Functional significance of external trap morphology in aquatic *Utricularia*

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Abstract

Utricularia is a genus of carnivorous plants with mechanically activated suction traps. Species are largely generalist, opportunistic predators with very plastic vegetative growth. They variously occupy terrestrial, aquatic and epiphytic habitats and can respond to changes in their environment by altering their investment in carnivory. Their traps are adorned with external appendages, the morphology of which varies greatly among species, carrying both phylogenetic and growth-habit specific signals. While this morphological variation is well documented, little is known about its functional significance. One hypothesis with limited support is that the appendage morphology of aquatic species is under selection for prey attraction. Previous work has shown that appendages of one aquatic clade, antennae and bristles, enhance the capture of one microcrustacean species. There has also been very little work done to quantify the plasticity of aquatic appendage expression, either among conspecifics or in response to environmental variation. Additionally, while studies have examined the effects of biotic and abiotic environmental variation on the growth and investment in carnivory of aquatic *Utricularia,* the effect of prey-derived mineral nutrition on plant growth has remained confounded with that of ambient nutrition. In this thesis I revisit the prey-capture enhancement hypothesis and look for plasticity in the appendage expression of aquatic *Utricularia*. Firstly, I conduct appendage ablation experiments on two aquatic *Utricularia* species with different growth habits, *U. australis* and *U. gibba,* to test the aquatic-appendage prey-capture hypothesis with a range of ubiquitous prey animals that exhibit differing feeding and locomotory behaviours. Aquatic appendages only enhance the trapping of prey taxa with specific feeding behaviour. Secondly, I conduct a growth experiment which produces the first experimental evidence of appendage expression changing in response to environmental variation, and demonstrate persistent differences in appendage expression between clones of the same species. Finally, with a second growth experiment, I examine the relative contributions of ambient and prey-derived nutrition to growth and investment in carnivory of *U. australis*. Prey capture plays a larger role in enhancing plant growth than ambient nutrition. I found little support for the aquatic prey-capture hypothesis. The capture rates of three ubiquitous prey taxa are unaffected by the presence of appendages. The degree of persistent appendage variation in between tested individuals is slight and therefore may not be functionally significant. Antennae and bristle expression is affected by environment but responses are not consistent with being an investment in carnivory.

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Contents

1. General Introduction

1.1 Carnivorous plants

Botanical carnivory, the capture and digestion of animals by plants, is a polyphyletic syndrome having evolved at least six times in the angiosperms, in five different orders: once in the Caryophyllales and Oxalidales, twice in the Ericales, three times in the Lamiales, and up to three times in the Poales (Albert *et al.*, 1992b; Bremer *et al.*, 2009; Ellison and Gotelli, 2009; Fleischmann, 2012b; Givnish, 2015). Carnivory is a way for plants to acquire limiting macronutrients (Thompson, 1981; Adamec, 1997b; Adamec, 2008a), allowing for niche exploitation and competitive advantage over other macrophytes, in extreme habitats with nutrient-poor substrates such as dystrophic waters, clay sands and soils impoverished by fire and heavy leaching (Givnish *et al.*, 1984; Juniper *et al.*, 1989). Carnivorous plants can be found on every continent except Antarctica (Ellison *et al.*, 2012).

To be considered 'legitimately' carnivorous, plants must nominally have morphological characters and physiological adaptations permitting the attraction, capture/retention, digestion and absorption of prey (Juniper *et al.*, 1989; Lloyd, 2008; Givnish, 2015), and obtain a demonstrable benefit from prey derived nutrients in terms of growth and/or reproduction (Adamec, 1997a; Płachno *et al.*, 2009). Many well recognised carnivorous taxa do not possess all of the aforementioned criteria. *Pinguicula* (Lentibulariaceae) exhibit no obvious prey attractants (Juniper *et al.*, 1989; Antor and Garcia, 1994). *Brocchinia* (Bromeliaceae), *Darlingtonia* (Sarraceniaceae), and some *Sarracenia* species (Sarraceniaceae) lack endogenous digestive enzymes and rely on commensalism or other symbioses for digestion by proxy (Anderson and Midgley, 2003; Fashing, 2005). Without digestive enzymes the obligatorily coprophagous *Roridula* spp. (Roridulaceae) do not gain nutrition directly from predation, but instead from the faeces of a hemipteran that preys upon the invertebrates they trap (Ellis and Midgley, 1996). *Roridula* also lack vascularised glands for absorption, but they are able to absorb the faecal nutrition though their leaves (Ellis and Midgley, 1996). This ability to utilise the nutrition directly or indirectly from prey capture in an ecologically significant way can serve as a fundamental criteria in the definition of carnivory and can be used to exclude 'protocarnivorous' taxa from the syndrome proper (Adamec, 1997a; Płachno *et al.*, 2009; Anderson *et al.*, 2012).

Complicating things further, plant species may meet all the requirements of carnivory and yet not be exclusively carnivorous. Three coprophagous species of *Nepenthes* (*N. lowii* Hook. *f.*, *N. rajah* Hook. *f.*, and *N. macrophylla* (Marabini) Jebb & Cheek*;* Nepenthaceae) have pitchers that function as latrines for tree shrews (Clarke *et al.*, 2010; Wells *et al.*, 2011), and *Nepenthes ampullaria* Jack is largely a herbivorous detritivore (Moran *et al.*, 2003; Pavlovič *et al.*, 2011). The traps of these species have structural modifications that facilitate their alternative diets. The subjects of this thesis, *Utricularia* spp. (Lentibulariaceae), do not feed exclusively on animal prey either, gaining nutrition from additional

sources such as algae, plant material and other detritus (Richards, 2001; Peroutka *et al.*, 2008; Koller-Peroutka *et al.*, 2014; Płachno *et al.*, 2015).

All carnivorous plants capture prey with trapping organs modified from leaves. Six trap styles can be distinguished: i) adhesive traps from sticky glandular leaves (*Byblis, Drosera, Drosophyllum, Philcoxia, Pinguicula, Roridula* and *Triphyophyllum*), ii) pitfall traps from tubular leaves (*Darlingtonia, Cephalotus, Heliamphora, Nepenthes, Sarracenia*), iii) pitfall traps from tubular rosettes (*Catopsis, Brocchinia*), iv) snap traps (*Dionaea, Aldrovanda*), v) lobster-pot traps from narrow tubular leaves (*Genlisea*) and vi) suction traps, modified tubular leaves that aspirate prey (*Utricularia*). The structural evolution of these trapping organs is a mixture of homology and analogy, with two clear patterns emerging at different scales: i) convergence from separate ancestry: form and function are tightly coupled at the level of the development of the carnivorous syndrome itself (e.g. between *Drosophyllum*, *Pinguicula* and *Byblis*) and ii) divergence from common ancestry: within monophyletic carnivorous lineages (e.g. the Droseraceae and Lentibulariaceae) trap form has diversified (Albert *et al.*, 1992b; Ellison and Gotelli, 2001). This diversification of trap forms within carnivorous clades consists of a repeated hierarchical pattern. Within carnivorous plant clades exhibiting a range of trapping styles, basal taxa often have adhesive traps. Processes such as episascidiation (inrolling of the leaf lamina) have then resulted in more complex trap types derived from adhesive traps (cf. the tubular pitfall traps of the enigmatic *Cephalotus follicularis* La bill. and the Sarraceniaceae, currently interpreted as basal within their respective carnivorous clades). Adhesive traps have arisen independently five times, those within the Caryophyllales and Lamiales from hypothetical ancestors pre-adapted for carnivory with multicellular, secretory glands (Cameron *et al.*, 2002; Fleischmann, 2012b). The stalked glands of hypothetical concestors within these clades formed the basis for the evolution of more complex structures, such as trigger hairs in the snap traps. Some of these derived trap types such as tubular leafed pitfalls (three origins) have evolved analogously within the unrelated carnivorous groups while others (e.g. bladders and eel traps) have evolved only once (Franck, 1976). Snap traps are also hypothesised to have arisen once in the Caryophyllales (Cameron *et al.*, 2002) from a terrestrial ancestor, but no fossil intermediates or *Dionaea muscipula* Sol. *ex* J Ellis. fossils exist to support this (Poppinga *et al.*, 2013).

Botanical carnivory has not evolved *de novo* within aquatic plant clades, rather aquatic carnivorous plants have arisen multiple times from terrestrial ancestors with pre-existing carnivorous habits (Arber, 2010). This has occurred only in two genera: once in the Droseraceae (the monospecific genus *Aldrovanda*), and multiple times within the genus *Utricularia.* Both *Aldrovanda* and *Utricularia* have trap morphologies (snap traps and bladders), that make the transition to an aquatic lifestyle feasible (unlike, for example, flypaper and pitfall traps). The morphologies of these plants have responded to the demands of aquatic environments in similar ways to non-carnivorous angiosperms (e.g. loss of anchoring

structures, loss of stomata, gain of air-filled cavities, dissection of leaves/stolons in response to the Prandtl boundary problem; Sculthorpe, 1971). The trap function of terrestrial and aquatic *Utricularia* remains largely similar (Taylor, 1989), but the traps of *Dionaea* close due to deformation of the two trap lobes, whereas trap closure in *Aldrovanda* involves the deformation of the trap midrib (Poppinga and Joyeux, 2011)

The number of carnivorous plants species recognised today ranges from a conservative 583 to c. 800 (Płachno *et al.*, 2009; Król *et al.*, 2011; Fleischmann, 2012a; Pereira *et al.*, 2012; Givnish, 2015) in up to 17 genera (**Table 1.1.**). The large disparity depends on whether (and how many) members of the genus *Stylidium* (Stylidiaceae) are considered carnivorous (Darnowski *et al.*, 2006; Płachno *et al.*, 2015). *Stylidium* species (triggerplants) have mucilage secreting glandular hairs on inflorescences that allow them surfaces to function as adhesive traps. Triggerplants have been identified as protocarnivorous because they trap the same number of insects per glandular surface area as other confirmed carnivorous plant species in their vegetative assemblages, and their glands show protease activity (Darnowski *et al.*, 2006). Further work is required to determine whether nutrient uptake occurs.

1.1.1 The costs and benefits of carnivory

The production and maintenance of carnivorous organs is an expense for plants as traps require structural (carbon), mineral and energetic investment (Friday, 1992; Knight, 1992; Adamec, 1997b; Ellison and Gotelli, 2009). Traps are also less efficient than leaves at photosynthesising (Benzinq, 1987; Knight, 1992; Ellison and Gotelli, 2002; Adamec, 2006). Investment in carnivory is therefore a trade off at the expense of producing more efficient photosynthetic surfaces; the evolution of plant carnivory can only occur when its attendant costs in the form of the maintenance and function of carnivorous structures are outweighed by gains from increases in the rate of photosynthesis (per unit of photosynthate invested in carnivorous structures). Put another way, the *marginal benefit* of carnivory must be positive, where marginal benefit is the difference between total photosynthetic increase resulting from nutrients gained from producing a new trap and the total photosynthetic cost of producing a trap compared with a photosynthetically more efficient structure (Ellison, 2006).

The original cost-benefit model for the evolution of carnivory by Givnish *et al.* (1984) predicts carnivory will become viable in nutrient-poor, moist and sunny conditions, whereas benefits from carnivory will decrease when ambient nutrient sources are high and when factors such as light and water are limiting (reduced photosynthetic ability makes the production of carnivorous structures too costly). As a result, carnivorous plants would compete poorly with noncarnivorous plants under dry and shaded conditions (Givnish *et al.*, 1984).

However, contrary to these predictions, the total photosynthetic (P_N) rates per unit of leaf mass are actually lower in carnivorous plants than non-carnivorous plants occupying similar niches (Ellison, 2006; Farnsworth and Ellison, 2008). Additionally, some perennial carnivorous plants occur in changeable habitats that undergo periods of drought and shade, despite incurring a reduction in the efficiency of photosynthetic yields (Ellison *et al.*, 2003). Aquatic carnivorous plants often grow optimally in comparatively light: 2-20% of incident photosynthetically active radiation (PAR) (Adamec, 2009).

How are carnivorous plants able to compete in these conditions? Perhaps surprisingly, photosynthetic gains from prey capture need not necessarily be particularly great for carnivory to yield a substantial marginal benefit. Recent work has shown that even though they are not as efficient at photosynthesising, traps are not always as expensive to produce as noncarnivorous structures (e.g. pitfall traps versus phyllodia, the non-trapping leaves produced by some pitcher plant species such as *Sarracenia*) photosynthesised ~20% faster than carnivorous pitchers (Ellison and Gotelli, 2002). Species with passive traps and even some 'active' traps such as those of *Dionaea muscipula* have functions that are comparatively inexpensive to maintain. Trap closure in *Dionaea muscipula* occurs through a passive release of elastic energy stored in fully hydrated leaves (Forterre et al., 2005). Although opening is an active process, this relatively cheap trap is rarely reset; rather, after one (rarely two or three) captures, the trap senesces (Darwin, 1875). The traps of aquatic carnivorous plants in the genus *Utricularia* are longer lived than flytraps, and expensive to maintain (Sasago and Sibaoka, 1985; Singh *et al.*, 2011). Aquatic *Utricularia* have a very high net photosynthetic rate compared to their non-carnivorous plant-competitors which may explain why they are able to tolerate lower light levels than predicted by Givnish *et al.* (1984).

A range of carnivorous plants exhibit plastic patterns in investment in carnivory, an ability that would be important to plant fitness in habitats that fluctuate between conditions that are favourable and unfavourable for the carnivorous habit (Ellison *et al.*, 2003). Lack of carbon in shaded conditions and excess of nutrition resulted in a reduction of mucilage production by the adhesive traps of *Pinguicula vallisneriifolia* Webb (Zamora *et al.*, 1998) and *Drosera rotundifolia* L. (Thorén et al., 2003) respectively. Nutrient additions to *Sarracenia purpurea* L. produced phyllodia (noncarnivorous leaves) that Similar results have been demonstrated for eight other species of *Sarracenia* with supplemented feeding (Farnsworth and Ellison, 2008). Trap production in *Utricularia* declines with increases in prey-derived or ambient nutrition (Knight and Frost, 1991; Friday, 1992; Guisande *et al.*, 2000; Englund and Harms, 2003; Guisande *et al.*, 2004). The costs and plasticity of the investment in carnivory of aquatic bladderworts is detailed further in sections **1.2.5-1.2.6**.

Givnish *et al.* (1984) specify three ways in which prey-derived nutrition could produce benefits for carnivorous plants: i) Mineral nutrient uptake increases P_N which allows for an increase in either photosynthesis per unit of leaf mass (A_{mass}) or in the total leaf mass that plants can support, ii) mineral nutrient uptake is allocated to reproduction, and iii) prey-derived carbon is used for producing sugars. The third benefit is particularly relevant for aquatic carnivorous plants which are otherwise dependant on, and limited by, CO_2 diffusion from the surrounding water (for detailed reviews see Ellison, 2006; Ellison and Gotelli, 2009). The positive effects of prey addition on plant growth (as proxy for elevated photosynthesis), and enhanced reproductive performance are well documented. Mineral nutrition from prey (chiefly N and P) enhances plant growth and reproduction (sexual and asexual) in a variety of ways, including biomass, length of shoots, leaf size, number of leaves, root growth, stimulation of mineral nutrition uptake by roots, number of flowers and seeds, size and nutrient content of seeds, size of propagules such as turions, and axillary budding (Darwin, 1875; Givnish *et al.*, 1984; Wilson, 1985; Thum, 1988; Friday and Quarmby, 1994; Zamora *et al.*, 1997; Otto, 1999; Adamec, 2002; Englund and Harms, 2003; Lenihan and Schultz, 2014). Measures of a direct, positive influence of feeding on photosynthetic enhancement are less conclusive. While a significant positive effect of feeding on the rate and efficiency of photosynthesis has been verified in some terrestrial carnivorous plants species including recent work with *Drosera capensis* L. (Pavlovic 2014) and *Nepenthes talangensis* Nerz & Wistuba (Pavlovič *et al.*, 2009), studies on *Drosera rotundifolia*, *Pinguicula villosa* L., *Pinguicula vulgaris* L. and *Sarracenia purpurea* found no significant increases in photosynthetic rates with prey addition (Ellison and Gotelli, 2009). The results for aquatic carnivorous plants are equally equivocal as a positive response of P_N per unit biomass to prey capture the has been demonstrated for *Aldrovanda vesiculosa* L., but not *Utricularia australis* R.Br. (Adamec, 2008a).

1.1.2 Prey attraction

Carnivorous plants are extreme examples of ambush predators (Harms, 1999). Unlike animal ambush predators such spiders (Janetos, 1986) and antlion larvae (Heinrich and Heinrich, 1984) carnivorous plants are entirely sedentary and cannot relocate to another site in response to a changes in environment such as a decline in food availability. If nutrition from prey is important for growth, then carnivorous plants should evolve mechanisms for attracting prey (Ellison and Gotelli, 2009). Evidence has been found for prey attraction in a range of different sensory modes including trap shape, visual cues and scent associated with the use of carbohydrate secretion (Joel *et al.*, 1985; Juniper *et al.*, 1989; Moran, 1996; Moran *et al.*, 1999; Di Giusto *et al.*, 2010). The nature and intensity of attractants can differ among species and species variants, between dimorphic traps within a single species, or with trap age (*Nepenthes*: Bauer *et al.*, 2009; Bauer *et al.*, 2011; Kurup *et al.*, 2013, Moran, 1996).

Visual attractants are reported for several groups. *Nepenthes*, *Sarracenia* and *Dionaea* all have blue fluorescence emissions on traps near spots where capture takes place. The masking of *Nepenthes khasiana* Hook. *f.* peristomes bearing these patterns reduces capture (Kurup *et al.*, 2013). Ultraviolet (UV) patterning stimulates insect activity (Tovée, 1995) and is a common feature of a number of carnivorous plants species, e.g. *Sarracenia flava* L., *Heliamphora nutans* Benth., *Cephalotus follicularis*, *Dionaea muscipula, Drosophyllum* (Joel *et al.*, 1985), and *Nepenthes* spp. (Moran *et al.*, 1999). The carnivorous tank bromeliads *Brocchinia hechtioides* Mez, *Brocchinia reducta* Baker and *Catopsis berteroniana* (Schult. & Schult. *f.*) Mez are all lined with UV-reflective wax (which also contributes to trapping mechanism as it creates a slipping surface) and have extremely conspicuous, bright yellow foliage (Frank and O'Meara, 1984; Gaume *et al*., 2004; Smith, 1997). Carnivorous plant traps also reflect patterns of visible light, but there is little evidence that these play a role in prey attraction. Bennett and Ellison (2009) demonstrated that the scent of nectar, not colour, was the prey attractant employed by a North American pitcher plant, *Sarracenia purpurea*. Foot *et al.* (2014) found no evidence that red coloration in *Drosera* attracted prey.

There is additional evidence of scent-mediated prey attraction in other carnivorous plant species, either mimicry of flower olfactory cues (Di Giusto *et al.*, 2010), or as a result of convergent evolution (Moran and Clarke, 2010). The upper pitchers of *Nepenthes rafflesiana* Jack mediate prey attraction through scent (Moran, 1996). The pitchers of *N. rajah* produce volatiles similar to those of fruit that attracts rats and tree shrews (Wells *et al.*, 2011). Nectaries from field collected leaves of *Heliamphora heterodoxa* Steyerm. and *Heliamphora tatei* Gleason also produce compounds that attract flies and ants (Jaffé *et al.*, 1995). Unlike the results for pitcher plants, the adhesive traps of *Drosera binata* La Bill and the traps of *Dionaea muscipula* emit weaker scents characteristic of green leaves that do not signal nectar rewards (Jürgens *et al.*, 2009). Chemical attraction has been proposed as a mechanism of prey attraction in *Utricularia*, but evidence is scant (Jobson and Morris, 2001) and possible mechanisms involving carbohydrate secretion have been falsified (Sanabria-Aranda *et al.*, 2006). Prey attraction in *Utricularia* is treated more fully in section **1.2.9**.

The nature of plant-prey relations in the tubular leaved pitcher plants (Cephalotaceae, Nepenthaceae, some members of the Sarraceniaceae) are best characterised as mutualistic rather than as an aggressive mimicry. Firstly, no models have been found for the traps, which would be required for the evolution an aggressive mimicry (where the traps are deceptive pollination mimics and prey are sensitive signal-receivers or operators) (Vane-Wright, 1976). Additionally, animals (chiefly insects) visiting the pitchers receive a legitimate reward in the form of nectar, with only a portion of them being trapped (Joel, 1988). Social insects such as ants, whose communities are robust to the sacrifice of individual members, form

close associations with *Nepenthes* species (Adlassnig *et al.*, 2011). In the case of the *Nepenthese bicalcarata* Hook. *f.* - *Camponotus schmitzi* Stärke mutualism the relationship is facultative for the plant but obligate for the ant (Bazile *et al.*, 2012). *Nepenthese albomarginata* feeds almost exclusively on termites which are baited with its own tissue: thatches of white trichomes growing under the pitcher rim (Merbach *et al.*, 2002). The traps of the tank bromeliad *Brocchinia reducta*, however, employ deceptive mimicry as they produce fragrance, but no nectar (Joel, 1988).

1.2 The Genus *Utricularia*

1.2.1 Taxonomy

Utricularia (the bladderworts) is one of three genera in the wholly-carnivorous plant family Lentibulariaceae. Along with two other genera, *Pinguicula* and *Genlisea*, the Lentibulariaceae contains c. 40% of all confirmed plant carnivores (Albert *et al.*, 1992a; Jobson *et al.*, 2003; Müller *et al.*, 2004; Müller and Borsch, 2005). The basal clade *Pinguicula* has adhesive traps. *Genlisea*, the 'corkscrew plant' is sister to *Utricularia* and traps with epiascidiate subterranean leaves called rhizophylls (Fleischmann, 2012b). *Utricularia* is the most speciose of all carnivorous plant genera, containing c. 228 members (Taylor, 1989; Jobson *et al.*, 2003; Albert *et al.*, 2010; Reut and Jobson, 2010; Fleischmann, 2012a) and has a global distribution, although it is generally absent from arid areas and oceanic islands (Taylor, 1989). The Neotropics have been reconstructed as a probable region of origin for the genus (Jobson *et al.*, 2003), supported by the comparatively high terrestrial species richness found there (Guisande *et al.*, 2007).

Utricularia is divided into three monophyletic subgenera, *Polypompholyx, Bivalvaria* and *Utricularia* (sensu Müller and Borsch, 2005). Subgenus *Polypompholyx,* sister to all other *Utricularia,* is further divided further into two sections, sect. *Pleiochasia* and sect. *Polypompholyx* and contains c. 40 species, all but one endemic to Australia. Subgenus *Bivalvaria* has 11 sections (excluding sect. Benjaminia) containing c. 93 species. Subgenus *Utricularia* is divided into 19 sections containing c. 111 species (Taylor, 1989; Müller and Borsch, 2005; Reut and Jobson, 2010; Fleischmann, 2012a; Jobson, 2012a).

1.2.2 Ecological types

All *Utricularia* species can be broadly classified into three categories based on growth habit: terrestrial (60% of species), aquatic and epiphytic. The terrestrial habit is considered pleisiomorphic with aquatic and epiphytic habits being derived conditions arising in terminal clades (Jobson and Albert, 2002; Jobson *et al.*, 2003; Müller and Borsch, 2005). Terrestrial *Utricularia* grow in moist to waterlogged soil, often peat and sand, where little surface water is present during flowering. Epiphytic *Utricularia* grow amidst bark, decaying leaves and moss on hillsides, cliff faces and trees (Taylor, 1989). Three 'epiphytic'

species, *Utricularia humboldtii* R.H. Schomb., *Utricularia quelchii* N.E.Br and *Utricularia nelumbifolia* Gardner, grow in the urns of tank bromeliads, many of which are epiphytes themselves. Within the urns these plants grow as aquatics but their stolons creep out onto the bromeliad leaves, bridge to neighbouring rosettes, and return to an aquatic habit. The association between *Utricularia nelumbifolia* and bromeliads from the genus *Vriesia* appears obligate, whereas *Utricularia quelchii* is often found in the tanks of *Brocchinia* spp., including *Brochinia reducta*, itself carnivorous! *Utricularia humboldtii* can be found in association with *Brocchinia* or growing as a terrestrial on marshy ground nearby (Arber, 2010). The majority of aquatic species live in lentic waters as either i) affixed aquatics that grow into the water column but anchor in benthic sediment or ii) suspended aquatics, freely suspended macrophytes capable of growing and flowering without any ground contact. Of the 33 suspended aquatic species 29 are found in section Utricularia. The remaining four species are found in sections Vesiculina (three species) and Pleiochasia (*Utricularia tubulata* F.Muell.). Approximately eight aquatic species exist as specialised rheophytes and occasionally suspended or affixed aquatics are also found growing atypically in lotic waters (Taylor, 1989).

This classification on the basis of growth habit is useful and justified by clearly identifiable morphological adaptations for life as epiphytes (leathery leaves and tubers), and aquatics, particularly rheophytes (anchoring organs, specialised trap orientation) and unequivocal suspended macrophytes (peduncle floats). However, as Taylor (1989) and Kamienski (1890) have noted, some caution is necessary. The distinction between terrestrials and affixed aquatics is the most arbitrary. “Terrestrial” species such as *Utricularia triflora* P. Taylor can be found as an affixed aquatic, while nearby growing exposed on soil and on tree stumps. *Utricularia uliginosa* Vahl will grow on stream banks with its vegetative organs extended underwater and into the stream bed (Taylor 1989). Several species from section Pleiochasia (subgenus *Polypompholyx*) have typical terrestrial vegetative growth, but occupy habitat that is seasonally flooded. The traps are borne on stalks of variable length with some extending up into the ephemeral water column (Taylor, 1989; Reut and Jobson, 2010). Taylor (1989) often refers to these species as “sub-aquatics”. The ecology of many terrestrial species needs therefore to be interpreted in the context of seasonal fluctuations in the water levels where inundation may occur (Reut and Jobson, 2010).

The growth habits of contemporary affixed aquatic species are suggestive of hypothetical intermediate forms that may have arisen during the adaptive radiation of *Utricularia* into aquatic habitats. *Utricularia stygia* G. Thor, *Utricularia ochroleuca* R.W.Hartm. and *Utricularia intermedia* Hayne are affixed aquatic species from section Utricularia with dimorphic shoots. They share characters with both suspended aquatic *Utricularia* bearing monomorphic, photosynthetic shoots and terrestrial species whose

traps grow on organs buried in substrate (Thor, 1988; Taylor, 1989). Shoots in the water column bear leaves and are clearly differentiated from the chlorophyll-free, trap bearing carnivorous shoots that grow in the substrate (Adamec, 2007a). Whether these species are truly representative of proto-aquatic ancestral forms is still unclear. Preliminary phylogenetic work by Jobson (in progress) on section Utricularia, using molecular characters, nests *Utricularia ochroleuca* and *Utricularia intermedia* within a clade of suspended aquatics, suggesting that the benthic growing carnivorous shoots of these plants represent a secondary gain. *Utricularia gibba* L., a member of another affixed aquatic clade, has extremely plastic growth that blurs the distinction between ecological types. *Utricularia gibba* has monomorphic shoots, but is often found anchored in benthic substrates, growing up into the water column in dense clouds or mats. This species will also grow as a suspended aquatic (although it rarely flowers in this circumstance unless it is able to anchor to floating mats of detritus), as a terrestrial growing in saturated soil, and as an epiphyte (Taylor, 1989; Chormanski and Richards, 2012).

1.2.3 Vegetative morphology

With very few exceptions, bladderworts form horizontal stolons. Flattened green organs arise from the nodes and by convention are called leaves. All *Utricularia* spp. lack true roots, but many species have organs termed rhizoids by Taylor (1989; but see Fleischmann, 2012b) that perform an anchoring function. Some aquatic species produce additional vegetative structures such as peduncle floats and air shoots. Peduncle floats are spongy, white and fusiform structures comprised of large air filled cells that occur in whorls at the base of the flowering stalk, holding the inflorescence above water level. Air shoots are filamentous, resembling elongate stolons with 'bract-like' scales and small intercellular air spaces at their tips that likely assist with gas exchange. (Taylor, 1989; Raynal-Roques and Jérémie, 2005; Lloyd, 2008). The unique trapping structures that characterise *Utricularia*, bladders (utricles), are foliar in origin (Juniper *et al.*, 1989; Lloyd, 2008) and arise from any or all of the other organs: stolons, rhizoids and leaves, where they are variously inserted, including the leaf tips (Taylor, 1989). Bladder morphology and function is discussed further in sections **1.2.4** and **1.2.5**.

It is important to qualify any discussion of *Utricularia* morphology by noting bladderworts have a plastic bauplan that resists the classical description more easily applied to most angiosperms. The principal distinctions between root/rhizoid, shoot and leaf that characterise this approach are not entirely appropriate for bladderworts whose modules cannot always be easily assigned to one category or the other, often appearing intermediate (Rutishauser and Isler, 2001; Chormanski and Richards, 2012). Some species of terrestrial and epiphytic *Utricularia* are clearly heterophyllous with distinct photosynthetic leaves and trapping leaves (bladders) arising independently from the stolons. However, for the affixed aquatics *Utricularia helix* P.Taylor and *Utricularia volubilis* R.Br. in section Pleiochasia this distinction

between leaf and trap is fuzzy, with trap stalks grading into forms indistinguishable from leaves, except for apices decurrent to the ventral surface of traps (Taylor, 1989). In many suspended aquatic species there is no clear leaf/shoot dichotomy and the leaf-like organs that are the main site of photosynthesis can be interpreted as modified stolons (Rutishauser and Isler, 2001; Chormanski and Richards, 2012; Fleischmann, 2012b). In the aquatic species from section Utricularia traps are inserted on these leaf-like organs as well as at the stolon nodes. In case of other species, stolons and rhizoids are not clearly distinguishable. Reut and Fineran (2000) describe the rhizoids (*sensu* Taylor, 1989) of the terrestrial *Utricularia dichotoma* La Bill. as "anchoring" stolons to distinguish them from the principle "runner" stolons, and also note the presence of intermediate types in some populations. The affixed aquatic *Utricularia gibba* produces indeterminate rhizoids that grade back into stolons, and in general airshoots are also capable of elongation and apical growth resulting in a similar transformation (Taylor, 1989; Chormanski and Richards, 2012). With the above in mind, the terms stolon, leaf, rhizoid and trap will still be employed throughout this thesis following Taylor (1989), both for the sake of convenience and because the leaf-like organs of aquatic section *Utricularia* aquatics are the primary sites of photosynthesis, fulfilling the functional definition of a leaf **.**

1.2.4 Trap Morphology

Utricularia have highly specialised, mechanically activated suction traps that require immersion in water to function (Skutch, 1928; Sydenham and Findlay, 1973; Juniper *et al.*, 1989; Lloyd, 2008) (**Fig. 1.1.**). All traps function in the same way regardless of the growth habit of a particular species. The traps of terrestrial species occupy saturated interstitial spaces; those of epiphytes are surrounded by a film of moisture. Traps are attached to other organs by stalks whose length and thickness varies, with the traps of some species being almost sessile. Bladders are roughly lenticular and contain a fluid filled lumen. In most species the bladder wall is two cells thick except at the threshold and vascular bundle (Compton, 1909; Reifenrath *et al.*, 2006). There is an aperture at the anterior end of bladder closed with a trap door (valve). The dorsal margin of the trap door is attached to the aperture while its free ventral margin rests against a thickened pavement of cells, the collar (threshold), creating a hermetic seal when the trap is set. The threshold is covered with a layer of tightly appressed glands called the pavement epithelium that secrete a membranous layer called the velum. The velum projects into the space of the trap entrance and acts as a door stop, supporting the sealing of the trap door. (Darwin, 1875; Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985; Reifenrath *et al.*, 2006). The position of the valve in relation to the traps stalk varies among- and within-species. Traps whose stalks are adjacent to the valve have "basal trap doors". Those with stalks opposite (at 180° to) the valve on the posterior end of the bladder have "terminal trap doors" and those with an intermediate position on the ventral side (c. 90°) have "lateral trap doors" (Taylor, 1989).

Both the internal and external trap surfaces bear various other trichomes of epidermal origin, many of them glandular. The functions of these glands are only partially resolved (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985; Juniper *et al.*, 1989). The outer surface of the trap door is often covered with two types of trichomes: stiff trigger hairs near the free ventral edge, and stalked secretory trichomes. Numerous button-like trichomes (paired cells) cover the entire exterior surface of the bladder. Button trichomes were historically proposed as an outlet for water transport from the bladder (Nold, 1934), but this is not the case (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985). More developed protuberances referred to as appendages surround the trap door and partially cover the exterior of the entrance. Appendages are discussed in more detail in **1.2.8**. Inside the bladder are conspicuous two- and four-armed glands, bifids and quadrifids. Bifids are found on the interior side of the threshold and are responsible for water extrusion. Quadrifids line the inner surface of the bladder and are involved with digestive enzyme secretion and the absorption of prey-derived nutrients (Meierhofer, 1902; Fineran and Lee, 1974; Thurston and Seabury, 1975; Taylor, 1989).

Some species of aquatic bladderworts exhibit trap dimorphism and polymorphism. About a quarter of the suspended aquatic species in section Utricularia (c. eight species) have two morphologically distinct trap types, lateral and basal (Taylor, 1989). Lateral traps are inserted on the filaments of the leaves and occasionally at the nodes, have a consistent shape, but vary in size at maturity. In species such as *Utricularia vulgaris* L. the size distribution of lateral traps falls into two distinct size classes, larger mid-line traps and smaller peripheral traps (Friday, 1991). In others such as *U. australis* and *Utricularia geminiscapa* Benj. lateral trap size decreases gradually from the base to the tip and the midline to the periphery of the leaf (See **Chapter 4**). Basal traps are found only at the base of the leaf, are smaller than the largest lateral traps, have different bladder shapes and longer trap stalks inserted closer to the trapdoor (basal trapdoors). They do not exhibit the same size plasticity as lateral traps. Historically the terms “trap di- and polymorphism” and appendage (as aspects of trap morphology) polymorphism have been conflated. In this thesis I make a clear distinction: Trap polymorphism relates only to only the shape-characters of the trap bladder such as trapdoor position. Appendage polymorphism relates to the variable expression of trap appendages. Hence, it is possible for a species to have dimorphic traps, with one form exhibiting appendage polymorphism and the other not (for example *Utricularia resupinata* Greene *ex* Bigelow; Taylor, 1989). Many of the “sub aquatics” of section Pleiochasia Taylor (1989) have polymorphic appendage expression (For more details see **1.2.8).**

1.2.5 Trap Firing

Trap action consists of two phases, a passive fast suction sequence followed by an active slow deflation. When the bladder is set, the hydrostatic pressure within the lumen is lower relative to the surrounding

water, about -16 kPa (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985; Juniper *et al.*, 1989; Singh *et al.*, 2011). This pressure gradient causes the bladder walls to assume a concave configuration, storing elastic energy. Aquatic microorganisms near the trap mouth may contact the trigger hairs, whose movement causes a reversible buckling (buckling/unbuckling) of the trapdoor in the form of a convex/concave inversion (Singh *et al.*, 2011; Vincent and Marmottant, 2011; Vincent *et al.*, 2011a). This trap activation signal is not propagated electrophysiologically, but mechanically by the trigger hairs acting as cantilevers (Adamec, 2012a). The elastic energy stored in the bladder is now converted to kinetic energy; the bladder walls relax, a strong in-rush of water aspirates the organisms into the expanding trap, and the trap door closes (Vincent *et al.*, 2011b). The trapdoor takes between 300 – 700 μs to open, then another 2-5 ms to close again (Singh *et al.*, 2011). This is one of the fastest movements by plant organs, and the fastest among carnivorous plant traps. Potential prey cannot respond fast enough to avoid capture, and escape from the bladder afterwards is not possible as the trap door is lodged firmly against the threshold.

