse between soil or water chemistry sampling and vegetation surveying. The correlation analysis showed some potential explanatory variables to be highly correlated (Appendix C). HCO<sub>3</sub>, Ca-water, Mg-water and EC were strongly correlated and only HCO<sub>3</sub> was retained for the initial full model.

#### 4. Discussion

Our results agree best with hypothesis B: *L. natans* was most abundant in water bodies subjected to physical disturbance and where taller species and shading remained limited. We found only limited support for hypothesis A. Although TP appeared relevant when taken in isolation, it was only marginally significant when combined with physical disturbance. Moreover, no interaction effect was observed between TP and physical disturbance.

##### 4.1 Water and soil conditions

Among the measured soil characteristics, only Ca-soil was found to relate slightly to *L. natans* abundance. Although Greulich et al. (2000b) mention good development on substrates with high calcium levels (Table 2), the species is more likely to be absent in Flanders in this case. This agrees with the findings of Cook (1983). However, it should be taken into account that soil calcium was reduced or even depleted by anthropogenic acidification in non-cultivated soils in north-eastern Belgium in the course of the 20<sup>th</sup> C (De Schrijver et al., 2006, Drouet et al., 2007) and for some populations, particularly those with strong recruitment from the seed bank, abundance may not yet have changed (i.e. decreased) accordingly. Also, even at very low calcium levels, breakdown of organic matter, sulphate reduction and denitrification generate in situ alkalinity (Rudd et al., 1986), which to some extent may compensate for a loss of carbonate-supported buffering.

Other measured soil characteristics were found to be of no importance for abundance or generative reproduction. But almost all sites - including those where the species disappeared – have sandy soils (Ronse et al., 2015; Figure 1). Consequently, inter-site differences are expected to be rather limited. Sites with large *L. natans* populations in Flanders present similar low soil TOC to those in Poland and France (Table 2). This may reflect the many negative consequences of organic matter accumulation demonstrated for isoetids (Spierenburg et al., 2012) and certain other aquatic plants (Silveira and Thomaz, 2015), as well as its indirect relation to the development of more productive competitors. High iron content is also assumed to characterize sites with *L. natans*, but the Flemish populations have lower Fe-soil than the Polish ones and no such relation appears to exist within the observed range

Water chemistry data show slightly more pronounced differences. In Flanders, large populations of *L. natans* are more likely to occur at low TP (Figure 5.B), but the range for attaining high abundance is considerably larger compared to Poland, and even The

Netherlands (Table 1). Orthophosphate concentrations, however, remain comparable to populations elsewhere. Also, the observed ranges for  $\text{NO}_3$  is wider in Flanders than in the Netherlands, and much broader compared to Poland. This indicates that, given certain conditions, the species may tolerate eutrophication quite well. Presumably, the relatively large nutrient tolerance in Flanders originates mainly from the partly amphibious nature of many populations. To some extent, broader apparent tolerance towards nutrients may also be due to a lagged response of population size to adverse conditions. For example, population size decreased from +500 rosettes in 2016 to zero in 2021 from the site with the highest ammonium level (0.49 mg  $\text{NH}_4/\text{L}$  in 2009 versus 1.26 mg  $\text{NH}_4/\text{L}$  in 2019). Models also indicate that absence of *L. natans* is associated with  $\text{O}_2$ -supersaturation. Except for a few extreme observations (once 137 % in Flanders and even 155.7 % in Poland according to Banas et al., 2023), most large populations in Flanders are limited to 100 %  $\text{O}_2$ , similar to France (Greulich et al., 2000b). Oxygen supersaturation usually develops in phytoplankton-turbid conditions, where the submerged growth form of *L. natans* is likely to be suppressed by light limitation (Nielsen et al., 2023).

Supply of iron-rich groundwater increases the immobilization of phosphorus, decreasing its availability, which apparently explains the distribution of *L. natans* in certain parts of the Netherlands (Lucassen et al., 2007). In Flanders, a number of sites also shows high values of dissolved iron, even higher than those observed in the Netherlands, but no apparent relation with either abundance or persistence of the species emerged, again pointing to independence of phosphorus levels, as such.

Observations from Poland suggest DOC to be of some importance, with optimal growth and reproduction occurring from 3.5 to 6 mg C/L. NPOC concentrations for large populations are much higher in Flanders. Negative effects of high DOC may result from light limitation in deeper water where growth is permanently subaquatic, which is less common in Flanders.

As elsewhere, the Flemish populations show a broad pH range, with high abundance occurring up to pH 8.1. Yet, extremes such as in the Netherlands (pH 3.6 or 9.5; Arts et al., 1990), which are unlikely to be supported by the submerged growth form that cannot utilize atmospheric  $\text{CO}_2$ , are lacking.

