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1 **Nutrient availability and the carnivorous habit in *Utricularia vulgaris*.**

2

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13

14 **Running title**

15 *Nutrients and carnivory in bladderwort*

1 **Summary**

- 2 1. Carnivory in plants is thought to enhance growth through an increased supply  
3 of nutrients, although there are considerable costs involved. It has been  
4 assumed that the relative investment of biomass in traps is inversely  
5 proportional to the availability of nutrients from non-carnivorous sources. Our  
6 aim was to test the effect of increasing nutrient concentration on investment in  
7 carnivory by *Utricularia vulgaris*.
- 8 2. Plants were grown under controlled conditions and nitrogen and phosphorus  
9 added at three loadings in a crossed design. Investment in carnivory was  
10 assessed as the proportion of i) leaf biomass and ii) leaf area comprising traps.
- 11 3. There was no effect of nutrient additions on plant growth or periphyton  
12 abundance. Investment in carnivory declined with increasing phosphorus  
13 loading. There was no effect of nitrogen, despite this being the nutrient  
14 commonly thought to be sought by carnivorous plants. Analysis of previously  
15 published data also indicated a decline in investment with increasing P  
16 availability.
- 17 4. Investment in carnivory in *U. vulgaris* is inversely proportional to the  
18 availability of phosphorus from non-carnivorous sources.

19

20 Key words: Bladderwort, carnivorous plant, investment, macrophyte, nutrients, prey,  
21 traps, *Utricularia vulgaris*.

1 **Introduction**

2 Carnivory in plants is a bizarre phenomenon, a complete reversal of the usual  
3 feeding relationship between plants and animals, and one that has fascinated scientists  
4 and natural historians for a very long time (Darwin, 1875). The unusual nature of  
5 these plants even lead to confusion over their classification, with carnivorous plants  
6 being put into a taxon separate from both plants and animals (Juniper, Robins & Joel  
7 1989). However, it is now generally assumed that carnivory has evolved to enhance  
8 the supply of mineral nutrients in habitats where growth is severely restricted by their  
9 supply (Heslop-Harrison, 1978).

10 Typically it is assumed that N is the most important nutrient derived from  
11 carnivory (Ellison & Gotelli, 2001; Guisande *et al.*, 2004). In the bladderwort  
12 *Utricularia vulgaris* L., 51.8% of the total nitrogen content has been estimated to  
13 come from insect-derived nitrogen (Friday & Quarmby, 1994). Such a substantial  
14 contribution of nitrogen from animal prey is not atypical of carnivorous plants, with  
15 estimates ranging from 10 to 87% dependent on taxa (Ellison & Gotelli, 2001).

16 Nevertheless, other nutrients could be gained through carnivory. Carnivorous  
17 plants release a variety of enzymes to digest prey, including esterase, protease,  
18 ribonuclease and acid phosphatase (Heslop-Harrison, 1978) and they take up other  
19 minerals from prey (Lollar, Coleman & Boyd, 1971).

20 A cost-benefit model of carnivory has been described where any gains, in  
21 terms of increased availability of nutrients, are offset by its costs, direct and indirect  
22 (Givnish *et al.*, 1984). Direct costs comprise those involved in the production and  
23 maintenance of the organs necessary for the attraction, capture and digestion of prey  
24 (Friday, 1992; Adamec, 1997; Mendez & Karlsson, 1999). Production costs will  
25 depend upon the size and complexity of the traps. Maintenance costs are likely to be

1 particularly high in those species that capture their prey by means of active traps [e.g.  
2 venus fly-trap (*Dionaea muscipula* Soland. ex Ellis), bladderworts (*Utricularia* spp.)],  
3 compared to species with passive traps [e.g. bromeliads (Bromeliaceae), pitcher plants  
4 (Sarraceniaceae)]. In *U. vulgaris*, for example, the traps take up to 40 minutes to reset  
5 once the prey is captured (Friday, 1991). Indirect costs of carnivory are a consequence  
6 of a reduced photosynthetic efficiency of traps compared to conventional foliage  
7 (Friday, 1992).

8         Thus, there is a trade-off between photosynthetic costs and benefits. The  
9 Givnish *et al.* cost-benefit model (1984) predicts that the net photosynthetic benefit of  
10 carnivory, and thus investment in it at the species or community level, should decline  
11 with increased availability of nutrients in the surrounding medium.

12         The common or greater bladderwort *Utricularia vulgaris* is a free-floating,  
13 submerged, aquatic plant that grows in low nutrient standing freshwaters. The plant  
14 gets its name from the traps, called bladders or utricles, on the finely dissected leaves.  
15 The number of traps on each leaf can be very high, but is variable, with leaf size, trap  
16 size and the density of traps all being plastic characters (Friday, 1992). Each bladder  
17 is capped by a small “trapdoor”, which is closed when the trap is primed and the ions  
18 pumped actively from within the bladder, causing water to leave, such that the walls  
19 of the bladder are flattened and under pressure (Sydenham & Findlay, 1975). The trap  
20 is activated when an animal disturbs the trigger bristles around the mouth of the trap,  
21 the trapdoor opens and the animal is sucked into the bladder as the walls revert to  
22 their rounded shape. Enzymes, glucosidases, aminidases and phosphatases are  
23 released from glands in the bladder wall and digest the prey (Sirová, Adamec & Vrba,  
24 2003).

1        *Utricularia vulgaris* plants are rootless and, other than the traps, have no  
2 specialised organs for the uptake of nutrients from the surrounding water. In this  
3 species, therefore, there are two routes by which nutrients can be taken up:  
4 a) by the capture and breakdown of prey in traps, and  
5 b) by the absorption of dissolved nutrients from the aquatic medium over the entire  
6 shoot surface (Friday, 1992).