The negative pressure gradient is restored in the traps as specialised glands pump water from the trap resulting in a smooth, continuous deflation (Juniper *et al.*, 1989; Lloyd, 2008) whose rate decreases over time (Vincent *et al.*, 2011a). By 25-35 minutes after firing, approximately 40% of the water is removed from the lumen and the trap is reset enough to fire (Sydenham and Findlay, 1973), but it takes 6-10 hours before deflation is complete (Adamec, 2011a). During trap resetting water is only exuded from the trapdoor region. The capital cells of the pavement epithelium provide the outlet for water outflow. They discharge water by bulk flow across the membrane due to intracellular pressure (Sasago and Sibaoka, 1985). By virtue of their location the bifid glands are the best candidates for the main inlet for water outflow. Electrical potential differences between the trap interior and both the ambient solution outside the trap and the inner cells of the trap wall have led to the hypothesis that the bifid glands actively take up Cl^- ions from the trap fluid, with water molecules following by osmosis (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985). Sydenham and Findlay (1973) proposed that traps may have an internal negative pressure sensor that regulates water pumping. However, there is no observed lag-time in water pumping within the first 2 s after trap firing, suggesting otherwise. In the “water recirculation” hypothesis water extrusion continues once the trap is fully deflated but is offset by an influx of water, perhaps due to the trapdoor being permeable or imperfectly sealed (Vincent *et al.*, 2011a). Alternatively, the mechanism of water pumping runs continually but becomes thermodynamically inefficient when the negative pressure gradient is high (Adamec, 2011c). Bladders can fire up to eight times without an appreciable effect on the resetting rate (Sydenham and Findlay, 1973; Rutishauser and Brugger, 1992).

Traps also fire spontaneously, without mechanical stimulation. This probably occurs when the negative pressure gradient is at its greatest (and trap thickness is minimal), and the trap door can no longer withstand the ambient water pressure (Adamec, 2011a; Vincent *et al.*, 2011b). Spontaneous firing has been observed in three different species of aquatic *Utricularia* species in traps of different ages, occurring 0.3-2.4 times over a one-day period with time between two such firings varying between 5-16h. During their active life traps can aspirate over 15 times their own volume in ambient water. There is no quantitative difference, in terms of the trap thickness increase due to firing/resetting rate, between spontaneous and mechanically stimulated firing (Adamec, 2011a)

1.2.6 Growth and ecophysiology of aquatic *Utricularia*

Aquatic *Utricularia* frequently grow in shallow, standing, dystrophic waters (high in humic acids and tannins) that are predominantly poor in N and P, and commonly poor in K. Free CO_2 is commonly high in these environments (>0.1mmol.l^{-1}), although sometimes is in short supply (below 0.05 mmol.l^{-1}), being highly dependent on the pH and Total Alkalinity (Adamec, 1997b, a; Adamec and Kovářová, 2006; Adamec, 2008b) and plants frequently grow in low oxygen concentrations (Guisande *et al.*, 2000; Guisande *et al.*, 2004) or even under complete anoxia (Adamec, 2007b). A correlation between the concentrations of total nitrogen (N_T) and humic acids with tannins at several Czech peaty sites indicates that the main pool of N_T available to aquatic carnivorous plants in dystrophic habitats comes from the organic nitrogen in the humic acids (Adamec, 2007a). Free CO_2 is the only source of inorganic carbon available to the aquatic carnivorous plants so far tested (Adamec, 1997a, 2009; Adamec and Pásek, 2009). While plants can also utilise organic carbon from prey carcasses, the amount is not significant compared to the carbon from free CO_2 (Adamec, 1997a).

Aquatic *Utricularia* have very rapid growth. It takes only 5-20 days for plants to double their biomass and they have apical shoot growth rates of 1-4.2 new leaf nodes per day. Basal segments senesce at the same rate (Friday, 1989; Adamec and Kovářová, 2006; Adamec and Pásek, 2009; Adamec, 2011d). The rapid apical shoot growth allows for a fast replacement of traps which may explain why traps are often short lived (Friday, 1989; Friday, 1992). Traps of *U. vulgaris* usually have a life of 30 d or less, being active for only 10-19 d or less, and their trapping efficiency (capture rate) declines rapidly as they age (Friday, 1989). Trap age must therefore be considered in any ecophysiological and capture rate studies of *Utricularia* traps (Friday, 1989; Sirová *et al.*, 2003).

Rapid growth rates are made possible by the plants' carnivorous habit, efficient nutrient recycling from senescent shoots, high capacity for bioconcentration of nutrients from water and high net photosynthetic rates (compared to other non-carnivorous, submerged plants) of 11.1 - 32.5 nmol g-1 s-1.a (Adamec, 1997b; Adamec, 2000; Englund and Harms, 2003; Adamec, 2006; Adamec, 2008a; Adamec, 2008b,

2009). Being rootless, aquatic *Utricularia* take up nutrients through their shoots, either directly from the ambient water or from captured prey. To date there has been no direct measurement of nutrient uptake by shoots or from prey, although estimates have been made. Adamec (2009), for example, used plants growing in oligotrophic waters with no prey availability to deduce very high shoot uptake affinities for mineral nutrients of at least 0.4 μM for NH_4^+ and 0.1 μM for phosphate. Prey carcasses are a poor source of K, Ca and Mg for plants but are relatively high in N and P tissue content, which therefore offer the greatest potential value from prey-capture in terms of mineral nutrition. Prey-derived N and P are both translocated to immature tissues although only N is retained in these younger tissues once they mature. Prey-derived P is back-translocated to tissues in sideshoot meristems and flowers arising from parts of the plant that are older than the leaf nodes where feeding takes place, whereas N is not (Friday and Quarmby, 1994).

Prey-derived nutrition enhances growth and photosynthetic efficiency in *Utricularia* (Adamec, 1997a; 2011e but see Adamec, 2008), however traps represent a huge structural and metabolic cost. Empty trap tissues are higher in P (53% of total) and K (51%) content per unit biomass than leaves, but lower in N (30%), Ca and Mg (Guisande *et al.*, 2004; Adamec, 2008b). Traps have a respiration rate two-three times greater than associated leaves or shoots (*U. australis* traps: 67% of total plant respiration rate, *U. intermedia* and *U. stygia* carnivorous shoots: 60-68% of total plant respiration rate: Adamec, 2006; Adamec, 2007a), whereas the photosynthetic rate of traps in six aquatic species measured by Adamec (2006 also see Knight, 1992) was 7-10 times smaller than that of leaves/shoots.

1.2.7 Investment in carnivory

Structural investment in carnivory (IIC) of aquatic *Utricularia* has been quantified in different ways including trap size, number of traps per leaf, number of traps per dry leaf dry weight (DW), and the ratio of trap DW to leaf DW. Recent work has acknowledged that the most appropriate methods are those considering trap weights or numbers relative to leaf biomass (Friday, 1992; Kibriya and Jones, 2007) and IIC is now commonly defined as the proportion (or percentage) of the total plant biomass that is made up of trap biomass (Kibriya and Jones, 2007; Adamec, 2011e). IIC is variable in aquatic *Utricularia*: *U. vulgaris*, 10-25% (Englund and Harms, 2003); *U. australis*, 23-61% (Adamec, 2008b); *U. australis*, *U. gibba* and *Utricularia reflexa* 11-20% (Porembski *et al.*, 2006); *U. intermedia* and *U. stygia* 18-29%; *Utricularia purpurea* Walter ca. 26% (Richards, 2001), and is considerably higher than that in terrestrial species: 0.14-0.85% (Porembski *et al.*, 2006). The comparatively high IIC associated with the aquatic habit may reflect a greater availability of prey or likelihood of prey capture than in terrestrial environments (Adamec, 2007a).

Aquatic *Utricularia* are able to change their IIC in response to variations in habitat factors (water chemistry, prey availability and irradiance) (Knight and Frost, 1991; Guisande *et al.*, 2004; Adamec, 2007a; Adamec, 2008b; Adamec *et al.*, 2010; Adamec, 2015, in press). Due to the expense of trapping structures it seems reasonable that low prey densities would result in high investment in carnivory through negative feedback in an attempt to catch what little prey is available (Englund and Harms, 2003; Adamec, 2007a). Historically tests of these predictions have been inconclusive (due in part to inconsistent measures of IIC), with prey density affecting IIC positively (Sorenson and Jackson, 1968: trap number only; Jobson *et al.*, 2000), negatively (Guisande *et al.*, 2000) or not at all (Knight and Frost, 1991) and ambient nutrition affecting IIC positively (Guisande *et al.*, 2000), negatively (Knight and Frost, 1991) or not at all (Jobson *et al.*, 2000). In a field study manipulating light and prey densities, Englund and Harms (2003) demonstrated that IIC is a product of trophic interactions between *U. vulgaris*, their animal prey and their periphyton. Prey density negatively affected the production of traps, particularly under conditions of low light (where the IIC was zero). However, prey addition was correlated with an increase in ambient nutrition, particularly the concentration of phosphorus, leading the authors to conclude that IIC was regulated exogenously by P rather than by prey derived nutrition. Other studies provide evidence for an ecophysiological regulation of structural IIC in aquatic *Utricularia.* Kibriya and Jones (2007) reported a significant negative correlation between investment in trap biomass and P availability in *U. vulgaris*. The IIC by *U. australis* was significantly inversely proportional to shoot N content (Adamec, 2008b; Sirová *et al.*, 2011), but positively correlated with free CO_2, with both factors acting partly independently of one another (Adamec, 2008b). Bern (as cited in Adamec, 2015 in press) found the IIC in *Utricularia foliosa* L. was inversely proportional to both N and P content.

A mechanism proposed by Adamec (2008b; Adamec, 2015, in press) points to control of IIC through two components: i) negative feedback from endogenous nutrient (N and P) content, ii) dominated by photosynthetic (CO_2) regulation. Under common photosynthetic conditions, with poor to medium concentrations of CO_2 (ca. 0.03-0.2 mM) a decline in shoot N and/or P stimulating trap production would enhance the rate of prey capture, leading in turn to an increase in shoot N and/or P content and a downregulation of trap production, beginning the cycle again. In highly favourable photosynthetic conditions with surplus CO_2 (>0.20 mM) and optimal light IIC is stimulated more by an excess of photosynthates than low shoot N or P content, and may even be positively affected by organic carbon uptake from prey, should this occur.

1.2.8 Feeding Ecology

Utricularia are generalist predators whose broad diets are limited by the availability of suitably sized prey (Harms, 1999; Mette *et al.*, 2000; Gordon and Pacheco, 2007). Large traps are more effective than

small ones, capturing greater numbers of prey that are larger and more species rich (Friday, 1991; Sanabria-Aranda *et al.*, 2006), but encounter rate with prey is a function of the number of traps (Harms 2002). Trap content surveys show both terrestrial and aquatic *Utricularia* utilise a similar range of meiofaunal prey whose availability overlaps for both growth plant habits. Common prey taxa include Copepoda (Cyclopoida, Calanoida, Harpacticoida); Ostracoda (*Cypris)*; Cladocera *(Daphnia, Chydorus);* dipteran larvae; Odonata nymphs; nematodes; rotifers. Acarina and Tardigrada *(Biapurtura)* have also been recorded in traps of the terrestrial *U. uliginosa* (Jobson and Morris, 2001; Harms, 2002; Guiral and Rougier, 2007; Martens and Grabow, 2011). Harms (2002) showed in a field experiment that predation by three aquatic *Utricularia* (*U. minor, U. intermedia/stygia, U. vulgaris*) was sufficient to depress microcrustacean densities and therefore influence prey community dynamics. *Utricularia* exhibit some selectivity, with prey characters such as mobility, size and habitat use being important determinants of diet (Harms, 1999; Harms and Johansson, 2000; Richards, 2001; Guiral and Rougier, 2007). Phytophilous species are more frequently trapped than highly mobile, planktonic ones (Harms, 1999) and larger zooplankton (cladocerans and copepods) are more frequently trapped than smaller ones, depending on the trap size and the seasonal availability of prey (Guiral and Rougier, 2007).

Attempts to determine whether aquatic *Utricularia* trap some phytophilous taxa in higher numbers relative to others have produced conflicting results. Two studies comparing densities of captured animals to those in the ambient water found a "*U. breviscapa*-like species" (Guiral and Rougier, 2007) and *U. vulgaris* (Harms and Johansson, 2000), fed on cyclopoid copepods over cladocerans. In a prey selection experiment Harms (2002) also found *U. vulgaris* captured a cyclopoid copepod (*Eucyclops serrulatus* Fischer) in preference to the cladoceran (*Polyphemus pediculus* O.F. Müller). In contrast, a trap-contents survey by Richards (2001) found higher numbers of *U. purpurea* traps contained cladocerans than copepods, but provided no estimate of the ambient density of available prey. Mette *et al.* (2000) recorded a 'mass occurrence' of one species of cladoceran, *Chydorus sphaericus* O.F. Müller in traps of *U. australis* as opposed to 'many' cyclopoid copepods, but in ambient water samples found only three *C. sphaericus* individuals per 10 l and >700 cyclopoid copepods. Jobson and Morris (2001) have conducted the only study to date on the feeding preferences of a terrestrial *Utricularia* species, *U. uliginosa.* They found nematodes were under-represented in traps, whereas a harpacticoid copepod, a species of *Elaphoidella*, was over-represented relative to numbers in the surrounding soil.

Phytoplankton and pollen may also be an important source of mineral nutrition for *Utricularia.* Allochthonous material starts to appear in traps once their trapdoors are fully developed but before leaves fully mature and increases exponentially with leaf age (Richards, 2001). Peroutka *et al.* (2008) found numerous traps of four aquatic *Utricularia* species (*U. vulgaris, U. australis, Utricularia minor* L. and

Utricularia bremii Heer *ex* Kölliker contained the same planktonic algae found in surrounding water, but around 90% were dead, fulfilling the role of prey. While the capture of algae and pollen appears to have little influence on the C/N ratio, it has been strongly correlated with other shoot growth parameters such as weight, length, budding and internode elongation in *U. australis*, *U. vulgaris* and *U. minor*, indicating algae and pollen provide other nutrients, perhaps phosphorus and trace elements (Koller-Peroutka *et al.*, 2014). This food source would be replenished not only in conjunction with meiofaunal prey capture, but on a regular basis by spontaneous trap firing, highlighting its ecological importance. For terrestrial species and aquatics with dimorphic shoots that produce subterranean shoots, aspirations of detritus could also provide nutrition (Adamec, 2011e).

In addition to prey, the traps of aquatic *Utricularia* are permanently inhabited by a live community of unspecialised commensal organisms: bacteria (including cyanobacteria), euglenoids, desmids, diatoms, dinophytes, green algae, protozoa and rotifers (Mette *et al.*, 2000; Richards, 2001; Gordon and Pacheco, 2007; Peroutka *et al.*, 2008; Alkhalaf *et al.*, 2009; Sirová *et al.*, 2009) These organisms, too small to mechanically stimulate trap firing, enter traps during spontaneous firings or during aspirations triggered by larger animals. They propagate inside traps and form miniature food webs (Sirová *et al.*, 2009). The nature of the interactions between *Utricularia* and trap commensals is unclear, but they may assist in prey digestion (Adamec, 2011b). Experiments with *U. australis* and *U. vulgaris* (Sirová *et al.*, 2010) show these plants allocate 20-25% of newly fixed carbon to traps, perhaps 'gardening' their commensals. This is affordable even in conjunction with the cost of a very rapid growth rate, as *Utricularia* shoots exhibit extremely high photosynthetic rates (Adamec, 2006). Cost benefit ratios under these circumstances should then depend on the proportion of traps capturing prey (Adamec, 2011c) and the density of their captures, supported by evidence for prey capture enhancement of growth in aquatic *Utricularia* (Englund and Harms, 2003; Adamec *et al.*, 2010; also Chapter Three). As high densities of commensal organisms are also found in traps empty of meiofaunal prey (Adamec, 2011e) a mutualistic interaction between plants and trap commensals maybe more important for mineral nutrition than prey capture for plants growing in nutrient poor waters with low prey densities (Alkhalaf *et al.*, 2009; Sirová *et al.*, 2009).

Algae, bacteria and rotifers use the exterior surface of *Utricularia* (shoots, traps and leaves) as attachment sites, where they receive nutrients lost from the plant traps during firing (Englund and Harms, 2003; Guisande *et al.*, 2007). *Utricularia* interacts with its periphyton directly, in the form of competition for nutrients and light, and loss of trap function when the trap door area is overwhelmed. They also act indirectly in that by capturing grazing animals, plants may regain nutrients both lost to the periphyton and those taken up by the periphyton from the water column. It is conceivable that grazing animals could

also increase the functional life of traps by reducing the rate of periphyton build-up around the trap door. However, such an advantage to the plant may be offset by an overall increase in periphyton as higher prey densities are associated increase in ambient nutrition, that in turn allows for increased algal growth (Englund and Harms, 2003).

1.2.9 Appendages

The exterior trapdoor region of *Utricularia* is adorned with external hairs and multicellular protuberances called appendages that project over the exterior of the trapdoor (Taylor, 1989; Reifenrath *et al.*, 2006). Appendages are classified by their position relative to the trapdoor, being referred to as dorsal, lateral or basal. There is a strong phylogenetic signal in appendage shape and positioning (**Fig. 1.2**) but similarities also exist among different ecological types (Lloyd, 1933; Taylor, 1989; Reifenrath *et al.*, 2006) which suggests they may evolve in response to habitat. Epiphytic species have comparatively uniform trap morphologies with dorsal appendages that curve down over the trap door; these are proposed to hold water over the trapdoor, to facilitate trap activation and provide suitable habitat for prey (Rutishauser and Brugger, 1992). Terrestrial *Utricularia* exhibit a wide variety of appendage forms, that range from elaborate projections positioned variously about the trap door region, to simple dorsal projections. In some species the appendages are reduced or even absent (Taylor, 1989; Rutishauser and Brugger, 1992). Terrestrial appendages may prevent trap door clogging by soil particles in terrestrial species (Lloyd, 1933), or assist in prey capture by acting as drift fences to steer prey towards the trap opening (Gardiner, unpublished data). Most aquatics inhabiting lentic waters have filiform appendages, sometimes extensively dendriform (Taylor, 1989). Rheophytes have simple, dorsal and lateral bristles or are exappendiculate (Taylor, 1989). Species specific examples of appendage morphologies are presented in **Table 1.2** and **Figs. 1.3-1.20**. The technique used to generate these images is present in **Appendix 2.**

The appendages of species belonging to one section of lentic aquatics, section Utricularia, have a highly conserved gross morphology (Taylor, 1989). They take the form of paired, dendriform, dorsal structures, that Darwin (1875) named antennae, as they reminded him of the sensory structures of cladoceran prey. These antennae are supplemented by varying numbers of simple, dorsal, lateral and ventral bristles (Taylor, 1989) (**Fig. 1.1**). While all section Utricularia species have antennae and some degree of supplementary bristling, both among- and within-species variation exists in the positioning and extent of their expression, in terms of bristle numbers, and the degree of antennal branching. *U. gibba*, for example, has comparatively robust and extensively branched antennae. Together with two closely related species, *Utricularia. striata* LeConte *ex* Torr. and *Utricularia floridana* Nash, *U. gibba* also has bristles along the dorsal margin of the trap door, a feature absent from the remaining 26 species in the section. Other section Utricularia species have forms where the antennae may be sparsely branched or unbranched (*U. vulgaris*,

Utricularia macrorhiza LeConte, *U. australis, Utricularia dimorphantha* Makino) or reduced to nubs with supplementary bristles entirely absent (*Utricularia aurea* Lour., *U. foliosa, Utricularia hydrocarpa* Vahl, *Utricularia inflexa* Forssk., *Utricularia muelleri* Kamiénski, *Utricularia stellaris* L.f.) (Taylor, 1989; Guiral and Rougier, 2007). Two other suspended aquatic species from the closely related section Vesiculina, *U. purpurea* and *Utricularia myriocista* A.St.-Hil. & Girard have traps without appendages of any kind (Lloyd, 1933). Aquatic appendages are thought to assist with prey attraction and capture enhancement (Darwin, 1875; Meyers and Strickler, 1979; Taylor, 1989 but see Lloyd, 1933). This hypothesis is discussed more fully in **1.2.10**.

Section Pleiochasia is predominantly terrestrial. Of the 34 species in the section Taylor (1989) considered 23 to be terrestrial, with a further two terrestrial species described since the publication of his monograph (Reut and Jobson, 2010). Eight others are described as affixed aquatics or 'sub-aquatics' (but see Reut and Jobson, 2010 regarding *U. triflora*), although three of these are also found as terrestrials, and one species, *U. tubulata* is a suspended aquatic (Taylor, 1989). Superficially, the appendage morphology of Pleiochasia species appears very heterogeneous (in contrast to section Utricularia); closer inspection reveals that they are variations on the same theme: a single dorsal appendage, a pair of lateral appendages on either side of the trap door, and a pair of ventral appendages that run along the belly of the trap from the ventral margin of the trap door to the trap stalk, termed ventral wings (Taylor, 1989). The difference in appendage morphology among species results from the variable expression of these three appendage types. The dorsal and ventral appendages of aquatic Pleiochasia are simplified and filiform in comparison to those of the terrestrials, and the ventral wings are often entirely suppressed (Taylor, 1989; Reut and Jobson, 2010). Appendage di- and polymorphism (in individuals of particular species) is also common in section Pleiochasia, especially, though not exclusively, among aquatics. Trap size within individuals of these species is also variable. Smaller traps with full appendage expression have relatively shorter trap stalks that hold them close to the stolon and substrate, while large traps on long stalks, presented to the water column often have more longer, more filiform dorsal and lateral appendages and reduced or absent ventral wings (Taylor, 1989; Reut and Jobson, 2010, *pers. obs.*).

1.2.10 Prey attraction

Utricularia selectively capture phytophilous and meiofaunal prey (see **1.2.8** for details). Refuge seeking and feeding behaviour have both been proposed as explanations for why these over-represented prey taxa may be drawn to traps, but a strategy of chemical attraction akin to nectar production (or its mimicry through scent) employed by pitcher plants has never been demonstrated. Cohen (1875) proposed that carbohydrate-containing mucilage secreted by the stalked glands around the trap door exterior might serve as a lure. However, Meyers and Strickler (1979) failed to find a link between mucilage secretion

and capture rate. While Sanabria-Aranda *et al.* (2006) found a positive relationship between the carbohydrate content of bladders and the number of prey per bladder, the ratio of trap carbohydrate content/bladder length was due to changes in periphyton abundance on the traps and not changes in production by the plant. A behavioural experiment by Jobson and Morris (2001) showed that traps of the terrestrial *U. uliginosa* attracted individual harpacticoid copepods from the genus *Elaphoidella* (in comparison to inert controls). Boiling of the control traps to remove any chemical attractants would also have removed adhering bacteria and the authors suggest either of the two elements could have exerted an attractive influence on the copepods.

The only prey attraction mechanism to be both elucidated and observed is the action of antennae and bristles, the appendages of species from the aquatic section Utricularia. Darwin (1875) suggested antennae and bristles function in concert as a funnel, enhancing the probability of a prey encounter by channelling them towards the trapdoor. This idea is particularly compelling taken in the context of trap insertion. Traps of all but two species in this section are born on comparatively short (for the genus) trap-stalks, inserted either on the capillary segments of the primary photosynthetic organs or less frequently in the angle between them (Taylor, 1989). When the plants are suspended in water, traps are not orientated in the same plane as the leaf segments, but are angled away from them. Traps are therefore positioned within three dimensional cells created by the capillary leaf segments, the area of which is effectively netted by antennae and bristles extending out from the trap door area.

An experiment by Meyer and Strickler (1979) demonstrated that the removal of either bristles or antennae from traps of one Section Utricularia species, *U. vulgaris*, resulted in reduced capture rates of one ubiquitous prey species, *Chydorus sphaericus*. *C. sphaericus* is a versatile cladoceran that can feed on bottom substrates and plant surfaces and is also found in the open water column (Vijverberg and Boersma, 1997). As a phytophilous grazer, *C. sphaericus* has two distinct feeding behaviours: i) Stationary filter feeding while grasping and ii) Traversing filamentous algal strands from tip to base. Meyers and Strickler (1979) observed *C. sphaericus* utilising the bristles and appendages of *U. vulgaris* in both ways, as well as stationary feeding on the trap bladder. Filament feeding behaviour on appendages positions *C. sphaericus* near the trap door and in contact with the trigger hairs. Manjarrés-Hernández et al (2006) found *U. foliosa* produced antennae 1.3 times longer, relative to bladder length, in low nitrogen environments. Their field-based study also assessed the role of habitat nutrient levels on the IIC. In so much as the appendages of aquatic species (antennae and bristle sets) function as lures for attracting prey, they too represent an investment in carnivory. The authors therefore concluded that plants may regulate the expression of these appendages in response to available nutrition, in a similar way to IIC.

1.3 Thesis content and structure

In this thesis I am concerned with further examination of aquatic appendage function and expression, with a particular focus on testing the prey attraction hypothesis. I address the following questions:

1. Do the appendages of species from Section Utricularia (antennae and bristles) increase the capture rate of prey with a range of feeding and locomotory behaviours, or are their effects limited to filament feeders such as *Chydorus sphaericus*? Put another way, is there any evidence that appendages really function in the sense Darwin (1875) envisaged, as a drift fence to funnel all potential, suitably-sized phytophilous prey towards the trap door, or are they acting as a kind of fishing lure targeting a specific set of feeding biases?

2. If appendages exploit specific behaviours, animals exhibiting these behaviours should be selectively trapped over others who do not. Is this the case?

3. Just how plastic is the expression of the 'morphologically conserved' (Taylor, 1989) antennae and bristles? If a filamentous appendage morphology has evolved in aquatic species, will the appendage expression of amphibious *Utricularia* species, with otherwise plastic bauplans, vary between aquatic and terrestrial phases? Could the appendages of *U. gibba* grown in a terrestrial phase more closely resemble subaquatic congeners than suspended aquatic species in the section? Do individuals (clones) of the same species exhibit consistent responses in appendage expression to changes in environmental conditions, both in direction and magnitude?

4. If appendages have been selected for their ability to enhance prey capture, they too represent a form of investment in carnivory. Therefore, is their expression affected by environmental factors, such as light and the availability of mineral nutrition, in a way similar to the structural investment in carnivory?

The remainder of this thesis is comprised of three data chapters (**Chapters 2-4**) written in the style of papers, followed by a general discussion (**Chapter 5**). The data chapters each present the results of an experiment (or series of experiments) that aim to address the questions raised above. However, there is no one-to-one correspondence between the research questions and the data chapters as, for logistical reasons, some experiments were used to address multiple questions. In **Chapter 2** I test the prey attraction hypothesis that antenna and bristles act as lures or guides. I repeat Meyer and Strickler's (1979) appendage ablation experiment with Cladoceran prey-species *Chydorus sphaericus*, using two different species from Section Utricularia, *U. gibba* and *U australis.* I then extend their work by testing the ability

of antennae and bristles to enhance the capture success of a range of aquatic microcrustaceans with differing feeding and locomotory behaviours. In **Chapter 3** I look for plasticity of appendage expression in response to environmental variation and persistent appendage variation between individuals (clones). I conduct a two-factor growth experiment with the amphibious *U. gibba* to assess the effects of light- and water-level on appendage expression. **Chapter 4** is again concerned with appendage plasticity in response to environmental variation. I conduct a second two-factor growth experiment, this time with *U. australis,* to assess the relative effects of two factors, feeding (prey consumption) and fertilisation (ambient nutrition), on plant growth and responses associated with investment in carnivory: IIC, appendage expression and the distribution of trap sizes within a leaf node. Here I also look for evidence of selective predation on microcrustacean prey-species. Finally, **Chapter 5** is a general discussion providing a synthesis and extension of the discussion points arising in **Chapters 2-4**, and suggesting future directions for research. To avoid repetition, the discussions in **Chapters 2-4** are truncated, with the broader implications of their results being deferred to **Chapter 5**.

Table 1.1. Carnivorous plant clades. Species numbers: *Aldrovanda* (Cross, 2012), *Byblis* (Lowrie and Robinson, 2013), *Genlisea* (Fleischmann, 2012b), Poales (McPherson, 2007), Sarraceniaceae (Ellison *et al.*, 2012), *Stylidium* (Darnowski *et al.*, 2006), *Utricularia* (Taylor, 1989; Fleischmann, 2012a),

Order	Family [number of genera/carnivorous genera]	Genus	Trap style	No. of species [species in genus]	Distribution
Poales	Bromeliaceae [/2]	*Brocchinia*	Pitfall	2 [19]	South America: Guiana Highlands
		Catopsis	Pitfall	1 [20]	Neotropics
Caryophyllales	Droseraceae [3/3]	*Drosera*	Adhesive	≥194	Cosmopolitan
		Dionaea	Snap trap	1	North America: Eastern US
		Aldrovanda	Snap trap	1	Old World, Australia
	Drosophyllaceae [1/1]	*Drosophyllum*	Adhesive	1	Western Mediterranean
	Dioncopyllaceae [3/1]	*Triphyophyllum*	Adhesive	1	Tropical Western Africa
	Nepenthaceae [1/1]	*Nepenthes*	Pitfall	c. 90*	Southeast Asia, India, Australia, Madagascar, Seychelles
Oxalidales	Cephalotaceae [1/1]	*Cephalotus*	Pitfall	1	Western Australia
Ericales	Roridulaceae [1/1]	*Roridula*	Adhesive	2	South Africa
	Sarraceniaceae [3/3]	*Darlingtonia*	Pitfall	1	North America western US
		Heliamphora	Pitfall	c. 18	Guiana Highlands: South America
		Sarracenia	Pitfall	11	North America: eastern US, Canada
Lamiales	Byblidaceae [1/1]	*Byblis*	Adhesive	8	Australia
	Lentibulariaceae [3/3]	*Pinguicula*	Adhesive	c. 100	Cosmopolitan
		Genlisea	Lobster-pot	≥ 32	Tropical Africa, Neotropics
		Utricularia	Suction Trap	≥ 228	Cosmopolitan
	Plantaginaceae	*Philcoxia*	flypaper	3	Brazil
Suspected of carnivory					
Poales	Eriocaulaceae	*Paepalanthus*	Pitfall	1 [300-400]	South America: Guiana Highlands
Asterales	Stylidaceae	*Stylidium*	Flypaper	? [>220]	Australia, Southeast Asia

Table 1.2. Growth habit and trap features (including appendage characters) of *Utricularia* species from **Figs. 3-10.** Bracketed trap-length ranges from Taylor (1989) and Thor (1988).

Section [species in section]	Species	Habit	Trap insertion	Trap dimorphism	Appendage types	di- or polymorphic appendages	Filiform appendages	Trap length mm	Location
Pleiochasia [40]	*U. antennifera*	T	P/N/I	0	Dr/L/V	0	1	1.03 (0.5-1.0)	Australia
	U. dichotoma	T	P/N/I	0	Dr/L/V	1 (± V)	0	1.59 (1.0-5.0)	Australia
	U. georgei	T	P/N/I	0	Dr/L/V	1 (±V, Dr shape)	0	1.30 (0.8-2.0)	Australia
	U. hamiltonii	AQ	P/N	0	Dr/L	0	1	2.7 (2.0-3.0)	Australia
	U. holtzei	SubA/T	P/N	0	Dr/L	0	1	1.55 (1.0-1.5)	Australia
	U. leptorhyncha	T	P/I	1	Dr/L/V	0,0	0	1.56 (0.5-0.8; c. 1.0)	Australia
	U. tubulata	SA	N	0	Dr/L	0	1	1.91(1.0-2.2)	Australia
Enskide [2]	*U. fulva*	T/SubA	R/L	0	Db	1(± papillose)	0	0.60 (0.3-0.5)	Australia
Orchidioides [16]	*U. reniformis*	T/E	I	0	PD	0	0	1.27 (0.7-1.5)	Brazil
Calpidisca [10]	*U. bisquamata*	T	R/I/L	0	Dt/G	0	0	0.58 (1.0-1.5)	Southern Africa
	U. livida	T	R/I/L	0	Dt/G	0	0	1.31(1.0-2.0)	Africa, Mexico
Foliosa [13]	*U. longifolia*	T/L	R/I/L	0	PD	0	0	0.84 (1.0-1.5)	Brazil
	U. praelonga	T	S	0	PD	0	0	1.10 (c. 1.0)	South America
Nelipus [3]	*U. limosa*	T/SubA	I/L	0	PD	0	0	0.92 (0.5-1.2)	Australia, Southeast Asia
Lecticula [2]	*U. resupinata*	SubA	I/L	±	A/B	1(±B),0	1	0.86 (c. 0.5; c. 1.0)	North and L. America
Australes [3]	*U. delicatula*	T	R/I/L	0	Dr	0	0	0.66 (0.5-0.7)	New Zealand
	U. lateriflora	T	R/I/L	0	Dr	0	0	0.49 (0.5-0.7)	Australia
Utricularia [37]	*U. aurea*	AQ	N/Lo	1	A	1(± A)	1	0.87 (1.0-4.0)	Asia, Australia
	U. bremii	AQ/SQ	Lo	0	A/B	0	1	1.42 (1.0-2.0)	Europe

Section [species in section]	Species	Habit	Trap insertion	Trap dimorphism	Appendage types	di- or polymorphic appendages	Filiform appendages	Trap length mm	Location
	U. foliosa	SA	Lo	0	A	1 (± A)	1	0.90 (1.0-2.0)	Africa, Madagascar, America
	U. stellaris	SA	Lo	0	A/B	1 (± A,± B)	1	0.79 (1.0-3.0)	Africa, Madagascar, Asia, Australia
	U. tenuicaulis	SA	N/Lo	1	A/B	1 (± A,± B),0	1,1	1.03 (0.5-2.5)	Japan
	U. stygia	AQ			A/B		1	1.92 (1.0-4.0)	Europe, North America
Vesiculina [3]	*U. purpurea*	SA	Lo	0	N	0	0	0.78 (1.0-2.0)	North and Central America

Growth habit: AQ: affixed aquatic; E: epiphyte; SA: suspended aquatic; SubA: sub-aquatic (seasonal inundation); T: terrestrial. **Trap insertion**: I: internode; N: node; P: peduncle base; R: rhizoid; L: leaf; Lo: leaf-like organ. The two leaf designations are used to distinguish between classically expressed leaf modules and the modified stolons of some aquatics. **Appendage types**: A: antennae; B: bristles; Db: dorsal bulge; Dr: dorsal rostrum; Dt: truncated dorsal; G: comb-like rows of glands; L: lateral projections; N: none or barely visible rudiments; PD: paired dorsal; V: ventral wings. Note: antennae are treated separately, but are a form of paired dorsal appendages. **Trap dimorphism**: where other aspects of trap morphology, notably bladder shape, fall into two distinct types, and are both expressed simultaneously within an individual of a species. In the case of trap dimorphism, appendage variability and trap length are specified for both types.

