Annals of Botany **109**: 1047–1053, 2012
doi:10.1093/aob/mcs021, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY Founded 1887

Combined use of leaf size and economics traits allows direct comparison of hydrophyte and terrestrial herbaceous adaptive strategies

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Received: 24 November 2011 Returned for revision: 10 January 2012 Accepted: 13 January 2012 Published electronically: 14 February 2012

• *Background and Aims* Hydrophytes generally exhibit highly acquisitive leaf economics. However, a range of growth forms is evident, from small, free-floating and rapidly growing Lemniden to large, broad-leaved Nymphaeiden, denoting variability in adaptive strategies. Traits used to classify adaptive strategies in terrestrial species, such as canopy height, are not applicable to hydrophytes. We hypothesize that hydrophyte leaf size traits and economics exhibit sufficient overlap with terrestrial species to allow a common classification of plant functional types, *sensu* Grime's CSR theory.
• *Methods* Leaf morpho-functional traits were measured for 61 species from 47 water bodies in lowland continental, sub-alpine and alpine bioclimatic zones in southern Europe and compared against the full leaf economics spectrum and leaf size range of terrestrial herbs, and between hydrophyte growth forms.
• *Key Results* Hydrophytes differed in the ranges and mean values of traits compared with herbs, but principal components analysis (PCA) demonstrated that both groups shared axes of trait variability: PCA1 encompassed size variation (area and mass), and PCA2 ranged from relatively dense, carbon-rich leaves to nitrogen-rich leaves of high specific leaf area (SLA). Most growth forms exhibited trait syndromes directly equivalent to herbs classified as R adapted, although Nymphaeiden ranged between C and SR adaptation.
• *Conclusions* Our findings support the hypothesis that hydrophyte adaptive strategy variation reflects fundamental trade-offs in economics and size that govern all plants, and that hydrophyte adaptive strategies can be directly compared with terrestrial species by combining leaf economics and size traits.

Key words: Aquatic plant, plant functional type, plant economics spectrum, universal adaptive strategy theory, worldwide leaf economics spectrum.

INTRODUCTION

The worldwide leaf economics spectrum (Wright *et al.*, 2004) describes a widespread gradient in leaf trait variability reflecting a trade-off between acquisitive and conservative leaf functioning. This relationship is hypothesized to be a universal characteristic of the plant kingdom, 'a tradeoff between attributes conferring an ability for high rates of resource acquisition in productive habitats and those responsible for retention of resource capital in unproductive conditions' (Grime *et al.*, 1997), and has been proposed as one of the key determinants of plant adaptive strategies (Grime, 2001). Leaf economics forms only a part of the overall plant economics spectrum (Grime *et al.*, 1997; Freschet *et al.*, 2010) that, in turn, is associated with only one of the main axes of trait variation evident for terrestrial plants (Díaz *et al.*, 2004; Cerabolini *et al.*, 2010*a*). Three main directions of evolutionary specialization exist, 'with extreme strategies facilitating the survival of genes via: (C). the survival of the individual using traits that maximise resource acquisition and resource control in consistently productive niches, (S). individual survival via maintenance of metabolic performance in variable and unproductive niches, or (R). rapid gene propagation via rapid completion of the lifecycle and regeneration in niches where events are frequently lethal to the individual' (reviewed by Grime and Pierce, 2012).

However, one of the practical difficulties in classifying and comparing organisms with contrasting life histories is the lack of common traits. For instance, Hodgson *et al.*'s (1999) CSR classification scheme, now applied to >1000 terrestrial herbaceous and woody species in a range of habitats throughout Europe (Caccianiga *et al.*, 2006; Pierce *et al.*, 2007*a*, *b*; Simonová and Lososová, 2008; Massant *et al.*, 2009; Cerabolini *et al.*, 2010*a*, *b*; Kilinç *et al.*, 2010; Navas *et al.*, 2010), assigns an index of competitive ability, or C adaptation, based in part on the trait canopy height. Weiher *et al.* (1999) suggest that 'height should be measured as the difference between the elevation of the highest photosynthetic tissue in the canopy and the base of the plant'. For aquatic macrophytes, canopy height is a difficult measure to apply where different growth forms position leaves equally at the air–water interface but may be free floating or anchored to the substrate. Hydrophytes are often classified in terms of CSR strategies (e.g. Kautsky, 1988; Murphy *et al.*, 1990; Lehmann *et al.*, 1997; Greulich and Bornette, 1999), but this has previously relied on inference of the degree of stress tolerance from measures of depth and light availability, which are not directly comparable with the leaf economics traits, size traits and phenological traits used in CSR classification (Hodgson *et al.*, 1999).

However, physical size, at least in productive niches, is a fundamental determinant of the ability to acquire resources (Grime and Pierce, 2012), and forms an axis of trait variability distinct from that of the plant economics spectrum (Díaz *et al.*, 2004; Cerabolini *et al.*, 2010*a*). Thus we hypothesize that economics and size traits (particularly area and mass) provide common points of reference, available from leaf material, which could potentially be used to compare the primary adaptive strategies of hydrophytes and terrestrial species directly.

