Plant traits and environment: floating leaf blade production and turnover of *Nymphoides peltata* (S.G. Gmel.) O. Kuntze (Menyanthaceae)

Peter F. Klok\(^1,2\) and Gerard van der Velde\(^1,3\)

\(^1\) Department of Animal Ecology and Physiology, Research Institute for Biological and Environmental Sciences (RIBES), Radboud University, Nijmegen, Netherlands
\(^2\) Department of Particle Physics, Institute for Mathematics, Astrophysics and Particle Physics, Radboud University, Nijmegen, Netherlands
\(^3\) Naturalis Biodiversity Center, Leiden, Netherlands

ABSTRACT

**Background:** Nymphaeid macrophytes, rooting in the sediment of water bodies and characterized by floating leaves, play an important role in wetland ecosystems. The present research deals with the effects of limited space, limited nutrient availability, water temperature and an unexpected inundation on the production, turnover and plasticity of floating leaves of the globally widespread species *Nymphoides peltata* (Fringed waterlily).

**Methods:** The effects of these environmental conditions were studied in two plots in outdoor concrete tanks (CT1, CT2, mesocosms simulating occurrence in small ponds) and in two plots in the floodplain oxbow lake Bemmelse Strang (BS1, BS2). Plot CT1 was situated in a stand coexisting with helophytes, plot CT2 in a monospecific stand, plot BS1 in the center and plot BS2 at the open water border of a monospecific stand. All floating leaf blades within the plots were marked at appearance at the water surface and subsequently length, width and damage of each leaf and maximum and minimum water temperatures were measured bi-weekly. Area and biomass of leaf blades were calculated based on leaf length and width and were used to calculate turnover rates and production.

**Results:** The growth period started in May and ended mid-October with continuous production of floating leaves during nearly the whole vegetation period. In the tanks the water level was very stable, but the lake underwent an inundation by river water, causing a sudden loss of existing leaves. Considering environmental conditions and based on the assumed ranking from low to high nutrient availability, the ranking of the plots was CT1, CT2, BS1, BS2. This order was found for maximum leaf life span and maximum leaf length, and the reverse order was found for number of leaves, new leaves per day and duration of the vegetation period. Turnover rates appeared to be relatively similar for plots CT1, CT2 and BS1, but for the deeper border plot BS2 lower ratios were found. These results indicate that increased enclosure with expected nutrient limitation causes (1) the production of high numbers of small leaves with larger totals for leaf area and biomass, (2) a shift towards increased sexual reproduction by the production of more flowering stem leaves.

How to cite this article Klok PF, van der Velde G. 2022. Plant traits and environment: floating leaf blade production and turnover of *Nymphoides peltata* (S.G. Gmel.) O. Kuntze (Menyanthaceae). PeerJ 10:e13976 DOI 10.7717/peerj.13976

Copyright 2022 Klok and van der Velde

Distributed under Creative Commons CC-BY 4.0
INTRODUCTION

Distribution

*Nymphoides peltata* (S.G. Gmel.) O. Kuntze is a globally widespread aquatic plant species occurring as native in central, western and southern Europe, northern and western Asia, Kashmir, the Himalayas and Japan ([Glück, 1924; Stuckey, 1974; Meusel, Jäger & Weinhardt, 1978](#)), and as invasive in North America ([Stuckey, 1974; Darbyshire & Francis, 2008](#)) and South Africa ([Cheek, 2018](#)). In the U.S. *N. peltata* was exterminated in drinking water reservoirs ([Lamb et al., 2021](#)). In New Zealand it was considered an invasive pest and it was exterminated ([CABI Invasive Species Compendium, 2005](#)). In Japan ([Takagawa, Nishihiro & Washitai, 2005](#)) and Poland ([Urbisz & Urbisz, 1998](#)) it is a protected species. The northern limit of occurrence of *N. peltata* coincides more or less with the 16 °C July isotherm ([Van der Velde, Giesen & Van der Heijden, 1979](#)).

Growth form and importance of floating leaves

*Nymphoides peltata* is a macrophyte with a nymphaeid growth form ([Luther, 1983; Den Hartog & Van der Velde, 1988](#)) ([Fig. 1](#)). The plant possesses short shoots (rhizomes) rooted in the sediment from where petioles develop which hold the nearly orbicular leaf blades (laminae) at the water surface, and long shoots creeping over the sediment surface which act as runners to create distant short shoots ([Van der Velde, Giesen & Van der Heijden, 1979](#)). In this way it can expand rapidly in a vegetative way. Flowering stems develop from leaf axils of a long shoot and grow towards the water surface, developing short internodes with floating leaves by sympodial branching, forming a platform that holds the flower buds near the water surface. From this platform the flowers rise above the water surface to be pollinated by insects for the production of seeds ([Van der Velde & Van der Heijden, 1981](#)). Floating leaves with their petioles contribute most to the biomass production of *N. peltata* ([Brock et al., 1983a](#)). Floating leaf blades are important for the plant by processes such as photosynthesis ([Smits et al., 1988; Yu et al., 2014](#)), gas ventilation ([Grosse & Mevischutz, 1987; Grosse & Bauch, 1991](#)), chemical accumulation (e.g., heavy metals by hydropotes ([Brock, 1983; Lavid et al., 2001](#)) and suppression of other aquatic macrophytes by shadowing ([Larson, 2007; Zhu et al., 2019](#)). Furthermore, floating leaf blades function as substratum for particular organisms (periphyton, sessile animal eggs), free moving species at and near the water surface, as isles in the open water for air-breathing animals, as substratum and nutrient for fungi and microbes and as food for more or less specialized invertebrates and for vertebrates. By their growth form, rooted plants with floating leaves structure the nymphaeid-dominated system in a particular way ([Van der Velde, 1980](#)), different from those by other growth forms of macrophytes ([Den Hartog & Van der Velde, 1988](#)).
Habitat and environmental conditions

According to Bornette & Puijalon (2011) environmental characteristics of aquatic habitats rule macrophyte species occurrence, life-history traits and community dynamics among aquatic macrophytes. Important factors mentioned are light, temperature, water nutrient content, substrate characteristics, water movements and disturbances like floods and drawdowns.

In the Netherlands *N. peltata* is commonly found in ditches, ponds, lakes, canals, lowland brooks and small rivers, but not in moorland pools as the plant needs calcium for leaf development (Van der Velde, Custers & De Lyon, 1986; Smits, Schmitz & Van der Velde, 1992). The plant grows in particular in eutrophic, alkaline fresh water bodies (Grote, 1980; Casper & Krausch, 1981; Smits et al., 1988) on mineral soils, in particular clay (Döhler, 1963; Westhoff et al., 1971), till a depth of about 3 m, with an optimum growth at a depth of 20–150 cm (Funke, 1951). By severe wave action the plant is often restricted to the edges of large water bodies (Brock, 1985). Although *N. peltata* is also found growing on mineral soils with organic matter (Grote, 1980; Van der Velde, Custers & De Lyon, 1986), its tolerance for organic matter is lower than that of waterlilies (Van der Voo & Westhoff, 1961; Westhoff et al., 1971; Smits et al., 1990). The species occurs commonly in backwaters of large rivers which are regularly flooded by river water in winter (Van der Voo & Westhoff, 1961). Extremely low water levels and emergence are tolerated by *N. peltata* and lead to massive germination of seeds present in the sediment. The seedlings can quickly grow towards full grown plants (Brock, Van der Velde & Van de Steeg, 1987). The plant also adapts easily to a terrestrial way of life (Li et al., 2010a; Li, Yu & Xu, 2010b; Yu et al., 2014) and can even be cultured as a pot plant (Heimans & Thijssie, 1956). Emergent leaves show a longer life span than floating leaves (Tsuchiya, 1988). Stress by main flow and turbulence is better tolerated by *N. peltata* than by completely submerged macrophytes (Asaeda & Rashid, 2017). The same is true for mechanical damage as a result of boating.
and other recreational activities, fishing and aquatic management regimes, and perturbations by propellers and rakes in the upper water layer. *Nymphoides peltata* shows a high capacity for foliage compensation at such disturbances (*Cao, Liu & Wang, 2016*) and it shows a high plasticity (*Xiao et al., 2011*).

**Research question and hypothesis**

The research question for this basic study is:

How are floating leaf blade characteristics of *N. peltata* influenced by different environmental conditions?

The hypothesis is posed that *N. peltata* recycles nutrients more frequently in dense stands than in pioneer or border stands, because a high consumption of nutrients during the growing season will lead to shortages for development and growth with consequences for leaf development. Similarities in development indicate stable traits, while differences must be interpreted as adaptations or responses to the environment. The study will furthermore be used as a basis for studying connected processes such as decomposition.

Floating leaves are indicated as leaves further in this article.

**MATERIALS AND METHODS**

**Sites**

Research took place in four plots with a size of 50 × 50 cm (0.25 m²) each. Pilot observations proved this size sufficient to follow the leaf development in a *Nymphoides* stand in a representative way. Plots CT1 and CT2 were situated in outdoor concrete tanks (CT) on the site of Radboud University Nijmegen (coordinates 51°49′21″N, 5°52′23″E) and plots BS1 and BS2 in Bemmelse Strang (BS), a fresh water oxbow lake north of the River Waal near Nijmegen (coordinates 51°52′43″N, 5°53′03″E).

