Effects of nitrogen and phosphorus upon the growth of some "Lemnaceae"

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Effects of nitrogen and phosphorus upon the growth of some *Lemnaceae*

by

Annamaria Lüönd

Contents

1. Introduction

Species of the duckweed family were investigated by numerous authors (e.g. LANDOLT 1957, 1980, 1980a; HILLMAN 1961). They proved to be particularly valuable for physiological studies for they can be easily grown in aseptic cultures and, because of their predominating vegetative reproduction, they mostly represent a genetically uniform material.

The *Lemnaceae* not only are important objects for physiological research but also attract more and more interest as crop plants. Their rapid growth, nutritional value and high biomass productivity suggest their use in waste water treatment (HILLMAN and CULLEY 1978).

To get a better understanding of physiological requirements of the duckweeds, the effect of the nitrogen and phosphorus uptake was observed in some strains from Central Europe. It was supposed that particular taxa may use in differential way various concentration of the nutritional salts.

Parallel to the experimental studies, the occurrence of the *Lemnaceae* in natural habitats of Switzerland and some adjacent regions (e.g. low plains of the Upper Rhine, ricefields of the Piedmont) was tested; a particular attention was given to the N- and P-content within various localities in view of possible relations between physiological behaviour of the duckweeds and their ecological requirements.

The present report deals with investigations on the physiological effects of N- and P-nutrient. The ecological problems will be discussed in a further paper.

Acknowledgements

Thanks are due to Prof. Dr. E. Landolt who stimulated me to undertake this study, provided the plant material and useful information. The help of Prof. Dr. K. Urbanska-Worytkiewicz who revised and improved the English text is greatly appreciated. Ms. A. Hegi helped with preparation of the culture mediums, Mr. H.-R. Binz advised on some statistical problems, Mr. B. Krüsi made some helpful suggestions, Ms. A. Honegger typed the manuscript; my sincere thanks are addressed to all these persons. Field assistance of numerous colleagues from the Geobotanical Institute is also gratefully acknowledged. The research was financially supported by the Swiss Federal Institute of Technology (SFIT), Zürich.

2. Material and methods

2.1. *Cultivation*

Four species of the *Lemnaceae* were chosen for the experiments: 1) *Spirodela polyrrhiza* (7344), 2) *Lemna minor* (6578), 3) *Lemna minuscula* (8370) and *L. gibba* (8428). The numbers given in parentheses refer to the strains forming the Institute's collection of live duckweeds that comprises about 800 clonal cultures (LANDOLT and URBANSKA-WORYTKIEWICZ 1980, see the last part of the present volume).

The Hutner's medium (HUTNER 1953, HILLMAN 1961) without sugar was used; it was diluted to 1/5 of its normal concentration. Depending on the respective concentration, the pH was adjusted to 5.5 with either 5n KOH or 5n HCL.

Three series of experiments were carried out:

a) *Experiments with different nitrogen concentrations*. The nitrogen was given in form of NH_4NO_3; to eliminate the other N-sources $Ca(NO_3)_2 \cdot 4H_2O$ was replaced by $CaCl_2 \cdot 2H_2O$ and $CoNO_3$ by $CoSO_4$. The concentrations of N ranged from $4.48 \cdot 10^{-3}$ to 8750.0 mg N/l; the ten concentration grades, differing from each other by factor 5, were codified in the following way:

N1 = $4.48 \cdot 10^{-3}$ mg N/l	N6 = 14.0
N2 = $2.24 \cdot 10^{-2}$	N7 = 70.0
N3 = 0.112	N8 = 350.0
N4 = 0.56	N9 = 1750.0
N5 = 2.8	N10 = 8750.0

The control cultures were grown on the Hutner's medium diluted to 1/5. The experiment consisted of three replicas.

b) *Experiments with various nitrogen forms*. On the whole six variants respectively codified as NF1 (KNO_3), NF_2 ($CaNO_3$), NF3 ($NaNO_3$), NF4 (NH_4Cl) and as NF5 $(NH_4)_2SO_4$) were used in the same molarity of NH_4NO_3 as in the 1/5 diluted medium; the control cultures containing NH_4NO_3 were codified as NF6. Four replicas were included.

c) *Experiments with different phosphorus concentrations*. Four concentrations

of phosphorus were used. They graded from $6.95 \cdot 10^{-4}$ to 10.86 mg P/l by factor 25: P1 = $6.95 \cdot 10^{-4}$ mg P/l P3 = 0.434 mg P/l

P2 = $1.73 \cdot 10^{-2}$ P4 = 10.86

This experiment consisted of six replicas. Two additional grades viz. P5 and P6 corresponded respectively to 271,5 and 6787.5 mg P/l. No replicas were made.

The duckweeds were grown in sterile cultures for 15 days in series comprising different concentrations of nitrogen; the series with various N-forms and those with different concentrations of phosphorus were grown for seven days.

Experiements were carried out in 500 ml Erlenmeyer flasks filled with 250 ml of medium and corked with cotton plugs, then autoclaved at 105 kPa during 20 minutes at 120°C. The flasks were subsequently inoculated with fronds and placed in a photothermostat with constant light intensity, temperature and moisture, a day-and-night rhythm being stimulated. Prior to the beginning of the actual experiments, the inoculations were repeated twice or three times at a seven-day-interval.

2.2. *Evaluation methods*

2.2.1. *Numbers of fronds and multiplication rate*

The growth of the clones was assessed by counting the fronds; the results were graphically expressed as number of fronds vs. time (Fig. 1) as well as in form of multiplication rate (Figs 5, 8, 11).

Has the multiplication rate be used as a specific characteristic of a species and its behaviour under certain conditions (e.g. the nutrient contents of the solution), the experiments are to be carried out within the phase of exponential growth (Fig. 2). It can be then assumed that the multiplication rate will not be influenced either by the reduced growth during the lag-phase or limiting factors (e.g. density of the plants, decrease of nutrients, change of pH-value). The increase of the fronds is thus proportional to the number of the existing fronds and can be expressed by the following equation:

$$\frac{dN(t)}{dt} = k \cdot N(t) \qquad (1)$$

t = time [d]
$N(t)$ = number of fronds at time t [-]
k = multiplication rate [d^{-1}]

The integration of eq. (1) yields:

$$\ln N(t) = \ln N(0) + k \cdot t \qquad (2)$$

$N(0)$ = number of fronds at time t = 0
ln = natural logarithm

which is an other form of the well-known formula for exponential growth:

$$N(t) = N(0) \cdot e^{kt} \qquad (3)$$

Eq. (2), a linear relation between the logarithm of N and the time, shows that the plotted points of the number of fronds in a logarithmical scale vs. time should theoretically lie on a straight line (Fig. 3). The multiplication rate could thus be evaluated from two counts at any time t_1 and t_2 by the following formula:

$$k = \frac{\ln N(t_2) - \ln N(t_1)}{(t_2 - t_1)} \qquad (4)$$

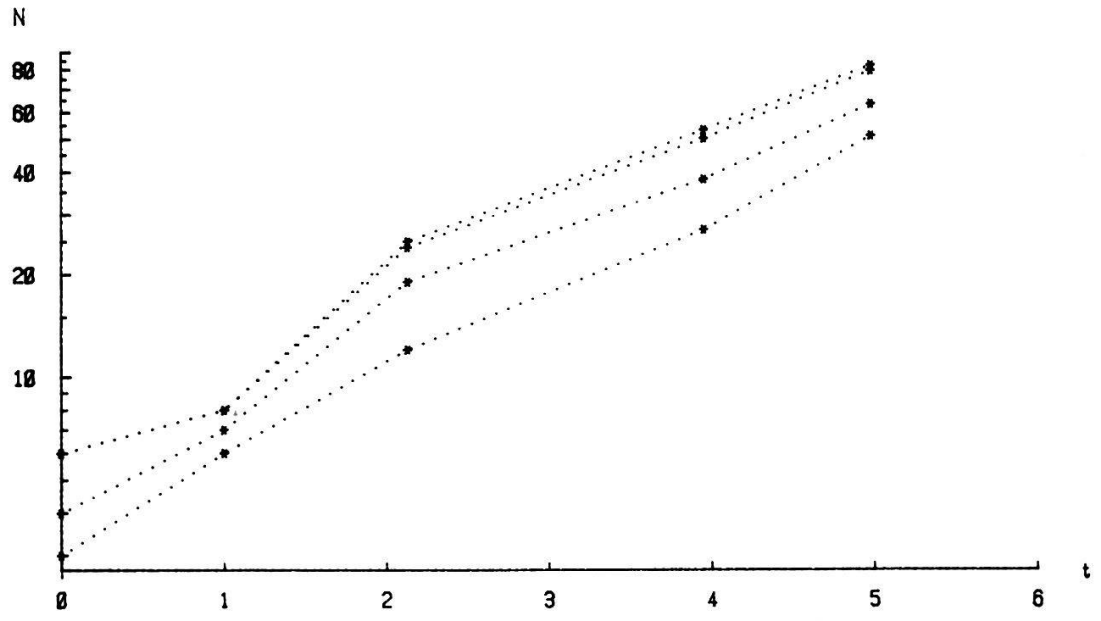

Fig. 1. Number of fronds vs. time

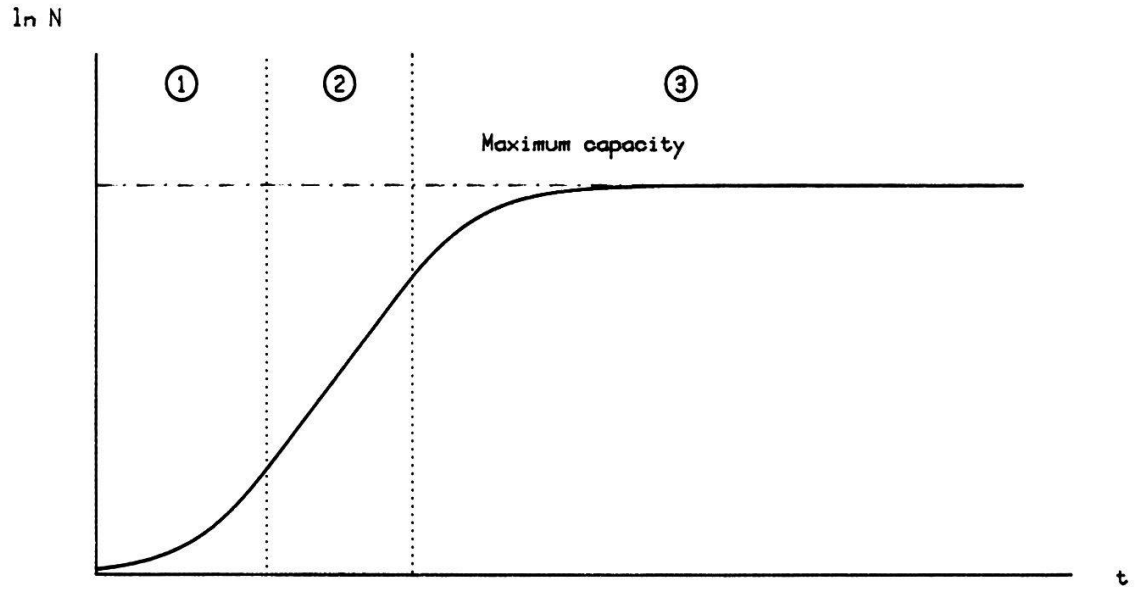

Fig. 2. Clonal growth of *Lemnaceae* in a limited area (e.g. Erlenmeyer flask).

1) *lag-phase*: The growth is reduced until the plants get used to their environment.
2) *Exponential growth*: As long as the number of fronds is small compared to the maximum capacity, the plants grow like in an unlimited environment (c.f. eq. (1)-(3)).
3) *Logistic growth*: The growth is influenced by limiting factors (e.g. area, decrease of nutrients) and becomes zero when the maximum capacity is reached. Later on, the number of fronds may decrease because of an augmented death rate.

In fact, however, the points are scattered around the theoretical line (Fig. 3.1.) which is due to: a) random deviations, b) to the fact that the fronds are counted by integer numbers and c) new fronds are not formed in regular intervals (HILLMAN 1961). If k is determined using eq. (4), all those errors may severely influence the result, because only two counts can be used at one time. The regression line g: $\ln \hat{N}(t) = a + bt$ (Fig. 3.2) is a statistical approximation to the real number-time relation. The regression coefficient b, represented by the slope of the line g, yields therefore a good estimate of the effective multiplication rate k. In the present paper, the multiplication rate was consequently evaluated by the latter method.

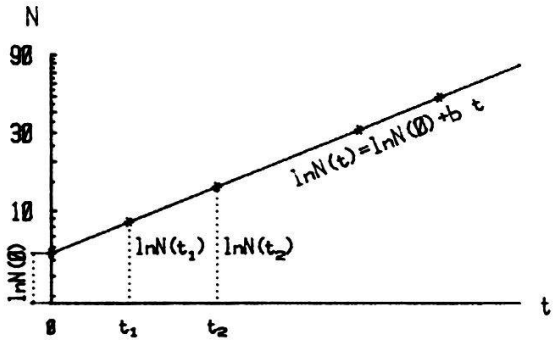

Fig. 3.1

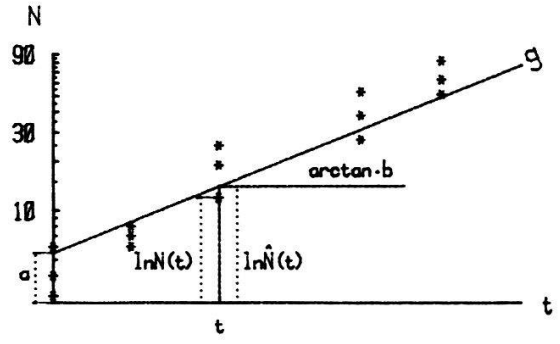

Fig. 3.2

Fig. 3. Theoretical (Fig. 3.1) and actual (Fig. 3.2) relation between the number of fronds and time.

2.2.2. *Root length and form*

A form coefficient was defined as the ratio of length to width of the fronds. The root length and the fronds were measured with the aid of copies (Fig. 4).

