

RESEARCH

## Non-destructive estimation of the leaf area in *Nuphar lutea* L. (Nymphaeaceae)

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### Abstract

*Nuphar lutea* (L.) Smith (Nymphaeaceae) is a widespread hydrophyte with leaves floating on the water. This highly productive plant forms a fairly large assimilating (photosynthesizing) surface. The purpose of this work was to develop a method for calculating the surface area of assimilating leaves of *N. lutea*. As a result, the formulas for calculating the surface area of a leaf (main photosynthetic organ of *N. lutea*) were obtained. The area formulas for the blade and petiole are based on the area formulas for geometrical figures and allow us to calculate the assimilating surface area of the yellow water-lily as precisely as possible. Therefore, the data obtained provide a better picture of this species productivity and will allow further to estimate the contribution of this species to the overall productivity of water bodies and watercourses

**Keywords:** Assimilating surface, photosynthesizing surface, hydrophytes, yellow water-lily

### Introduction

Plant leaves are the main photosynthetic organ. The leaf shape depends on the plant species and correlates strongly with temperature and abiotic environment. The leaf area (LA) is the main parameter associated with the assimilating surface area, photosynthesis, respiration, transpiration, specific leaf area, and productivity (Filbin & Hough 1983; Klok & van der Velde 2017; Liu M.et al. 2017). The LA of a specified plant species indicates the performance of such mechanisms as radiation interception, water, and energy exchange. LA has been proven to be of great significance in plant growth studies and has helped with the understanding of plant-environment interactions (Gong et al. 2013; Costa et al. 2016). Exact and fast determination of this parameter is of great importance

There are several methods for determining LA. Direct measurements are based either on leaf harvesting and include grid count and gravimetric analysis or use of scanning area meter (e.g. LI-3000, Licor, NE, USA). Direct measurements of LA are considered to be the most accurate, but at the same

time, they are destructive to plants, very time-consuming, and expensive (Jonckheere et al. 2004; Bréda 2008; Liu Z.et al. 2017). A portable scanning planimeter is only suitable for small plants with few leaves and not feasible for large leaves (Nyakwende et al. 1997; Rouphael et al. 2010). Moreover, these methods do not allow us to study the seasonal dynamics of leaf growth.

Indirect measurements are those methods based on observations and measurements of allometric parameters (leaf length and width) which are then used as input data for regression modeling (Blanco & Folegatti 2003; Jonckheere et al. 2004; Liu Z.et al. 2017). Such models are based on the correlation between allometric parameters of plants and their leaf areas (Bréda 2003; Jonckheere et al. 2004). These methods are non-destructive, fast and are suited for automation of all calculations (Costa et al. 2016). Mathematical modeling for dry land plants is widely applied by different investigators; thus, there are regression models for fast estimation of leaf area and weight of some broad-leaved species (Liu Z.et al. 2017). Regression models have been widely used to estimate the area and weight of leaves in a variety of crops such as maize (Birch et al. 1998), peach (Espinoza-Espinoza et al. 1998), coffee (Antunes

et al. 2008), pepper (Rojas-Lara et al. 2008), avocado (Calderón et al. 2009), papaya (Cardona et al. 2009), mango (Ghoreishi et al. 2012), rose (Fascella et al. 2013), cocoa (Salazar et al. 2018) and olive (Koubouris et al. 2018). The equations obtained in each specific case approach only for a specific species.

Indirect methods also include photography and remote sensing with subsequent image interpretation. Nowadays, special software (Igathinathane et al. 2006) and mobile applications (Gong et al. 2013; Tech et al. 2018) are being developed for leaf area measurements.

Methods described above substantially belong to dry land plants. Unfortunately, there is a lack of published works for aquatic plants. There are only fragmentary data on LA of several hydrophytic species (Brock et al. 1983; Filbin & Hough 1983; Boese et al., 2008; Sinden-Hempstead & Killingbeck 1996).

At present, there is undoubtedly a need for obtaining LA data for aquatic plants. It is particularly important when studying the productivity of macrophytes in water bodies and watercourses and overgrowing of waters. This work is aimed to obtain the formulas for calculating the leaf area of hydrophytes with floating leaves. In this work, the yellow water-lily was used as a model species.