In agreement with observations of Greulich et al. (2000b) and Willby and Eaton (1993), water and soil chemistry of sites with *L. natans* show broad ranges for most of the measured components. Yet, future monitoring should point out whether some of the most extreme concentrations encountered in our study, e.g. for TP, TN,  $\text{NO}_3$  and DOC truly support viable populations.

#### **4.2 Water body and vegetation features**

Population size as well as reproduction clearly depend on water body as well as vegetation characteristics. Large populations occur where accretion of organic sediment and development of shading vegetation are slowed down by physical processes or sporadically set back by more dramatic events. Our study included too few observations to differentiate between different types of disturbance, and we could only assess their incidence, not their frequency or physical effects, so we are limited in their appreciation to what they may have in common. Water-level fluctuations and water-level regulation result in temporary drying and mineralization of organic matter along the shore, maintaining favorable conditions for seed germination (Nielsen et al., 2006) and rooting of dislodged rosettes. Whereas the winnowing action of waves removes organic particles, lowers nutrient retention and improves soil oxygenation, reducing availability of phosphorus and slowing down development of canopy vegetation. Erosional events, such as caused by storms or channel scouring, also rejuvenate



soil conditions, revive dormant seed banks and set vegetative propagules afloat where organic deposits have accumulated more strongly (O'Donnell et al., 2014). Both phenomena thus create new habitat, slow down natural succession and spread propagules. Time since more drastic disturbance events (e.g. water body creation or restoration) is also important: large populations are associated with relatively recent disturbance events (2-5 years), which is also in line with behavior expected of early-colonizing species. Alternative deployment of different growth forms (isoetid, nymphaeid or terrestrial), clonal reproduction and seed longevity allow flexible adaptation to changing physical conditions and enable the species to maximally exploit available physical niches and colonize new suitable habitat. The combination of these traits also constitutes an effective escape mechanism to survive adverse conditions, allowing formation of more resilient metapopulations. It also explains why *L. natans* depends less on a permanently nutrient or carbon limited chemical environment, and extends successfully beyond low-productive soft water. Notably, physical disturbance was found to be more important than nutrient availability for its abundance, but did not affect its nutrient range. This is likely due to the relative indifference of the species to trophic status.

In spite of its ruderal and stress-tolerant character, the species is a poor competitor (Willby and Eaton, 1993; Greulich and Bornette, 1999). Large populations were observed mainly where the percentage cover of tall vegetation and degree of shading were low and *L. natans* was often absent in water bodies with much tall vegetation or shading. This suggests *L. natans* is quite light-demanding and rapidly out-competed when light conditions deteriorate.

Generative reproduction dominated in water bodies with a high percentage of submerged vegetation (PVI), indicating increased investment in seed production in order to survive unfavorable conditions or to facilitate spreading to more suitable sites when needed. Vegetative reproduction, on the other hand, was associated with less submerged vegetation and high water transparency, conditions most suited for isoetid growth. Greulich et al. (2001) demonstrated that the plasticity in growth form from isoetid to nymphaeid relates to a nutrient and light gradient, but it appears that the reproduction strategy is also linked to this gradient.

Somewhat surprisingly, higher coverage of invasive species was observed for large *L. natans* populations, indicating congruent conditions for establishment or expansion of such species. Physical disturbance creates favorable conditions for *L. natans* as well as new, unoccupied niches allowing invasive species to establish themselves successfully. Although extirpation of *L. natans* due to competitive exclusion by non-natives remains to be shown unequivocally, negative effects on population size and seed recruitment are not unlikely. Only *Crassula helmsii*, *Elodea nuttallii* and *Lemna minuta* were noted several times with more considerable abundance in the presence of *L. natans*, and for most populations and potential habitat, *C. helmsii* is probably the most problematic species, considering that it often colonizes bare mineral substrates in recently restored water bodies and rapidly develops dense swards on exposed banks and in shallow water (Smith and Buckley, 2020). At one site (Langdonken) where *L. natans* covered 5-50 m<sup>2</sup> in 2009 in the absence of *C. helmsii*, less than 5 m<sup>2</sup> remained in 2019 when *C. helmsii* reached 30 % cover. *Elodea* primarily resides in deeper water where it potentially inhibits submerged growth of *L. natans*. However, whereas *Elodea* occupies a distinct niche within the water body, *L. natans* extends into the shallowest and periodically inundated parts, suggesting lower competitive pressure. This partly holds for *L. minuta* as well, which moreover does not form a dense cover in the presence of *L. natans* and is unlikely to cause light exclusion. Overall, the impact of invasive species on the survival of *L. natans* and the mechanisms through which they may interact are still insufficiently known, compromising development of effective management strategies to ensure its long-term viability.