Poorter *et al.* (2009) included hydrophytes in their review of leaf mass per area (LMA – a key indicator of leaf economics) and found that hydrophytes exhibited the lowest LMA values (i.e. highly acquisitive physiologies) compared with a range of terrestrial plant growth forms. However, all freshwater species were amalgamated into a single growth form category that actually masks a range of highly divergent life history strategies. These include free-floating leafy forms, such as *Lemna minor* [the species with the highest relative growth rate (RGR) ever measured; Grime *et al.*, 2007], and large species anchored to the substrate with extensive rhizome systems and with slower growth rates, such as the water lilies (e.g. *Nymphaea alba*). The variation in economics between these diverse hydrophyte groups, and specifically its relationship to contrasting hydrophyte growth forms, is not understood. A number of growth form classification systems exist that can bring order to studies of hydrophyte functional biology, the most recent and comprehensive being that of Wiegleb (1991), summarized in Table 1. This system classifies hydrophytes based on a small number of key criteria, such as whether the plant is anchored to the substrate by roots or is free floating, whether the leaves are submerged, float or emerge from the water, and leaf form and arrangement.

The present study aims to compare variation in a range of traits to determine whether hydrophyte leaf characteristics co-vary in a manner consistent with terrestrial species, allowing a consistent CSR classification system for hydrophytes, and whether differences in primary adaptive strategy are apparent between hydrophyte growth forms.

TABLE 1. *Hydrophyte growth forms according to Wiegleb (1991)*

Growth form	Characteristics
Batrachiden	Anchored plants with both floating and submerged leaves that are entire or compound.
Ceratophylliden	Free-floating plants with submerged finely divided leaves.
Elodeiden	Anchored submerged plants with whorls of small, undivided leaves.
Herbiden	Anchored herbaceous plants similar in phenotype to terrestrial herbs.
Hydrochariden	Free-floating plants with large leaves.
Isoetiden	Anchored plants with basal buds and stiff, narrow leaves.
Lemniden	Floating plants composed mainly of small leaves.
Nymphaeiden	Anchored plants with floating leaves attached to a submerged rhizome by an elongate petiole.
Magnopotamiden	Anchored submerged plants with large, entire leaves.
Myriophylliden	Anchored submerged plants with long stems and finely divided leaves.
Parvopotamiden	Anchored submerged plants with small, entire leaves and sympodial shoots.
Pepliden	Anchored plants with elongated or spathulate leaves forming a terminal rosette adapted for emergence into the atmosphere.
Riccielliden	Free-floating but submersed plants with small, entire leaves.
Stratiotiden	Free-floating plants with emerging leaves.
Vallisneriden	Anchored plants with long, floating basal leaves.

MATERIALS AND METHODS

Plant material was collected from 47 water bodies (12 lakes, four marshes, four peat bogs, 19 irrigation canals, seven ponds and one spring) over a wide range of bioclimatic zones spanning alpine to lowland sites in northern Italy, between the months of July and September 2009. Whenever necessary, plant material was collected using a rowing boat. Ten fully expanded, intact leaves of each species were collected from separate individuals of 61 species representing 21 families (for species list see Table 2; species nomenclature follows Pignatti, 1982), with each species collected from a single site.

The most distal fully expanded leaves along the rhizome or stem were sampled. For the special case of the carnivorous *Utricularia* species, the area of the distal 4 cm of each shoot (including photosynthetic stems and stem-like leaves) were sampled and bladder traps were removed prior to area and mass measurements. Plant material was transported to the laboratory and stored underwater in the dark overnight at 4 °C. Following the standardized methods of Cornelissen *et al.* (2003), turgid leaf fresh weight (LFW) was determined from these saturated organs. Leaf area was determined using a digital leaf area meter (Delta-T Image Analysis System; Delta-T Devices Co. Ltd, Burwell, Cambridgeshire, UK). Leaf dry weight (LDW) was then determined following drying for 24 h at 105 °C, and parameters such as SLA [i.e. leaf area (LA) divided by LDW] were calculated. Leaf dry matter content (LDMC) was calculated as the proportion of LFW accounted for by LDW. Leaf nitrogen concentration (LNC) and leaf carbon concentration (LCC) were quantified from dried plant material using a CHN-analyzer [NA-2000 NProtein; Fisons Instruments S.p.A., Rodano (MI), Italy] following the method outlined by Cerabolini *et al.* (2010*a*).

Data gathered for aquatic species were compared against data, measured using precisely the same methods, for terrestrial herbaceous species already published in the FIFTH database (the Flora d'Italia Functional Traits Hoard; Cerabolini *et al.* 2010*a*), downloadable from: www.springerlink.com/content/21535l25m82×7076/supplementals.

The FIFTH database includes 506 terrestrial species from geo-climatically diverse regions of northern Italy (from lowland, mid-elevation and alpine sites), and encompasses the full range of leaf economics values so far recorded for herbaceous species, providing an appropriate and readily available 'control' spectrum against which to compare the leaf traits of hydrophytes measured from the same latitudes (Cerabolini *et al.*, 2010*a*). The FIFTH database also includes whole-plant traits and CSR strategies for each species, the latter calculated following the method of Hodgson *et al.* (1999) and which we have described and justified extensively in previous

