Plant Ecology and Evolution 147 (2): 202–212, 2014
http://dx.doi.org/10.5091/plecevo.2014.902

REGULAR PAPER

An autecological investigation of *Desmodesmus*: implications for ecology and taxonomy

Elliot Shubert[1,*] Elżbieta Wilk-Woźniak[2] & Sławomir Ligęza[3]

[1]University of Westminster, London, W1W 6XH, United Kingdom
[2]Institute of Nature Conservation PAS, Kraków, PL-31-120, Poland
[3]Institute of Soil Science and Environment Management, Lublin, PL-20-069, Poland
*Author for correspondence: e.shubert@nhm.ac.uk

Background and aims – Phenotypic plasticity is the morphological response of a single genotype to produce several different physiological and/or morphological types under changing environmental conditions. *Desmodesmus*, a green alga, has been well documented for its phenotypic responses (e.g. colony transformation to unicells, and spiny transformation to spineless). Two oxbow lakes (R1 and R2) and one artificial dam reservoir (R3) were used to evaluate the quantity and diversity range of *Desmodesmus* species occurring during four seasons of one-year.
Methods – Water samples were quantified for *Desmodesmus* species and identified using the Scanning Electron Microscope (SEM). To better understand the morphological responses of *Desmodesmus*, an axenic culture of *Desmodesmus abundans*, CCAP 258/299, was cultured in sterile-filtered water from each water body collected from each season.
Key results – Not surprisingly, different *Desmodesmus* species inhabited each water body and exhibited different densities of growth, contributing 77% of the total density in R1, 22% in R2 and only 1% in R3. Summer and autumn were the seasons when *Desmodesmus* showed the highest density in the shallow and polymictic oxbow lakes (R1 and R2). In the deep and dimictic reservoir (R3), the highest density of *Desmodesmus* was during autumn. Phenotypic plasticity was observed in the experiments, with unicells being formed from colonies, some with shorter spines. *Desmodesmus* grown in a high concentration of nitrogen (SE, Medium 7 +3N and BBM) had a higher percentage of unicells compared to the other culture conditions.
Conclusions – Our studies are important from both taxonomical and ecological points of view, since our results showed that there may be new possibilities for using the phenotypic plasticity of *Desmodesmus* to assess water quality and as a potential bioindicator of nutrient availability in natural ecosystems.

Key words – Autecological, bioassays, *Desmodesmus*, green algae, phenotypic plasticity, Polish oxbows and reservoirs.

INTRODUCTION

Phenotypic plasticity is the ability of a single genotype to produce several different physiological and/or morphological types under changing environmental conditions. "It is now known, for example, that plasticity is simply a modification in the ability of a genotype to express a particular character. Such modification may occur before or after a character has been expressed and it is always the response to a change in either intra or extracellular environments, or both" (Morales et al. 2002). Thus, some taxa have the ability to modify their morphology according to the environment, e.g. in response to water chemistry and/or predator pressure (for water chemistry see: Trainor 1998, for *Daphnia* and infochemicals see: Hessen & van Donk 1993, Lampert et al. 1994, Lürling et al. 1997).

It has been documented that many algal species exhibit phenotypic plasticity (Trainor et al. 1971, Gómez et al. 1995, Pigliucci 1996, 2001, Pigliucci et al. 1996, Graham & Wilcox 2000, Zirbel et al. 2000, Morales et al. 2002). When identifications are made from live or fixed specimens after collection of field samples, a particular morph is identified to genus and/or species. However, when field samples are not fixed and algae are subsequently isolated and cultured in the laboratory, they may produce a variety of morphs. There is empirical evidence from laboratory experimentation and *in*

All rights reserved. © 2014 Botanic Garden Meise and Royal Botanical Society of Belgium – ISSN 2032-3921

situ field experiments that some algae can change their morphology under changing environmental conditions (Trainor 1998).

It is important to give an historical perspective. Phenotypic plasticity in *Scenedesmus* Meyen (including *Desmodesmus* An, Friedl & Hegewald) has been known since the middle of the 1800s (Grunow 1858, Wood 1873, Wolle 1887, Chodat & Malinesko 1893, Grintzesco 1902a, 1902b, Chodat 1913, 1926). Grunow (1858) observed phenotypic plasticity, and speculated that filamentous forms of *Dactylococcus* might be stages of development in *Scenedesmus*. Wood (1873) observed and described a multi-spined unicell, which he described as a new species, *S. rotundus* Wood. Wolle (1887) commented that this "unicellular form is not infrequently intermingled with other species of *Scenedesmus*; it certainly appears related, and whether a distinct species, or merely connected in some way in a process of development, has not been made clear". "Thus a relationship between the unicellular morph and *Scenedesmus* colonial morphs was made well over 100 years ago" (Trainor 1998). Using an experimental approach, Chodat & Malinesko (1893) made the connection that filaments (*Dactylococcus*) and colonies were related to *S. acutus* Meyen. These observations were confirmed by Grintzesco (1902a, 1902b) and Chodat (1913, 1926). Chodat (1913, 1926) laid the groundwork for understanding phenotypic plasticity in *Scenedesmus* using cultures and proposed the concept of polymorphism in microalgae. However, his observations were criticized by Smith (1914a, 1914b), who did culture algae and could not find the *Dactylococcus* stage in *Scenedesmus*. Furthermore, Smith focused on the identification of algae fixed from field material and did not want to include modifications that occurred in culture (which he called teratology). Phenotypic plasticity remained ignored until the 1960s when Trainor (1963) 'rediscovered' phenotypic plasticity in *Scenedesmus* (= *Desmodesmus*) by demonstrating that spiny colonies could produce spiny unicells in an axenic culture. Due to the work of Trainor (1969), Shubert & Trainor (1974), Siver & Trainor (1981), Trainor (1998), and Morales et al. (2002), we now know a great deal about phenotypic plasticity in *Desmodesmus*.

Separation of non-spiny and spiny *Scenedesmus* into different genera was first postulated by Trainor et al. (1976) and later by Komárek & Fott (1983) and Kessler et al. (1997). The validity of this suggestion was confirmed by molecular analysis; *Scenedesmus* was retained for non-spiny forms and a new genus, *Desmodesmus*, was erected for the spiny forms (An et al. 1999). There are distinct and consistent morphological differences between *Scenedesmus* and *Desmodesmus*; the former does not produce spines or true unicells, whereas the latter does. In addition, the cell wall morphology (as viewed with the SEM) is entirely different; *Desmodesmus* walls are highly ornamented, whereas *Scenedesmus* walls are not (Shubert & Wilk-Woźniak 2003). Wall ornamentation came to light when empty cell walls were viewed first with the Transmission Electron Microscope (TEM) (Bisalputra & Weier 1963, Bisalputra et al. 1964, Massalski et al. 1974) and later with the SEM (Komárek & Ludvík 1971, Hegewald 1989, Hegewald et al. 1990). Surface wall morphology as seen with the SEM varies, e.g. warty [*D. armatus* (R.Chodat) E.Hegewald], net-like [*D. quadricauda* (R.Chodat) E.Hegewald] and star-like [*D. serratus* (Corda) An, Friedl & E.Hegewald].