### 4.3 Threats and possible causes of decline

*Luronium natans* is vulnerable and faces several threats that could lead to its decline. Our results indicate that the species can tolerate a wide range of water and soil conditions, but further degradation may exacerbate its decline. This process may be hidden due to a lag between environmental changes and their impact on the populations. Still, eutrophication and acidification are most mentioned threats to affect macrophytes (Murphy, 2002). Also indirect consequences caused by eutrophication can affect *L. natans*. This includes the growth of fast-growing aquatic plants that can outcompete the less competitive species (Nielsen et al., 2023), as is also confirmed by our results. Therefore, the above mentioned 'high abundance class' of > 100 rosettes or > 5m<sup>2</sup> (based on Huck and Michl, 2006) may not be stringent enough to support sustainable populations.

Climate change, with increased temperatures, altered precipitation patterns, and more frequent extreme weather events, can affect water bodies and their macrophytes by altering water levels or their fluctuations (Lind et al., 2022). Both excessively high and low water levels can disrupt the growth cycle of *L. natans*, resulting in population declines.

The presence and growth of invasive species, both plant and animal, may outcompete *L. natans* for space and resources. While this was not confirmed in our study, likely due to the limited examples of invasive species dominance in the dataset, it remains a concern as it was observed to be important for macrophytes (Murphy 2002; Gallardo et al., 2016).

In addition, increased erosion from deforestation and agricultural activities can increase sediment levels in water bodies. Sedimentation can smother *L. natans*, block sunlight, and alter the substrate, making it difficult for the plant to survive. This is also observed by macrophytes in Northern Irish soft water lakes (McElarney et al., 2010).

Observed changes in the quality of populations assessed annually, as well as those observed in some repeated assessments (results not shown), can be attributed to restoration efforts or the absence of such efforts. In cases where there was no management, some populations declined or even disappeared, often because they were outcompeted by more successful species or because they faced increasing competition for light. Conversely, other populations either emerged or reappeared following restoration activities, often due to the germination of seed from the seed bank (e.g. Heuvelsven).

### 4.4 Future research

Although we attempted to include most of the important factors affecting *Luronium natans* at a regional scale, our survey could not address a number of mechanisms and processes possibly affecting its population characteristics in more specific circumstances. These include, for instance, animal interactions (invertebrates, fish, birds), seed longevity in relation to environmental conditions and the role of landscape resistance (Bakker et al., 2016; Wood et al., 2017a).

Furthermore, the genetic diversity of the remaining populations in Europe remains to be explored further, in order to better understand the genetic constraints for sustainable populations, and to elucidate whether genetic make-up influences realized niches and population resilience to environmental pressures. The observed difference with the Central European niche may also be traced back to a genetic difference with our populations. Therefore, it is also interesting to investigate the relationship between genetics and environmental characteristics more closely.

Population dynamics also need better understanding in order to assess the effectiveness of conservation efforts over time, which is crucial for assessing population size trajectories and

identifying potential threats that may arise. Also, monitoring frequency of the site specific characteristics need to be evaluated.

Our results indicate physical disturbances to be important for this species. Although currently only recorded at incidence level, it would be interesting to explore this in a more (semi-) quantitative approach in future monitoring.

Furthermore, research is required to ascertain the significance of population connectivity and to determine whether criteria can be formulated to quantify and monitor this. This is primarily aimed at monitoring or estimating the appropriate size of a population or metapopulation.

## 5. Conclusion

Our comprehensive survey of the habitat conditions of *Luronium natans* reveals several key findings for its effective conservation management. Although emphasis is often placed on maximally restoring nutrient or buffering conditions, our study indicates that physical disturbance, either resulting from natural processes or induced by active management, may have more bearing on population size in many cases because it creates or sustains favorable conditions for vegetative development as well as recruitment of the species. Indirectly, physical-chemical soil and water characteristics, in particular high Ca-soil and TP-water, constrain its occurrence by their relation to stronger growth of competing macrophytes of more robust posture, - or in case of isoetid habitus - turbidity of the water column or epiphyton development.

*Luronium natans* demonstrates a remarkable adaptability to varying abiotic conditions, including nutrient levels. Furthermore, both physical water body and vegetation characteristics strongly determine its population size and reproductive strategies, including physical disturbance, shading and tall vegetation.

Long-term monitoring initiatives are essential for monitoring population dynamics and identifying emerging threats (O'Hare et al., 2018). This is also important for assessing the effectiveness of conservation efforts and designing targeted management strategies to protect *L. natans* populations.

