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The duckweed *Lemna minor* compared with the alga *Selenastrum capricornutum* for bioassay of pond-water richness

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Abstract

In an initial comparison of *Lemna* and *Selenastrum* bioassay, on water from two ponds, both organisms made greater growth in the more nutrient-rich water. However, when the comparison was extended to 57 diverse ponds, the two bioassay organisms responded to different aspects of water quality. *Lemna* and *Selenastrum* growth were not correlated, and were not necessarily related to the same water-quality variables: e.g. *Lemna* dry weight was correlated with Ca, Mg and K while *Selenastrum* cell concentration was correlated with PO_4, NH_4, NO_2 and (negatively) with pH. Neither *Lemna* nor *Selenastrum* growth were related to the number of hydrophyte species or their average trophic ranking score. Thus, bioassay to assess pond richness, either using *Lemna* or *Selenastrum*, is not straightforward and is probably best used alongside water analysis and/or vegetation survey. © 1998 Elsevier Science B.V.

Keywords: Bioassay; *Lemna*; Ponds; *Selenastrum*; Trophic status

1. Introduction

The allocation of standing fresh waters to their appropriate position in the dystrophic–oligotrophic–mesotrophic–eutrophic continuum is operationally defined, and depends on the criteria chosen as the basis of classification. Classification is sometimes on the basis of water chemistry, often with emphasis on phosphorus and nitrogen content (Vollenweider, 1971; Forsberg and Ryding, 1980). Reliable classification of ponds by water analysis is, however, a substantial undertaking. Many variables need to be

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measured and date of sampling is important. Sample collection in summer, for example, may be inappropriate since nutrients (e.g. NO_3–N; Goulder and Boatman, 1971) may be removed from pond water by plants early in the year and stored in tissues to support summer growth.

Alternatively, aquatic macrophytes can be used to classify ponds, since the distribution of macrophyte species is related to habitat richness (Haslam, 1978; Newbold and Palmer, 1979). Thus, Palmer (1992) described the allocation of a trophic ranking score (TRS) to each of the species of aquatic plants found in Great Britain. The average TRS of a pond site (i.e. the sum of TRS for all species present/number of species) is a numerical indicator of its trophic status. This approach requires extensive field survey, in a narrow seasonal window when all species are recognizable, together with specialized taxonomic knowledge.

A simpler approach is bioassay, using water samples collected in winter when nutrient conditions are potentially optimum because of high inputs and low biological demand. Algal bioassay, usually using *Selenastrum capricornutum* Printz (Chlorophyceae, Chlorococcales), has successfully indicated richness of waters, often highlighting high phosphate availability (e.g. Forsberg, 1979; Carr and Goulder, 1990; Lieberman, 1995).

Microalgae are, however, not necessarily appropriate indicators of suitability for macrophyte growth. The aim of this study, therefore, was to evaluate the aquatic angiosperm *Lemna minor* L. as a tool for bioassay of pond-water richness and the objective was to develop a simple procedure for its use in bioassay. This species was chosen because it is free-floating and, therefore, customarily derives its nutrients from the surrounding water. Furthermore, it has been used in toxicity testing (Taraldsen and Norberg-King, 1990; Clément and Merlin, 1995), and Elster et al. (1995) found relationships between root length and pond-water quality. An initial evaluation of *Lemna* bioassay, and comparison with *Selenastrum* bioassay, was made using water from two adjacent ponds which had markedly different aquatic vegetation and inorganic-nutrient content. *Lemna* bioassay was then carried out using water from 57 ponds in North–East England. The results were compared with paralleled *Selenastrum* bioassay, with aquatic macrophyte average TRS values from summer vegetation surveys, and with the results of water analysis on winter samples.

2. Materials and methods

2.1. Sites and sampling

The two small, shallow (mostly < 1 m deep) ponds used for initial evaluation of *Lemna* bioassay are about 100 m apart, in the East Yorkshire village of Bishop Burton in North–East England. The larger pond (0.4 ha), National Grid Reference SE 990 398, is adjacent to a road and supported abundant semi-tame ducks and large carp, much fed by the public. It had, in summer 1996, little aquatic vegetation, i.e. limited marginal cover of the emergents *Acorus calamus* L., *Alisma plantago–aquatica* L. and *Epilobium hirsutum* L. The smaller pond (0.06 ha), SE 990 399, is 50 m away from the road.