The concrete tanks were sunken in the ground, surrounded by a paved area and covered by a frame with chicken wire to prevent leaves and animals falling into the water adding nutrients. The inner surface area of the tanks measured 80 × 150 cm with a depth of 60 cm. The tanks were filled with river water. A heavy river clay mud layer on the bottom resulted in a water depth of 40–50 cm and an alkaline and eutrophic water quality. The water level fluctuated slightly due to precipitation and evaporation and there was no wave and wind action. Plot CT1 contained a mixed culture of *N. peltata*, *Glyceria fluitans* (L.) R. Br. and *G. maxima* (Hartm.) Holmb. and plot CT2 a monoculture of *N. peltata*. The *Glyceria* species spontaneously occurred and all plants inclusive *N. peltata* in the tanks developed for 3 years prior to this research. No additional water or nutrients were added to the tanks.

Bemmelse Strang is a shallow, isolated, alkaline (2.6–4.8 meq.L⁻¹), and eutrophic oxbow lake (pH 7.6–8.6), a former branch of the River Waal. The bottom of a large *Nymphoides* stand consisted of sand and a detritus layer with increasing thickness towards the littoral border of the lake. The hydrology was influenced by precipitation, evaporation, upward seepage and river water overflow. Plot BS1 was situated in the center of the stand and plot BS2 at the open water margin of the stand. The water depth fluctuated for BS1 from 30–106 cm (mean 67 cm, standard deviation 22 cm) and for BS2 from 82–154 cm (mean
117 cm, s.d. 22 cm). Minimum depth was measured on 20 October 1980, maximum depth on 7 August 1980. The *N. peltata* stands were exposed to moderate wave and wind action.

**Field data**

A non-destructive leaf-marking method was used to mark all leaves originating within a plot at appearance, which enabled data collection during the complete life span of each leaf. During data collection in the tanks a square PVC tube frame was placed on the vegetation in a fixed corner of the tank (Fig. 2). In Bemmelse Strang a similar perforated tube frame was held permanently in a fixed position approximately 15 cm below the water surface by cork floaters and anchored by four bricks. By keeping the frame under water, the unrolling of leaves in the plot was not hindered. All leaves having their petioles within the frame were used for data collection.

Leaves were tagged by numbered Rotex tapes (width 12 mm) in the tanks and by small numbered aluminum strips in Bemmelse Strang. Tags were fixed around the petiole just below the leaf. Two leaf types were distinguished: short shoot leaves and flowering stem leaves.

A leaf was considered gone when the leaf had disappeared or when the remaining lamina totally sank under water. The presence over time of a floating leaf is called the leaf life span, calculated in days from the date of appearance at the water surface until the date it was gone. Terms used in other studies are leaf persistence (*Brock et al., 1983a*) and longevity (*Chabot & Hicks, 1982*).

Measurements and observations of all leaves within a plot took place twice a week during the growing season. It included tagging newly unrolled leaves, counting the actual number of leaves, measuring leaf length and width and visually estimating both leaf damage and decomposition as percentage of the potential leaf area of each leaf. Leaf length was measured from the leaf tip to one of the basal lobe tips.

A distinction was made between potential, actual and photosynthetic leaf area. The potential area is defined as the area of an entirely intact leaf, the actual area as the potential area minus the area missing (loss caused by consumption and other animal activities, by mechanical damage, by decay causing disintegration) and the photosynthetic area.
area as the remaining green part of the actual area. The same distinction was made for leaf biomass.

Maximum and minimum water temperatures were collected bi-weekly in the plots by means of maximum-minimum thermometers at a depth of 10 cm as part of the standard data taking procedure. After each read-out the thermometers were reset and installed again in the water.

Data for CT1 and CT2 were collected in 1978 and 1979, data for BS1 and BS2 in 1980. Since some data of the first weeks in 1978 for CT1 and CT2 were missing and the growing season of *N. peltata* started on the same date in 1978 and 1979, leaf data and water temperature data of these weeks in 1979 have been used to complete the picture.

**Growth, vegetation and flowering stem leaf periods**

The growth period of *N. peltata* of a plot was calculated as the number of days during which new leaves appeared at the water surface. The vegetation period was calculated as the number of days between the day of first leaf emergence and the day that all leaves had disappeared from the water surface. The flowering stem leaf period was calculated as the vegetation period for flowering stem leaves only.

**Regression equations for calculating leaf area and biomass**

The leaf area was calculated from the leaf length and width by Eq. (1), determined by previous research (Van der Velde et al., 1982). The leaf biomass was calculated from the leaf area by regression Eq. (2) with coefficients determined from data of undamaged, randomly harvested green leaves outside the plots. Biomass is given in grams ash-free dry weight (g AFDW). Mathematically, the equations for potential area and for biomass are described by:

\[
A(L, W) = 1.028 \times \pi \times \left( \frac{L + W}{4} \right)^2
\]  
\[
B_i(A) = d_i A + e_i
\]

where:

- \(A(L, W)\) = potential leaf area at length \(L\) and width \(W\) (mm\(^2\))
- \(L\) = leaf length (mm)
- \(W\) = leaf width (mm)
- 1.028 = correction factor (the leaves are not circular)
- \(B_i(A)\) = potential leaf biomass in plot \(i\) for potential leaf area \(A\) (g AFDW)
- \(A\) = potential leaf area (mm\(^2\))
- \(i\) = plot
- \(d_i, e_i\) = coefficients for plot \(i\)

Since leaf samples for the calculation of biomass were collected monthly during this research, the regression Eq. (2) were calculated per month. Annual regression equations, including all monthly harvested leaves, were also calculated. Separate sets of regression equations were made for tank plots and for Bemmelse Strang plots (Table 1).
For each sampling date the total potential area (leaf area index or LAI) and biomass of a plot were calculated by summation of the potential area and biomass values of the individual leaves in the plot on that date, respectively.

The total annual production of potential area was calculated by summation of the final potential areas after leaf growth of all produced leaves per plot. For conversion to potential biomass both monthly and annual regression equations were used. Actual and photosynthetic area and biomass were calculated from potential area and biomass by subtracting the field data loss percentages of leaf area per leaf per plot.

**Statistics**

Since all data were stored in MS Excel (version 2009), this package was used to compute mean values, standard deviations, trend lines and perform single factor (or one-way) ANOVA tests.

**RESULTS**

Results are described by values of leaf blade characteristics, by the influence of water temperature, by the impact of an unexpected inundation on the characteristics, by development in time of characteristics and by ratios based on characteristics.

**Leaf blade characteristics**

Leaf blade characteristics consist of data about number of leaves, leaf life span, vegetation period, growth period, flowering stem period, leaf length, leaf width and leaf area. Values of leaf blade characteristics of the four plots are listed in Table 2.

---

Table 1  Regression equations to calculate biomass from leaf area. Regression equations to calculate biomass from leaf area for *Nymphoides peltata* in tank and lake plots per leaf. Where \( n \) = number of leaves, \( A \) = area (cm\(^2\)) and \( B \) = biomass (mg AFDW). Equations have been tested with the Student t-test. Coefficients are computed both for use in monthly periods and for annual use (*Van der Velde et al.,* 1982).