Many of the strains/species of *Desmodesmus* exhibit extensive phenotypic plasticity (Trainor et al. 1971, Shubert & Trainor 1974, Trainor 1993, 1998, Shubert & Wilk-Woźniak 2003) alternating between colonies and unicells and/or spiny and spineless forms.

It has been well documented that some species of *Desmodesmus* [e.g. *D. subspicatus* (R.Chodat) E.Hegewald & A.Schmidt and *D. armatus*] produce spiny colonies when nutrient availability is low, whereas unicells are produced when phosphorus or nitrogen are elevated (Shubert & Trainor 1974, Siver & Trainor 1981, Trainor 1998). Colonies and unicells become spineless when iron availability is low or lacking (Trainor 1969, Trainor 1998).

The purpose of this study was: (i) to determine the diversity and abundance of *Desmodesmus* in different aquatic ecosystems in Poland using the SEM, and (ii) to conduct preliminary experiments on the use of *Desmodesmus* as a potential bio-indicator of nutrient availability in aquatic ecosystems.

MATERIALS AND METHODS

Study area

Samples were collected from three different aquatic ecosystems (R1, R2, R3) located in southern and central Poland (electronic appendix 1, table 1):

- R1 (ZA Puławy, 21°53'E 51°28'N) – A shallow old river bed (polymictic oxbow) of the Vistula River transformed into an industrial water reservoir, which is highly polluted by municipal and industrial waste.
- R2 (Wiślisko Kobyle, 20°25'E 50°06'N) – A shallow old river bed (polymictic oxbow) of the Vistula River, where chemicals are deposited in the bottom sediments and are subjected to resuspension. The main problem for water quality is the creation of organic (DOC) and organogenic elements.
- R3 (Dobczyce reservoir, 20°05'E 49°52'N) – A deep submountain dimictic reservoir, having chemicals deposited in the bottom sediments, which are excluded from biogeochemical cycles resulting in rather good water quality. However, the main problem is water quality and eutrophication, because it is impacted by agriculture wastes.

Collection, identification and water analysis

Water samples (10 dm^3) were collected for one-year during spring, summer, autumn and winter for taxonomical identification by filtering through a 10 µm mesh plankton net. Some *Desmodemus* spiny morphs can be smaller than 10 µm; however despite the mesh size the morphs can be trapped in detritus or entangled in other microorganisms. From this concentrate, microscopic slides were prepared for the identification of live *Desmodesmus* taxa using a Zeiss Jenaval light microscope (LM), at 250x, 400x and 1,000x oil immersion magnification. This procedure included observation of 20 fields from three separate slide preparations. In addition, another 1 dm^3 of water from the original 10 dm^3 sample was preserved with Lugol's solution. Algae from this sample was

Table 1 – Chemical properties of water samples of the oxbow lakes and reservoir.
Sterile-filtered water was used for the bioassay experiments.

	R1 Spring	R2 Spring	R3 Spring	R1 Summer	R2 Summer	R3 Summer	R1 Autumn	R2 Autumn	R3 Autumn	R1 Winter	R2 Winter	R3 Winter
Chlorophyll *a* [µg L^{-1}]	288.9	1941.8	13.7	97.02	132.61	2.37	59.2	85.3	10.1	11.8	11.8	7.1
EC [mS]	0.95	1.74	0.27	1.77	0.37	0.28	1.24	0.52	0.27	1.79	0.80	0.37
NH_4-N [mg L^{-1}]	18.70	5.90	1.30	4.20	2.80	0.30	0.51	0.35	0.23	0.80	1.90	0.40
NO_3-N [mg L^{-1}]	5.10	1.70	0.80	1.60	0.40	0.60	6.80	1.00	0.50	7.90	1.30	1.10
P_{tot} [mg L^{-1}]	1.04	1.63	0.12	0.9	1.00	0.30	1.25	0.15	0.15	1.22	2.10	0.04
PO_4-P [mg L^{-1}]	0.17	0.05	0.08	0.36	0.34	0.09	0.31	0.10	0.10	0.68	1.87	0.02
Cl [mg L^{-1}]	107	586	12	215	13	10	191	9	9	164	20	16
Fe [mg L^{-1}][3]	16	87.5x10^3	25	66	116	68	12	15	10	22	56	5
Na [mg L^{-1}]	88	13	8	90	5.2	8.8	56	9	11	123	21	23
K [mg L^{-1}]	8	11	2	8.7	5.3	3.2	9	5	2	11	12	3
Ca [mg L^{-1}]	70	230	39	69	62	39	80	61	45	96	90	55
Mg [mg L^{-1}]	17	24	8	17	6	6	18	8	7	19	10	9
SO4 [mg L^{-1}]	18	76	12	21	24	11	13	12	7	22	17	17
DOC [mg L^{-1}]	18.6	21.6	3.6	17.4	10.2	1.2	9.6	3.9	1	8.1	6.6	3.0
Ni [µg L^{-1}]	5	38	3	3.6	2.6	2.2	5	2	2	5	4	1
Pb [µg L^{-1}]	3	41	4	12	11	13	8	8	8	4	9	8
Cu [µg L^{-1}]	6	18	4	6	5	6	5	4	4	7	6	5
Cd [µg L^{-1}]	0.10	4.70	0.15	0.05	0.00	0.10	0.15	0.15	0.20	0.05	0.25	0.20
Zn [µg L^{-1}]	44	666	25	56	64	21	37	31	24	48	26	39
Cr [µg L^{-1}]	0.70	1.9	0.3	0.7	0.7	0.7	0.4	0.4	0.4	0.9	0.2	0.0
Mn [µg L^{-1}]	30.10	17200	0.0	4	1105	0.0	8.9	1.9	37	18.6	13433	19.6

Table 2 – **Density of *Desmodesmus* in two oxbow lakes and one reservoir (R1, R2, R3).**
Total density of phytoplankton (ind. ml^{-1}) during all four seasons in two oxbow lakes and one reservoir.