It had extensive vegetation, including 10 species of aquatic macrophyte, and few ducks or fish.

The wider trials of *Lemna* bioassay were made on water from 57 ponds in East Yorkshire. The ponds ranged in area from 0.003 ha to 3.2 ha, were of diverse age and origin, and included ancient moats, village ponds, clay pits, gravel pits, and borrow pits excavated during nineteenth-century railway construction.

Surface water was collected in acid-washed polythene bottles and was transported on ice. Water for initial bioassays was collected in November 1995, and for later bioassays and chemical analysis in January–March 1996; i.e. all samples were collected during winter when inorganic nutrient concentrations should be at their highest.

2.2. Lemna and Selenastrum bioassays

Pond water for both *Lemna* and *Selenastrum* bioassays was filtered through GF/C glass microfibre filters, nominal pore size 1.2 μm (Whatman International, Maidstone, UK), boiled for 2 min to kill remaining native microalgae, cooled and 50 ml aliquots were dispensed into acid-washed, sterile 100-ml conical flasks.

Algae-free *L. minor* was donated by Dr Craig Turner of the Environment Agency, Newcastle upon Tyne and stocks were cultured in quarter-strength Hoagland's solution (Hewitt, 1966). Five *Lemna* fronds were transferred to each culture flask using a sterile wire loop. In preliminary trials fronds were counted daily over 18 days incubation; new fronds were counted irrespective of whether they were detached from parent fronds. Root length (Elster et al., 1995) was not measured since the procedure is time consuming and potentially destructive of the plants. The water was either unchanged or changed every three days. After 18 days the fronds were rinsed with pure water, dried to constant weight at 100°C, cooled in a desiccator and weighed. In later *Lemna* bioassays, on water from 57 ponds, the water was changed at three-day intervals and number and dry weight of fronds were determined after 18 days.

Selenastrum bioassay was based on Miller et al. (1978). Stock cultures of *S. capricornutum* (CCAP 278/4; Culture Centre of Algae and Protozoa, Ambleside, UK) were grown to stationary phase in Jaworski's medium (Tompkins et al., 1995) and then, to minimize carry-over of nutrients, were thrice centrifuged (1000 RCF for 3 min) and resuspended in pure water. Culture flasks were inoculated with 1 ml of the resuspended stock culture. In preliminary bioassays flasks were swirled and cells were counted daily over 18 days of incubation. Counting was at $\times 100$ magnification in a Fuchs–Rosenthal haemacytometer (Fisons Scientific Equipment, Loughborough, UK). At least 350 cells were counted per culture. In later bioassays, flasks were swirled daily and cells were counted after 18 days.

All bioassays using *Lemna* and *Selenastrum* were carried out in triplicate; incubations were at 20°C under continuous illumination by white fluorescent lamps (PAR 76 μmol m^{-2} s^{-1}).

2.3. Vegetation survey and water analysis

The species of emergent, natant and submerged aquatic macrophytes present in the 57 ponds were recorded on two visits to each pond, one in May–June and one in

July–August 1996. Submerged plants were retrieved with a grapnel. The average trophic ranking score (TRS) was calculated for each pond as described by Palmer (1992).