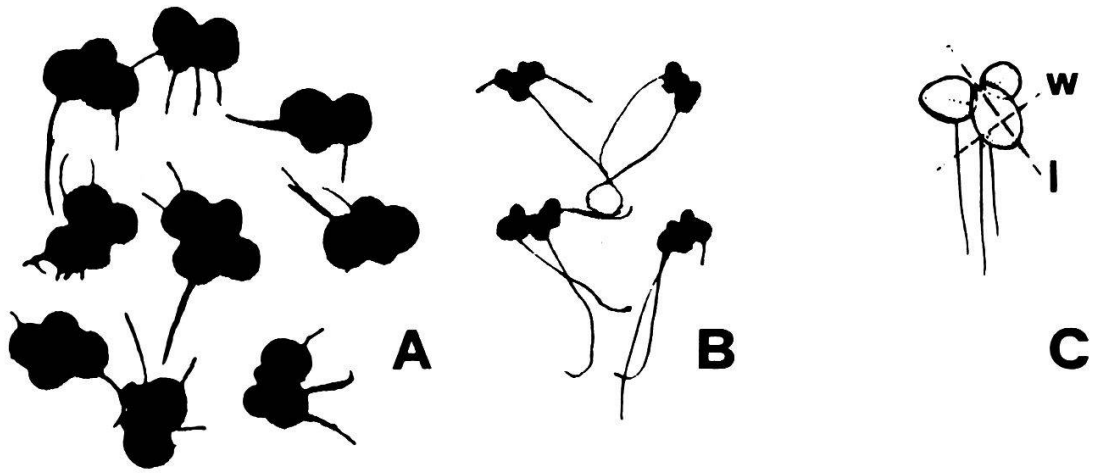

Fig. 4. A-B. Copies made from the frond groups of *Spirodela polyrrhiza* (A) and *Lemna minor* (B). C. Measurements for calculation of the form coefficient.

3. Results

3.1. *Different concentrations of nitrogen in the culture medium*

3.1.1. *Multiplication rates and some morphological aspects*

Increasing concentrations of NH_4NO_3 resulted in increasing multiplication rates until an optimum was reached (Fig. 5). This tendency was observed in all studied strains; however, optimal concentration grades in *Spirodela polyrrhiza, Lemna gibba* and *L. minor* were N6-N8 corresponding to 14.0-350.0 mg N/l, whereas in *L. minuscula* a rather well-defined optimal concentration was considerably lower viz. 0.12 mg N/l (grade N3)
Multiplication rates increased in a different way in the studied material. *Spirodela polyrrhiza* and *Lemna gibba* were comparable in this respect: up to

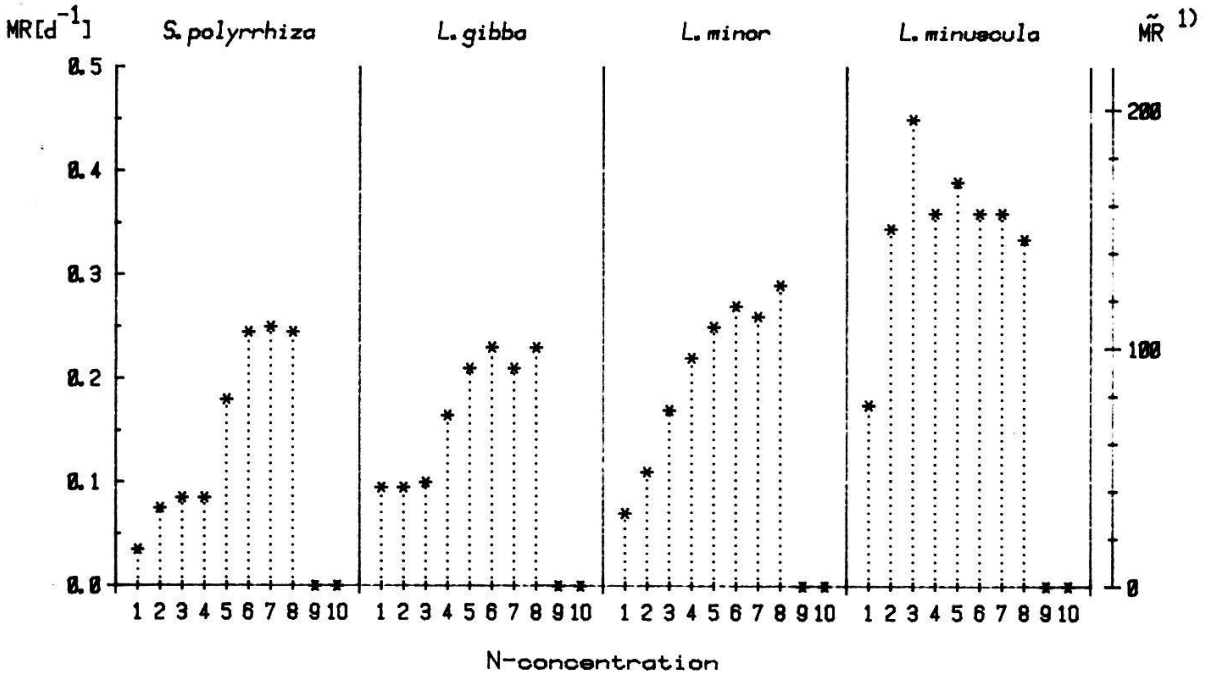

Fig. 5. Multiplication rates MR in the studied taxa following the ten concentration grades of nitrogen in the culture medium.
1) Multiplication rate $\tilde{MR}$ calculated after LANDOLT (1957), HILLMAN (1961) etc.

the concentration grades N3-N4 a rather static behaviour was observed, the multiplication rates increasing only within higher concentrations i.e. N5-N8. The response of *L. minor* towards increasing concentrations was fairly regular. On the other hand, *L. minuscula* proved to be rather sensitive towards low N-concentrations and, after having reached the optimal concentration grade N3, decreasing multiplication rates were observed.
The two highest concentration grades i.e. N9 and N10 corresponding respectively to 1750.0 and 8750.0 mg N/l resulted invariably in death of all plants already during their accommodation phase.
As far as morphological observations are concerned, *Spirodela polyrrhiza* studied in concentration grades N1-N4 mostly formed turions but occasionally also some new fronds. The newly-formed fronds were dark green with a strong development of anthocyanin on the lower surface; the older ones turned yellow. In concentration grades N5-N8 new fronds were still produced, but turions were not formed anymore. The fronds developed at the concentration grade N5 were still tinged with anthocyanin; however, in the three higher concentration grades anthocyanin was not observable.
Lemna gibba corresponded to increasing concentration of nitrogen in the same way as *Spirodela polyrrhiza*, bar the formation of turions.
Lemna minor manifested no particular morphological changes throughout the experiment, save for the differences in frond coloration occurring between plants grown on medium with lower and higher concentrations of nitrogen. In concentration grades N1-N4, the colour of fronds changed from the initial light-green to yellow; no anthocyanin was observed. Within concentration grades N5-N8, fronds of *L. minor* regained their habitual green colour.
Lemna minuscula behaved generally in a way comparable to that of *L. minor*, but the change of colour in its fronds was observable a little earlier viz. at the concentration grade N4. At the concentration grade N8, numerous light-green fronds as well as dead ones appeared within the culture.

3.1.2. *Root length*

Interspecific differences in the root length are of a common occurrence within the duckweed family. The intra-individual variation observed in the course

Table 1. Maximum root length and the corresponding N-concentrations in the culture medium

Taxon	Maximal root length (cm)	Concentration (mg/l)
Spirodela polyrrhiza	2.54	0.112
Lemna gibba	4.98	2.8
Lemna minor	2.34	0.112
Lemna minuscula	0.88	0.112/0.56

of the present study was influenced, on the other hand, by the nitrogen concentration. The root length was expected to decrease with increasing nutrients content (WHITE 1936b, 1937a,b; WHITE and TEMPELMANN 1937, PIRSON and GÖLLNER 1953). The present experiments suggest, however, that the nitrogen concentrations optimally influencing the root growth do not necessarily represent low values; our results point out as well that the optimal nitrogen concentrations may vary from one taxon to another (Table 1).

The results of the experiments are graphically presented in Fig. 6. It should be noted that numerous roots fell off in cultures grown on medium containing low nitrogen concentrations. The presented values being thus subject to some errors, the most doubtful points are referred to by question marks.