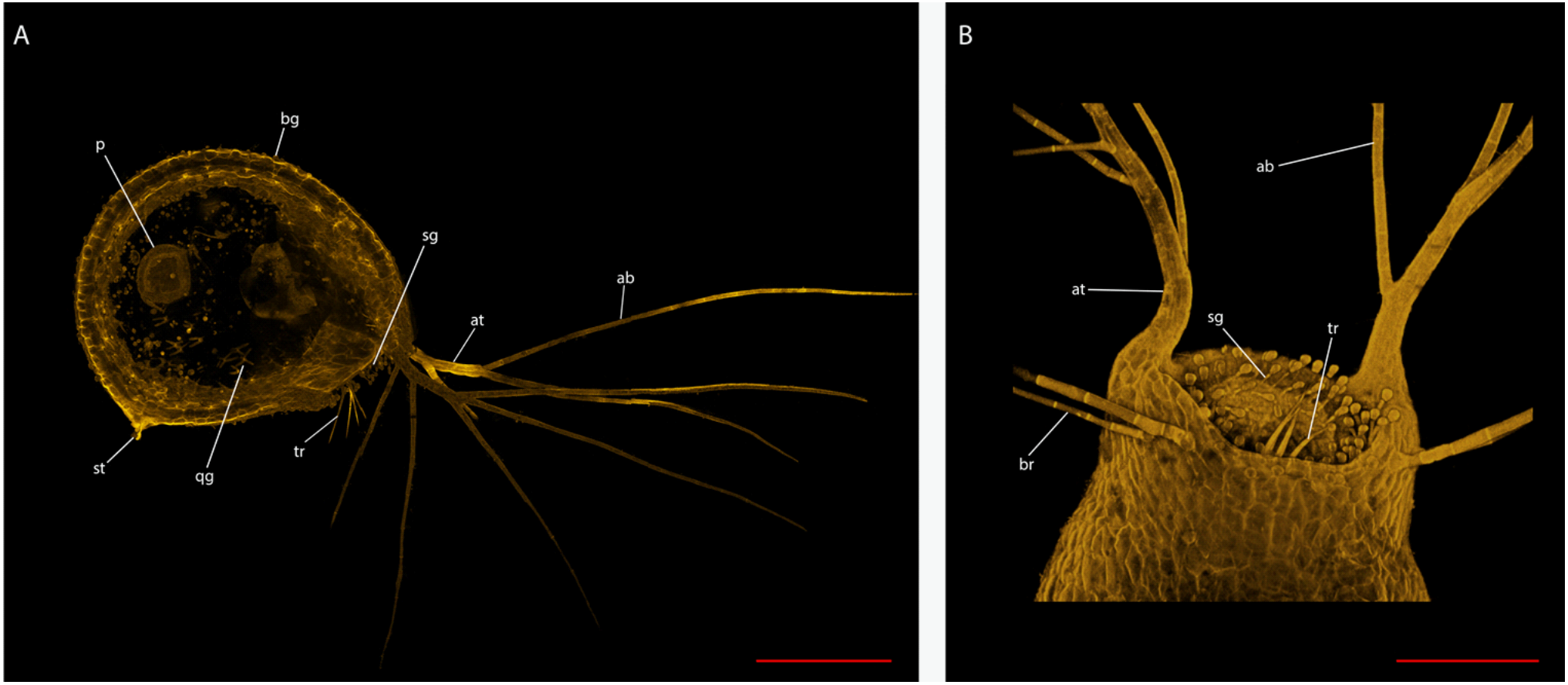

Fig. 1.1. Images of *Utricularia* traps generated by laser scanning confocal microscopy. **A:** *U. breviscapa* (lateral view), composite of optical sections showing internal and external bladder morphology. Scale = 500 µm; **B:** *U. minor* (ventral view), orthographic projection of trapdoor area. Scale = 200 µm. Both species are aquatics from the infra-generic section Utricularia, having paired dorsal appendages called antenna. (at) antenna trunk, (ab) antenna branch, (bg) button gland, (br) supplementary bristle, (p) cladoceran prey-carapace, (qg) quadrifid gland, (sg) stalked gland, (st) stalk, (tr) trigger hair. Bifid glands not shown.

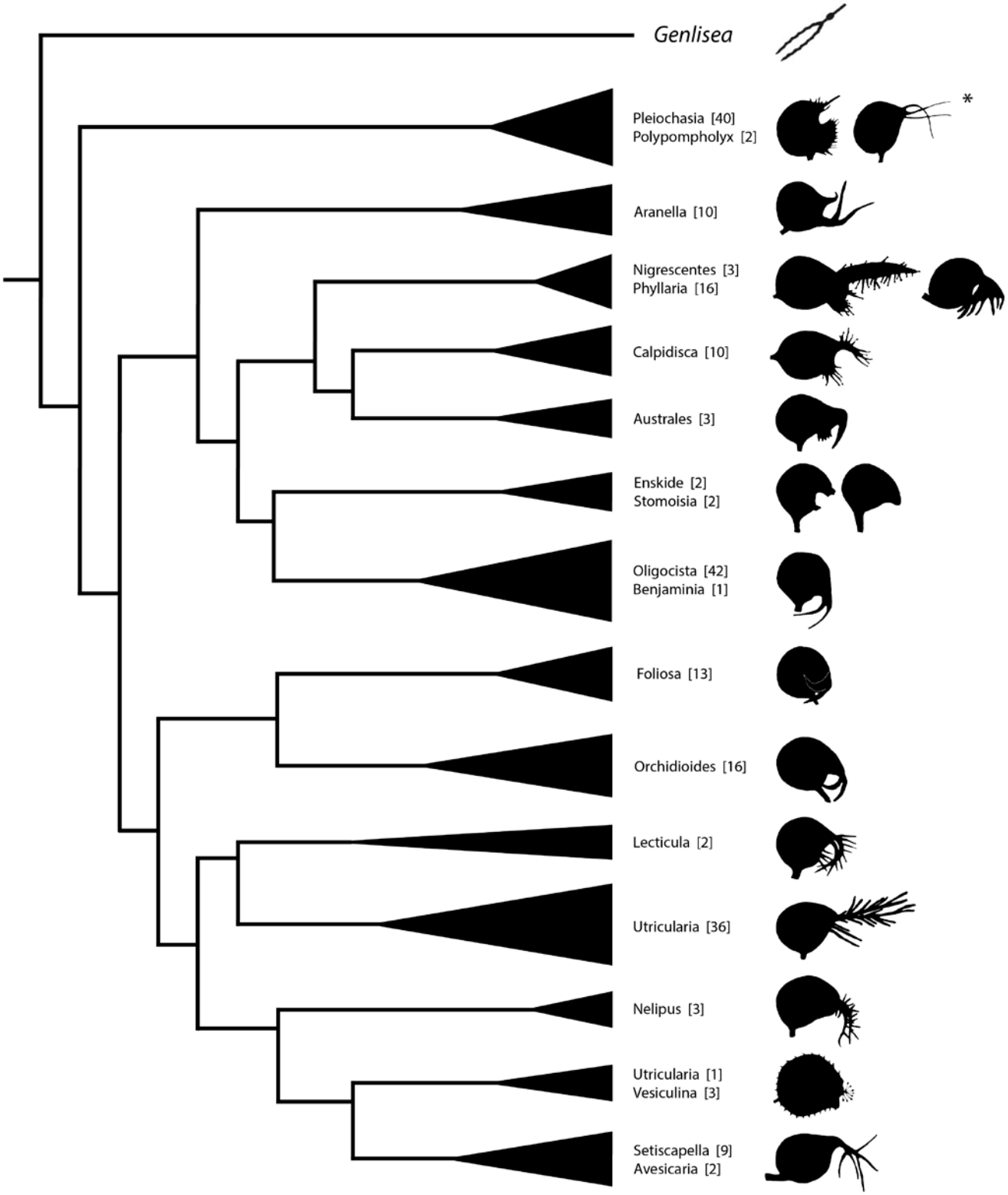

Fig. 1.2. Diagrammatic representation of *Utricularia* phylogeny showing 21 of the 34 infra-generic sections. The carnivorous genus *Genlisea*, sister to *Utricularia*, is included as an outgroup. Tree modified from Jobson *et al.* (2003). Square brackets show number of species in each section; triangle sizes are only relative approximations. Silhouettes are generalised representations of lineage-specific trap forms, presented as lateral orthographic projections emphasising variation in appendages. Trap silhouettes are derived from drawings in Taylor (1989) and are not to scale. Where two sections are combined into a single clade and traps from both are illustrated, the silhouette on left corresponds to the first section listed. Section Utricularia *sensu* Taylor (1989) is polyphyletic. * Silhouettes show extremes of intra- and inter-specific appendage expression from the very variable section Pleiochasia. Traps from section Polypompholyx not shown.

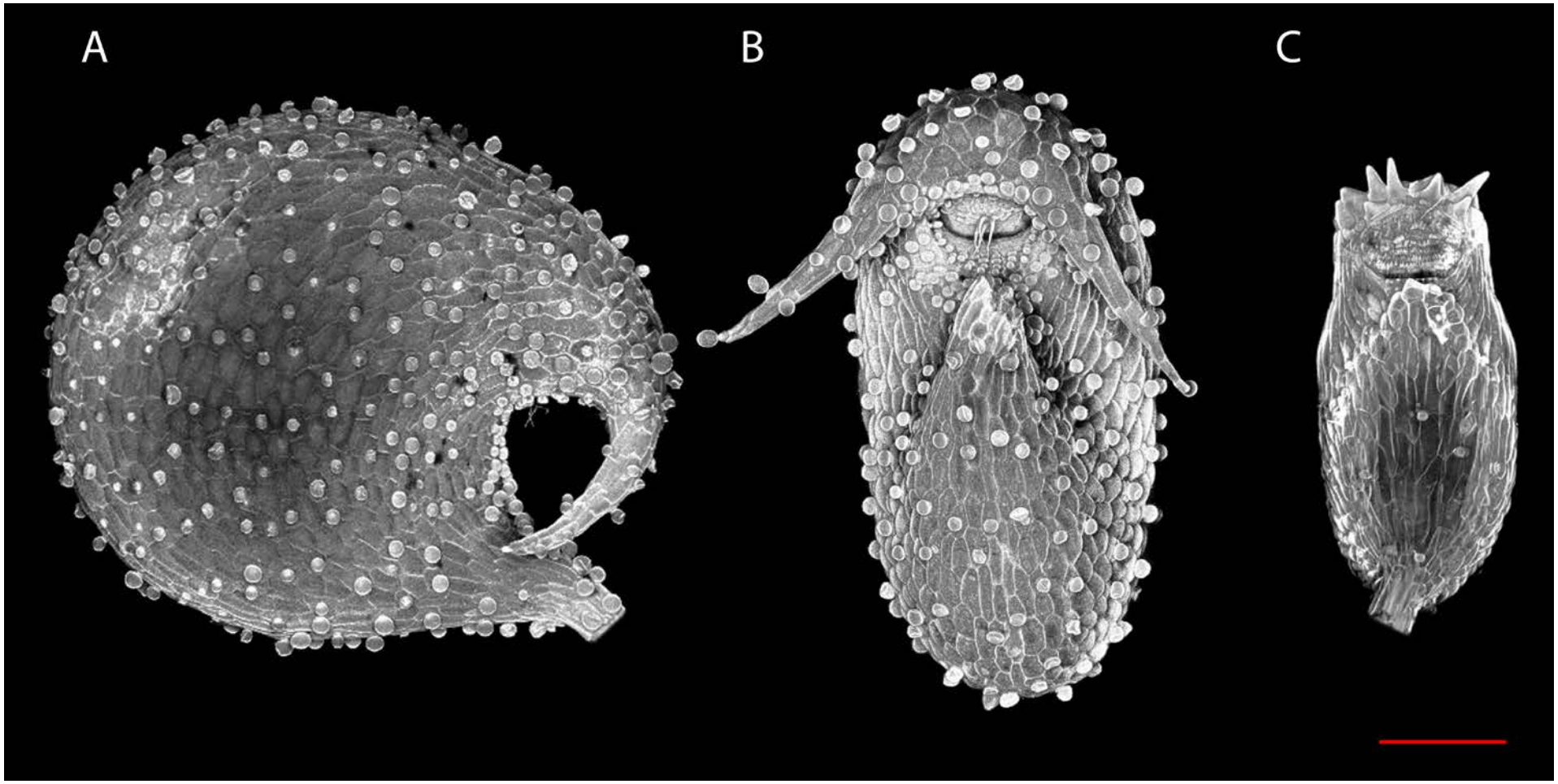

Fig. 1.3. Orthographic projections of traps. **A-B:** Section Orchidioides, *U. reniformis* (lateral and ventral views); **C:** Section Enskide, *U. fulva* (ventral view). Scale = 200 μm.

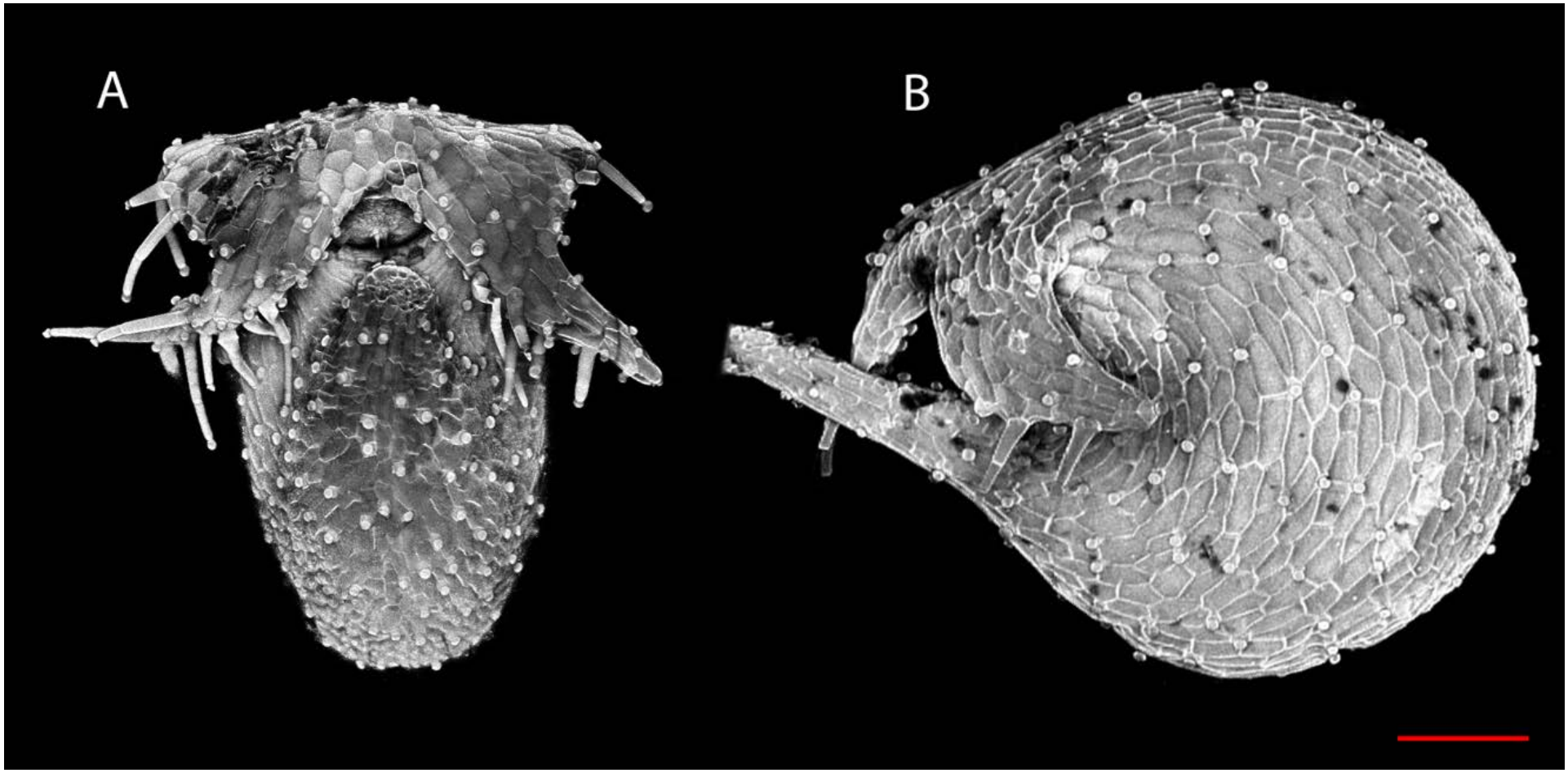

Fig. 1.4. Orthographic projections of traps from Section Foliosa. **A:** *U. longifolia* (ventral view); **B:** *U. praelonga* (lateral view). Scale = 200 μm.

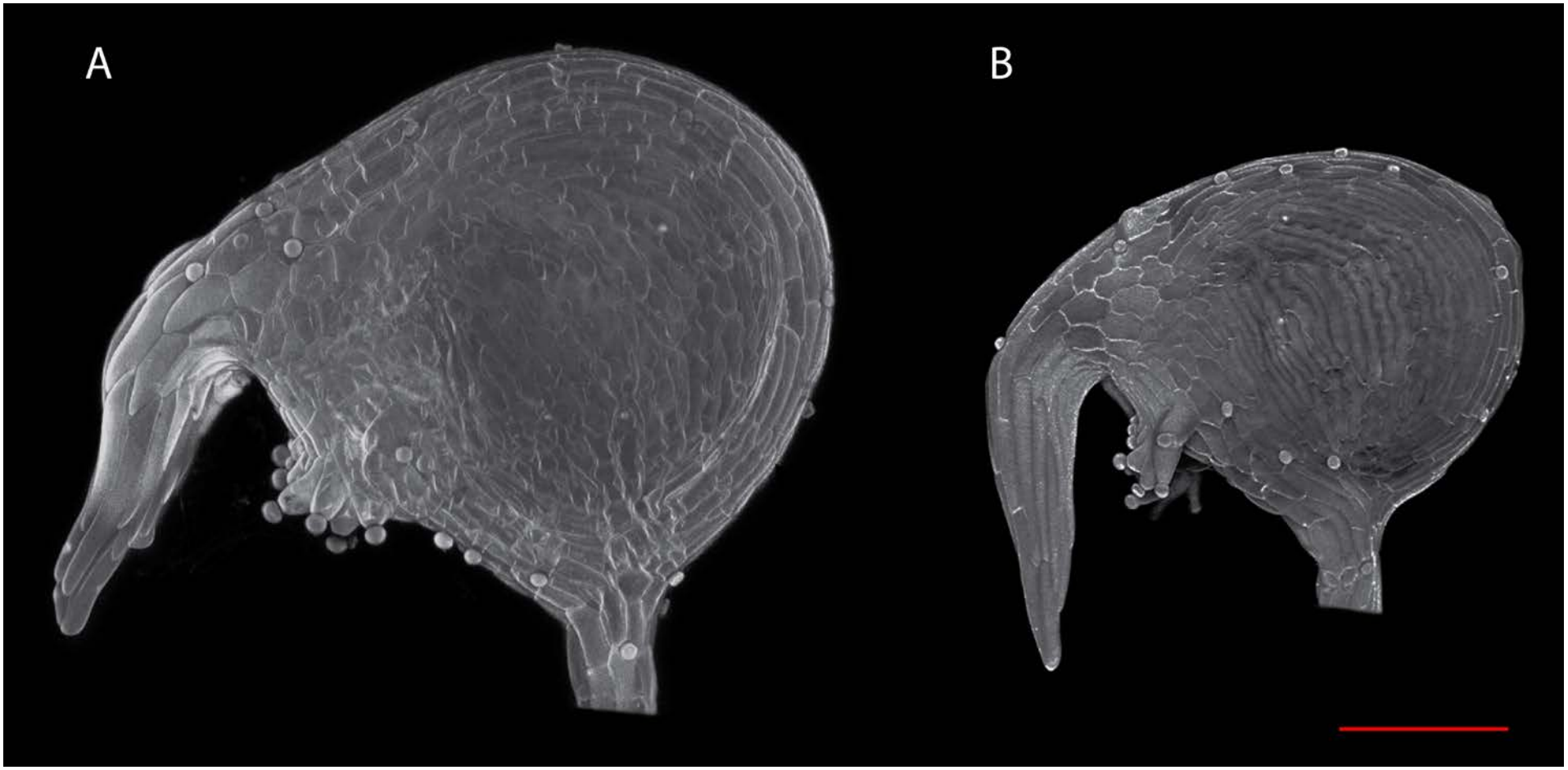

Fig. 1.5. Lateral orthographic projections of traps from Section Australes. **A:** *U. delicatula*; **B:** *U. lateriflora*. Scale bar = 200 μm.

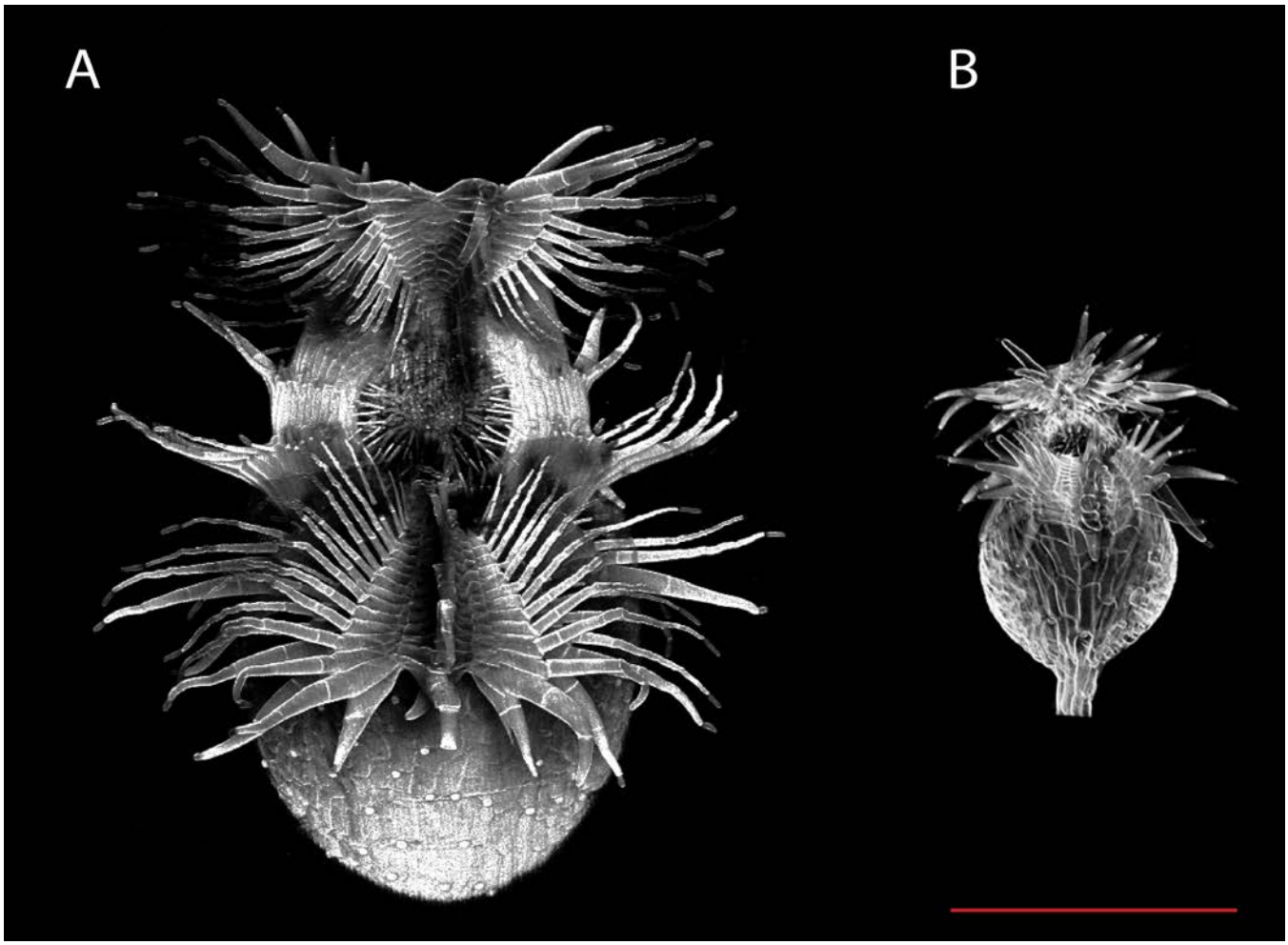

Fig. 1.6. Ventral orthographic projections of traps from Section Calpidisca. **A:** *U. livida*; **B:** *U. bisquamata*. Scale bar = 500µm.

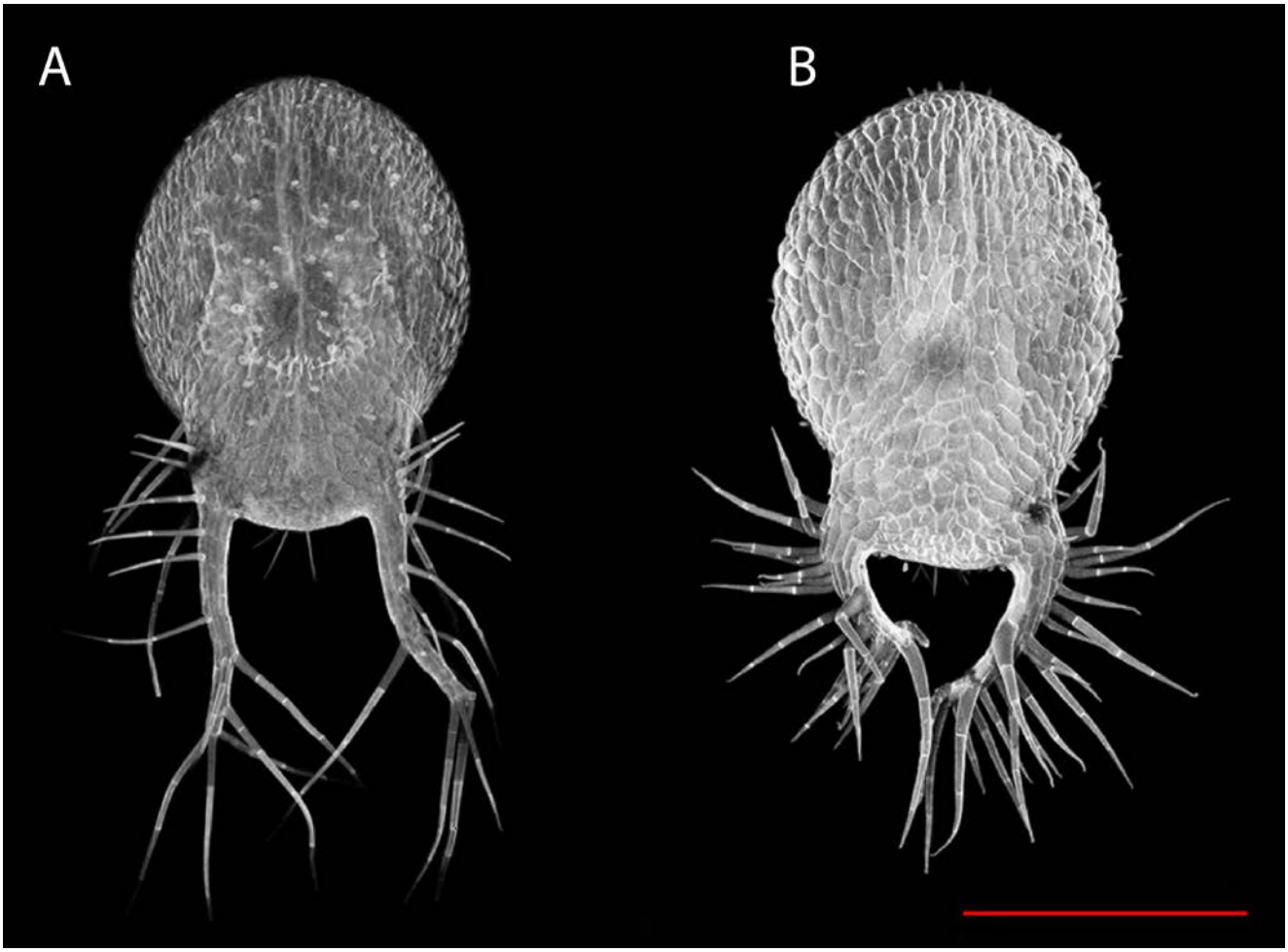

Fig. 1.7. Dorsal orthographic projections of traps. **A:** Section Lecticula, *U. resupinata*; **B:** Section Nelipus, *U. limosa*. Scale bar = 500 µm.

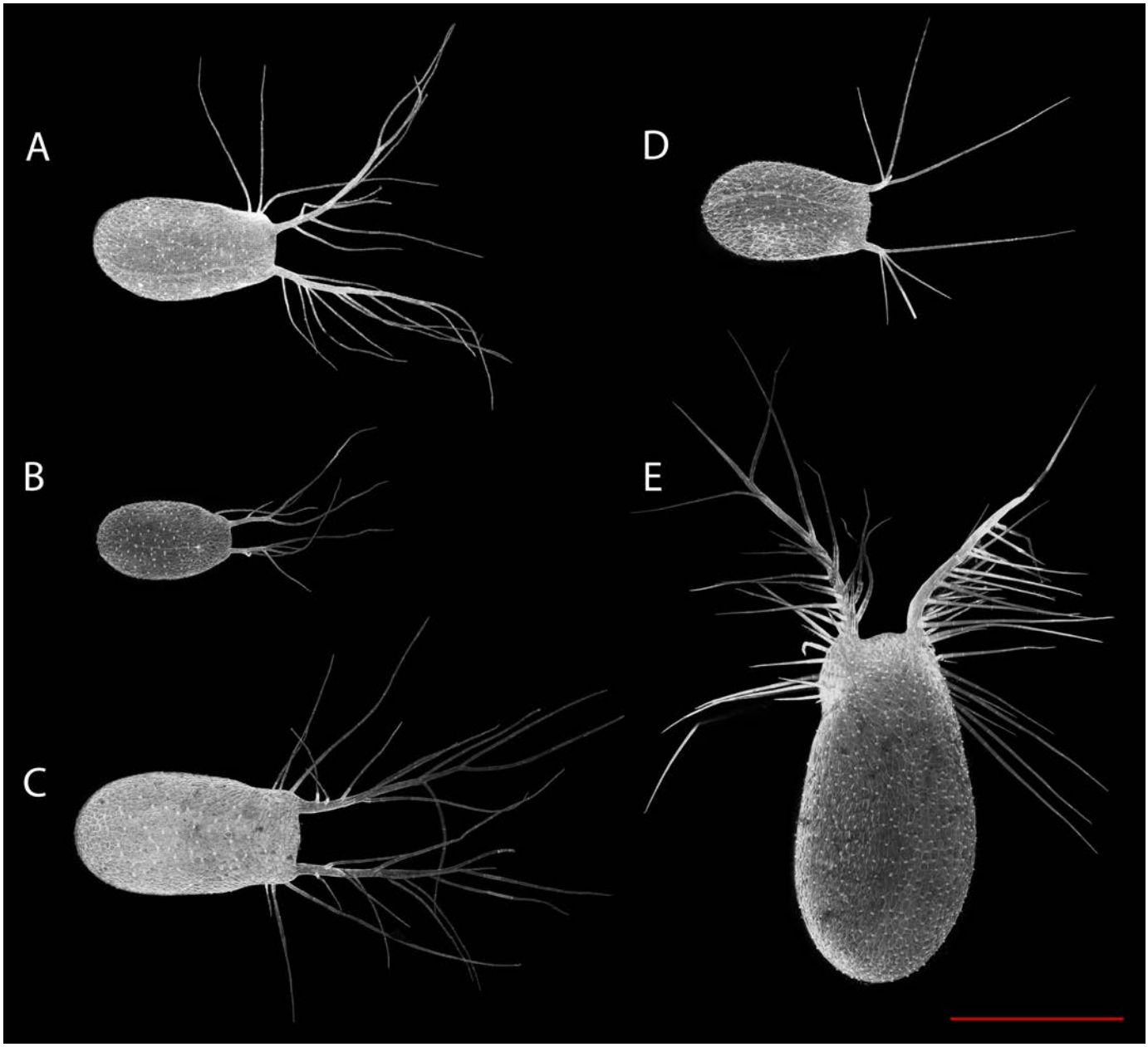

Fig. 1.8. Dorsal orthographic projections of traps from Section Utricularia. **A:** *U. tenuicaulis*; **B:** *U. stellaris*; **C:** *U. bremii*; **D:** *U. foliosa*; **E:** *U. Stygia*. Scale = 1 mm.

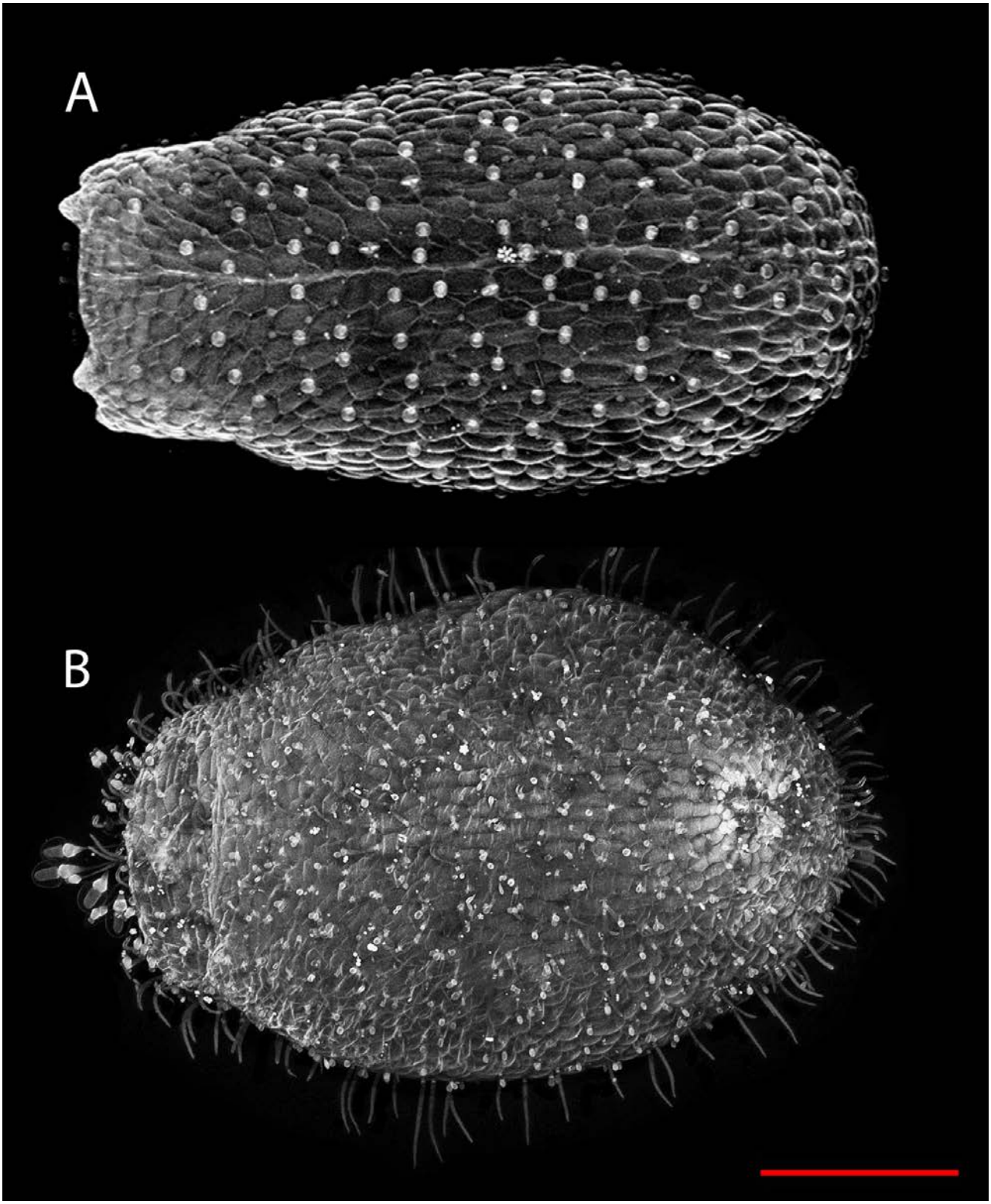

Fig. 1.9. Orthographic projections of traps. **A:** Section Utricularia, *U. aurea* (dorsal view); **B:** Section Vesiculina, *U. purpurea* (ventral view). The appendages of *U. aurea* have polymorphic expression, ranging from none to dendriform antennae. Scale = 500 μm.