Inorganic phosphate, ammonium, nitrite and nitrate, in winter-collected water samples, were determined spectrophotometrically using the methods of McCullough (1967), Stainton et al. (1977) and Mackereth et al. (1978). Calcium and magnesium were determined by AAS; sodium and potassium by flame photometry. Alkalinity was obtained by acid titration (Mackereth et al., 1978); pH and conductivity were measured

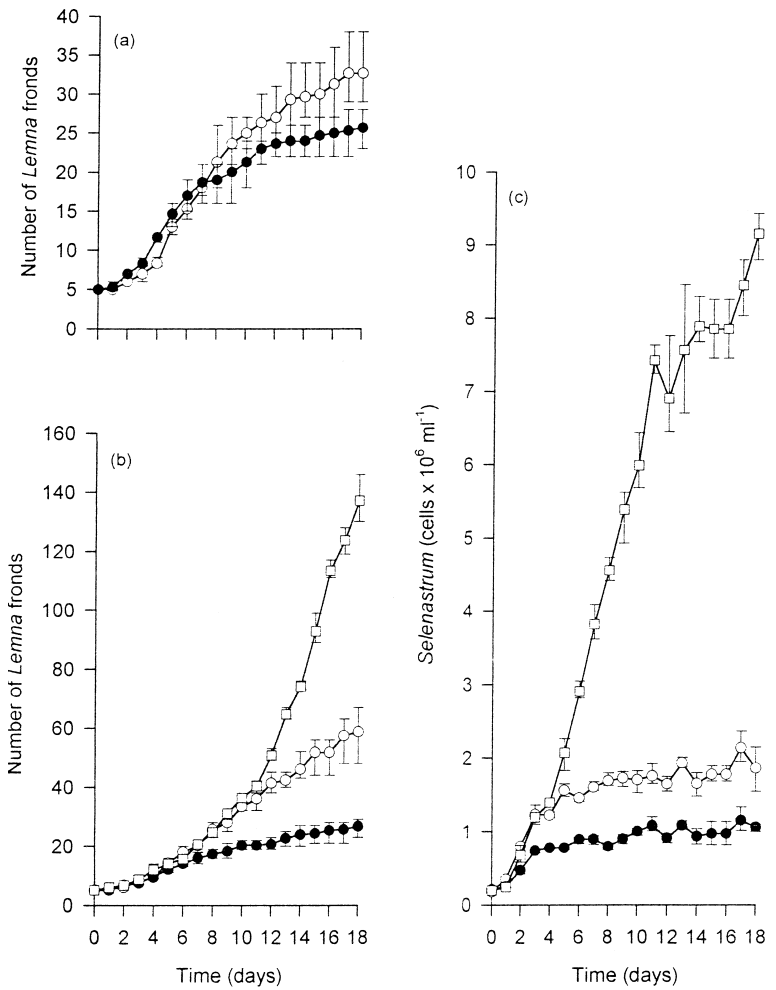

Fig. 1. Results of initial *Lemna* and *Selenastrum* bioassays using water from two ponds at Bishop Burton, November 1995. (a) 18 days growth of *Lemna* without change of water, and (b) with change of water at three-day intervals. (c) 18 days growth of *Selenastrum.* Values are means from three replicate incubations (vertical bars indicate range) in water from the large pond (open circles), the small pond (closed circles), and in nutrient-rich reference media (open squares), i.e. quarter-strength Hoagland's solution changed at three-day intervals (b) or Jaworski's medium (c).

using pH and conductivity meters. All variables, except ammonium and pH, were determined after GF/C filtration. Phosphate, ammonium, alkalinity, pH and conductivity were determined on the sampling day or the following day. Other determinations were after sample storage at −20°C.

3. Results

3.1. Initial bioassays on two ponds

In the initial *Lemna* bioassay, using water from the two ponds at Bishop Burton, without change of water during incubation, there was little between-pond difference in growth (Fig. 1a). Mean frond number after 18 days incubation was 33 per flask in water from the large pond and 26 per flask for the small pond. Mean dry weights were 12.2 (range of three replicates 11.9–12.7) mg per flask and 9.5 (8.7–10.7) mg per flask respectively. In contrast, when the water was changed during incubation, growth was substantially greater in water from the large pond (Fig. 1b). After 18 days, mean frond number was 59 per flask compared to 27 per flask, and mean dry weight was 20.7 (17.8–26.3) mg per flask compared to 10.7 (8.4–12.2) mg per flask.