Spirodela polyrrhiza reached a maximal root length i.e. 2.8 cm at the concentration grade N3; a decreasing tendency was observed within grades N4-N6, but from this point on an apparently stabilized root length of about 0.7-0.77 cm was noted. *Lemna gibba* developed the longest roots (4.9 cm) at the concentration grade N5; in higher concentrations, a clear decrease was observed, the lowest value of 1.2 cm occurring at the concentration grade N8. *L. minor* manifested a pattern comparable to that of *Spirodela polyrrhiza*, the maximal root length being, however, 3.8 cm. *Lemna minuscula* showed in general not a very pronounced variation, but the differences were statistically significant; the maximal root length observed at the concentration grade N3 was 0.88 cm, whereas the shortest roots (0.24 cm) appeared at the concentration grade N6.

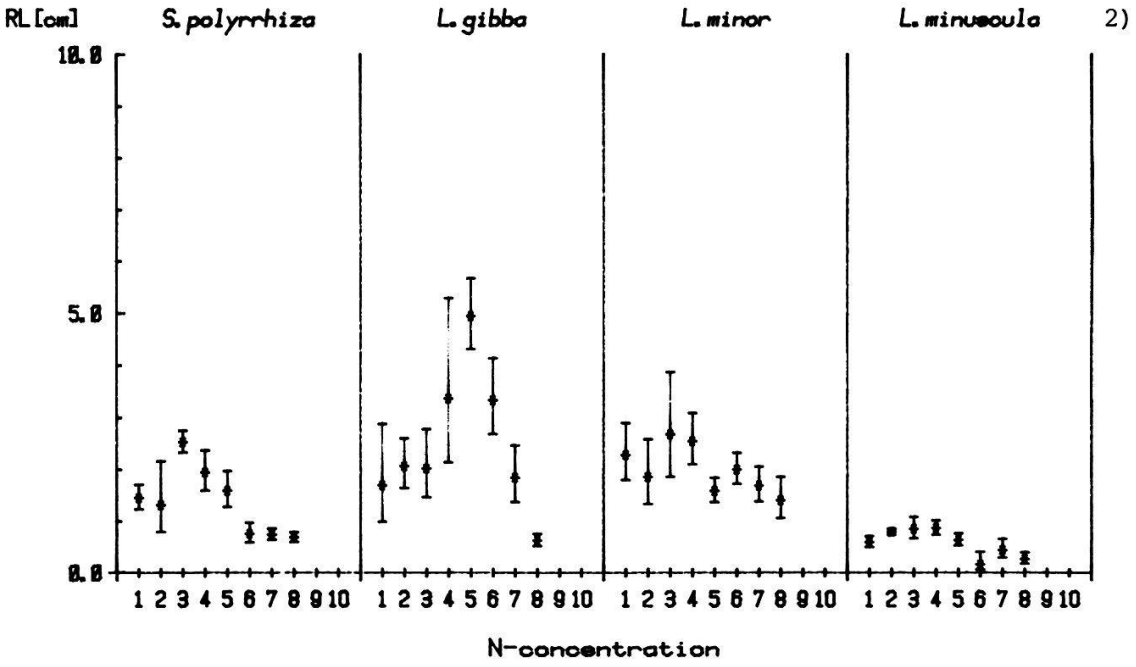

Fig. 6. Root length in the studied taxa following the eight concentration grades of nitrogen in the culture medium.
2) Means and standard deviations were calculated by log-data.

3.1.3. *Form coefficient*

As mentioned before, the form coefficient was worked out as the length/width ratio of the fronds. The results are presented in Fig. 7. The concentration of nitrogen apparently had no effect upon the form of the fronds. The differences between the obtained values were not statistically significant.

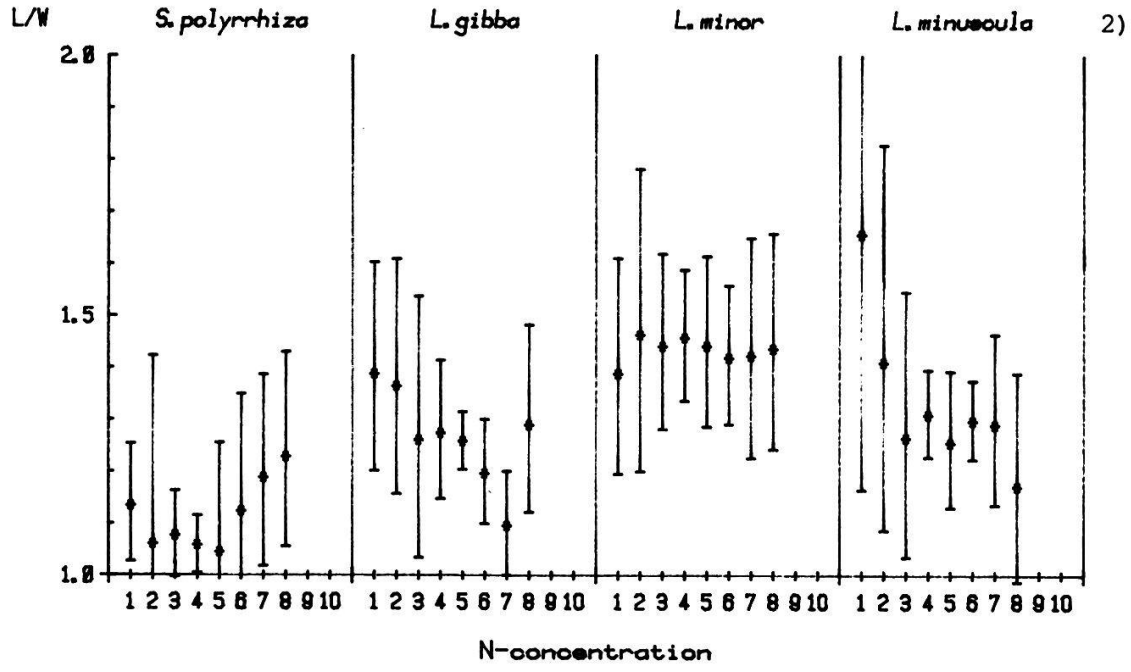

Fig. 7. Form coefficient of the studied taxa following the eight concentration grades of nitrogen in the culture medium.

3.2. *Experiments with various forms of nitrogen*

3.2.1. *Multiplication rate*

Multiplication rates in all species grown in medium with nitrate were significantly lower than those of strains feeding on ammonium, the differences being of about 20 per cent. Comparable differences were observed between cultures with NO_3-N and the control ones containing NH_4NO_3. It should be noted, however, that each of the four studied taxa showed a little different pattern of variation related to different nitrogen forms (Fig. 8). Observations on the morphology of fronds did not reveal any aberrations, both the form and the colour being typical of particular taxa.

