Ecological differentiation of *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleiden populations

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(Received: September 9, 1976)

Abstract

The subject of the present study are 19 natural, ecological populations of *Lemna minor* and *Spirodela polyrrhiza* coming from sixteen habitats in Lower Silesia. The study showed the essential differences between ecological populations of both species, conditioned on chemistry (pH, level N, P, K, Ca, Mg and Fe) and the natural water radioactivity. It was shown experimentally that these populations, in nature, establish a set of ecotypes or ecophenè variability. Ecotypic differentiation of populations of *Lemna minor* and *Spirodela polyrrhiza* is based upon individual features and population characteristics such as: shape and size of plants, plant frequency of two, three, and four fronds, the ratio of right- to left-handed plants, and the dynamics of growth and biomass production.

INTRODUCTION

Among the current tasks of aqueous plant ecology outlined in the programme of the International Hydrobiological Decade (IHD 1965—1974) which have not been fully implemented so far, is the investigation of the ecological constitution of macrohydrophytes (Mitchell 1974). The possibility of utilization of *Lemna minor* and *Spirodela polyrrhiza* as bioindicators of chemical and radioactive pollution of surface waters (Rzewuska and Wernikowska-Ukleja 1972; Włodek 1966) and in fodder production (Taubaev et al. 1972, Narmukhamedov and Vassigov 1972) requires the knowledge of ecological differentiation of natural populations of these species. The study undertaken is aimed to solve the following problems:

1. What are the ecological differences between *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleiden populations, with regard to individual and group characteristics?

2. Will the possible differences between individual characteristics of natural populations be also present in sterile cultures, and are they dependent on edaphic factors?
3. Do ecological populations coming from the radioactive waters of the Sudeten radioactive anomaly area differ from the remaining populations?

The assumptions of the present study are justified by the occurrence of *Lemna minor* and *Spirodela polyrrhiza* over a wide range of ecological conditions and over a great geographical area (Hegelmaier 1868; Landolt 1957; Daubs 1965). Moreover, ecotypal differentiation of various plant species, resulting from natural steady low-level radiation in radioactive anomaly areas is already known (McCormick and Platt 1963; Osburn 1961, Sarosiek and Wożakowska-Natkaniec 1967).

METHODOLOGY

Choice of habitats and populations

In selecting the natural populations of *Lemna minor* and *Spirodela polyrrhiza*, the criterion of mass appearance of these plant species was adopted, since only in such cases was it possible to take any number of samples from pure populations. 14 ecological* populations of *Lemna minor* and 5 populations of *Spirodela polyrrhiza* were selected. Among the water bodies selected, referred to hereafter as habitats of the studied populations, only 3 are common for populations of both species. Populations of *Lemna minor* come from habitats listed in Table 1 (items 1—14). Populations of *Spirodela polyrrhiza* come from the following habitats:

1. A pond in the Wrocław Botanical Garden (item 15, Table 1),
2. A pond in Szczodre near Wrocław (item 16, Table 1),
3. A pond in Jelcz (item 11, Table 1),
4. A pond in Paulinum near Jelenia Góra (item 9, Table 1),
5. A pond in Stanisławów (item 8, Table 1).

With the exception of the pond in Jelcz, where the populations of studied species are components of *Salvinio-Spirodeletum polyrrhizae* Slavnic 1956 association, in all habitats they are a part of *Lemna minor*-*Lemna trisulca* R. Tx 1955 community (Scamoni 1967).

Selection and determination of basic characteristics of habitats (water)

According to Hutchinson (1957), as well as Just and Hermanowicz (1964), the following indicators were adopted in evaluation of

* Term introduced by Naumov in 1956.

water which is the habitat of the development of pleustonet type hydrophytes (Starmach 1963): pH factor, total nitrogen content, content of phosphorus, potassium, calcium, magnesium and iron, as well as the content of dry residue and residue after ignition of water. In all habitats i.e., in 16 water bodies, samples were taken for analysis three times during the vegetative period, following the methodological recommendations of Just and Hermanowicz (1964). The chemical analyses of water were done as follows: the pH value was measured by potentiometer, nitrogen by the Kjeldhal method (Just and Hermanowicz 1964), phosphorus by the Fiske-Subbarow method (Mejbaum-Katzenellenbogen 1965), potassium in a flame photometer using the Johanson-Ulrich method (Nowosielski 1968), calcium and magnesium by the complex Barrous and Simpson method (1962), iron by the colorimetric method with alpha-phenanthroline according to Piper (1957), and the dry residue and residue after ignition, according to Just and Hermanowicz (1964).

Selecting plant samples

Considering the nature of relatively continuous area synusia of *Lemna minor* and *Spirodela polyrrhiza* plants among other macrohydrophytes, adopting the criteria of distribution of sample points over a flat surface, according to Zubrzycka (1960), the six samples each of the 19 ecological populations were taken randomly. The samples were taken at one time, in the middle of the vegetative periods of 1971, 72 and 73.

Selection of properties characteristic of natural populations of *Lemna minor* and *Spirodela polyrrhiza*

The following properties were chosen as characteristic for ecological populations of *Lemna minor* and *Spirodela polyrrhiza*: 1) Shape index of the parent frond, i.e., the ratio of its length to its width. 2) The top angle of the parent frond. 3) The area of two, three, and four-frond plants and the average area of frond in mm². 4) The ratio of number of right-handed plants to lefthanded (R : L). 5) The frequency of occurrence of two-, three-, and four-frond plants, in %. 6) The frequency of occurrence of anomalous plants, in %.

Properties 1 (length: width) and 2 (top angle) characterize the shape of fronds. From direct observation, the relatively regularly oblong fronds of *Lemna minor* and the asymmetric frond of *Spirodela polyrrhiza* do not vary significantly within populations. In order to define the shape of

frond within specific populations, I have employed the shape index, i.e., the ratio of length to width, as did Czopek (1959). The index does not express, however, the degree of pointedness of the top part of the frond, a quality which, in direct observation appeared to differ between populations. The pointedness of frond ending is customarily expressed by the so-called top angle of the frond (property 2). Plant size was expressed in mm². However, the area of two-, three-, and four-frond plants were measured separately, because of the known relationship between the great fronds of a plant and the number of sister fronds (Kasimov, 1968). The measurements were made by the photographic-planimetric method, used by Gorham (1941) and Czopek (1959) in evaluating the growth of *Lemna minor* and *Spirodela polyrrhiza* in cultures. The photographs, in suitable enlargements were also used for measurements of properties 1 and 2. Planimetric error did not exceed $\pm 0.2\%$.

In nature, *Lemna minor* and *Spirodela polyrrhiza* plants occur in two forms, differing among themselves in the steady developmental sequence of left-, and right-handed fronds (Hegelmaier 1868, Kandler 1955, Landolt 1957). With vegetative propagation, the quality was preserved in all descended plants (Kasimov 1969).

In our case, left- and right-handed of plants was assessed on the basis of appearance of actual configuration of descendant fronds in relation to the parent frond, i.e., a plant currently having a larger right descendant frond (regardless of the fact whether it is the first one growing out the parent frond), is recognized as right-handed; while a plant having a larger left descendant frond is recognized as left-handed. That property was determined from direct observation of plants (property 4).

Each of the 19 populations was represented in analysis, in the case of properties 1, 2 and 3, by 300 plants from 6 random samples of 50 plants each. In the analysis of the R:L ratio (property 4), each of the 19 populations was represented by 1200 individual plants all of them three-frond, randomly taken in 6 samples. The percentage of two-, three-, and four-frond plants in a population (property 5) was determined on the basis of an analysis of 1000 plants taken in 5 random samples. The percentage of anomalous plants (property 6) was determined on the basis of the same samples as property 5.

Experiments with cultures *in vitro*

Plants coming from natural populations were sterilized in 0.1% solution of sublimate, and 50% solution of alcohol, by submerging them for 30 to 60 seconds, depending on the species, according to Kandler (1955) and Czopek (1963). The plants were then transferred to Erlenmayer flasks containing sterile culture medium, proceeding all the time

under aseptic conditions, in inoculation chamber. After several days of growth in a light thermostat, the dying parent fronds gave rise to descendant fronds initiating a sterile culture. Plants obtained in this manner from each population were used in further experiments.

The plants were raised on liquid Hoagland medium, modified by Czerwinski, of the following composition: $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ — 0.252 g, KCl — 0.447 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ — 0.493 g, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ — 1.653 g, iron citrate — 0.005 g (in 1 l of distilled H_2O). To each liter of medium, 1 ml of microelements was added in the Gorham set (Landolt 1957), as well as 1% of saccharose. The pH level of medium was set at 5.9. The medium was sterilized in an autoclave for 45 min., at 0.5 atm. The experiments were carried out under stable light and temperature conditions: 1200 lux continuous illumination, temperature of $26^\circ \pm 1^\circ \text{C}$. Plants were placed in Erlenmayer flasks of 100 ml capacity, containing 50 ml of medium. The experiments lasted 14 days.