	spring	summer	autumn	winter
R1 (ind. ml^{-1})				
D. abundans	118	0	36	0
D. armatus	94	272	201	0
D. intermedius	129	71	225	0
D. opoliensis	130	520	130	0
D. quadricauda	12	153	178	0
D. spinosus	60	450	344	35
D. subspicatus	71	0	12	0
Desmodesmus sp.	496	626	59	0
Total *Desmodesmus* density	1110	2092	1185	35
Total phytoplankton density	39059	54695	14808	1241
R2 (ind. ml^{-1})				
D. abundans	0	0	0	0
D. armatus	12	12	36	0
D. intermedius	0	12	0	0
D. opoliensis	83	142	236	0
D. quadricauda	0	0	0	0
D. spinosus	107	0	166	0
D. subspicatus	0	201	0	0
Desmodesmus sp.	118	83	24	0
Total *Desmodesmus* density	320	450	462	0
Total phytoplankton density	39059	12327	10566	343
R3 (ind. ml^{-1})				
D. abundans	0	0	0	0
D. armatus	0	0	0	0
D. intermedius	0	0	60	0
D. opoliensis	0	0	0	0
D. quadricauda	0	0	0	0
D. spinosus	0	12	0	0
D. subspicatus	0	0	0	0
Desmodesmus sp.	0	0	0	24
Total *Desmodesmus* density	0	12	60	24
Total phytoplankton density	1441	1052	3675	7387

allowed to settle for 48 h to form a concentrate in a settling chamber and were examined under 250x and 400x oil immersion magnification (Ligęza & Wilk-Woźniak 2011). Samples for the quantitative analysis of specimens were fixed with Lugol's solution and concentrated by sedimentation from a 1 dm^3 sample (Starmach 1955). Algae were counted according to the method of Lund et al. (1958).

Fresh samples of *Desmodesmus* were fixed in 2.5% glutaraldehyde for 1hr and prepared for the SEM as described by Shubert & Wilk-Woźniak (2003) and viewed with a Philips XL 30 SEM. Measurements were made on the SEM image. Up to 25 cells were measured for each morphological feature; although some measurements of cells and features are based on less than 25 (measurements were based only on fixed field material) (fig. 1).

For taxonomic analyses of *Desmodesmus* we used Komárek & Fott (1983) and Hegewald (2000). The species identifications in this paper were based on clear and reliable taxonomic features.

Physico-chemical properties of water were analyzed immediately after sampling and were measured according to Hermanowicz et al. (1999) with modifications (Prince 1955, van Reeuwijk 1995).

Bioassay experiments

For bioassays, water samples were pre-filtered, sterile filtered and stored in sterile bottles at 4°C. The experimental design used 4-well sterile titer plates. Each water sample was inoculated aseptically into duplicate wells and each well was inoculated with 1×10^5 cells ml^{-1} of 100% colonial and

axenic *D. abundans* (Kirchner) E.Hegewald (isolated from the Vistula River in 2004; CCAP 258/299). Control cultures (medium 7 (Trainor 1998); medium 7 with P or N additions and N+P additions; Bold's Basal Medium (BBM) and soil extract were also inoculated with *D. abundans* strain CCAP 258/299. The cultures were maintained at 24°C and 16 h L: 8 h D. The cultures were counted after 72 hours and 6 days for percentage of spiny and/or spineless colonies and/or unicells. The bioassay experiments were conducted three times.

RESULTS

Environmental conditions of R1, R2 and R3

The largest biomass (chl *a*) of algae occurred in spring in R2 (1941 μgL^{-1}) and the lowest algal biomass occurred in the summer in R3 (2.37 μgL^{-1}). The highest dissolved ions (EC) occurred in the spring in R2, summer in R1 and winter in R1 (1.74–1.79 mS) and the lowest in the spring and summer in R3 and autumn in R3 (0.27–0.28 mS). Overall, there was a large variation in the water chemistry between oxbow lakes (R1, R2, R3) and seasons of the year. A very high manganese concentration (17200 μgL^{-1}) was measured in the spring in R2 (table 1). For a more detailed analysis, see Wilk-Woźniak et al. (2014).

Desmodesmus: occurrence and abundance

R1 was the oxbow lake in which different species and morphs of *Desmodesmus* were observed in the greatest density. Of all reservoirs sampled, 77% of the total density of *Desmodesmus* was observed in R1, 22% in R2 and only 1% in R3. Summer and autumn were the seasons when *Desmodesmus* showed the highest density in the shallow and polymictic oxbow lakes (R1 and R2). In the deep and dimictic reservoir (R3), the highest density of *Desmodesmus* was during the autumn season (table 2).

We identified seven different species of *Desmodesmus* in the shallow oxbow lakes (R1, R2) and two species in R3.

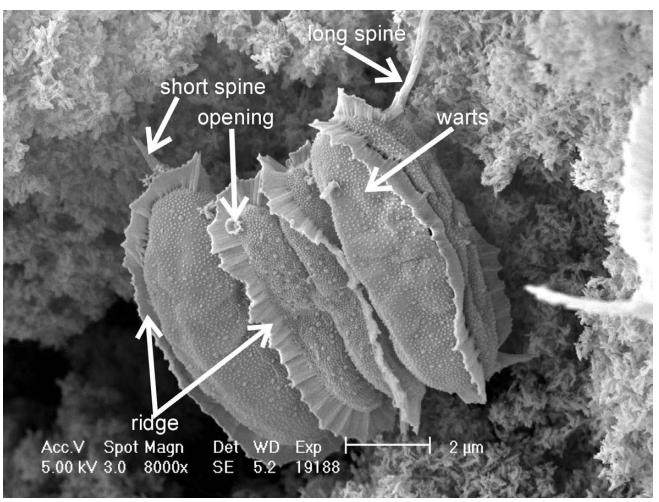

Figure 1 – SEM of outer wall of *Desmodesmus* illustrating ultrastructural features such as a short spine, long spine, warts, ridge and opening.