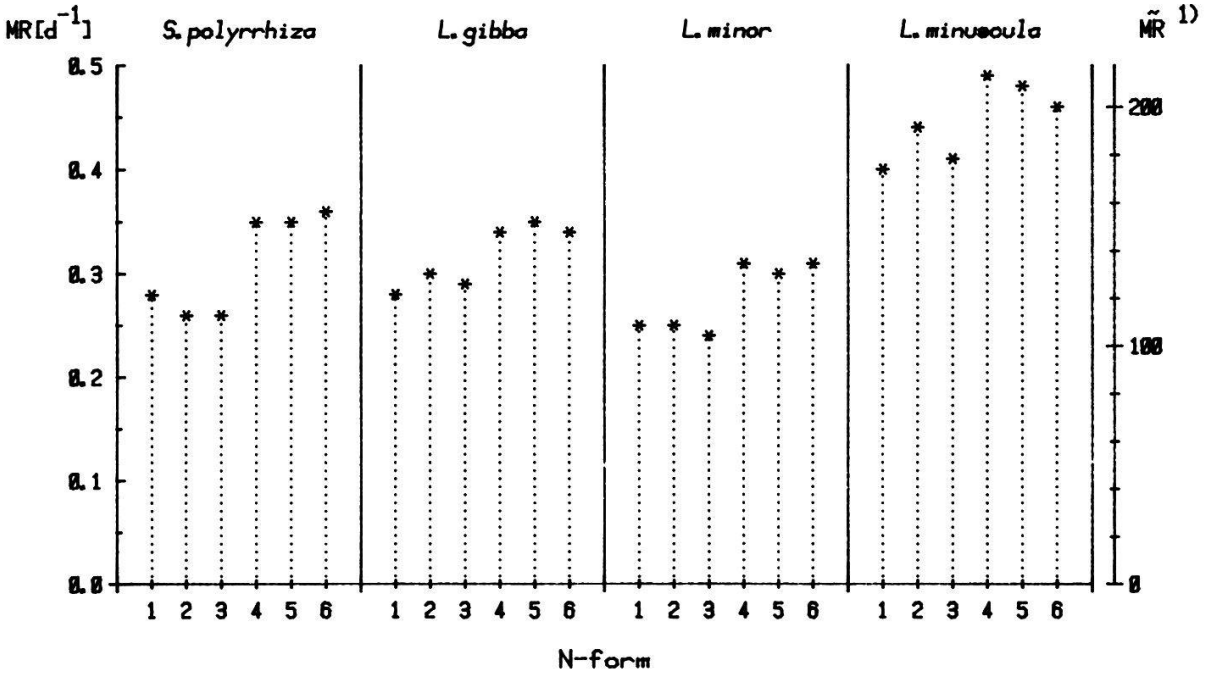

Fig. 8. Multiplication rates MR in the studied taxa following six various forms of nitrogen in the culture medium.

3.2.2. *Root length*

Compared to the multiplication rates, an opposite tendency was observed in influence of various N-forms upon the root length: roots produced by clones feeding on NH_4-N were about one-half to one-third shorter than those observed in cultures with NO_3-N (Fig. 9). The longest roots comporting 8.1 cm were produced by *Lemna gibba*, the shortest ones (0.42 cm) appeared in *Lemna minuscula*.

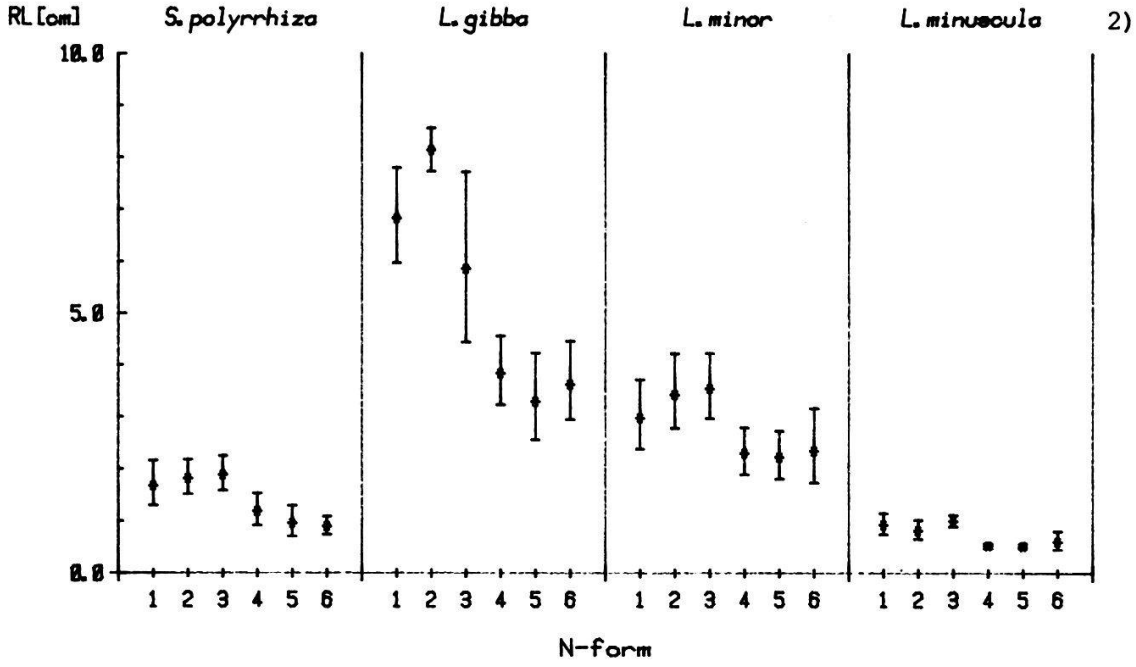

Fig. 9. Variation in root length in the studied taxa following the six various forms of nitrogen in the culture medium.

3.2.3. *Form coefficient*

The results obtained in this respect clearly suggest no apparent relation between a particular nitrogen form contained in the culture medium and the form of the fronds (Fig. 10).

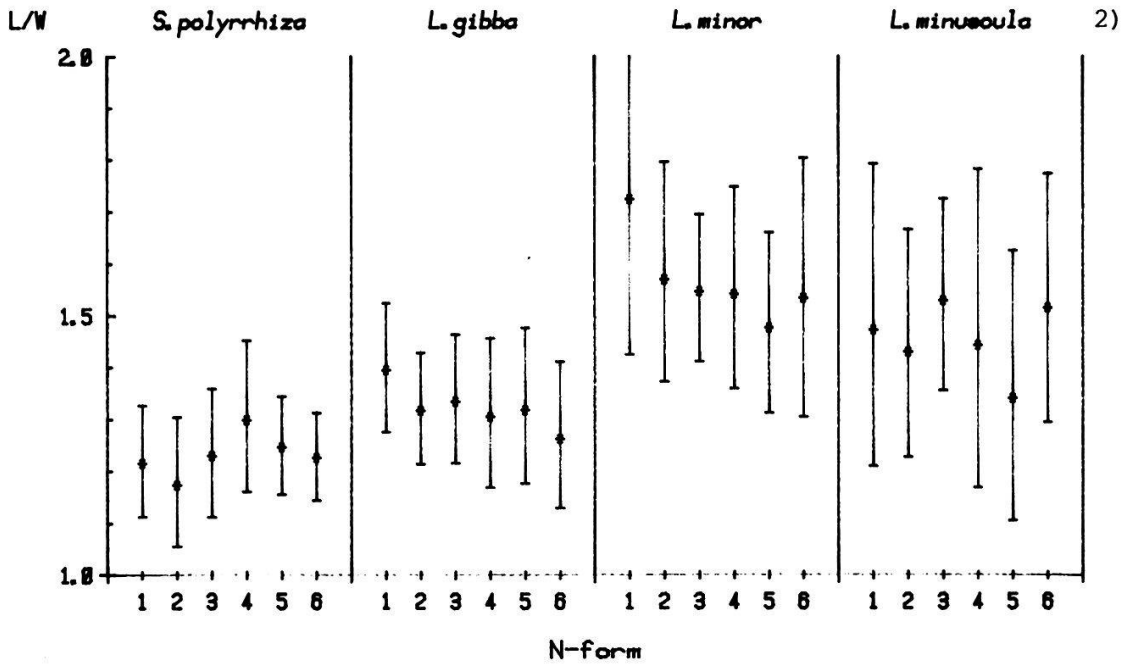

Fig. 10. The form coefficient in the studied taxa following the six various forms of nitrogen in the culture medium.