Selenastrum bioassay, similarly, gave greater growth in water from the large pond (Fig. 1c). Mean cell density after 18 days was 1.9×10^6 ml^{-1} compared to 1.1×10^6 ml^{-1}. The growth of both *Lemna* (Fig. 1b) and *Selenastrum* (Fig. 1c), however, was greatest in nutrient-rich reference media (i.e. quarter-strength Hoagland's solution and Jaworski's medium respectively).

Water analysis showed that the large pond was the more nutrient rich, with notably greater concentrations of inorganic phosphate and nitrogen (Table 1).

3.2. Bioassays on 57 ponds

The results of *Lemna* and *Selenastrum* bioassay on water from 57 ponds are given in Fig. 2. There was substantial between-pond variation in growth of *Lemna* (both frond number and dry weight) and *Selenastrum*. Thus, the mean number of *Lemna* fronds

Table 1
Results of chemical analysis of water from the two ponds at Bishop Burton, January 1996

Variable	Large pond	Small pond
PO_4–P ($\mu g\ l^{-1}$)	507	< 20
NH_4–N ($\mu g\ l^{-1}$)	437	274
NO_2–N ($\mu g\ l^{-1}$)	17	< 1
NO_3–N ($\mu g\ l^{-1}$)	397	61
Calcium ($mg\ l^{-1}$)	37	35
Magnesium ($mg\ l^{-1}$)	8	5
Sodium ($mg\ l^{-1}$)	80	28
Potassium ($mg\ l^{-1}$)	19	9
Alkalinity ($meq\ l^{-1}$)	3.9	3.4
pH	8.0	8.2
Conductivity ($\mu S\ cm^{-1}$)	829	543

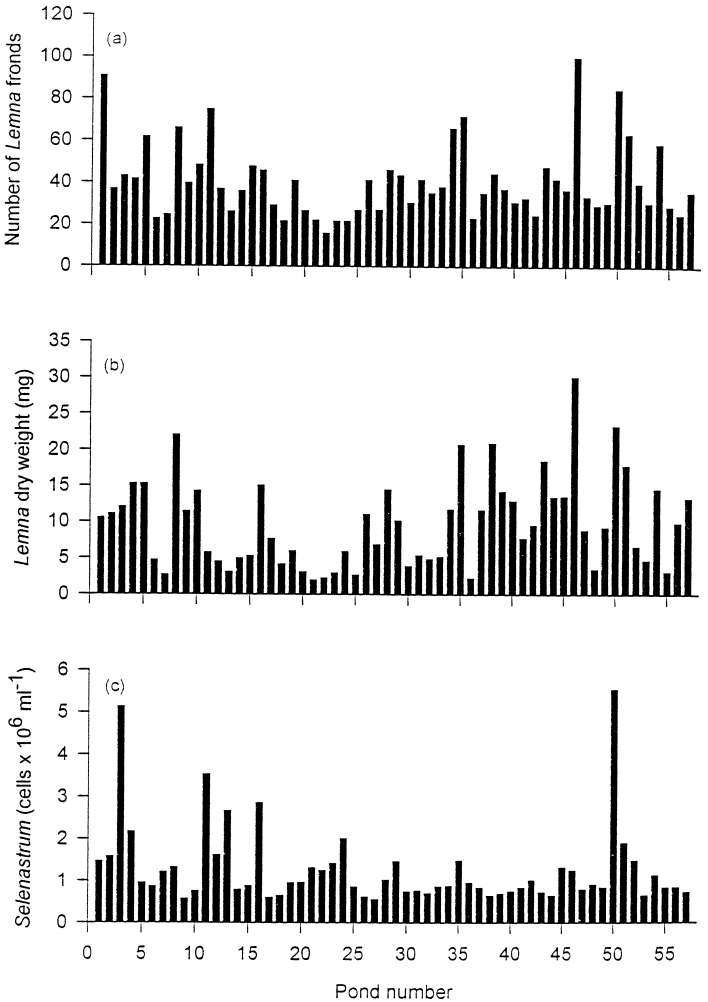

Fig. 2. Results of *Lemna* and *Selenastrum* bioassays using water from 57 ponds, February–March 1996. (a) Mean *Lemna* frond number per flask, (b) mean *Lemna* dry weight per flask, and (c) mean *Selenastrum* cell concentration. Values are means from three replicate incubations of 18 days duration. The water in the *Lemna* cultures was changed at three-day intervals. Ponds 1–5 were ancient moats, 6–17 were village ponds, 18–26 were clay pits, 27–37 were gravel pits, 38–47 were borrow pits, and 48–57 were of miscellaneous origin.