Table 3 – Morphometrics of *Desmodesmus* species measured with the SEM.
Observations based on field material. Up to 25 cells were measured for each species/morph.

	surface wall morphology	cell length	cell width	ridge height	ridge width	spine length	opening diam.
4-celled (min-max):							
D. abundans	hexagonal net	5.05 µm	3.09 µm	600 nm	656 nm	566 nm	434–514 nm
D. armatus	scattered warts	5.05–12.1 µm	1.59–3.92 µm	838 nm–2.59 µm	484 nm–1.02 µm	374 nm–4.49 µm	327–711 nm
D. opoliensis	scattered warts	10.4–14.9 µm	2.87–5.25 µm	453 nm–1.1 µm	578–973 nm	5.98–12.2 µm	278–382 nm
D. quadricauda	net	11 µm	6.03 µm	not present	not present	8.88 µm	434–514 nm
D. subspicatus	scattered warts	6.78–7.98 µm	3.89–4.87 µm	not present	not present	1.64–5.49 µm	278–318 nm
unicell (min-max):							
D. abundans	hexagonal net	4.84–5.64 µm	3.66–4.68 µm	600 nm	407-532 nm	not observed	not observed
D. armatus	scattered warts	4.46–9.06 µm	3.16–6.87 µm	not observed	not observed	not observed	not observed
D. opoliensis	scattered warts	5 µm	1.8 µm	not observed	not observed	not observed	300 nm
D. quadricauda	net	no unicells	no unicells	no unicells	no unicells	no unicells	no unicells
D. subspicatus	scattered warts	5.66–7.32 µm	4.72–4.99 µm	not present	not present	1.08 - 5.25 µm	315 nm

The most commonly found species were: *D. abundans*, *D. armatus*, *D. intermedius* (R.Chodat) E.Hegewald, *D. opoliensis* (P.G.Richt.) E.Hegewald, *D. quadricauda*, *D. spinosus* (R.Chodat) E.Hegewald, *D. subspicatus* (R.Chodat) E.Hegewald & A.Schmidt (figs 2 & 3). We also identified unicells belonging to the following taxa: *D. abundans*, *D. armatus*, *D. opoliensis* and *D. subspicatus*. Unfortunately, *Desmodesmus* unicells are usually identified as species of *Chodatella* Lemmermann, *Lagerheimia* Chodat or *Franceia* Lemmermann despite the extensive literature published on phenotypic plasticity in the past sixty years (Trainor 1998, Shubert 2003: 255–256).

During different seasons in the water bodies, we observed the following changes in *Desmodesmus* species composition (ranked according to individuals/ml share of total density of *Desmodesmus*) (table 2):

R1
- Spring: *D. intermedius* = *D. opoliensis* > *D. abundans* > *D. armatus* > *D. subspicatus* > *D. spinosus* > *D. quadricauda*
- Summer: *D. opoliensis* > *D. spinosus* > *D. armatus* > *D. quadricauda* > *D. intermedius*
- Autumn: *D. spinosus* > *D. intermedius* > *D. armatus* > *D. quadricauda* > *D. opoliensis* > *D. abundans* > *D. subspicatus*
- Winter: *D. spinosus*

R2
- Spring: *D. spinosus* > *D. opoliensis* > *D. armatus*
- Summer: *D. subspicatus* > *D. opoliensis* > *D. armatus* = *D. intermedius*
- Autumn: *D. opoliensis* > *D. spinosus* > *D. armatus*

R3
- Summer: *D. spinosus*
- Autumn: *D. intermedius*

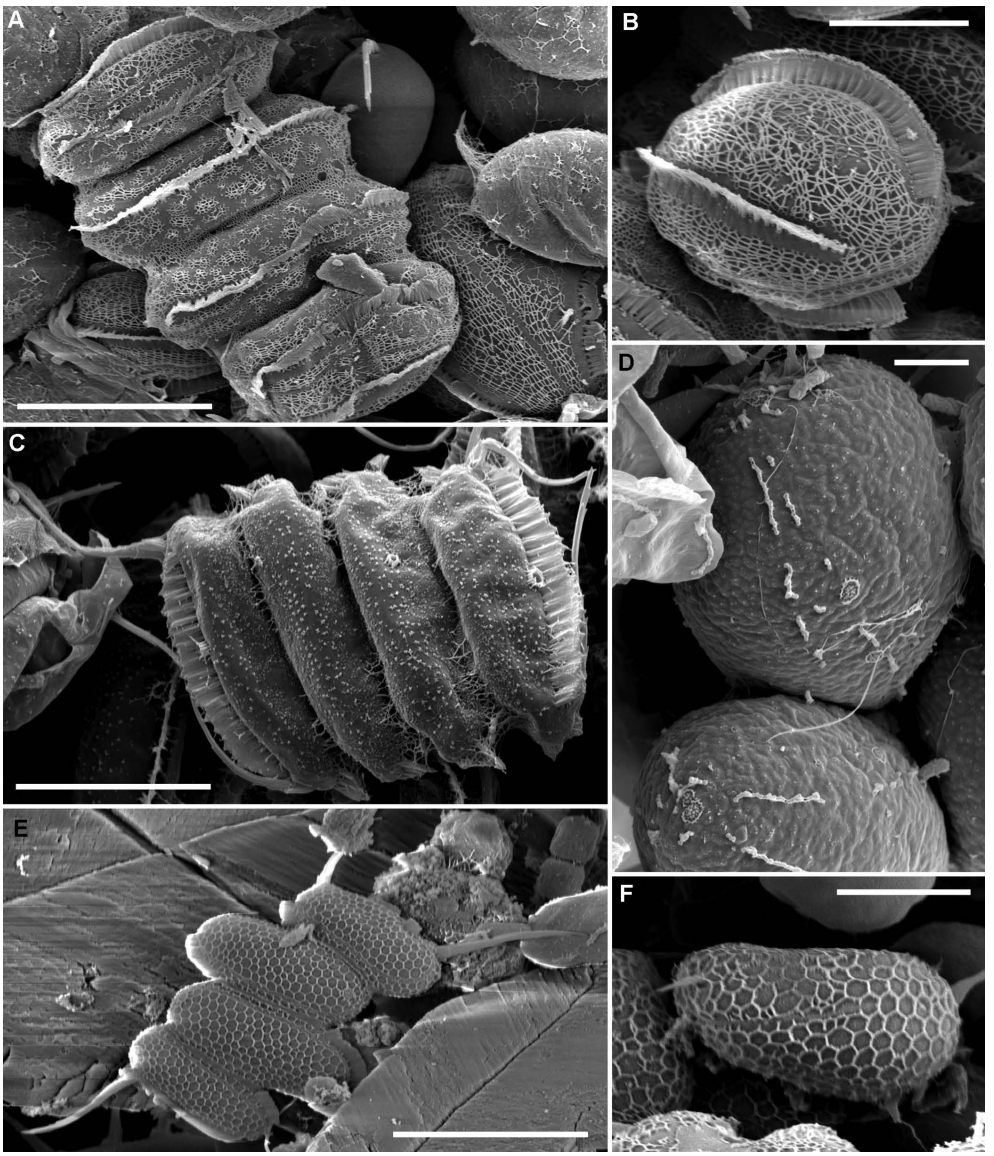

Figure 2 – Scanning electron micrographs of *Desmodesmus* species: A, *D. abundans* colony; B, *D. abundans* unicell; C, *D. armatus* colony; D, *D. armatus* unicells; E, *D. intermedius* colony; F, *D. intermedius* unicell. Scale bars: A, C & E = 5 µm; B, D & F = 2 µm.