Methodological assumption: will plants of both species, coming from different natural populations, preserve their characteristics in cultures *in vitro*? The experiment involves 14 populations of *Lemna minor* and 5 populations of *Spirodela polyrrhiza*, designated by names and numbers of the ecological populations from which they were taken. The experiment was carried out three times under the same conditions, in 6 repeats. A repeat consisted of one three-frond plant, inoculated on medium in one flask. Experimental plants came from a two-week culture, counting from the moment of sterilization. 14 days after the start of the experiment, the number of fronds was checked (property 6), dry mass was designated (property 8), (dry mass value was calculated for 10 issuing fronds). Plants were then photographed, and on the basis of the photographs the same properties were determined which were considered in characterizing the natural populations (properties 1—5). Moreover, the multiplication rate of plants (V) was calculated in this experiment (property 7), according to the Landolt formula (1957):

$$V = 0.4343 \lg \frac{N_t}{N_0} \cdot \frac{1}{t} \cdot 1000,$$

where: N_0 — initial number of fronds, N_t — final number of fronds following a given period of time t , expressed in days.

Statistical-mathematical methods

The following statistical methods were employed in obtaining empirical data: analysis of variance with two and three factors, two-variable correlation, and multiple correlation.

The variability of habitats and populations in relation to selected characteristics was investigated by the method analysis of variance. The error of mean, 1/2 confidence interval and last significant difference were determined by calculating total variation, various sources of variation, caused by the factors under consideration, their interaction and residual. The significant difference was determined by the F-Snedecor test, with the probability level of 0.05 (Scheffe 1959).

In biologically justified cases, the relationships between characteristics of different habitats, between characteristics of different plant populations, and between habitat characteristics and population characteristics were determined. Correlation and regression coefficients were determined by the smallest square method, according to the algorithm described by Beale, Kendall and Manna (1967). The significance of the correlation coefficients was checked by the F Snedecor test, and that of the regression coefficients, by the T Student test (the method of calculating multiple correlation coefficients and testing of significance is given by Rao 1965).

RESULTS

Habitat characteristics

1. Habitats of *Lemna minor*

The basic characteristics of habitats (water) of the studied *Lemna minor* populations are given in Table 1. Analysis of variance has shown significant differences between the 16 analyzed habitats.

The pH factor of the habitats shows wide variation, within the range of 6.6 to 9.9. Only two of the habitats were characterized by a slight acidity, the remaining ones were weakly alkaline or alkaline. The highest pH factor was observed in the waters of the Proszówka (7) and Złotniki (14) habitats. The lowest pH was observed in the water of the Radech (1) and Wojszyce (13) habitats.

A significant positive correlation was observed between the pH level of a habitat and its phosphorus content. Moreover, there was significant correlation between the pH value of a habitat and its joint magnesium and phosphorus content (Table 4).

The studied habitats exhibit a wide variety with respect to the nitrogen content of water (from 2.03 to 19.90 mg in l). The greatest nitrogen content was observed in the Proszówka habitat (7), the lowest in Radech (1). Relatively high nitrogen content was observed in habitats 10, 11 and 12 (ca. 12 mg in l of water). Low nitrogen content was characteristic of habitats 3, 5 and 6.

The phosphorus content of the studied habitats varies within the range of 0.18 to 2.70 mg/l of water. The Proszówka (7) habitat was distinguished by the highest phosphorus content, and Jelcz (11) by the lowest. Habitats 14 and 9 were also characterized by a relatively high phosphorus content, while habitats 1, 2, 3, 6, 10 and 13 — by relatively low phosphorus content.

A significant positive correlation was observed between the phosphorus and potassium contents of habitats (Table 4).

The potassium content of the studied habitats falls within the range of 0.84—66.92 mg/l of water. The highest potassium level was observed in the Proszówka (7) habitat, the lowest in Jajkowo (3). A rather high level of potassium was also observed in Złotniki (14). Fairly low potassium levels were observed in habitats 1, 2, 6 and 13.

The habitats exhibit a high degree of variance with respect to the calcium content (from 18.04 to 101.37 mg/l of water). The Proszówka habitat (7) is characterized by the highest calcium content, while the Radech habitat (1) — by the lowest. High calcium levels were observed in Widawka (12), Wojszyce (13) and Lipa Piotrkowska (4), while relatively low levels were observed in habitats 3, 8 and 9.

A significant positive correlation was observed between the calcium content of studied habitats and their nitrogen content. Moreover, significant correlation was found between the calcium content of habitats and the combined nitrogen and magnesium content (Table 4).

The magnesium content of the studied habitats falls within the range of 4.64 to 43.78 mg/l of water. The highest magnesium content was observed in Wojszyce (13) habitat, also high was the level at Proszówka (7), though significantly lower than the previous one. The lowest magnesium levels were observed at Jajkowo (3). Relatively high magnesium levels were observed at Radoniowo (6) and Widawka (12).

A significant positive correlation was stated between the magnesium content of studied habitats and their calcium content (Table 4).

In the studied habitats, iron occurs within the value range of 0.12 to 3.24 mg/l of water. The highest iron content was stated in the Radoniowo (6) habitat, the lowest in the Jajkowo (3) habitat. A relatively high iron content was noted in habitats 8, 7 and 14, which differed significantly with respect to this characteristic from the Radoniowo (6) habitat, as well as from the remaining habitats. Habitats 1 and 4 were characterized by low iron levels.

Dry residue in water differs among the studied habitats within the range of 167.0 to 745.0 mg/l. The highest level of dry residue was observed in the water of the Proszówka (7) habitat, the lowest in Jajkowo (3) and Radech (1). Residue after ignition of water vary within the range of 87.5 to 620.0 mg/l. Differences among habitats with respect to this cha-

racteristic are analogous to the differences with respect to the differences in dry residue in water.

In addition to habitat characteristics presented in Table 1, the Radoń habitats (5 and 6) differ from the remaining habitats by a heightened, i.e., higher than the background *, natural radioactivity. Those are radon waters. The average radioactivity of the studied waters amounts to around 20 μCi and is conditioned by the presence of radon and the products of its decay (S a r o s i e k 1972). The concentration of radon in the studied waters varied over the period of one year from 50 to 2000 emanations ** (K a c z m a r e k and L u c z e j k o 1961). Uranium concentration did not exceed the value of 5×10^{-6} g/l.

Considering the differentiation of the studied *Lemna minor* habitats, with respect to all of the analyzed characteristics (Table 1) the Proszówka (7) habitat stands out most clearly among the 14 habitats. It is characterized by the highest levels of nitrogen, phosphorus, potassium, calcium, a high level of magnesium, and the highest dry residue and residue after ignition. Diametrically opposed to this habitat are the habitats in Radeck (1) and Jajkowo (3). They are characterized by lowest levels of macroelements; the Radeck habitat is also distinguished by the lowest pH value. Also distinct was the Wojszyce (13) habitat, with its low pH, low potassium and phosphorus content, the highest magnesium content, and a high level of calcium.

2. Habitats of *Spirodela polyrrhiza*

The basic characteristics of habitats of the studied ecological populations of *Spirodela polyrrhiza* are given in Table 1. The variation analysis carried out has shown significant differences among the habitats.

The studied populations occur in nature within the pH range of 7.7 to 8.5. The lowest pH factor was observed at Jelcz (11) habitat, the highest at Paulinum (9) and at Szczodre (16).

The nitrogen content of the studied habitats differs within the range of 3.6 to 12.5 mg/l of water. The highest nitrogen content was stated at Jelcz (11), while the lowest at Szczodre (16). A low nitrogen content was also observed in the Wrocław (15) habitat.

The phosphorus content of *Spirodela polyrrhiza* habitats falls within the range of 0.18 to 1.2 mg/l of water. The richest in phosphorus were habitats in Paulinum (9) and Szczodre (16), the poorest in phosphorus were habitats in Jelcz (11) and Wrocław (15).

With regard to the potassium content, the studied habitats show a lar-

* background = 10 to 15 μR

** 1 μCi = emanations

Table 1
Characteristics of habitats of populations of *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleiden

Habitats of populations of <i>Lemna minor</i> and <i>Spirodela polyrrhiza</i>	pH	Contents in mg/l of water						Dray residue	Residue after ignition
		N	P	K	Ca	Mg	Fe		
1. Radez — pond I	6.6	2.0	0.19	4.0	18.0	20.2	0.29	204.7	132.3
2. Radez — pond II	7.6	6.9	0.36	5.8	39.3	11.3	0.88	286.0	180.0
3. Jajkowo — pond	7.6	3.1	0.22	0.8	32.4	4.6	0.12	167.4	87.5
4. Lipa Piotrkowska — pond	7.9	10.4	0.58	27.6	52.4	13.9	0.60	467.5	306.7
5. Radoniów — pond I	8.3	3.4	0.86	25.1	46.0	14.0	1.10	354.7	264.3
6. Radoniów — pond II	7.8	2.9	0.36	4.5	41.4	23.7	3.24	262.2	132.3
7. Proszówka — pond	9.0	19.9	2.70	66.9	101.4	36.1	1.81	745.4	620.0
8. Stanisów — pond	8.1	8.4	0.82	21.7	26.3	12.3	1.04	280.6	211.0
9. Paulinum near Jelenia Góra — pond	8.5	8.1	1.20	15.9	33.5	16.1	1.64	385.0	247.8
10. Jelenia Góra — pond	7.5	11.8	0.37	12.1	39.3	9.3	0.75	227.8	190.9
11. Jelcz — pond	7.7	12.5	0.18	9.4	46.4	10.4	1.25	461.7	391.3
12. Widawka by Wrocław — river	8.5	12.8	0.68	9.2	66.5	22.7	0.84	242.5	191.0
13. Wojszyce — melioration ditch	6.8	7.9	0.26	3.1	64.5	43.8	1.17	403.2	299.8
14. Złotniki — pond	8.8	6.4	1.67	42.9	44.1	7.5	1.82	360.8	225.8
15. Wrocław — pond	7.9	4.0	0.19	1.5	21.4	3.6	0.44	221.3	185.4
16. Szczodre — pond	8.5	3.6	1.20	8.0	62.5	15.9	0.32	250.0	215.7
Total mean	7.9	7.44	0.74	16.2	46.0	16.6	1.08	332.5	242.6
Error of mean	0.14	0.70	0.04	1.39	1.57	1.12	0.09	2.87	2.54
Last significant difference	0.42	2.0	0.11	4.0	4.5	3.2	0.26	83.0	73.0
F-Snedecor-test	15.9	49.6	313.9	153.7	168.4	88.0	73.3	23.3	21.8
	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×

where : $x-xxx = F$ tabulated value of the F-Snedecor statistic

ge degree of differentiation, within the range of 1.5 to 21.67 mg/l of water. The highest potassium level was observed in the Staniszków (8) habitat, the lowest- in Wrocław (15).