TABLE 2. *Leaf traits of 61 hydrophyte species*

Binomial	Growth form	LA (mm^2)	LFW (mg)	LDW (mg)	LDMC (%)	SLA (mm^2 mg^{-1})	LNC (%)	LCC (%)
Alisma gramineum Lej. subsp. *gramineum*	Vallisneriden	4825·5 ± 994·43	3452·81 ± 614·98	209·44 ± 39·12	6·1 ± 0·27	23·0 ± 1·86	3·3 ± 0·06	35·9 ± 0·37
Azolla filiculoides Lam.	Lemniden	0·9 ± 0·14	0·08 ± 0 .02	0·02 ± 0·00	29·5 ± 7·26	41·4 ± 11·76	3·5 ± 0·03	35·4 ± 0·16
Berula erecta (Huds.) Coville	Herbiden	1112·4 ± 260·61	153·92 ± 38·70	17·64 ± 4·58	11·5 ± 0·62	63·5 ± 4·61	4·0 ± 0·03	37·2 ± 0·24
Callitriche obtusangula LeGall	Pepliden	26·8 ± 2·42	3·57 ± 0·36	0·34 ± 0·03	9·4 ± 0·38	79·8 ± 5·45	4·7 ± 0·19	41·2 ± 0·21
Callitriche platycarpa Kütz.	Pepliden	32·0 ± 4·63	3·70 ± 0·54	0·48 ± 0·11	12·8 ± 1·19	68·5 ± 8·65	2·8 ± 0·01	37·4 ± 0·07
Ceratophyllum demersum L.	Ceratophylliden	41·1 ± 8·46	9·49 ± 1·96	0·66 ± 0·13	7·1 ± 0·43	61·5 ± 5·15	4·2 ± 0·05	39·9 ± 0·20
Egeria densa Planch.	Elodeiden	104·0 ± 13·90	7·98 ± 1·14	1·13 ± 0·20	14·1 ± 0·63	92·6 ± 6·92	5·1 ± 0·05	42·9 ± 0·26
Elodea canadensis Michx.	Elodeiden	26·3 ± 3·71	2·02 ± 0·31	0·35 ± 0·06	17·5 ± 2·92	76·4 ± 14·29	4·5 ± 0·07	39·2 ± 0·60
Elodea nuttallii (Planch.) H.St.John	Elodeiden	27·7 ± 4·26	2·03 ± 0·49	0·46 ± 0·12	22·5 ± 1·45	62·3 ± 8·10	3·3 ± 0·11	37·8 ± 0·74
Groenlandia densa (L.) Fourr.	Parvopotamiden	39·9 ± 8·06	2·07 ± 0·45	0·36 ± 0·09	17·3 ± 1·51	112·1 ± 9·22	3·1 ± 0·07	38·5 ± 0·33
Helosciadium nodiflorum (L.) W.D.J. Koch	Herbiden	3362·4 ± 974·29	717·73 ± 235·11	56·58 ± 18·00	7·9 ± 0·58	60·2 ± 7·50	5·0 ± 0·01	39·1 ± 0·10
Hippuris vulgaris L.	Elodeiden	52·3 ± 8·10	5·73 ± 1·05	0·72 ± 0·15	12·5 ± 0·88	73·7 ± 5·84	3·4 ± 0·03	38·2 ± 0·13
Hottonia palustris L.	Myriophylliden	257·7 ± 11·40	31·02 ± 1·80	5·82 ± 1·24	18·7 ± 3·74	45·9 ± 8·64	2·0 ± 0·06	41·2 ± 0·53
Hydrocharis morsus-ranae L.	Hydrochariden	1466·6 ± 170·53	283·93 ± 42·79	41·49 ± 6·20	14·6 ± 0·38	35·5 ± 1·93	4·1 ± 0·05	44·0 ± 0·15
Juncus bulbosus L.	Isoetiden	91·6 ± 17·71	15·64 ± 4·52	4·07 ± 1·19	26·5 ± 6·54	23·5 ± 4·94	1·4 ± 0·02	43·3 ± 0·37
Lagarosiphon major (Ridl.) Moss.	Parvopotamiden	17·6 ± 2·69	1·56 ± 0·24	0·38 ± 0·06	24·4 ± 0·84	46·2 ± 2·24	3·0 ± 0·04	40·6 ± 0·10
Lemna gibba L.	Lemniden	18·8 ± 2·09	8·27 ± 1·13	0·34 ± 0·08	4·1 ± 0·96	56·9 ± 6·85	3·7 ± 0·03	41·6 ± 0·24
Lemna minor L.	Lemniden	5·8 ± 0·77	0·60 ± 0·10	0·07 ± 0·01	12·3 ± 1·02	80·0 ± 8·37	2·8 ± 0·01	37·4 ± 0·19
Lemna minuta Kunth	Lemniden	2·4 ± 0·54	0·16 ± 0·04	0·02 ± 0·00	10·1 ± 0·90	155·5 ± 30·07	2·7 ± 0·01	35·3 ± 0·05
Lemna trisulca L.	Riccielliden	18·0 ± 3·95	2·51 ± 0·48	0·33 ± 0·08	13·3 ± 2·22	55·0 ± 8·58	2·7 ± 0·03	36·9 ± 0·36
Marsilea quadrifolia L.	Magnonymphaeiden	534·2 ± 123·93	70·72 ± 17·84	16·01 ± 3·76	22·7 ± 1·27	33·5 ± 1·74	3·2 ± 0·05	44·3 ± 0·12
Myriophyllum aquaticum (Velloso) Verdc.	Myriophylliden	455·1 ± 57·73	32·79 ± 3·22	2·24 ± 0·27	6·8 ± 0·33	203·2 ± 11·52	3·0 ± 0·02	38·4 ± 0·10
Myriophyllum spicatum L.	Myriophylliden	160·0 ± 56·09	18·45 ± 5·76	2·22 ± 0·70	12·1 ± 0·97	71·4 ± 6·87	3·3 ± 0·00	42·1 ± 0·11
Myriophyllum verticillatum L.	Myriophylliden	278·3 ± 58·21	38·68 ± 10·56	2·96 ± 0·83	7·6 ± 0·47	96·5 ± 12·54	2·7 ± 0·02	41·2 ± 0·20