We also observed some taxa only once, e.g. *D. armatus* var. *spinosus* (F.E.Fritsch & Rich) E.Hegewald, *D. serratus*, and *D. costato-granulatus* (Skuja) E.Hegewald. Overall, *D. opoliensis* and *D. spinosus* had the greatest densities (520 ind/ml and 450 ind/ml respectively) and *D. armatus*, *D. spinosus* and *D. subspicatus* had the lowest densities (12 ind/ml).

Morphological variation/phenotypic plasticity

Different species of *Desmodesmus* from different populations were measured for length and width of cells, length of spines, presence/absence of ridges, height and width of ridges, diameter of openings, and height of opening using the SEM (fig. 1). We observed a range in size of different structures of the cells, which indicates that *Desmodesmus* is responding to variable environmental conditions (e.g. light, temperature and/or nutrients) (table 1) and/or genetic variation. Comparing the five species, *D. opoliensis* colonies (fig. 3A, table 3) had the longest cells and nearly the largest width (14.9 µm × 5.25 µm), whereas *D. abundans* colonies (fig. 2A, table 2) had the shortest cell length and width (5.05 µm × 3.09 µm). *D. opoliensis* also had the longest spine (12.2 µm) and *D. abundans* had the shortest spine (514 nm). The other five species: *D. armatus*, *D. intermedius*, *D. quadricauda*, *D. serratus* and *D. subspicatus* (figs 2C, 2E, 3C, 3E and 3F respectively) were mid-range in cell size and length of spine (table 2). The unicells of *D. armatus* (fig. 2D, table 3) had the largest cell dimensions and *D. opoliensis* unicells (fig. 3B, table 2) had the smallest cell dimensions. The unicells of *D. abundans* (fig. 2B, table 2) and *D. subspicatus* (fig. 3G, table 2) were mid-range.

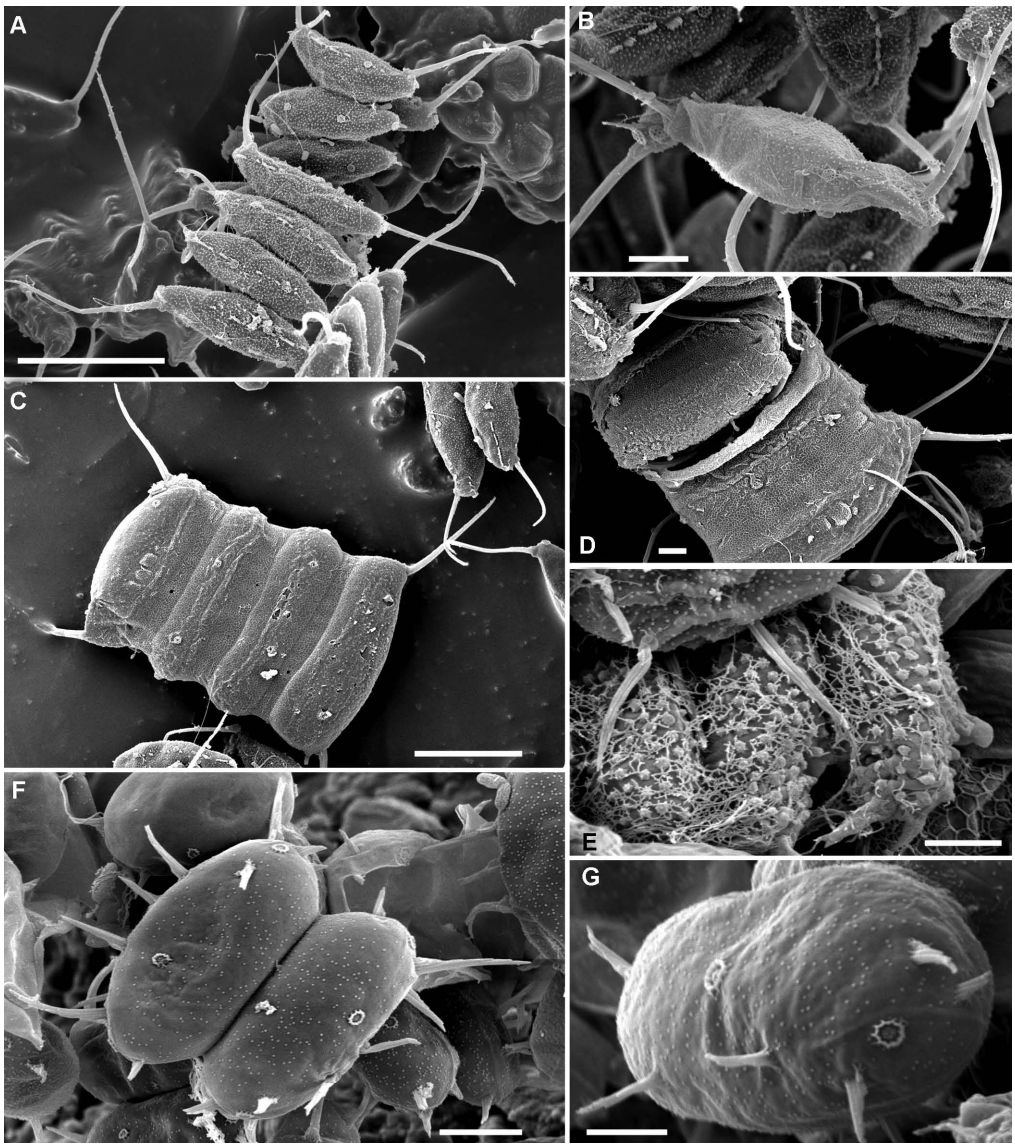

Figure 3 – Scanning electron micrographs of *Desmodesmus* species: A, *D. opoliensis* colony; B, *D. opoliensis* unicell; C, *D. quadricauda* colony; D, *D. quadricauda* colony (showing progeny colony before release from parent cell); E, *D. serratus* colony; F, *D. subspicatus* colony; G, *D. subspicatus* unicell . Scale bars: E & G = 1 µm; B, D, F = 2 µm; A & C = 10 µm).

Table 4 – Mean percentage of unicells and colonies produced in the water bodies.
Cultures were grown under controlled environmental conditions; the experiments were conducted three times; numbers 1 to 12 ((R1, R2, R3) correspond to the same numbers in table 1.