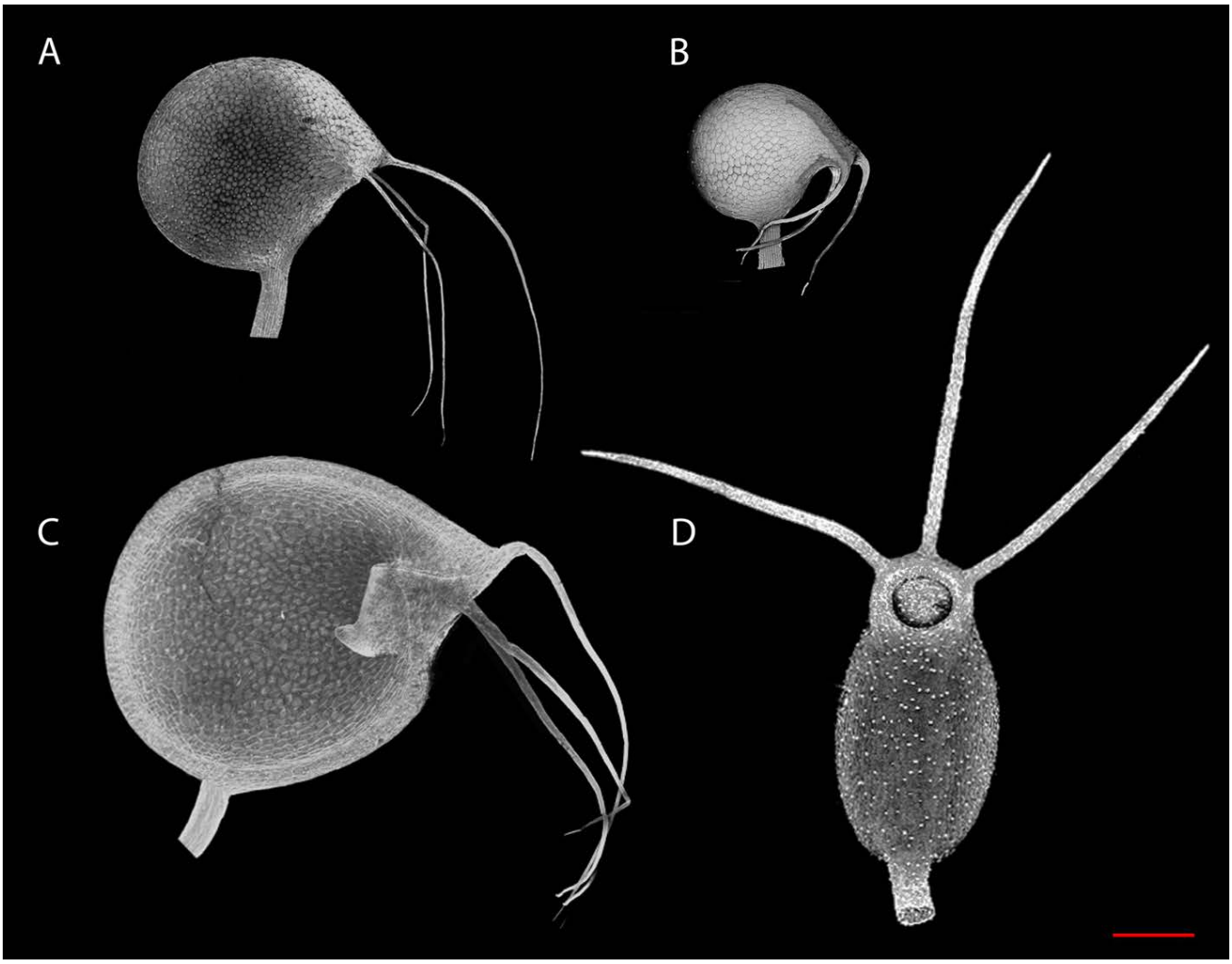

Fig. 1.10. Orthographic projections of traps from Section Pleiochasia. Lateral views, **A:** *U. holtzei*; **B:** *U. antennifera*; **C:** *U. hamiltonii*; **D:** *U. tubulata* (ventral view). Scale = 500 μm.

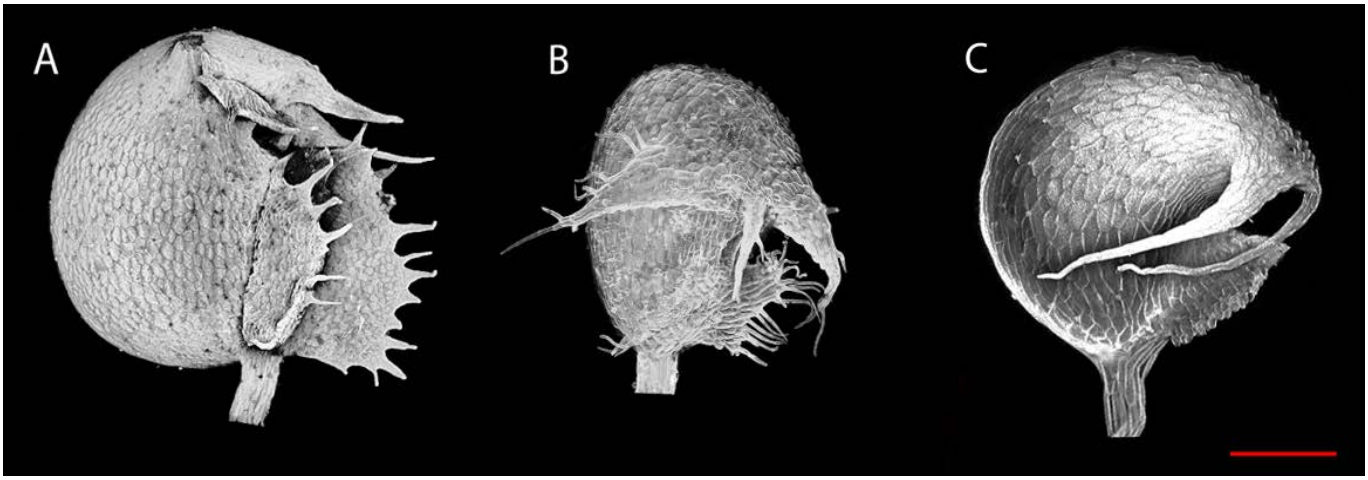

Fig. 1.11. Orthographic projections of traps from Section Pleiochasia. Latero-ventral views, **A:** *U. dichotoma*; **B:** *U. georgei;* lateral view; **C:** *U. leptorhyncha*. Scale = 500 μm

2. Do appendages enhance prey capture? The role of antennae and bristles in two aquatic *Utricularia* species

2.1 Introduction

Nutrition from prey is an important supplement to the poor nutrient availability of carnivorous plant habitats, assisting in plant growth and reproduction (Darwin, 1875; Givnish *et al.*, 1984; Wilson, 1985; Thum, 1988; Friday and Quarmby, 1994; Zamora *et al.*, 1997; Otto, 1999; Adamec, 2002; Englund and Harms, 2003; Lenihan and Schultz, 2014), and allows botanical carnivores to compete effectively with non-carnivorous plants (Givnish *et al.*, 1984). As plant carnivores are extreme ambush predators and are not able to actively forage or relocate in response to prey abundance, prey attraction mechanisms should evolve (Guisande *et al.*, 2007; Ellison and Gotelli, 2009). For carnivorous plants, the trap and the trapping organism are one; traps are aspects of carnivorous plant bauplans. Therefore, to be most effective, attraction mechanisms are located close to point of capture, the trapping organs themselves, and prey attraction should be an important driver of trap structure. Carnivorous plant traps have evolved a range of prey attraction strategies including floral-scent mimicry and UV patterning that exploit insect preferences (Joel *et al.*, 1985; Bennet and Ellison, 2009; Jürgens *et al.*, 2009).

Utricularia is a carnivorous plant genus whose members have evolved epiphytic, water-logged terrestrial and aquatic growth habits. The terrestrial habit in the genus is ancestral with aquatic invasions having occurred independently at least four times (Jobson and Albert, 2002; Jobson *et al.*, 2003; Müller and Borsch, 2005). All *Utricularia* spp. catch prey with complex, mechanically activated suction traps derived from leaves (Juniper *et al.*, 1989; Lloyd, 2008). Meiofauna contacting trigger hairs that protrude from the exterior trapdoor surface mechanically activate the trap (Adamec, 2012a). The trapdoor buckles inwards and the inrushing water draws the prey into the bladder (Singh *et al.*, 2011; Vincent and Marmottant, 2011; Vincent *et al.*, 2011a). Traps also fire spontaneously when the negative pressure gradient is at its greatest (Adamec, 2011c; Vincent *et al.*, 2011b).

While *Utricularia* are generalist predators with diets limited by the availability of suitably sized prey, they also exhibit some selectivity. Prey Characteristics such as mobility, size and habitat use being important determinants of diet (Harms, 1999; Mette *et al.*, 2000; Gordon and Pacheco, 2007). Microcrustacea are common prey (Skutch, 1928; Mette *et al.*, 2000; Jobson and Morris, 2001; Harms, 2002; Guiral and Rougier, 2007; Martens and Grabow, 2011). Phytophilous species are more frequently trapped than highly mobile, planktonic ones (Harms, 1999). Larger zooplankton (cladocerans and copepods) are selected over smaller ones, depending on their seasonal availability (Guiral and Rougier, 2007). Some field data and prey selection experiments show cyclopoid copepods are trapped more frequently than cladocerans when both taxa are present (Harms, 1999; Harms and Johansson, 2000; Guiral and Rougier, 2007). Other studies show the opposite (Mette *et al.*, 2000; Richards, 2001). Phytoplankton may also provide an important source of nutrition (Richards, 2001) and traps permanently

house live communities of unspecialised commensal organisms. (Mette *et al.*, 2000; Richards, 2001; Gordon and Pacheco, 2007; Peroutka *et al.*, 2008; Alkhalaf *et al.*, 2009; Sirová *et al.*, 2009)

The exterior of the trap entrance is surrounded by trichomes and protuberances called appendages whose morphology varies greatly among species (Taylor, 1989; Reifenrath *et al.*, 2006; **Section 1.3.4**). The function of appendages is currently under investigation and may serve different purposes depending on ecological type. Species in the wholly aquatic, infrageneric section Utricularia (Taylor, 1989; Jobson *et al.*, 2003; Albert *et al.*, 2010; Reut and Jobson, 2010; Fleischmann, 2012a) have paired, branched dorsal appendages, named antennae which are supplemented by further lateral and sometimes dorsal bristles (**Fig. 2.1**). Darwin (1875) hypothesised that these appendages acted in concert to enhance capture rates by funnelling prey towards the trapdoor, as antennae and bristles effectively net the area around the trap (Taylor, 1989).

Meyer and Strickler (1979) demonstrated bristle and antennae removal reduced capture rates of one ubiquitous cladoceran prey species, *Chydorus sphaericus* O.F. Müller, 1785 by the suspended aquatic *U. vulgaris*. *C. sphaericus* has two phytophilous feeding modes: Stationary filter feeding while grasping, and traversing filamentous algal strands from tip to base. Meyers and Strickler (1979) observed *C. sphaericus* feeding on the bristles and appendages of *U. vulgaris* in both ways, as well as stationary feeding on the trap bladder. Filament feeding on antennae often brings *C. sphaericus* in contact with the trigger hairs. While these results provide support for the thesis that aquatic appendages have a role in enhancing prey capture, this experiment has never been replicated with other prey species employing differing feeding and locomotory behaviours. It is therefore unclear to what degree appendages may increase trapping efficiency when functioning as a funnel, defined here in the stricter sense as exploiting locomotory behaviour, as opposed to lures that exploit a specific feeding behaviour employed by a single prey species.

In this chapter I address the following question: i) Do the appendages of species from Section Utricularia (antennae and bristles) increase the capture rate of prey with a range of feeding and locomotory behaviours, or are their effects limited to filament feeders such as *Chydorus sphaericus*? Put another way, is there any evidence that appendages really function in the sense Darwin (1875) envisaged, as a drift fence to funnel all potential, suitably-sized phytophilous prey towards the trap door, or are they acting as a kind of fishing lure targeting a specific set of feeding biases? ii) Do section Utricularia species (with conserved appendage morphology) exhibit a uniform response to appendage removal? ii) Is context important? Does the surrounding leaf matrix influence capture enhancement by aquatic appendages?

I make a more comprehensive examination of trap appendage function in section *Utricularia,* assessing the role of aquatic appendages in prey capture with a series of experimental manipulations using two section *Utricularia* species, the suspended aquatic *U. australis* and the affixed aquatic (and amphibious) *U. gibba*. In addition to conducting new experiments with *C. sphaericus*, I tested the more general hypothesis of the efficacy of the appendages as a funnel using three other microcrustacean taxa, the phytophilous and sediment foraging ostracod *Cypridopsis vidua* O.F. Müller, 1785, the pelagic cladoceran *Scapholebris kingi* G. O. Sars, 1888 and two predatory cyclopoid copepod species, *Acanthocyclops robustus* G.O. Sars, 1863 and *Mesocyclops* cf. *leuckarti* Claus, 1857. I also tested for an interactive effect between the surrounding leaf matrix and aquatic appendages of *u. australis* on prey-capture enhancement of *A. robustus* and *Mesocyclops* cf. *leuckarti*.

2.2 Materials & Methods

2.2.1 Plants

I conducted experiments on mature lateral traps of two aquatic species from section *Utricularia*, *Utricularia australis* and *U. gibba. U. australis* is a rhizoidless, freely-suspended macrophyte with filamentous, multi lobed leaves organised in whorls on non-differentiated monomorphic shoots. During the growing season shoots have continuous, very rapid, apical growth with corresponding basal decay (Taylor, 1989; Adamec and Kovářová, 2006). Traps are dimorphic with i) Numerous lateral traps (0.5-2.5 mm long) borne on leaves from capillary leaf segments (Taylor, 1989) or in place of a leaf filament in a dichotomy (depending on perspective), with a single leaf incorporating a range of trap sizes while ii) smaller, basal traps, with truncated antennal trunks, uniform in size, grow in the angle between the shoot and primary leaf segment (Taylor, 1989) (**Fig. 2.2A**). *U. gibba* is an affixed aquatic. Groups of highly ramified individuals, densely intertwined, form mats of vegetation (ramified colonies). *U. gibba* grows more robustly on and just below substrate as an amphibious plant, but can grow as a suspended aquatic. Taylor (1989) refers to this suspended form as sterile, as flowering will not normally take place unless *U. gibba* is anchored, although flowering can occur in deeper water when plants use floating mats of detritus as scaffolding. I used this suspended form in all experiments. The traps of *U. gibba* are uniform in shape, being of the lateral kind found in *U. australis*, although less variable in size (1-2.5 mm long). They arise on sparsely lobed leaves singularly, in pairs or occasionally more numerous (Taylor, 1989; Guiral and Rougier, 2007) (**Fig. 2.2B**). Traps of both species share the branched, filiform antennae characteristic of section *Utricularia,* although *U. gibba* has considerably more supplementary bristles (Taylor, 1989; Guiral and Rougier, 2007) including a set arising on the dorsal margin of the trap door which is absent from *U. australis.*

I conducted this experiment using one clone each of *U. australis* and *U. gibba.* Stolons of both species were collected from the Upper North Island, New Zealand. Prior to experimentation plant material used in this experiment was pre-cultivated from the field collected plants for no less than 12 months in an indoor, rooftop greenhouse at the School of Biological Sciences, University of Auckland. *U. gibba* was cultivated in a 60 l plastic tank with a water column of 400 mm. The tank contained one clone derived from a single stolon fragment. Multiple stolon lengths of *U. australis* were to produce a single cultivation in a 300 l glass aquarium, filled almost to the brim. As the *U. australis* stolons used were collected from a single population and *U. australis* seldom flowers and produces sterile seed (Taylor, 1989), this cultivation was assumed to be a single clone. All tanks/aquarium contained a 30 mm base layer of 1:1 peat moss/sand mix. Water depth was maintained through the addition of deionised water, steeped in identical substrate and filtered prior to addition. The pH within the tanks varied between 4.8 and 6.8. Light in the greenhouse was approximately 40% of the outside light in the open. All traps used in the experiments were positioned near the base of the leaf, arising either from primary or initial secondary leaf filaments (see **Chapter One** for detail on leaf anatomy). For these traps, the two species had different, non-overlapping size distributions, with *U. gibba* having smaller traps. Mean trap bladder lengths were determined from a randomly selected subsample of the traps used in the experiments: *U. gibba* 1.32 mm (0.97–1.69 mm, n= 23), *U. australis* 2.03 mm (1.81–2.38 mm, n=22).

2.2.2 Prey

Five species of micro-crustaceans from three orders were in the appendage removal experiments, the cladoceran *C. sphaericus* (Chydoridae, Diplostraca), the ostracod *C. vidua* (Cyprididae, Podocopida), the daphnid *S. kingi* (Daphniidae, Diplostraca) and two cyclopoid copepods, *A. robustus* (Cyclopidae, Cyclopoida) and *M.* cf. *leuckarti* (Cyclopidae, Cyclopoida). All prey species used are of a cosmopolitan distribution and were cultivated in the rooftop greenhouse of the School of Biological Sciences, University of Auckland (**Fig. 2.3**).

C. sphaericus and *C. vidua* are both phytophilous and benthic feeding but unlike *C. sphaericus, C. vidua* does not filament feed (Roca and Danielopol, 1991). While adept at penetrating the interstitial spaces of substrate, *C. vidua* preferentially seeks out and scrapes periphyton on plant surfaces (Roca *et al.*, 1993). *S. kingi* is associated with weedy littoral; areas but is an epineustic feeder, applying its ventral side to the water surface and moving about upside-down (Chapman *et al.*, 2011). *A. robustus* and *M.* cf. *leuckarti* are both predatory, similar sized, cyclopoid copepods, occupying a wide range of habitats in sympatry, primarily littoral and benthic (Maier, 1990).

C. sphaericus, A. robustus and *M.* cf. *leuckarti* have previously been recorded in content surveys of field collected traps (Meyers and Strickler, 1979; Andrikovics, 1988; Mette *et al.*, 2000; Kurbatova and Yershov, 2009). Although *C. vidua* has never been specifically identified, ostracods are preyed upon by aquatic *Utricularia* (Guisande *et al.*, 2004; Walker, 2004; Kurbatova and Yershov, 2009) but accurate identification beyond class is infrequent. *Scapholeberis mucronata,* a cladoceran with similar morphology and behaviour to *S. kingi* has been surveyed with *Utricularia* (Pokyi; Marazanof, 1967; Mahoney *et al.*, 1990; Kuczyńska-Kippen and Nagengast, 2006)

2.2.3 Antennae and bristle removal

I performed a series of three appendage manipulation experiments in the rooftop greenhouse at of the School of Biological Sciences, University of Auckland. All experiments were conducted Nov – Jan (summer). I tested the effect of appendage (bristles and antenna) ablation on the following combinations of aquatic *Utricularia* and prey species: 1) *U. gibba* and *U. australis* with *C. sphaericus*, to confirm the role of appendages in enhancing the capture rate of phytophilous, epibiont grazers and assess the effect of trap size on the ability to enhance capture probability, 2) *U. gibba* and *U. australis,* with *C. sphaericus, C. vidua , S. kingi* to compare the effects of differing prey locomotory and feeding behaviour on capture probability, and 3) *U. australis* with a mixture of *A. robustus* and *M.* cf. *leuckarti* to test whether appendages affected the rate of capture of phytophilous but predatory animals, and to test if appendages interact with the matrix of leaf filaments surrounding traps to enhance capture of a prey species.

In all experiments I pipetted animals into 25 ml cell culture dishes (60x15mm) under a stereoscope. Dishes were filled with 20 ml of plant cultivation water filtered through 150 µm nylon mesh. I used the trap door sizes of randomly selected exemplar traps for both *Utriculaia* species to help select only animals small enough to be successfully trapped. The exemplar traps were taken from the same stolon-position as those selected for ablation (specified above), from additional randomly selected stolon lengths, at nodes as specified for each experiment (below). The size distributions of animals in the *U. australis* treatments were larger than those in *U. gibba* treatments. The number of animals varied among experiments and between each dish (see below for counts for each experiment). Animals were occasionally added during the experiment by way of live birth as it was not always feasible to use only non-gravid animals. All dishes were inoculated to the point of saturation, however, so animals remained available for capture throughout the duration of the experiment (see below for ranges in the number of animals per dish for each experiment).

I removed trap-bearing plant modules (the type of modules, the number of traps per dish and stolon positions from which that traps were derived varied among experiments, for details see below) from

cultivation tanks to an additional set of filtered cultivation water filled 25 ml culture dishes and randomly allocated each dish to a treatment. To reduce the likelihood of air bubbles forming in the traps I moved plant material using a cut-down 3 ml Pasteur pipette. Stolon fragments and excised leaves are relatively autonomous organs able to survive for weeks in ambient water with traps continuing to function for at least four days (Sirová *et al.*, 2003). The use of multiple fragments allowed for up to ten traps per treatment while ensuring they were of a similar age, as Friday (1989) showed trapping efficiency declines rapidly in older traps. The position of the trap bearing node on the stolon, in relation to the growth tip, served as a crude approximation for trap age. As *U. australis* and *U. gibba* were each represented by a single clone, for both species I considered traps taken from different stolon lengths to come from a single plant.

I ablated appendages from submerged traps under a stereoscope (Leica) between x10 to x25 magnifications with a pair of 2mm cutting edge spring scissors (Vannass). Micro-surgical ablation of appendages does not noticeably affect trap function (Meyers and Strickler, 1979), but care is still required not to damage trigger hairs, the trapdoor, or its margin. After 24 hours I checked dishes and replaced any dead or injured animals (< 5% in all cases). I also replaced any traps not obviously resetting (< 1%). Finally, between 10:00 and 14:00 hrs, I relocated the plant fragments to the dishes containing prey, beginning the experiment. Traps were exposed to prey for 24 hrs, which for all experiments included a light period of c.a. 15 h. Water temperatures in the culture dishes across all three experiments ranged between 20.5 – 26.2 °C (only one temperature reading taken per experiment, from a randomly selected culture dish), reflecting the ambient temperature of the greenhouse on the day each experiment was conducted. At the termination of the experiment I added c. 2 ml of 98% ethanol to all treatments and recorded both captured and uncaptured animals, pooling results for each dish.

Experiment one: *U. gibba and U. australis* with *C. sphaericus.*

Treatments consisted of 20-30 *C. sphaericus* and 10 traps of either, *U. gibba* or *U. australis*. I subjected traps attached to shoot fragments to one of four appendage manipulations: i) All appendages removed, ii) Antennae removed, iii) Bristles removed, iv) All appendages intact (control). I replicated each treatment block six times (6x2x4=48 dishes in total). For the *U. gibba* treatments I used 10 traps attached to the 7^{th} to 12^{th} nodes of two shoots. For *U. australis* the 10 traps came from the excised 10^{th} nodes of two shoots. I removed any basal traps and all but the five largest lateral traps. I trimmed the capillary leaf segments on the *U. australis* leaves until the ratio of leaf to traps was similar to that on the *U. gibba* nodes. Water in the culture dishes was diluted to 50:50 mix with deionised water.

Experiment two: *U. gibba and U. australis* with *C. sphaericus, C. vidua and S. kingi.*
Treatments consisted of 15-25 animals from one crustacean species and 10 traps of either *U. gibba* or *U. australis.* I treated traps in one of two ways: i) All appendages intact, ii) All appendages removed. Animals and traps were selected and prepared as for experiment one. I replicated each treatment block six times (6x2x3x2=72 dishes in total). I conducted a subsequent trial using only *U. gibba* and *S. kingi* with each treatment block replicated 12 times (12x2=24 dishes in total). Water in the culture dishes was undiluted.

Experiment three: *U. australis* with *A. robustus and M. cf. leuckarti.*
Treatments consisted of six lateral traps and 12-20 copepods drawn from mixed cultivation of *A. robustus* and *M.* cf. *leuckarti.* I used the largest trap from the 7^{th} to 13^{th} nodes of a single stolon fragment. Animals were selected as for experiment one. Traps had either: i) all appendages intact, ii) all appendages removed. In order to assess the effect of the surrounding leaf matrix on the effectiveness of appendages, leaves were also manipulated in one of two ways: i) capillary leaf segments around the traps trimmed away, ii) capillary leaf segments around the trap intact. In the latter case I trimmed the tips of any capillary leaf segments not directly surrounding the traps to ensure all nodes were subject to similar types of damage. I replicated each treatment block 6 times (6x2x2=24 dishes in total). Water in the culture dishes was undiluted.

2.2.4 Statistical treatment

I conducted all analyses in R 3.1.1 (Team, 2014).
For all experiments I analysed the proportion of available prey captured after 24 hrs (termed rate of capture) using Generalised Linear Models (GLMs) with binomial distributions and logit link functions. The logit link function is the canonical link function for the Bernoulli distribution. The response variables for all experiments were two column matrices of the number of captured and remaining animals. Experiment one had two factors: appendage ablation at four levels and plant species at two levels. Experiment two had three factors: appendage ablation at two levels, plant species at two levels, and prey species at three levels; the supplementary experiment had one factor, appendage ablation at two levels. Experiment three had two factors: appendage ablation at two levels and leaf ablation at two levels. When model fitting, I removed non-significant interactions providing the subsequent model (the model without the non-significant interaction) had a smaller Akaike Information Criterion (AIC) than the previous one (the model including the non-significant interaction). For chosen models I assessed the statistical significance of model terms (main effects and interactions) for factors with more than two levels using a chi-squared test based on the reduction of residual deviance. Tukey contrasts for multiple comparisons were performed using the multcomp package (Hothorn *et al.*, 2008). As the logit function gives the

logarithm of the odds *p/*(1-*p),* where *p* = the probability of an event (in this case, a capture), I reported odds ratios in addition to probabilities. Graphs of model estimated capture probabilities (effects sizes) and 95% confidence intervals, created using the effects package (Fox, 2003) are also provided. These provide Tabulated logistic regression, ANOVA and Tukey outputs for all analyses are provided in **Appendix 1**. For all experiments the critical value for rejecting the null hypothesis was taken as $\alpha = 0.05$.

2.3 Results

2.3.1 Experiment one: *Utricularia gibba* and *U. australis* with *Chydorus sphaericus.*

U. gibba trapped *C. sphaericus* in higher proportions than *U. australis* irrespective of the presence or absence of appendages ($z = 4.105$, $p < 0.0001$) (**Fig. 2.4**). Appendage ablation significantly reduced capture in the *U. gibba* treatments only. Only the removal of all appendages resulted in a significant reduction in capture rate compared to the control treatments that had all appendages intact ($z = 5.047$, $p < 0.0001$). For the pairwise comparisons between the other levels of the ablation factor (e.g. bristles only-all appendage and antenna only-all appendages) for *U.* gibba and all pairwise comparisons for *U. australis*, see, **Table 1.1.4** in **Appendix 1**. The odds of *C. sphaericus* being captured by traps with a full complement of appendages (control) is 5.11 times higher than those with none.

2.3.2 Experiment two: *U. gibba* and *U. australis* with *C. sphaericus, Cypridopsis vidua* and *Scapholebris kingi.*

As there was no significant three-way interaction between plant species, prey species and appendages, or two-way interaction between plant species and appendages, these two terms were removed from the model. The interaction between plant species and prey species was also insignificant (χ^2 test, df = 2,65; $p = 0.056$), but retained as the removal of this term did not lower the AIC of the subsequent model. *U. gibba* captured all prey species in higher proportions than *U. australis* ($z = 8.658$, $p < 0.0001$) (**Fig. 2.5**). Appendages impacted prey capture, but their effect differed significantly among prey species (χ^2 test, df = 3,63; p= 0.0001) (**Fig. 2.6**). Appendages had no effect on the capture of *C. vidua* in either *U. gibba* ($z = 0.912$, $p = 0.740$) or *U. australis* ($z = -1.615$, $p = 0.286$) treatments. When considering both plant species collectively, the odds of *C. sphaericus* being caught by traps with appendages (without consideration of plant species) was 2.04 times higher than that of being caught by traps without them. This difference was significant only in the *U. gibba* treatments ($z =- 2.915$, $p = 0.011$), but not in the *U. australis* treatments ($z = 2.222$, $p = 0.077$). Appendages of both *Utricularia* species negatively affected the capture of *S. kingi.* In the *U. gibba* treatments the odds of *S. kingi* being captured by traps without appendages was 2.23 higher than those with, although this difference was not significant (*U. gibba*: $z = -2.190$, $p =0.083$). In the subsequent trial, conducted only using *U. gibba* and *S. kingi* with increased replication, this effect was significant ($z = -4.815$, $p < 0.0001$). Here, the odds of *S. kingi* being captured by traps without appendages was 3.87 times higher than those with them.

2.3.3 Experiment three: *U. australis* with *Acanthocyclops robustus* and *Mesocyclops* cf. *leuckarti*

The leaf matrix surrounding traps did not significantly interact with appendages to affect the capture of the cyclopoid copepods so this term was dropped from the model. Neither did appendages nor a surrounding matrix of leaf segments have any individual effect on the probability of prey capture (appendages: z = -1.558, p = 0.119; leaf matrix: z = -0.230, p=0.818).

2.4 Discussion

It seems reasonable that traps of aquatic *Utricularia* would be under selection for mechanisms that enhance trapping success. In aquatic species, traps are in competition with other plant modules for the attention of phytophilous microcrustaceans engaged in feeding and ovipositing, and taking refuge from other predators. Prey species who are themselves predatory, such as carnivorous cyclopoid copepods, hunt throughout the plant. Lures or funnels steering animals through the competing plant matrix to a trap encounter should confer an adaptive advantage (Darwin, 1875; Meyers and Strickler, 1979; Guisande *et al.,* 2007).

Appendages lure filament feeders…

Appendage removal resulted in a significant reduction in the capture of the filament-feeding cladoceran *C. sphaericus*. Unlike Meyers and Stickler (1979), who used a similar level of replication, I was unable to demonstrate an increase in appendage effectiveness with successive additions of bristle sets and antenna. Only fully ablated traps captured lower numbers of *C. sphaericus* than those with a full complement of appendages.

…but do not act as funnels

I found no evidence that appendages funnel in a stricter sense, acting as a locomotory pathway that guides pelagic animals to the trap door. The absence of appendages affected the capture of *C. sphaericus* (**Fig. 2.5**), but not *Cypridopsis vidua,* an animal also phytophilous and associated with the benthos, but without the filament feeding behaviour of *C. sphaericus* (**Figs. 2.6-2.7**). Possessing appendages actually deterred the capture of the pelagic cladoceran, *Scapholebris kingi* whose interactions with the plants were limited to passing through them between episodes of epineustic feeding. While *C. vidua* and *C. sphaericus* move among plants, *S. kingi*'s vertical traversal of the water column results in movement perpendicular to the stolon. It is possible that with this angle of approach to traps appendages deflect these animals from contact with trigger hairs.

Plant matrix has no effect on the ability of appendages to enhance prey capture

Appendages do not exist in isolation. The traps of *U. australis* are surrounded by a leaf matrix whose filamentous nature resembles the bristles and antennae branches themselves. While appendage ablation did not reduce the capture rate of capture of the cyclopoid copepods *Acanthocyclops robustus* and *Mesocyclops* cf. *leuckarti,* irrespective of the presence or absence of a surrounding plant matrix, these animals were being tested in isolation from prey species.

Copepods do not predate blindly, searching in an undirected fashion (Kerfoot, 1978; Williamson, 1983) and change their behaviour in relation to prey density (Williamson, 1981). Copepod behaviour (locomotion) may change in the presence of their own prey, in such a way as to increase the likely hood of a trap encounter. The same also applies to the prey of copepods and other predatory microcrustaceans. The presence of predatory animals causes their prey to seek refuge (Lima, 1990) and appendages may offer such shelter, increasing the chance of an encounter with the trap.

Prey also aggregate in response to nearby predators (Lima, 1990; Sparrevik and Leonardsson, 1995) and some microcrustaceans aggregate in response to changes I their population density (Harms and Johansson, 2000). Changes in density lead in turn to changes in locomotion which can effect encounter rates with *Utricularia* traps (Harms and Johansson, 2000). Harms and Johansson (2000) demonstrated that patterns in prey selection in a two prey system can reverse with changes in density of the respective prey species. In the future ablation trials should be conducted with species assemblages and where prey density is varied.

The effectiveness of appendages varied between aquatic Utricularia species.

While the general pattern of capture probabilities (the central tendency) was the same for both *Utricularia* species, appendages had a statistically significant effect on capture rates in only plant species, *U. gibba.* The *U. australis* traps used in my experiments, however, were more similar to those of *U. vulgaris* used in experiments by Meyers and Stickler (1979), who demonstrated larger effects than those of *U. gibba*. The reason for this disparity is unclear; why I failed to observe capture enhancement by *U. australis* appendages, while similar work on the similarly large traps of *U. vulgaris* demonstrated such a clear affect. The trap resetting rates of aquatic *Utricularia* are too similar to be responsible for a difference in capture rate, moreover, Adamec (2011c) recorded *U. australis* having slightly *faster* resetting rates than other aquatic *Utricularia* (including *U. vulgaris* and *U. floridana,* an affixed aquatic species closely related to *U. gibba*).

Guiral and Rougier (2007) noted that the *U. gibba* traps analysed in their study not only had a greater density of bristling and branching than the ´*U. breviscapa*-like´ species, but also a more closed appendage architecture with the antenna trucks recurving in different directions. Those of the *breviscapa*-like species curved back over the top of the trap bladder whereas those of *U. gibba* were recuved towards the stalk. It is possibe that such three-dimensional shape differences in appendage architecture between the traps of *U. gibba* and *U. australis* may also have influenced capture rates. In future studies it would be useful to record variation in such charaters using morphometric methods.