7        As the production of traps by the plant is plastic, and there is no cost involved in  
8 the production of roots to obtain nutrients from the medium, *U. vulgaris* should be an  
9 ideal species to test the impact of increasing nutrients on investment in carnivory.  
10 However, previous attempts to use *Utricularia* species to assess the investment in  
11 carnivory have been unsuccessful due to the use of poor measures of relative  
12 investment (e.g. Knight, 1992, see Friday, 1991), or poorly controlled experiments  
13 (Knight & Frost, 1991). Nevertheless, it has been suggested that the investment in  
14 traps varies in response to season (Friday, 1992), prey density (Guisande *et al.*, 2000),  
15 periphyton density and nutrient availability (Knight & Frost, 1991). There are further  
16 complications in that the growth of the plants, and leaf size in particular, also appears  
17 to be affected by several of these factors (Kosiba, 1992a; Kosiba, 1992b), highlighting  
18 the importance of using relative measures of investment in carnivory rather than total  
19 investment per leaf (e.g. Knight, 1992; Guisande *et al.*, 2004).

20        Our aim was to quantify the effects of increasing nutrient availability on the  
21 investment in carnivory of *U. vulgaris* under controlled conditions using appropriate  
22 measures of relative investment in trap production. We tested the hypothesis that the  
23 relative investment of biomass in traps is negatively influenced by the availability of  
24 limiting nutrients available from non-carnivorous sources. We did not attempt to  
25 investigate the influence of prey availability or nutritional quality on relative

1 investment of biomass in traps, but it is possible that these factors may have an  
2 influence also.

3

#### 4 **Methods**

##### 5 Site Description

6 *Utricularia vulgaris* plants were collected from Wicken Fen National Nature  
7 Reserve, Cambridgeshire, UK (52 18' N 0 17' E), and transported to the laboratory in  
8 water. Wicken Fen is a calcareous lowland fen exceptionally rich in vascular plants  
9 with over 400 species present. Additional water was also collected for use as a growth  
10 medium and for the estimation of nitrogen and phosphorus concentrations using a  
11 Skalar San<sup>++</sup> autoanalyser (Breda, The Netherlands).

##### 12 Experimental Design

13 On return to the laboratory, plants were cut to a standard length of 10 cm from  
14 the growing point (comprising approximately 25 expanded nodes). Only unbranched  
15 shoots were used; any shoots with developed side branches less than 10 cm from the  
16 growing point were discarded. Each plant was then placed in a plastic bucket (20 cm  
17 diameter) containing 1L of unfiltered Wicken Fen water. The water was well mixed  
18 prior to use, in order to keep prey density similar between the all replicates.

19 The plants were incubated for 5 weeks (4<sup>th</sup> May to 8<sup>th</sup> June, 2004) under  
20 controlled temperature ( $12 \pm 1^\circ \text{C}$ ) and light conditions ( $130 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR at plant  
21 surface) on a 16 hour light: 8 hour dark cycle. Additions of nitrogen, as ammonium  
22 nitrate ( $\text{NH}_4\text{NO}_3$ ), and phosphorus, as sodium dihydrogen orthophosphate ( $\text{NaH}_2\text{PO}_4$ ),  
23 were added weekly to buckets at one of three loadings in a fully crossed design:

24 Phosphorus

25 i)  $0 \mu\text{g P L}^{-1}$  ( $0 \mu\text{M}$ ),

1 ii) 25  $\mu\text{g P L}^{-1}$  (0.78  $\mu\text{M}$ ),

2 iii) 50  $\mu\text{g P L}^{-1}$  (1.56  $\mu\text{M}$ )

3 and nitrogen

4 i) 0  $\mu\text{g N L}^{-1}$  (0  $\mu\text{M}$ ),

5 ii) 400  $\mu\text{g N L}^{-1}$  (28.6  $\mu\text{M}$ ),

6 iii) 800  $\mu\text{g N L}^{-1}$  (56.1  $\mu\text{M}$ )

7 Each of the nine treatments was replicated five times arranged within five blocks. Any  
8 water loss due to evaporation was replaced with deionised water.

9 The investment in carnivory was assessed at the start of the experiment, prior to  
10 any nutrient addition, and at the end of the five week growing period, using two  
11 methods.

12 a) The proportion of the leaf area comprising traps (assessed using image analysis).

13 This measure of the investment in carnivory takes into account both the number  
14 and the size of individual traps, relative to the size of the leaf. Large traps are  
15 more effective than small (Friday 1991), but encounter rate with prey is a function  
16 of the number of traps (Harms 2002).

17 b) The proportion of the leaf dry mass comprising traps. This measure of the  
18 investment in carnivory takes into account the amount of matter used to construct  
19 the traps, relative to that used to construct the leaf. There is not a direct  
20 relationship between this measure of investment and investment measured as area,  
21 because large traps have a higher mass per unit area than small traps (Friday,  
22 1991).

23 Although the proportion of biomass comprising traps is the most useful measure of a  
24 plant's investment in carnivory, the way in which the biomass is partitioned among a



1 variable number of traps of different sizes may also be important (Friday, 1992).

2 Hence, both methods were used.

3

4 *a) Image analysis*

5 A single leaf attached to the shoot 3 cm from the growing point was carefully  
6 removed from the stem of each plant using forceps and a scalpel. Each leaf was then  
7 placed individually into a glass Petri-dish, using a paintbrush and needle to ensure that  
8 the leaf and traps were spread out and did not overlap. A computerised image of each  
9 leaf was produced and analysed using Optimas 6.5 software to calculate surface area.

10 Once the image had been produced all the traps were carefully removed and the  
11 process repeated. The percentage of the leaf surface area that comprised traps was  
12 then calculated by the difference between the two images.

13

14 *b) Dry weight*

15 After the leaves had been used for image analysis, the dry mass of the leaves  
16 and traps were determined separately after drying at 70 °C to constant mass.