The level of calcium falls within the range of 21.64 to 62.53 mg/l of water. The Szczodre habitat (16) was characterized by the highest level of calcium, the Wrocław (15) habitat — by the lowest level of this element.

The studied habitats show magnesium levels within the range of 3.59 to 16.14 mg/l of water. The highest magnesium levels were observed in the habitats at Paulinum (9) and at Szczodre (16) the the lowest — in Wrocław (15).

The studied habitats exhibit iron content within the range of 0.32 to 1.64 mg/l of water. The highest iron level was stated in the habitat at Paulinum (9), the lowest — in the habitats at Szczodre (16) and Wrocław (15).

The amount of dry residue in water of the studied habitats occurs within the range of 221.3 to 461.7 mg/l of water. The greatest amounts of dry residue were stated in the Jelcz (11) habitat, the smallest in Wrocław (15).

The amount of residue after ignition of water of the studied habitats varies within the range of 185.4 to 391. 2 mg/l. With respect to this property the studied habitats show differentiation analogous to these in the case of dry residue in water.

Considering the differentiation among habitats of *Spirodela polyrrhiza* with respect to the analyzed properties (Table 1), the Wrocław habitat (15) should be noted as clearly standing out among other habitats, in being characterized by low pH, low content of studied macroelements, low amounts of dry residue in water and residue after ignition. In contrast, the Staniszków (8) and Paulinum (9) habitats were rich in macroelements had high amounts of dry residue in water and residue after ignition, and they were alkaline.

Characteristic properties of ecological populations

1. Populations of *Lemna minor*

With respect to the studied properties, natural population of *Lemna minor* coming from habitats exhibit significant differences. The values of studied properties are shown in Table 2.

Adult fronds of *Lemna minor* in the studied populations fall within the dimension range: length 3—5 mm, width 2—4 mm. Their sizes fall within the range given by D a u b s (1965): width 1.5—4.0 length 3.0—6.0 mm.

Table 2

Characteristics of ecological populations of *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleiden

Characteristics of plants	1.	2. Top	3. Area of plants (in mm ²)				4. Ratio of right-to left-handed plants	5. Frequency of plants (%)			6. Frequency of anomalous plants (%)
	Index of shape	angle of frond	a. 2—fronds	b. 3—fronds	c. 4—fronds	d. Mean 1—fronds		a. 2—fronds	b. 3—fronds	c. 4—fronds	
Populations			<i>Lemna minor</i> L.								
1	1.52	126	8.4	12.1	15.1	4.0	1.30	10.2	60.9	28.9	2.6
2	1.57	120	10.4	14.7	25.0	5.9	1.80	15.9	55.9	28.2	2.0
3	1.68	127	10.8	16.1	19.9	5.2	2.30	26.0	36.0	38.0	0.7
4	1.32	133	9.6	12.4	19.0	4.6	0.45	28.2	49.8	22.0	1.2
5	1.54	140	8.8	13.9	20.0	4.7	6.80	4.2	41.0	54.8	17.2
6	1.65	130	9.9	16.0	22.3	5.3	5.60	5.0	44.6	50.4	19.5
7	1.53	121	6.7	9.8	13.2	3.3	0.90	67.3	21.3	11.4	7.3
8	1.48	139	11.3	14.8	25.4	5.6	2.00	29.5	55.1	15.4	0.9
9	1.42	144	8.6	10.1	14.3	3.8	3.00	26.2	51.4	22.4	1.4
10	1.59	128	6.2	8.6	12.7	3.1	0.20	12.2	40.7	47.1	1.7
11	1.51	142	10.0	14.0	19.5	4.8	0.71	20.0	48.6	31.7	0.8
12	1.48	134	11.5	18.5	26.3	6.2	0.60	4.5	57.2	38.3	0.8
13	1.31	151	12.0	18.8	26.6	6.3	2.10	18.0	69.2	12.8	0.6
14	1.41	143	10.4	14.4	22.8	5.2	0.10	54.0	30.2	15.8	1.2
Total mean $\bar{x}$	1.50	134.2	9.6	13.9	20.1	4.8	1.97	22.9	47.3	29.8	4.1
Error of mean	0.04	0.77	0.8	0.83	1.02	0.12	0.24	0.37	0.54	0.38	0.12
Last significant difference	0.09	2.2	2.25	2.35	2.89	0.33	0.64	1.03	1.52	1.06	0.33
F-snedecor-test	9.5	144.5	4.9	15.7	18.9	71.8	64.7	2522.7	556.0	1415.8	2944.5
	× ×	× × ×	× ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×
Populations			<i>Spirodela polyrrhiza</i> L. Schleiden								
1	1.17	150	36.4	53.4	71.6	18.4	0.40	7.2	29.7	63.1	1.1
2	1.17	150	32.3	52.0	70.8	17.3	0.39	8.2	31.4	60.4	0.8
3	1.19	145	26.4	38.8	62.9	14.5	0.48	10.7	49.1	40.2	0.8
4	1.23	141	37.6	55.8	81.7	19.4	0.49	12.4	44.2	43.4	2.1
5	1.14	151	28.0	41.4	66.3	15.0	2.03	13.0	38.0	48.7	4.2
Total mean $\bar{x}$	1.18	147.5	32.1	48.2	71.6	16.9	0.75	10.3	38.5	51.2	1.8
Error of mean	0.02	0.55	1.9	2.21	3.21	0.43	0.01	0.9	0.28	0.22	0.06
Last significant difference	0.05	1.62	5.6	6.4	9.3	1.26	0.03	0.53	0.8	0.65	0.17
F-snedecor-test	1.06	78.89	8.51	12.81	6.25	19.42	4785.5	197.3	901.0	207.96	623.1
	—	× × ×	× ×	× × ×	× ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×

The plant shape index of the studied populations varies within the range of 1.31 to 1.68. The highest shape index was observed in plants from Jajkowo (3). High values of that index were also observed in plants from Radoniowo (6), Jelenia Góra (10), Radech (2) and Proszówka (7). The fronds of these plants are noticeably oblong. The lowest values of shape index were stated in populations from Wojszyce (13) and Lipa Piotrkowska (4). The fronds of those plants are almost rounded in shape (suborbicular).

The adult fronds of studied populations have the top frond angle (property 2) of 120 to 151°. The population from Wojszyce (13) is distinguished by the largest top angle. The smallest top angle was observed in the populations from Radech (2) and Proszówka (7).

On the basis of the data given in Table 2, a relationship may be stated between the top angle value and the shape index. This significant negative correlation has been illustrated by a graph (Table 4, Fig. 1).

Fig. 1. Relationship between shape index (length/width of frond) and the value of frond top angle

Two frond plants of the studied natural populations of *Lemna minor* have area (property 3a) within the range of 6.25 to 12.0 mm². The greatest area in two-frond plants was stated in the population from Wojszyce (13). Relatively high values of this property were also observed in the populations from Stanisław (8) and Widawka (12), the lowest- in the populations from Jelenia Góra (10) and Proszówka (7). The three-frond plants of studied populations have area (property 3b) within the range of 8.6 to 18.8 mm². The populations from Widawka (12) and Radech (2) were distinguished by greatest area of three-frond plants. A relatively large area of three-frond plants was also observed in the populations from Wojszyce (13), Stanisław (8), Radoniów (6) and Złotniki (14). The smallest area of three-frond plants was observed in populations from Jelenia Góra (10) and Proszówka (7). The four-frond plants of the studied populations have area (property 3c) of 12.7 to 26.5 mm². The greatest area of four-frond

plants was observed in plants from populations in Wojszyce (13) and Wi-
dawka (12), as well as in Radech (2) and Staniszków (8). The smallest area
of four-frond plants was observed in populations from Jelenia Góra (10)
and Proszówka (7).

In general, populations with large area of two-frond plants were also
characterized by large areas of three- and four-frond plants. Among po-
pulations with large plants were, in descending order, the populations
from: Widawka (12), Wojszyce (13), Radech (2) and Staniszków (8). Small
area plants were characteristic of populations from Jelenia Góra (10), Pro-
szówka (7) and Paulinum (9).