The results of *C. sphaericus* trials in experiment one (*U. gibba* with *C. sphaericus*) are not directly comparable with those from experiment two (*U. gibba* and *U. australis* with *C. sphaericus, C. vidua* and *S. kingi*). The noticeable decline in the rate of capture between the experiments for both *Utricularia* species was likely due to the decreased trapping effort as the number of traps in each dish was halved. The difference in the water quality of the culture dish environments could have also been significant. As *C. vidua* and *S. kingi* are far more sensitive to water chemistry than *C. sphaericus* (pers. obs.), deionised water was not used to the dilute culture dish water for any of the three prey species in experiment two (cf. experiment one). This resulted in greater amounts of fine particulate matter settling on the bottom of the dishes, providing a competing source of food for the benthic feeding *C. sphaericus*. For the same reason the observed probability of capture for *C. vidua* should not be taken as an absolute measure of this animal's susceptibility to entrapment.

No evidence for interference between trap bladder and antennae

Meyers and Stickler (1979) observed that trap bladders interfere with antennae for the attention of *C. sphaericus,* who utilise both substrates for feeding with different behavioural modes. The appendages of *U. gibba* traps used in these experiments were more robust than those of *U. australis,* in terms of the number of supplementary bristle sets, the number of bristles within bristle sets common to both, and antennal branching in relation to the size of the trap bladder. Their more effective trapping in the control treatments could therefore be interpreted as resulting from larger appendage to bladder size ratios (in the context of prey being scaled to trap size), than *U. australis*.

However, the *U. gibba* clone used in this experiment had smaller trap bladders than that of *U. australis*; therefore *U. gibba* having more robust appendages is contrary to the inference that appendages under selection for an adaptive value in enhancing prey capture would be comparatively more numerous (bristles) and more branched (antennae) on larger traps (positive isometric scaling) to offset increasing interference from the alternative feeding substrate provided by trap bladder (Meyers and Strickler, 1979). Additionally, no statistically significant reduction in capture rates of *C. sphaericus* was recorded for *U.*

australis. Under the bladder interference hypothesis these larger traps should have been at a greater disadvantage than those of *U. gibba* without the assistance of appendages and therefore *U. australis* should have shown a stronger response negative response in capture rate than *U. gibba*.

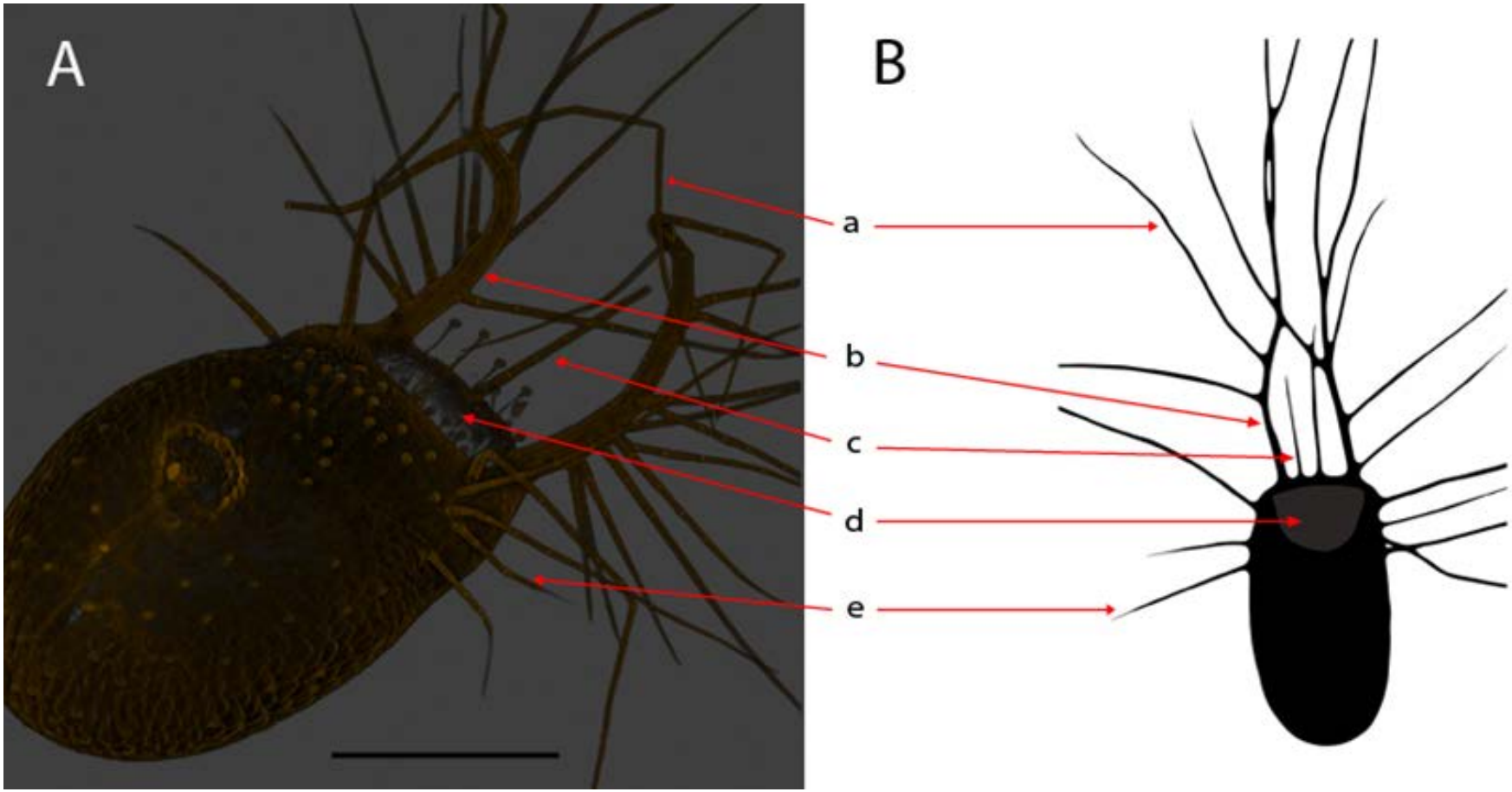

Fig 2.1. Ventral views of a *U. gibba* trap showing appendages typical of suspended aquatic species from Section *Utricularia.* **A**: Laser scanning confocal microscope image. Scale = 500µm. **B**: Stylised silhouette. Appendage types: a = antenna branch; b = antenna trunk; c = dorsal bristle; d = trap door; e = lateral bristle. Trigger hairs not shown.

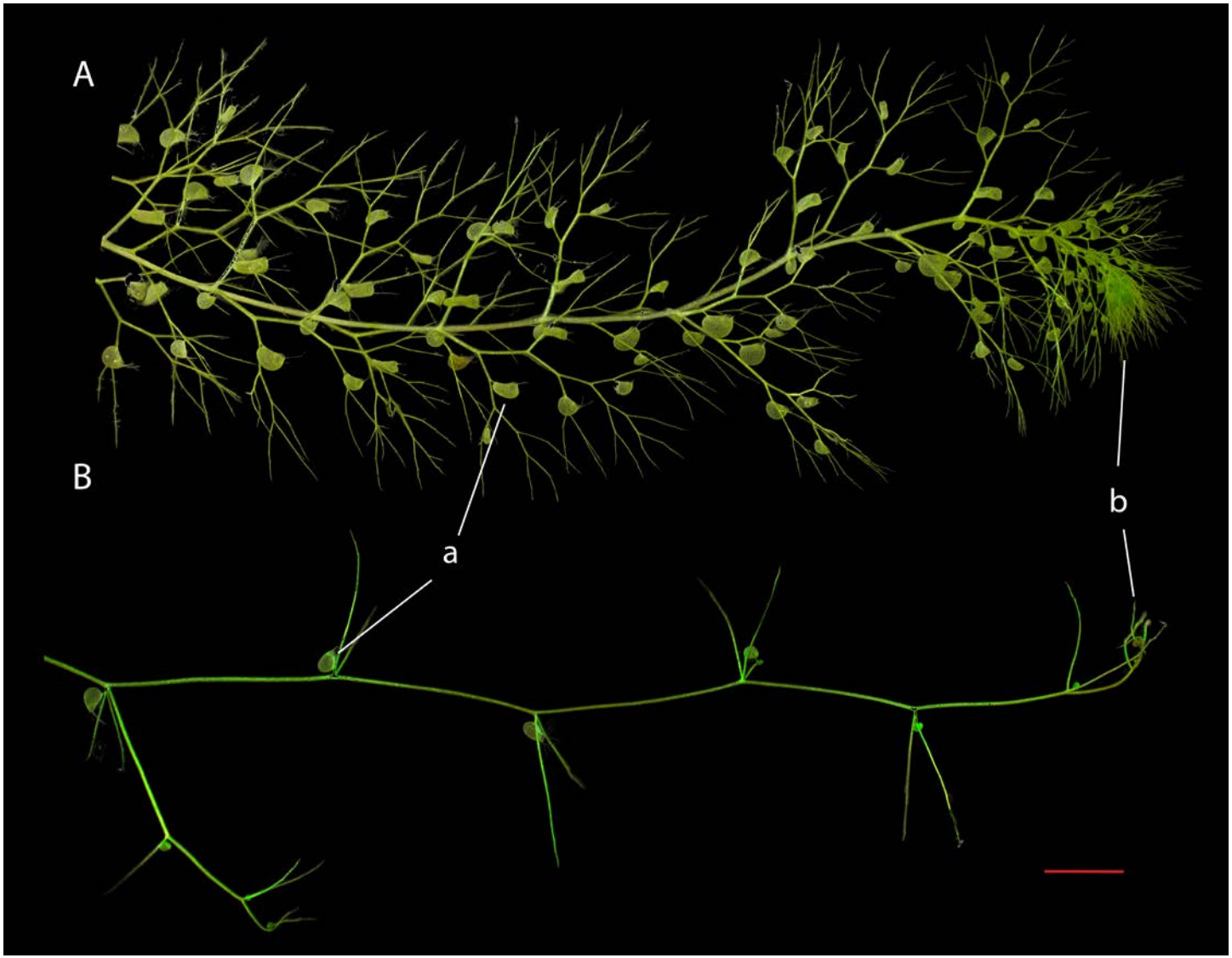

Fig 2.2. Typical shoot lengths of the two Section *Utricularia* species used in appendage ablation experiments, showing differences in trap size and number per node. **A:** *U. australis*, suspended aquatic; a= trap, b= shoot apex **B:**.*U. gibba* is of the 'sterile' form. Scale = 5mm. *U. gibba*, affixed aquatic,

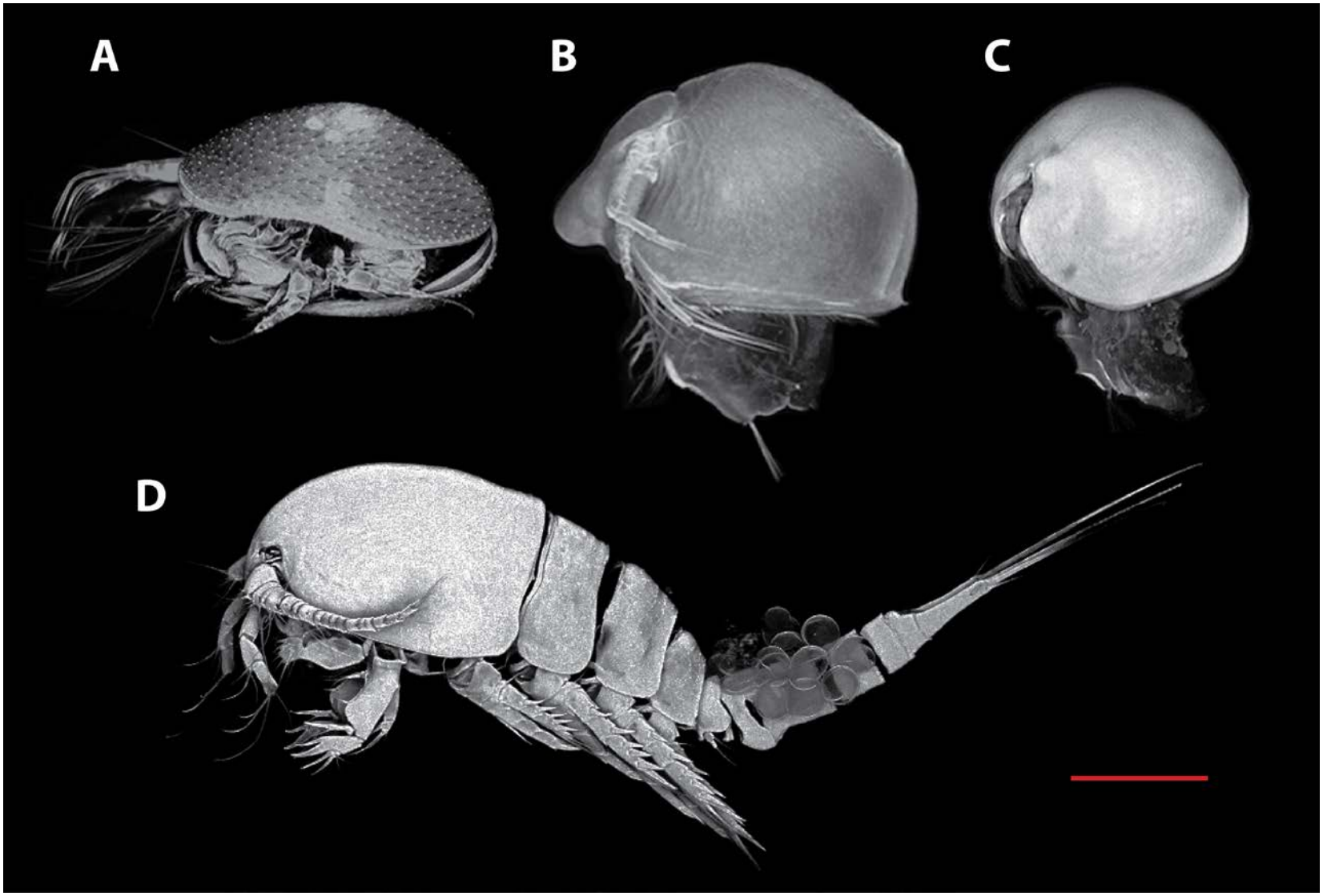

Fig 2.3. Orthographic projections generated from laser scanning confocal microscope image stacks showing lateral views of four microcrustacean species used in appendage manipulation experiments. **A**: *Cypridopsis vidua*, phytophilous and benthic forager; **B**: *Scapholebris kingi*, planktonic, epineustic feeder; **C**: *Chydorus sphaericus*, phytophilous and benthic, filament feeder **D**: *Acanthocyclops robustus* littoral and benthic predator. Scale = 200 μm.

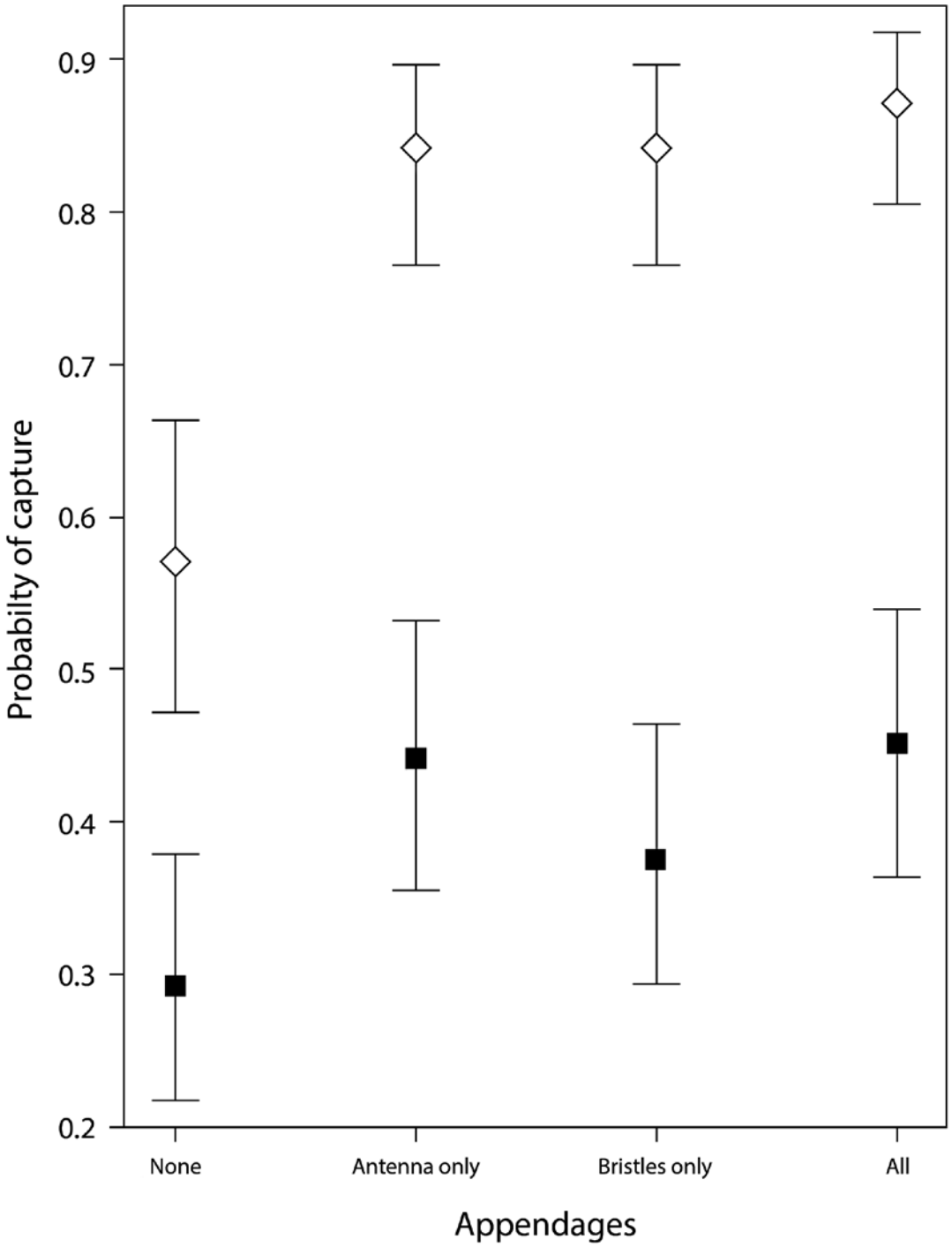

Fig 2.4. Effects plot showing the model estimated probability of capture of the cladoceran *Chydorus sphaericus* over 24 hrs by *U. australis* (■) and *U. gibba* (◊) traps subject to one of four appendage manipulations: None (all appendages removed), Antenna only (bristles removed), Bristles only (antenna removed), All (all appendages intact, control). Bars indicate 95-percent confidence intervals.

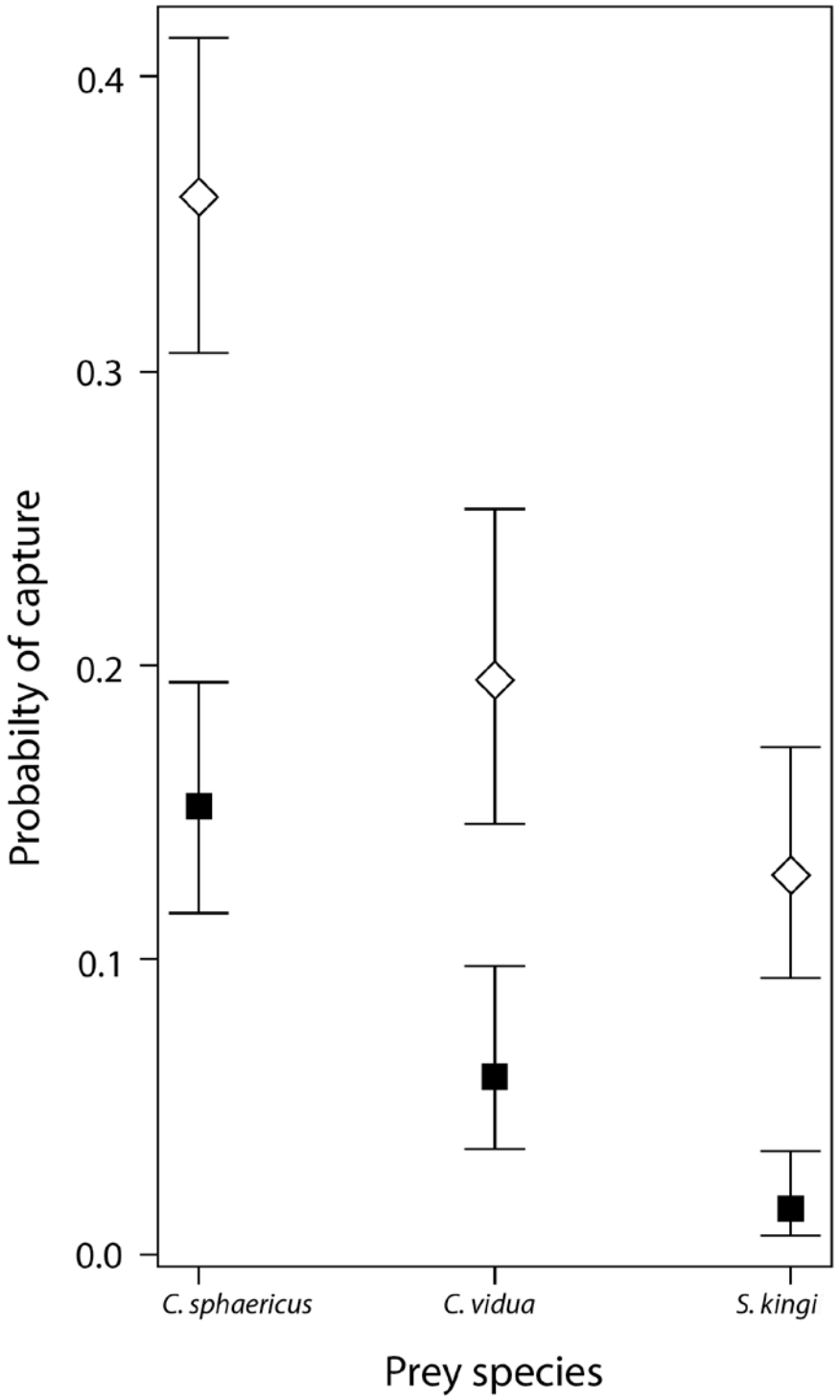

Fig 2.5. Effects plot showing the model estimated probability of capture of three freshwater micro-crustaceans *C. sphaericus*, *C. vidua* and *S. kingi* over 24 hrs by *U. australis* (■) and *U. gibba* (◊). Bars indicate 95-percent confidence intervals.

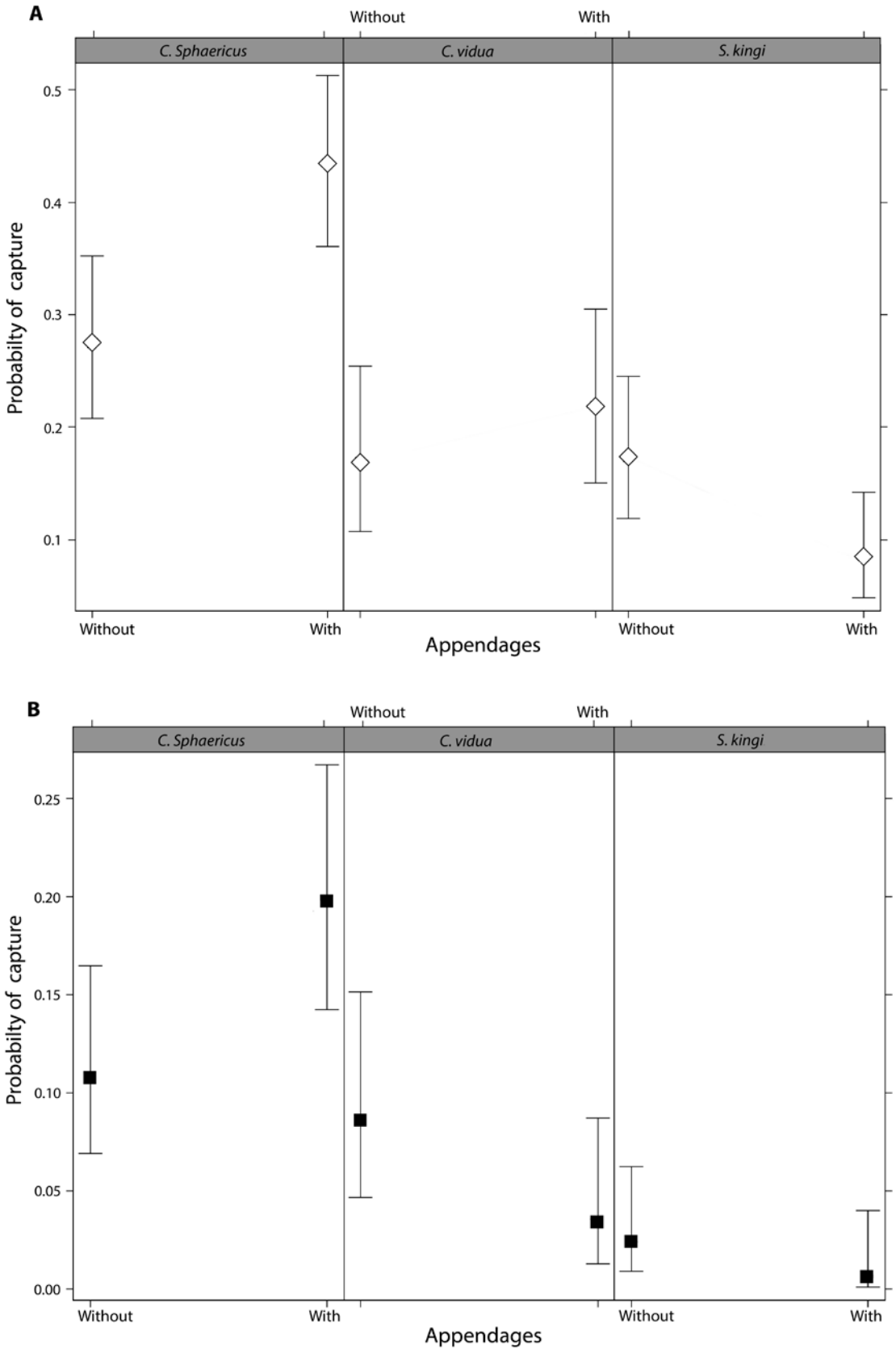

Fig 2.6. Effects plot showing the model estimated probability of capture of three freshwater micro-crustaceans *C. sphaericus*, *C. vidua* and *S. kingi* over 24 hrs by *Utricularia* traps without or with appendages. **A**: *U. gibba* (◊) and **B**: *U. australis* (■). Bars indicate 95-percent confidence intervals. Note the different scales on the y-axes.

3. The effect of light and water level on trap size and appendage expression of the amphibious *Utricularia gibba*

3.1 Introduction

Utricularia is the largest and most derived genus within the wholly carnivorous Lentibulariaceae with ~ 220 species occupying waterlogged-terrestrial, epiphytic, and aquatic growth habits (Taylor, 1989; Jobson *et al.*, 2003; Albert *et al.*, 2010; Reut and Jobson, 2010; Fleischmann, 2012a). The aquatic lifestyle is a homoplastic trait, having arisen at least four times (Jobson and Albert, 2002; Jobson *et al.*, 2003; Müller and Borsch, 2005). Some aquatics are freely suspended macrophytes, the majority of which are found in one clade, the infra-generic section Utricularia. Other aquatic species anchor in the benthos with rhizoids or specialised stolons and grow up into the water column (Taylor, 1989). These "affixed" aquatics may be representative of hypothetical, ancestral intermediates during the radiation into aquatic habitats. Most terrestrial carnivorous plants are exposed to high levels of light, in keeping with the classical model of carnivorous plant evolution (Givnish *et al.*, 1984). Although some taxa, including *Utricularia* with terrestrial habits, can be considered facultatively sciophilous, with many growing within a herbaceous understory (Taylor, 1989; Adamec, 2011b). In contrast, aquatic carnivorous plants (all but one are *Utricularia* spp.) are shade adapted like other suspended macrophytes, and are exposed to lower irradiance than terrestrial species (Adamec, 2008b).

One of the most striking cases of convergence in aquatic plants is their reliance upon clonality for reproduction and dispersal, and the perpetuation of a specific, stable genotype within water bodies (Barrett *et al.*, 1993; Kameyama and Ohara, 2006b). Aquatic *Utricularia* are no exception and, despite niche restrictions arising from their poor competitive ability, are models for the syndrome; they self-propagate and disperse through shoot fragmentation, and some suspended aquatic species with subtropical to polar distributions form turions, vegetative, overwintering buds that also function as propagules (Winston and Gorham, 1979; Adamec, 1999). Work by Kameyama and Ohara (2006b; 2006a) found the within-population genotypic diversity of the sexually reproducing *U. tenuicaulis* (as *U. australis* f. *tenuicaulis*) was extremely low, in contrast to different genotypes among populations.

All *Utricularia* have mechanically activated suction traps (Lloyd, 2008; for more details see **Chapter 1**). The appendages of section Utricularia consist of a pair of branched, setiform, dorsal appendages (antennae), supplemented by bristles lateral and ventral to the trap door. (Taylor, 1989; Guiral and Rougier, 2007; for more details in Chapter 2). The antennae of section Utricularia are hypothesised to have evolved in response to aquatic conditions, principally as a strategy for enhancing prey capture (Darwin, 1875; Meyers and Strickler, 1979). The appendage morphology among members of section Utricularia appears conserved in comparison to other sections. However, inter-specific variation in appendage expression does exist: the size of supplementary bristle sets, extent of antennal branching, and the size of antennae relative to the trap bladder (Taylor, 1989; Friday 1991; Guiral and Rougier, 2007).

Additionally, some species (e.g. *U. aurea)* have otherwise monomorphic traps that exhibit appendage polymorphism, where antennae of bristle sets may be entirely absent (Taylor, 1989). Antennae and bristles are also variably expressed among individuals of a particular species, in allometry with other trap characters such as trap bladder sizes (Friday 1991; such variation is due to both genetics and in response to environmental factors (Taylor, 1989; Friday, 1991; Guiral and Rougier, 2007; pers. obs.). Evidence for the latter is largely anecdotal, or inferred from the nature of changes in the investment in carnivory (IIC) in response to environment (light, CO_2 levels, prey availability, availability of ambient nutrients); Changes in environment are reliably correlated with changes in trap-to-vegetative organ weight ratios, but not trap number per leaf. Therefore, the sizes of individual traps, relative to the vegetative organs, are changing in response to environment.

The amphibious *U. gibba* (section Utricularia) is one of the few affixed aquatic species that is capable of growing in both terrestrial and aquatic phases. Plants grow as terrestrials in/on wet, solid substrate or in slurry, and as affixed aquatics they anchoring in the benthos; yet *U. gibba* does not produce dimorphic shoots. Those growing within the substrate are not clearly differentiated from those in the water, and bear traps in both instances. *U. gibba* will occasionally produce "rhizoids" or un-pigmented, rhizoid-like shoots when attached to substrate. These modules are indeterminate and will grade back into green, leaf bearing shoots. *U. gibba* can also grow fully suspended without any anchoring structures (Taylor, 1989; Chormanski and Richards, 2012). *U. gibba* is a widespread species with a pan-tropical distribution, and is in general highly plastic (Taylor, 1989; historically, the species has over 60 synonyms). *U. gibba* reproduces from seed and through extensive fragmentation but does not produce turions characteristic of other obligate, suspended aquatic species from temperate climates. Traps are born singly or in pairs on sparsely lobed leaves, are variable in size (1-2.5 mm long) but uniform in shape. Trap appendages are characteristic of section Utricularia. The antennae of *U. gibba* are extensively branched in comparison to other sect. Utricularia species (Guiral and Rougier, 2007). They may recurve over the trap door area in a fashion reminiscent of the sub-aquatic *U. resupinata* from the closely related section Lecticula (Thurston and Seabury, 1975, as *U. biflora*; pers. obs.). *U. gibba* is also part of a small clade of affixed aquatics in section Utricularia that also have dorsal supplementary bristles between the antenna trunks (Taylor, 1989). Although lacking true appendage polymorphism, the amphibious and extremely plastic growth of *U. gibba* makes this species a good model for testing the response of trap characters to environmental variation reflecting the difference between aquatic and terrestrial habitats.

To date no experiments have been conducted that examine the relative contribution of environmental variation to appendage expression. In this two-factor fully crossed study I assess the effects of two environmental variables, light and water level on the size of *U. gibba* traps. I also test the following

hypotheses regarding appendage expression: i) clones will exhibit persistent differences in appendage expression, ii) the expression of trap appendages (antenna and bristles) will vary between terrestrial and aquatic phase with iii) plants growing in terrestrial phase will produce less dendriform antennae and reduced bristle sets.

3.2 Materials & Methods

3.2.1 Plants

I used shoots of *Utricularia gibba* L. collected from two locations in the upper North Island of New Zealand, The Whangamarino river (37°18'23.92"S, 175°5'12.00"E) and Lake Rotokawau (35°1'3.91"S, 173°12'17.55"E). I pre-cultivated plants for 12 months in an indoor, rooftop greenhouse at the School of Biological Sciences, University of Auckland, where the experiment was also conducted. Each clone was grown in a single 60 l plastic tank layered with 30 mm of 2:1 peat moss and sand substrate and filled with water to a depth of 400 mm. I inoculated the tanks with fine zooplankton including ostracod, cladoceran, and cyclopoid and harpacticoid copepod species sourced from an outdoor pond.

3.2.2 Growth experiment

The experimental apparatus consisted of a nested series of plastic, rectangular receptacles hereafter referred to as trays, tubs and containers. Pairs of trays were connected by pumps (more below for more details). Connected trays allowed for a single volume of water to be exposed to two different light levels, tubs were placed within trays to permit (pseudo)replication. Containers were paced within tubs to allow the addition of extra substrate, producing treatments with reduce water columns (**Fig. 3.1**).

Receptacles were prepared as follows: I drilled circular holes in the bottoms and sides of sixteen 1.8 l containers and 16 12 l tubs. Containers had a rectangular arrangement of twenty-four 5 mm holes their bottoms and sides (121 holes per container). Tubs had a similar arrangement of 91 five mm holes in each side and 182 in each bottom (546 holes per tub). I covered the holes in the tubs with 150 µm mesh nylon monofilament screen (Nitex) using structural plastic adhesive (Scotch-Weld DP8005, 3M) and clear, glass silicone-sealant (Selleys). I placed two containers side by side in eight of the 16 tubs. Four tubs, two with containers and two without, sat within one 35 l plastic tray. In summary, there were four trays, holding 16 tubs with half of the tubs holding in turn 16 containers. I then placed two trays under a 600W metal halide lamp (SON-T) and two under a suspended square of shade cloth.

On 4 April 2012 I filled containers with 1.5 kg of 2:1 peat moss and sand mix. I covered the bottoms of the empty tubs with the same amount of substrate. I then filled the surrounding trays with 25L of deionised water. This was sufficient to saturate the substrate in the containers, creating the low water

level treatments, while providing a ~15cm water column in the container-free tubs creating the high water level treatments. I used a pair of seven Watt, 220 L/hour pumps (Aleas) to couple one tray under light and one under shade to form a treatment block. There were consequently two treatment blocks or replicates (**Fig 3.1.**). The holes in the containers and tubs allowed for an exchange of water between receptacles within the trays. With the pumps circulating water between trays, this allowed the water chemistry to equalise within a treatment block in a manner similar to Adamec *et al.* (2008a). I added additional deionised water throughout the experiments as necessary to offset evaporation. Glutaraldehyde in the form of a liquid (API) was added to treatment blocks via the trays every three days at 1ml/25L to boost CO_2 levels. This concentration was well below toxic levels for aquatic plants and animals (Pereira *et al.*, 2014). Additional CO_2 was supplied to each treatment block by 88g pressurised kits (Fluval), bubbled into the tray water.