Najas marina ssp. *intermedia* (Wolfg. ex Gorski) Casper	Parvopotamiden	94·3 ± 18·93	50·58 ± 14·10	2·43 ± 0·63	4·8 ± 0·30	39·8 ± 5·11	2·4 ± 0·06	36·2 ± 0·49
Najas minor All.	Parvopotamiden	6·2 ± 1·29	0·69 ± 0·15	0·08 ± 0·02	12·1 ± 1·61	76·3 ± 16·21	3·7 ± 0·08	40·9 ± 0·52
Nasturtium officinale R.Br. subsp. *officinale*	Herbiden	339·3 ± 160·47	56·39 ± 29·70	3·48 ± 1·82	6·2 ± 0·50	101·0 ± 12·97	6·7 ± 0·08	39·2 ± 0·22
Nuphar lutea (L.) Sm.	Magnonymphaeiden	27701·7 ± 4930·16	13464·90 ± 2803·01	2688·60 ± 559·88	20·0 ± 1·52	10·4 ± 0·98	2·7 ± 0·03	44·8 ± 0·22
Nymphaea alba L.	Magnonymphaeiden	44608·0 ± 6206·08	23801·20 ± 3389·24	4980·90 ± 894·25	20·8 ± 0·97	9·0 ± 0·58	1·9 ± 0·02	45·2 ± 0·08
Nymphaea candida C. Presl	Magnonymphaeiden	35576·6 ± 6193·01	14390·00 ± 3148·49	2998·00 ± 878·03	20·6 ± 2·32	12·3 ± 2·09	3·1 ± 0·04	45·4 ± 0·20
Nymphaea odorata subsp. *tuberosa* (Paine) Wiersema & Hellquist	Magnonymphaeiden	25388·1 ± 5011·12	11098·00 ± 2652·61	2053·00 ± 419·81	18·7 ± 2·12	12·5 ± 1·86	2·8 ± 0·03	45·4 ± 0·16
Nymphaea × *marliacea* Wildsmith cv. Carnea	Magnonymphaeiden	43936·7 ± 8548·06	18817·80 ± 4797·76	3309·90 ± 1111·52	17·4 ± 3·27	13·9 ± 2·20	2·4 ± 0·02	45·2 ± 0·14
Nymphoides peltata (S.G. Gmel.) Kuntze	Magnonymphaeiden	6894·3 ± 021·33	2243·07 ± 694·13	268·04 ± 86·74	11·9 ± 0·78	26·1 ± 3·19	2·8 ± 0·01	44·6 ± 0·10
Persicaria amphibia (L.) Delarbre	Magnonymphaeiden	1347·6 ± 198·90	242·48 ± 3 7·41	44·24 ± 8·14	18·2 ± 1·03	30·7 ± 2·30	3·9 ± 0·05	45·0 ± 0·34
Persicaria dubia (Stein.) Fourr.	Herbiden	821·9 ± 58·78	131·87 ± 9·81	14·98 ± 0·89	11·4 ± 0·46	54·9 ± 2·71	5·6 ± 0·06	41·2 ± 0·39
Persicaria hydropiper (L.) Delarbre	Herbiden	1017·0 ± 346·47	100·42 ± 33·24	12·33 ± 3·64	12·4 ± 0·77	81·5 ± 6·84	5·4 ± 0·12	42·8 ± 0·43
Potamogeton berchtoldii Fieber	Parvopotamiden	60·5 ± 6·88	3·51 ± 0·43	0·63 ± 0·13	17·9 ± 2·57	98·3 ± 14·89	3·5 ± 0·07	39·1 ± 0·46
Potamogeton crispus L.	Parvopotamiden	499·9 ± 39·12	56·05 ± 4·73	11·08 ± 0·83	19·9 ± 1·88	45·3 ± 4·80	4·2 ± 0·02	45·2 ± 0·34
Potamogeton lucens L.	Batrachiden	1686·2 ± 220·79	329·95 ± 45·08	40·92 ± 5·33	12·4 ± 0·45	41·3 ± 1·91	4·7 ± 0·05	42·5 ± 0·08
Potamogeton natans L.	Batrachiden	3736·9 ± 754·83	644·56 ± 140·91	119·66 ± 26·34	18·6 ± 1·52	31·7 ± 5·06	4·1 ± 0·09	44·6 ± 0·14
Potamogeton nodosus Poir.	Batrachiden	4068·4 ± 702·53	932·10 ± 200·07	183·67 ± 62·22	19·6 ± 4·82	24·2 ± 8·30	3·5 ± 0·06	45·2 ± 0·18
Potamogeton pectinatus L.	Parvopotamiden	40·4 ± 6·63	8·65 ± 1·68	1·22 ± 0·23	14·1 ± 0·89	33·8 ± 6·03	3·7 ± 0·14	43·8 ± 1·20
Potamogeton perfoliatus L.	Magnopotamiden	654·4 ± 137·03	99·96 ± 22·78	16·26 ± 3·25	16·4 ± 0·99	40·2 ± 2·98	2·4 ± 0·06	40·9 ± 0·18
Potamogeton polygonifolius Pourr.	Batrachiden	1529·0 ± 229·32	309·36 ± 55·52	102·31 ± 15·13	33·3 ± 1·78	15·0 ± 1·07	2·3 ± 0·04	45·0 ± 0·55
Potamogeton trichoides Cham. & Schltdl.	Parvopotamiden	24·4 ± 5·01	1·39 ± 0·32	0·31 ± 0·07	22·1 ± 1·75	80·2 ± 5·68	4·6 ± 0·11	41·5 ± 0·59
Ranunculus aquatilis L.	Batrachiden	169·5 ± 31·35	37·76 ± 8·20	4·02 ± 0·81	10·7 ± 0·33	42·4 ± 2·21	5·3 ± 0·04	41·9 ± 0·25
Ranunculus fluitans Lam.	Myriophylliden	638·8 ± 118·09	195·71 ± 42·38	26·08 ± 6·84	13·2 ± 1·25	25·2 ± 3·54	3·1 ± 0·02	42·1 ± 0·47