Water samples	Season	Day 0		After 3 days		After 6 days	
		unicells	colonies	unicells	colonies	unicells	colonies
1 (R1)	Spring	0%	100%	20%	80%	0%	100%
2 (R1)	Summer	0	100%	40	60	0	100
3 (R1)	Autumn	0	100%	35	65	30	70
4 (R1)	Winter	0	100%	40	60	0	100
5 (R2)	Spring	0	100%	10	90	0	100
6 (R2)	Summer	0	100%	5	95	0	100
7 (R2)	Autumn	0	100%	10	90	0	100
8 (R2)	Winter	0	100%	10	90	0	100
9 (R3)	Spring	0	100%	10	90	0	100
10 (R3)	Summer	0	100%	5	95	0	100
11 (R3)	Autumn	0	100%	10	90	0	100
12 (R3)	Winter	0	100%	5	95	0	100
M7		0	100%	0	100	0	100
M7+P		0	100%	5	95	0	100
M7+N		0	100%	0	100	0	100
BBM		0	100%	50	50	10	90
SE		0	100%	90	10	100	0
M7+3N		0	100%	95	5	95	5

Bioassay experiments

D. abundans CCAP 258/299 exhibited phenotypic plasticity when grown in water collected from the three different ecosystems. All unicells and colonies produced spines, except for those in the R2 (7) spring sample, which supported 90% colonies with very short spines (table 4). Experimental results showed that there was a higher percentage of spiny unicells (up to 40%) in eutrophic R1 compared to R2 and R3 water (table 4). Spiny colonies dominated in R2 and R3. *Desmodesmus* grown in high nitrogen controls (SE, Medium 7 +3N and BBM) had a higher percentage of unicells compared to the other cultures (table 4). Most cultures were transformed to 100% colonies by day 6, except for: (R1), BBM, SE and M7+3N.

DISCUSSION

Desmodesmus species are very common in freshwater habitats, especially nutrient rich ecosystems. This was the situation in our investigation, with the greatest variability and density of *Desmodesmus* in the oxbow lake polluted by municipal and industrial discharges (R1). Cronberg (1982) observed a similar result from samples taken from the nutrient rich sediments from Lake Växjösjön, Sweden, which indicates that this genus might be useful as an indicator of nutrient loading in aquatic ecosystems. We know that low nutrient concentrations stimulate colony formation and elevated levels of nitrogen or phosphorus stimulate unicell formation (Trainor 1998).

The density of *Desmodesmus* species was highest in oxbow lakes (R1 and R2), because the lakes were shallow and allowed for light penetration, and they had the highest nutrient concentrations. The reservoir (R3) had a lower density of *Desmodesmus* species, because the reservoir was deeper and lower in nutrients. The relationship of cell density to environmental parameters will be explored.

Unfortunately, because of their phenotypic plasticity, *Desmodesmus* species are very difficult to define taxonomically. Many of the morphological features, which might be used as taxonomic characters, are not visible with the LM or are unstable. For example, spine number and spine length cannot be used as stable characters for taxonomic differentiation, because they are variable within a clone. This has been shown for numerous clones (Trainor 1998). Thus, a clone can be spineless and spiny (2 to 4 to 8 spines per colony) depending on the age of the culture (Trainor 1998). In addition, presence or absence of ridges is sometimes also unclear. As we have shown in a range of measurements of cell morphology, the ridges are sometimes so small that it is not possible to see them in LM, especially in unicells or young cells. Using a 100x oil immersion objective, some features can be visualized, such as ridges on the edge of cells and openings. However, most investigators rely on identifications using a 40x objective, which is too low for visualizing finer detail. Some authors indicated that the size of some elements of ultrastructure might depend on the age of cells (Kováčik 1975, Trainor 1996). However, as Komárek & Ludvík (1972) indicated, there are some stable ultrastructural features that might be used as identification characters, such as the pat-

tern on the surface of cells (presence/absence of warts, nets, etc.), and presence/absence and pattern of openings, type of ridges, ribs, etc. Unfortunately these features are not readily visible with the LM, which is the main reason why we relied on SEM observations. This is also the reason why unicells were not identifiable to their colonial morph, since we could not see the wall ultrastructure with the LM. SEM observations allow one to see features in more detail and they may be used for taxonomic analysis (Komárek & Ludvík 1972).

Presence of ecomorphs adds to the taxonomic conundrum, since "The unicell is demonstrated to be one of the more important morphs produced; it will aid in determining species limits. The shortcomings of numerous publications in the last two decades, with an absence of, or incomplete data on individual ecomorphs, now become clear" (Trainor & Egan 1990a).

Our experiments showed that the *D. abundans* CCAP 258/299 was sensitive to higher nitrogen concentrations, which resulted in unicell formation. For example, 95% unicells and only 5% colonies were formed in the samples with SE and M7 + 3N (Siver & Trainor 1981). Whereas in samples with higher phosphate concentrations, we observed the formation of 95% colonies, but only 5% unicells formed. In investigations of other strains, phosphorus has been shown to stimulate unicell formation (Shubert & Trainor 1974). This suggests that differential gene expression is "triggered" by the growth stimulating nutrients N or P (Shubert, unpubl. res.)

In future investigations, numerous *Desmodesmus* strains will be isolated from the Vistula River and tested for their response to phosphorus. A colonial strain may be found that responds to phosphorus by producing unicells.

The cyclomorphosis theory proposed by Trainor (1996) stated that, in nature, unicells are produced when growth stimulating nutrients are available in high concentration (spring). This was demonstrated in laboratory experiments where different ecomorphs were produced using *D. armatus* (Trainor & Egan 1990b). Phenotypic plasticity is affected by different nutrients (not only one) and different species respond differently. It is known that some *Desmodesmus* populations are unable to produce unicells (Shubert, pers. obs.). Our laboratory experiments showed that lack of iron availability resulted in no spines or very short spines. There were no Cladocera or Copepoda species present in the oxbow lakes. Thus, infochemicals could not have had an effect on spine or colony formation (Hessen & van Donk 1993, Lürling & van Donk 1997). In the samples with a high concentration but low availability of iron (probably due to binding with phosphorus), we observed cells with very short spines (e.g. *D. opoliensis* 5.98 nm), which are sometimes difficult to see with the light microscope (compare measurements in table 1). In such a situation, the taxon might be classified as "cells without spines" and possibly misidentified as *Scenedesmus* or some other taxon.

Why has phenotypic plasticity evolved? Morales et al. (2002) postulated that "Phenotypic plasticity may be viewed as a way of maintaining fitness during changes in habitat conditions, and thus, as a way of buffering evolutionary change, making the species less vulnerable to erratic climatic shifts (natural selection)". Although phenotypic plasticity creates a conundrum for taxonomy, it provides additional characters for systematic analysis.