A significant positive correlation was stated between average frond
size and the frequency of occurrence of three-frond plants (Table 4,
Fig. 2).

Fig. 2. Relationship between mean value of frond area and frequency of three-frond
plants

The ratio of right- to left-handed plants in populations of *Lemna mi-
nor* occurs with the value range of 0.10 to 6.80. The populations from Ra-
doniów (6 and 5) were distinguished by highest values of this property,
while the populations from Złotniki (14) and Jelenia Góra (10), by the
lowest. Among populations with clear majority of left-handed plants are
the populations 4, 11 and 12. In the population from Paulinum (9), three
times as many right-handed plants were found as left-handed.

The percentage of right- and left-handed plants in the studied popu-
lations of *Lemna minor* is shown below:

% of plants	No. of populations													
	1	2	3	4	5	6	7	8	9	10	11	12	18	14
R	57	63	70	31	87	85	48	67	75	17	43	37	68	9
L	43	37	30	69	13	15	52	33	25	83	57	63	32	91

A significant positive correlation was found between the value of the ratio of right- to left-handed plants (R : L) and the frequency of occurrence of anomalous plants in a population (Table 4).

The percentage of two-frond plants in natural populations of *Lemna minor* amounts to 4.2—67.3 (property 5a). The percentage of three-frond plants in these populations is 21.3 to 69.2, 47.1 on the average (property 5b). The percentage of four-frond plants in these populations is 11.4 to 54.8, 30 on the average (property 5c). With respect to this property (5), the following populations stand out: Proszówka (7) — by the highest frequency of two-frond plants and the lowest frequency of three and four-frond plants; populations 5 and 6 from Radoniów — by the lowest frequency of two-frond plants and the highest frequency of four-frond plants; and the population from Wojszyce (13) by the highest frequency of three-fronds plants and low frequency of four-frond plants.

There is a significant positive correlation between the frequency of occurrence of two-frond plants and the amount of the potassium in water (Table 4, Fig. 3). A negative, though also significant correlation occurs between the frequency of three-frond plants and amounts of potassium in water (Table 4, Fig. 4).

The frequency of anomalous plant development in natural populations of *Lemna minor* is 0.6 to 19.7%. Populations from Radoniów (5 and 6) and Proszówka (7) are distinguished by very high frequencies of anomalous plant development. A very low frequency of anomalous plants, not exceeding 1% of the total population, was observed in populations from Wojszyce (13), Jajkowo (3), Jelcz (11) and Widawka (12).

In natural populations of *Lemna minor*, the following developmental anomalies were observed (teratological forms): 1) changes in the body

Fig. 3. Relationship between frequency of two-frond plants and the potassium content in habitat

Fig. 4. Relationships between frequency of three-frond plants and the potassium content in habitat

shape, most often asymmetry of extremities is clearly visible, 2) dwarfed fronds, 3) abnormal direction of frond growth — heterotaxy *, 4) inappropriate location of induction of descendant fronds and 5) wrinkling or folding of frond area. With the exception of heterotaxy, which was observed only in the populations from Radoniów (5 and 6), all of the developmental anomalies were present in all populations.

2. Populations of *Spirodela polyrrhiza*

With respect to studied properties, the natural populations of *Spirodela polyrrhiza* coming from 5 habitats showed significant differences among themselves (Table 2).

Adult fronds of *Spirodela polyrrhiza* in natural populations are 4.5 to 8.5 mm long, and 2.5 mm wide, almost rounded in shape, often more or less egg-shaped. The range of dimensions of the studied plants is in agreement with the data given by Daubs (1965) (3.0—10.0 mm in length, more often 5.0—8.0 mm, and 2.5—8.0 mm in width).

Populations of *Spirodela polyrrhiza* exhibit relatively small variations in shape. The shape index of adult fronds of these plants varies within a small range from 1.14 to 1.23. The populations from Paulinum (9) is distinguished by the highest value of the frond shape index (plants with largest fronds). The lowest value of this index characterized plants from the population from Stanisław (5), which had the broadest fronds. Moreover, the Stanisław population was characterized by plants having the

* Term introduced by Bloch in 1965 (St. Muszyński 1970).

largest top angle of fronds. The smallest top angle of fronds was observed in plants from population from Paulinum (4).

Two-frond *Spirodela polyrrhiza* plants in natural populations (property 3a) have areas of 26.4 to 37.7 mm². The greatest area of two-frond plants was observed in the populations from Wrocław (1) and from Paulinum (4). The latter population was also distinguished by the greatest areas of three, and four-frond plants and greatest average area of frond (properties 3a, b, c and d). The population from Jelcz (3) was characterized by two-, three-, and four-frond plants of the smallest areas.

The ratio of right-, to left-handed plants (R : L) in the natural populations of *Spirodela polyrrhiza* varies within a narrow range, from 0.39 to 2.03%. Among the analyzed populations, the population from Stanisławów (5) stands out by having twice as many right-handed plants as left-handed ones. In the remaining populations, the number of left-handed plants exceed the number of right-handed ones.

The percentage of two-frond plants in natural populations of *Spirodela polyrrhiza* is from 7.2 to 13.0% (property 5a). The percentage of three-frond plants in those populations amounts to from 29.7 to 49.1% (property 5b). The percentage of four- and more frond plants in the same populations is from 40.2 to 63.1% (property 5c).

Among populations of *Spirodela polyrrhiza*, the following ones stand out with respect to the frequency of occurrence of two-, three-, and four-frond plants: the population from Wrocław (1), having the lowest frequency of two- and three-frond plants and the highest of four-frond plants; the population from Jelcz (3), having the highest frequency of three-frond plants and the lowest of four-frond plants; and the population from Stanisławów (5), having the highest frequency of two-frond plants.

The frequency of anomalous plant development in natural populations of *Spirodela polyrrhiza* is from 0.8 to 4.2%. The highest frequency of anomalous plants was observed in the population from Stanisławów (5).

Characteristics of experimental populations, determined on the basis of results of experiments

1. Populations of *Lemna minor*

Analysis of variance of the results of measurements of analyzed properties has shown the differences between all 14 populations to be significant (Table 3).

The index of the shape of plant fronds (property 1) of the studied populations of *Lemna minor* in culture *in vitro* has values within the range of 1.28 to 1.65. The highest value for this property was observed in the population from Radoniów (6). Plants of this population are distinguished by strongly elliptically elongated frond. High values of this property were

Table 3
Characteristics of experimental populations of *Lemna*

Characteristics of plants	1.	2.	3. Area of plants (in mm ²)			
	Index of shape	Top angle of frond	2— fronds	3— fronds	4— fronds	mean 1— frond
			a	b	c	d
Populations					<i>Lemna minor</i> L.	
1	1.44	131	12.8	18.4	23.1	6.1
2	1.60	120	10.4	13.4	20.3	4.9
3	1.48	141	15.5	18.7	25.0	6.6
4	1.47	143	11.8	15.6	23.4	5.6
5	1.45	144	13.0	18.5	23.5	6.1
6	1.64	131	10.9	14.4	19.6	5.0
7	1.60	125	13.1	18.8	27.5	6.6
8	1.42	148	12.6	17.4	22.8	5.9
9	1.45	147	11.1	15.4	19.5	5.1
10	1.53	134	12.4	17.2	23.1	5.8
11	1.47	146	12.5	16.5	22.4	5.7
12	1.50	135	13.8	19.4	24.6	6.4
13	1.28	154	15.9	22.2	31.0	7.7
14	1.42	146	11.4	15.2	20.3	5.2
Total mean $\bar{x}$	1.48	139	12.7	17.2	23.3	5.9
Error of mean	0.02	2.40	0.66	0.84	1.10	0.11
Last significant difference	0.05	6.72	1.8	2.4	3.09	0.30
F-Snedecor test	33.5	32.1	6.2	7.7	8.2	51.8
	× × ×	× × ×	× ×	× ×	× ×	× × ×
Populations	<i>Spirodela polyrrhiza</i>					
1	1.16	140	40.2	61.7	80.4	20.2
2	1.17	140	35.4	51.6	69.4	17.2
3	1.18	142	30.2	45.7	64.7	15.8
4	1.21	148	42.6	64.7	86.8	21.5
5	1.14	147	31.4	48.6	67.8	16.1
Total mean $\bar{x}$	1.17	143.4	36.0	54.0	73.8	18.2
Error mean	0.02	1.83	0.38	0.42	0.41	0.15
Last significant difference	0.06	5.3	1.13	1.22	1.20	0.43
F-Snedecor test	1.38	5.36	193.8	406.3	529.5	295.8
	—	× ×	× × ×	× × ×	× × ×	× × ×

also observed in populations from Radech (29) and Proszówka (7). The lowest value of the shape index is characteristic for Wojszyce population (13). Low values of this property were also observed in plants from the Stanisławów (8) and Złotniki (14) populations.