ranged from 16–101 per flask and mean dry weight from 2.0–30.2 mg per flask, while mean *Selenastrum* cell density ranged from $0.56–5.6 \times 10^6$ ml^{-1}.

There was a highly significant correlation between frond number and dry weight of *Lemna* (Spearman's rank-correlation coefficient, $r_s = 0.76$, $n = 57$, $P < 0.001$). However, neither the number of *Lemna* fronds nor the dry weight of *Lemna* were correlated with *Selenastrum* cell density ($r_s = 0.22$ and 0.08 respectively, $n = 57$, $P > 0.05$).

Table 2
Summary of data from 57 ponds; results of vegetation survey (summer 1996) and chemical analysis of water (January–February 1996), and relationships between the results of *Lemna* and *Selenastrum* bioassay and macrophyte and water chemistry variables

	Mean	(Range)	CV (%)	r_s		
				Lemna frond number	*Lemna* dry weight	*Selenastrum*
Macrophyte variables						
Average TRS	8.5	(7.4–9.5)	5	NS	NS	NS
Number of species	10.0	(1–24)	55	NS	NS	NS
Water chemistry variables						
PO_4–P (μg l^{-1})	100	(<20–1118)	223	0.27 *	NS	0.50 * * *
NH_4–N (μg l^{-1})	833	(<5–8357)	197	0.31 *	NS	0.45 * * *
NO_2–N (μg l^{-1})	20.8	(<1–259)	200	NS	NS	0.28 *
NO_3–N (μg l^{-1})	2151	(<1–49 100)	313	NS	NS	NS
Calcium (mg l^{-1})	73.2	(9–250)	73	NS	0.33 *	NS
Magnesium (mg l^{-1})	27.3	(2–90)	81	NS	0.40 * *	NS
Sodium (mg l^{-1})	96.5	(20–515)	120	NS	NS	NS
Potassium (mg l^{-1})	21.6	(<1–268)	172	NS	0.41 * *	NS
Alkalinity (meq l^{-1})	3.7	(1.1–9.0)	29	NS	NS	NS
pH	8.0	(6.4–9.2)	8	−0.43 * *	NS	−0.31 *
Conductivity (μS cm^{-1})	1080	(383–3060)	56	NS	NS	NS

Average trophic ranking score (TRS) was calculated using the macrophyte species present which are listed as British aquatic plants and given a trophic ranking score by Palmer (1992). Number of species includes only those which were used in calculation of average TRS. For average TRS, and number of species, $n = 54$ ponds, because no listed species were present in three of the ponds. For chemical variables $n = 57$ ponds. r_s = Spearman's rank-correlation coefficient; * $P < 0.05$, * * $P < 0.01$, * * * $P < 0.001$, NS = $P > 0.05$, CV = coefficient of variation.

Data on average TRS, number of hydrophyte species, and results of water analysis are summarized in Table 2. These indicated substantial variation in the richness of the ponds.

There were relatively few significant correlations between the bioassay variables and the macrophyte and water chemistry variables (Table 2). *Lemna* frond number was significantly correlated with phosphate, ammonium and (negatively) with pH; *Lemna* dry weight was correlated with calcium, magnesium and potassium; *Selenastrum* cell density was correlated with phosphate, ammonium, nitrite and (negatively) with pH.

4. Discussion

The initial *Lemna* bioassays, using water from the two ponds at Bishop Burton, showed differential growth over 18 days incubation, provided that the water was changed at intervals (Fig. 1a,b). The replenishment probably prevented nutrient depletion during incubation. Hence in later *Lemna* bioassays, 18 days of incubation with

regular change of culture water was standard procedure. Both frond number and dry weight proved to be good indicators of growth, hence either is potentially suitable for use in routine bioassay. Frond number is, however, quicker to assess.