Conclusion

Our results indicated that there was a differential phenotypic response to the concentration of nutrients from each of the three water bodies. The phenotypic response from the titer plate experiments was compared to the chemical analyses of the water samples. *Desmodesmus abundans* CCAP 258/299 produced 40% unicells and 60% colonies in the spring samples from R1 (high availability of nutrients: NH_4^+ 18.7 mg N/L and NO_3^- 5.1 mg N/L), whereas in the samples from R2 and R3 (lower availability of nutrients) *D. abundans* CCAP 258/299 produced only 10% unicells and 90% colonies. The percentage of unicells is thus a good indicator of nutrient availability. Similarly, spine development is inhibited when iron is lacking or unavailable (e.g. sequestered with phosphorus).

Our studies are important from both ecological and taxonomical points of view, since our results showed that there may be new possibilities for using the phenotypic plasticity of *Desmodesmus* for the assessment of water quality, and using morphs of *Desmodesmus* as bioindicators of nutrient availability (N, P and/or Fe) in natural aquatic ecosystems.

SUPPLEMENTARY DATA

Supplementary data are available in pdf at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo.supp-data), and consists of a diagram of the locality of the oxbow lakes (http://maps.geoportal.gov.pl).

ACKNOWLEDGEMENTS

Studies were supported by MNiI 2PO4F04572, BC/ MNiI WAR/ 341/ 251, BC/ MNiI WAR 342/48, Synthesys GB-TAF 1515. We thank Alex Ball, NHM, for his assistance with the SEM, and E.J. Cox for critically reading a draft of the manuscript and preparing the photographic plates. We also thank Alix Le Marois, who was a student volunteer from France, for her assistance in the laboratory, and two anonymous reviewers for their critical evaluation.

REFERENCES

An S.S., Friedl T., Hegewald E. (1999) Phylogenetic relationships of Scenedesmus and Scenedesmus-like coccoid green algae as inferred from ITS-2rDNA sequence comparisons. Plant Biology 1: 418–428. http://dx.doi.org/10.1111/j.1438-8677.1999.tb00724.x

Bisalputra T., Weier T.E. (1963) The cell wall of Scenedesmus quadricauda. American Journal of Botany 50: 1011–1019. http://dx.doi.org/10.2307/2439909

Bisalputra T., Weier T.E., Risley E., Englebrecht A. (1964) The pectic layer of the cell wall of Scenedesmus quadricauda. American Journal of Botany 51: 548–551. http://dx.doi.org/10.2307/2440285

Chodat R. (1913) Monographies d'algues en culture pure. Berne, Matériaux pour la Flore Cryptogamique Suisse.

Chodat R. (1926) Scenedesmus. Étude de génétique, de systématique expérimentale et d'hydrobiologie. Revue d'Hydrobiologie 3: 71–258.

Chodat R., Malinesko O. (1893) Sur le polymorphisme de Scenedesmus acutus. Bulletin de l'Herbier Boissier I: 184–190.

Cronberg G. (1982) Pediastrum and Scenedesmus (Chlorococcales) in sediments from Lake Växjösjön, Sweden. Archiv für Hydrobiologie Supplement 60,4/ Algological Studies 29: 500–507.

Gómez N., Riera J.L., Sabater S. (1995) Ecology and morphological variability of Aulacoseira granulata (Bacillariophyceae) in Spanish reservoirs. Journal of Plankton Research 17: 1–16. http://dx.doi.org/10.1093/plankt/17.1.1

Graham L.E., Wilcox L.W. (2000) Algae. Upper Saddle River, New Jersey, Prentice-Hall.

Grintzesco J. (1902a) Recherches expérimentales sur la morphologie et la physiologie de Scenedesmus acutus Meyen. Bulletin de l'Herbier Boissier II: 217–264.

Grintzesco J. (1902b) Recherches expérimentales sur la morphologie et la physiologie de Scenedesmus acutus Meyen. Bulletin de l'Herbier Boissier II: 406–429.

Grunow A. (1858) Die Desmidiaceen und Pediastreen einiger österreichischen Moore, nebst einigen Bemerkungen über beide Familien im Allgemeinen. Verhandlungen des Zoologisch-Botanischen Vereins in Wien 8: 489–502.

Hegewald E. (2000) New combinations in the genus Desmodesmus (Chlorophyceae, Scenedesmaceae). Archiv für Hydrobiologie Supplement 60/ Algological Studies 96: 1–18.

Hegewald E., Schmidt A., Schnepf E. (1990) Studies on the genus Scenedesmus Meyen (Chlorophyceae, Chlorococcales) from South India, with special reference to the cell wall ultrastructure. Beiheft Nova zur Hedwigia, Heft 99.

Hermanowicz W., Dojlido J., Dożańska W., Koziorowski B., Zerbe J. (1999) Fizyczno-chemiczne badanie wody i ścieków. Warszawa, Arkady.

Hessen D.,van Donk E. (1993) Morphological changes in Scenedesmus induced by substances released from Daphnia. Archiv für Hydrobiologie 127: 129–140.

Kessler E., Schäfer M., Hümmer C., Kloboucek A., Huss V. (1997) Physiological, biochemical, and molecular characters for the taxonomy of the subgenera of Scenedesmus (Chlorococcales, Chlorophyta). Botanica Acta 110: 244–250. http://dx.doi.org/10.1111/j.1438-8677.1997.tb00636.x

Komárek J., Ludvík J. (1971) Die Zellwandultrastruktur als taxonomisches Merkmal in der Gattung Scenedesmus. 1. Die Ultrastrukturelemente. Archiv für Hydrobiologie Supplement / Algological Studies 39: 301–333.

Komárek J., Ludvík J. (1972) Die Zellwandultrastruktur als taxonomisches Merkmal in der Gattung Scenedesmus. 2. Taxonomische Auswertung der untersuchten Arten. Archiv für Hydrobiologie Supplement / Algological Studies 41: 11–47.

Komárek J., Fott B. (1983) Chlorophyceae (Grünalgen), Ordnung: Chlorococcales. In: Huber-Pestalozzi G. (ed.) Das Phytoplankton des Süßwassers 7, 1: 101–137, Systematik und Biologie. 7. Teil, 1. Hälfte. Stuttgart, E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller).

Kováčik L. (1975) Taxonomic review of the genus Tetraedron (Chlorococcales). Archiv für Hydrobiologie Supplement 46/ Algological Studies 13: 354–391.