For management and restoration purposes of this species, the creation of early-successional conditions and prevention of vegetation succession seem to be the main focus in Flanders. Nevertheless, improving other abiotic characteristics remains important to improve habitat quality and ensure long-term sustainable populations.

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## List of figures, tables and appendices

### Figures

Figure 1 – Distribution of *Luronium natans* in Flanders since 2000 (status 2021). Most recent abundance class is shown on the map; discriminating between: species was present in 2000, but is now absent (white circle); low abundance (grey circle); high abundance (black circle).

Figure 2 – Boxplots and bar charts for selected soil, water column, water body and vegetation characteristics. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green). Graphs of other variables are included in Appendix D.

Figure 3 – Modelled ordinal responses for abundance classes with 95 % CI for water body characteristics: conditional predictions for (A) temporary drying, (B) time since last disturbance, (C) water-level fluctuations, (D) water-level regulation and (E) wind action. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).

Figure 4 – Modelled ordinal responses for abundance classes with 95 % CI for vegetation characteristics: conditional predictions for (A) acidification, (B) eutrophication, (C) invasive species, (D) shading and (E) tall vegetation. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).

Figure 5 – Modelled ordinal responses for abundance classes with 95 % CI for soil and water characteristics: conditional predictions for (A) Ca-soil, (B) TP total phosphorus and (C) O<sub>2</sub>-saturation. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).

Figure 6 – Modelled binomial responses for generative reproduction with 95 % CI for water body and vegetation characteristics: conditional predictions for (A) circularity, (B) eutrophication, (C) mud thickness, (D) PVI Plant Volume Infested, (E) water depth and (F) water transparency.



## Tables

	Poland (1)	The Netherlands (2)	Germany (3)	France (4)	Flanders (this study)
NPOC (mg C/L)	3.5-6*	-	-	-	13.2 (3.55-28.71)* 13.2 (3.55-40.56)
Fe-water (mg/L)	0-0.24	0.03-0.21	0.033	-	0.64 (0.04-4.74)* 1.24 (0.04-18.72)
NH <sub>4</sub> (mg/L)	0.20 (0.06-0.32)	0.01-0.49	0.025	0.02-0.04*	0.18 (0.03-1.26)* 0.15 (0.03-1.26)
NO <sub>3</sub> (mg NO <sub>3</sub> /L)	0.18 (0.13-0.18)	0-7.92	1.23	3.1-22.2	0.87 (0.05-10.74)* 0.50 (0.05-10.74)
pH	6-7*	6.1* 3.6-9.5	5.8-7.2	6.82-7.1*	6.66 (4.91-8.10)* 6.71 (4.91-8.62)
PO <sub>4</sub> (mg/L)	0.01 (0.01-0.04)	0.02 (0-0.06)	0.084	< 0.05	0.02* 0.02 (0.02-0.05)
TP (mg/L)	0.01-0.02*	0-0.07	-	-	0.11 (0.01-0.28)* 0.19 (0.01-2.21)
TN (mg/L)	0.76 (0.27-2.5)	-	-	-	1.84 (0.64-7.87)* 1.93 (0.61-7.87)

Table 1 – Comparison of reported water chemistry for occurrence and optimal (indicated by \*) conditions of *Lurionium natans* in different regions: (1) Szankowski and Klosowski (2001); Bazydło (2004); Bazydło and Szmeja (2004); Szmeja and Bazydło (2005); Szankowski and Klosowski (2006); Banas et al. (2023). (2) Roelofs (1983); Arts et al. (1990); Lucassen et al. (2007). (3) Pietsch (1986) (4) Greulich et al. (2000b). Mean values, with range if available. NPOC non purgeable organic carbon, TP total phosphorus, TN total nitrogen.

	Poland (1)	The Netherlands (2)	France (3)	Flanders (this study)
Ca-soil (g/kg d.w.)	0.9-3.2	0.28 (0-0.681)	9.56-12.1	0.639 (0.044-2.223)* 0.848 (0.044-2.691)
Fe-soil (g/kg d.w.)	1 (0.25-2.8)	-	0.2 (<0.107)	0.086 (0.07-0.161)* 0.164 (0.07-0.879)
TOC (% mass d.w.)	5.1-10*	-	2.59-6.72*	1.91 (0.26-10.34)* 3.77 (0.21-26.23)

Table 2 – Comparison of reported soil requirements for occurrence and optimal (indicated with \*) conditions of *Lurionium natans* in different regions: (1) Szankowski and Klosowski (2001); Bazydło (2004); Bazydło and Szmeja (2004) (2) Roelofs (1983) (3) Greulich et al. (2000b). Mean values with ranges are given when available. TOC total organic carbon.