17

18 Only one leaf was sampled as bladders are only active for a short time after  
19 leaf expansion (Friday, 1989). We chose this position as this was within the region of  
20 the stem where 100% of the bladders are active. Once the leaves are more than 10  
21 days old the bladders stop being active and start to break down. After 21 days, >90%  
22 of the bladders are missing (Friday, 1989). An additional advantage of using young  
23 leaves is that they are generally clear of periphyton, thus improving the accuracy of  
24 measurements.

1           At the end of the five-week growth period the plants were harvested and the  
2 investment in carnivory assessed as at the start. To estimate plant growth, the length  
3 of the main axis of each plant was measured and the dry mass of the plants measured  
4 after the removal of periphyton. Periphyton was removed from each plant by vigorous  
5 shaking it for 1.5 minutes in a sealed vessel containing 150ml of tap water, sufficient  
6 to remove nearly all attached algae (Zimba & Hopson, 1997; Jones *et al.*, 2000). The  
7 plant, and any fragments, was removed from the algal suspension and dried at 70°C to  
8 constant mass. One 50ml aliquot of the resultant suspension was passed through a pre-  
9 weighed Whatman GF/C filter. The filter was checked for any plant fragments, which  
10 were removed with forceps, and dried at 70°C to constant mass to estimate total mass  
11 of matter, including all live and dead organic matter and inorganic material. Another  
12 50 ml aliquot of the periphyton suspension was passed through a second Whatman  
13 GF/C filter in a similar manner, and chlorophyll-*a* determined after cold extraction in  
14 90% acetone. In order to make valid comparisons between plants of differing size,  
15 measures of periphyton were standardized to per unit area of plant surface, calculated  
16 from known surface area: dry mass relationships (following Jones *et al.*, 2000).

17

#### 18 Published data

19           The data given in Kosiba (1992a & b) were used to assess the effect of varying  
20 nutrient conditions across sites in the field. The data were collected from various sites  
21 in Poland in 1983. Leaves varied in size among the sites. As area varies in proportion  
22 to the square of length, the number of traps per unit leaf length squared was used as a  
23 measure of investment in carnivory to correct for variations in leaf size. Kosiba  
24 (1992a & b) also include data on the elemental composition of the plants (N, P, K, Ca,  
25 Mg, Na, Fe, S) and the water chemistry parameters measured at the sites (pH, PO<sub>4</sub><sup>3-</sup>

1 (orthophosphate),  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Ca}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Fe}^{3+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ , total hardness,  
2 total organic carbon and humic acid concentration). A further chemical variable, the  
3 atomic N:P in the water at each site, was calculated from these data to determine if the  
4 relative availability of nutrients was important. Both sets of data, water chemistry  
5 variables and plant elemental composition, were used to determine the effect on  
6 investment in carnivory.

## 7 Statistics

8 A general linear model two-way analysis of variance (GLM ANOVA) was  
9 used to test for differences between nutrient treatments using MINITAB. Data on the  
10 proportion of leaf comprising traps, determined both by image analysis and by dry  
11 weight, were transformed using arcsin transformation before use. The relationship  
12 between investment in carnivory and water chemistry variables in the Polish data set  
13 was analysed using stepwise regression (SAS).

14

## 15 **Results**

### 16 Water

17 The water from Wicken Fen contained  $58 \mu\text{g l}^{-1}$  ( $3.4 \mu\text{M}$ ) ammonium,  $3.6 \mu\text{g l}^{-1}$   
18 ( $0.06 \mu\text{M}$ ) nitrate,  $1.8 \mu\text{g l}^{-1}$  ( $0.04 \mu\text{M}$ ) nitrite and  $1.2 \mu\text{g l}^{-1}$  ( $0.01 \mu\text{M}$ ) soluble reactive  
19 phosphate.

### 20 Growth

21 All plants grew during the five week incubation period, with a mean length of  
22 the main axis of 30.88 cm (*cf.* 10 cm at start). However, there was no effect of  
23 nitrogen loading on the growth of the plants ( $p = 0.75$ ), and the influence of  
24 phosphorus was also (though only marginally) insignificant ( $p = 0.08$ ; Fig. 1). This  
25 may have been due to the growth period being of insufficient time for significant

1 differences to develop between the treatments. Similar results were found for final dry  
2 mass.

### 3 Periphyton

4         The mean density of periphyton, as dry mass per unit plant surface area,  
5 ranged from 0.017 mg cm<sup>-2</sup> to 0.022 mg cm<sup>-2</sup> and, as chlorophyll-*a* per unit plant  
6 surface area, from 0.0052 µg chl*a* cm<sup>-2</sup> to 0.0102 µg chl*a* cm<sup>-2</sup>. However, the amount  
7 of periphyton growing on the plants was not significantly affected by phosphorus,  
8 nitrogen or by the interaction between phosphorus and nitrogen, using either measure  
9 (Fig. 2).

### 10 Investment in Carnivory

11         Although variable among plants, the percentage of the total surface area of the  
12 leaf that comprised traps was not significantly different among the treatments at the  
13 start of the experiment (Fig. 3a). Neither was there any difference among treatments  
14 in the percentage of the leaf mass that comprised traps (Fig. 4a). Therefore, there was  
15 no difference in investment in carnivory between treatments before the nutrient  
16 manipulation began.

17         In contrast, after five weeks incubation the percentage of the total surface area  
18 comprising traps was significantly lower in those plants that had been exposed to  
19 higher phosphorus loading (Fig. 3b,  $p = 0.007$ ). However, there was no significant  
20 effect of nitrogen, or of the interaction between phosphorus and nitrogen (Fig. 3b).  
21 The same result was found when investment in carnivory was estimated as the  
22 proportion of the leaf mass comprising traps (Fig. 4b). It is possible that the observed  
23 experimental effect of phosphorus on investment in carnivory was indirect, via  
24 changes in the quantity or quality of prey. Nevertheless, it is clear that the availability

1 of phosphorus in the water has a significant impact, either direct or indirect, upon the  
2 investment in carnivory of *U. vulgaris*.

