# ON THE OCCURRENCE OF THE FLAT FORM OF LEMNA GIBBA L. IN NATURE

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### **SUMMARY**

Lemna gibba vegetations which remain flat throughout the summer season have not been observed in the field. However, a flat "winterform" could be found above a hydrosoil of clay until May and above a hydrosoil of sand or peat until July/August. It has been concluded that flat Lemna vegetations in The Netherlands consist either of pure L. minor (mostly above sand), a mixture of L. minor and (temporarily) flat L. gibba, or exceptionally, (temporarily) flat L. gibba only.

#### 1. INTRODUCTION

The occurrence of flat forms of Lemna gibba L. was first reported by HEGELMAIER (1868). Subsequently Guppy (1894) observed that flat plants are budded off in the autumn, while in spring flat plants produce gibbous daughter plants. This phenological nature of the flat form of L. gibba was also mentioned by Bowen (1958), Clapham et al. (1962) and Schultz (1962). However, apart from these winterforms, flat modifications of L. gibba were described, occurring under the influence of, e.g., shade, stagnant water and low temperature (Van Horen 1869), low pH (Segal & Groenhart 1967), and a low mineral content of the water (Bowen 1958; Segal 1966; De Lange & Segal 1968). In addition it was suggested that the flat form of L. gibba is genetically based (Mason 1957; De Sloover 1966; Den Hartog 1968).

It has been postulated that in The Netherlands many reports on *L. minor* in fact should refer to flat forms of *L. gibba* (SEGAL 1966; DE LANGE & SEGAL 1968). Although morphological criteria to distinguish between the two taxa had been given by HEGELMAIER (1868), DE LANGE & SEGAL (1968), KANDELER (1975), LANDOLT (1975), DE LANGE (1975) and DE LANGE & WESTINGA (1979), it was stated in the latter two publications that there exists a large overlap and inconsistency in these characteristics.

PIETERSE et al. (1970) and PIETERSE (1975) reported that by means of the chelating agent EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid) it can be

tested under laboratory conditions whether flat plants are potentially gibbous. By menas of this diagnostical method it was shown that supposedly flat forms of L. gibba did not become gibbous and it was concluded that these plants belonged to L. minor (DE Lange & Pieterse 1973). Moreover, translocation in the field of a supposedly flat form of L. gibba to a site where gibbous forms occur, did not result in a transition to the gibbous form (DE Lange 1974). For field identifications, where the EDDHA test cannot be applied, the term L. gibba/L. minor complex was suggested (DE Lange & Pieterse 1973).

Recently, it was demonstrated by means of the EDDHA test that in The Netherlands most *Lemna* populations consist of a mixture of *L. gibba* and *L. minor* (DE LANGE et al. 1981). This suggests that the habitat of the two species overlap more than previously had been assumed (DE LANGE & SEGAL 1968) and a synsystematic recognition of this finding was suggested (DE LANGE et al. 1981). Moreover, on the basis of in vitro studies it was proposed that in nature gibbosity is regulated by ethylene which is produced by the hydrosoil (PIETERSE 1976; ELZINGA et al. 1980).

The purpose of the present study was to ascertain whether the flat form of *L. gibba* is a "winter form", or that, under certain conditions in The Netherlands, it is persistent throughout the summer season.

# 2. MATERIALS AND METHODS

In the period May/October 1982, 351 Lemna (i.e. plants which are either L. gibba or L. minor) vegetations were studied in different areas in The Netherlands. The research areas are located in the Provinces of Noord-Holland, Zuid-Holland, Utrecht, Gelderland, Overijssel and Drenthe. From 29 vegetations which consisted exclusively of flat plants a sample was taken (samples 1–29). In addition flat plants were sampled from 7 mixed vegetations (i.e. vegetations of gibbous and flat plants (samples 30–36). For details about the location of the 36 sites sampled is referred to Wetsteyn (1983). On each collection site ambient water was collected for chemical analysis in the Hugo de Vries Laboratory.

In the laboratory length, width and gibbosity of 20–30 specimens in each plant sample were measured and subsequently 10 single plants were grown axenically on M-medium (HILLMAN 1961) supplemented with 10 g/l sucrose and 10 mg/l EDDH (ethylenediamine-di-o-hydroxyphenylacetic acid) in 100 ml Erlenmeyer flasks (one plant per flask). If there were morphologically different flat forms in a plant sample, 10 plants were taken of each form. Sterilization of the plant material was according to De Lange & Pieterse (1973). The Erlenmeyer flasks were kept in a growth cabinet at a temperature of 25° ± 2°C under a continuous illumination of 8000 erg. cm<sup>-2</sup>. sec<sup>-1</sup> at plant level from Gro-Lux fluorescent tubes (F40 T12/gro). After a period of 18–40 days a sample of 20–30 plants was used for recording morphological data. Measuring was performed with a sliding gauge with nonius (accuracy 0.05 mm). On the basis of previous studies (De Lange & Pieterse 1973; De Lange et al. 1981) a gibbosity of 1 mm was used as a yardstick for the distinction between the comceptions "gibbous" and

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"flat", and a marked reaction on EDDHA – i.e. gibbosity attained > 1 mm – for the distinction between L. gibba and Lemna minor.