## Appendices

Appendix A – Description and laboratory procedure of environmental characteristics. Lab procedures according to ISO-standards, Belgian standards (NBN) or Flemish standards (CMA, 2023).

Appendix B – Raw data.

Appendix C – Spearman rank-correlation of soil and water column variables.

Appendix D – Plots for soil, water, water body and vegetation characteristics of *Luronium natans* abundance.

Appendix E – Results for model selection based on AICc of generalized linear models for *Luronium natans* abundance and logistic regression for models of reproduction classes.

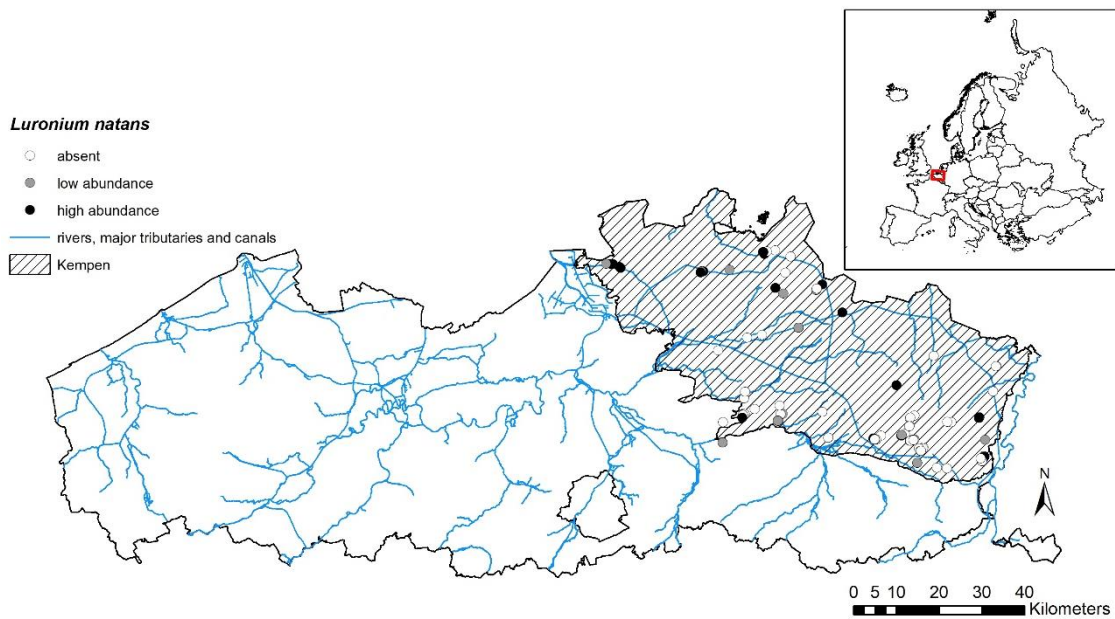


Figure 1 – Distribution of *Lurionium natans* in Flanders since 2000 (status 2021). Most recent abundance class is shown on the map; discriminating between: species was present in 2000, but is now absent (white circle); low abundance (grey circle); high abundance (black circle).