3 Over the five weeks incubation the percentage of the total surface area that  
4 comprised traps declined overall ( $p = 0.0001$ ), but the percentage of the leaf mass that  
5 comprised traps did not ( $p = 0.11$ ). The difference in response between these two  
6 measures of investment is due to an increase in average trap size; small traps have a  
7 lower mass per unit area than large traps (Friday, 1991). Distribution of the same  
8 mass among a smaller number of large traps will result in a reduced encounter rate  
9 with prey, which may reflect an increased prey density in all experimental treatments  
10 relative to the field.

11

## 12 Published data

13 The biomass of *U. vulgaris* increased with increasing phosphorus  
14 concentration in the surrounding water (Fig. 5a), as noted by (Kosiba, 1992a).  
15 Although there was no relationship between biomass of *U. vulgaris* and nitrogen  
16 concentration (either as ammonium, nitrate or both), high biomass was not achieved at  
17 high nitrogen concentrations. It was also apparent that leaf size increased with  
18 increasing phosphorus concentration (Fig. 5b), which had an influence on the number  
19 of traps per leaf (Fig. 5c). Hence, the number of traps per unit leaf length squared was  
20 used as a measure of relative investment, to take into account variations in leaf size.  
21 There was no relationship between the investment in carnivory and the concentration  
22 of nitrogen in the water, either as nitrate, ammonium, or total nitrogen (Fig. 5d). Of  
23 the water chemistry variables, the best predictor of relative investment in carnivory  
24 was phosphorus concentration in the surrounding water, although its effect was  
25 marginally insignificant (Fig. 5e). The poor correlation between measured

1 orthophosphate and investment in carnivory may reflect the difficulties of estimating  
2 phosphorus availability with this measure (a small and rapidly recycled portion of the  
3 total phosphorus pool). When plant elemental composition was included, the best  
4 predictor of relative investment was phosphorus content (Fig. 5f). Again there was a  
5 decline in relative investment with increasing phosphorus content, but the relationship  
6 was highly significant using this measure of phosphorus availability (Fig. 5f).

7

## 8 **Discussion**

### 9 Plant Growth

10 It is generally assumed that carnivory enhances the supply of nutrients in  
11 habitats where growth is severely restricted by their supply. In this experiment,  
12 however, increased availability of phosphorus or nitrogen in the water surrounding the  
13 *Utricularia* plants did not affect their growth. Nevertheless, the field data of Kosiba  
14 (1992a) show a strong relationship between phosphorus and growth. Other workers  
15 have suggested that the growth of *Utricularia* is better explained by prey density  
16 (Englund & Harms, 2003), but this seems highly unlikely unless nutrient acquisition  
17 is solely via carnivory.

18 It is possible that growth of the *Utricularia* plants was restricted by some other  
19 factor, with the most likely explanation being that the density of periphyton (Jones &  
20 Sayer, 2003). However, there was no relationship between periphyton, either as dry  
21 weight or chlorophyll per unit plant surface area, and plant growth. Also, the amount  
22 of periphyton was not significantly affected by the addition of P, N or the interaction  
23 between P and N. These findings suggest that in this experiment, the periphyton was  
24 not responsible for the lack of a relationship between nutrient addition and the growth  
25 of *Utricularia*. This is contrary to the theory proposed by Phillips *et al.* (1978) who

1 suggested that periphyton biomass is related to nutrient concentration. Laboratory  
2 experiments under controlled conditions have also shown an increase in periphyton  
3 with nutrients (for example, Jones *et al.*, 2000) and there is a well described negative  
4 interaction between periphyton and plants (Jones *et al.*, 1999; Jones & Sayer, 2003).  
5 However, there is also evidence that the main constraint on periphyton biomass is the  
6 grazing action of invertebrates (Cattaneo, 1983; Kairesalo & Koskimies, 1987; Jones,  
7 Moss & Young, 1998; Jones & Sayer, 2003) and it is likely that the prey, chydorids  
8 and ostracods, were feeding upon the periphytic algae.

9       Most importantly, the lack of a significant effect of the added nutrients on the  
10 growth of the plants and periphyton, and a lack of a significant effect of periphyton on  
11 plant growth, removes the influence of these confounding variables on the  
12 relationship between nutrient availability and investment in carnivory. The test of this  
13 relationship was the prime objective of this work.

#### 14 Investment in Carnivory

15       At the start of the experiment the proportion of leaf biomass represented by  
16 traps was not significantly different among the treatments, measured using either  
17 image analysis or dry weight. There was variation among the plants, but this was  
18 expected as the proportion of biomass invested in traps can vary widely within  
19 populations of *U. vulgaris* (Friday, 1992), presumably as each individual plant  
20 experiences different conditions dependent upon microhabitat.

21       By the end of the growth period, however, there was a significant relationship  
22 between phosphorus addition and the investment in carnivory, measured both as  
23 surface area and dry mass. Both the image analysis and the dry weight measurements  
24 showed that the proportion of the leaf dedicated to carnivory declined with increasing  
25 availability of phosphorus in the surrounding medium, but was not influenced by

1 nitrogen additions or by the interaction between phosphorus and nitrogen. This is  
2 somewhat surprising as it is commonly assumed that nitrogen is the nutrient “sought  
3 after” by carnivorous plants. However, the concentration of nitrogen in the water at  
4 Wicken Fen, from where the plants were collected and was used as the growth  
5 medium during the experiment, was far greater than that of phosphorus. Assuming  
6 that only ammonium was used by the plants, as it is energetically favoured, there was  
7 260-fold more N than P ( $3.4\mu\text{M NH}_3$  *cf.*  $0.013\mu\text{M PO}_4^-$ ) in the water at Wicken Fen.  
8 The habitats where the plants and water were collected were typical of the stagnant  
9 pools in this peaty fen. The conditions of low oxygen would tend to encourage  
10 mineralization and the production of ammonia, a characteristic common to many of  
11 the sites where *U. vulgaris* grows (Kosiba, 1992a). *Utricularia vulgaris* cannot make  
12 direct use of the sediment pool of phosphate, which is the principle source for rooted  
13 macrophytes, suggesting that, at the start of the experiment the plants depended on  
14 prey as virtually the sole source of phosphorus. Friday & Quarmby (1994) found this  
15 to be the case in their investigation of *U. vulgaris* growing at Wicken Fen.