3.3. *Experiment with different concentrations of phosphorus in the culture medium*

3.3.1. *Multiplication rate and morphological aspects*

On the whole, multiplication rates observed in this experiment were comparable to those found in series with different concentrations of nitrogen (see p. 125 and 126).

Spirodela polyrrhiza cultivated in two deficient solutions P1 and P2 with respective content of phosphorus $6.95 \cdot 10^{-4}$ and $1.74 \cdot 10^{-2}$ mg/P1 formed mostly turions and also some fronds that were strongly coloured with anthocyanin. The multiplication rate of this taxon reached its maximum of $0.34[d^{-1}]$ in the P3 series corresponding to 0.43 mg/P1 (Fig. 11); the fronds were normally green. As far as the control series P4 containing 10.68 mg P/1 is concerned, the appearance of the fronds was also quite normal. In experiments carried

out with medium grade P6 containing 6787.5 mg/P1 i.e. a concentration of phosphorus that was 625 times higher than that in the control cultures, fronds of all taxa died already during the accommodation phase. The behaviour of plants grown at the grade P5 was not yet studied. The tendency observed in *Lemna gibba* was fairly different from all other studied taxa. The multiplication rate appearing in both deficient solutions P1 and P2 was low and the anthocyanin occurred in the fronds. The two higher concentrations i.e. P3 and P4 resulted in a distinct increase of the multiplication rate; no anthocyanin was observed in the fronds. In *Lemna minor*, high concentrations of phosphorus in the culture medium were positively correlated with increased multiplication rates, the colour of the fronds being normally green. On the other hand, fronds in the P1 and P2 series changed their colour from green to light-green. The variation in multiplication rate occurring in *Lemna minuscula* corresponded in general to the pattern observed in *Spirodela polyrrhiza*; the values, however, were higher (Fig. 11).

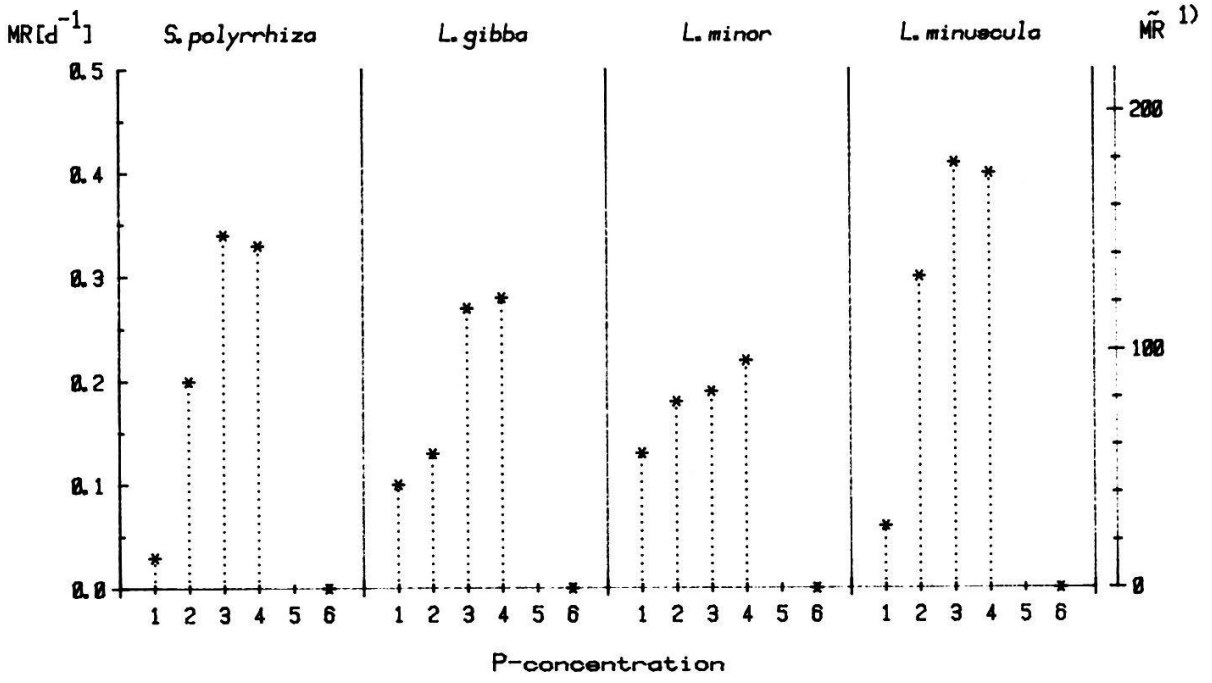

Fig. 11. Multiplication rates in the studied taxa following the five different concentrations of phosphorus in the culture medium.

3.3.2. *Root length*

The influence of different phosphorus concentrations upon the root length was not uniform. Two tendencies observed in the studied material were represented, on the one hand, by *Spirodela polyrrhiza* as well as *Lemna gibba* and, on the other hand, by *Lemna minor* and *Lemna minuscula*.

In *Spirodela polyrrhiza* an initial root length remained invariable in the two deficient solutions P1 and P2 and increased only in the P3 series, but then decreased again reaching in the P4 solution virtually the same value as that in P1 and P2. A comparable pattern occurred in *Lemna gibba* (Fig. 12). On the other hand, *Lemna minor* and *L. minuscula* produced the longest roots in both deficient solutions, whereas a well-marked decrease in root length appeared in series with higher phosphorus concentrations.

It should be added that the longest roots in the present experiment were produced by *Lemna minor* (6.4. cm), whereas in the series with different nitrogen concentrations maximal root length (4.98 cm) was observed in *Lemna gibba*.

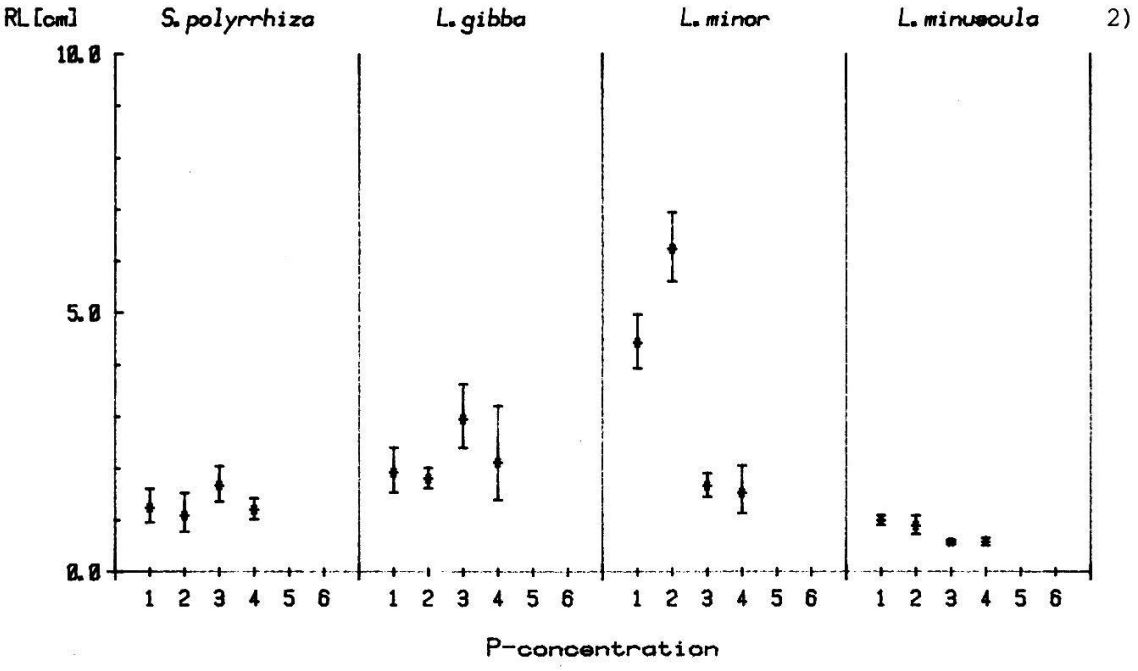

Fig. 12. Variation in root length in the studied taxa following four concentrations of phosphorus in the culture medium.