Continued

TABLE 2. *Continued*

Binomial	Growth form	LA (mm^2)	LFW (mg)	LDW (mg)	LDMC (%)	SLA (mm^2 mg^{-1})	LNC (%)	LCC (%)
Ranunculus trichophyllus Chaix subsp. *eradicatus* (Laest.) C.D.K. Cook	Myriophylliden	107·0 ± 48·97	14·89 ± 7·00	2·85 ± 0·93	20·5 ± 5·18	36·7 ± 7·73	3·0 ± 0·08	44·1 ± 0·11
Ranunculus trichophyllus Chaix subsp. *trichophyllus*	Myriophylliden	974·3 ± 180·00	226·01 ± 48·03	20·31 ± 4·36	9·0 ± 1·08	48·3 ± 3·98	4·2 ± 0·13	41·3 ± 0·31
Salvinia natans (L.) All.	Lemniden	126·5 ± 18·01	31·74 ± 6·40	2·29 ± 0·52	7·2 ± 0·71	56·7 ± 8·77	3·1 ± 0·04	39·0 ± 0·40
Sparganium emersum Rehmann	Vallisneriden	5247·5 ± 1757·44	1324·50 ± 578·09	125·69 ± 49·29	9·6 ± 0·99	42·5 ± 3·60	3·7 ± 0·03	41·3 ± 0·24
Sparganium natans L.	Vallisneriden	3042·4 ± 302·53	677·27 ± 88·03	141·49 ± 13·69	21·0 ± 1·43	21·6 ± 1·73	3·7 ± 0·02	45·4 ± 0·04
Spirodela polyrhiza (L.) Schleid.	Lemniden	40·0 ± 2·53	8·59 ± 0·73	0·94 ± 0·08	11·0 ± 0·75	42·7 ± 3·30	4·7 ± 0·05	42·1 ± 0·28
Trapa natans L.	Magnonymphaeiden	3640·7 ± 467·05	1430·81 ± 199·74	319·35 ± 46·73	22·3 ± 1·17	11·4 ± 0·63	2·8 ± 0·01	42·5 ± 0·04
Utricularia australis R.Br.	Ceratophylliden	106·5 ± 12·48	10·21 ± 1·95	0·82 ± 0·16	8·0 ± 0·36	133·3 ± 19·57	4·0 ± 0·06	44·3 ± 0·29
Utricularia vulgaris L.	Ceratophylliden	46·3 ± 11·62	3·49 ± 0·84	0·28 ± 0·07	8·1 ± 0·59	164·0 ± 9·31	3·5 ± 0·11	39·8 ± 0·80
Vallisneria americana Michx.	Vallisneriden	21861·6 ± 4590·41	8990·60 ± 1991·34	509·17 ± 153·22	5·6 ± 0·60	43·9 ± 4·64	2·8 ± 0·02	37·7 ± 0·11
Vallisneria spiralis L.	Vallisneriden	4095·9 ± 1062·65	1080·80 ± 325·96	62·13 ± 21·34	5·7 ± 0·39	68·1 ± 8·82	3·5 ± 0·01	35·6 ± 0·09
Veronica beccabunga L.	Herbiden	280·9 ± 63·58	44·36 ± 11·17	2·36 ± 0·60	5·3 ± 0·19	120·1 ± 6·08	5·0 ± 0·01	42·5 ± 0·05
Wolffia arrhiza (L.) Horkel ex Wimm.	Lemniden	0·8 ± 0·09	0·19 ± 0·04	0·01 ± 0·00	4·4 ± 0·73	103·4 ± 25·81	4·3 ± 0·08	36·6 ± 0·55
Zannichellia palustris L. subsp. *palustris*	Parvopotamiden	19·3 ± 3·60	1·73 ± 0·39	0·23 ± 0·04	13·2 ± 0·83	85·6 ± 8·66	2·8 ± 0·04	36·8 ± 0·17

Data represent the means ± s.e. of ten replicates (LNC and LCC; $n = 3$). Traits are: LA, leaf area; LFW, leaf fresh weight; LDW, leaf dry weight; LDMC, leaf dry matter content; SLA, specific leaf area; LNC, leaf nitrogen concentration; LCC, leaf carbon concentration. Growth forms follow Wiegleb (1991), as summarized in Table 1.

publications (Caccianiga *et al.*, 2006; Pierce *et al.*, 2007*a*, *b*; Cerabolini *et al.*, 2010*a*, *b*). The 'GLOPNET leaf economics dataset' available as part of the publication of Wright *et al.* (2004) has a wider coverage, in terms of the number of species and geographic range, but does not include CSR strategies, or basic leaf size traits such as area or mass (only transformed values of traits derived from these measurements, such as logLMA, are available).