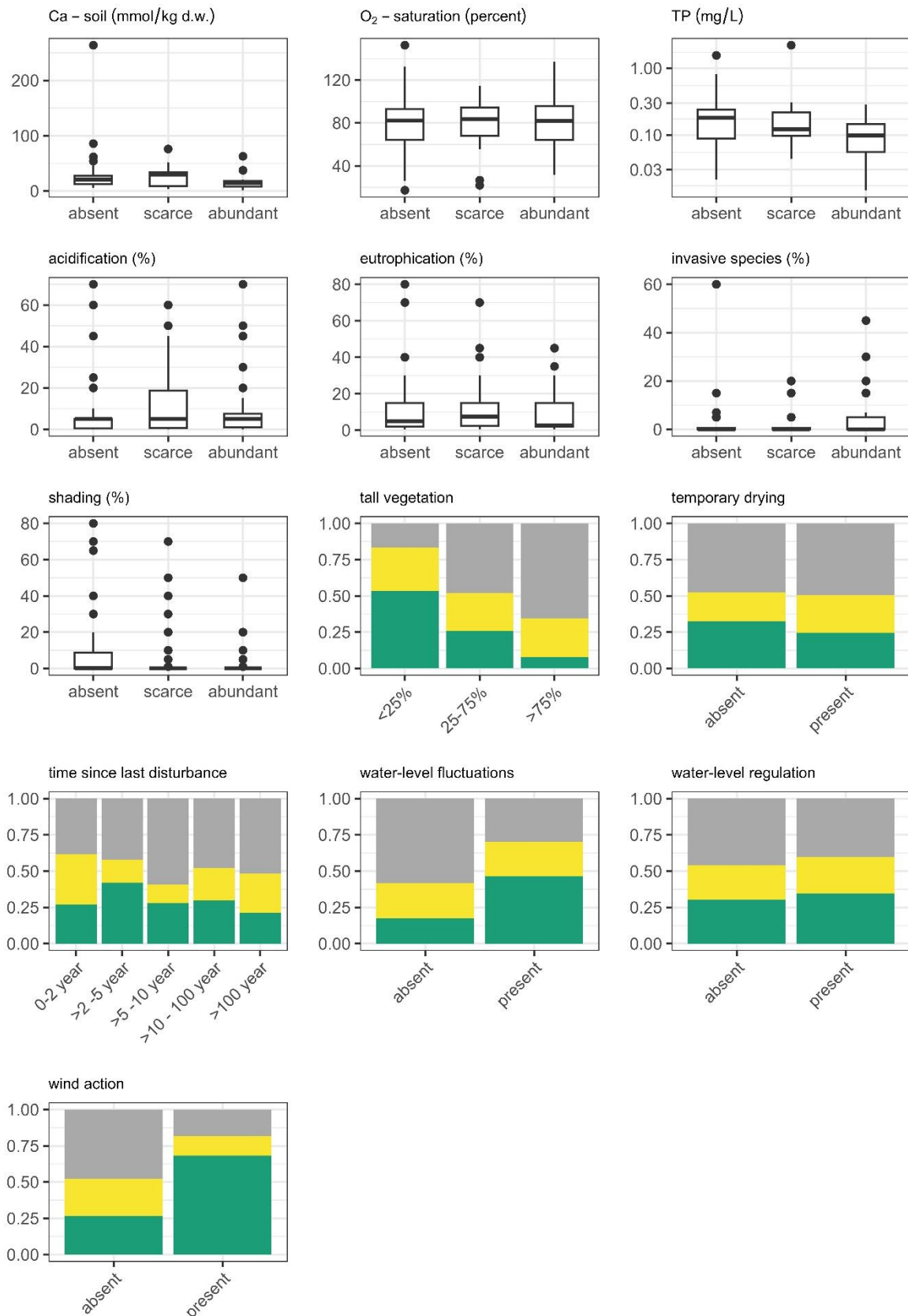


Figure 2 – Boxplots and bar charts for selected soil, water column, water body and vegetation characteristics. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green). Graphs of other variables are included in Appendix D.