The top angle of frond of plants of the studied populations in cultures *in vitro* (property 2) varied from 120° to 154°. The largest top angle was

minor L. and *Spirodela polyrrhiza* (L.) Schleiden

4. Ratio of right to left-handed plants	5. Frequency of plants (%)			4.	5.	6.
	2— fronds	3— fronds	4— fronds	Number of fronds	Multi- plication rate	Biomass (mg)
	a	b	c			
1.25	4.0	61.8	34.2	30.2	48.9	30.9
1.84	15.0	64.4	20.6	29.7	48.7	28.3
2.40	26.8	37.9	35.3	36.7	51.7	34.3
0.4	38.0	35.5	26.5	26.3	46.9	28.0
6.5	4.3	45.7	50.0	27.6	47.6	27.6
4.9	8.4	40.6	51.0	21.0	43.7	27.0
0.9	12.0	43.5	44.5	44.5	54.5	46.5
2.0	25.1	58.7	16.2	30.7	48.9	29.2
3.2	24.0	62.0	14.0	42.0	53.7	46.7
0.4	20.0	31.2	48.8	40.7	53.0	36.2
0.7	17.0	44.2	38.8	42.7	53.9	39.5
0.61	21.0	42.0	37.0	28.7	48.2	32.3
2.3	18.8	70.5	10.7	36.8	51.8	39.0
0.07	58.5	27.5	14.0	33.5	50.4	36.2
1.95	20.9	47.6	31.6	33.6	50.2	34.4
0.23	0.38	0.46	0.43	2.18	0.38	2.8
0.65	1.08	1.28	1.23	6.16	1.07	7.98
64.7	1374.7	873.0	934.8	10.4	65.81	5.25
× × ×	× × ×	× × ×	× × ×	× × ×	× × ×	× ×

(L.) Schleiden

0.41	8.3	19.2	72.5	79.7	62.3	110.7
0.40	6.6	27.9	65.5	71.5	61.3	74.0
0.47	6.5	52.0	41.6	89.0	64.5	135.3
0.48	10.9	35.8	53.3	95.8	65.0	146.8
1.52	12.6	39.6	47.8	76.0	62.1	98.3
0.66	8.9	35.0	56.1	82.4	62.8	113.0
0.13	0.17	0.26	0.41	0.13	0.23	5.72
0.37	0.51	0.79	1.18	1.9	0.66	16.5
12.1	147.6	352.2	901.2	229.8	50.3	25.7
× ×	× × ×	× × ×	× × ×	× × ×	× × ×	× × ×

observed in plants coming from the Wojszyce population (13). Relatively large top angles were observed in plants of the population from Stanisław (8), Paulinum (9), Jelcz (11), and Złotniki (14). The smallest top angle was characteristic for plants coming from the Radech (2) population; also small was the top angle in the plants from Proszówka (7).

Two-frond plants of *Lemna minor* in the studied experimental popula-

tions have areas (property 3a) within the range of 10.4 to 15.9 mm², the three-frond plants have area (property 3b) within the range of 13.4 to 22.2 mm²; and four-frond plants have area (property 3c) within the range of 19.5 to 31.0 mm².

In experimental populations, as well as in the case of the studied natural populations, the following regularity is present in all cases: populations with large areas of two-frond plants are also characterized by larger areas in three- and four-frond plants.

Among the experimental populations with large areas of two-, three-, and four-frond plants, may be included, in descending order: the population from Wojszyce (13), Proszówka (7), Widawka (12) and Jajkowo (3). Analogically, the populations with small area of plants are: Radoniów (6), Paulinum (9), Radecz (2) and Złotniki (14).

The ratio of right- to left-handed plants (property 4) in the studied experimental populations of *Lemna minor*, has values within the range from 0.07 to 6.5. The highest value of this property was observed in the populations from Radoniów (5); it was also high in the population 6 from Radoniów. In those populations, right-handed plants are definitely in majority. The lowest values of this property were stated in the population from Złotniki (14). Low values were also observed in populations from Jelenia Góra (10) and Lipa Piotrkowska (4) majority of left-handed plants.

After 14 days of culture, the number of plants representing each of the analyzed populations (property 6) reached the value of 21.0 to 44.5 of fronds. The greatest number of fronds was observed in the population from Proszówka (7), a fact resulting from the most rapid multiplication rate of plants from this population (property 7). The smallest number, of fronds, and slowest propagation, were characteristic for the population from Radoniów (6). Moreover, small number of fronds and slow multiplication rate were observed also in populations from Lipa Piotrkowska (4) and Radoniów (5). A rather large number of fronds and relatively rapid propagation distinguished the populations from Jelenia (10) and Jelcz (11).

Dry mass of plants after 14 days of culture attained, for particular populations, has values within the range of 27.0 to 46.7 mg, calculated per 10 fronds of starter culture. The highest values of dry mass were observed in the following populations: Proszówka (7) and Paulinum (9). The lowest dry mass values were observed in populations from Radoniów (5 and 6). Low values were also observed in populations 2, 4, 8 and 1. Relatively high dry mass values were observed in populations from Wojszyce (13) and Jelcz (11). There are significant positive correlation between the amount of dry mass and the number of fronds, and between the amount of dry mass number of fronds on the one hand, and the iron content of water on the other. Moreover, correlation was stated between dry mass and frond number on the one hand, and the combined activity of iron and magnesium (Table 4).

Table 4

Correlations between characteristics of habitats, characteristics of populations, and between characteristics of habitats and populations of *Lemma minor* and *Spirodela polyrrhiza*

No.	Relationships	Multiple regressions coefficients				Equation of regression $y = b_0 + b_1x_1 + \dots + b_nx_n$	Multiple correlation coef. (R^2)	F test for R^2
		b_0	b_1	b_2	b_3			
1.	pH and level of P in water	7.32	0.76	—	—	$y = 7.32 + 0.76 x$	0.5956	100.2 signif.
2.	pH and level of P and Mg in water	7.6	0.82	—0.018	—	$y = 7.6 + 0.82 x_1 - 0.018 x_2$	0.67	68.0 signif.
3.	P and K level in water	0.12	0.036	—	—	$y = 0.12 + 0.036 x$	0.8335	340.3 signif.
4.	Ca and N level in water	20.34	3.11	—	—	$y = 20.34 + 3.11 x$	0.5697	90.0 signif.
5.	Ca, N, and Mg level in water	11.69	2.67	0.7	—	$y = 11.69 + 2.67 x_1 + 0.7 x_2$	0.699	77.9 signif.
6.	Mg and Ca level in water	3.69	0.30	—	—	$y = 3.69 + 0.30 x$	0.5115	30.76 signif.
7.	Shape index and top angle of frond	2.45	—0.0068	—	—	$y = 2.45 - 0.0068 x$	0.60	102.76 signif.
8.	Mean value of frond area and frequency of three-frond plants	2.81	0.042	—	—	$y = 2.81 + 0.042 x$	0.358	23.7 signif.
9.	Ratio of right-to left-handed plants and frequency of anomalous plants	0.89	0.26	—	—	$y = 0.89 + 0.26 x$	0.6523	127.6 signif.
10.	Frequency of two-frond plants and the potassium level in habitat	8.21	0.83	—	—	$y = 8.21 + 0.83 x$	0.682	146.1 signif.
11.	Frequency of three-frond plants and the potassium level in habitat	55.77	—0.48	—	—	$y = 55.77 - 0.48 x$	0.988	63.76 signif.
12.	Biomass and number of fronds	6.41	0.83	—	—	$y = 6.41 + 0.83 x$	0.775	234.1 signif.
13.	Biomass and number of fronds and the Fe level in habitat	2.57	0.86	2.28	—	$y = 2.57 + 0.86 x_1 + 2.28 x_2$	0.845	182.2 signif.
14.	Biomass and number of fronds and Fe and Mg levels in habitat	1.88	0.85	0.11	1.82	$y = 1.88 + 0.85 x_1 + 0.11 x_2 + 1.82 x_3$	0.872	149.3 signif.

In the experimental populations of *Lemna minor*, the percentage of two-frond plants (property 5a) varies from 4.0 to 58.5%. The percentage of three-frond plants (property 5b) in those populations varies from 27.5 to 70.5%, and the percentage of four-frond plants (property 5c) varies between 10.7 to 51.0%. Outstanding among the studied populations were: the populations from Radoniów (5 and 6) by low frequency of two-frond plants and the highest frequency of four-frond plants; the population from Wojszyce (13), by the highest frequency of three-frond plants and lowest frequency of four-frond plants; the population from Złotniki (14), by the highest frequency of two-frond plants and lowest frequency of three-frond plants; and the population from Radech (1), by the lowest frequency of two-frond plants and relatively high frequency of three-frond plants.

2. Populations of *Spirodela polyrrhiza*

The shape index of plant fronds from experimental populations of *Spirodela polyrrhiza* amounts to 1.14 to 1.21 (property 1). The populations from Paulinum (4) is distinguished by the highest value of plant frond shape index. Plants from that population are hence distinguished by the narrowest fronds. Plant coming from the Staniszków population (5) are characterized by the lowest value of frond shape index. These plants are widest, most rounded in shape, orbicular (Table 3).

The population from Paulinum (4) is distinguished by the widest top frond angle (property 2), while the narrowest top angle of fronds is characteristic of the population from Szczodre (2).

Two-frond plants of *Spirodela polyrrhiza* in experimental populations have areas of 30.2 to 42.6 mm² (property 3a). Three-frond plants of those populations have areas of 45.7 to 64.7 mm² (property 3b). Plant with four and more fronds coming from the same populations have areas of 64.7 to 86.8 mm² (property 3c). With respect to the area of all plants, i.e., two-, three-, and four-fronds, among the studied populations stands out the one from Paulinum (4). Analogically, the population from Jelcz (3) stands out as having the smallest area of all plants.