For each trait, data were normalized and the spectrum of mean values was compared between aquatic and terrestrial species using Student's *t*-test. Normalization of percentage data was carried out by arcsine transformation (for the traits LDMC, LNC and LCC), and logarithmic transformation was used for LA, LFW, LDW and SLA. Co-variation between traits was determined from non-normalized data using principal components analysis (PCA; Multi-Variate Statistical Package v3·13o; Kovach computing Services, Anglesey, UK). Data were also compared between aquatic plant growth forms, *sensu* Wiegleb (1991).

RESULTS

Trait means for the 61 species are presented in Table 2 (a version of this table in Microsoft Excel format including values for the 506 terrestrial species is available as Supplementary Data Table S1). Hydrophytes exhibited significantly greater mean SLA and LNC than terrestrial species, and significantly lower mean LDMC, LCC, LA, LFW and LDW (Fig. 1). Specifically, a mean SLA of $59{\cdot}6 \pm 5{\cdot}1\ mm^2\ mg^{-1}$ for hydrophytes was significantly greater ($P < 0{\cdot}0001$) than the $26{\cdot}0 \pm 0{\cdot}6\ mm^2\ mg^{-1}$ mean of terrestrial species, and hydrophyte SLA values ranged from a moderately low $9{\cdot}0 \pm 0{\cdot}58\ mm^2\ mg^{-1}$ in *Nymphaea alba* to the extremely fine and soft leaves of *Myriophyllum aquaticum* ($203{\cdot}2 \pm 11{\cdot}52\ mm^2\ mg^{-1}$; Fig. 1). Hydrophytes included much higher SLA values and a greater overall SLA compared with terrestrial species (Fig. 1). Mean LNC was 3·6 % for hydrophytes vs. 2·7 % for terrestrial plants; LCC, 41·1 % (hydrophytes) vs. 46·0 % (terrestrial); LDMC, 14·2 % (hydrophytes) vs. 20·7 % (terrestrial) – all statistically different at the $P \leq 0{\cdot}001$ level (Fig. 1).

The first two axes of the PCA accounted for 72·1 % of the total variability in the data (Fig. 2) and included: PCA1, an axis of variability in size-related traits, such as LA, LFW and LDW; and PCA2, an axis of leaf economics running from high LMDC and LCC at one extreme to high SLA and LNC at the other extreme. Traits were highly significantly correlated with PCA scores as determined by Spearman's correlation coefficient (Fig. 2). Most hydrophytes were ordinated within the same triangle of multidimensional space occupied by terrestrial species, but nine species with particularly high SLA, high LNC leaves extended the triangle negatively along the PCA2 axis (*Helosciadium nodiflorum*, *Lemna minuta*, *Myriophyllum aquaticum*, *Nasturtium officinale*, *Utricularia australis*, *U. vulgaris*, *Vallisneria spiralis*, *Veronica beccabunga* and *Wolffia arrhiza*). No hydrophytes exhibited high LMDC and LCC equivalent to terrestrial species at the positive extreme of PCA2 (Fig. 2).

Differences were evident between growth forms. Most growth forms were comprised of species with small, high

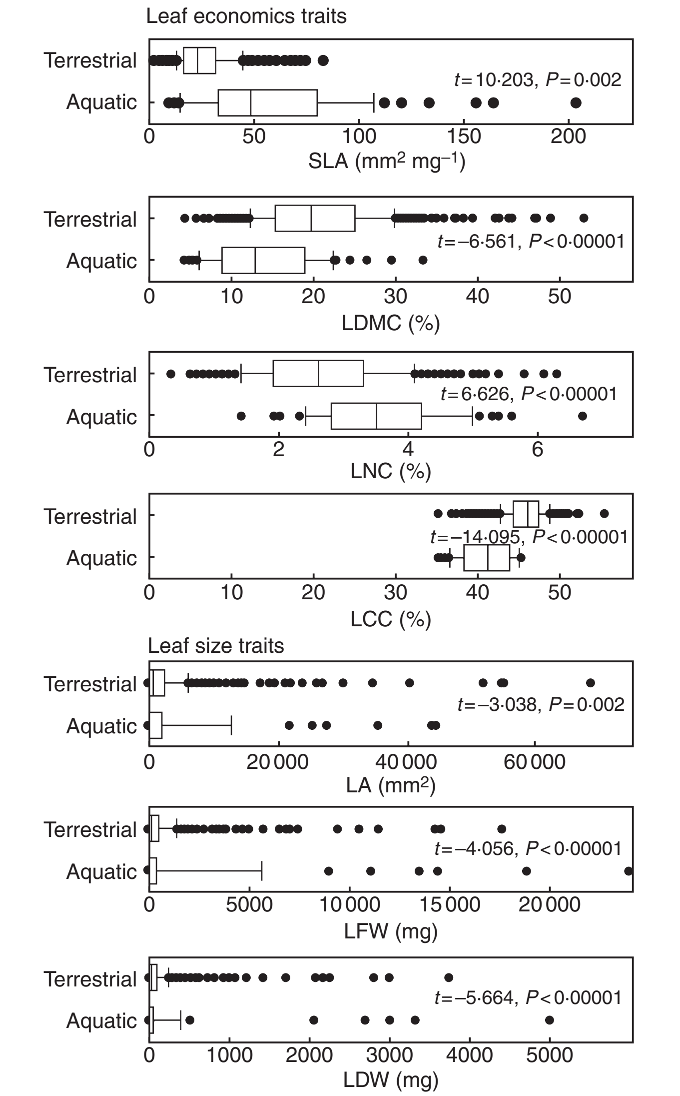

FIG. 1. Comparison of leaf economics traits (LCC, leaf carbon concentration; LDMC, leaf dry matter content; LNC, leaf nitrogen concentration; SLA, specific leaf area) and leaf size traits (LA, leaf area; LDW, leaf dry weight; LFW, leaf fresh weight) between terrestrial herbs ($n = 506$) and aquatic species ($n = 61$). Data represent the mean of ten replicates, and means of the two groups are compared by Student's *t*-test, following normalization for each trait as detailed in the text.