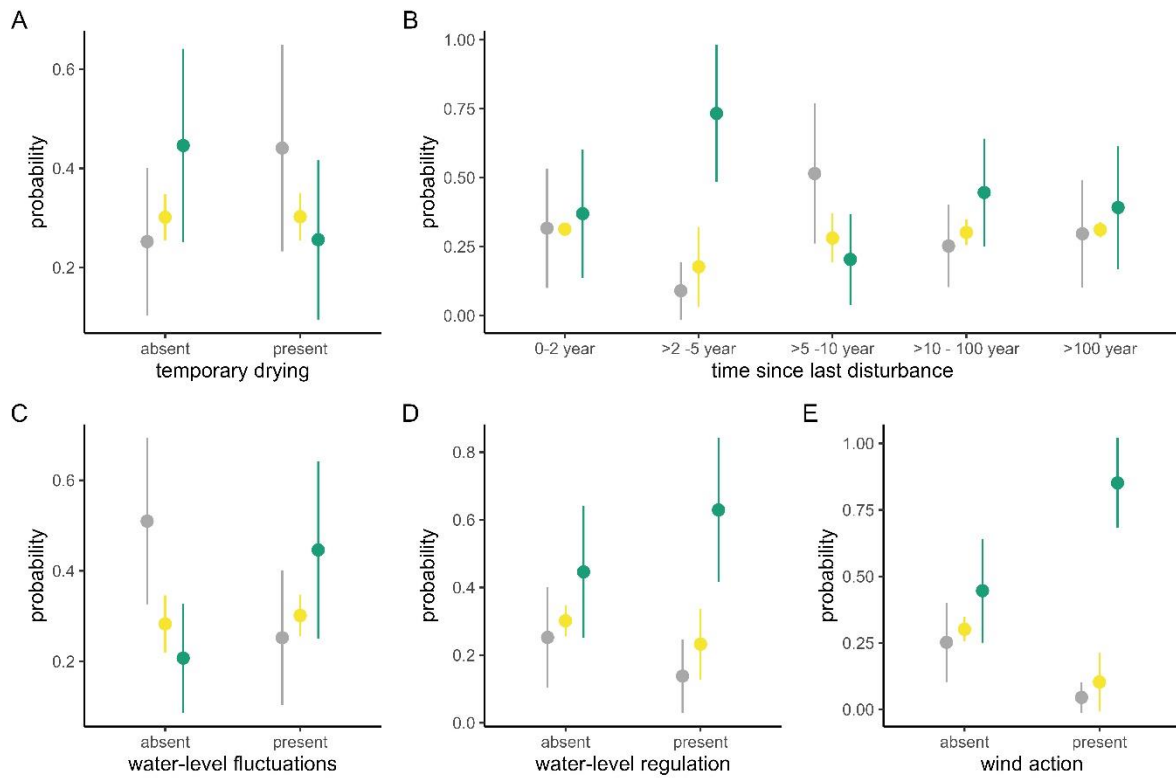


Figure 3 – Modelled ordinal responses for abundance classes with 95 % CI for water body characteristics: conditional predictions for (A) temporary drying, (B) time since last disturbance, (C) water-level fluctuations, (D) water-level regulation and (E) wind action. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).

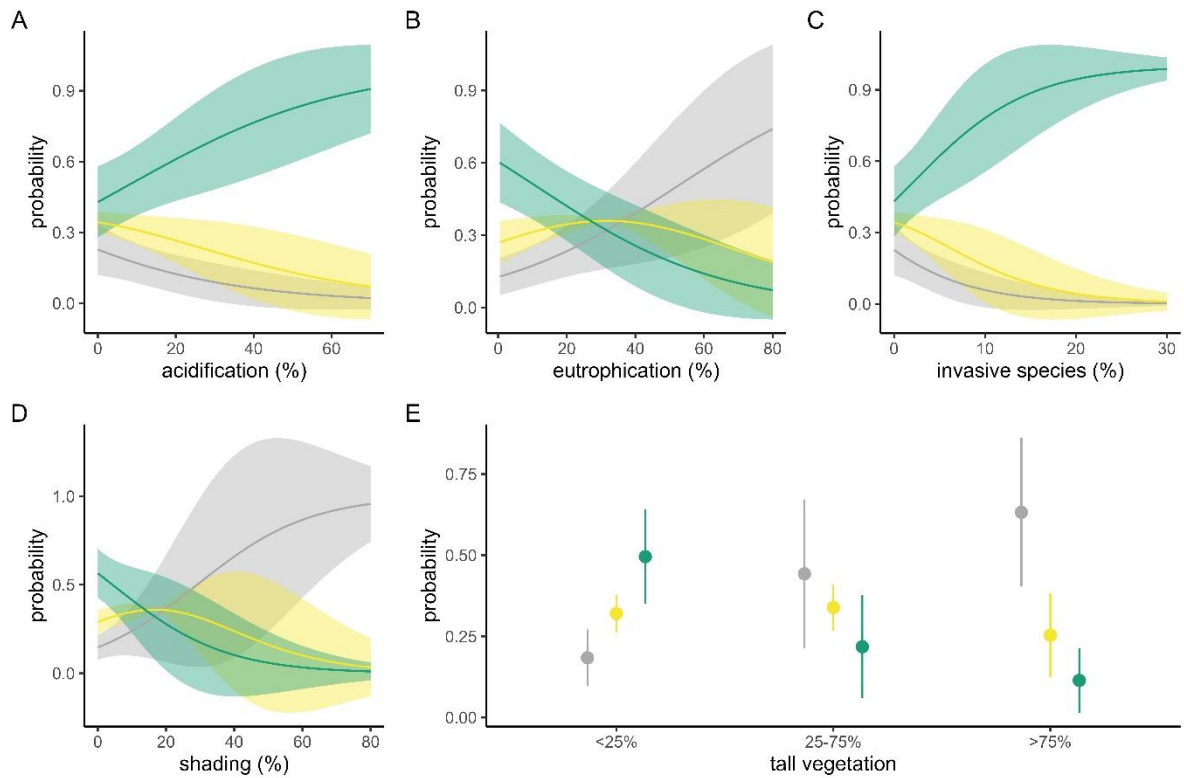


Figure 4 – Modelled ordinal responses for abundance classes with 95 % CI for vegetation characteristics: conditional predictions for (A) acidification, (B) eutrophication, (C) invasive species, (D) shading and (E) tall vegetation. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).