The ratio of right- to left-handed plants (R : L), in the experimental populations of *Spirodela polyrrhiza* varies from 0.4 to 1.52. With respect to this property, outstanding among the studied experimental populations is the population from Staniszków (5), in which right-handed plants are far in excess, numerically, of left-handed plants (around 1.5 times as many). In the remaining populations there was a numerical majority of left-handed plants.

The percentage of two-frond plants in the experimental populations of *Spirodela polyrrhiza* amounts to 6.5 to 12.6% (property 5a). The percentage of three-frond plants in those populations is 19.2 to 52.0% (property

5b). The percentage of plants with four and more fronds, in the analyzed populations, amounts to 41.6 to 72.5% (property 5c). With respect to the frequency of occurrence of two-, three-, and four-frond plants, the following populations stand out: Wrocław (1) — by the lowest frequency of three-frond plants and the highest of four-frond plants; Jelcz (3) — by the highest frequency of three-frond plants and the lowest of four-frond plants; Paulinum (4) — by the highest frequency of two-frond plants.

After 14 days of culture, the number of plants (property 6) representing each of the analyzed populations could be expressed by the value of 71.5 to 95.8 frond. The largest number of fronds was observed in the population from Paulinum (4), resulting from the most rapid vegetative propagation of plants in this population (property 7). The smallest number of fronds and slowest multiplication rate was characteristic for the population from Szczodre (2).

Dry mass of plants (property 8), after 14 days of culture, reached, for different populations, the values of 74.0 to 146.8 mg calculated per 10 fronds of starter culture. Population from Paulinum (4) was distinguished by the highest dry mass value; while the population from Szczodre (2) — by the lowest.

A comparison of natural and experimental populations of *Lemna minor* and *Spirodela polyrrhiza* with respect to the analyzed characteristic properties

I am assuming, in accordance with Stebbins (1958), that the observed variations within species (differences among ecological populations) of *Lemna minor* and *Spirodela polyrrhiza*, though difficult to evaluate directly, were confirmed by detailed analysis of selected properties (Table 2) and are conditioned by differences in habitats, particularly with respect to the levels of basic macroelements (Table 1). I could have also expected, according to K o v a l s k i et al. (1967), that in the case of radioactive waters (in our case, only the habitat of the development of ecological populations of *Lemna minor*) a particular set of variances within this species will be present. The distinguished biotypes of the studied species (natural populations outstanding with respect to analyzed properties, particularly with respect to several properties simultaneously) are also to be expected (according to T u r e s s o n 1922) with such a wide differentiation of studied habitats, considering the fact that these are popular species, with broad geographical range and wide scope of ecological habitats (their geographical races are known, L a n d o l t 1957). It only remained to ascertain the rank of outstanding systems of intra-species variance, observed for particular natural populations. Will plants for the studied populations, differing with respect to selected characteristics, preserve their habitual

uniqueness, and to what degree, when transferred to a system of optimum, stenotope conditions such as the sterile cultures *in vitro*? Will the differences between biotypes, confirmed by analysis, with respect to the analyzed properties of both species, be still evident in cultures *in vitro*? Variation analysis of the values of the studied properties between the natural and experimental populations of *Lemna minor* and *Spirodela polyrrhiza* permitted the determination, in the case of populations outstanding with respect to selected properties, and constituting unquestionable separate biotypes, which of them constitute ecotypes and which are only ecophenes.

1. Natural and experimental populations of *Lemna minor*

The significance of differentiation between the natural and the experimental populations of *Lemna minor* with respect to the analyzed properties is presented in Table 5.

The majority of natural populations (9 out of 14 populations) do not differ significantly from experimental populations with respect to values of the shape index (populations 2, 6, 7, 8, 9, 10, 11, 13, 14). Moreover, 9 out of the 14 populations in culture have preserved the characteristic value of the top angle (no significant differences were proven to occur between natural and experimental populations). No natural or experimental populations show significant differences with respect to one property (4) — the ratio of right- to left-handed plants. With respect to the remaining characteristics (area of fronds and the frequency of two-, three-, and four-frond plants) only some of the natural populations differ significantly from experimental populations.

Outstanding populations, characterized by the same values for most properties in nature as well as *in vitro* (no proven differences) are the populations from Radech (2), Radoniów (5 and 6), Widawka (12), Wojszyce (13) and Złotniki (14). In nature, these populations were even more outstanding in comparison with the other populations (Table 2). The outstanding properties of these populations were preserved *in vitro* for a long time with proper cultivation and regular passaging (the last checking was done after 16 months of cultivation). According to D a u b e n m i r e (1973), this allows us to define the above populations as ecotypes of the species*, conditioned in nature by different degrees of water trophism, and in the case of populations from Radoniów (5 and 6), by the increased radioactivity of water.

The remaining populations (1, 3, 4, 7, 8, 9 and 10) differ significantly with respect to the majority of analyzed properties in nature and in cul-

* Ecotypes — synonyms: ecological races (Caussen J., Keck D. D. and Hissey 1974); physiological races (Gregor J. W. 1946).

Table 5

Comparison of characteristics of natural (N) and experimental (E) populations of *Lemna minor* and *Spirodela polyrrhiza*