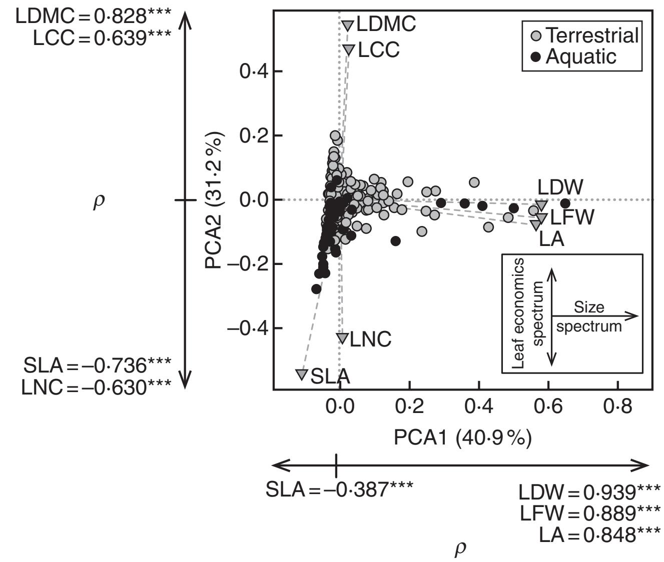

FIG. 2. Principal components analysis (PCA) biplot showing the first two principal axes of variation in mean leaf trait data for 506 herbaceous (grey circles) and 61 aquatic (black circles) plant species from alpine, sub-alpine and lowland continent bioclimatic zones of northern Italy. PCA axis 1 and axis 2 together account for 72·1 % of variability in the data set. Significant correlations between trait scores and PCA axes were determined using Spearman's correlation coefficient (ρ), where *** denotes a significant correlation at the $P \leq 0·001$ level. Traits are: LA, leaf area; LCC, leaf carbon content; LDW, leaf dry weight; LDMC, leaf dry matter content; LFW, leaf fresh weight; LNC, leaf nitrogen content; SLA, specific leaf area.

SLA, high LNC leaves, and some growth forms were restricted to this suite of traits (e.g. Elodeiden, Herbiden, Lemniden and Parvopotamiden) (Fig. 3). However, the Batrachiden spanned a range of moderate leaf economics trait values, all with small leaves, and the Nymphaeiden all exhibited intermediate leaf economics trait values but encompassed the full variation in leaf size evident for terrestrial herbs (Fig. 3). Growth forms represented by only one or two species are presented, not in Fig. 3, but together in Supplementary Data Fig. S1.

DISCUSSION

Our data suggest that there is nothing fundamentally different about the adaptive trade-offs faced by hydrophytes and terrestrial plants. Firstly, with regard to plant economics, most hydrophytes simply lie at one extreme of the acquisitive/conservative economics spectrum. Indeed, hydrophytes exhibit the lowest LMA values ever recorded (Poorter *et al.*, 2009): Gerber and Les (1994) determined a value of 3 g m^{-2} within the genus *Myriophyllum*, and in the present study a value of 4·9 g m^{-2} (when converted from SLA) was recorded for *Myriophyllum aquaticum*. The low LMA/high SLA leaves of most hydrophytes act to minimize resistances to the diffusion of resources (particularly CO_2) between the environment and the chloroplasts, and are thus highly acquisitive, thin (including thin cuticles) and may orient chloroplasts towards the epidermis to maximize photosynthetic rates (Mommer *et al.*, 2004, 2005*a*, *b*; Voesenek *et al.*, 2006). Indeed, there is now evidence that many of the characteristics of hydrophytes, particularly those with emergent leaves that must acclimate to flooding, may simply be co-opted from the responses typical of terrestrial plants: low LMA may be a response to low photosynthate concentrations, and a thin cuticle a response to high humidity (Mommer *et al.*, 2007). Thus we can have a high degree of confidence in the statement that hydrophytes extend the leaf economics spectrum to include the most acquisitive leaves so far measured.

However, our data also demonstrate that not all hydrophytes lie at the acquisitive extreme of the leaf economics spectrum, and not all share the same adaptive strategy. When the principal directions of adaptive specialization were examined by PCA (Fig. 2) we found that many hydrophyte growth forms, particularly Elodeiden, Herbiden, Lemniden, Myriophylliden and Parvopotamiden, achieved a position in the PCA also