| Location                        | Period          | \( n \) | Equation                  | SE   | \( r^2 \) | \( p \)     |
|---------------------------------|-----------------|--------|---------------------------|------|-----------|------------|
| Concrete tanks (CT1, CT2)       | May–June        | 25     | \( B = 2.956 \times A – 2.120 \) | 13.66| 0.96      | <0.001     |
|                                 | July            | 25     | \( B = 4.265 \times A – 7.135 \) | 9.00 | 0.95      | <0.001     |
|                                 | August          | 25     | \( B = 3.267 \times A + 1.033 \) | 11.79| 0.85      | <0.001     |
|                                 | September–October| 25    | \( B = 3.610 \times A + 1.573 \) | 3.06 | 0.97      | <0.001     |
|                                 | Annual          | 100    | \( B = 3.018 \times A + 6.506 \) | 13.81| 0.91      | <0.001     |
| Lake Bemmelse Strang (BS1, BS2) | May–June        | 20     | \( B = 3.625 \times A – 21.815 \) | 14.02| 0.93      | <0.001     |
|                                 | July            | 18     | \( B = 4.439 \times A – 37.529 \) | 15.81| 0.96      | <0.001     |
|                                 | August          | 19     | \( B = 4.375 \times A – 21.450 \) | 30.99| 0.95      | <0.001     |
|                                 | September       | 28     | \( B = 3.901 \times A – 27.868 \) | 15.40| 0.95      | <0.001     |
|                                 | October         | 21     | \( B = 3.386 \times A – 21.746 \) | 21.97| 0.93      | <0.001     |
|                                 | Annual          | 106    | \( B = 3.925 \times A – 26.572 \) | 26.06| 0.91      | <0.001     |
| Location | Year | CT1 1978 | CT2 1978 | BS1 1980 | BS2 1980 |
|----------|------|----------|----------|----------|----------|
| Number of leaves | | | | | |
| Total $m^{-2} \cdot yr^{-1}$ | | 2,552 | 2,492 | 1,712 | 1,108 |
| Short shoot $m^{-2} \cdot yr^{-1}$ | | 2,148 | 1,644 | 1,664 | 1,096 |
| Flowering stem $m^{-2} \cdot yr^{-1}$ | | 404 | 848 | 48 | 12 |
| Mean new per day $m^{-2}$ | | 14.92 | 14.92 | 11.05 | 7.01 |
| Maximum $m^{-2}$ | | 608 | 832 | 392 | 380 |
| Date of maximum | | Aug. 15 | Aug. 18 | Jun. 16 | Sep. 25 |
| Leaf life span | | | | | |
| Maximum $d$ | | 43 | 54 | 59 | 63 |
| Minimum $d$ | | 3 | 2 | 3 | 3 |
| Mean $d$ | | 25.30 | 22.51 | 22.85 | 29.59 |
| Standard deviation $d$ | | 7.64 | 9.06 | 10.93 | 13.26 |
| Vegetation period | | | | | |
| Length $d$ | | 202 | 184 | 169 | 169 |
| Begin date | | May 2 | May 6 | May 18 | May 18 |
| End date | | Nov. 20 | Nov. 6 | Nov. 3 | Nov. 3 |
| Growth period | | | | | |
| Length $d$ | | 171 | 167 | 155 | 158 |
| Begin date | | May 2 | May 6 | May 18 | May 18 |
| End date | | Oct. 20 | Oct. 20 | Oct. 20 | Oct. 23 |
| Flowering stem leaf period | | | | | |
| Length $d$ | | 107 | 114 | 66 | 62 |
| Begin date | | Jun. 20 | Jun. 13 | Jul. 15 | Aug. 26 |
| End date | | Oct. 5 | Oct. 5 | Sep. 19 | Oct. 27 |
| Leaf length (pot.) | | | | | |
| Maximum $cm$ | | 9.2 | 9.2 | 12.9 | 14.7 |
| Minimum $cm$ | | 1.0 | 0.8 | 2.6 | 3.1 |
| Range $cm$ | | 8.2 | 8.4 | 10.3 | 11.6 |
| Mean begin $cm$ | | 4.09 | 4.17 | 6.11 | 6.84 |
| Standard deviation $cm$ | | 1.46 | 1.84 | 1.59 | 1.60 |
| Mean end $cm$ | | 4.41 | 4.42 | 7.22 | 8.14 |
| Standard deviation $cm$ | | 1.57 | 2.00 | 1.87 | 2.08 |
| Range $cm$ | | 0.32 | 0.25 | 1.12 | 1.30 |
| Leaf width (pot.) | | | | | |
| Maximum $cm$ | | 8.0 | 9.0 | 10.9 | 11.3 |
| Minimum $cm$ | | 0.8 | 0.7 | 2.4 | 2.9 |
| Range $cm$ | | 7.2 | 8.3 | 8.5 | 8.4 |
| Mean begin $cm$ | | 3.68 | 3.79 | 5.40 | 5.92 |
| Standard deviation $cm$ | | 1.27 | 1.58 | 1.32 | 1.26 |
The growth period of *N. peltata* started in May at a daily water temperature max(min) of 12/7 °C in the tanks and of 20/17 °C in Bemmelse Strang and lasted until the second half of October when minimum water temperatures dropped below 10 °C (Fig. 3, Tables 3, and 4). In the tank plots the growth period started 2 weeks earlier than in the Bemmelse Strang plots, while the end date of the growth periods was similar for all plots. The production of new leaves during the growth period continued almost the whole vegetation period. Growth/vegetation period ratios for *N. peltata* were 84–94% (Fig. 4, Table 2).

The vegetation period of all plots showed a max(min) daytime water temperature of 30/10 °C and a nighttime water temperature of 23/3 °C. The average water temperatures did not differ very much between the plots and ranged for daytime between 19.4–20.8 °C (growth period) and 18.9–19.7 °C (vegetation period) and for nighttime between 14.7–15.5 °C (growth period) and 13.9–14.8 °C (vegetation period) (Table 3).

### Inundation and leaf loss

On June 23, 1980, Bemmelse Strang was inundated unexpectedly by overflow of the River Waal by 0.5 m. The inundation period lasted until July 29, 1980, and led to water temperatures of on average 7 °C lower during the day and 4 °C during the night and to water level fluctuations with impact on leaf loss and production in plots BS1 and BS2. The loss of leaves of *N. peltata* by drowning was 49% of the leaves present in BS1 and 55% in BS2. The percentage of lost leaf area on June 23 compared to the total leaf area produced, was 17.01% for BS1 and 8.75% for BS2. The leaf loss peak of June 23 was followed by a month of decreased numbers of newly developed leaves. The concrete tanks

---

**Table 2 (continued)**

| Location Year | CT1 1978 | CT2 1978 | BS1 1980 | BS2 1980 |
|---------------|----------|----------|----------|----------|
| Mean end cm   | 3.97     | 4.02     | 6.54     | 7.18     |
| Standard deviation cm | 1.36     | 1.71     | 1.55     | 1.68     |
| Range cm      | 0.29     | 0.23     | 1.14     | 1.26     |
| Leaf area     |          |          |          |          |
| Tot. pot. m².m⁻².yr⁻¹ | 3.5270   | 3.8204   | 6.9103   | 5.5477   |
| Max. pot. m²  | 1.4295   | 1.4274   | 1.7741   | 2.2719   |
| Max act. m²   | 1.4287   | 1.4168   | 1.5760   | 2.0489   |
| Max. phot. m² | 0.9528   | 0.9326   | 1.4180   | 1.6752   |
| Mean pot. per day m².m⁻².d⁻¹ | 0.0175   | 0.0208   | 0.0409   | 0.0328   |
| Mean pot. per leaf m².m⁻² | 0.0016   | 0.0017   | 0.0040   | 0.0050   |
| Standard deviation m².m⁻² | 0.0010   | 0.0013   | 0.0019   | 0.0023   |
| Max. pot. date | Jul. 11  | Jun. 30  | Sep. 5   | Oct. 20  |
| Max act. date  | Jul. 11  | Jun. 30  | Sep. 5   | Sep. 16  |
| Max. phot. date | Jun. 9   | Jun. 30  | Jun. 23  | Sep. 16  |
CT1 and CT2, with only small fluctuations in water level, showed leaf loss peaks in July and at the end of August, respectively (Fig. 5).

Development of leaves

Development of leaf blades is shown in figures by plots over time and by scatter plots of two variables with accompanying data in tables.

Cumulative numbers of leaves per plot over time showed a regular increase over nearly the whole vegetation period. The actual numbers of leaves showed a rapid increase from the start of the growing season in May towards a relatively high number in June. Decrease of numbers started in September (BS1, BS2) or after a peak number in August (CT1, CT2). The actual number of all leaves, with separate contributions of short shoot and flowering stem leaves, is shown per plot over time in combination with the observed maximum and minimum water temperature over time. The number of leaves and the total potential leaf area in time showed a clear correlation with the course of the water temperature (Figs. 6

Figure 3 Water temperatures in the *Nymphoides peltata* plots. Water temperatures in the *Nymphoides peltata* plots, combined with the number of leaves per plot over time. Temperatures are blue for maximum (day) and red for minimum (night) values. The contribution to leaves per plot over time is divided in short shoot leaves (green) and flowering stem leaves (yellow).
Table 3: Water temperature data of measuring dates for growth and vegetation periods. Water temperature data of measuring dates for growth and vegetation periods of *Nymphoides peltata* in concrete tank and Bemmelse Strang plots. With CT1 (1978–1979), CT2 (1978–1979), BS1 (1980), BS2 (1980).

| Plot  | Daytime water temperature (°C) | Nighttime water temperature (°C) |
|-------|-------------------------------|----------------------------------|
|       | Growth period | | | Vegetation period | | |
| max   | min | range | average | s.d. | max | min | range | average | s.d. |
| CT1   | 28.50 | 12.00 | 16.50 | 20.84 | 4.67 | 23.00 | 6.50 | 16.50 | 14.73 | 3.72 |
| CT2   | 30.00 | 12.00 | 18.00 | 20.26 | 4.51 | 22.50 | 6.50 | 16.00 | 14.72 | 3.67 |
| BS1   | 27.00 | 11.00 | 16.00 | 19.52 | 3.37 | 21.00 | 7.00 | 14.00 | 15.48 | 3.11 |
| BS2   | 27.00 | 10.00 | 17.00 | 19.40 | 3.66 | 21.00 | 7.00 | 14.00 | 15.31 | 3.27 |

Table 4: Water temperatures of the last three measuring dates before the end of the growth and vegetation period. Water temperatures of the last three measuring dates before the end of the growth and the vegetation period of *Nymphoides peltata* as maximum(minimum) °C for plots CT1 (1978), CT2 (1978), BS1 (1980), BS2 (1980).

| Plot  | Temperatures at end of growth period | Temperatures at end of vegetation period |
|-------|-------------------------------------|----------------------------------------|
|       | Last-but-two date | Last-but-one date | Last date | Last-but-two date | Last-but-one date | Last date |
|       | CT1 | CT2 | BS1 | BS2 | CT1 | CT2 | BS1 | BS2 | CT1 | CT2 | BS1 | BS2 |
| Last-but-two date | 18(13) | 18(13) | 13(8) | 14(8) | 13(8) | 13(9) | 10(8) | 10(8) |
| Last-but-one date | 16(13) | 16(13) | 14(8) | 11(8) | 13(8) | 11(8) | 11(10) | 11(10) |
| Last date | 15(9) | 15(9) | 11(8) | 10(8) | 9(7) | 10(8) | 11(3) | 11(3) |

Figure 4: Numbers of floating leaves per plot over time. Number of floating leaves present (red line) and cumulative number of leaves (blue line) of *Nymphoides peltata* per plot over time. The dash-dotted black line indicates missing data in (A) and (B).