# 3. RESULTS

Most vegetations, i.e. 306 out of 351, were partly or exclusively gibbous from May/June onwards. At many sites a transition could be observed from flat to gibbous during the investigation period. Above a hydrosoil of clay this transition always occurred before the end of May; above sand or peat gibbosity occasionally appeared later, but not after July/August. In September the gibbous plants in the field became less inflated and eventually produced flat daughter plants.

In table 1 the dimensions of the 36 plant samples are presented, measured directly after collection and after cultivation in vitro in the presence of EDDHA. As far as the 29 vegetations are concerned, which were exclusively flat at the day of collection, it turned out that plants from 23 samples (1–23) remained flat on the EDDHA medium and consequently should be considered as vegetations of L. minor. It should be noted, however, that in the course of the summer gibbous plants were observed in the field at the sites where samples no. 1 and 14 were collected. In samples 1 and 6 there were two morphologically different strains of L. minor. Sample 24 apparently consisted only of L. gibba plants, all the plants turning gibbous after EDDHA treatment. Samples 25–29 appeared to be mixtures of L. minor and L. gibba, as part of the plants became gibbous in the presence of EDDHA. In July/August a mixture of gibbous and flat plants could also be observed at the sampling sites. The flat components of samples 30–36 consisted of L. minor only.

Flowering was not observed in the field, but after cultivation on EDDHA many plants of both *L. gibba* and *L. minor* flowered (table 1) and generally in samples collected late in summer or in the autumn.

No consistent differences were found in nutrient composition of the water in which *L. minor* only, or mixed stands of *L. minor* and *L. gibba* occurred. Pure *L. minor* stands generally grew above a sand bottom.

# 4. DISCUSSION

In the light of the results of the present study it may be assumed that flat forms of *L. gibba* do not persist throughout the summer season. Apparently flat *L. gibba* plants are winter forms, although they may occur until July/August. In the literature the flat form of *L. gibba* is frequently described as common in The Netherlands. However, as now becomes evident, it is questionable whether it has always been correctly identified, especially in connection with the statements of De Lange & Pieterse (1973) and De Lange & Westinga (1979), that flat forms of *L. gibba* do not consistently differ from *L. minor* in their morphology. As a consequence it seems that there is hardly justification for the distinction of an ecologically determined flat modification of *L. gibba*.

Table 1. Dimensions of *Lemna* strains, that were flat on the day of collection, prior to and after ADDHA treatment and flowering % after this treatment; m = month of sampling; L, B, G = mean length, width and gibbosity (mm);  $\delta = standard$  deviation

(mm); fl. = flowering %. Gibbosity figures referring to L. gibba in bold type.