Ligęza S., Wilk-Woźniak E. (2011) The occurrence of a Euglena pascheri and Leponcinclis ovum bloom in an oxbow lake in southern Poland under extreme environmental conditions. Ecological Indicators 11: 925–929. http://dx.doi.org/10.1016/j.ecolind.2010.10.008

Lampert W., Rothhaupt K., von Elert E. (1994) Chemical induction of colony formation in a green alga (Scenedesmus acutus) by grazers (Daphnia). Limnology and Oceanography 39: 1543–1550. http://dx.doi.org/10.4319/lo.1994.39.7.1543

Lund J.W.G., Kipling G., Le Cren E.D. (1958) The inverted microscope method of estimating algae numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170. http://dx.doi.org/10.1007/BF00007865

Lürling M., De Lange H.J., van Donk E. (1997) Changes in food quality of the green alga Scenedesmus induced byDaphnia infochemicals: biochemical composition and morphology. Freshwater Biology 38: 619–628. http://dx.doi.org/10.1046/j.1365-2427.1997.00225.x

Lürling M., van Donk E. (1997) Life history consequences for Daphnia pulex feeding on nutrient-limited phytoplankton. Freshwater Biology 38: 693–709. http://dx.doi.org/10.1046/j.1365-2427.1997.00242.x

Massalski A., Trainor F.R., Shubert L.E. (1974) Wall ultrastructure of Scenedesmus culture N46. Archiv für Microbiologie 96: 145–153. http://dx.doi.org/10.1007/BF00590171

Morales E.A., Trainor F.R., Schlichting C.D. (2002) Evolutionary and ecological implications of plastic responses in algae [online]. Constancea 83.4. Available from http://ucjeps.berkeley.edu/constancea/83/morales_etal/plasticity.html [accessed 13 May 2014].

Pigliucci M. (1996) How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). Trends in Ecology and Evolution 11: 168–173. http://dx.doi.org/10.1016/0169-5347(96)10008-2

Pigliucci M. (2001) Phenotypic Plasticity. Beyond Nature and Nurture. Baltimore, The John Hopkins University Press.

Pigliucci M., Schlichting C.D., Jones C.S., Schwenk K. (1996) Developmental reaction norms: Interactions among allometry, ontogeny and plasticity. Plant Species Biology 11: 69–85. http://dx.doi.org/10.1111/j.1442-1984.1996.tb00110.x

Prince A.L. (1955) Appendix – Methods in soil analysis. In: Bear F.E. (ed.) Chemistry of the soil: 328–362. ACS Monograph 126. Reinhold Publishing Corporation.

Shubert L. E. (2003) Nonmotile Coccoid and Colonial Green Algae. In: Wehr J.D., Sheath R.G. (eds) Freshwater Algae of North America: 253–309. New York, Academic Press.

Shubert L.E., Trainor F.R. (1974) Scenedesmus morphogensis. Control of the unicell stage with phosphorus. British Phycological Journal 8: 1–7. http://dx.doi.org/10.1080/00071617400650011

Shubert L.E., Wilk-Woźniak E. (2003) SEM investigation of several non-motile coccoid green algae isolated from aquatic habitats in Poland. Biologia 58: 459–466.

Siver P., Trainor F.R. (1981) Morphological control and physiology of Scenedesmus strain 170. Phycologia 22: 127–131. http://dx.doi.org/10.2216/i0031-8884-20-1-1.1

Smith G.M. (1914a) The organization of the colony in certain four-celled algae. Transactions of the Wisconsin Academy of Sciences 17: 1165–1220.

Smith G.M. (1914b) A monograph of the algal genus Scenedesmus based upon pure culture studies. Transactions of the Wisconsin Academy of Sciences 32: 278–297.

Starmach K. (1955) Metody badań plankton. Warszawa, PWRiL.

Trainor F.R. (1963) The morphology of a Scenedesmus in pure and contaminated culture. Bulletin of the Torrey Botanical Club 90: 137–138. http://dx.doi.org/10.2307/2483011

Trainor F.R. (1969) Scenedesmus morphogenesis. Trace elements and spine formation. Journal of Phycology 5: 185–190. http://dx.doi.org/10.1111/j.1529-8817.1969.tb02601.x

Trainor F.R. (1993) Cyclomorphosis in Scenedesmus subspicatus (Chlorococcales, Chlorophyta): stimulation of colony development at low temperature. Phycologia 32: 429–433. http://dx.doi.org/10.2216/i0031-8884-32-6-429.1

Trainor F.R. (1996) Reproduction in Scenedesmus. Algae (The Korean Journal of Phycology) 11: 183–201.

Trainor F.R. (1998) Biological aspects of Scenedesmus (Chlorophyceae) – phenotypic plasticity. Nova Hedwigia, Beihefte 117.

Trainor F.R., Cain J.R., Shubert L.E. (1976) Morphology and nutrition of the colonial green alga, Scenedesmus: 80 years later. The Botanical Review 42: 5–25. http://dx.doi.org/10.1007/BF02860860

Trainor F.R., Egan P.F. (1990a) The implications of polymorphism for the systematics of Scenedesmus. British Phycological Journal 25: 275–279.

Trainor F.R., Egan P.F. (1990b) Phenotypic plasticity in Scenedesmus (Chlorophyta) with special reference to S. armatus unicells. Phycologia 29: 461–469. http://dx.doi.org/10.2216/i0031-8884-29-4-461.1

Trainor F.R., Rowland H.L., Lylis J.C., Winter P.A., Bonanomi, P.L. (1971) Some examples of polymorphism in algae. Phycologia 10: 113–119. http://dx.doi.org/10.2216/i0031-8884-10-1-113.1

van Reeuwijk L.P. (1995) Procedures for soil analysis. Wageningen, ISRIC & FAO.

Wolle F. (1887) Freshwater algae of the United States. Bethlehem, Pennsylvania, The Comenius Press.

Wood H. (1873) A contribution to the history of the fresh-water algae of North America. Smithsonian Contributions to Knowledge 19. http://dx.doi.org/10.5962/bhl.title.27413

Wilk-Woźniak E., Ligęza S., Shubert E. (2014) Effect of water quality on phytoplankton structure in oxbow lakes under anthropogenic and non-anthropogenic impacts. Clean - Soil, Air, Water 42: 421–427. http://dx.doi.org/10.1002/clen.201200214

Zirbel M.J., Veron F., Latz M.I. (2000) The reversible effect of flow on the morphology of Ceratocorys horrida (Peridinales, Dinophyta). Journal of Phycology 36: 46–58. http://dx.doi.org/10.1046/j.1529-8817.2000.98088.x

Manuscript received 3 Jul. 2013; accepted in revised version 9 May 2014.

Communicating Editor: Bart Van de Vijver.