Klok and van der Velde (2022), *PeerJ*, DOI 10.7717/peerj.13976
Development of leaves took place above 15 °C and leaves disappear when the water temperature dropped below 10 °C.

Leaf life span and leaf size

Both leaf length frequencies and leaf life span frequencies of the four plots have been tested for differences by a single-factor ANOVA. Neither showed significant differences (leaf length p-value = 0.318 and leaf life span p-value = 0.548).

The relation between the maximum potential leaf area and the leaf life span for all leaves per plot is shown by means of scatter plots with linear trend lines, showing a correlation of leaf size with leaf life span in all cases. Larger leaves existed generally longer than smaller leaves (Fig. 8).
Figure 6 Relation between number of leaves and maximum water temperature. Relation between number of leaves and maximum water temperature on measuring dates with linear trend lines. Per plot each figure is divided in increasing temperatures from May through July (left half) and decreasing temperatures from August through November (right half). DOI: 10.7717/peerj.13976/fig-6
Figure 7  Relation between potential leaf area and maximum water temperature. Relation between potential leaf area and maximum water temperature on measuring dates with linear trend lines. Per plot each figure is divided in increasing temperatures from May through July (left half) and decreasing temperatures from August through November (right half). DOI: 10.7717/peerj.13976/fg-7
Short shoot leaves were in each plot generally much larger than flowering stem leaves. The mean potential leaf area values of the plots ranking from low to high were CT1, CT2, BS1 and BS2 (Table 5). Confidence interval values have been computed for all leaves, and separately for short shoot and for flowering stem leaves (Table 6).

### Leaf growth at the water surface

Begin vs. end values of leaf area per leaf are shown per plot in scatterplots with trend lines, showing a linear relationship (Fig. 9). Begin and end values of potential leaf area and biomass are shown for all plots over time, indicating growth at the water surface during the vegetation season. Those values are also shown in scatter plots with a second-order polynomial trend line with trend line equations and confidence intervals (Fig. 10, Tables 7

---

**Table 5** Mean potential leaf area values of *Nymphoides peltata*. Mean potential leaf area values of *Nymphoides peltata* with standard deviations (s.d.) for all leaves, short shoot leaves and flowering stem leaves per plot.

| Plot (year) | CT1 (1978) | CT2 (1978) | BS1 (1980) | BS2 (1980) |
|------------|------------|------------|------------|------------|
| All        | 0.0016     | 0.0017     | 0.0040     | 0.0050     |
| s.d.       | 0.0010     | 0.0013     | 0.0019     | 0.0023     |
| Short shoot| 0.0018     | 0.0023     | 0.0041     | 0.0050     |
| s.d.       | 0.0009     | 0.0013     | 0.0019     | 0.0023     |
| Flowering stem | 0.0006 | 0.0007     | 0.0020     | 0.0029     |
| s.d.       | 0.0005     | 0.0006     | 0.0009     | 0.0018     |

---

![Figure 8](https://example.com/figure8.png)

**Figure 8** Maximum potential leaf area vs leaf life span. Scatter plots of maximum potential leaf area vs. leaf life span of all *Nymphoides peltata* leaves per plot with linear trend lines and 95% confidence interval. See Table 7 for confidence interval values.
The trend lines show optima in May/June (CT2), June/July (CT1, BS1) and July/August (BS2) for begin values and in June (CT2), July/August (CT1), August (BS1) and August/September (BS2) for end values.

Average potential begin and end values of leaf area and leaf biomass for all plots are shown in Table 9.

Leaves in the tank plots were smaller than those in the lake plots (Table 2), and showed a lower increase in total potential leaf area by growth on the surface: 14% for CT1, 12% for CT2, 30% for BS1 and 31% for BS2. The graphs displaying the potential begin and end leaf area (Fig. 11) clearly show differences in growth: the mean growth increase is 18.77% for CT1, 15.62% for CT2, 41.89% for BS1, 45.00% for BS2, respectively. The leaf area also shows that the tank plots contained smaller leaves than the lake plots (Fig. 8).

**Table 6**  Confidence interval values of trend lines for maximum potential leaf area vs. leaf life per leaf.

| Plot | Leaves | Mean (mm²) | SE  | 95% conf int | Lower limit | Upper limit |
|------|--------|------------|-----|--------------|-------------|-------------|
| CT1  | all (555) | 1,588.611 | 0.303 | 1.964 | 24.710 | 25.901 |
|      | ssl (454) | 1,806.827 | 0.319 | 1.965 | 26.025 | 27.280 |
|      | fsl (101) | 607.720 | 0.714 | 1.984 | 17.832 | 20.666 |
| CT2  | all (558) | 1,711.665 | 0.330 | 1.964 | 21.859 | 23.154 |
|      | ssl (346) | 2,344.753 | 0.407 | 1.967 | 24.440 | 26.041 |
|      | fsl (212) | 678.417 | 0.505 | 1.971 | 17.053 | 19.044 |
| BS1  | all (428) | 4,036.374 | 0.486 | 1.965 | 21.891 | 23.803 |
|      | ssl (416) | 4,095.216 | 0.494 | 1.966 | 21.956 | 23.899 |
|      | fsl (12)  | 1,996.510 | 2.819 | 2.228 | 13.805 | 26.367 |
| BS2  | all (277) | 5,006.969 | 0.627 | 1.969 | 28.358 | 30.826 |
|      | ssl (274) | 5,030.387 | 0.614 | 1.969 | 28.131 | 30.549 |
|      | fsl (3)  | 2,868.123 | 1.600 | 12.706 | 26.069 | 66.736 |

The trend lines show optima in May/June (CT2), June/July (CT1, BS1) and July/August (BS2) for begin values and in June (CT2), July/August (CT1), August (BS1) and August/September (BS2) for end values.

Average potential begin and end values of leaf area and leaf biomass for all plots are shown in Table 9.

Leaves in the tank plots were smaller than those in the lake plots (Table 2), and showed a lower increase in total potential leaf area by growth on the surface: 14% for CT1, 12% for CT2, 30% for BS1 and 31% for BS2. The graphs displaying the potential begin and end leaf area (Fig. 11) clearly show differences in growth: the mean growth increase is 18.77% for CT1, 15.62% for CT2, 41.89% for BS1, 45.00% for BS2, respectively. The leaf area also shows that the tank plots contained smaller leaves than the lake plots (Fig. 8).

**Leaf area and biomass**

Leaf area data are shown in Table 2. The mean potential leaf area values with standard deviations have been computed over all measuring dates per plot for all leaves, for short shoot leaves and for flowering stem leaves (Table 5).

Leaf biomass data per plot were calculated by monthly and by annual regression equations based on the leaf area of each leaf as calculated from the leaf lengths measured in the plots (Tables 1 and 10).

Changes over time of potential, actual and photosynthetic leaf area and leaf biomass based on both regression equations demonstrated in the plots CT1 and CT2 a clear peak at the end of June which is maximal for CT1 and CT2 with a lower peak in August after which there is a further decrease. In BS1 and BS2 a clear peak of June is visible, after which a strong loss of leaves is visible due to a flooding after which a second peak was reached in September which was about equal in size with the first peak in the case of BS1 and much higher than that in the case of BS2 (Fig. 12).
Ratios

Based on leaf characteristics (Table 2), various ratios were calculated for the plots (Table 11) to provide information on the plasticity of the species.

The turnover rate Vegetation period/Mean leaf life span was high for CT1, CT2 and BS2, with a very low value for BS2, which may indicate that BS2 suffered least of nutrient limitation and of inundation. The turnover rate Total potential biomass/Maximum
Figure 10 Observed begin and end area of *Nymphoides peltata* leaves. Scatter plots of observed begin (green) and end (brown) area of *Nymphoides peltata* leaves per plot over time with second order curve fitting polynomial. Axes with biomass values are shown on the right side of the plots. See Table 8 for trend line equations with $r^2$ values and Table 9 for confidence interval values.