*Nuphar lutea* (L.) Smith (Nymphaeaceae Salisb.) is a species native to temperate regions of Europe and western Asia (Tcvelev 2000). Yellow water-lily is a hydrophyte with a thick (up to 15 cm) rootstock, floating and submerged leaves, triquetrous petiole, and large blades that are wide-elliptical to oval in shape. Flowers are yellow, up to 6.5 cm wide, on a cylindrical flower spike. Fruits are green, large, and have numerous seeds (up to 400 seeds per one fruit) which are olive-green and egg-shaped (Padgett 2007).

Associations with the yellow water-lily are typical for lakes, reservoirs, oxbows, and small and medium rivers. *N. lutea* forms both monodominant communities and communities with hydrophytes (*Lemna minor* L., *Ceratophyllum demersum* L., *Nymphaea alba* L., *Potamogeton lucens* L., *Potamogeton natans* L.) and helophytes (*Sagittaria sagittifolia* L., *Butomus umbellatus* L., *Sparganium erectum* L.). The aboveground form is an important part in the cenoses of hygrophelophytes and even mesophytes but it occurs only in dry shallow waters and less often on sandbanks. Due to its high ecological valency, *N. lutea* makes a considerable contribution to the total productivity of

waterbodies and watercourses (Papchenkov 2001, Chernova 2015, Klok & van der Velde 2017).

*N. lutea* plays an important role in aquatic ecosystems-its high phytoncidal activity prevents water blooming, inhibits the development of pathogenic organisms (Dubyna 1982; Negrobov & Khmelev 1999) and fungi (Vergeer & van der Velde 1997), and has an allelopathic effect on other aquatic plants (Elakovich & Wooten 1991; Elakovich & Yang 1996; Macías et al. 2008). It is also an important food resource for animals (Heslop-Harrison 1955; Smits et al. 1989).

*N. lutea* is very important for the aquatic carbon cycle as it promotes the elimination of methane from benthic deposits (Dacey & Klug 1979). It accumulates heavy metals, such as Cu (Aulio 1980), Cd (Thompson et al. 1997), and some micronutrients (Klink 2004, 2005; Tomaszewicz 2009; Tomaszewicz & Ciecierska 2009). Studies of low-molecular-weight volatile organic compounds of aquatic macrophytes (including above-ground organs of *N. lutea*) are being conducted (Kurashov et al. 2014).

## Materials and Methods

### Plant material

The plant material for morphological studies and measurements of major parameters was collected during the growing season of 2010 (May to October) in the model rivers Ild (N 57°53'32,0" E 038°03'41") and Latka (N 58°04'24,8" E 038°07'50") which are tributaries of Rybinsk Reservoir (Fig. 1).

Cameral treatment of collected leaves included washing with tap water and separation leaf blades from petioles. In total, 1441 leaves were analyzed in terms of four parameters for leaf blades and in terms of three parameters for petioles.

### Data analysis

For leaf blades, the blade length between the point of petiole attachment and the blade apex ( $l_1$ ), total blade length ( $l_2$ ), blade width ( $w$ ), and height of the blade wing ( $h$ ) (Fig. 2) were measured. To analyze morphometric parameters, correlation and regression analyses were used.

## Results and Discussion

Based on the results of correlation analysis, the type of



Figure 1. Model rivers Ild (left) and Latka (right).

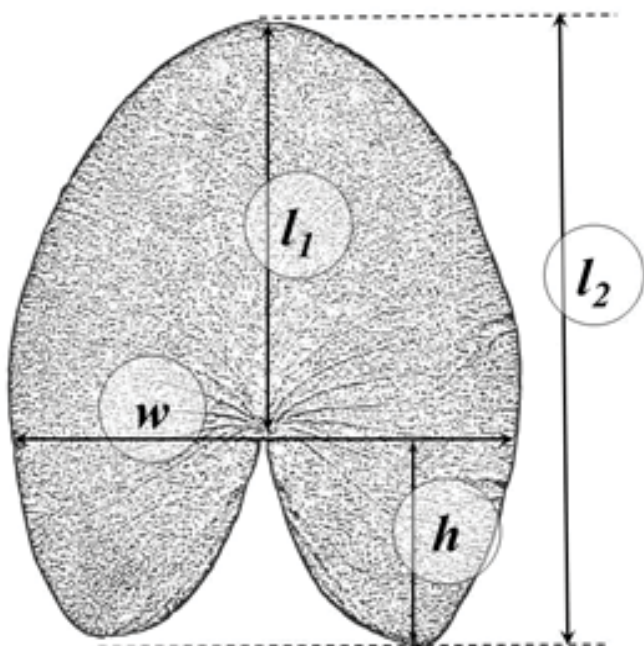


Figure 2. Major measurements parameters of leaf blade of *N. lutea*.