Characteristics		1. Index of shape			2. Top angle of frond			3. Area of plants (in mm ²)								4. Ratio of right-to left-handed plants			5. Frequency of plants (%)												
Populations (P)		Populations		Mean of populat.	Populations		Mean of populat.	a. 2—fronds		Mean of populat.	b. 3—fronds		Mean of populat.	c. 4—fronds		Mean of populat.	d. 1—frond		Mean of populat.	N	E	Mean of populat.	a. 2—fronds		Mean of populat.	b. 3—fronds		Mean of populat.	c. 4—fronds		Mean of populat.
		N	E		N	E		N	E		N	E		N	E		N	E					N	E		N	E				
<i>Lenaea minor</i>	1	1.52	1.44	1.48	126	131	128.5	8.4	12.8	10.6	12.1	18.4	15.2	15.1	23.1	19.1	4.0	6.1	5.0	1.3	1.25	1.28	10.2	4.0	7.1	60.9	61.8	61.4	28.9	34.2	31.6
	2	1.57	1.60	1.59	120	120	120	10.4	10.4	10.4	14.7	13.4	14.0	25.0	20.3	22.6	5.9	4.9	5.4	1.8	1.8	1.8	15.9	15.0	15.5	55.9	64.4	60.1	28.2	20.6	24.4
	3	1.68	1.48	1.59	127	141	134	10.8	15.5	13.1	16.1	18.7	17.4	19.9	25.0	22.4	5.2	6.6	5.9	2.3	2.4	2.35	26.0	26.8	26.4	36.0	37.9	36.95	38.0	35.3	36.65
	4	1.32	1.47	1.40	133	143	138	9.6	11.8	10.7	12.4	15.6	14.0	19.0	23.4	21.2	4.6	5.6	5.1	0.45	0.4	0.42	28.2	38.0	33.1	49.8	35.5	42.65	22.0	26.5	24.25
	5	1.54	1.45	1.50	140	144	142	8.8	13.0	10.9	13.9	18.5	16.2	20.0	23.5	21.7	4.7	6.1	5.4	6.8	6.5	6.65	4.2	4.3	4.25	41.0	45.7	43.4	54.8	50.0	52.4
	6	1.65	1.64	1.645	130	131	130.5	9.9	10.9	10.2	16.0	14.4	15.2	22.3	19.6	20.9	5.3	5.0	5.15	5.6	4.9	5.25	5.0	8.4	6.7	44.6	40.6	42.6	50.4	51.0	50.7
	7	1.53	1.60	1.565	121	125	123	6.7	13.1	9.9	9.8	18.8	14.3	13.2	27.5	20.35	3.3	6.6	4.9	0.9	0.9	0.9	67.3	12.0	38.7	21.3	43.5	32.4	11.4	44.5	27.9
	8	1.48	1.42	1.45	139	148	143	11.3	12.5	11.9	14.8	17.4	16.1	25.4	22.8	24.1	5.6	5.9	5.8	2.0	2.0	2.0	29.5	25.1	27.3	55.1	58.7	56.9	15.4	16.2	15.8
	9	1.42	1.45	1.435	144	147	145.5	8.6	11.1	9.9	10.1	15.4	12.7	14.3	19.5	16.9	3.8	5.1	4.5	3.0	3.1	3.05	26.2	24.0	25.1	51.4	62.0	56.7	22.4	14.0	18.2
	10	1.59	1.53	1.56	128	134	131	6.2	12.4	9.3	8.6	17.2	12.9	12.7	23.1	17.9	3.1	5.8	4.45	0.2	0.4	0.3	12.2	20.0	16.1	40.7	31.2	33.95	47.1	48.8	47.9
	11	1.51	1.47	1.49	142	146	144	10.0	12.5	11.25	14.0	16.5	15.2	19.5	22.4	20.95	4.8	5.7	5.3	0.7	0.7	0.7	20.0	17.0	18.5	48.6	44.2	46.4	31.4	38.8	35.1
	12	1.48	1.50	1.49	134	135	134.5	11.5	13.8	12.7	18.5	19.4	18.95	26.3	24.6	25.5	6.2	6.4	6.3	0.6	0.6	0.6	4.5	21.0	12.75	57.2	42.0	49.6	38.3	37.0	37.65
	13	1.31	1.28	1.30	151	154	152.5	12.0	15.9	13.9	18.8	22.2	20.5	26.6	31.0	28.3	6.3	7.7	7.0	2.1	2.3	2.2	18.0	18.8	18.4	69.2	70.5	69.85	12.8	10.7	11.8
	14	1.41	1.42	1.415	143	146	144.5	10.4	11.4	10.9	14.4	15.2	14.8	22.8	20.3	21.55	5.2	5.2	5.2	0.1	0.07	0.09	54.0	58.5	56.25	30.2	27.5	28.85	15.8	14.0	14.9
$\bar{x}$		1.50	1.48	1.49	134	139	136.5	9.6	12.7	11.15	13.9	17.2	15.5	20.1	23.3	21.7	4.8	5.9	5.4	1.97	1.95	1.96	22.9	20.9	21.9	47.3	47.6	47.5	29.8	31.5	30.6
Analysis of variance for		NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P
Error of mean		0.007	0.018	0.025	0.38	1.0	1.42	0.19	0.52	0.73	0.22	0.59	0.84	0.28	0.74	1.05	0.03	0.08	0.11	0.06	0.15	0.21	0.10	0.26	0.37	0.13	0.35	0.50	0.11	0.29	0.41
Last significant difference		0.02	0.05	0.07	1.05	2.8	4.01	0.54	1.43	2.02	0.62	1.64	2.32	0.79	2.1	2.9	0.08	0.22	0.31	0.15	0.41	0.58	0.28	0.73	1.04	0.37	0.98	1.39	0.31	0.81	1.15
F-snedecot-test		3.79	25.01	6.14	80.7	87.4	3.84	123.5	6.4	3.9	113.2	13.6	7.28	62.6	16.5	13.8	709.8	67.6	57.7	0.15	164.6	0.46	197.8	2878.6	967.2	—	1173.3	201.4	40.52	1942.6	330.8
		—	×××	×××	×××	×××	×××	×××	××	××	×××	×××	××	×××	×××	×××	×××	×××	×××	—	×××	—	×××	×××	×××	—	×××	×××	×××	×××	×××
Populations (P)		1			2			3								4			5												
<i>Spirodela polyrrhiza</i>	1	1.17	1.16	1.165	150	140	145	36.4	40.2	38.3	53.4	61.7	57.6	76.1	80.4	78.1	18.4	20.2	19.3	0.4	0.41	0.405	7.2	8.3	7.75	29.7	19.2	24.5	63.1	74.5	67.8
	2	1.17	1.17	1.17	150	140	145	32.3	35.4	33.8	52.0	51.6	51.8	70.8	69.4	70.1	17.3	17.2	17.2	0.39	0.4	0.395	8.2	6.6	7.4	31.4	27.9	29.7	60.4	65.5	63.0
	3	1.19	1.18	1.185	145	142	143.5	26.4	30.2	28.3	38.8	45.7	42.3	62.9	64.7	63.8	14.5	15.8	15.15	0.48	0.47	0.475	10.7	6.5	8.6	49.1	52.0	50.6	40.2	41.6	40.9
	4	1.23	1.21	1.22	141	148	144.5	37.6	42.6	40.2	55.8	64.7	60.3	81.7	86.8	84.2	19.4	21.5	20.5	0.49	0.48	0.485	12.4	10.9	11.7	44.2	35.8	40.1	43.4	53.3	48.35
	5	1.14	1.14	1.14	151	147	149	28.0	31.4	29.1	41.1	48.6	44.8	66.3	67.8	67.05	16.9	16.1	15.55	2.03	1.52	1.76	13.0	12.6	12.8	38.0	39.6	38.8	48.7	47.8	48.25
$\bar{x}$		1.18	1.17	1.175	147.5	143	145	32.1	36.0	34.3	48.2	54.5	51.3	71.6	73.8	72.7	16.9	18.2	17.6	0.76	0.66	0.72	10.3	8.9	9.6	38.5	35.0	36.9	51.2	56.1	53.5
Analysis of variance for		NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	E	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P	NE	P	Inter-action NE×P
Error of mean		0.01	0.014	0.02	0.6	0.94	1.33	0.63	0.98	1.38	0.71	1.1	1.58	1.02	1.61	2.28	0.14	0.23	0.32	0.06	0.10	0.14	0.08	0.12	0.17	0.13	0.18	0.26	0.14	0.23	0.33
Last significant difference		0.03	0.042	0.06	1.79	2.83	4.0	1.85	2.93	4.14	2.12	3.35	4.74	3.06	4.84	6.84	0.43	0.68	0.97	0.18	0.29	0.41	0.36	0.4	0.51	0.35	0.56	0.79	0.44	0.69	0.98
F-snedecot-test		—	3.15	—	60.8	—	22.8	14.2	29.3	—	39.4	48.9	3.91	—	26.1	3.13	30.3	92.2	4.38	—	42.7	—	549.4	204.2	147.5	348.8	3001.8	325.2	712.8	2313.3	71.3
		—	×	—	×××	—	×××	×××	×××	—	×××	×××	×	—	×××	×	×××	×××	×	—	×××	—	×××	×××	×××	×××	×××	×××	×××	×××	×××

ture. Hence, values of properties of these populations in culture differ from the analogous values in nature. It has been proven statistically, that in nature, some of these populations differed significantly among themselves, while some were outstanding e.g. the populations from Proszówka (7) and Jelenia Góra (10) — Table 2. Special attention should be given the Proszówka (7) populations, which stands out strongly in nature as well as in cultures. Of the distinguishing properties, in culture it preserves the value of shape index. The particularly small area of frond in nature changes in culture; in culture that population is distinguished by large area of fronds. Other distinguishing characteristics of that culture are: the number of fronds, the multiplication rate, dry mass value. In nature that populations was also characterized by a predominance of two-frond plants, while in culture, there was a clear predominance of three- and four-frond plants.

According to Daubenmire (1973), populations of *Lemna minor* differentiated in nature due to habitat conditions, but losing their distinguishing properties under stenotopic conditions of *in vitro* cultures, may be described as ecophenes (synonyms: habitat forms, epharmones- Cockayne 1933, ecades-habitat variants).

2. Natural and experimental populations of *Spirodela polyrrhiza*

The significance of differentiation between the experimental and the natural populations of *Spirodela polyrrhiza* with respect to analyzed properties is presented in Table 5.

Only two properties, the index of shape (property 1) and the ratio of right- to left-handed plants (property 4), do not differ significantly between the natural and the experimental populations. However, with respect to the frequency of two-, three-, and four-frond plants (property 5a, b and c) all populations, except the one from Stanisław (5) were changed in culture, i.e., differed significantly from natural populations. With respect to the remaining properties, only a few experimental populations did not significantly differ from natural ones. The greatest number of properties characteristic of natural population were preserved in culture by the population from Stanisław (5). That population was also outstanding among other natural populations. The population from Paulinum (4), as well as the one from Wrocław (1), exhibited the greatest degree of change in properties in culture.

DISCUSSION

The species *Lemna minor* and *Spirodela polyrrhiza*, being eurytopic even in the small area of Lower Silesia as compared to their general geographic range (acc. to Dąb 1965) appear over a wide scope of analyz-

ed ecological habitat conditions (Table 1). It should be noted, however, that in our case, with the exception of one factor the presence of nitrogen in maximum amounts and having a limiting influence (according to the Shelford tolerance law — Odum 1963) on the development of *Lemna minor* in one single habitat at Proszówka, none of the remaining factors are determinant of tolerance thresholds for the studied species. We may quote as an example the pH factor of water of the studied habitats; the lowest pH in our case was 6.6 (Radeck habitat), while according to Steinberg (Landolt 1957), *Lemna minor* plants may still grow at pH 3.8, and *Spirodela polyrrhiza* stops growing at pH 5.0. In the Proszówka habitat, where conditions for the development of *Lemna minor* were not optimum with respect to only one factor, i.e., nitrogen level, which was confirmed by observed symptoms of ammonia poisoning in studied plants, we should expect the narrowing of tolerance limits of the studied species in relation to other factors, as has been shown by, among others, Penman (1956) in the case of nitrogen in the soil as the factor limiting the growth of grasses. The recognition of diminishing size of *Lemna minor* fronds as one of the symptoms of the limiting influence of high nitrogen concentrations at the Proszówka habitat is in agreement with White's experiments (1936, 1937). Under unfavourable conditions, according to Kasinov (1968), descendant fronds of *Lemna minor* separate more rapidly from parent fronds. Hence, it is not surprising that the *Lemna minor* population from the Proszówka habitat is characterized by a predominance of two-frond plants. This also results in diminishing of frond size, since Kasinov has shown that premature separation of descendant frond from the parent frond causes a decrease in the latter's adult length as well as in its growth rate, from which we may conclude that the contact between parent and descendant fronds increases the growth of the latter. The positive correlation observed in the present study between the amount of potassium in a habitat and the frequency of two-frond plants, and the negative correlation with the frequency of three-frond plants (Table 4, Fig. 3 and 4) points to the fact that in our case also, potassium may be the factor limiting the development of *Lemna minor* plants, if we consider the dependency of descendant frond growth on their ties to parent fronds, pointed out by Kasinov (1968).

