Lemna minor cultivation in the climatic conditions of Saint Petersburg

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Abstract. Cultivation of duckweed Lemna minor in conditions of St.Petersburg was carried out. Two cultivation variants were studied: 1- in a natural reservoir of Petergof; 2 - in an artificial reservoir at the yard of Peter the Great St. Petersburg Polytechnic University (Russia) during a period from May to July 2017. The following cultivation conditions were determined: lighting, temperature. The population growth intensity was estimated by increase of plant cover area of the pond. The most favourable conditions appeared to be at natural pond in Petergof. Daylight features, lightning intensity of the Leningrad Region are less favourable than that for south Russian regions, so biomass production rate is drastically lower. However, the climatic characteristics (moderate climate, transient from continental to marine) allows increasing L. minor cultivation period: from May to November inclusive. We carried out atomic-physiological investigation of fronds in summer and autumn, as well as epidermis and chlorenchyma cell analysis, which allow one to characterize photosynthesys system of the plants.

1. Introduction
Duckweed is quickly vegetatively divided in summer in fresh ponds with ditch-water, and form a green carpet on pond whole surface, it duplicates its' mass in 6 days [1]. L. minor is a typical species at middle-Russian area. It may produce 200 tons of biomass from a hectare during a season at multiple gathering. At Uzbekistan 276 tons were gathered from 1 hectare during 8 months [2].

Literature analysis have shown that the major directions of L. minor cultivation investigation are defined by further usage of its biomass:
- usage of L. minor in ecotoxicological investigations and tests [3–13];
- directed cultivation for obtaining valuable components in Lemna minor tissues. Duckweeds accumulate in fronds flavonoids, diterpenoids, tannins, B1, B2 and C vitamins, steroids, unsaturated fatty acids, including Omega-3, giving rise to its' medicinal properties and its' wide application in medicine [14].
- cultivation of L. minor with further usage as a lifestock feed (duckweed has a protein content up to 45% in dry weight) [15,16];
- L. minor cultivation for biomass production as a source for bioethanol and biogas obtaining. These technological cycles are based on enzymatic hydrolysis of starch, which is contained in duckweed [15,16];
- duckweeds are of interest as a potential biofactory for fused proteins production, such as vaccine proteins, serum albumin, hemoglobin, collagen; moreover, a genetic transformation of duckweeds might be made in order to enhance its stability to pollutants, for example, heavy metals [17,18].

2. Materials and methods

2.1 Determination of intensity of L. minor population growth
The evaluation of L. minor growth intensity in natural conditions was carried out in accordance with pond surface incrustation area. For this purpose, square frames were cut from expanded polyethylene: the inner enclosure is 20×20 cm (400 cm$^2$), the external dimensions are 26×26 cm (676 cm$^2$), Figure 1.

In order to achieve high cultivation efficiency, one should use pure culture. Storage of L. minor stock culture is performed at 4-10°C, using MS-environment, pH is 5.7 [19].

Duckweed is gathered using fork or shovel devices with mesh-tip.

![Figure 1. A scheme of enclosing frame.](image)
The frame is placed at the pond surface. It should not sink. 10 plants are placed in the inner part of the frame. At intense population growth the frame is photo-recordered once a day (Figure 2).

![Figure 2. Photographs of L. minor population cultivated in enclosing frame.](image)

15 Days
34 Days
The photographs are contrasted using Levenhuk ToupView software. The software instruments are used for total area estimation \( S_{\text{tot}} \) in pixels (px), which is enclosed by the inner frame. Also, it is used for determination of total area, which is occupied by plants \( S_p \) in pixels (px). Pond incrustation ratio \( W, \% \) is determined according to the formula (1):

\[
W = \frac{S_p}{S_{\text{tot}}} \times 100, \% \tag{1}
\]

Pond incrustation ratio is determined minimum in three frames and, using methods of statistical data processing, the average value of pond incrustation ratio and confidence range \( (x_{av} \pm S_x) \) are determined. Using the obtained data the cultivation curve is plotted: relation between pond surface incrustation area in enclosing frame (\%) and cultivation time (days).