16         The relative importance of nitrogen and phosphorus for carnivorous plants has  
17 been debated for some time (Lollar *et al.*, 1971; Heslop-Harrison, 1978), and it would  
18 seem reasonable that under conditions of nutrient limitation, carnivory could provide  
19 a route for the supply of whatever nutrient was limiting growth. Although *U. vulgaris*  
20 grows in waters over a range of nitrogen and phosphorus conditions (Knight & Frost,  
21 1991; Friday, 1992), both the laboratory experiment and the data of Kosiba (1992a &  
22 b) show that investment in carnivory in *U. vulgaris* was inversely correlated to  
23 phosphorus availability, and particularly the amount of phosphorus acquired by the  
24 plants. The availability of nitrogen in the medium or acquired by the plant, both as  
25 absolute concentration or relative to phosphorus, had little influence on either the



1 growth of the plants or the investment in carnivory. Furthermore, it is apparent that  
2 growth and flowering are directly related to the availability of phosphorus (Kosiba,  
3 1992a). Many carnivorous plants release phosphatases to digest their prey and, in  
4 *Utricularia* at least, they appear to be more important than other enzymes (Sirová *et*  
5 *al.*, 2003). Phosphorus uptake via carnivory has been identified using radioisotopes  
6 (Lollar *et al.*, 1971). Phosphorus often limits the growth of phytoplankton in  
7 freshwaters, and the influence of phosphorus on carnivory may reflect the aquatic  
8 habit of *U. vulgaris*. However, these findings may be more applicable to all  
9 carnivorous plants, as phosphorus limitation has been linked to the occurrence of  
10 carnivory in the terrestrial cobra lily *Darlingtonia californica* Torr. (Ellison &  
11 Farnsworth, 2005).

12         The findings here support the assumption that increasing availability of  
13 nutrients from external sources leads to a reduction in the investment in carnivory  
14 (Givnish *et al.*, 1984). As *U. vulgaris* does not have any specialised organs for the  
15 uptake of nutrients from the surrounding water, there are no costs involved in the  
16 production of roots to obtain nutrients from the medium, and the reduction in the  
17 proportion of the leaves that comprise traps is a reduction in investment in carnivory  
18 in terms of reduced commitment of biomass. What is somewhat surprising is that they  
19 indicate that phosphorus is the element sought after by this species of carnivorous  
20 plant, not nitrogen as is commonly assumed.

21

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1 Figure 1. Influence of nutrient addition on the growth of *U. vulgaris* measured as  
2 mean length of the main axis ( $\pm$  SE, n = 5). From ANOVA phosphorus (p = 0.08),  
3 nitrogen (p = 0.75), interaction between phosphorus and nitrogen (p = 0.53).

4

5 Figure 2. Periphyton density expressed as mean dried weight per unit plant surface  
6 area after 5 weeks ( $\pm$  SE, n = 5). From ANOVA phosphorus p = 0.096, nitrogen p =  
7 0.76, and the interaction between phosphorus and nitrogen p = 0.794.

8

9 Figure 3. The mean percentage ( $\pm$  SE, n = 5) of the leaf surface area that comprised  
10 traps, **(a)** at the start of the experiment (phosphorus p = 0.37, nitrogen p = 0.38,  
11 phosphorus\*nitrogen p = 0.13), and **(b)** after 5 weeks incubation under the  
12 experimental treatments (phosphorus p = 0.007, nitrogen p = 0.55,  
13 phosphorus\*nitrogen p = 0.89). Between the two time intervals p = 0.0001.

14

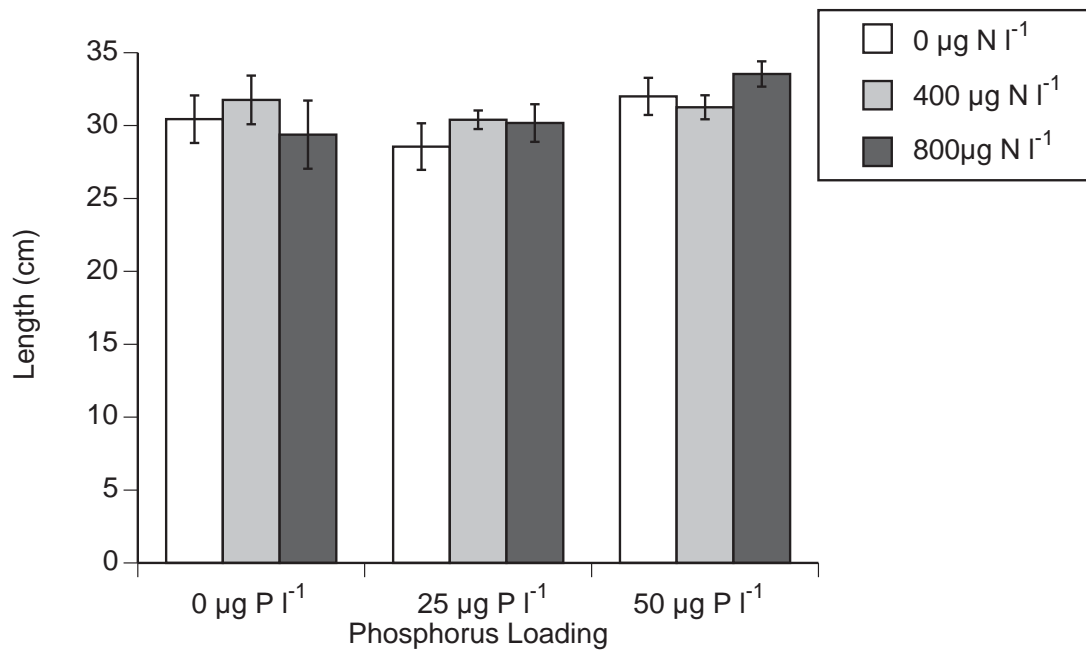
15 Figure 4. The mean percentage ( $\pm$  SE, n = 5) of the leaf dry mass that comprised traps,  
16 **(a)** at the start of the experiment (phosphorus p = 0.66, nitrogen p = 0.98,  
17 phosphorus\*nitrogen p = 0.14), and **(b)** after 5 weeks incubation under the  
18 experimental treatments (phosphorus p = 0.03, nitrogen p = 0.32,  
19 phosphorus\*nitrogen p = 0.80). Between the two time intervals p = 0.11.