I left the substrate soaking for two weeks. On 18 April 2012 I randomly selected 32 pieces of each *U. gibba* clone for a total of 64 shoot segments. Each shoot segment consisted of a length of stolon complete with an apex. While shoot segments of each clone came from the same tank and therefore shared the same general growing conditions prior to the experiment, growth histories among them varied dependant on microclimate within the tank. This resulted in initial variation among shoot segments in internode length, mean trap size and the position of the first node bearing mature traps relative to the apex.

I prepared each shoot segment as follows: i) Removal from cultivation tank, rinsed with deionised water and placed in cell culture dishes containing filtered tank water, ii) Trimming at the halfway point along the internode between the eighth and ninth nodes prior to the shoot apex, iii) Removal of any traps and side shoots using 2x 0.15mm cutting edge spring scissors (Vannass) under a stereoscope (Leica) at x25 and x40 magnification, iv) Any remaining algae and detritus gently rinsed and scraped away and v) Cotton tied carefully between the shoot apex and the first node, colour coded to distinguish between clones.

I then randomly allocated two prepared shoots from each clone to each tub for a total of four shoots per tub. These shoots grew independently as “clonal units”. In low water treatments two fragments were laid on the substrate in each container and gently watered in. In high water treatments two shoots were anchored at either end of the tub by burying the length of stolon between the sixth and eighth nodes in the substrate. The remaining shoot was left to float suspended in the water column. The plants were left to grow for 21 days. Each day I wiped the monofilament mesh to remove algae and lifted tubs to allow 80-90% of their water to drain into the trays through the mesh. I then depressed the tubs to refill them through the mesh.

Incident light levels were measured using SQ-120 and SQ-110 standard quantum sensors (Apogee) for high and low light treatments. Mean midday light under the high light treatment was 41% (37-47%) of adjacent outdoor incident light. at 492 (±3.9) µmol m^{-2} s^{-1}. Under the low light treatment midday irradiance was 8% (6-10%) of open outdoor levels at 54 (±5.1) µmol m^{-2} s^{-1}. I measured pH and water temperature Portable Meter, (HQd, HACH) in water sampled between 11:00 and 13:00 hours. TA estimates were provided by Hill Laboratories, Hamilton, New Zealand. I calculated dissolved CO_2 from pH and TA (after Helder, 1988). Water temperature, pH, TA and free CO_2 were similar in between replicates (replicate one: 19.8–21.7°C, 5.90-6.50, 0.2-0.4 meq.l^{-1}, 0.130-0.256 mM; replicate two: 19.9-21.6°C, 5.87-6.80, 0.2-0.4meq.l^{-1}, 0.198-0.259 mM).

3.2.3 Data collection

After 21 days I removed all traps from the leaves and stolons and fixed both separately in 50% ethanol. Traps were then stained in an aqueous, 1% weight per volume, solution of Congo Red to enhance visibility. I measured the following responses for each trap: i) Number and position of supplementary bristles ii) Number of branches on the antennae, iii) Trap length, iv) Trap depth, perpendicular to length (**Fig. 3.2**.). All mature, undamaged traps produced during the growth experiment were used in analyses. An exception was made in the case of one large clonal unit appendage counts where I used a subsample of 50 traps from. These 50 traps were selected at random after staining. To be considered mature, traps were required to have fired (particulate matter present inside the trap bladder). To meet the criteria of undamaged, traps had to exhibit the following characteristics: i) For appendage counts traps, the antenna trunk had to be intact (the bases of antenna branches and supplementary bristles were still visible even after mechanical damage), ii) For linear-morphometric measurements of the trap body, trap bladders had to be entire, without obvious dorso-ventral and lateral compression. Some traps rejected for appendage counts were suitable for linear morphometrics, and vice versa. Appendages were not counted for traps growing in the low-light, low-water treatments (see **3.3.1** for details). For logistical reasons, appendage counts and linear morphometric measurements were preformed independently on traps. Within each treatment block, traps from replications of each combination of light and water level (from identically treated tubs) were pooled for analysis. Traps from four independently growing clonal units, subjected to the same water and light levels within each treatment block, were therefore also pooled for analysis. For sample sizes see **Tables 3.2-3.3**. I conducted all counts under a stereoscope (Wild M3C, Leica) at x25–x40 magnifications. Linear measurements were made in ImageJ (Abramoff *et al.*, 2004) from digital photographs taken with a stereo macroscope (Leica).

3.2.4 Statistical treatment

Analyses were conducted in R 3.1.1 (Team, 2014).

For continuous response variables (trap length and depth), I used Linear Mixed Models (LMMs), were used created with **nlme** (Pinheiro *et al.*, 2014). The use of mixed models allowed me to include 'treatment block' (coupled trays with shared water chemistry, n=2) as a random factor in the models. Spatial limitations for the placement of treatment blocks within the growth apparatus, in combination with the nature of the available greenhouse space, resulted in an unquantified positional effect (light, temperature variations) associated with each treatment block. The organisation of treatment block components (the trays) were representative of a range of possible configurations. Additionally, it is assumed that in the absence of control over the water chemistry in each treatment block, the nutrient-level trajectories between them will have differed at random. Therefore, treatment blocks can be seen as 'experimental sites' (lentic water bodies) drawn from a population of possible experimental sites – their effect being 'random' (Little *et al*., 1996). The component of variance attributable to treatment block in the final models for each continuous response variable are provided in **Appendix 1**, (trap length: **Table 2.1.1**; trap height: **Table 2.2.1**). Shapiro-Wilk tests of normality for the residuals of LMMs are provided in **Appendix 1**. In the cases where these tests rejected the null hypothesis that the residuals were normally distributed, the assumption of normality was still made if the normal Q-Q plots for the LMMs passed the fat pencil test (were centred) on inspection. Ratios were log transformed.

Bristle and antennal branching count data were underdispersed, so data were modelled with GLMs with quasipoisson distributions were used. As functions for conducting Generalised Linear Mixed Models (GLMM's), such as glmer from the **lme** package, cannot use quasi-families, treatment was not included as a random factor for analyses of appendage counts. Counts were pooled across treatment blocks and GLMs were fitted with **lme4** (Bates *et al.*, 2014).

In the case of both continuous and count response variables, models were fitted sequentially. Non-significant interactions were removed from models when i) In the case of continuous response variables, the subsequent model (the model without the non-significant interaction) had a smaller AIC than the previous one (the model including the non-significant interaction) or ii) arbitrarily for count data modelled with a quasipoisson distribution. For chosen models I assessed the statistical significance of model terms (main effects and interactions) for factors with more than two levels using ANOVA (LMMs) and χ^2 (GLMs) tests based on the reduction of residual deviance. Tukey contrasts of multiple comparisons were performed using **multcomp** (Hothorn *et al.*, 2008). For all experiments the critical value for rejecting the null hypothesis was taken as $\alpha = 0.05$. Tabulated regression outputs are provided in **Appendix 2**.

3.3 Results

Both *Utricularia gibba* clones grew poorly in the low water, low light treatment. Only three plants survived and produced traps in very low numbers (< three per plant). The low water, low light treatment was therefore excluded from analysis and water and light levels combined into a single factor ('environment') at three levels: high water & high light, high water & low light, and low water & high light. Trap length, depth, antenna branching, and lateral bristle expression were all significantly affected by clone, environment and the interaction between these two factors. Dorsal bristling was significantly affected by clone and environment but not by their interaction, so in this instance the interaction term was dropped from the model (**Table 3.1**).

3.3.1 Trap characters

Bladder length, height and area

There were persistent differences between clones for all trap size and appendage expression parameters. One clone (Clone two, represented by white diamonds in figures) produced significantly larger (longer and deeper) traps than the other (Clone one, represented by black squares in figures) at all three levels of environment (combination of light and water level) (**Fig. 3.3**). Trap length and height were very strongly correlated ($F_{1,550}$ = 5973, p < 0.0001, adjusted r^2 = 0.916) (**Fig. 3.4**). Clone two had a larger log(height:depth) ratio than Clone one (ANOVA, $F_{1,540}$ = 68.614; p < 0.0001), irrespective of environment (ANOVA, $F_{2,540}$ = 0.940; p =0.328).

In the case of both clones it was the reduction of water level and not light that had a significantly negative effect on bladder length and height (see **Appendix 2.4-2.5** for significance values). The interactions between the factors clone and environment (trap length: $F_{2,540}$ = 3.689, p =0.026; trap height: $F_{2,540}$ = 3.714, p =0.035) occurred because the effect of water reduction on the traps of Clone two (the clone with the more robust traps) was significantly greater than for Clone one. For the height of Clone one, the only significant pairwise difference between levels of the environment factor was between high water & high light, and low water & high light (z=-2.629, p=0.045). Whereas for Clone two, bladder height in the high water & low light treatments was not only significantly lower than bladder height in high water & high light treatments (z=-6.231, p<0.0001), but also that in the low water & high light treatments (z=-3.642, p=0.002).

3.3.2 Appendages

Supplementary bristles, antenna branching, and relative antenna length

Clone two had more supplementary bristles in both lateral and dorsal bristle sets, and had more branched antenna than Clone one, regardless of the environmental conditions (**Fig. 3.5**). Reducing either the light or the water level caused a corresponding significant decline in the number of antenna branches, and

lateral and dorsal bristles of Clone two. The effects of environment on the appendages of Clone one were less consistent. The expression of dorsal and lateral bristles followed the same pattern as with Clone two. However, the number of branches of Clone one antennae only decreased significantly with a reduction in light level. For significance values see **Appendix 2.1-2.3**.

3.4 Discussion

Consistent clonal variation in trap size, shape and appendage expression

The two randomly selected clones of *U. gibba* used in this experiment exhibited consistent differences not only in bladder size and shape, and appendage expression, but also in the magnitude of their responses to changes in environment. The differences between clones in response to environment demonstrate the importance of replicating with multiple clones (genotypes) when conducting laboratory-based growth experiments, due the low within-population genotypic diversity of suspended macrophytes in general (Sculthorpe, 1971), including sexually-reproducing aquatic *Utricularia* species (Kameyama and Ohara, 2006b; *U. tenuicaulis*). These results also emphasise the need for replicating across multiple water bodies with similar environmental conditions in order to avoid a correlation between environmental and genotypic variation.

The traps of Clone two also had more setae (bristles and branches) than those of Clone one. Clone two also had uniformly larger traps than Clone one, so differences in appendage expression may reflect isometric scaling. In contrast, Friday (1991) found *U. vulgaris* trap characters showed allometry in an alternative characterisation of appendage expression, antennae length:bladder length ratios. The larger midline traps of *U. vulgaris* had relatively shorter antennae and taller bladders (very slight difference) than the smaller peripheral traps (See section **4.3** where smaller bladders of *U. australis* have more antennae branches compared to the larger ones**;** but note the comparison within, not among or between individuals). Friday (19991) also found allometric variation in the bladder height:length ratio (where height is referred to as "depth") between the midline and peripheral traps of *U. vulgaris,* with the height of the midline traps being greater in relation to their length than the peripheral traps. This same pattern was observed between traps of the two *U. gibba* clones; the larger bladders of Clone two were more rounded, being taller in relation to length, than those of Clone one, irrespective of environment.

Averaging across all *U. gibba* traps (irrespective of clone or treatment) the height of bladders was ~72% that of their length. The lateral traps (midline and peripheral combined, basal traps excluded) of *U. vulgaris* measured by Friday (1991) were more globose with height ~79% of length. Although the lateral traps of *U. vulgaris* and the traps of *U. gibba* have the same general shape dictated by the positioning of the trap door relative to the trap stalk, the morphology of their bladders is slightly different. The *U. gibba*

traps produced in this experiment were smaller than the lateral *U. australis* traps surveyed by Friday (1991), suggesting that the bladder shape allometry is shared among section Utricularia species; that larger traps are more globose that smaller ones. It would be interesting to compare trap length to height ratios for additional aquatic and terrestrial species that exhibit trap size polymorphism, to see if this pattern is consistent of bladder size and shape relations across section Utricularia.

Appendage expression reduced with environment…

U. gibba does not exhibit appendage polymorphism in the strict sense (defined in section **1.2.3**), as antennae and bristle sets are always present (Taylor, 1989). Nonetheless, the appendage expression (as the amount of bristling and antennae branching) of this species is plastic. Both *U. gibba* clones showed a reduction in the degree of bristling and antenna branching (setae counts) when the plants were growing in a terrestrial phase, reflecting a tendency towards the less dendriform appendage morphology of closely related terrestrial and sub-aquatic species in sections Lecticula and Nelipus (**Fig 1.3)**. The appendages of one *U. gibba* clone also had fewer setae in response to reduced light (~ 8% of open levels).

Variable appendage expression has also been observed in another sect. Utricularia species, *U. foliosa*, where relative antennae length varied in response to ambient nutrition (NO_3^-: Manjarrés-Hernández *et al.*, 2006). Unlike *U. gibba*, however, *U. foliosa* does display appendage polymorphism; the comparatively sparsely branched antennae of this species may be entirely absent. Darwin (1875) accounted for the appendage morphology of the aquatics in sect. Utricularia by hypothesising that they functioned in concert to funnel phytophilous prey towards the trap door. This was later demonstrated by Meyers and Strickler (1979) for one cladoceran species (but see section **2.3**). In this type of attraction strategy antennae length may not be the only important quality of appendage expression. In so much as the antennae and bristles function to guide prey to the trap door, the number of setae (in a particular the amount of antennal branching) would alter the mesh size of the net formed by these appendages and therefore influence the size class of trapped prey.

However, it does not seem plausible that the increase in appendage expression observed in the high water treatments was a functional response to enhance prey capture. Firstly, although setae counts were significantly higher in plants growing as aquatics, numerically the differences were very small. Between levels of the environment, antennae varied by only ~ three branches and most bristle sets by less than one seta; differences that are unlikely to be functionally significant. Simple setae counts are also not the most appropriate characterisation of the mesh size. A more comprehensive measure of these reticula will require branch counts relative to antennae length, or measurements of the areas formed by the mesh.

Secondly, trap placement in shoot-dimorphic affixed-aquatics from sect. Utricularia (e.g. *U. stygia*) occurs on stolons within the substrate (Taylor, 1989), yet these traps are large and extensively bristled (Taylor, 1989). However, unlike *U. gibba* grown in the low water treatments of this experiment (whose traps were also embedded in the substrate), these species also have the benefit of large, filamentous shoots, suspended in water, to boost photosynthesis. The stolons and fine, sparsely-branched leaves of *U. gibba* (See figure **2.2B**) may photosynthesise efficiently while suspended. In a water column their shape is optimised for but CO_2 diffusion (Sculthorpe, 1971) but less so when growing in a terrestrial phase. Terrestrial or sub-aquatic *Utricularia* typically have broader, simple leaves, arising from nodes as modules distinct from the traps (Taylor, 1989). Given that appendage expression scaled isometrically with bladder size, the reduced appendages probably resulted from poor carbohydrate production in the low water and low light treatments where marginal PAR may have been compounded by a lack of free CO_2. Poor photosynthesis, perhaps compounded by a lack of ambient mineral nutrition (in the absence of a water column), may have also led to the poor growth and subsequent death of many of the replicates. A repeat of this experiment would need to quantify differences in CO2 availability between aquatic and terrestrial treatments throughout the duration of the experiment. Trap and appendage shape data could also be gathered from field populations of *U. gibba* growing in various phases in ephemeral water bodies, both from a range of sites and over the course of several seasons.

Table 3.1. Trap bladder and appendage characteristics of two *Utricularia gibba* clones grown under high & low light, and high & low water level over a 21-day greenhouse experiment. Length and height taken from orthogonal projections of trap bladder in lateral presentation. Means are reported for all treatment combinations. ± SE are reported for all treatment combinations except –light, –water treatments, where sample sizes were extremely low (See **Table 3.2**). Letters in bold denote statistically significant differences between variants within a clone (column) at p<0.05 (LMM, simultaneous tests for general linear hypotheses). Significance of main effects and interactions from F-tests (continuous responses) and χ^2 tests (counts and proportions) on the reduction of residual deviance for final models: *** – P < 0.001; ** – P < 0.01; * – P < 0.05; ns – 0.1 > P > 0.05; **ns** – P > 0.1.

		Trap Length (mm)		Trap Height(mm)		Antenna branching		Lateral bristles		Dorsal bristles	
		Clone 1	Clone 2	Clone 1	Clone 2	Clone 1	Clone 2	Clone 1	Clone 2	Clone 1	Clone 2
Treatments											
- Light	- water	0.8	1.0	0.6	0.7	18.0	21.5	6.0	9.0	0.7	2.5
	+ water	1.0±0.02**a**	1.3±0.02**a**	0.7±0.01	0.9±0.01**a**	20.3±0.3**a**	26.8±0.3**a**	7.2±0.2**a**	8.5±0.2**a**	1.3±0.1**a**	2.8±0.1**a**
+ Light	- water	1.0±0.02**b**	1.1±0.02**b**	0.7±0.01**a**	1.0±0.1**b**	24.2±0.4**b**	27.5±0.5**a**	7.4±0.2**a**	8.9±0.2**a**	1.4±0.1**a**	2.7±0.1**a**
	+ water	1.2±0.01**a**	1.3±0.01**a**	0.9±0.04**b**	1.0±0.06**a**	23.5±0.3**b**	29.6±0.3**b**	7.8±0.2**b**	10.41±0.2**b**	1.8±0.1**b**	3.88±0.1**b**
Factor											
Clone		*******		*******		*******		***		*******	
Environment		*******		*******		*******		***		*******	
Clone x Environment		*****		*****		*******		**		**ns**	

Table 3.2. Number of traps sampled for linear morphometric measurements (trap length and height) from two *Utricularia gibba* clones grown under high & low light, and high & low water levels over a 21-day greenhouse experiment. Traps from disconnected clonal units within each treatment were pooled for analyses

		Environment			
		- Light		+ Light	
		- water	+ water	- water	+ water
Treatment block					
1	Clone 1	0	21	30	31
	Clone 2	2	19	32	33
2	Clone 1	3	26	43	100
	Clone 2	0	64	37	111

Table 3.3. Number of traps sampled for appendage counts from two *Utricularia gibba* clones grown under high & low light, and high & low water levels over a 21-day greenhouse experiment. Appendages were not counted for traps growing in the low light, low water treatments. Traps from disconnected clonal units within each treatment were pooled for analyses. Treatment blocks were pooled for analyses (see text in **3.2.4** for details.)

		Environment			
		- Light		+ Light	
		- water	+ water	- water	+ water
Treatment block					
1	Clone 1	-	23	29	24
	Clone 2	-	33	20	27
2	Clone 1	-	27	47	69
	Clone 2	-	36	41	73

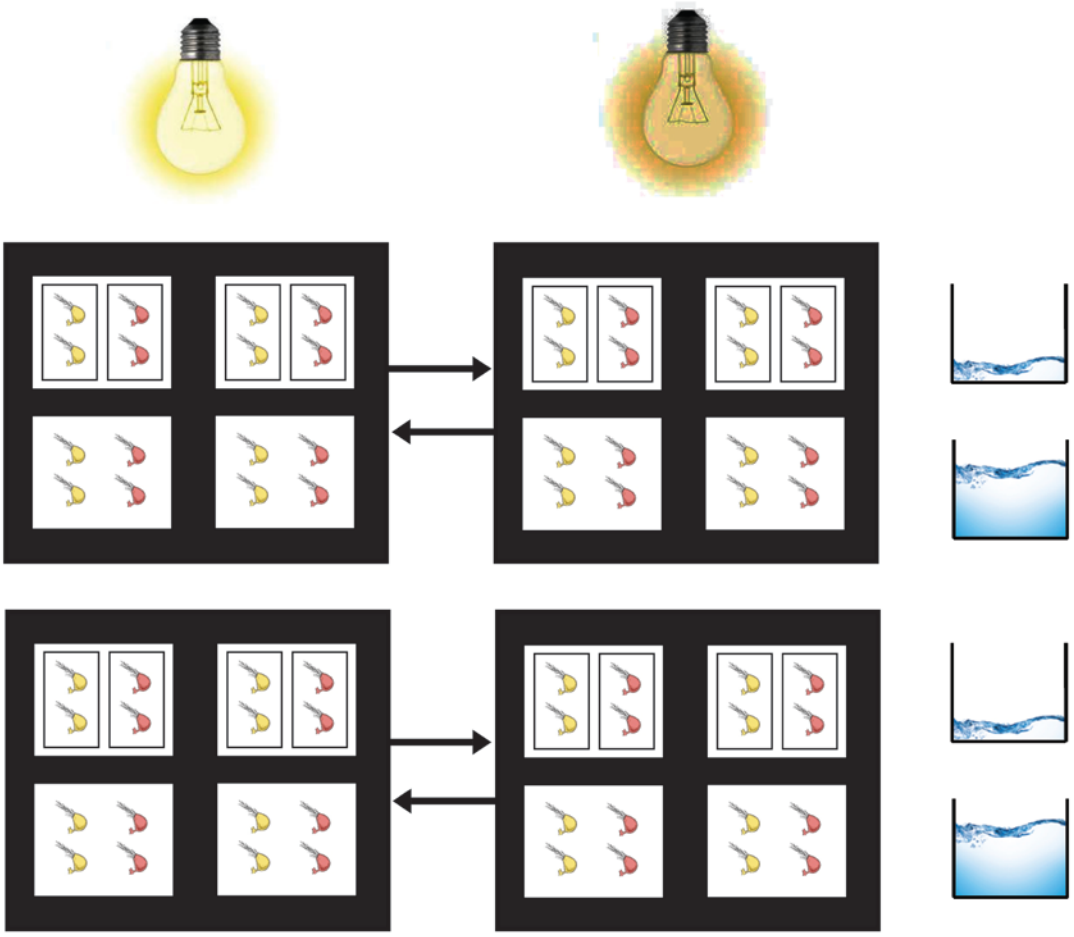

Fig. 3.1. Schematic diagram of a two factorial experiment testing the effects of light (top) and water level (right) on the trap size, expression of external trap morphology and investment in carnivory of *U. gibba*. Receptacles used in the experiment are as follows, from outside in: trays (black), tubs (white) and containers (black lines). Trap icons represent individual plants, colours indicate different clones. Randomised placement of plants within tubs and containers not illustrated. Arrows indicate water circulation via pumps.

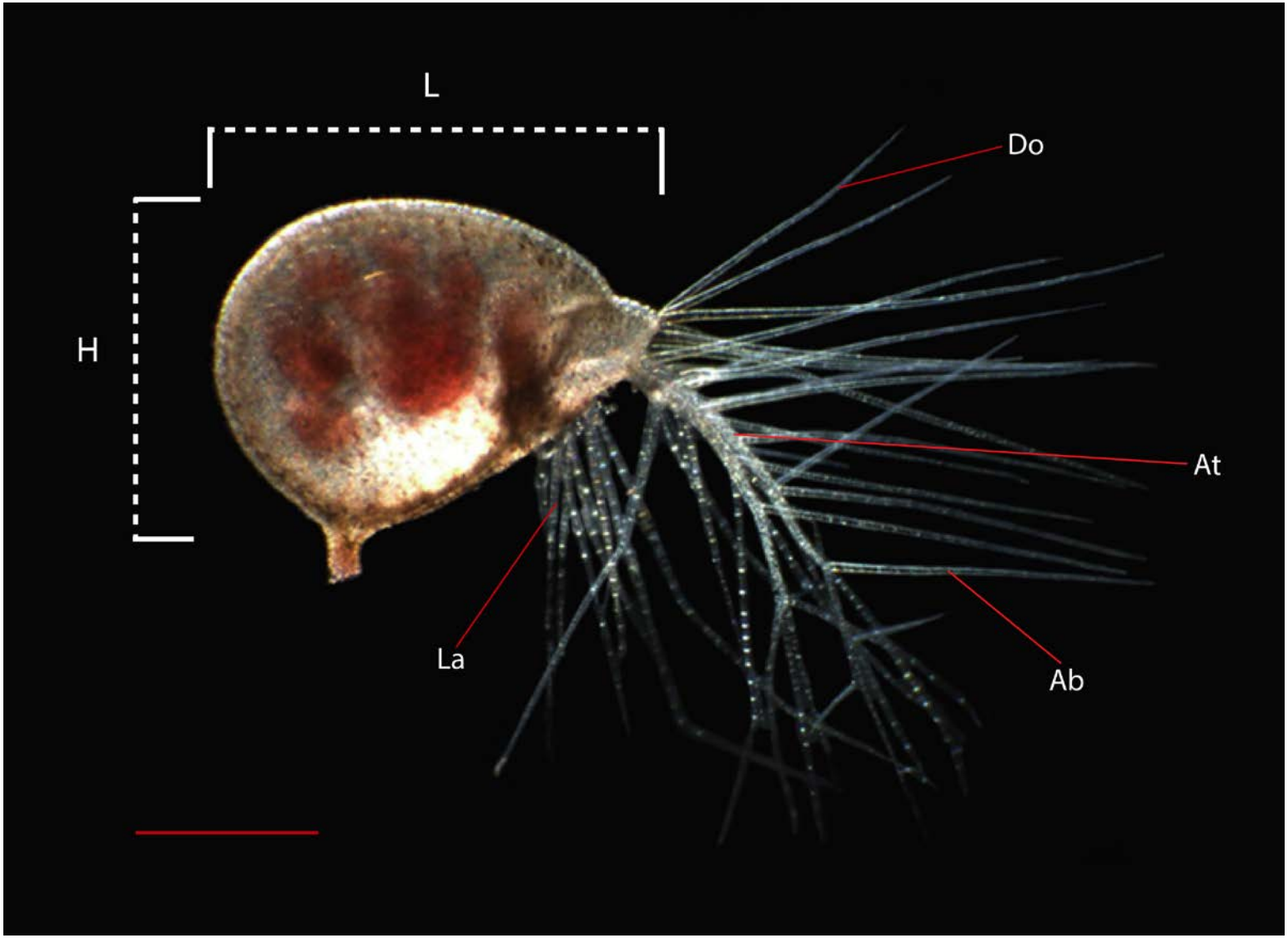

Fig. 3.2. Lateral view of a *U. gibba* trap. **L** = length; **H** = height; At = antenna trunk; **Ab** = antenna branch; **La** = lateral bristle; **Do** = dorsal bristle. Scale = 500 μm.

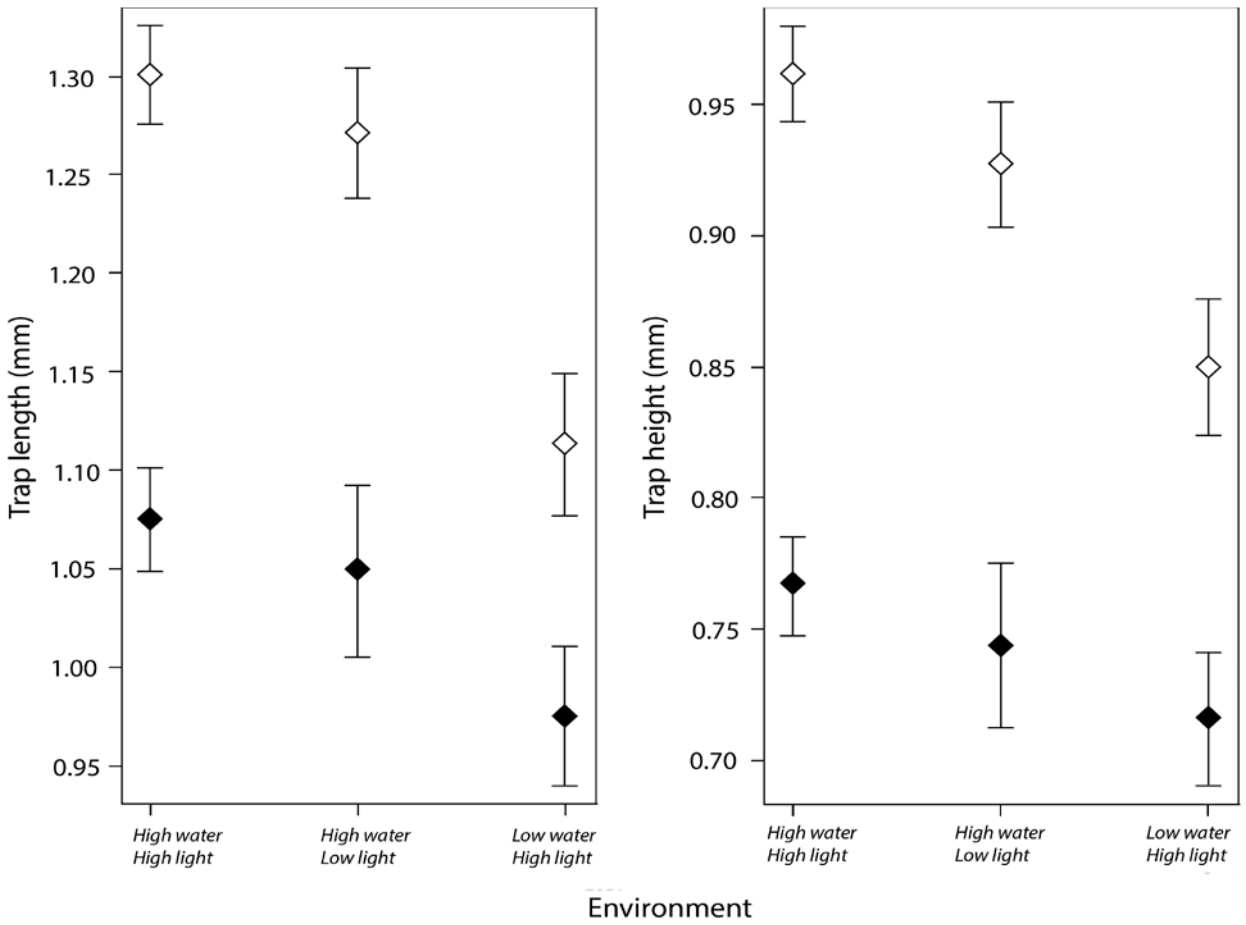

Fig. 3.3. Model estimated trap bladder length and height of two *U. gibba* clones (♦&◊) after a 21-day experiment where plants were grown under three sets of environmental conditions: High water level & High light, High water level & Low light, and Low water level & Low light. High water treatments had a ~30 cm water column; In low water treatments the substrate was saturated and there was no water column. High light and low light levels were 41% and 8% of adjacent outdoor incident light, respectively (see text for details). Bars indicate 95-percent confidence intervals.

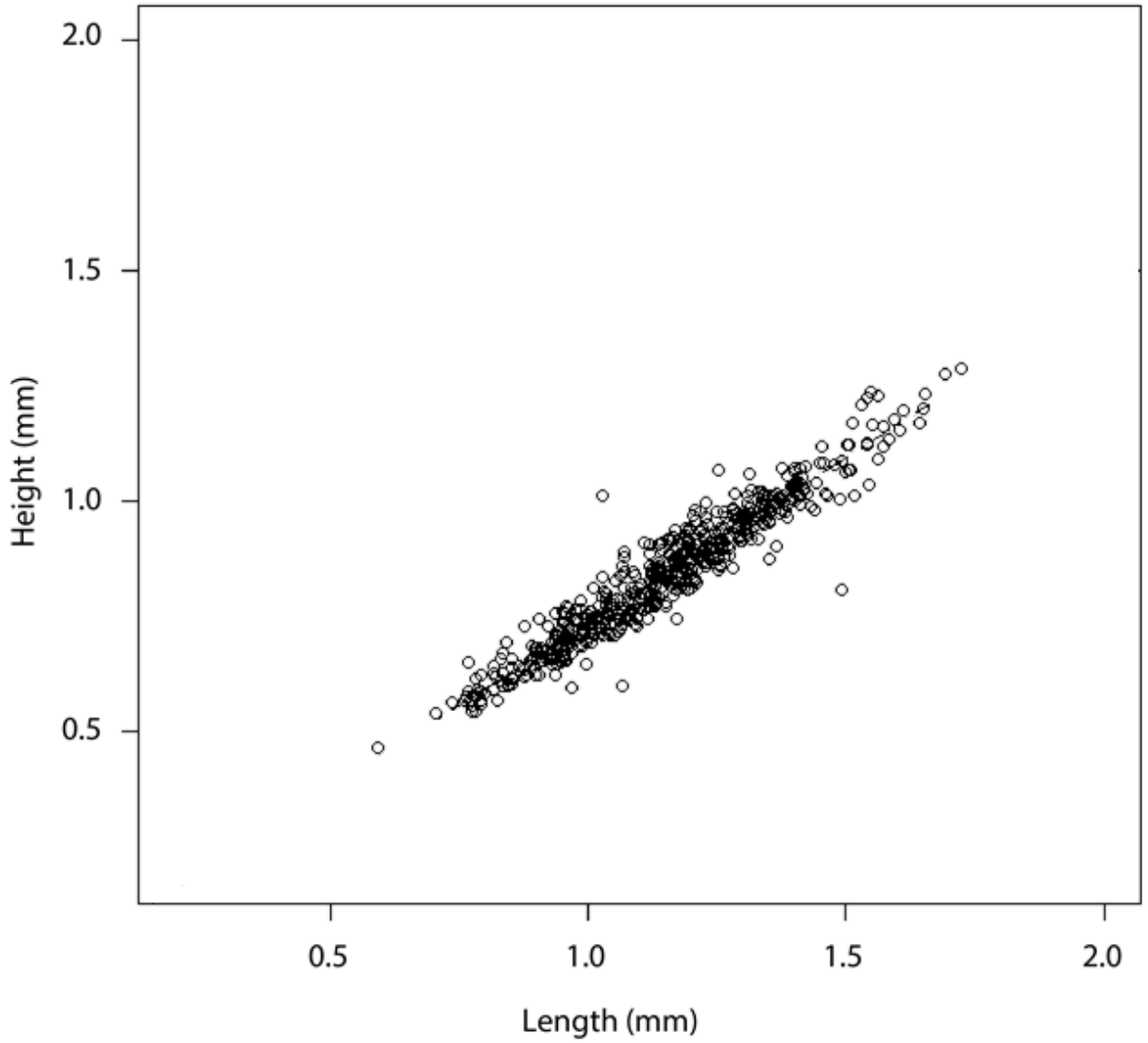

Fig. 3.4. Bivariate plot of height *vs* length of laterally presented *U gibba* traps from a 21-day growth experiment. Scaling relationship: Height = 0.017 + 0.717 Length. n =552.