1.2 Morphological-anatomical investigation of L. minor

Morphological-anatomical study of L. minor are carried out by microscopy of intravital cells (colored and non-colored). Magnification power is 640 times. Epidermis investigation is carried out without its separation from parenchyma. In order to investigate chlorenchyma and chloroplast cells, we cut fronds by a thin blade at low magnification (\( \times 7 \)).

For describing shape and dimensions of epidermis and parenchyma, and chloroplast dimensions, we made microphotographs using digital camera IS-500 and "Microanalysis FOTO" software for 10 fields of vision. Microphotographs analysis (colored and non-colored photographs) was performed using Levenguk software, which allows one to adjust contrast and brightness and additionally magnify the image.

In order to characterize major epidermis cells we determined its' area and perimeter, in order to characterize the degree of tortuosity of anticlinal walls, we determined the coefficient (2):

\[
K_d = \frac{P}{S} \times 100, [\mu m^{-1}] \tag{2}
\]

where \( P \) is anticlinal wall projection perimeter of major epidermis cells, \( \mu m \);

where \( S \) is anticlinal wall projection area of major epidermis cells, \( \mu m^2 \);

The diagnostic indicator which characterize metabolism (photosynthesis) is stomata condition: open stomata is a necessary condition for high metabolism intensity; the degree of stomata openness can be derived from formula (3):

\[
\text{SOD} = \frac{S_2}{S_1} \times 100, [\%] \tag{3}
\]

where \( \text{SOD} \) is stomata openness degree (\%);

\( S_1 \) is stomata projection area, \( \mu m^2 \);

\( S_2 \) is stomata cleft area, \( \mu m^2 \);

Stomata projection area, and stomata cleft area can be determined using microphotographs analysing software, for example, Levenguk software. Chlorenchyma cell and chloroplast dimensions were determined according to its' projection area at frond cuts. Projection area was determined from the photographs by Levenguk software instruments.

3. Results and discussion

3.1 L. minor cultivation conditions

The experiment was conducted in Saint Petersburg (Russia) during the period from May, 29 to July, 3, Figure 3: variant 1 - in natural pond in southern Saint Petersburg (Petergof); variant 2 - in an artificial pond in northern Saint Petersburg (Saint Petersburg State Polytechnical University named after Peter the Great, SPBPU, Novorossiyskaya Str., 48) Colonies consisting of 2-4 green plates are taken from stock culture and moved into the pond for cultivation.
In natural conditions of Leningrad region *L. minor* cultivation may be performed from April to November inclusive. The most efficient constant temperatures are 19-26 °C. The plants withstand short-term frosts. However, at long-term water temperature decrease below 3 °C we observed slowdown of plant growth rate. Day average temperatures of *Lemna minor* cultivation places are reliably equal, Figure 4.

Possible pH ranges in which *L. minor* is cultivated are 3.5-8.5 [20], at this optimal pH values are 6.3-8.0.

Cultivation took place at natural light. Lightning intensity in day time is the following: South of Saint Petersburg 405-1570 lx; North of Saint Petersburg 1350-4000 lx. Optimum range was from 405 to 9000 lx (3000 lx in average).
3.2 *L. minor* cultivation curves

The most intensive growth of *L. minor* was observed in a natural reservoir (in pond in Petergof, Figure 5) in comparison with population in artificial pond (SPBPU). The time of *L. minor* population duplication is 20 days.

![Figure 5. L. minor population growth at Saint Petersburg conditions (natural reservoir in Petergof, South of Saint Petersburg; artificial reservoir in SPBPU, North of Saint Petersburg).](image)

Minimum sum of daily-average temperatures, which is necessary for population growth up to 100% of pond area in natural conditions is 406 °C at day lightning intensity 405-1570 lx.

3.3 *Lemna minor* plant characteristics at cultivation in Saint Petersburg conditions

Dry substance content in plant tissues (was determined by a commonly used method [21]) was 14.20±0.34%, water content was 85.79±0.34%.

Morphological characteristics were determined for *L. minor* population intensive-growth phase for natural conditions of Saint Petersburg (Table 1).

The features of fronds ultrastructure allow one to characterize the condition of photosynthesysing apparatus [21,22], which is directly relevant to accumulation of organic substances in tissues and biomass growth.