field **EDDHA** No. m L δ В δ G δ L δ В δ Ġ δ fl. n 1 6 30 3.95 0.27 3.04 0.22 0.62 0.09 3.18 0.20 2.16 0.13 0.57 0.06 0 30 3.84 0.15 2.63 0.11 0.62 0.06 0 2 6 30 0.35 3.80 0.31 0.56 0.10 4.11 0.21 2.72 0.17 0.59 0.08 0 5.03 0.50 3 30 3.37 0.24 2.29 0.18 0.07 4.28 0.17 2.95 0.20 0.71 0.06 0 4 6 30 4.95 0.41 3.58 0.35 0.38 0.11 3.86 0.18 2.57 0.16 0.54 0.06 0 5 6 30 3.18 0.23 2.12 0.18 0.35 0.05 3.55 0.19 2.45 0.12 0.52 0.05 0 0.48 6 4.57 0.24 3.21 0.22 0.09 0.17 2.96 6 30 4.13 0.15 0.56 0.08 0 6 30 3.28 0.19 2.20 0.17 0.43 0.09 3.52 0.18 2.34 0.14 0.51 0.07 0 7 6 30 4.43 0.32 2.85 0.21 0.35 0.06 3.72 0.28 2.46 0.18 0.52 0.07 0 8 6 30 4.28 0.29 3.02 0.22 0.60 0.09 3.62 0.26 2.36 0.16 0.57 0.07 0 9 6 30 4.31 0.27 3.05 0.21 0.57 0.08 3.75 0.22 2.50 0.18 0.07 0 0.60 10 6 30 4.35 0.26 3.05 0.19 0.60 0.06 3.63 0.17 2.42 0.16 0.57 0.08 0 5.37 0.42 3.59 0.28 2.38 11 6 30 0.24 0.57 0.08 3.58 0.16 0.54 0.06 0 12 30 3.84 0.24 2.62 0.19 0.50 0.06 3.44 0.20 2.30 0.17 0.58 6 0.06 0 13 6 30 4.55 0.27 3.22 0.19 0.60 0.06 3.53 0.19 2.27 0.16 0.54 0.05 0 7 30 3.26 14 4.27 0.24 0.16 0.73 0.09 4.25 0.18 2.92 0.18 0.68 0.04 0 7 15 30 2.79 0.54 3.95 0.18 0.16 0.08 4.39 0.17 3.02 0.18 0.64 0.07 3 7 16 30 3.49 0.20 2.65 0.17 0.58 0.07 3.73 0.14 2.41 5 0.14 0.53 0.05 17 7 30 3.87 0.30 2.64 0.22 0.41 0.07 3.80 0.17 2.57 0.18 0.60 0.05 20 18 7 30 3.92 0.25 2.80 0.18 0.52 0.07 3.30 0.09 2.21 0.55 5 0.10 0.06 7 19 30 3.08 0.22 2.16 0.16 0.53 0.06 4.00 0.19 2.61 0.12 0.62 0.07 0 7 20 30 4.17 0.26 2.95 0.22 0.52 0.07 4.48 0.12 2.67 0.12 0.55 0.05 5 21 8 20 2.73 0.21 1.78 0.15 0.35 0.05 3.85 0.07 2.51 0.09 0.49 0.06 0 22 8 3.49 0.15 2.39 45 20 0.14 0.65 0.07 4.30 0.18 3.07 0.15 0.75 0.07 23 8 20 0.23 1.84 0.28 0.05 0.12 0.50 35 3.17 0.10 2.65 1.72 0.12 0.05 24 6 7 4.34 0.18 3.41 0.28 0.60 0.20 4.71 0.30 3.44 0.19 1.66 0.16 0 25 6 30 4.94 0.32 3.62 0.28 0.60 0.11 5.30 0.46 4.11 0.44 2.12 0.37 0 7 3.62 0.21 2.63 0.51 0.05 2.48 30 0.14 3.86 0.15 0.14 0.56 0.04 5 26 4.32 2.92 0.32 6 30 0.34 0.36 0.08 5.12 0.19 3.87 0.21 1.49 0.12 0 8 20 4.23 0.15 2.93 0.15 0.46 0.05 3.94 0.13 2.48 0.12 0.55 0.04 0 7 3.74 0.32 0.73 0.08 27 30 5.26 0.39 4.43 0.18 3.22 0.18 1.59 0.11 10 2.32 7 20 0.21 1.51 0.18 0.34 0.07 3.60 0.14 2.43 0.56 O 0.12 0.06 28 7 20 3.49 0.13 2.48 0.11 0.49 0.06 3.91 0.11 3.05 0.19 1.57 0.16 0 7 20 2.33 0.23 1.56 0.19 0.49 0.05 4.04 0.18 2.66 0.15 0.59 0.05 0 29 7 20 1.90 0.13 0.37 0.03 0.22 2.94 0.13 5.51 4.21 0.18 2.10 0.20 0 7 20 3.63 0.26 2.32 0.24 0.51 0.05 4.43 0.21 3.06 0.22 0.76 0.07 5 30 2.87 0.38 27 6 30 3.97 0.30 0.21 0.13 4.30 0.15 3.11 0.15 0.61 0.07 31 6 30 3.58 0.27 2.58 0.20 0.42 0.12 3.99 0.19 2.75 0.20 0.58 0.06 0 32 6 30 3.64 0.24 2.44 0.13 0.60 0.06 3.31 0.20 2.26 0.21 0.58 0.07 0 33 6 30 3.49 0.20 2.38 0.14 0.56 0.06 3.94 0.18 2.72 0.17 0.54 0.05 0 34 3.49 0.15 0.06 3.78 2.70 0.12 0.55 0.05 0 6 30 0.17 2.41 0.61 0.11 15 35 8 20 3.47 0.19 2.45 0.12 0.55 0.08 4.18 0.14 2.75 0.20 0.63 0.07 36 8 20 4.61 0.15 3.33 0.12 0.73 0.08 4.27 0.21 3.09 0.16 0.67 0.07

The hydrosoil seems to influence the phenology of *L. gibba*, as the flat forms were more persistent above sand or peat than above clay. This difference could not, however, be correlated unambiguously with the mineral content of the water.

Pursuant to the report of De Lange et al. (1981) it is obvious that in The Netherlands L. gibba generally occurs in a mixed vegetation with L. minor, a fact which should be syntaxonomically recognized. Vegetations which consist exclusively of L. gibba, such as no. 24, seem to be rare. Pure stands of L. minor have been observed mainly above a hydrosoil of sand which suggests that this species stands a larger variety in ecological conditions than L. gibba. Although the chemical analyses do not show a significant difference between the sites of L. minor and L. gibba it should be noted that in other areas L. minor has been described to have the largest ecological and geographical range which overlaps that of L. gibba (LANDOLT 1982).