Table 7 Trend line equations for begin and end area ($m^2$) and biomass (g AFDW) of *Nymphoides peltata* leaves. Trend line equations for begin and end area ($m^2$) and biomass (g AFDW) of *Nymphoides peltata* leaves in plots CT1 (1978, 1979), CT2 (1978, 1979), BS1 (1980) and BS2 (1980). See Table 8 for confidence intervals of the trend lines and Fig. 11 for scatter plots with trend lines.

| Plot | Trend line begin area | $r^2$ | Trend line end area | $r^2$ |
|------|-----------------------|-------|---------------------|-------|
| CT1  | $y = -0.2174x^2 + 12470x - 2E+08$ | 0.2205 | $y = -0.1993x^2 + 11437x - 2E+08$ | 0.1744 |
| CT2  | $y = -0.1717x^2 + 9836.8x - 1E+08$ | 0.2372 | $y = -0.1484x^2 + 8506.9x - 1E+08$ | 0.1790 |
| BS1  | $y = -0.3251x^2 + 19127x - 3E+08$ | 0.1556 | $y = -0.3868x^2 + 22782x - 3E+08$ | 0.1438 |
| BS2  | $y = -0.4737x^2 + 27882x - 4E+08$ | 0.2685 | $y = -0.5126x^2 + 30210x - 3E+08$ | 0.2163 |
|      | Trend line begin biomass/annual | $r^2$ | Trend line end biomass/annual | $r^2$ |
| CT1  | $y = -0.6562x^2 + 37634x - 5E+08$ | 0.2205 | $y = -0.6013x^2 + 34516x - 5E+08$ | 0.1744 |
| CT2  | $y = -0.5181x^2 + 29687x - 4E+08$ | 0.2372 | $y = -0.4479x^2 + 25674x - 4E+08$ | 0.1790 |
| BS1  | $y = -1.2760x^2 + 75075x - 1E+09$ | 0.1556 | $y = -1.5182x^2 + 89419x - 1E+09$ | 0.1438 |
| BS2  | $y = -1.8593x^2 + 103439x - 2E+09$ | 0.2685 | $y = -2.0121x^2 + 118576x - 2E+09$ | 0.2163 |
potential biomass ($P/B_{\text{max}}$) showed similar values for CT1, CT2 and BS2 and a clearly higher value for BS1. Total number of leaves/Maximum number of leaves showed differences between CT1, BS1 and CT2, BS2, which may have been caused by limitation of space and nutrients. Mean leaf life span/Total number of leaves was similar for CT1 and CT2, showed a much higher value for BS1 and a value twice as high for BS2. Growth period/Vegetation period showed similar values for the four plots. Ratios for Leaf area and biomass, i.e., Maximum leaf area or biomass (pot., act. and phot.)/Total leaf area or biomass (pot.) generally showed higher values for CT1 and BS2 compared to CT2 and the

Table 8  Confidenc intervals for begin and end area (cm$^2$) and biomass (mg AFDW) of *Nymphoides peltata* leaves. Confidence intervals for begin and end area (cm$^2$) and biomass (mg AFDW) of *Nymphoides peltata* leaves in plots CT1 (1978, 1979), CT2 (1978, 1979), BS1 (1980) and BS2 (1980). The biomass is based on annual regression equations. See Table 7 for trend line equations and Fig. 11 for scatter plots and trend lines.

| Plot | Leaf area |  |  |  |  |
|------|-----------|---|---|---|---|
|     | Mean      | St deviation | 95% conf int | Lower limit | Upper limit |
| CT1  | begin     | 1,257.00     | 840.50 | 64.52 | 1,192.60 | 1,321.64 |
|      | end       | 1,493.00     | 970.22 | 74.47 | 1,418.15 | 1,567.10 |
| CT2  | begin     | 1,472.00     | 1,115.58 | 87.04 | 1,885.06 | 1,559.15 |
|      | end       | 1,702.00     | 1,299.33 | 101.38 | 1,600.29 | 1,803.05 |
| BS1  | begin     | 2,844.66     | 1,360.42 | 128.88 | 2,715.78 | 2,973.55 |
|      | end       | 4,036.37     | 1,865.72 | 176.76 | 3,859.62 | 4,213.13 |
| BS2  | begin     | 3,453.00     | 1,447.83 | 170.50 | 3,282.35 | 3,623.35 |
|      | end       | 5,007.00     | 2,284.98 | 269.09 | 4,737.88 | 5,276.05 |

Table 9  Average potential begin and end values for leaf area (cm$^2$) and leaf biomass (mg AFDW). Average potential begin and end values for leaf area (cm$^2$) and leaf biomass (mg AFDW) of *Nymphoides peltata* in plots CT1 (1978), CT2 (1978), BS1 (1980) and BS2 (1980). See Fig. 10 for scatter plots of begin vs. end leaf area.

| Plot | Average potential leaf area |  |  |  |  | Average potential leaf biomass |  |  |  |
|------|-----------------------------|---|---|---|---|--------------------------------|---|---|---|
|      | Begin | End | End-Begin | Begin/End | Begin | End | End-Begin | Begin/End |
| CT1  | 1,364.12 | 1,603.05 | 238.94 | 0.85 | 4,123.41 | 4,844.58 | 721.17 | 0.85 |
| CT2  | 1,511.62 | 1,711.67 | 200.04 | 0.88 | 4,568.58 | 5,172.31 | 603.73 | 0.88 |
| BS1  | 2,844.66 | 4,036.37 | 1,191.71 | 0.70 | 11,138.70 | 15,816.20 | 4,677.47 | 0.70 |
| BS2  | 3,452.90 | 5,007.00 | 1,554.10 | 0.69 | 13,526.00 | 19,626.00 | 6,099.90 | 0.69 |
lowest for BS1. Leaf area/Leaf biomass showed low values for BS1, similar values for CT1 and CT2 and slightly lower values for BS1 and BS2.

**DISCUSSION**

**Water temperature and leaf development**

The difference in start date of floating leaf development between tank plots and lake plots can be explained by the measurement of water temperature at a depth of 10 cm, because water exchange and the great water mass in the lake delay the warming up of the water at
the bottom and therefore the development of leaves from the bottom. In contrast to the growth period of other nymphaeids (*Nuphar lutea* (L.) Sm., *Nymphaea alba* L., *Nymphaea candida* Presl), which ended about halfway the vegetation period (Klok & Van der Velde, 2017), the production of new leaves of *N. peltata* continued over nearly the whole growth period. Growth and vegetation periods showed a high similarity in all plots and seemed to be regulated by water temperature. Development of leaves takes place above a maximum water temperature of 15 °C and at the end of the season leaves disappear when the maximum water temperature drops below 10 °C. Markovskaya et al. (2019) noted the high invasive capacity at high air temperatures at the northern limit of its occurrence with an increase in leaf area.

### Influence of inundations or floods

Inundations, causing a sudden and considerable rise of the water level in the growing season, can diminish the vitality of *N. peltata* or even cause its complete disappearance (Brock, Van der Velde & Van de Steeg, 1987; Yu & Yu, 2011). A rapid increase in water depth has a stronger negative impact than a gradual increase during the growing season.

| Location | Year | CT1 1978 | CT2 1978 | BS1 1980 | BS2 1980 |
|----------|------|----------|----------|----------|----------|
| Leaf biomass (monthly) | | | | | |
| Total pot. | g AFDW.m⁻² yr⁻¹ | 133.24 | 137.31 | 272.18 | 208.59 |
| Max. pot. on date | g AFDW.m⁻² | 60.92 | 58.14 | 74.32 | 90.20 |
| Max. act. on date | g AFDW.m⁻² | 60.89 | 58.05 | 63.08 | 83.91 |
| Max. phot. on date | g AFDW.m⁻² | 38.15 | 38.98 | 54.70 | 72.31 |
| Mean pot. per day | g AFDW.m⁻².d⁻¹ | 0.06596 | 0.7463 | 1.6105 | 1.2342 |
| Mean pot. per leaf | g AFDW.m⁻² | 0.0600 | 0.0615 | 0.1590 | 0.1883 |
| Standard deviation | g AFDW.m⁻² | 0.0408 | 0.0513 | 0.0776 | 0.0905 |
| Max. pot. date | Jul. 4 | Jul. 4 | Aug. 26 | Aug. 29 |
| Max. act. date | Jul. 4 | Jul. 4 | Aug. 29 | Aug. 29 |
| Max. phot. date | Jul. 4 | Jul. 9 | Aug. 29 | Aug. 29 |
| Leaf biomass (annual) | | | | | |
| Total pot. | g AFDW.m⁻² yr⁻¹ | 106.58 | 115.45 | 270.77 | 213.49 |
| Max. pot. on date | g AFDW.m⁻² | 43.18 | 43.11 | 69.52 | 89.07 |
| Max. act. on date | g AFDW.m⁻² | 43.18 | 42.79 | 61.77 | 80.33 |
| Max. phot. on date | g AFDW.m⁻² | 28.78 | 28.16 | 55.57 | 65.68 |
| Mean pot. per day | g AFDW.m⁻².d⁻¹ | 0.5276 | 0.6274 | 1.6022 | 1.2632 |
| Mean pot. per leaf | g AFDW.m⁻² | 0.0480 | 0.0517 | 0.1582 | 0.1927 |
| Standard deviation | g AFDW.m⁻² | 0.0299 | 0.0401 | 0.0733 | 0.0898 |
| Max. pot. date | Jul. 11 | Jun. 30 | Sep. 5 | Oct. 20 |
| Max. act. date | Jul. 11 | Jun. 30 | Sep. 5 | Sep. 16 |
| Max. phot. date | Jun. 9 | Jun. 30 | Jun. 23 | Sep. 16 |
Species with a rapid, adaptive morphological response to fluctuating water levels have continuous and rapid leaf recruitment (Ridge & Amarasinghe, 1984; Yu & Yu, 2011). Leaf life spans of 20–30 days (Tsuchiya, 1991; Kunii & Aramaki, 1992) and a high productivity relative to biomass (Brock et al., 1983a; Twilley et al., 1985; Kunii & Aramaki, 1992) represent a strategy of morphological adjustments to water level fluctuations. *N. peltata* mainly relies on morphological plasticity by petiole elongation to adapt to water level rise, since it cannot escape like free floating plants and it does not develop underwater leaves for survival (Cao & Mei, 2015). Continuous leaf recruitment ensures that young radical leaves, growing and elongating faster than old ones, are always present in the canopy (Ridge, 1985). Nohara (1991) found that biomass of *N. peltata* in sites of more than 1.5 m depth increased in late June and decreased after a flood causing a water level rise of 1 m and lasting 1 week in Lake Kasumigaura. Tsuchiya, Nohara & Iwakuma (1990) found that when leaves were lost by wave action, the plants soon recovered by vegetative growth producing leaves from the runners which are perennial. *Nymphoides*
"peltata" in the Oude Waal showed a low degree of flooding tolerance when a summer flood rose the water level by 3 m with a duration of several weeks. When the rates of elongation and leaf recruitment do not match the rise in water level, plants die prematurely (Brock et al., 1983a; Brock, Van der Velde & Van de Steeg, 1987; Tsuchiya, 1991; Cooling, Ganf & Walter, 2001).