**Table 1.** Morphological characteristics of *L. minor* population in intensive-growth phase for natural conditions of Saint Petersburg, July 2017 (n=20).

| Rootlet length, cm | Rootlet number | Diameter of frond rosette, cm | Frond number |
|--------------------|----------------|-----------------------------|--------------|
| 1.64±0.13          | 7.20±0.68      | 0.89±0.06                   | 3.55±0.39    |

The indicators of intensive metabolism are the following:

- large open stomata, its high concentration (which characterizes intensive gaseous exchange), Figure 6a.
- large chlorenchyma cells (accumulation of storage polysaccharides, intensive photosynthesis), Figure 6b.
- large chloroplasts (which characterize photosynthesis intensity), Figure 6b.
Figure 6. Microscopy image of L. minor fronds: a - epidermis (1 - stoma); b - photosynthesising parenchyma cells (2 - chloroplasts).

We have carried out an anatomical investigation of *Lemna minor* fronds, which was cultivated in natural conditions of Saint Petersburg in July and October 2017. The population growth in July is intensive, while it slows down in October.

Chlorenchyma cell projection area in the phase of intensive growth is reliably equal to the data presented in literature [22], in autumn chlorenchyma cells increase for four times (Table 2), which is driven by intensive cell vacuolization (small vacuoles merge in a whole) and accumulation of reserved nutrients.

Table 2. Anatomical characteristics of *L. minor* fronds in natural St. Petersburg conditions, July-October 2017, n=50.

| Cultivation period | S of chlorenchyma cell under epidermis | S of stoma projection | SOD, % | S of basic epidermis cells | Ka |
|--------------------|---------------------------------------|-----------------------|--------|---------------------------|----|
| summer             | 153.87±14.89                           | 219.32±11.49          | 11.29±1.23 | 388.86±66.84           | 1.73±0.18 |
| autumn             | 625.42±33.62                           | 252.53±15.36          | 18.38±1.26 | 316.46±54.62          | 2.15±0.079 |

Note: SOD is stomata openness degree; Ka is coefficient of tortuosity of anticlinal walls of major epidermis cells.

Chloroplast dimensions in plant frond chlorenchyma at population intensive growth is somewhat less than literature data (Ekaterinburg, Russia) [22]. This might be connected with lower Sun intensity in St.Petersburg. However, at toxical phenolic impact we observed more intensive decrease of chloroplast dimensions.

Epidermis cells are more sensitive to changes of environmental factors, so its' characteristics are the most informative. Stomata of the upper epidermis are rather large and have a tendency to growth in autumn (Table 2).

Open stomata is a necessary condition of metabolism high intensity [23]. In autumn stomata dimensions and the degree of its' openness increase (Table 2), which states for high intensity of metabolic processes and accumulation of storage metabolites in tissues. This may be used for prediction of storage substances content in *L. minor* biomass.

By autumn major epidermis cells are formed smaller and with more tortuous anticlinal walls. Cell wall of young cells is rather elastic, so in such conditions cells can grow in size. At ageing or negative factors impact, induration of cell wall takes place, the cell stops growing, but non-uniform induration of cell tissue various regions results in edge tortuosity increase. Consequently, in autumn the major cells
have stronger cell wall, which is driven by impact of unfavorable factors (low temperatures, short-term frosts) on *Lemna minor* fronds. These plants become less able to reproduction.

### 4. Conclusions

1. *Lemna minor* growth conditions appeared to be more favorable at natural pond of Peterhof, rather than at artificial reservoir at the yard of Peter the Great St. Petersburg Polytechnic University.

2. Daylight features, lightning intensity of the Leningrad Region are less favourable than that for south Russian regions, so biomass production rate is drastically lower. However, the climatic characteristics (moderate climate, transient from continental to marine) allows increasing *L. minor* cultivation period: from May to November inclusive.

3. Anatomic-morphological characteristics of *L. minor* fronds are in good agreement with literature data (obtained for south regions of Russia). The most informative characteristics are the following: dimensions and openness degree of stomata, chlorenchyma cell dimensions, chloroplast dimensions. In autumn we observe a drastical increase of chlorenchyma cells, which is driven by vacuolizations of this cells and accumulation of nutrients. SOD remains high both at summer and at autumn, which is a necessary condition for intensive metabolic processes, including photosynthesis.

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