The known experimental morphological and developmental conditioning of plants from the *Lemnaceae* family, described by Landolt in his monograph (1957) attest to the fact that the significant differentiation among natural populations of both studied species with respect to their characteristic properties (Table 2), which was confirmed by the present study, is not incidental, but significantly conditioned by the different sets of ecological conditions present in each water habitat. The ecological differentiation of the studied natural populations of *Lemna minor* and *Spirodela polyrrhiza* should be attributed to, above all, differing water chemi-

stry, since it would be difficult to attribute it to varying climatic conditions when we consider the fact of both species naturally ranging over different climatic zones. (L and o l t 1957 distinguishes two geographical races of *Lemna minor*, occurring in different climatic zones, one of these races shows high tolerance for the salt content of water). Already H e g e l m a i e r (1868), pointing out the great changeability of species within the *Lemnaceae* family, suspected the existence of lower taxones — varieties and races — within those species. H i c k s (1932), in his studies with experimental populations of several species of the *Lemnaceae* family and determining their tolerance of medium pH, noted the possibility of occurrence of physiological races (synonyms: ecological races, ecotypes).

The majority of studied natural populations of *Lemna minor* and *Spirodela polyrrhiza* significantly differing among themselves with respect to plant size, i.e., the size, index shape, number of fronds and the multiplication rate, lose those differences after being transferred to stenotopic conditions (in our case, the conditions of sterile *in vitro* cultures). Hence, the differentiation is exclusively the result of differing configurations of complex environment, and such plants, known as ecophenes "belong, in reality to a homogenous genetic line" — according to C o c k a y n e (1933) and D a u b e n m i r e (1973). Of the 19 natural populations analyzed, under stenotopic conditions, only 7 preserve their characteristic properties (Table 5), therefore, the significant differences among plants of those 7 different natural populations are, in this case, conditioned by heredity (they could be defined as ecotypes).

We conclude, that each configuration of the complex environment (the analyzed water bodies of Lower Silesia, i.e., habitats of both species) modifies "the properties of *Lemna minor* and *Spirodela polyrrhiza*, especially so, since these tendencies are not counteracted by sexual propagation" (as it is known, plants from the studied populations propagate in our conditions almost exclusively vegetatively and their blossoming is an extremely rare phenomenon). In the case of the 7 natural populations, the recognition of a system of intraspecies variability in the rank of ecotypes is also justified by the fact that, in the control experiment checking the durability of characteristics of particular populations, we are dealing with clones i.e., groups of plants obtained from a single parent plant, characterized by the same genotype. The necessity of using clones or pure strains in ecological experiments of this type was noted by S t e b b i n s (1958) and D a u b e n m i r e (1973). Similarly, Z u r z y c k i (1957 and 1962), in studies of *Lemna trisulca* physiology, observed large ecological differentiation among plants of this species in natural conditions, while W c i s ł o (1963) has doubtlessly singled out 3 ecotypes from three different clones of this species, in sterile cultures originating from separate ecological populations in the vicinity of Kraków.

CONCLUSIONS

1. Development habitats of *Lemna minor* and *Spirodela polyrrhiza*, exhibiting different degrees of eutrophication, proven by the significant differentiation among them with respect to chemical properties (pH, N, Ca, Mg, Fe and organic substance — Table 1), constitute different configurations of a complex environment, which condition — to a greater or lesser degree — the particular character of ecological populations of both species.

2. The particular character of ecological populations of *Lemna minor* and *Spirodela polyrrhiza* is expressed in the shape and size of plants, the frequency of two-, three-, and four-frond plants, the ratio of right-handed to left-handed plants (Table 2), as well as the dynamics of population growth and biomass production (Table 3).

3. The intra-species variability of *Lemna minor* and *Spirodela polyrrhiza* has the character of two systems, confirmed experimentally (Table 5) and significantly influenced by different environmental configurations, namely:

- system of ecophene variability (12 ecological populations), and
- system of ecotype variability (7 ecological populations).

4. The ecological differentiation of populations of *Lemna minor* and *Spirodela polyrrhiza* under the influence of chemical properties of their habitats is confirmed by the interdependence between the characteristics of plants and their habitats (Table 4):

- a) The correlation between the frequency of two-frond plants and potassium level in water (Fig. 3),
- b) The correlation between frequency of three-frond plants and the potassium level in water (Fig. 4),
- c) The correlation between the biomass and the number of fronds on the one hand, and the iron level in water on the other,
- d) The correlation between the biomass and number of fronds on the one hand, and the iron and magnesium levels in water, on the other.

5. Increased natural radioactivity of the Radoniowo habitats (radon waters) plays a particular role in the ecotypal differentiation of *Lemna minor* populations, which is evident in the high frequency of developmental anomalies in those populations. Moreover, those populations are distinguished by predominance of right-handed and four-frond plants (Table 2), by least numerous populations, lowest propagation rate, and lowest biomass production (Table 3), hence, they are characterized by a relatively low biotic potential.

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Ekologiczne zróżnicowanie populacji *Lemna minor* L. i *Spirodela polyrrhiza* (L.) Schleiden

Streszczenie

Do przeprowadzenia niniejszych badań analitycznych i eksperymentalnych wybrano 14 populacji *Lemna minor* i 5 populacji *Spirodela polyrrhiza* z 16 siedlisk na Dolnym Śląsku celem stwierdzenia, jak zróżnicowane są ekologiczne populacje obu badanych gatunków pod względem charakterystyk osobniczych i grupowych i czy różnice te są uwarunkowane czynnikami edaficznymi.

Trofizm siedlisk określono oznaczając odczyn wody, zawartość makroelementów i substancji organicznej. Wyniki wskazują na bardzo istotne zróżnicowanie siedlisk pod względem badanych charakterystyk (tabela 1), co pozwala wyróżnić siedliska o różnym stopniu trofizmu. Ponadto siedliska *Lemna minor* z Radoniowa odznaczały się podwyższoną, naturalną radioaktywnością, uwarunkowaną radonem i produktami jego rozpadu (średnio radoczynność wody wynosi $20 \mu\text{Ci/l}$). W siedliskach stwierdzono następujące zależności (tabela 4): pH a zawartość P w wodzie, pH a zawartość P i Mg, P względem K, Ca względem N, Ca a łączna zawartość N i Mg w wodzie oraz Mg względem Ca w wodzie.

Do charakterystyki ekologicznych populacji obu badanych gatunków roślin przyjęto następujące cechy: wskaźnik kształtu członu macierzystego, kąt wierzchołkowy członu macierzystego, powierzchnia roślin (dwu-, trój-, czteroczłonowych), stosunek liczby roślin prawo- do lewostronnych, frekwencja roślin dwu-, trój- i czteroczłonowych, frekwencja roślin anomalnych. Wyniki opracowane statystycznie (tabela 2) wskazują, że naturalne populacje *Lemna minor* i *Spirodela polyrrhiza* pochodzące z 16 różnych siedlisk różnią się między sobą istotnie pod względem badanych cech.

Stwierdzono ujemną zależność między wartością wskaźnika kształtu a kątem wierzchołkowym oraz między średnią powierzchnią członu a frekwencją roślin trój- i czteroczłonowych (tabela 4, ryc. 1 i 2), co potwierdza słuszność wyboru charakterystyk.

W doświadczeniu, którego celem było stwierdzić, czy rośliny z różnych naturalnych populacji zachowują swe odrębne cechy w kulturze *in vitro* (warunki stenotopowe), oprócz cech osobniczych, zbadano również liczbę członów, szybkość rozmnażania roślin oraz biomasę po 14 dniach hodowli, jako właściwości grupowe. Wyniki wskazują (tabela 3) na istotne zróżnicowanie populacji pod względem badanych cech w kulturze *in vitro*.

Na podstawie przeprowadzonej analizy porównawczej naturalnych i doświadczalnych populacji obu badanych gatunków pod względem charakterystyk osobniczych (tabela 5), stwierdzono, które z nich zachowują jednakże wartości swych cech w hodowli (warunki stenotopowe). Pozwoliło to określić te populacje jako ekotypy.

uwarunkowane w naturze różnym trofizmem wód. Populacje, które wykazały istotne różnice w wartościach analizowanych cech podczas hodowli uznano za ekofeny.

O zróżnicowaniu ekologicznym populacji *Lemna minor* i *Spirodela polyrrhiza* pod wpływem chemicznych właściwości siedlisk, świadczą stwierdzone zależności (tabela 4) między charakterystykami roślin i siedlisk: Korelacja między frekwencją roślin dwuczłonowych oraz trójczłonowych a zawartością potasu w wodzie (ryc. 3 i 4), korelacja między biomasą a liczbą członów roślin i zawartością żelaza w wodzie, korelacja między biomasą i liczbą członów a łączną zawartością żelaza i magnezu w badanych siedliskach.

Naturalna radioaktywność siedlisk z Radoniowa ma szczególną rolę w ekotypowym zróżnicowaniu populacji *Lemna minor*, co wyraża się niskim potencjałem biotycznym i wysoką ilością anomalii rozwojowych.