20

21 Figure 5. The relationship between phosphorus concentration and **(a)** biomass ( $R^2 =$   
22 0.488, p = 0.005), **(b)** maximum leaf length ( $R^2 = 0.333$ , p = 0.019), and **(c)** traps per  
23 leaf ( $R^2 = 0.315$ , p = 0.015) of *U. vulgaris* from sites in Poland. The relationship  
24 between investment in carnivory in the same plants, measured as traps per cm<sup>2</sup> leaf  
25 length, and **(d)** nitrogen concentration ( $R^2 = 0.008$ , p = 0.74) and **(e)** phosphorus

- 1 concentration ( $R^2 = 0.192$ ,  $p = 0.079$ ) in the surrounding water, and (f) phosphorus
- 2 content in the plant tissue ( $R^2 = 0.349$ ,  $p = 0.013$ ). (All data from Kosiba, 1992a & b)
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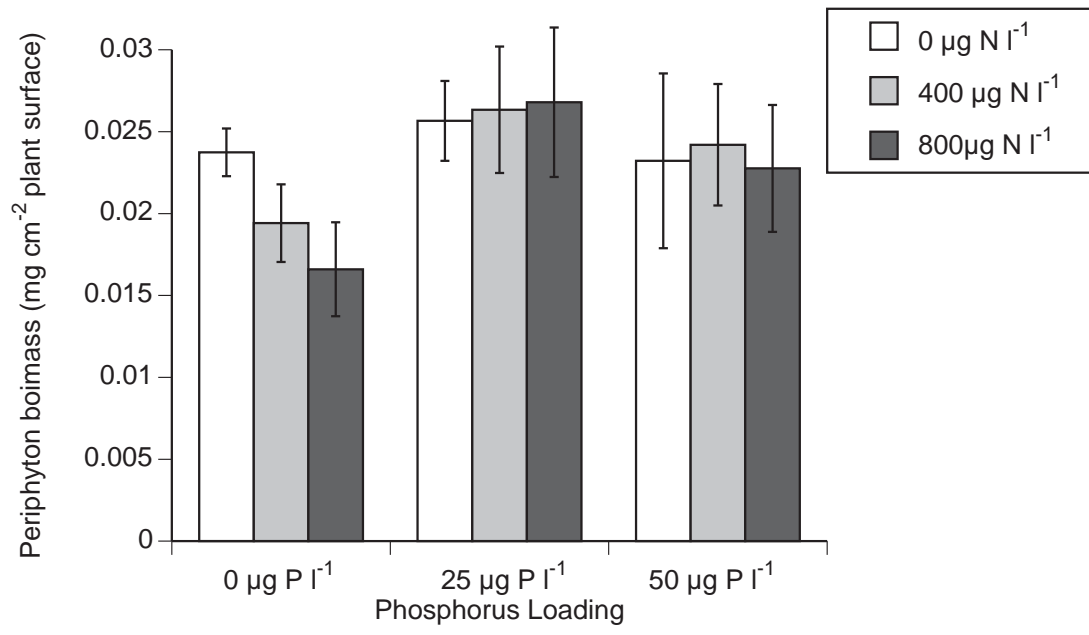
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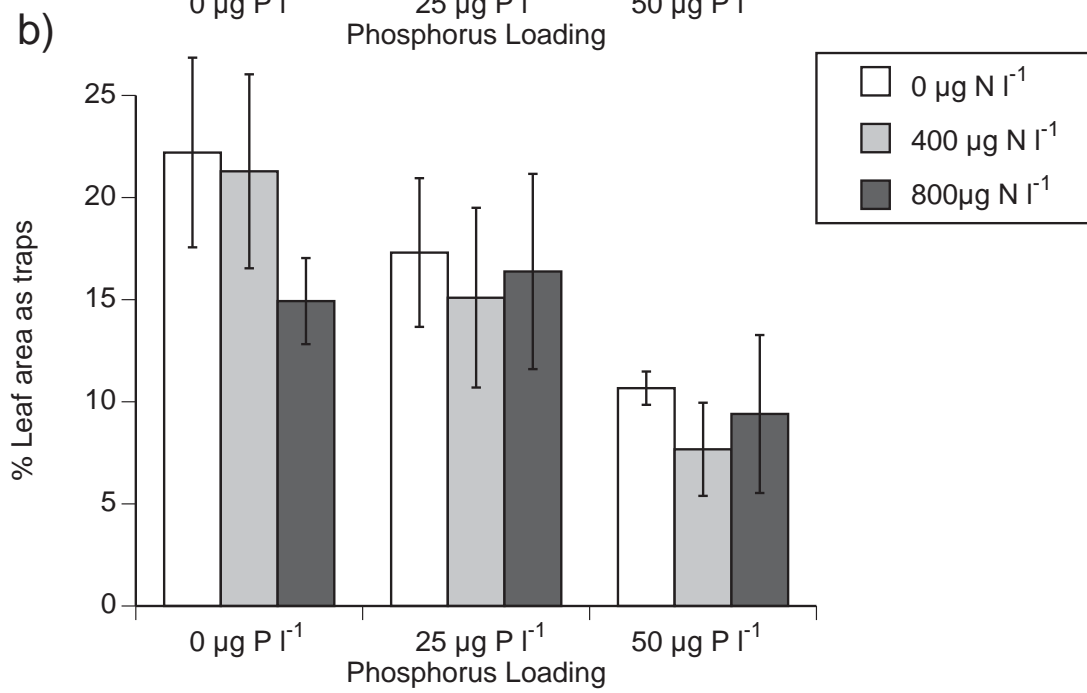
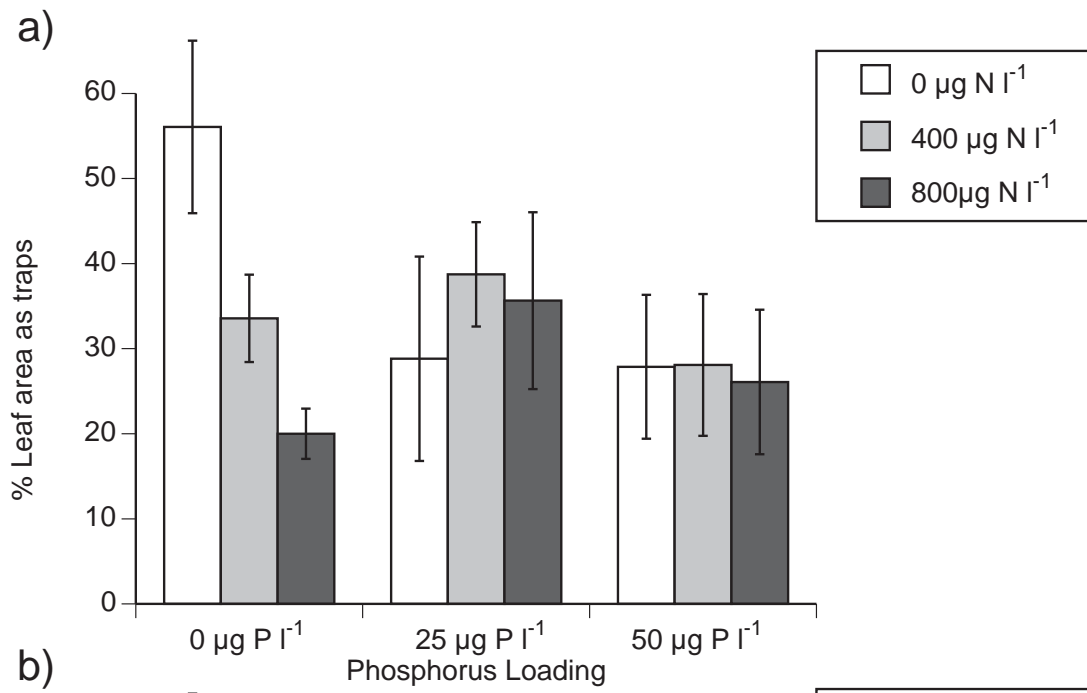
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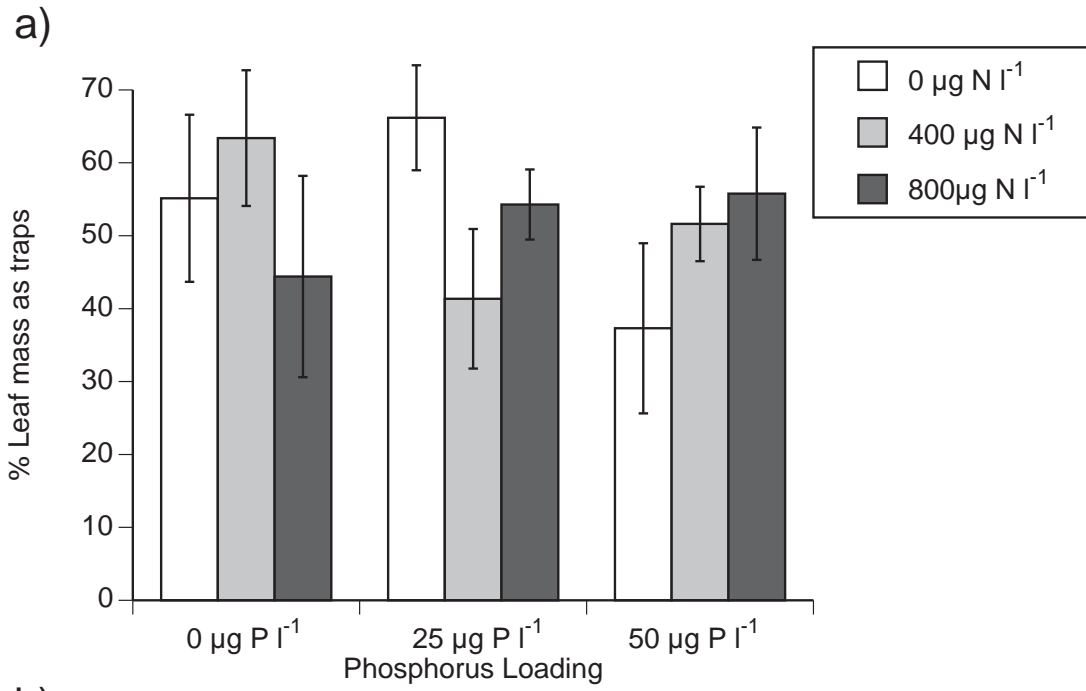