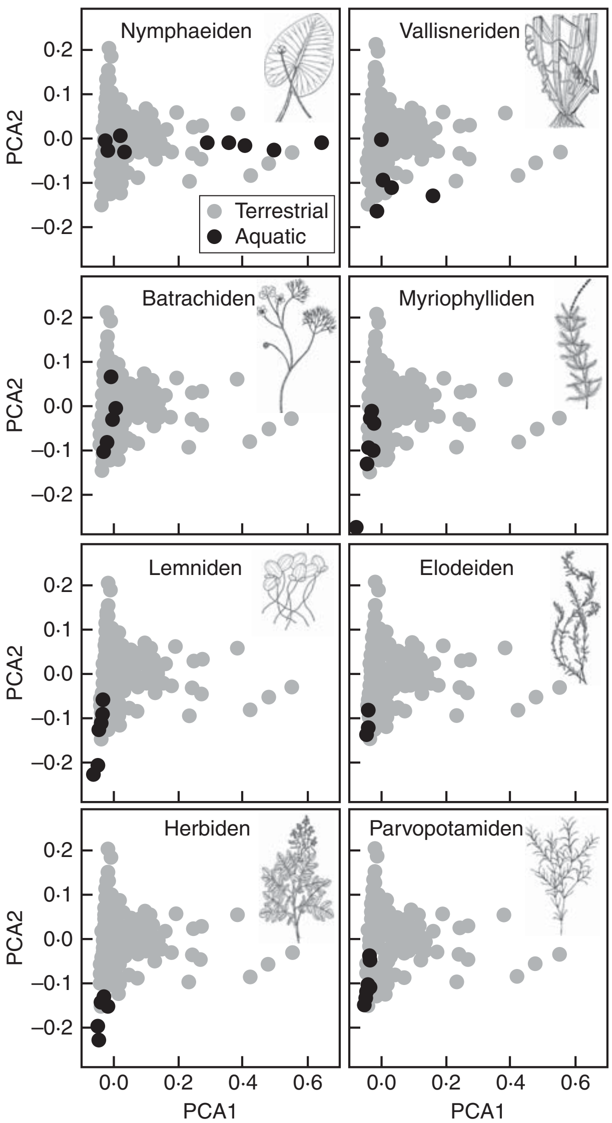

FIG. 3. A comparison of the PCA ordinations of eight of the most frequently represented hydrophyte growth forms (black circles) within the context of terrestrial herbaceous plant trait variation (grey circles). Line drawings are copyright-free material made available by the USDA-NRCS PLANTS Database (http://plants.usda.gov), originally by Britton and Brown (1913).

occupied by highly ruderal, R-selected herbaceous terrestrial plants. Cerabolini *et al.* (2010*a*) provide precise CSR co-ordinates for the terrestrial species, so we can be certain of the classification of these hydrophytes as R selected. In fact, nine species of Herbiden and Lemniden (listed previously in the Results section) went beyond the degree of R selection evident for the most ruderal of terrestrial species, such as *Arabidopsis thaliana*, *Poa annua* and *Stellaria media*. Thus many aquatic species are R selected in the extreme, in keeping with a lifestyle based around rapid regeneration in the face of disturbance. Many are typical of disturbed habitats, colonizing where seasonal flooding scours away vegetation (e.g. *Nasturtium officinale* and *Zannichellia palustris*; Bornette *et al.*, 2008) and some, such as *Potamogeton pectinatus*, germinate after scouring events due to natural scarification of the seeds (Teltscherova and Hejny, 1973). *Hippuris vulgaris*, *Myriophyllum spicatum* and *Alisma* species have seeds that can float for extended periods, sometimes for more than a year, to allow colonization of fresh sites (Guppy, 1906; Praeger, 1913).

In contrast, species such as *Nuphar lutea* and *Nymphaea alba* (Nymphaeiden) exhibit a range of traits suggesting a different adaptive strategy based on the evolution of size variation (Fig. 3) and differing competitive ability between species. Other traits that may form part of this C-selected syndrome for Nymphaeiden include moderate relative growth rates, limited vegetative dispersal and seeds that sink immediately, with strict light/water quality requirements for germination (Bornette *et al.*, 2008). Indeed, it is evident from Fig. 3 that the Nymphaeiden encompass a spectrum of strategies equivalent to highly C-selected to SR-selected terrestrial species, such as *Pteridium aquilinum*, *Aruncus dioicus*, *Filipendula ulmaria* and *Laserpitium halleri* (C selected), and *Hieracium glaciale*, *Lotus alpinus* and *Gentiana brachyphylla* (SR selected; Cerabolini *et al.*, 2010*a*).

The most S-selected hydrophytes were *Juncus bulbosus* (Isoetiden), *Potamogeton polygonifolius* (Batrachiden) and *Trapa natans* (Magnonymphaeiden), although in absolute terms these were SR selected, occupying positions on the PCA plot that overlapped with terrestrial SR-selected species such as *Aira caryophyllea*. Thus no hydrophyte species in our study exhibited the extremely conservative leaf economics typical of S-selected species in low productivity terrestrial habitats, such as *Erica carnea* and *Carex curvula* from the positive extreme of PCA2 (Fig. 2). This confirms Kautsky's (1988) suggestion that hydrophytes may not include stress tolerators *sensu* Grime (1979).

In conclusion, our data demonstrate that together the leaf economics spectrum and leaf size traits provide a dependable common reference frame for the quantitative comparison of the wider primary adaptive strategies of plants from highly contrasting habitats.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: comparison of the PCA ordinations for hydrophyte growth forms represented by only one or two species within the context of terrestrial herbaceous plant trait variation. Table S1: trait means for the 61 aquatic species examined in this study together with values for the 506 terrestrial species taken from the FIFTH database, in the form of an Excel spreadsheet.

ACKNOWLEDGEMENTS

S.P. and G.B. were funded by the Native Flora Centre of the Lombardy Region (Centro Flora Autoctona; CFA) via the University of Insubria.

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