Normally winter and spring floods occur each year outside the vegetation period in Bemmelse Strang after which the water level gradually decreases without influence on later development of N. peltata (Brock, Van der Velde & Van de Steeg, 1987). Paillisson & Marion (2011) noted that early spring water level fluctuations have a poorly related influence on the biomass of N. peltata in contrast to Nymphaea alba L. and Nuphar lutea (L.) Sm. The leaf loss by the early summer inundation of lake plots BS1 and BS2 during this research was compensated by N. peltata by developing new leaves of which the petioles adapted to the new water levels. In the case of border plot BS2 the inundation also led to an increased growth nearly till the end of the vegetation period (Growth period/Vegetation period 93.5%).

**Impact of nutrient availability**

This research further investigated the consequences for leaf blade characteristics and leaf development from the posed hypothesis that dense enclosed Nymphoides stands recycle nutrient sources more frequently than less dense open stands. High consumption of nutrients by growth may lead to limited resources for further development and growth,

| Location | Turnover rate, or Vegetation period/Mean leaf life span | Turnover rate (P/B\(_{\text{max}}\)), or Tot. pot. leaf biomass/Max. pot. leaf biomass | Total number of leaves/Max. number of leaves | Mean leaf life span/Total number of leaves | Growth period/Vegetation period | Leaf area of Max pot. | Leaf area of Max act. | Leaf area of Max phot. |
|----------|--------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------|-------------------------------------------|---------------------------------|----------------------|----------------------|----------------------|
| CT1 (1978) | 7.98 | 2.47 | 4.20 | 0.010 | 0.847 | 0.4053 | 0.4051 | 0.2700 |
| CT2 (1978) | 8.17 | 2.68 | 3.00 | 0.009 | 0.908 | 0.3736 | 0.3709 | 0.2441 |
| BS1 (1980) | 7.40 | 3.91 | 4.37 | 0.013 | 0.917 | 0.2567 | 0.2281 | 0.2052 |
| BS2 (1980) | 5.71 | 2.40 | 2.92 | 0.027 | 0.935 | 0.4095 | 0.3693 | 0.3020 |

Klok and van der Velde (2022), *PeerJ*, DOI 10.7717/peerj.13976
which influences leaf development. Experiments with herbaceous terrestrial plants under conditions of lowered nutrient levels demonstrated that plants developed smaller leaves with a shorter leaf life span, leading to a higher leaf turnover, a lower biomass and the development of more flowers (El Hinnawy, 1956). Brock et al. (1983b) studied the nitrogen and phosphorus accumulation and cycling in and outside a N. peltata stand in Bemmselse Strang during 1980 and found that during the growing season most of the nutrients were translocated to petioles and leaves, that uptake of nutrients was through the roots from the interstitial water of the sediment and that phosphorus was taken up for at least 80% from the sediment. Harvesting of N. peltata has a positive effect by decreasing the total nitrogen, ammonium nitrogen and chemical oxygen demand, though it may also result in an increase of phosphorus causing algal blooms (Zhu et al., 2019).

**Plot ranking by nutrient availability**

Leaf development of N. peltata was followed in four plots representing different situations that may influence nutrient availability: volume of growing area, thickness of rooting layer, water depth, water exchange and competition with other macrophyte species. The tanks with plots CT1 and CT2 provided a limited volume for growing, while the lake with plots BS1 and BS2 had an “unlimited” volume. The average depth of CT1, CT2 and BS1 was similar (45–67 cm), in contrast to BS2 (117 cm). BS1 had a thicker detritus layer compared to BS2, which may have enlarged the nutrient availability for BS1. Water flow in the lake as well as the unexpected inundation by river water will have improved the nutrient availability in the water layer for BS1 and BS2. The center plot BS1 of the lake stand had a much thicker vegetation than border plot BS2. In contrast with the monospecific N. peltata vegetation in CT2, CT1 contained a mixed culture of N. peltata and some helophytes (Glyceria spp.). Less nutrient availability is expected for N. peltata in CT1 due to competition with the helophytes. Considering these differences, the ranking of the plots from low to high nutrient availability was expected to be CT1, CT2, BS1, BS2.

**Plot ranking for characteristics**

An increasing order was found for maximum leaf life span and maximum leaf length, and a decreasing order for number of leaves, number of short shoot leaves, new leaves per day, and the duration of the vegetation period. Comparing CT with BS plots showed higher CT-values for flowering stems, maximum number of leaves, duration growth period, duration flowering stem period, and lower CT-values for maximum leaf length, minimum leaf length, begin and end leaf length, leaf area, leaf biomass.

The total number of leaves per plot showed a decreasing order for the expected ranking. However, if a distinction between short shoot leaves and flowering stem leaves is made, the number of short shoot leaves showed a similar order: CT1, CT2 and BS1 (about the same), BS2, but the number of flowering stem leaves showed a large difference between tank and lake plots and a different order: CT2 (848), CT1 (404), BS1 (48) and BS2 (12). The difference in flowering stem leaves between CT1 and CT2 might be caused by the mixed culture in CT1 leaving less volume for development of long shoots. This means more leaves and more flowers are an indication of nutrient shortage.
The total potential leaf area of plots, a measure for leaf production, shows the increasing order CT1, CT2, BS2, BS1. The total potential biomass shows the same sequence using both monthly and annual regression equations for tank and lake plots. Since the annual equations are “averaging”, the monthly equations clearly show a higher biomass production in July for CT1 and CT2, while, as a result of the flooding of June 23 in BS1 and BS2, only a slightly higher production is shown in these plots. Consequence of nutrient limitation may be the production of smaller leaves and less biomass.

The maximum leaf life span increased with the expected ranking (CT1, CT2, BS1, BS2), but the mean leaf life span shows the order CT2 and BS1 (about the same), CT1, BS2 from low to high. Here, sexual reproduction might be an important factor that reduces the leaf life span, since CT2 and BS1 showed more flowering stems than CT1 and BS2. Although the trend is clear, the leaf life span frequency of all leaves showed no significant differences between the plots (single-factor ANOVA test; $p \geq 0.05$), so leaf life span frequency can be considered a stable trait.

Differences between plots

Wu & Yu (2004) and Wu et al. (2006) found in microcosm experiments that at increasing competition by rice (Zizania latifolia (Griseb.) Turcz ex Stapf), N. peltata showed lower leaf numbers, leaf areas and water surface coverage. Such effects on N. peltata due to the presence of Glyceria in CT1 were not observed in the tank plots, as differences in leaf blade characteristics of the two CT plots were not significant (Table 2). A possible explanation might be that competition intensity was not strong enough to make N. peltata produce morphological differences, apart from the lower number of flowering stem leaves compared to CT2.

The peak density of leaves in the tank plots was reached in August and in Bemmelse Strang in June (center plot, BS1) and September (border plot, BS2), respectively. After the peak in June and the flooding, the production of new short shoot leaves in BS1 had to compete with the development of flowering stems caused by nutrient shortage by enclosed growth, which may have prevented a higher peak later in the season. The flooding had less impact on the number of leaves in BS2, which reached a later and higher peak in September, also where there were no limiting nutrients at the border of the stand as the bare sediment could be colonized without competition.

Consequence of nutrient limitation may be the development of more, but smaller leaves. Mean leaf length was similar in the tank plots, but much larger in the lake plots, where less, but larger leaves and also less flowering stem leaves developed. The largest leaves occurred in border plot BS2. The leaf area in the tank plots showed a rapid increase to a peak level after which a lower level was established, an indication of nutrient shortage preventing a further increase.

Leaf area and biomass in the lake plots reached a later peak (September), compared to the tank plots (July). The late peaks in leaf area and biomass of border plot BS2 were relatively very high. This might be explained by the lower impact of the June 23 inundation at BS2, compared to BS1 as in the latter plot the peak of leaf area and biomass in June was already very high when the flooding started. In BS1 the later peak in leaf area and biomass
in September was even lower than in June. The graphs displaying the potential biomass for begin and end leaf size show very small differences between begin and end biomass values for CT2 and BS1. This might be due to lack of nutrients resulting in mainly growth by cell stretching causing low leaf biomass values. The decrease after the early optima for CT1 and CT2 may indicate the growing influence of nutrient limitation in time.