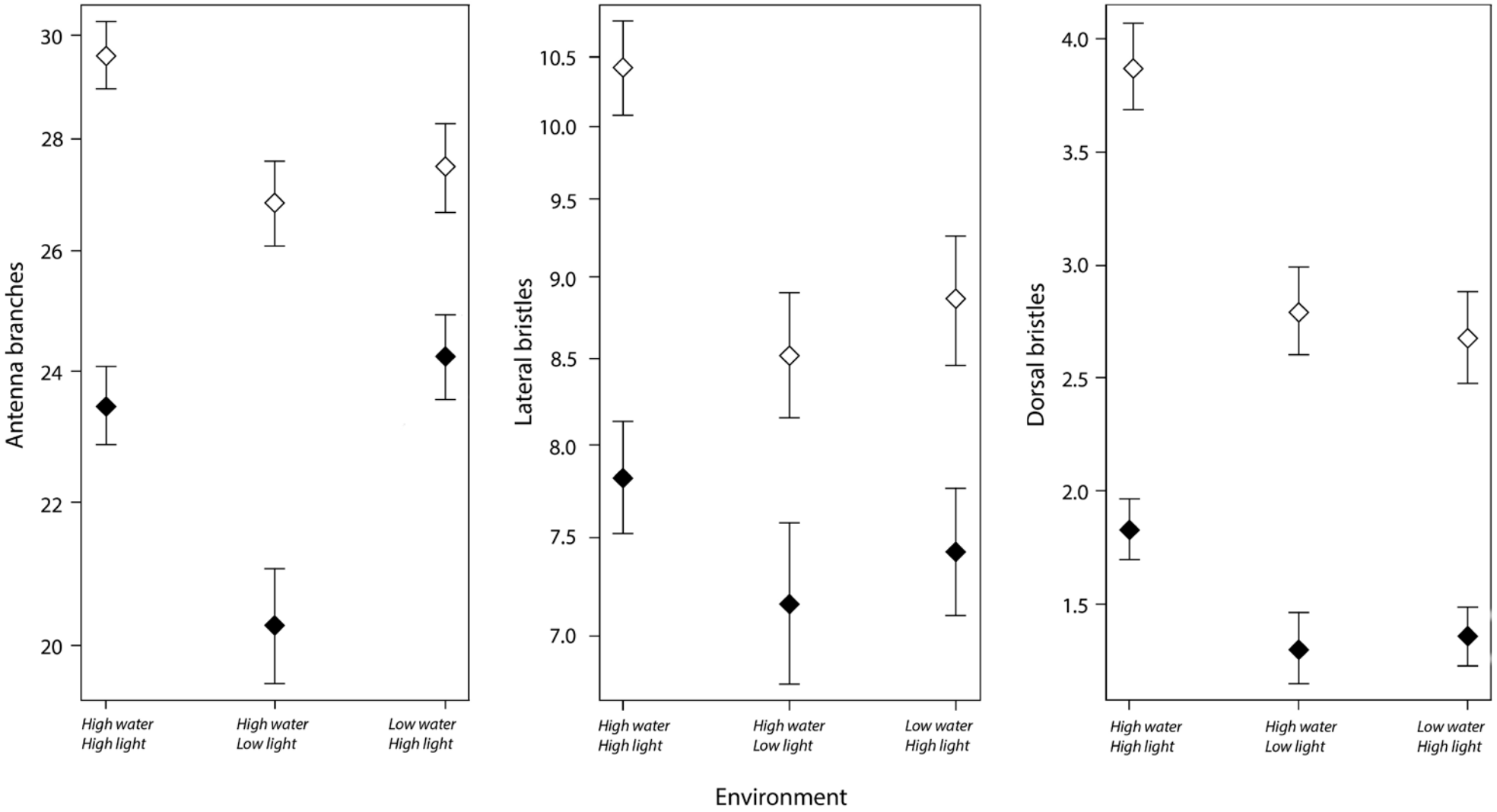

Fig. 3.5. Effects plot showing the model estimated appendages counts of two *U. gibba* clones (♦&◊) after a 21-day experiment where plants were grown under three sets of environmental conditions: High water level & High light, High water level &low light, and Low water level & low light. High water treatments had a ~30 cm water column; in the low water treatments the substrate was saturated, no water column. High light and low light levels were 41% and 8% of adjacent outdoor incident light, respectively (see text for details). Bars indicate 95-percent confidence intervals.

4. The effect of feeding and fertilisation on growth, trap size and appendage expression of the suspended aquatic *Utricularia australis*

4.1 Introduction

The production and maintenance of carnivorous organs is costly and requires structural, mineral and energetic investment from plants. (Friday, 1992; Knight, 1992; Adamec, 1997b). Mineral nutrition from prey (chiefly N and P) enhances plant performance in a variety of ways including growth (biomass, length of shoots, leaf size, number of leaves, root growth, stimulation of mineral nutrition uptake by roots) and sexual and asexual reproduction (number of flowers and seeds, size and nutrient content of seeds, size of propagules such as turions, axillary budding) (Darwin, 1875; Givnish *et al.*, 1984; Wilson, 1985; Thum, 1988; Friday and Quarmby, 1994; Zamora *et al.*, 1997; Otto, 1999; Adamec, 2002; Englund and Harms, 2003; Lenihan and Schultz, 2014). *Utricularia* spp. catch prey with mechanically activated suction traps – hollow, fluid filled bladders with an inward flexing trapdoor at one end (Lloyd, 2008). Traps have a short active life (Friday, 1989), but their structural, mineral and energetic costs are considerable (Adamec, 2006; Adamec, 2007a). The exterior of *Utricularia* L. (Lentibulariaceae) traps is adorned with trichomes of epidermal origin (Meierhofer, 1902), including robust structures around the trap door margin referred to as appendages. Appendage position and morphology, which vary greatly among species (Taylor, 1989), show a strong phylogenetic signal. Similarities also occur across independently derived habits (Lloyd, 1933; Taylor, 1989; Reifenrath *et al.*, 2006), suggesting they also evolve in response to habitat specific demands.

About 50 *Utricularia* species have an aquatic habit and their appendages are more conserved than those of terrestrial congeners. Appendages from the infra-generic section *Utricularia* take the form of paired branched dorsal structures called antennae, supplemented by sets of bristles (Darwin, 1875; Taylor, 1989). Meyers and Strickler (1979) provided evidence that antennae and bristles function as prey attractants, increasing capture rates. A high proportion of aquatic *Utricularia* (>80%) are found in the infra-generic section Utricularia. Of these, 29 are fully suspended (submerged) macrophytes (Taylor, 1989), the others are affixed aquatics and/or amphibious. Aquatic *Utricularia* grow in shallow, dystrophic (high in humic acids and tannins), lentic waters, usually with a high concentration of free CO_2 (>0.1mmol.l^{-1}), but poor in N and P, and often K (Adamec, 1997b, a; Adamec and Kovářová, 2006; Adamec, 2008b). The plants absorb nutrition through their shoots, either from captured prey or directly from the ambient water. Prey carcasses are high in N and P while being a relatively poor source of K, Ca and Mg for plants. While plants can also utilise organic carbon from prey carcasses the amount is not significant compared to the carbon acquired from free CO_2 (Adamec, 1997a).

Trap firing is not only triggered by meiofaunal prey but also occurs autonomously (Vincent *et al.*, 2011a). Phytoplankton drawn into the traps during spontaneous firing are utilised in the same way as animal prey and can provide adequate nutrition in environments where animal prey are absent or in low densities (Richards, 2001; Peroutka *et al.*, 2008; Koller-Peroutka *et al.*, 2014). Traps are also phytotelmata,

permanently hosting a community of unspecialised commensal organisms including algae, bacteria and *Paramecium* spp. (Mette *et al.*, 2000; Richards, 2001; Gordon and Pacheco, 2007; Peroutka *et al.*, 2008; Alkhalaf *et al.*, 2009; Sirová *et al.*, 2009). Prey capture may not be obligatory for *Utricularia* but it always (though variably) enhances plant growth (Sorenson and Jackson, 1968; Kosiba, 1992; Englund and Harms, 2003; Adamec, 2008a; Adamec *et al.*, 2010). Trap contents surveys have shown that aquatic *Utricularia* exhibit selective feeding, but the disproportionately trapped taxa vary among studies. Some have reported disproportionate trapping of cladocerans (Meyers and Strickler, 1979; Mette *et al.*, 2000; Richards, 2001), others of cyclopoid copepods (Harms, 1999; Harms and Johansson, 2000; Guiral and Rougier, 2007). Earlier studies have not always measured estimates of ambient prey densities to control for prey availability.

Aquatic *Utricularia* are able to change their structural investment in carnivory (IIC; the ratio of trap dry weight to leaf/stolon dry weight), in response to variations in habitat quality, such as water chemistry, prey availability and irradiance (Knight and Frost, 1991; Guisande *et al.*, 2004; Adamec, 2007a; Adamec, 2008b; Adamec *et al.*, 2010; Adamec, 2015, in press). Recent work by Adamec (2008b; 2015, in press) proposes the IIC is regulated by through two components: negative feedback from endogenous nutrient (N and P) content, dominated by photosynthetic (CO_2) regulation. Under common photosynthetic conditions of shaded growth, with poor to medium concentrations of CO_2, a decline in shoot N and/or P stimulates trap production and enhances prey capture. This leads to an increase in shoot N and/or P content and a downregulation of trap production, beginning the cycle again. However, when light is optimal and CO_2 is not limiting, the IIC is upregulated by excess photosynthates (see **1.2.6** for more details). Manjarrés-Hernández *et al*. (2006) propose that, due to their role in prey attraction, aquatic appendages (antennae and bristles) also constitute an investment in carnivory, and that their expression may be regulated by the same mechanisms as the IIC.

A field study (Englund and Harms, 2003) has demonstrated a negative relationship between IIC and prey addition. However, the authors were unable to separate the effect of prey capture (feeding) from a rise in ambient nutrition in the surrounding waters due to the presence of prey. Aquatic plants frequently grow in water that are high in free CO_2 but poor in N and P, and often K (Adamec, 1997b, a; Adamec and Kovářová, 2006; Adamec, 2008b). Aquatic carnivorous plants have a high affinity for nutrient uptake from the ambient medium (Adamec, 2009), but so do other suspended macrophytes (Sculthorpe, 1971). Therefore, after radiating into the fully aquatic environment, nutrition from prey would still be important for *Utricularia* in maintaining a competitive edge against other non-carnivorous plants. Here I test the hypothesis that increases in prey derived nutrition and ambient nutrition both positively affect plant growth.

In this two-factor, fully-crossed study I aim to quantify the relative contribution of prey capture (feeding) and/or ambient mineral nutrition (fertilisation) on variety of plant growth responses in the submerged aquatic *U. australis,* including ramification, biomass production, shoot to leaf dry weight (DW) ratio, and IIC. I examine the effect of feeding and fertilisation on trap characters such as size, number per leaf and appendage expression in the context of the relative trap-insertion position within a leaf node, to better understand whether and how the distribution of trap sizes per leaf changes in response to environment, and its relationship to the number of traps produced per leaf. *U. australis* is an ideal model for addressing such questions as lateral traps exhibit a gentle size gradient, as opposed to e.g. *U. vulgaris,* where size distributions of the midline and peripheral lateral traps are very disjoint (Friday, 1991). I also test for a correlation between trap size and capture rate (number of captures per trap).

In addition, I take the opportunity afforded by controlled prey addition to address the question of selective predation. I testing the hypothesis that *U. australis* feeds on the filament feeding cladoceran *C. sphaericus* in preference to another cladoceran, the filter feeding *Simocephalus* cf. *vetulus* and the predatory cyclopoid copepods *Acanthocyclops robustus* and *Mesocyclops* cf. *leuckarti.*

Utricularia australis R.Br is widely distributed in Europe and Australasia, tolerating variable water chemistry (Kosiba, 2004; Adamec and Kovářová, 2006; Adamec, 2008b, 2009). Fast growing, it can produce 2.6-3.5 leaf whorls a day and propagates by branching (Adamec and Kovářová, 2006; Adamec *et al.*, 2010; Adamec, 2011d). The monomorphic shoots have a modular structure of asymmetric, two-lobed leaves, separated by internodes (**Fig. 4.1A)**. Each leaf lobe is dichotomously divided into further filaments. Traps are dimorphic with i) numerous lateral traps (0.5-2.5 mm long) inserted on capillary leaf segments (Taylor, 1989) or in place of a leaf filament in a dichotomy (depending on perspective), with a single leaf incorporating a range of trap sizes while ii) smaller basal traps, with truncated antennal trunks, uniform in size, grow in the angle between the shoot and primary leaf segment (Taylor, 1989) (**Fig. 4.1B**). Like other suspended aquatics in section *Utricularia* the gradient of trap sizes within a leaf is not a function of age or developmental duration as most traps on a leaf mature on the same day (Friday, 1989; Friday, 1991). Appendage expression is typical for section *Utricularia* consisting of paired, branched dorsal antennae and supplementary bristles sets (left and right lateral sets only) (Taylor, 1989). *U. australis* has been used in a number of ecophysical experiments investigating growth and IIC (Adamec, 2011b; Adamec, 2012b).

4.2 Materials and Methods

4.2.1 Plants

I used shoots of a single *Utricularia australis* clone originally collected from Lake Ohia in the upper North Island of New Zealand (34°58'53.36"S, 173°21'50.24"E). All plant material was derived from two-year-old indoor greenhouse cultivations. Prior to the experiment, plants were cultivated from overwintered turions in a 150 l glass aquarium, layered with *Carex* litter and washed river sand, and filled with deionised water. Plants were unshaded and exposed to clear midday mean PAR of 873 µmol m^{-2} s^{-1} (712-996 µmol m^{-2} s^{-1}, n=3). No prey or additional CO_2 were provided.

4.2.2 Growth experiment

The experimental design was similar to that used for the growth experiment in Chapter Three and was again conducted in the rooftop greenhouse of the School of Biological Sciences, University of Auckland. In this instance only two types of receptacles were used, 12 l tubs and 35 l trays. The tubs were drilled and meshed as in Chapter Three. Four tubs were allocated to each of the six trays (**Fig. 4.2**). All trays were placed under two 60W metal halide lamps. On 20 January 2014 I added 25 g DW of rinsed, autoclaved and oven-dried *Carex secta* Bott leaf litter to each tub and filled the trays with 25 l of deionised water, which in turn filled the tubs. I then connected the trays into two sets of three with six 7 Watt, 220 l/h pumps (Aleas), one set to provide fertilised treatments, the other unfertilised treatments. CO_2 was supplied to each treatment block by 88g pressurised kits (Fluval). Glutaraldehyde in the form of a liquid (API) was also added every three days at 80 µl per litre to boost CO_2 levels. This concentration was well below toxic levels for aquatic plants and animals (Pereira *et al.*, 2014). I cleaned the mesh windows and exchanged approximately 80% of the water in the tubs every two days, by stirring the substrate and slowly lifting the tubs to let water drain into the trays through the mesh. Gently depressing the tubs caused them to refill. Additional deionised water was added trays over the duration of the experiment to offset evaporation.

On 28 January 2014 I created fed treatments by inoculating one tub in each tray (six in total) with a mix of pre-cultivated microcrustaceans: two cladoceran species, *Chydorus sphaericus* (Chydoridae, Diplostraca) and *Simocephalus* cf. *vetulus* (Daphniidae, Diplostraca), an ostracod *Cypridopsis vidua* (Cyprididae, Podocopida) and two cyclopoid copepod species, *Acanthocyclops robustus* (Cyclopidae, Cyclopoida) and *Mesocyclops* cf. *leuckarti* (Cyclopidae, Cyclopoida). I introduced animals by pipetting 3ml amounts of water from zooplankton cultivation tanks using clear Pasteur pipettes. Pipettes were checked under a stereoscope to ensure the presence of animals. With the exception of *C. vidua*, I added additional prey in this way every seven days for the duration of the experiment. The remaining six tubs functioned as unfed treatments. Trays were also inoculated with cyclopoid copepods to help reduce

ambient nutrient differences between fed and unfed treatments. I inspected the unfed tubs at six to seven day intervals for unwanted animals. Sticky traps (Biobest) were suspended above the tubs to reduce numbers of aquatic fly larvae.

On 04 February 2014, after allowing the substrate to pre-soak for 14 days, I randomly selected 67 starved *U. australis* shoots and prepared them as follows: i) Removal from plant-cultivation tank, rinsing with deionised water and placement in cell culture dishes containing filtered tank water, ii) Trimming at the halfway point along the internode between the eighth and ninth adult nodes behind the shoot apex, iii) Removal of any side shoots using 2x 0.15mm cutting edge spring scissors (Vannass) under a stereoscope (Leica) at x25 and x40 magnification, iv) Any remaining algae and detritus gently rinsed and scraped away and v) Cotton tied carefully between the shoot apex and the first preceding node. No traps were removed. Five shoots, hereafter referred to as plants, were then randomly allocated to each tub, for a total of 60 plants. The remaining seven plants were dried and weighed to provide a baseline for calculating biomass production.

I added mineral nutrients to each tray in the fertilised treatment block four times during the experiment at five day intervals. For the first loading I added nitrogen, phosphorus and potassium in the form of 0.5mg l^1 NH_4Cl, 0.15 mg l^{-1} NaH_2PO_4 and 3 mg l^{-1} KCl respectively (all salts from Sigma Technologies). The next two nutrient loadings were identical to the first. For the fourth and final round of fertilisation, only NH_4Cl, 0.15 mg l^{-1} and NaH_2PO_4 were added at 0.25 and 0.15 mg l^{-1} respectively (**Table 4.1A**). The following water chemistry parameters for fertilised and unfertilised water were estimated at seven day intervals throughout the experiment, from tray water: total phosphate (persulphate digestion and colorimetry/discrete analyser), nitrate (Anions by Ion Chromatography), and K (ICP-MS trace, default digest). Total alkalinity or TA (titration to pH 4.5, M-alkalinity, autotitrator, modified for alkalinity <20), was estimated at three day intervals. To compare the chemistry of the tray water with that inside individual tubs, I took additional water samples from two pairs of randomly selected tubs within the fertilised treatment (with and without prey added), on the final day of water sampling. Nutrient analyses were conducted by Watercare Laboratory Services, Auckland New Zealand. TA estimates were provided by Hill Laboratories, Hamilton, New Zealand.

I measured pH and water temperature (HQd Portable Meter, HACH) and calculated dissolved CO_2 from pH and TA (after Helder, 1988), also at three day intervals. I sampled water for analyses between 11:00 and 13:00 hours. Water temperature, pH, and TA were similar for both fertilised (21.3 – 24.8°C; 6.17 - 6.85; 0.3-0.4 meq.l^{-1}) and unfertilised treatments (22.5 -26.3°C; 5.80-6.95; 0.3-0.4 meq.l^{-1}) treatments. Free CO_2 was similar in both fertilised (0.138-0.235mM) and unfertilised (0.107- 0.329 mM) treatments

(**Table 4.1A**). The mean midday irradiance at plant level (~5 mm below the water level) during the course of the experiment was 317 (± 3.3) µmol m^{-2} s^{-1} (226-383 µmol m^{-2} s^{-1}), 41% (34-44%) of adjacent outdoor incident light.

On 17 February, after 13 days, halfway through the experiment, I removed and gently rinsed all plants. I removed and filtered 12 l of water from each tub, and rinsed the substrate to remove algal build-up and dislodge copepods and chironomid larvae. I returned the water to the trays. I then cleaned the mesh windows thoroughly using 10 % ethanol and rinsed the tubs before replacing them in the trays and returning the substrate and plants. Animals filtered from the fed (prey added) treatment water were also returned. I terminated the experiment after 21 days on 25 February. I removed all plants from treatment, fixing them in 25% ethanol for preservation during the lengthy data collection process. I collected and filtered the water separately from each tub, then rinsed the substrate, also collecting and filtering the rinsate. I preserved filtrates in 25% ethanol. Prior to data collection all plant material was stained with an aqueous, 1% weight per volume solution of Congo red to improve visibility of trap structures and of captured prey.

4.2.3 Data collection

Prey capture and disproportionate representation of taxa within traps.

I identified and counted all whole animals present in tub filtrates using a stereo microscope to estimate ambient zooplankton densities for each tub at time of termination (number of animals of each species per 12 l). I examined all traps for evidence of feeding and recorded the proportion of traps per plant with captured prey. After taking linear measurements, but before drying and weighing (see below), I opened all traps from the fed treatments, from 10 nodes after the cotton tag to the FMN using a 0.2 mm thick micro knife with 4mm cutting edge (Sharpoint) to make an incision along the trap midline from the dorsal margin of the trap door to stalk. I carefully removed the contents, and counted and identified the captured animals. Partial animals were not considered. Easy identification was possible due to the high affinity of crustacean exoskeletons for Congo red (Michels and Büntzow, 2010) and the limited number of species present. I tested for a Relationship between the number of prey per trap and trap size (as trap length) for traps with one or more captures. For this analysis all whole animals were counted. To test over representation of particular prey taxa in traps (as compared to their abundance in the ambient environment), I compared the number of animals of each species per plant trapped, with the total number collected from the tub water at the termination of the experiment. Only a subset of four crustacean species was considered: *C. sphaericus*, *S.* cf. *vetulus* and collectively, as cyclopoid copepods, *A. robustus* and *M. leuckarti*. *C. vidua* was apparently extirpated in all treatments by the 13th day of the experiment and any populations of chironomid and culicidae larvae arising from uncontrolled inoculations were ephemeral.

Shoot and node characters

I dismantled plants into their component nodes (leaf nodes) which I stored separately in 25% ethanol. I numbered the node immediately subsequent to the cotton tag node one, the second node two, and so forth until the first mature node (FMN) before the shoot apex (**Fig. 4.2A**). I defined the FMN as the first node where all traps exhibited unfurled appendages (Friday, 1989). Any following younger nodes were left combined with the shoot apex. Mature nodes were then assigned a relative shoot position as a proportion: node number/total number of mature nodes subsequent to the tag cotton. I inventoried each node for the number of: i) basal traps, ii) lateral traps and their trap insertion positions relative to the leaf base iii) shoot initiation in leaf dichotomies, and iv) branches (side shoots). Side shoots were considered 'developed' if primordial traps were visible in their apex. I registered lateral traps according to their insertion within a leaf whorl, based on an arbitrary classification of leaf filament architecture (after Friday, 1991) that preserved the latitudinal and longitudinal position of traps within the leaf lobe (**Fig. 4.1B**).

Trap characters

Using the nodes 10 - FMN I conducted the following analyses: I measured three responses for laterally orientated traps at all trap-insertion positions (including basal traps) using digital photographs taken using a macroscope (Leica) i) trap length, ii) trap height, perpendicular to length and iii) area of laterally presented bladder surface (hereafter referred to as trap area) (**Fig. 4.1C).** For analyses of length and height only the following traps were used as they were common to all treatments: i) lateral traps inserted on the primary, first four secondary filaments, and first tertiary filament, ii) basal traps (n=5507). For the analysis of area, basal traps were not considered (n= 4738) as their shape in lateral projection was markedly different to that of the lateral traps and therefore not directly comparable (**Fig. 4.1C**). I quantified appendage expression by counting the number of supplementary bristles (left and right lateral bristle sets were pooled) and the number of antenna 'branches' (antennal bristling) for trap-insertion positions 11 and 21, on every plant. I counted the same bristle sets for traps at all other positions on a subsample of two plants per treatment. For a selection of four nodes from a subsample of two plants per treatment (relative shoot positions 0.5 – 0.8), I measured the trunk length and total length of one randomly chosen antenna of traps at positions one and two, using photographs taken with a Ti-E inverted microscope (Nikon). The ratio of antenna length to trap length provided a metric for appendage investment. I made linear measurements using ImageJ (Abramoff *et al.*, 2004) from digital photographs taken with a stereo macroscope (Leica) and counts using a Wild M3C stereoscope (Leica) at 25–40 x magnifications.

Biomass production, module DW and investment in carnivory

I pooled the nodes of the main stolon of each plant into three groups: i) Nodes one – nine, ii) Nodes 10 – FMN, and iii) The shoot apex (**Fig. 4.1A**). For nodes 10 – FMN the stolon, leaves, lateral and basal traps were considered separately. For nodes one - nine and the shoot apex all modules were combined. Any side shoots were pooled and their modules combined. I compared the dry weights of plant modules and ratios (for example stolon:leaf), among treatments, and used the total dry weight of each plant in conjunction with the averaged dry weight of the seven pre-treatment shoots lengths to derive biomass production. I made two measures of investment in carnivory using dry weight ratios: i) Trap number to dry weight of combined leaf and stolon and ii) Trap dry weight to combined leaf and stolon dry weight. I conducted drying and weighing as follows. I weighed empty 157µl tin capsules or standard sized tin foil cones (Elemental Microanalysis) using a micro-balance (UMX5 Comparator, Mettler Toledo, Greifensee Switzerland). Reported weights for all empty receptacles stabilised in under a minute. I transferred plant modules to the weighing receptacles for drying in an anhydric incubator (Gallenkamp) at 65°C degrees for four hours. I then determined the dry weight to the nearest 10 µg by subtracting the weight of the empty receptacle. I added silicon beads to the foil cones and the mouths of capsules between removal from the oven and weighing to minimise rehydration. As there was no dehumidification in the balance chamber, the plant material began to hydrate immediately at point of weighing. As a compromise I recorded all weights after three minutes. For 20 plants all weights were recorded three times and averages taken. The range of standard errors generated by this replication was used to determine the accuracy of reporting. Dry weights cannot be taken as absolute measures, as storage in ethanol prior to weighing would have resulted in leaching of alcohol-soluble metabolites, affecting weight (Howmiller, 1972; invertebrate examples). Under the assumption of a similar rate of leaching across all samples dry weights are still suitable for comparative measures.

4.2.4 Statistical treatment

Analyses were conducted in R (Team, 2014) using mixed models (unless otherwise specified). In many analyses the tub and tray were included in models as nested random effects to account for the pseudoreplication within tubs, the lack of independence between tubs sharing the same tray, and any positional effect arising from the placement of receptacles (see **3.2.4** for more details). Linear Mixed Models (LMMs), used for continuous responses, were created with **nlme** (Pinheiro *et al.*, 2014). Shapiro-Wilk tests of normality for the residuals of LMMs are provided in **Appendix 1**. In the cases where these tests rejected the null hypothesis that the residuals were normally distributed, the assumption of normality was still made if the normal Q-Q plots for the LMMs passed the fat pencil test (were centred) on inspection. Ratios were log transformed.

GLMMs using other distribution families were created with **lme4** (Bates *et al.*, 2014) or **MASS** (Venables and Ripley, 2002). For LMMs I assessed statistical significance of model terms (main effects and interactions) for factors with more than two levels using ANOVAs based on the reduction of residual deviance. Tukey contrasts of multiple comparisons were performed using **multcomp** (Hothorn *et al.*, 2008) For tabulated regression output see **Appendix 2**.

In the case of both continuous and count response variables, models were fitted sequentially. Non-significant interactions were removed from models when i) In the case of continuous response variables, the subsequent model (the model without the non-significant interaction) had a smaller AIC than the previous one (the model including the non-significant interaction) or ii) arbitrarily for count data modelled with a quasipoisson distribution. For all experiments the critical value for rejecting the null hypothesis was taken as $\alpha = 0.05$.

Prey capture and selection:

I used GLMMs with binomial distributions and the random effects of tubs nested within trays to check for a difference in the proportion of traps with captures between i) fed and unfed treatments, ii) fertilised and unfertilised treatments, within the fed treatments, and iii) basal and lateral traps. I also used GLMMs with negative binomial distributions to test: i) whether the number of animals caught per trap was correlated to trap size (laterally presented trap area), and ii) for prey selection on counts of three microcrustacean taxa (the cyclopoid copepod species were combined) in two locations, trapped by plants and within the ambient tub water. I used a prey species-by-location interaction in the model to test for a difference in the pattern of counts, by species, between the traps and ambient water. For the first analysis I included shoot position as a random factor as the number of captures per trap varied depending on the position of the trap-bearing node in relation to the shoot apex. For the second analysis I included plants nested in tubs as random factors to allow for variation due to prey availability and species composition between tubs. Negative binomial distributions were used in preference to Poisson because the counts were overdispersed (Lindén and Mäntyniemi, 2011) See **Appendix 1.3** for dispersion parameters generated by preliminary quasi-Poisson modelling).

Plant growth characters

I used LMMs for dry weights and dry weight ratios. For counts (node number) and proportions (number of nodes with side shoots) I used GLMMs with Poisson and binomial distributions, respectively. In all cases feeding and fertilisation were fixed factors, tubs nested within trays were included as random factors.

Trap characters.

Linear morphometric measurements of the trap bladders (length, height, area), and ratios of antenna length to trap length were analysed with LMMs (Fixed factors: feeding, fertilisation, trap-insertion position; random factors: tubs nested within trays). Relative shoot position (relative position of the trap-bearing node along the main shoot axis) was included as a covariate in the case of the trap bladder characters. The statistical significance of model terms (main effects and interactions) for factors with more than two levels was assessed using ANOVAs. The data for supplementary bristle and antenna bristle counts were underdispersed and therefore modelled with feeding, fertilisation and trap-insertion position as fixed factors, and without random effects, using a General Linear Model (GLM) with a quasipoisson distribution, as functions from **lme4** do not accept quasi-distributions. For the analysis of appendage expression, the statistical significance of model terms for factors with more than two levels was assessed using a chi-squared test based on the reduction of residual deviance.

4.3 Results

4.3.1 Fertilisation

Despite initial loading of fertilised treatment water with 0.5mg l^{-1} NH_4Cl, 0.15 mg l^{-1} NaH_2PO_4 and 3 mg l^{-1} KCl, 24 hrs later (also 24 hours after the introduction of plants) there was little detected difference between the fertilised and unfertilised treatments in the concentrations of PO_4^{3-} (high at 0.93 mg l^{-1} and 0.92 mg l^{-1}, respectively) and NH_4^+ (both below the detection limit of 0.01 mg l^{-1}). Total K was 2.6 mg l^{-1} higher in the fertilised water. By the 14th day of the experiment (after two more nutrient loadings) both fertilised and unfertilised waters were barely mesotrophic (0.02 mg l^{-1} PO_4^{3-}-P; 0.02 mg l^{-1} NH_4^+ -N). At the termination of the experiment the unfertilised treatment water had become oligotrophic (0.01 mg l^{-1} PO_4^{3-}-P; <0.01 mg l^{-1} NH_4^+ -N) (nutrient concentration differences among trophic levels after Adamec, 2008b) (**Table 4.1B**). The single instance of sampling comparing tray and individual treatment water revealed little difference among treatments and between treatments and the tray water in pH, temperature, and TA. There was no difference in PO_4^{3-}P concentrations between the fed and unfed treatment tubs (both 0.03 mg l^{-1}), but these were lower than the 0.05 mg l^{-1} recorded in the tray. NH_4^+ -N concentrations in the treatment tubs were slightly less than those in the tray water (**Table 4.1B**).

4.3.2 Prey capture

The unfed treatments did not remain free of animals throughout the course of the experiment. Chironomid and culicid flies were not completely deterred by the sticky traps and their larvae hatched within a range of treatment tubs. Copepod nauplii in their smallest instars seemed able to penetrate the mesh screen and enter the unfed treatments. However, prey densities in the unfed treatments remained extremely low, with 20 of the 30 plants showing no evidence of prey capture (**Table 4.2**). The proportion of traps with

captures was significantly lower in the unfed treatments than the fed treatments (z=6.486, p<0.001). For fed treatments, there was no significant difference in the proportion of traps with captures between fertilised and unfertilised treatments (z=1.275, p=0.202; **Fig. 4.3**). Basal traps contained a significantly lower proportion containing trapped animals (z =11.17, p=<0.001; **Fig. 4.4**).

C. sphaericus, *S.* cf. *vetulus*, *A. robustus* and *M.* cf. *leuckarti* made up 95% (± 0.4%) of captures within each trap (n = 1467). The remainder were chironomid and culicid larvae and *C. vidua* (two individuals). Nauplii and copepod eggs (with no accompanying female) were also recorded. The number of captures per trap increased with trap size (θ=10.815, t=4.378, p<0.0001), with a multiplicative increase of 1.1 animals trapped for every 1 mm^2 increase in trap area. After allowing for the variance in the number of prey per trap attributable to trap size, 19% of remaining residual variance in prey counts was attributable to the position of the trap-bearing leaf on the shoot (the remaining due to the difference between plant fragments). After shoot positions 0.6-0.7 the number of animals per trap generally decreased the closer the associated leaf was to the shoot apex (**Fig 4.5**).

4.3.3 Prey selection

U. australis preyed on cyclopoid copepods in disproportionately larger numbers to both *C. sphaericus* and *S.* cf. *vetulus* when the number of animals of each species trapped was compared to their abundance in the surrounding waters at the end of the experiment. The mean numbers (±se) of each prey species trapped per plant over 21 days were: *C. sphaericus*, 21 (±0.7); *S.* cf. *vetulus*, 2 (±0.1); cyclopoid copepods, 88 (±2.9). The mean numbers present in the tub water at the end of the experiment were: *C. sphaericus*, 187 (±25.3); *S.* cf. *vetulus*, 53 (±11.3); cyclopoid copepods, 60 (±2.9). There were 2.44 times more *C. sphaericus* than cyclopoid copepods in the tubs but 4.27 times more cyclopoid copepods than *C. sphaericus* trapped by the plants. This difference in ratios was highly significant (θ=1.023, t=4.267, p<0.0001). The difference in ratios between *S.* cf. *vetulus* and the cyclopoid copepods was also significant (θ=1.023, t=6.509, p<0.0001) with 1.25 times more cyclopoid copepods in the tubs than *S.* cf. *vetulus* but plants captured 49.7 times more cyclopoid copepods than *S.* cf. *vetulus*. After allowing for the variance of microcrustacean counts attributable to the main effects and their interaction, 26% of remaining residual variance in prey counts was attributable to differences between tubs; the remainder was due to the difference between plants.

4.3.4 Shoot and node characters

Ramification, module DW's, biomass gain and IIC

While prey addition (feeding) and/or fertilisation both produced increases in the mean values of most plant growth parameters, feeding had the greatest effect (**Table 4.2**). Almost no branching occurred in

plants from the unfed, fertilisation-free treatments. In other treatments a primordial shoot apex was always visible in the leaf axil of each node, often showing signs of initiation (elongation of the first internode) and more occasionally developed into a side shoot with mature nodes. In the fertilised treatments, primordial shoot apices could also be seen on the leaves themselves, forming in the dichotomies between filaments. The amount of ramification per plant increased with both fertilisation and feeding as measured by the following three parameters: i) Number of side shoots plant^{-1}, ii) Proportion of nodes with side shoots plant^{-1} and iii) Side shoot dry weight (DW) biomass as % of total plant DW biomass. Fertilisation significantly increased the proportion of nodes with side shoots ($z=2.375$, $p=0.018$) but feeding did not ($z=1.883$, $p=0.060$). Conversely, feeding significantly increased the DW biomass of side shoots relative to total plant DW biomass ($t=2.262$, $p=0.050$) whereas fertilisation did not ($t=1.395$, $p=0.235$) (**Table 4.2**). There was no significant interaction between feeding and fertilisation for either parameter and these terms were removed from the models. Only feeding significantly increased the number of mature nodes produced by plants ($t=2.262$, $p<0.0001$) (fertilisation: $t=1.395$, $p=0.235$).