3.3.3. *Form coefficient*

The study revealed no significant correlations between the form coefficient and the concentration of phosphorus in the culture media (Fig. 13). In this respect, the presented results corroborate our data on nitrogen concentration and various nitrogen forms as being of no influence upon the form coefficient, presented in a former part of this report (see p. 128 and 131).

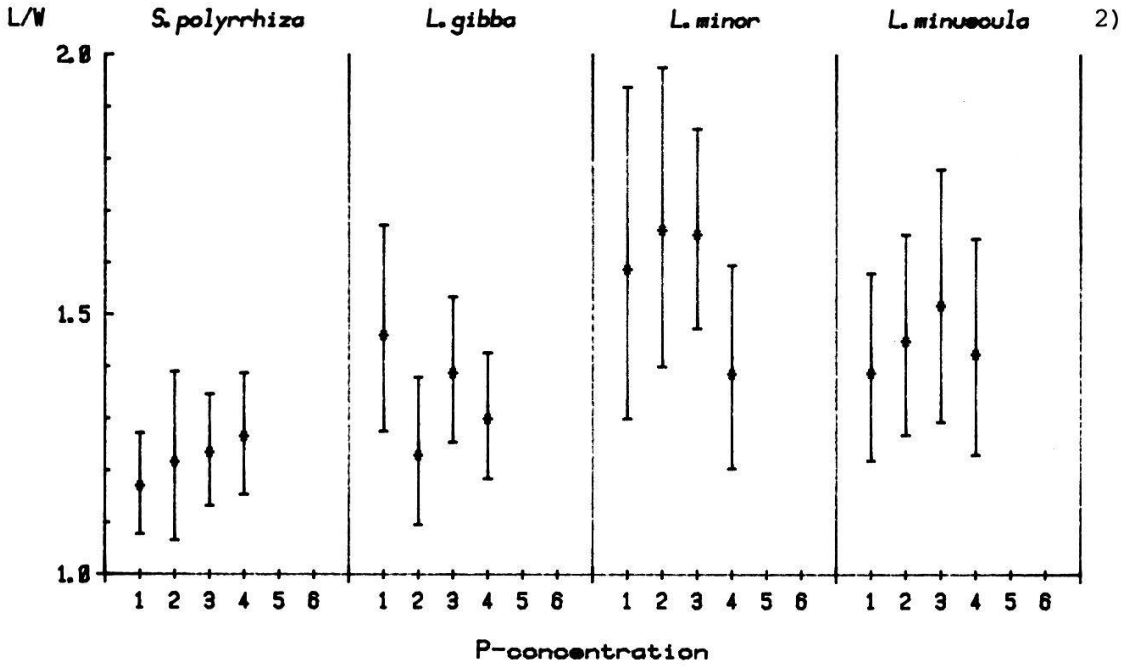

Fig. 13. Form coefficient of the studied taxa following the four different concentrations of phosphorus in the culture medium.

4. Discussion

Nitrogen and phosphorus play a particularly important rôle in the nutrition of the duckweeds. Not only do they influence protein and oxalat synthesis and dry matter production (BORNKAMM 1965), the content of phytic acid (SCHEINER et al. 1978), as well as the ageing of the fronds (BöSZöRMENYI and BöSZöRMENYI

1957), but also the multiplication rates and the morphology (LANDOLT 1957). The present results corroborate the previous data, especially as far as the two latter aspects are concerned. In this respect, three categories of the nutrient solutions viz. deficient, optimal and exceeding ones could have been distinguished in relation to different N- and P-concentrations. Deficient solutions resulted in low multiplication rates and elongation of roots. They also affected various morphological aspects (e.g. turion formation, content of anthocyanin, colour of fronds). In *Spirodela polyrrhiza, Lemna gibba* and *L. minor*, this category of nutrient solutions corresponded to the N-concentrations ranging from $4.48 \cdot 10^{-3}$ to 0.56 mg/N1 i.e. grades N1-N4, whereas in *L. minuscula* a more limited concentration bracket of $4.48 \cdot 10^{-3}$ to 0.112 mg N/1 (N1-N3) resulted in deficiency symptoms. As far as the phosphorus concentrations are concerned, the deficient solutions observed in all four studied taxa corresponded to $6.95 \cdot 10^{-4}$ to $1.73 \cdot 10^{-2}$ mg/P1 (grades P1-P2). Comparable results with P-deficient solutions were obtained by REID and BIELESKI (1970) in *Spirodela punctata* (=*S. oligorrhiza*) as well as by JUNGNICKEL (1978) in *Spirodela polyrrhiza, Lemna minor, L. minor* f.*leptophylla, L. gibba, L. trisulca* and *Wolffia arrhiza*. Similar responses to N-deficient medium were obtained by HUMPHREY et al. (1977) in *L. minor*. According to SARAWEK and DAVIES (1977a, b), activity and aldolase in N-deficient cultures was critically hampered by increased amount of low-molecular proteins. Our results can be considered as yet another contribution to the general knowledge of response patterns occurring within the duckweed family; it should be most interesting to include further taxa in experiments with various nutrient concentrations.

Optimal solutions were characterized by high and constant multiplication rates, the appearance of fronds being typical of given taxa. The optimal nitrogen concentrations observed in our study were those of 14.0 to 350.0 mg N/1 (N6-N8) in *Spirodela polyrrhiza, Lemna gibba* and *L. minor*, whereas optimal response of *L. minuscula* was observable already at lower concentrations i.e. 0:56 and 2.8 mg N/1; the latter taxon revealed thus a fairly good performance within a rather broad concentration spectrum of nitrogen (N4-N7). As far as the optimal phosphorus concentrations are concerned, the respective values in the studied material comprised 0.434-10.86 mg P/1 (grades P3-P4) in *Spirodela polyrrhiza, Lemna gibba* and *L. minuscula*, whereas in *L. minor* one lower

concentration grade i.e. P2 corresponding to 1.73 10^{-2} mg P/l caused comparable effects.

Exceeding solutions were referred to when multiplication rates decreased, the cultures desisted from growing and eventually died. In experiments with different nitrogen concentrations, exceeding solutions in *Lemna minuscula* contained 350.0-8750.0 mg N/l (grades N8-N10); in three remaining taxa, they corresponded to grades N9-N10. Our results remain open to further verifications, no data on the subject being unfortunately available in the literature. As to the influence of the phosphorus, effects of the highest concentration used in the present study viz. P6 grade were obviously lethal. However, the series P5 being not studied, further investigations are required.

Morphological observations of the previous authors (WHITE 1936b, REID and BIELESKI 1970, JUNGNICKEL 1978) were confirmed by the present results. In our experiments, deficiencies in nitrogen and phosphorus affected forming of turions in *Spirodela polyrrhiza*, anthocyanin in *Sporodela polyrrhiza* and *Lemna gibba* and colour alterations in all four taxa. The variation in the root length did not reflect any particular influence of the nutritional components; the only exception was *L. minor*, which produced exceedingly long roots in P-deficient solutions, although multiplication rates of this species were rather regularly increasing from low to high concentrations. It seems that *L. minor* manifested its P-deficiency more on root length than in multiplication rate.