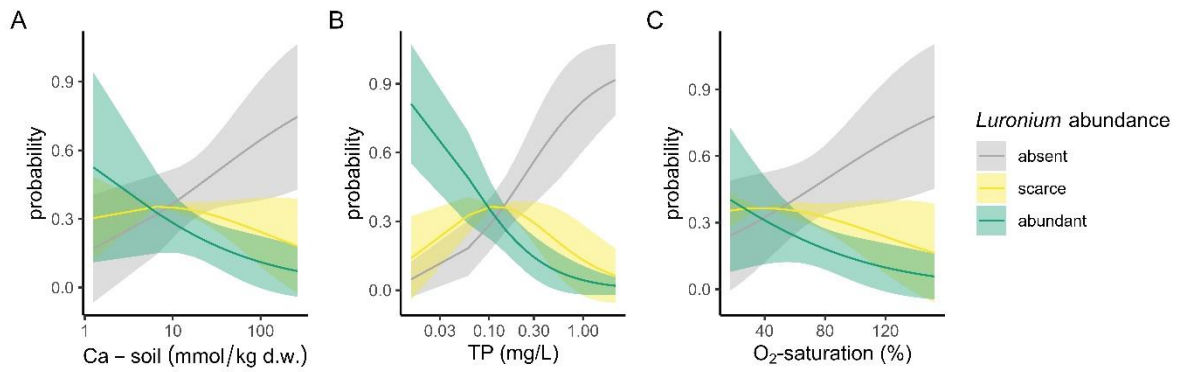


Figure 5 – Modelled ordinal responses for abundance classes with 95 % CI for soil and water characteristics: conditional predictions for (A) Ca-soil, (B) TP total phosphorus and (C) O<sub>2</sub>-saturation. *Luronium natans* abundance classes: absent (grey), scarce (yellow), abundant (green).



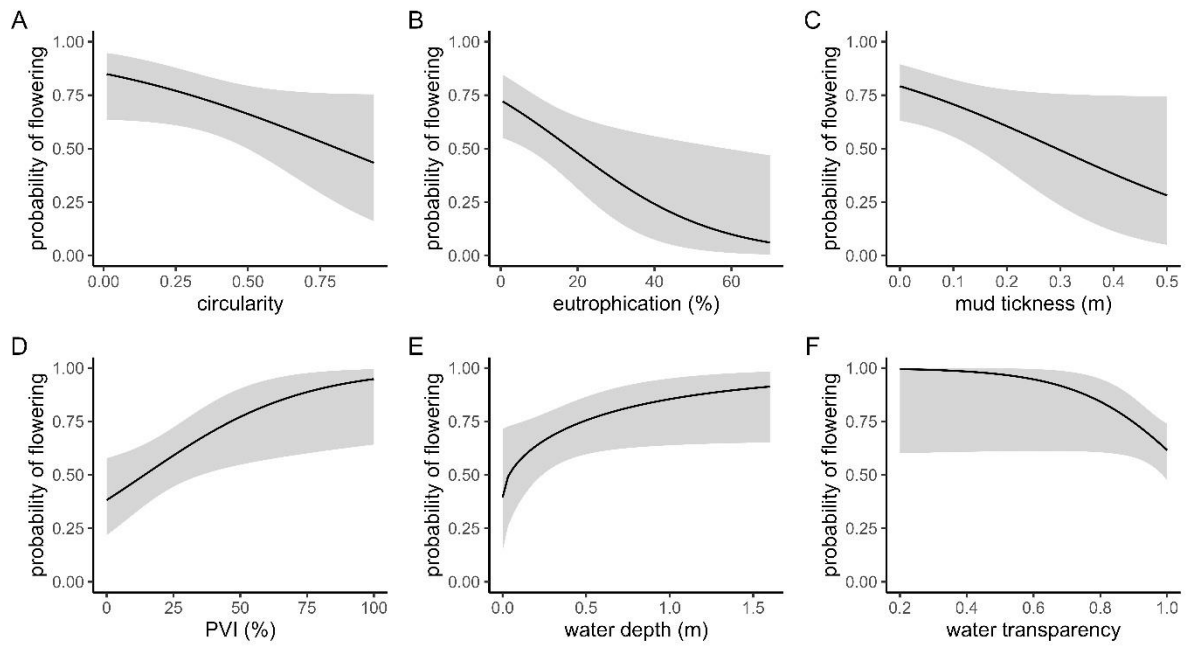


Figure 6 – Modelled binomial responses for generative reproduction with 95 % CI for water body and vegetation characteristics: conditional predictions for (A) circularity, (B) eutrophication, (C) mud thickness, (D) PVI Plant Volume Infested, (E) water depth and (F) water transparency.