The high leaf area and biomass production in border plot BS2 can be explained by a higher nutrient availability and room for expansion at the border of the stand favoring vegetative growth, while in center plot BS1 high densities of short shoots and roots lead to competition of plant organs for nutrients inducing sexual reproduction by producing flowering stems with flowers at the expense of leaf production. The high production of flowering stems is shown clearly in the tank plots and can be the result of nutrient poor conditions as found for terrestrial plants (El Hinnawy, 1956).

Comparing CT with BS plots showed higher CT-values for flowering stems, maximum number of leaves, duration of growth period, duration flowering stem period, and lower CT-values for maximum leaf length, minimum leaf length, begin and end leaf length, leaf area, leaf biomass.

**Ratios**

Based on leaf blade characteristics, various ratios were calculated for the plots. The turnover rate Vegetation period/Mean leaf life span was high for CT1, CT2 and BS2, with a very low value for BS2, which may indicate that BS2 suffered least of nutrient limitation and of inundation. The turnover rate Total potential biomass/Maximum potential biomass (P/B_max) showed similar values for CT1, CT2 and BS2 and a clearly higher value for BS1. Total number of leaves/Maximum number of leaves showed differences between CT1, BS1 and CT2, BS2, which may have been caused by limitation of space and nutrients. Mean leaf life span/Total number of leaves was similar for CT1 and CT2, showed a much higher value for BS1 and a value twice as high for BS2. Growth period/Vegetation period showed similar values for the four plots. Ratios for Leaf area and biomass, *i.e.*, Maximum leaf area or biomass (pot., act. and phot.)/Total leaf area or biomass (pot.) generally showed higher values for CT1 and BS2 compared to CT2 and the lowest for BS1. Leaf area/Leaf biomass showed low values for BS1, similar values for CT1 and CT2 and slightly lower values for BS1 and BS2. From the differences in ratios it can be concluded that the ratio growth/vegetation period is most stable over the plots, which indicates that this is a characteristic of the plant species. Unfortunately, we have data of only four plots, which is insufficient to allow further generalizations based on the ratios. We need wide scale investigations to interpret these results.

**CONCLUSIONS**

The results of this study lead to the following conclusions:

(a) Growth and vegetation periods of *N. peltata* seemed to be regulated by water temperature (10–30 °C): growth stops when the water temperature drops below 10 °C and this indicates a stable trait.
(b) The growth period started in May and ended mid-October with continuous production of floating leaves during nearly the whole vegetation period indicating its capacities for rapid colonization and spread in contrast to waterlilies. The petioles of the floating leaves adapt to the water level. The water level in the tanks was very stable, but the inundation of the lake plots caused the sudden loss of existing leaves and a lower production of new leaves for about a month, then followed by an increase. The deeper border plot suffered less from the inundation and showed an absolute leaf maximum in September.

(c) Resources in the tanks were limited compared to the lake which resulted in the production of a very high number of smaller leaves with lower totals for leaf area and biomass and in abundant sexual reproduction via flowering stems.

(d) Turnover rates and other ratios calculated, appeared to be relatively similar for plots CT1, CT2 and BS1, but for the deeper border plot BS2 lower ratios were found.

e) All these results suggest that established dense enclosed stands, have to recycle nutrients more frequently than stands in pioneer or border situations with consequences for leaf development and leaf blade characteristics.

ACKNOWLEDGEMENTS
We thank L. van der Heijden, P. M. M. Bexkens and P. A. J. van Grunsven for collecting and working out field data, P. H. M. Charpentier for providing all necessary literature, T. C. Oor (†) for making Fig. 1, W. J. Metzger for checking and correcting the English text, and two reviewers (Marina Satika Suzuki and one anonymous reviewer) as well as the editor (Carlos Zuniga Gonzalez) for constructive remarks.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The authors received no funding for this work.

Competing Interests
The authors declare that they have no competing interests.

Author Contributions
• Peter F. Klok conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
• Gerard van der Velde conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
    The raw data is available in the Supplemental File.
Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.13976#supplemental-information.

REFERENCES

Asaeda T, Rashid MH. 2017. Effects of turbulence motion on the growth and physiology of aquatic plants. Limnologica 62:181–187 DOI 10.1016/j.limno.2016.02.006.

Bornette G, Puijalon S. 2011. Response of aquatic plants to abiotic factors: a review. Aquatic Sciences 73(1):1–14 DOI 10.1007/s00027-010-0162-7.

Brock TCM. 1983. The distribution of elements in a Nymphoides peltata-dominated system. In: Proceedings International Symposium on Aquatic Macrophytes, Nijmegen, 18–23 September 1983, Nijmegen.

Brock TCM. 1985. Ecological studies on nymphaeid water plants with emphasis on production and decomposition. PhD Thesis, Catholic University Nijmegen.

Brock TCM, Arts GHP, Goossen ILM, Rutenfrans AHM. 1983a. Structure and annual biomass production of Nymphoides peltata (Gmel.) O. Kuntze (Menyanthaceae). Aquatic Botany 17:167–188 DOI 10.1016/0304-3770(83)90056-6.

Brock TCM, Bongaerts MCM, Heijnen GJMA, Heijthuijsen JHFG. 1983b. Nitrogen and phosphorus accumulation and cycling by Nymphoides peltata (Gmel.) O. Kuntze (Menyanthaceae). Aquatic Botany 17:189–214 DOI 10.1016/0304-3770(83)90057-8.

Brock TCM, Van der Velde G, Van de Steeg HM. 1987. The effects of extreme water level fluctuations on the wetland vegetation of a nymphaeid-dominated oxbow lake in the Netherlands. In: Pokorný J, Lhotský O, Denny P, Turner EG, eds. Waterplants and Wetland Processes. Archiv für Hydrobiologie Beihefte, Ergebnisse der Limnologie/Advances in Limnology. Vol. 27. Stuttgart: E Schweitzerbart’sche Verlagsbuchhandlung (Naegel und Obermiller), 57–73.

CABI Invasive Species Compendium. 2005. Datasheet Nymphoides peltata. Available at www.cabi.org/isc/datasheet/107746.

Cao Q-J, Liu N, Wang L. 2016. Relative response to mechanical stress of co-existing aquatic species, floating-leaved Nymphoides peltata and submerged Myriophyllum spicatum. Pakistan Journal of Botany 48(3):935–943.

Cao Q-J, Mei F-F. 2015. Growth of floating-leaved and submerged plants in artificial cultured microcosms: morphological responses to various water level fluctuation regimes. Pakistan Journal of Botany 47(1):141–148.

Casper SJ, Krausch H-D. 1981. Süßwasserflora von Mitteleuropa. Begründet von A. Pascher. Band 24: Pteridophyta und Anthophyta, 2. Teil: Saururaceae bis Asteraceae. Stuttgart, New York: Gustav Fischer Verlag, 409–942.

Chabot BF, Hicks DJ. 1982. The ecology of leaf life spans. Annual Review of Ecology and Systematics 13:229–259 DOI 10.1146/annurev.es.13.110182.001305.

Cheek MD. 2018. First confirmed record of Nymphoides peltata (SG Gmel.) Kuntze (Menyanthaceae) naturalised in southern Africa. Bothalia 48(1):1–4 DOI 10.4102/abc.v48i1.2258.

Cooling MP, Ganf GG, Walter KF. 2001. Leaf recruitment and elongation: an adaptive response to flooding in Villarsia reniformis. Aquatic Botany 70:281–294 DOI 10.1016/S0304-3770(01)00153-X.
Darbyshire SJ, Francis A. 2008. The biology of invasive alien plans in Canada. 10. Nymphoides peltata (S.G. Gmel.) Kuntze. Canadian Journal of Plant Science 88(4):811–829 DOI 10.4141/CJPS07208.

Den Hartog C, Van der Velde G. 1988. Structural aspects of aquatic plant communities. In: Symoens JJ, ed. Vegetation of Inland Waters. Handbook of Vegetation Science. Vol. 15. Dordrecht: Kluwer Academic Publishers, 113–153.

Döhler H. 1963. Die Seekanne, unser einziges wasserbewohnendes Enziangewächs. Kosmos 59:254–257.

El Hinnawy EI. 1956. Some aspects of mineral nutrition and flowering. Mededelingen van de Landbouwhogeschool te Wageningen/Nederland 56(9):1–51.

Funke GL. 1951. Waterplanten. In: Noorduijn's Wetenschappelijke Reeks. Vol. 38. Gorinchem: J. Noorduijn and son, 1–250.

Glück H. 1924. Biologische und morphologische Untersuchungen über Wasser- und Sumpf-gewächse IV. In: Untergetauchte und Schwimmblattflora. Jena: Gustav Fischer Verlag, 746.

Grosse W, Bauch C. 1991. Gas transfer in floating-leaved plants. Vegetatio 97:185–192 DOI 10.1007/BF00035391.

Grosse W, Mevischutz J. 1987. A beneficial gas-transport system in Nymphoides peltata. American Journal of Botany 74(6):947–952 DOI 10.2307/2443876.

Grote E. 1980. Zur Verbreitung der Seekanne im Gebiet von Ems und Hase mit Bemerkungen zu den Biotopansprüchen. Inf. Naturschutz und Landschaftspfllege 2:142–153.

Heimans E, Thijsse JP. 1956. In sloot en plas. Seventh Edition. Amsterdam: Uitgeverij Ploegsma, 1–168.