Since the differential growth of *Lemna* in water from the two Bishop Burton ponds was paralleled by similar differential growth of *Selenastrum* (Fig. 1c), and since both made greater growth in water from the large pond which was more nutrient rich (Table 1), it follows that *Lemna*, like *Selenastrum*, is a potential tool for bioassay of trophic status of ponds. Since both assay organisms showed greatest growth in high-nutrient reference media (Fig. 1b,c) both had scope to be efficient indicators in richer waters than the Bishop Burton ponds. The uncoupling of the results of bioassay (Fig. 1) and water analysis (Table 1) from aquatic macrophyte distribution (i.e. the small pond had the richer vegetation) was possibly caused by the water fowl and fish. Thus, the large pond had the potential to support abundant aquatic vegetation but, although we have no direct evidence for this, any new growth was probably grazed by ducks and carp.

When *Lemna* bioassay was extended to 57 diverse ponds, frond number and dry weight were closely correlated, thus the suitability of either frond number or dry weight as a measure of growth was confirmed.

The extension of *Lemna* and *Selenastrum* bioassay to 57 ponds also suggested that the use of these organisms for assay of pond richness is not straightforward. Growth of both showed substantial between-pond variation (Fig. 2) but *Lemna* and *Selenastrum* apparently did not necessarily respond to the same water-quality variables, since (1) neither frond number nor dry weight of *Lemna* were significantly correlated with *Selenastrum* cell density, and (2) *Lemna* frond number and *Selenastrum* growth were largely correlated with the same water-chemistry variables, but *Lemna* dry weight was correlated with different water-chemistry variables (Table 2).

Especially relevant is that both *Lemna* and *Selenastrum* growth were not obviously related to pond vegetation, i.e. there were no correlations with number of plant species or average TRS. This was probably because the diversity and species composition of aquatic vegetation in the 57 ponds were not solely controlled by water chemistry.

Other potentially relevant variables included the following. (1) Availability of seeds or vegetative propagules (Spence, 1967): age of pond and the proximity of other macrophyte-bearing water bodies are potentially important. (2) The nutrient content of the pond sediments: for some hydrophytes the substratum can be a major nutrient source (Denny, 1972; Moeller et al., 1988; Rattray et al., 1991). (3) Shading: marginal trees can substantially repress hydrophyte vegetation (Dawson and Kern-Hansen, 1979; Jorga et al., 1982). (4) Competition with phytoplankton: submerged macrophytes can be replaced by phytoplankton, sometimes in response to decrease in zooplankton grazing pressure rather than gross change in nutrient availability (Mitchell et al., 1988; Irvine et al., 1989). (5) Grazing: wild fowl or herbivorous fish may suppress macrophytes (ten Winkel and Meulemans, 1984; Søndergaard et al., 1996). (6) Pond size: the ponds sampled in this study ranged in area over about three orders of magnitude and species diversity is known to increase with pond area (Helliwell, 1983).

It follows that *Lemna* bioassay, like *Selenastrum* bioassay, cannot be used as the sole indicator of trophic status of ponds. Both *Lemna* and *Selenastrum* can, however, indicate differences in water quality (Figs. 1 and 2). Moreover, since the two organisms

do not necessarily respond to the same water-quality variables (Table 2), the two assays are potentially complementary. Thus, *Lemna* bioassay, as well as *Selenastrum* bioassay, and also water chemistry and macrophyte species composition are potential indicators of pond richness which might be used in combination, to the extent allowed by available resources.

The results raise the question of how far it is worthwhile pursuing the use of further plant species as potential indicators of water quality and pond richness. Extra bioassays might add work without leading to greater understanding. Choice of further potential indicator species should be based on the particular interest or concern to the researcher or water manager. Specifically, if the distribution of rooted hydrophytes is of concern then bioassays utilizing rooted plants grown in pond sediments rather than water might be more appropriate.

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