leaf blade growth (isometric or non-isometric), as well as dependencies between  $l_1$  and  $l_2$ , between  $l_1$  and  $w$  and between  $l_2$  and  $w$  were determined. In the present research, a linear correlation coefficient ( $r$ ) which shows the strength of the relationship and how changes of one parameter are due to the changes of another one was used (Tab. 1). Calculated paired correlation coefficient are considered significant at significance level  $\alpha=0.05$ .

Such high correlation coefficients demonstrate a strong relationship between parameters of interest. On this basis, it was concluded that the growth is isometric and the leaf shape changes insignificantly in the growth process (Tab. 1).

Earlier it was determined that the height of the blade wing is slightly more than half of the blade length between the point of petiole attachment and the blade apex. The  $h/l_1$  is constant and is equal to 3/5 (Chernova 2013).

The blades of *N. lutea* are oval to heart-shaped and conditionally consist of three parabolic segments ABC, AEO and CDO (last two-lateral wings-are fairly of the same area) (Fig. 3).

Therefore, the blade area ( $S_B$ ) is a sum of the areas of three parabolic segments (1):

$$S_B = S_{ABC} + S_{AEO} + S_{CDO} = S_{ABC} + 2 \cdot S_{AEO} \quad (1)$$

The area of a parabolic segment is two-thirds multiplied by the product of the length of the line segment between the points of intersection and the distance from the horizontal line to the parabola vertex. Then the formula (1) will be as follows (2):

$$S_B = \frac{2}{3} \cdot AC \cdot BO + 2 \cdot \frac{2}{3} \cdot AO \cdot KE = \frac{2}{3} \cdot l_1 \cdot w + \frac{2}{3} \cdot h \cdot w \quad (2)$$

If express  $h$  in formula (2) in terms of  $l_1$  (3):

$$S_B = \frac{2}{3} \cdot l_1 \cdot w + \frac{2}{3} \cdot \frac{3}{5} \cdot l_1 \cdot w = \frac{16}{15} \cdot l_1 \cdot w \approx l_1 \cdot w \quad (3)$$

Therefore, the blade area is approximately equivalent to the blade length (between the point of petiole attachment and the blade apex) multiplied by the blade width (measured through the point of petiole attachment to the blade). To determine the assimilating surface area, it is necessary to multiply the blade area by 2.

Table 1. Linear correlation coefficients ( $r$ ) for parameters,  $l_1$ ,  $l_2$  and  $w$ .

Parameters	Ild river	Latka river
$l_1$ and $l_2$	0.97	0.96
$l_1$ and $w$	0.94	0.86
$l_2$ and $w$	0.94	0.86

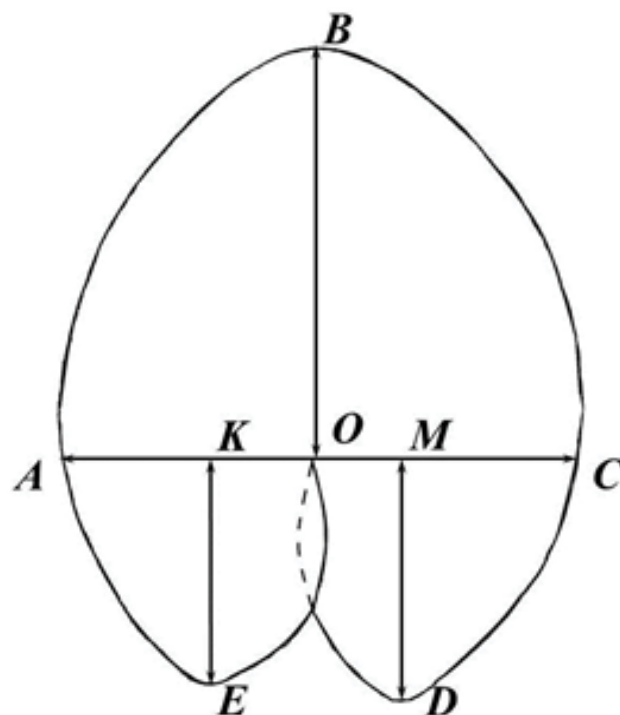


Figure 3. Schematic illustration of the leaf blade of *N. lutea*, conditionally divided into parabolic segments; O-point of petiole attachment to the blade, BO ( $l_1$ )-blade length between the point of petiole attachment and the blade apex; AC ( $w$ )-blade width measured through the point of petiole attachment to the blade, KE and MD- heights ( $h$ ) of the blade wings.