#### **ACKNOWLEDGEMENTS**

The authors wish to express their gratitude to Prof. Dr. A. D. J. Meeuse of the Hugo de Vries Laboratorium, University of Amsterdam, for his stimulating interest in the investigations of the morphology and ecology of the Lemnaceae.

## REFERENCES

- BOWEN, H. J. M. (1958): Variations in Lemna gibba. Proc. B.S.B.I. 3: 86-88.
- CLAPHAM, A. R., T. G. TUTIN & E. F. WARBURG (1962): Flora of the British Isles. Cambridge, 2nd edn. 1296 pp.
- ELZENGA, J. T. M., L. DE LANGE; A. H. PIETERSE (1980): Further indications that ethylene is the gibbosity regulator of the Lemna gibba/Lemna minor complex in natural waters. *Acta Bot. Neerl.* 29: 225-229.
- GUPPY, H. B. (1894): On the habits of Lemna minor, L. gibba and L. polyrrhiza. J. Linn. Soc. Bot. 30: 323-330.
- HARTOG, C. DEN (1968): De platte vorm van Lemna gibba, nog steeds een probleem. Gorteria 4: 90-92.
- HEGELMAIER, F. (1968): Die Lemnaceen, eine monographische Untersuchung. Verl. Engelmann, Leipzig, 169 pp.
- HILLMAN, W. S. (1961): Experimental control of flowering in Lemna. III. A relationship between medium composition and the opposite photoperiodic responses of L. perpusilla 6746 and L. gibba G3. Amer. J. Bot. 48: 413-419.
- HOREN, F. VAN (1869): Observations sur la physiologie des Lemnacées. Bull. de la Soc. Roy. de Bot. de Belgique 8: 15-88.
- KANDELER, R. (1975): Species delimitation in the genus Lemna. Aq. Bot.: 365-376.
- LANDOLT, E. (1975): Morphological differentiation and geographical distribution of the Lemna gibba-Lemna minor group. Aq. Bot. 1: 345-363.
- (1982): Distribution pattern and ecophysiological characteristics of the European species of the Lemnaceae. Ber. Geobot. Inst. ETH, Stiftung Rübel 49: 127-145.
- Lange, L. DE (1974): Translocation experiments in the field with the Lemna gibba-Lemna minor complex. Acta Bot. Neerl. 23: 109-112.
- (1975): Gibbosity in the complex Lemna gibba/Lemna minor: literature survey and ecological aspects. Aq. Bot. 1: 327-332.

- & A. H. Pieterse (1973): A comparative study of the morphology of Lemna gibba L. and Lemna minor L. Acta Bot. Neerl. 22: 510-517.
- —, A. H. PIETERSE & I. VAN BAARSEN-BECKERS (1981): The occurrence of mixed populations of different genotypes of the Lemna gibba-Lemna minor complex. Acta Bot. Neerl. 30: 191-197.
- & S. SEGAL (1968): Over het onderscheid en de oecologie van Lemna minor en Lemna gibba.
  Gorteria 4: 5-12.
- & E. Westinga (1979): The distinction between Lemna gibba L. and Lemna minor L. on the basis of vegetative characters. Acta Bot. Neerl. 28: 169-176.
- MASON, H. L. (1957): A flora of the marshes of California. Univ. Calif. Press, Berkeley, Calif., 331 pp.
- PIETERSE, A. H. (1975): Physiological, morphological and anatomical aspects of gibbosity in Lemna gibba. Aq. Bot. 1: 333-344.
- (1976): Specific interactions in the physiology of flowering and gibbosity of Lemna gibba G3. *Plant & Cell Physiol.* 17: 713–720.
- —, P. R. BHALLA & P. S. SABHARWAL (1970): Control of gibbosity in Lemna gibba G3 by ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA). Acta Bot. Neerl. 19: 521-524.
- SCHULTZ, B. (1962): Wasserlinsen. Ziemsen Verlag, Wittenberg, 95 pp.
- SEGAL, S. (1966): Oecologie van hogere waterplanten. Vakbl. Biol. 46: 138-149.
- & M. C. GROENHART (1967): Het Zuideindigerwiede, een uniek verlandingsgebied. Gorteria 3: 165-181.
- SLOOVER, J. L. DE (1966): La fronde, la graine et la germination d'un Lemna. Natur. Belg. 47: 443-356.
- WETSTEYN, L. P. M. J. (1983): Een onderzoek naar het voorkomen van vlakke vormen van Lemna gibba L. in Nederland. *Int. Rapp. Hugo de Vries Lab., Univ. Amsterdam* 143: 81 p.