Klok PF, Van der Velde G. 2017. Plant traits and environment: floating leaf blade production and turnover of waterlilies. PeerJ 5:e3212 DOI 10.7717/peerj.3212.

Kunii H, Aramaki M. 1992. Annual net production and life span of floating leaves in Nymphaea tetragona Georgi: a comparison with other floating-leaved macrophytes. Hydrobiologia 242:185–193 DOI 10.1007/BF00019967.

Lamb BT, McCrea AA, Stoodley SH, Dzialowski AR. 2021. Monitoring and water quality impacts of an herbicide treatment on an aquatic invasive plant in a drinking water reservoir. Journal of Environmental Management 288:112444 DOI 10.1016/j.jenvman.2021.112444.

Larson D. 2007. Growth of three submerged plants, below different densities of Nymphoides peltata (S.G. Gmel.) Kuntze. Aquatic Botany 86(3):280–284 DOI 10.1016/j.aquabot.2006.10.007.

Lavid N, Schwartz A, Lewinsohn E, Tel-Or E. 2001. Phenols and phenol oxidases are involved in cadmium accumulation in the water plants Nymphoides peltata (Menyanthaceae) and Nymphaeae (Nymphaeaceae). Planta 214:189–195 DOI 10.1007/s004250100610.

Li Z, Xu J, Cao T, Ni L, Xie P. 2010a. Adaptive responses of a floating-leaved aquatic plant, Nymphoides peltata, to a terrestrial habitat. Journal of Freshwater Biology 25(3):481–486 DOI 10.1080/02705060.2010.9664392.

Li Z, Yu D, Xu J. 2010b. Adaptation to water level variation: Responses of a floating leaved macrophyte Nymphoides peltata to terrestrial habitats. Annales de Limnologie-International Journal of Limnology 47:97–102 DOI 10.1051/limn/20100029.

Luther H. 1983. On life forms, and above-ground and underground biomass of aquatic macrophytes. Acta Botanica Fennica 123:1–23.

Markovskaya EF, Novichonok EV, Dachkova TY, Morozova KV. 2019. Nymphoides peltata (S.G. Gmel.) O. Kunze at the northern limit: eco-physiological peculiarities. Botanica Pacifica 8(2):41–49 DOI 10.17581/bp.2019.08205.
Meusel HE, Jäger E, Weinert E. 1978. Vergleichende Chorologie der Zentraleuropäischen Flora. Vol. 1: Text 583 pp., Vol. 2: Karten, 1–258. Vol. 1. Jena: VEB Gustav Fischer Verlag.

Nohara S. 1991. A study on annual changes in surface cover of floating-leafed plants in a lake using aerial photography. *Vegetatio* 97:125–136 DOI 10.1007/BF00035386.

Paiillisson J-M, Marion L. 2011. Water level fluctuations for managing excessive plant biomass in shallow lakes. *Ecological Engineering* 37:241–247 DOI 10.1016/j.ecoleng.2010.11.017.

Ridge IR. 1985. Ethylene and petiole development in amphibious plants. Chapter 19: 229–240. In: Roberts JA, Tucker GA, eds. *Ethylene and Plant Development*. Butterworths, London.

Ridge I, Amarasinghe I. 1984. Ethylene and growth control in the fringed water lily (*Nymphoides peltata*): Stimulation of cell division and interaction with buoyant tension in petioles. *Plant Growth Regulation* 2:235–249 DOI 10.1007/BF00124772.

Smits AJM, De Lyon MJH, Van der Velde G, Steentjes PLM, Roelofs JGM. 1988. Distribution of three nymphaeid macrophytes (*Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and *Nymphoides peltata* (Gmel.) O. Kuntze) in relation to alkalinity and uptake of inorganic carbon. *Aquatic Botany* 32:45–62 DOI 10.1016/0304-3770(88)90087-3.

Smits AJM, Laan P, Thier RH, Van der Velde G. 1990. Root aerenchyma, oxygen leakage patterns and alcoholic fermentation ability of the roots of some nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. *Aquatic Botany* 38:3–17 DOI 10.1016/0304-3770(90)90095-3.

Smits AJM, Schmitz GHW, Van der Velde G. 1992. Calcium-dependent lamina production of *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae) Implications for distribution. *Journal of Experimental Botany* 43(254):1273–1281 DOI 10.1093/jxb/43.9.1273.

Stuckey RL. 1974. The introduction and distribution of *Nymphoides peltatum* in North America. *Bartonia* 42:14–23.

Takagawa S, Nishihiro J, Washitai I. 2005. Safe sites for the establishment of *Nymphoides peltata* seedlings for recovery the population from the soil seed bank. *Ecological Research* 20:661–667 DOI 10.1007/s11284-005-0079-0.

Tsuchiya T. 1988. Comparative studies on the morphology and leaf life span of floating and emerged leaves of *Nymphoides peltata* (Gmel.) O. Kuntze. *Aquatic Botany* 29:381–386 DOI 10.1016/0304-3770(88)90082-4.

Tsuchiya T. 1991. Leaf life span in floating leaved plants. *Vegetatio* 97:149–160 DOI 10.1007/BF00035388.

Tsuchiya T, Nohara S, Iwakuma T. 1990. Net primary production of *Nymphoides peltata* (Gmel.) O. Kuntze at Edosokitii Bay in Lake Kasumigaura, Japan. *Japanese Journal of Limnology* 51:307–312.

Twilley RR, Blanton IR, Brinson MM, Davis GJ. 1985. Biomass production and nutrient cycling in aquatic macrophyte communities of the Chowann River, North-Carolina. *Aquatic Botany* 22(3–4):231–252 DOI 10.1016/0304-3770(85)90002-6.

Urbisz A, Urbisz A. 1998. Rosliny chronione potudniowo-zachodniej czesci Wyzyny Slaskiej. *Acta Biologia Silesiana* 33(50):113–1142 (English Abstract).

Van der Velde G, Custers CPC, De Lyon MJH. 1986. The distribution of four nymphaeid species in the Netherlands in relation to selected abiotic factors. In: *Proceedings EWRS/AAB 7th Symposium on Aquatic Weeds*. 363–368, 1986.

Van der Velde G. 1980. Studies in nymphaeid-dominated systems with special emphasis on those dominated by *Nymphoides peltata* (Gmel.) O. Kuntze (Menyanthaceae). PhD Thesis Nijmegen University, 1–163.
Van der Velde G, Giesen TG, Van der Heijden L. 1979. Structure, biomass and seasonal changes in biomass of Nymphoides peltata (Gmel.) O. Kuntze (Menyanthaceae), a preliminary study. Aquatic Botany 7:279–300 DOI 10.1016/0304-3770(79)90029-9.

Van der Velde G, Van der Heijden LA. 1981. The floral biology and seed production of Nymphoides peltata (Gmel.) O. Kuntze (Menyanthaceae). Aquatic Botany 10:261–293 DOI 10.1016/0304-3770(81)90027-9.

Van der Velde G, Van der Heijden LA, Van Grunsven PAJ, Bexkens PMM. 1982. Initial decomposition of Nymphoides peltata (Gmel.) O. Kuntze (Menyanthaceae), as studied by the leaf-marking method. Hydrobiological Bulletin 16(1):51–60 DOI 10.1007/BF02255412.

Van der Voo EE, Westhoff V. 1961. An autecological study of some limnophytes in the area of the large rivers. Wentia 5:163–258 DOI 10.1111/j.1438-8677.1961.tb00008.x.

Westhoff V, Bakker PA, Van Leeuwen CG, Van der Voo EE. 1971. Wilde planten, Flora en Fauna in onze natuurgebieden. Deel 2, Het lage land. Vereniging tot Behoud van Natuurmonumenten in Nederland. 1–304.

Wu Z, Yu D. 2004. The effects of growth and biomass allocation in Nymphoides peltata (Gmel.) O. Kuntze growing in microcosm. Hydrobiologia 527:241–250 DOI 10.1023/B:HYDR.0000043187.37838.f6.

Wu Z, Yu D, Tu M, Wang J, Li Z. 2006. Competitive performance of Nymphoides peltata (Gmel.) O. Kuntze growing in microcosm. Hydrobiologia 571:41–49 DOI 10.1007/s10750-006-0215-z.

Xiao K, Yu D, Huang Y, Wang H. 2011. Plastic and non-plastic variation in clonal morphology of Nymphoides peltata along a water depth gradient in Liangzi Lake, China. Journal of Freshwater Ecology 21(3):371–378 DOI 10.1080/02705060.2006.9665012.

Yu H, Niu Y, Hu Y, Du D. 2014. Photosynthetic response of the floating-leaved macrophyte Nymphoides peltata to a temporary terrestrial habitat and its implications for ecological recovery of Lakeside zones. Knowledge and Management of Aquatic Ecosystems 412:08 DOI 10.1051/kmae/2013090.

Yu L, Yu D. 2011. Differential responses of the floating-leaved aquatic plant Nymphoides peltata to gradual versus rapid increases in water level. Aquatic Botany 94:71–76 DOI 10.1016/j.aquabot.2010.11.004.

Zhu J, Peng Z, Liu X, Deng J, Zhang Y, Hu W. 2019. Response of aquatic plants and water quality to large-scale Nymphoides peltata harvest in a shallow lake. Water 11(1):77 DOI 10.3390/w11010077.