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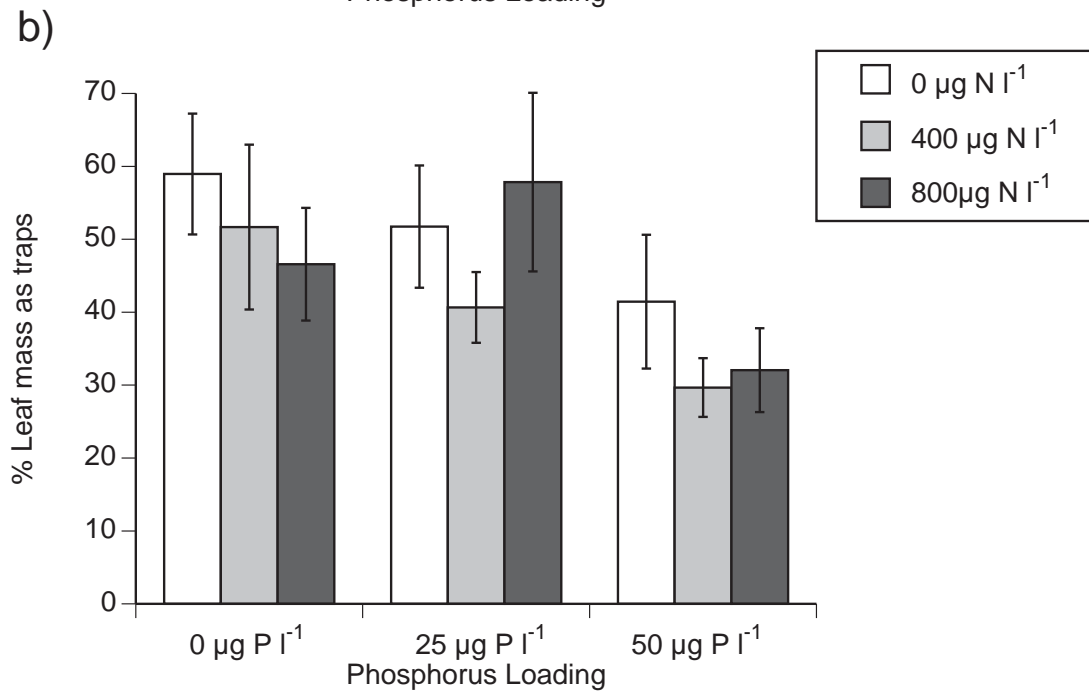


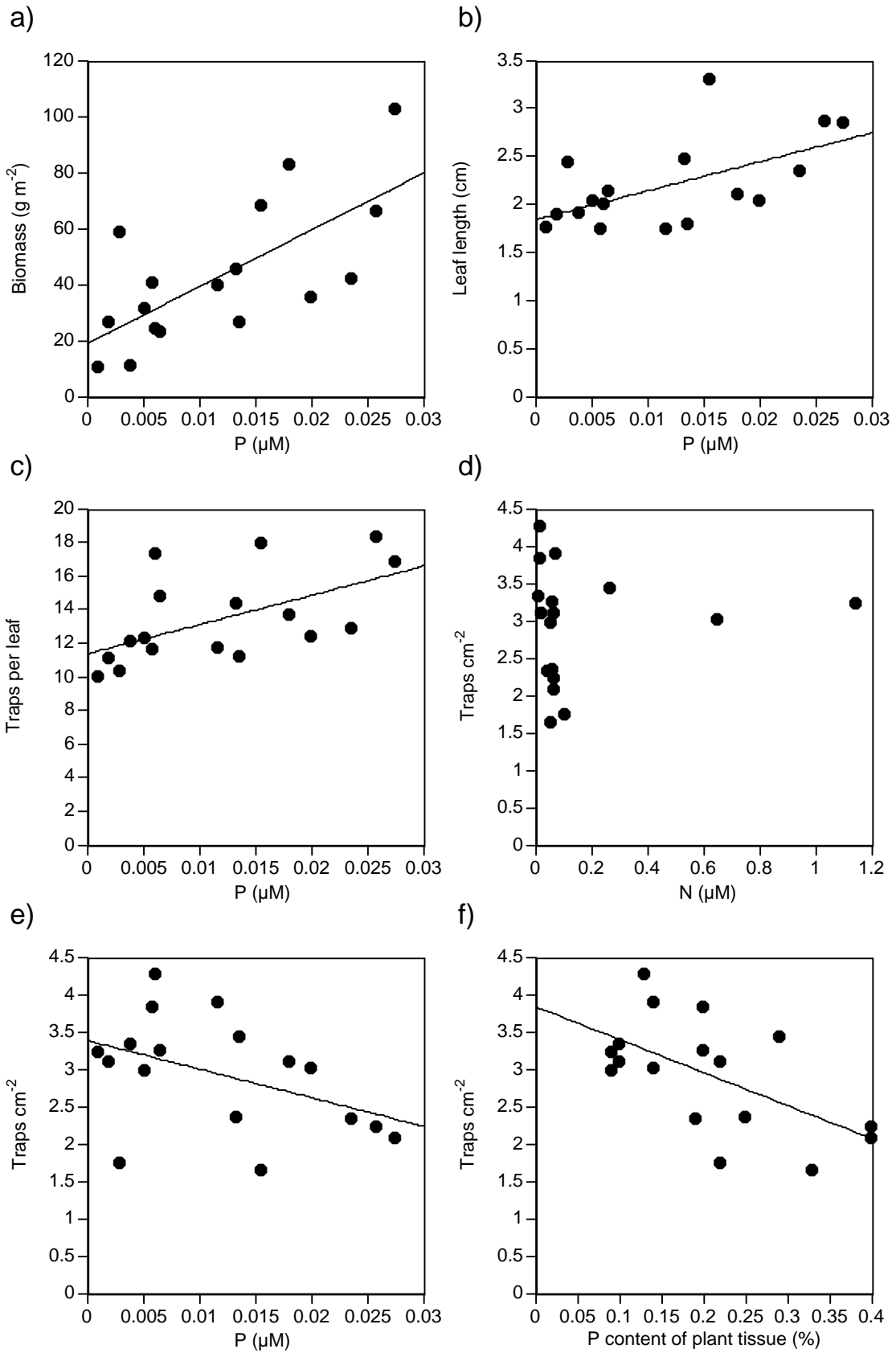
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