The multiplication rate appeared to depend generally on the size of the fronds, larger plants (*Spirodela polyrrhiza, Lemna gibba*) being more sensitive to nitrogen deficiencies than the smaller ones (*L. minor, L. minuscula*); in the present study, *L. minor* and *L. minuscula* showed a rather regularly increasing multiplication rate from one concentration grade to the other, this tendency being not observable in *Spirodela polyrrhiza* and *Lemna gibba*. In the experiment with different concentrations of phosphorus, the pattern was not very pronounced, greater differences between particular concentration grades being perhaps responsible.

As far as various forms of nitrogen are concerned, the present study revealed higher multiplication rates in solutions containing NH_4-N than in those with NO_3-N in normal CO_2-concentration (300 ppm), no differences in pH being observed at the end of the experiment between the NO_3- and NH_4-medium. MüLLER

et al. (1977) found that in NH_4-containing medium with high CO_2-concentration (9000 ppm) in air, multiplication rates were higher than those in NO_3-medium; however, no difference was obtained in low CO_2-concentration (100 ppm) and constant pH-values. The differences between the previous and the present data could be influenced by different mediums and different pH; MüLLER et al. (1977) used a special solution prepared according to ERISMANN and FINGER (1968) with pH = 6.5. In our experiment Hutner's medium was used (pH = 5.5). It should be added that LANDOLT (unpubl.) observed in some duckweeds (e.g. *Spirodela polyrrhiza, Lemna minor, L. gibba* etc.) much longer roots grown in cultures with NO_3-N than in those fed on NH_4-N-medium as sole nitrogen source.

The present results suggest that the duckweeds might prefer relatively high concentrations of nitrogen and phosphorus for their nutrition; however, maximal concentrations seem to be limited as indicated by the experiment with more than 350.0 mg N/l in which all the fronds died (see p. 127). Our study, however, was limited to four clones, each representing a different taxon; it should be most desireable to investigate experimentally the nutritional requirements of the *Lemnaceae* not only in various strains of a given taxon, but also in more species of the family. Our study is in progress.

Summary

Influence of nitrogen and phosphorus uptake on multiplication rates, root length and length/width ratio of the fronds was studied in four duckweed taxa. Under constant conditions, clonal cultures of *Spirodela polyrrhiza, Lemna gibba, L. minor* and *L. minuscula* were grown on mediums containing different N- or P-concentrations; the third series of experiments was carried out with various forms of nitrogen in the medium.

Three categories of the nutrient solutions viz. deficient, optimal and exceeding ones were distinguished in relation to different N- and P-concentrations. Deficiencies in nitrogen and those in phosphorus resulted in diminished multiplication rates. Deficient N-solutions in *Spirodela polyrrhiza, Lemna minor* and *L. gibba* corresponded to concentration grades N1-N4 ($4.48\ 10^{-3}$-0.56 mg N/l), the variation spectrum in *L. minuscula* being a little more limited (N1-N3). Phosphorus deficiency appeared in concentration grades P1-P2 ($6.95\ 10^{-4}$-$1.73\ 10^{-2}$ mg P/l) in all four studied taxa. Optimal nitrogen concentrations observed in *Spirodela polyrrhiza, Lemna gibba* and *L. minor* ranged from 14.0 to 350.0 mg N/l (N6-N8), a slight shift towards

lower concentrations (N4-N7) being noted in *L. minuscula*. Optimal phosphorus concentrations corresponded to P3-P4 grades (0.434-10.86 mg P/l) in *Spirodela polyrrhiza, Lemna gibba* and *L. minuscula*, whereas *L. minor* manifested an optimal performance already at the P2 grade ($1.7\ 10^{-2}$ mg P/l). As far as the N-exceeding solutions are concerned, *L. minuscula* proved to be more sensitive than the three other studied taxa; its exceeding solutions corresponded to the grades N8-N10 (350.0-8750.0 mg N/l), whereas in *Spirodela polyrrhiza, Lemna minor* and *L. gibba* they were identified as N9-N10. Effects of the one studied solution with exceedingly high P-concentration were lethal; however, further experiments with P-exceeding solutions are required.

N- and P-deficient solutions resulted in turion formation in *Spirodela polyrrhiza*, as well as colour alterations in all four taxa. In higher concentrations, the plants regained their normal appearance. The variation in root length did not reflect any particular influence of nutritional components, save for *Lemna minor* that formed exceedingly long roots in P-deficient solutions.

Multiplication rates in solutions containing NH_4-N proved to be higher than those in media containing NO_3-N.

Zusammenfassung

Bei vier *Lemnaceae*-Arten wurde der Einfluss von Stickstoff und Phosphor auf die Wachstumsrate, die Wurzellänge und das Längen/Breiten-Verhältnis untersucht. Unter konstanten Bedingungen wurden Kulturen von *Spirodela polyrrhiza, Lemna gibba, L. minor* und *L. minuscula* auf verschieden konzentrierten N- und P-Medien herangezogen; eine dritte Serie wurde mit verschiedenen Stickstoffformen ausgeführt.

Drei Kategorien von Nährlösungen konnten bezüglich dem N- und P-Angebot unterschieden werden: Mangel-, optimale und übermässige Nährlösungen.

Stickstoff- und Phosphormangel äusserten sich in verrringerten Wachstumsraten. Bei *Spirodela polyrrhiza, Lemna minor* und *L. gibba* war das in den vier Konzentrationen zwischen $4.48 \cdot 10^{-3}$ und 0.56 mg N/l (N1-N4) der Fall. *Lemna minuscula* wies nur in den ersten drei Konzentrationen $4.48 \cdot 10^{-3}$, $2.24 \cdot 10^{-2}$ und 0.112 mg N/l einen Mangel auf. Phosphormangel wurde bei allen vier Arten in den Konzentrationen $6.95 \cdot 10^{-4}$ und $1.73 \cdot 10^{-2}$ mg P/l (P1-P2) beobachtet.

Optimale Stickstoffernährungen wurden bei *Spirodela polyrrhiza, Lemna gibba* und *L. minor* in den Konzentrationen von 14, 70 und 350 mg N/l (N6-N8), bei *L. minuscula* in 0.56, 2.8, 40 und 70 mg N/l (N4-N7) gefunden. Optimale P-Konzentrationen entsprechen 0.434 und 10.86 mg P/l (P3-P4) bei *Spirodela polyrrhiza, Lemna gibba* und *L. minuscula*; bei *L. minor* war bereits in $1.73\ 10^{-2}$ mg P/l (P2) eine optimale Ernährung zu beobachten.

Gegenüber zu hohen Stickstoffgaben verhält sich *Lemna minuscula* etwas anders als die übrigen Arten: bereits bei 350 mg N/l (N8) weist sie eine niedrigere Vermehrungsrate auf, bei noch höheren Konzentrationen stirbt sie, wie die drei anderen Arten, ab. Die Auswirkung von zu hohen Phosphorwerten wurde in

einem parallelen Experiment mit 6787.5 mg P/l untersucht: in diesen extrem hohen Konzentrationen starben die Glieder aller vier Arten. Weitere Untersuchungen sind im Gange.

N- und P-Mangellösungen riefen Turionenbildung bei *Spirodela polyrrhiza* und Farbveränderungen bei allen vier Arten hervor. In höheren Konzentrationen zeigten die Pflanzen ein normales Aussehen. In der Wurzellänge konnte kein Unterschied bezüglich N- und P-Ernährung festgestellt werden; ausgenommen bei *Lemna minor*, die in P-Mangellösungen extrem lange Wurzeln bildete.

Die Vermehrungsraten in NH_4-N enhaltenden Medien waren höher als jene mit nur NO_3-N.

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