The surface area of the petiole of *N. lutea* consists of three approximately equal geometric figures (trapezoids) (4)

$$S_p = 3 \cdot \frac{d_p + D_p}{2} \cdot p \quad (4)$$

where  $S_p$  - petiole surface area,  $d_p$  -average size of the petiole in the point of its attachment to the blade,  $D_p$  -average size of the petiole at the bottom,  $l_p$  -petiole length.

To obtain comparable data, the author of the present article proposes considering the petiole size in the point of its attachment to the blade and the petiole size at the bottom as constant values (for example, 0.7 cm and 1.7 cm). Then, to calculate the photosynthesizing surface area of the leaf, the petiole length only is needed.

As it was determined, the growth type of leaf blades of *N. lutea* is isometric (like of many other plants). In the growth process, *N. lutea* leaves change in size, but not in shape. Based on this fact, the formula for calculating such important parameter as LA was obtained. The proposed model is an indirect measurement of LA and has a measuring error.

When calculating the leaf area of *N. lutea*, it is necessary

to realize that area formulas for geometrical figures are used. Therefore, there are some deviations from the actual values from the very beginning. However, this is a method error and this error is always the same. The formulas provided are suitable for calculating the photosynthesizing surface areas of corrugated submerged and floating leaves but not applicable in case of new rolled-up leaves. As a rule, the amount of such leaves is quite small (Chernova 2015) and their assimilating surface areas may be disregarded in case of field researches of large water bodies.

Previously, the regression method was applied to different fruit and vegetable crops (Montero et al. 2000; Buttaro et al. 2015; Blanco & Folegatti 2003; De Swart et al. 2004; Bakhshandeh et al. 2011; Silva et al. 2008; Zhang & Liu 2010; De Maria 2018; Tsialtas & Maslaris 2007; Firouzabadi et al. 2015) as well as to some woody plants (Cristofori et al. 2007; Koubouris et al. 2018). Authors of these studies carried out the regression analysis of leaf length, width, and area. The most of obtained regression equations are linear with general form  $y=ax+b$ . This is correct mathematically, but incorrect biologically-at zero leaf length and zero leaf width, leaf area is not equal to zero but is equal to  $b$ . probably, the authors would specify the leaf length and width ranges in which obtained formulas are correct. Nevertheless, the contribution to indirect measurements of LA made by these researchers is a basis for further studies.

The studies of LA of aquatic plants presented are some of the first. The formulas that were obtained can be applied in the field and are not destructive for phytocenoses allowing us to carry out seasonal studies of growth dynamics using the same plants and within the same sites. It is possible to determine the LA using two parameters only-the length and the width of the leaf blade.

The formulas obtained can be used to calculate the assimilating surface area of *N. lutea*. To do that it is necessary to know the number of leaves per unit area and the main morphometric parameters of leaves: the blade length measured between the point of petiole attachment and the blade apex, blade width measured through the point of petiole attachment to the blade, and petiole length.

If the number and size of leaves used for LA determination are large, the method can be time-consuming (Costa et al. 2016). However, such an approach is fast, reliable, and cost-effective.

## Conclusion

The proposed indirect measurement of LA is a simple method that can be repeated by any researcher. This method allows us to obtain reliable LA values, identify the dynamics of plant growth and development. The only equipment required for this method is a simple ruler.

It may be considered, that the formulas obtained can be adapted to other Nymphaeaceae species as well as to other hydrophytes with floating leaves.

The author plans to continue studies to find formulas for indirect measurement of LA of other aquatic plants. Precise measurement of leaf area and leaf weight is necessary for

a greater understanding of plant growth processes. In the future, the data obtained can form the basis for the modeling of aquatic ecosystems productivity.

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