The addition of prey also significantly increased the following DW parameters: stolon ($F_{1,5}=16.997$, $p=0.009$), leaf ($F_{1,5}=23.250$, $p=0.005$), lateral trap ($F_{1,5}=17.134$, $p=0.009$) and basal trap ($F_{1,5}=17.865$, $p=0.008$) dry weights, and biomass gain ($F_{1,5}=10.118$, $p=0.025$). Fertilisation did not significantly affect these parameters (see **Appendix 3.2**). The percentage of the total DW of vegetative organs (traps excluded) made up of stolon was significantly affected by fertilisation ($F_{1,5}=8.979$, $p=0.040$). The investment in carnivory (IIC, % of the total plant biomass made up of trap biomass) was high across all treatments (40-50%) and was little affected by either feeding ($t=1.680$, $p= 0.154$) or fertilisation ($t=2.528$, $p= 0.065$), although pairwise tests showed either adding prey or nutrient loading did result in a small, significant increase in the IIC compared to the unfed, unfertilised treatments (feeding: $z=2.485$, $p=0.041$; fertilisation: $z=3.036$, $p=0.009$). In contrast neither feeding ($z=2.175$, $p=0.088$) nor fertilisation ($z=-0.087$, $p=0.9999$) affected the number of traps per leaf DW (**Table 4.2**). The addition of prey to the fertilised treatments caused a significant reduction in the number of traps per leaf DW ($z=-2.989$, $p=0.009$), but this result was skewed by comparatively poor shoot growth in the pseudoreplicates of one of the unfed, fertilised treatments.

4.3.5 Trap characters

Bladder length, height and area

Mature leaves of all plants bore multiple lateral traps on primary (the main axis of a leaf lobe), secondary and tertiary filaments (**Fig. 4.1B**). 87.5% (SE± 0.85) of nodes per plant had at least one basal trap. Lateral trap size parameters (length, height and area) varied within leaves. On any given leaf lobe, the size

distribution of the traps conformed to the same pattern: Traps inserted closer to the base of the leaf lobes were larger than those closer to leaf apices, and traps closer to the axis of the leaf lobe were larger than those closer to the margins at corresponding latitudes (**Table 4.3**). Trap length and height were correlated for both lateral ($F_{1,4840}$ = 1.387*10^4, p < 0.0001, adjusted r^2 = 0.741), and basal ($F_{1,761}$ = 97.540, p < 0.0001, adjusted r^2 = 0.112) traps. The predicted height of lateral traps in mm was equal to 0.058 + 0.781 length. The predicted height of basal traps in mm was equal to 0.101 + 0.689 length.

Prey addition rather than fertilisation had the strongest effect on trap size, producing increases in length, height and area. There were no significant three-way interactions between feeding, fertilisation, and trap-insertion position for trap length (ANOVA: $F_{6, 5470}$=1.860, p=0.084), trap height ($F_{6, 5470}$=0.234, p=0.966) and lateral trap area ($F_{5, 4705}$=0.738, p=0.595). As there was no evidence of a multiplicative effect of feeding and fertilisation working together on these three parameters that differed depending on the trap-insertion position, this term was dropped from the models. Similarly, there were no significant interactions between feeding and fertilisation on length (Test from ANOVA: $F_{1,4}$=0.004, p=0.952), height ($F_{1,4}$=1.161, p=0.709) or lateral trap area ($F_{1,4}$=0.213, p=0.668) therefore this term was also dropped from the models. There were significant interactions between feeding and trap-insertion position, and fertilisation and trap-insertion position for all three parameters. Relative shoot position significantly interacted with all independent variables (including trap insertion position) making it unsuitable as a covariate, but the term was retained in the model for interest. A one percent increase in shoot position towards the apex was associated with a 340 μm (+/- 12 μm) increase in length, 260 μm (+/- 21 μm) increase in height, and 682 μm^2 (+/- 29 μm^2) increase in area. ANOVAs for the final models are shown in **Table 4.4A-C**.

The main effects of feeding and fertilisation on trap length, height and area differed depending on the position of the traps within the leaf. The significance of the effects of feeding and fertilisation at different trap-insertion positions for these parameters are examined in **Table 4.5A-C**. Feeding had a significant effect on length, height and area at all positions. For length and height, feeding had the greatest effect at positions 11 and 21 and the smallest effect on the basal traps. For lateral trap area, feeding had the greatest effect at positions 11 and 21. Fertilisation did not have a significant effect on length, height or area at any position. The differences in estimated effects of both feeding and fertilisation on the length, height and area of traps were significant between some trap-insertion positions. This was true even though the main effect of fertilisation was non-significant at all trap positions, for all three parameters. The length and height of basal traps were significantly less affected by both sources of nutrition than those at all other positions. There was no significant difference in the effect of feeding or fertilisation on length,

height and area between traps at positions 11 and 21, but their effects were significantly higher at these two positions than others (see **Tables 4.6 – 4.11** for estimated effects and significance values).

4.3.6 Appendages

Supplementary bristles, antenna branching, and relative antenna length

There was considerable variation in the number of lateral supplementary bristles (4-14, n=2828) and antenna branches (6-26, n=2826) per trap (See **Table 4.12** for means ± SE). There was no significant effect of a three-way interaction between feeding, fertilisation and trap-insertion position on either bristles or antenna branching so these terms were dropped from both models. All main effects and their two-way interactions, for both appendage types, were significant. Feeding and fertilisation differed depending on trap insertion position, and with each other (**Table 4.12**). Generally, larger traps inserted closer to the base of the leaf lobe had more supplementary bristles, compared with traps closer to the leaf apex, but their antennae were less branched (**Table 4.12**). Feeding increased lateral bristle number but had a greater effect in unfertilised treatments than fertilised treatments. For the significance of feeding and fertilisation at different trap-insertion positions on bristles and antenna branching, see **Table 4.13A-B**. The parameters had the greatest effect on basal appendages and the least on traps inserted at positions 11 and 21. In all cases the estimated effect sizes were very small at less than two bristles (**Table 4.14A-B)**. The length of the antenna trunk (for details of antenna morphology see **Chapter 1**) was used in preference to total antenna length when calculating ratios, as the later character was unreliable due frequent mechanical damage that shortened the terminal branches. Neither feeding nor fertilisation affected relative antenna length, but traps at position 21 had significantly longer antenna relative to trap length than the larger sized traps (within the context of individual leaves) at position 11 ($t=1.994$, $p=0.048$) (**Table 4.11**). There were no significant three-way or two-way interactions between trap-insertion position, feeding or fertilisation on relative antenna length, therefore these terms were dropped from the model.

4.4 Discussion

The mineral nutrition available to plants differed between fertilised and unfertilised treatments, with the disparity growing over the course of the experiment. The only sources of mineral nutrition available to plants were either prey capture or the ambient water because i) the shortening of shoots prior to the experiment ensured there would be little re-utilisation of mineral nutrients from senescing shoot bases, and ii) the experiment was terminated prior to the death of basal shoot segments so there would be little loss of mineral nutrients from shoot to the ambient medium (Adamec, 2008b; Adamec *et al.*, 2010). The concentrations of K^+ in both fertilised and unfertilised treatments were high compared to common growth conditions for aquatic carnivorous plants (Guisande *et al.*, 2007; Adamec, 2008b) and were not limiting.

The low levels of NH_4^+ -N and PO_4^{3-}P detected in the fertilised treatment water after nutrient loadings indicate uptake by plants and algae of all supplemented N and P to support growth.

In a field experiment, Englund and Harms (2003) found differences in the concentration of phosphorus between fed and unfed treatment water in meshed tubs suspended in a lake, even when 80-90% of the water in both treatments was replenished from the surrounding lake every 48 hrs. They therefore concluded that the positive effect of prey addition on plant growth was as much attributable to an increase in ambient nutrition resulting from prey life histories as to trapped prey. Adamec (Adamec *et al.*, 2010), in contrast, describes the nutrient differences between the fed and unfed treatments separated by mesh frames, in a controlled growth experiment on *U. australis* and *Aldrovanda vesiculosa,* as negligible. In this current study, care was taken to regularly exchange water between the tubs and trays and a single test of water from both fed and unfed tubs in the fertilised trays, immediately prior to exchanging the tub and tray water, revealed only marginal differences in water chemistry parameters between treatments. It is still possible, even likely, for temporary fluctuations in nutrient levels to have occurred within the treatment tubs (as opposed to surrounding trays) between water exchange.

Prey capture enhances growth more than ambient mineral nutrition

Prey capture had the greatest effect on enhancing plant performance, supporting previous studies that have demonstrating a positive effect of feeding on the growth of *Utricularia* (Sorenson and Jackson, 1968; Kosiba, 1992; Jobson *et al.*, 2000; Englund and Harms, 2003; Adamec, 2008a; Adamec *et al.*, 2010), and other carnivorous plants (Darwin, 1875; Givnish *et al.*, 1984; Wilson, 1985; Thum, 1988; Zamora *et al.*, 1997; Otto, 1999; Lenihan and Schultz, 2014). Prey addition enhanced plant growth by producing a significant increase in biomass production, dry weight increases of all plant modules, and the relative biomass of side shoots in comparison with unfed treatments. Fertilisation also increased the mean values of some plant growth parameters, although of these only two were significant: an increase in the number of mature nodes with side shoots per plant, and the DW biomass of stolon relative to all vegetative organs. In the case of the relative stolon DW, the difference between fertilised and unfertilised treatments was driven by one group of poorly performing pseudo-replicates in an unfed, fertilised treatment tub that produced comparatively little DW leaf biomass.

There was a great deal of variation in growth among plants within the same treatments. These differences could be partly explained by intraspecific competition for the limited ambient mineral nutrition available in the trays, arising as a trade-off for the increased replication provided by five plants per tub. Smaller plants are at an accumulating disadvantage under a ‘hierarchy of dominance and suppression’, and increasing size inequality occurs until the onset of self-thinning (Westoby, 1984; Weiner and Thomas,

1986). The treatment water was shared within each level of the fertilisation factor, acting as a single water body. Therefore, any effects of intraspecific competition for ambient nutrition on plant growth may have extended to all plants from both fed and unfed treatments within a system of coupled trays, exacerbating the effect of prey capture by giving unfed plants an accumulating disadvantage to those that captured prey. This would be particularly true if prey capture stimulated shoots to take up mineral nutrition from the ambient water, in an analogous fashion to the increase in root uptake of nutrients caused by feeding in terrestrial carnivorous plants (Adamec *et al.*, 2010).

Mineral nutrition increases the structural investment in carnivory under favourable photosynthetic conditions

Both prey addition and fertilisation caused significant increases in the investment in carnivory (IIC). Most studies show aquatic *Utricularia* optimise cost-benefit ratios by downregulating IIC in response to greater availability of mineral nutrition, from prey capture and ambient NH_4^+-N, NO_3^--N, and PO_4^{3-}P – "nutrient regulation" (Knight and Frost, 1991; Friday, 1992; Guisande *et al.*, 2000; Richards, 2001; Englund and Harms, 2003; Guisande *et al.*, 2004; Adamec and Kovářová, 2006; Manjarrés-Hernández *et al.*, 2006; Adamec, 2007a; Kibriya and Jones, 2007; Adamec, 2008b; Adamec *et al.*, 2010). However, Adamec (2015, in press) reports CO_2 concentration also regulates IIC – "photosynthetic regulation", and dominates endogenous nutrient regulation. The negative feedback of nutrient regulation prevails only in conditions with a low-moderate CO_2 concentration (0.03-0.2 mM), whereas in more favourable photosynthetic conditions IIC is not downregulated by increases in shoot N and P and is *upregulated* by prey capture. In this study, CO_2 concentrations in both fertilised and unfertilised treatment water exceeded 0.2 mM and plants received light of ~ 40%, within the probable optimum range for *U. australis* (Adamec and Kovářová, 2006). Moreover, plants in the fed treatments also formed primordial shoot apices in the dichotomies of their "leaves". Recently work by Mason (2014) on two terrestrial plant species supports a theory of apical dominance where auxiliary bud outgrowth is regulated not by auxin, but by the growth tip's high demand for sugars. Some species of aquatic *Utricularia* can regenerate from severed leaves (Adamec, unpubl. res.) but signs of apical release between the leaf filaments of *attached* leaves (as opposed to leaf (-like organ) nodes) points to a satiated shoot apex and a surplus of carbohydrates. Therefore the increase in structural investment in carnivory occurring with either the addition of prey or fertilisation (similar to that occurring with the addition of prey in the high CO_2 *U. australis* treatment of Adamec, 2015, in press) supports the hypothesis of positive feedback under conditions of excess photosynthates, resulting in the upregulation of the IIC.

Mineral nutrition alters the size relationships of traps within a leaf.

Traps at all insertion positions exhibited significant size increases (length, height and area) in response to the addition of mineral nutrition, particularly prey addition. The increase in basal trap size was much weaker than that of lateral traps, pointing to them having a more restricted size distribution. The size increases of lateral traps were not uniform over all insertion positions. The traps at positions 11 and 21, closest to the base of the leaf, exhibited disproportionate increases in all size parameters (length, height and area), exacerbating the size differences between these and other lateral traps. *Utricularia* are generalist predators. Lifestyle differences make some animals more susceptible to capture than others, but prey capture is chiefly limited by the ability of potential prey to fit through the trap door (Harms, 1999; Guiral and Rougier, 2007). A larger trap door provides opportunities for trapping a greater size range of prey, but requires an attendant increase in trap size (Friday, 1991; Guiral and Rougier, 2007) and therefore costs more in terms of carbon, N and P (Friday, 1991). Traps also have species-specific maximum sizes (Taylor, 1989). While this study did not attempt to quantify the size or nutritive value of individual prey items, the number of captures per trap increased with trap size. A number of animals caught by position 11 and 21 traps were clearly too large to have been trapped by the smaller traps (*pers. obs*), and Harms (1999) found the bigger traps of *U. vulgaris* caught larger prey disproportionately. Trapping such larger prey, or even comparatively smaller animals, could provide sufficient nutrition to offset the increased mineral (N and P) and structural (C) costs of producing and maintaining a larger trap. When allocating additional resources to trap production it could therefore be advantageous for plants to first develop a few traps to their maximum possible size as opposed to smaller across the board increases to all traps. Why traps at positions 11 and 21 should be the recipients of the disproportion increase is unclear; their proximity to the base of the leaf (hence the stolon) may give them preferential access (during maturation) to any increase in photosynthates.

Cyclopoid copepods are trapped in greater numbers than Chydorus sphaericus.

The test for prey selectivity in this study showed that the predatory cyclopoid copepods *Acanthocyclops robustus* and *Mesocyclops* cf. *leuckarti* were preferred over *C. sphaericus*, contrary to the expectation generated by the prey capture hypothesis for appendage evolution. The appendages (antennae and bristles) of aquatic *Utricularia* increase the capture rate of filament feeding cladocerans (Meyers and Strickler, 1979; **Chapter 2**) by acting as lures. This supports the hypothesis that aquatic appendage morphology provides an adaptive advantage, and is under selection for prey attraction, albeit exploiting a more specific set of prey behaviours than those envisioned by Darwin (1875) and Meyers and Strickler (1979). Appendages do not appear to assist in the capture of all prey species, having no effect and have a detrimental effect on the capture of animals that do not filament feed (**Chapter 2**). Therefore, for prey capture enhancement to be a strong selective force, filament feeders (e.g. *Chydorus sphaericus*) should

be preferred over prey with other feeding and locomotory behaviours (e.g. predatory copepods). Field studies have produced conflicting results. Mette *et al* (2000) found *U. australis* trapped *C. sphaericus* in preference to cyclopoid copepods and other animals, while Guiral and Rougier (2007) and Harms and Johansson (2000) found that cyclopoid copepods were trapped in greater numbers than cladocerans.

Basal traps have lower capture rates than lateral traps

Basal traps caught less prey than lateral traps. The lower captures rates could be due to less exposure to the prey species on offer during the experiment. The position of basal traps at the base of a "leaf node", the position of the trap door in relation to the stalk and their more recurved antennae could reduce encounters with free swimming or stationary feeding phytophillous prey. Aquatic larvae that crawl along the stolons have also been recorded in *Utricularia* traps (Jobson, 2001; Guiral and Rougier, 2007). These animals may come into contact with basal traps more frequently as they frequently utilise the central axis of the shoot when navigating the plant matrix (Pers. obs.)

Appendage expression

Manjarrés-Hernández *et al.* (2006) found the length of antennae relative to the trap bladder was 1.3 times higher in field sites with low NO_3^-, raising the possibility that like the structural IIC, appendage expression represents an investment in carnivory and may be subject to environmental regulation. However, I found no effect of either ambient mineral nutrition or prey capture on the antenna/bladder length ratio, for lateral traps inserted at positions 11 and 21. Relative antenna length did differ significantly between the insertion positions. The traps inserted at position 21 had relatively longer antennae than those at position 11. On any given leaf, the bladders of these traps were always smaller. This is in keeping with the findings of Friday (1991) who found the smaller peripheral traps of *U. vulgaris* had proportionally longer antenna. Larger lateral traps also had less developed antenna in terms of branching, but more numerous supplementary bristle sets. Suggesting bristle expression is isometric, whereas appendage branching is allometric.

There is no immediately obvious reason why antenna branching and supplementary bristling would have opposing patterns of expression if they have both evolved in concert as a 'net' to enhance prey capture. As Friday (1991) notes, the allometry of relative antenna-length (*U. vulgaris)* probably has little functional significance when absolute sampling area of the appendages is taken into account; though smaller traps have relatively longer antennae than larger one, the larger traps still sample over a greater area. The effect sizes (model estimated differences between the numbers of bristles and antenna branching induced by treatment) were also very small. Bristle density may be important, however, as it directly affects the retention of prey within the confines of the 'net' created by the appendages. Smaller

traps have smaller trap doors (Friday, 1991; Guiral and Rougier, 2007) and therefore a comparatively restricted size-class of prey from which to trap. It would be advantageous for smaller traps to have a reduced effective mesh-size to maximise the entrapment of all size suitable prey available to them. On the other hand, an increase in the mesh-size of larger traps would result in smaller, less cost effective prey having the opportunity to escape the funnel.

Table 4.1. A: Values of four nutrient loadings, and water chemistry parameters sampled at 6-7 day intervals, during a 20-day greenhouse experiment with *Utricularia australis* grown with and without prey and with and without additional fertilisation. Nutrient loading for fertilised treatments only. Water was circulated between treatment blocks with the same level of fertilisation factor. **B:** Water chemistry parameters for four randomly selected treatment tubs with fertilised water, two fed and two unfed, sampled on day 20.

A

Day 0	Day 5	Day 10	Day 15	Day 21
Plants added				Plants removed
NH_4Cl, 0.50 mg l^{-1} NaH_2PO_4 0.15 mg l^{-1} KCl 3.00 mg l^{-1}	NH_4Cl, 0.50 mg l^{-1} NaH_2PO_4 0.15 mg l^{-1} KCl 3.00 mg l^{-1}	NH_4Cl, 0.50 mg l^{-1} NaH_2PO_4 0.15 mg l^{-1} KCl 1.50 mg l^{-1}	NH_4Cl, 0.25 mg l^{-1} NaH_2PO_4 0.15 mg l^{-1}	

	Day1		Day 8		Day 14		Day 20	
Parameter (mg l^{-1})	Unfertilised	Fertilised	Unfertilised	Fertilised	Unfertilised	Fertilised	Unfertilised	Fertilized
Total K	3.00	5.60	2.90	7.00	2.60	7.80	2.80	7.30
NH_4^+-N	< 0.010	< 0.010	< 0.010	< 0.010	0.02	0.02	< 0.010	0.015
Dissolved reactive Phosphorus	0.30	0.30	0.13	0.16	0.01	0.01	0.01	0.02
PO_4 -P	0.92	0.93	0.38	0.50	0.02	0.02	0.01	0.05

B

	Treatment Tubs				Tray water
Parameter	Fed 1	Fed 2	Unfed 1	Unfed 2	
Total K ($mg1^{-1}$)	7.3	7.3	7.5	7.3	7.30
NH_4^+ ($mg1^{-1}$)	<0.010	0.011	0.011	0.013	0.015
Dissolved reactive Phosphorus ($mg1^{-1}$)	0.009	0.011	0.01	0.01	0.02
PO4 ($mg1^{-1}$)	0.028	0.035	0.03	0.03	0.05
pH	6.56	6.3	6.38	6.49	6.27
Total alkalinity $CaCO_3$ g/m^3	15.7	15.7	16.2	15.5	16.6

Table 4.2. Means (± SE) of shoot and trap growth responses of *Utricularia australis* grown with or without prey and with or without fertilisation over a 20-day greenhouse experiment. Different letters within each row denote statistically significant differences (Tukey HSD test) between the four treatments at $P < 0.05$. Significance for main effects and interactions from F-tests (continuous responses) and χ^2 tests (counts and proportions) on the reduction of residual deviance for final models: *** – $P < 0.001$; ** – $P < 0.01$; * – $P < 0.05$; ns – $0.1 > P > 0.05$; **ns** – $P > 0.1$.

	Treatment						
	- Prey		+ Prey				
	- fertilisation	+ fertilisation	- fertilisation	+ fertilisation			
Parameter	a	b	c	d	Prey	Fert	Prey x Fert
Basal traps with prey (%)	0	0.4±0.4	1.6±0.7	6.2±1.6	***	ns	ns
Lateral traps with prey (%)	1.0±0.7	5.3±1.3	29.5±2.1	39.0±3.5			
Number of side shoots	0.5 (0-2)	1.3 (1-2)	2 (0-3)	3.1 (1-6)	-	-	-
Nodes with side shoots per plant (%)	5.1±1.1	6.0±0.4	7.3±0.7	10.7±1.0	ns	*	ns
Side shoots per total plant biomass plant (%DW)	0.0	0.5±0.3	2.3±1.1	8.2±2.0	*	ns	ns
Number of nodes main shoot^{-1}	18.5±0.6	20.9±0.6	27.2±1.0	28.5±1.4	***	ns	ns
Stolon DW (µg)	160.0±18.7^{d}	196±17.1^{d}	388.7±48.2	484.7±58.0^{ab}	**	ns	ns
Leaf DW (µg)	400.0±55.4^{cd}	298.7±29.6^{cd}	1010.0±141.1^{ab}	1076.0±171.9^{ab}	**	ns	ns
Lateral trap DW (µg)	314.7±39.8^{d}	418.0±46.8^{d}	1097.3±133.9	1434.0±232.0^{ab}	**	ns	ns
Basal trap DW (µg)	52.0±6.8^{cd}	63.3±5.7^{d}	125.3±11.0^{a}	124.7±13.4^{ab}	**	ns	ns
Stolon per vegetative organs (% DW)	30.0±1.8^{b}	40.1±0.7	28.3±0.4^{b}	32.5±0.8^{b}	ns	*	ns
Biomass production (% DW)	166.9±13.9^{cd}	170.4±13.0^{d}	321.5±32.5^{a}	430.1±71.0^{ab}	**	ns	ns
IIC- basal & lateral traps (% DW)	40.3±1.6^{bcd}	48.9±0.5^{a}	47.1±06^{a}	49.4±0.8^{a}		-- ns --	
Lateral traps µg leaf $^{-1}$ DW	0.13±0.02^{bd}	0.20±0.0^{acd}	0.13±0.01^{bd}	0.15±0.01^{ab}		-- ns --	

Table 4.3. Means (± SE) of length and area of *Utricularia australis* traps at different points of insertion within leaf whorls, grown with or without prey and with or without fertilisation over a 20-day greenhouse experiment. Traps from an additional insertion position, 24, were included in analysis but their means and errors are omitted here. Pairwise comparisons are show in **Tables 5-10**. Significance for main effects and interactions from F-tests (continuous responses) and χ^2 tests (counts and proportions) on the reduction of residual deviance for final models: *** – P < 0.001; ** – P < 0.01; * – P < 0.05; ns – 0.1 > P > 0.05; **ns** – P > 0.1.

		Trap Length (mm)						Trap height (mm)					
Trap insertion position		(basal)	11	21	22	23	21,31	(basal)	11	21	22	23	21,31
Treatments													
- Prey	- fert	1.07±0.007	1.56±0.013	1.38±0.012	1.09±0.006	1.01±0.006	0.92±0.011	0.88±0.006	1.19±0.011	1.03±0.010	0.82±0.032	0.74±0.005	0.66±0.010
	+ fert	1.16±0.008	1.76±0.013	1.55±0.014	1.22±0.007	1.15±0.011	1.11±0.041	0.88±0.061	1.32±0.011	1.13±0.011	0.89±0.027	0.81±0.008	0.74±0.045
+ Prey	- fert	1.27±0.006	1.99±0.012	1.79±0.015	1.43±0.008	1.38±0.009	1.29±0.024	0.97±0.005	1.53±0.010	1.35±0.012	1.06±0.007	1.02±0.007	0.95±0.026
	+ fert	1.33±0.010	2.17±0.019	2.00±0.022	1.57±0.016	1.52±0.017	1.46±0.040	1.01±0.008	1.65±0.015	1.49±0.019	1.19±0.031	1.11±0.013	1.07±0.029
Prey				**						*			
Fertilisation				NS						NS			
Node Position				***						***			
Prey x Fertilisation				NS						NS			
Prey x Node position				***						***			
Fertilisation x Node position				***						***			

Table 4.4. F-tests tests on the reduction of residual deviance for final models of the effect of feeding (prey addition), fertilisation, shoot position and trap-insertion position on **A**: Trap length, **B**: Trap height, **C**: Lateral trap area.

A

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	5476	1230.07	<.0001
Feeding	1	5	17.61	0.009
Fertilisation	1	4	2.28	0.206
Trap-insertion position	6	5476	2728.82	<.0001
Shoot position	1	5476	859.99	<.0001
Prey by trap-insertion position interaction	6	5476	54.12	<.0001
Fertilisation by trap-insertion position interaction	6	5476	16.54	<.0001

B

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	5476	1217.56	<.0001
Feeding	1	5	19.29	0.007
Fertilisation	1	4	1.34	0.311
Trap-insertion position	6	5476	511.65	<.0001
Shoot position	1	5476	162.12	<.0001
Prey by trap-insertion position interaction	6	5476	3.27	0.003
Fertilisation by trap-insertion position interaction	6	5476	15.33	<.0001

C

	Numerator DF	Denominator DF	F-value	p-value
(Intercept)	1	4705	252.67	<.0001
Feeding	1	5	13.86	0.014
Fertilisation	1	4	1.601	0.275
Trap-insertion position	6	4705	1766.53	<.0001
Shoot position	1	4705	550.094	<.0001
Prey by trap-insertion position interaction	6	4705	47.69	<.0001
Fertilisation by trap-insertion position interaction	6	4705	19.28	<.0001

Table 4.5. The significance of feeding (prey addition) and fertiliser addition on trap bladder parameters at different trap-insertion positions. **A**: Trap length, **B**: Trap height, **C**: Lateral trap area. Significance values are extracted by looking at the main effect tests in the final regression model, with each level of trap insertion set as the reference category. Note: effect sizes calculated from model estimates.

A

	Feeding (prey addition)					Fertilisation				
Trap-insertion Position	**Size of effect[1] (µm)**	**SE**	**DF**	**t-value**	**p-value**	**Size of effect[2] (µm)**	**SE**	**DF**	**t-value**	**p-value**
basal	206	0.077	5	2.69	0.044	65	0.082	4	0.79	0.472
11	439	0.076	5	5.74	0.002	181	0.082	4	2.20	0.092
21	456	0.076	5	5.97	0.002	193	0.082	4	2.36	0.078
21,1	347	0.080	5	4.31	0.008	120	0.084	4	1.43	0.226
22	376	0.076	5	4.93	0.005	130	0.082	4	1.59	0.188
23	378	0.077	5	4.95	0.004	123	0.082	4	1.50	0.209
24	358	0.093	5	3.86	0.012	121	0.084	4	1.43	0.227

B

	Feeding (prey addition)					Fertilisation				
Trap-insertion Position	**Size of effect[1] (µm)**	**SE**	**DF**	**t-value**	**p-value**	**Size of effect[2] (µm)**	**SE**	**DF**	**t-value**	**p-value**
basal	129	0.058	5	2.22	0.077	14	0.064	4	0.23	0.823
11	353	0.058	5	6.11	0.002	116	0.064	4	1.82	0.143
21	362	0.058	5	6.26	0.002	120	0.064	4	1.89	0.132
21,1	302	0.073	5	4.13	0.009	62	0.071	4	0.87	0.435
22	286	0.056	5	5.07	0.004	91	0.062	4	1.45	0.221
23	297	0.058	5	5.13	0.004	66	0.063	4	1.04	0.358
24	356	0.100	5	3.57	0.016	112	0.090	4	1.25	0.227

C

	Feeding (prey addition)					Fertilisation				
Trap-insertion Position	**Size of effect[1] (µm^2)**	**SE**	**DF**	**t-value**	**p-value**	**Size of effect[2] (µm^3)**	**SE**	**DF**	**t-value**	**p-value**
11	939	0.153	5	6.12	0.002	358	0.157	4	2.29	0.084
21	881	0.153	5	5.75	0.002	349	0.157	4	2.23	0.090
21,1	461	0.164	5	2.81	0.038	115	1.162	4	0.71	0.517
22	577	0.152	5	3.78	0.013	173	0.156	4	1.11	0.328
23	561	0.154	5	3.65	0.015	124	0.157	4	0.79	0.473
24	356	0.100	5	3.57	0.016	112	0.090	4	1.25	0.227

[1]Mean difference in length between fed and unfed groups (yes – no)
[2]Mean difference in length between fertilised and unfertilised groups (yes – no)

Table 4.6. Tests of pairwise differences in the effect of prey addition on trap length at different trap-insertion positions. The effect of prey addition at the basal position was significantly less than the effect of prey addition at all other positions *(highlighted blue)*; The effect at position 11 was significantly more than at basal, 21x31, 22 and 23 positions *(highlighted yellow - but the comparison with basal position in blue);* The effect at position 21 was significantly more than at the basal, 21x31, 22 and 23 positions *(highlighted green - but the comparison with the basal position in blue).* Note: significance values are from unadjusted comparisons. Effects sizes in mm.

Comparison of Effects of Feeding

Group 1	Group 2	Difference in Size of Effect (Group 2 – Group 1)	SE	DF	t-value	p-value
Basal	Position 11	0.233	0.015	5476	15.29	<.0001
Basal	Position 21	0.251	0.015	5476	16.48	<.0001
Basal	Position 21x31	0.141	0.029	5476	4.83	<.0001
Basal	Position 22	0.170	0.014	5476	12.58	<.0001
Basal	Position 23	0.173	0.015	5476	11.26	<.0001
Basal	Position 24	0.152	0.055	5476	2.79	0.005
Position 11	Position 21	0.017	0.015	5476	1.17	0.243
Position 11	Position 21x31	-0.092	0.029	5476	-3.18	0.002
Position 11	Position 22	-0.063	0.013	5476	-4.83	<.0001
Position 11	Position 23	-0.061	0.015	5476	-4.04	0.0001
Position 11	Position 24	-0.081	0.055	5476	-1.49	0.137
Position 21	Position 21x31	-0.110	0.029	5476	-3.78	0.0002
Position 21	Position 22	-0.081	0.013	5476	-6.18	<.0001
Position 21	Position 23	-0.078	0.015	5476	-5.22	<.0001
Position 21	Position 24	-0.098	0.055	5476	-1.80	0.071
Position 21x31	Position 22	0.029	0.028	5476	1.03	0.303
Position 21x31	Position 23	0.032	0.029	5476	1.09	0.274
Position 21x31	Position 24	0.011	0.060	5476	0.19	0.852
Position 22	Position 23	0.003	0.013	5476	0.21	0.836
Position 22	Position 24	-0.018	0.054	5476	-0.33	0.742
Position 23	Position 24	-0.021	0.055	5476	-0.38	0.707

Table 4.7. Tests of pairwise differences in the effect of fertilisation on trap length at different trap-insertion positions. The effect of fertiliser at the basal position was significantly less than the effect of fertilisation at all other positions *(highlighted blue);* The effect at position 11 was significantly more than at the basal, 21x31, 22, 23, and 24 positions *(highlighted yellow - but the comparison with the basal position in blue;* The effect at position 21 was significantly more than the effect at the basal, 21x31, 22, 23, and 24 positions *(highlighted green - but the comparison with the basal position in blue).* Note: significance values are from unadjusted comparisons. Effects sizes in mm.

Comparison of Effects of Fertilisation

Group 1	Group 2	Difference in Size of Effect (Group 2–Group 1)	SE	DF	t-value	p-value
Basal	Position 11	0.116	0.015	5476	7.84	<.0001
Basal	Position 21	0.128	0.015	5476	8.72	<.0001
Basal	Position 21x31	0.055	0.023	5476	2.39	0.017
Basal	Position 22	0.065	0.013	5476	4.96	<.0001
Basal	Position 23	0.058	0.014	5476	4.01	0.0001
Basal	Position 24	0.055	0.025	5476	2.23	0.026
Position 11	Position 21	0.013	0.014	5476	0.88	0.380
Position 11	Position 21x31	-0.061	0.023	5476	-2.66	0.008
Position 11	Position 22	-0.051	0.013	5476	-4.07	<.0001
Position 11	Position 23	-0.058	0.014	5476	-4.15	<.0001
Position 11	Position 24	-0.060	0.025	5476	-2.45	0.014
Position 21	Position 21x31	-0.073	0.023	5476	-3.22	0.001
Position 21	Position 22	-0.064	0.013	5476	-5.09	<.0001
Position 21	Position 23	-0.071	0.014	5476	-5.07	<.0001
Position 21	Position 24	-0.073	0.025	5476	-2.96	0.003
Position 21x31	Position 22	0.010	0.022	5476	0.44	0.660
Position 21x31	Position 23	0.003	0.022	5476	0.12	0.907
Position 21x31	Position 24	0.000	0.030	5476	0.01	0.989
Position 22	Position 23	-0.007	0.012	5476	-0.57	0.569
Position 22	Position 24	-0.009	0.024	5476	-0.39	0.699
Position 23	Position 24	-0.002	0.024	5476	-0.09	0.927

Table 4.8. Tests of pairwise differences in the effect of prey addition on trap height at different trap-insertion positions. The effect of prey addition at the basal position was significantly less than the effect of prey addition at all other positions *(highlighted blue)*; The effect at position 11 was significantly more than at basal, 21x31, 22 and 23 positions *(highlighted yellow - but the comparison with basal position in blue);* The effect at position 21 was significantly more than at the basal, 21x31, 22 and 23 positions *(highlighted green - but the comparison with the basal position in blue).* Note: significance values are from unadjusted comparisons. Effects sizes in mm.

Comparison of Effects of Feeding (prey addition)

Group 1	Group 2	Difference in Size of Effect (Group 2 – Group 1)	SE	DF	t-value	p-value
Basal	Position 11	0.233	0.015	5476	15.29	<.0001
Basal	Position 21	0.251	0.015	5476	16.48	<.0001
Basal	Position 21x31	0.141	0.029	5476	4.83	<.0001
Basal	Position 22	0.170	0.014	5476	12.58	<.0001
Basal	Position 23	0.173	0.015	5476	11.26	<.0001
Basal	Position 24	0.152	0.055	5476	2.79	0.005
Position 11	Position 21	0.017	0.015	5476	1.17	0.243
Position 11	Position 21x31	-0.092	0.029	5476	-3.18	0.002
Position 11	Position 22	-0.063	0.013	5476	-4.83	<.0001
Position 11	Position 23	-0.061	0.015	5476	-4.04	0.0001
Position 11	Position 24	-0.081	0.055	5476	-1.49	0.137
Position 21	Position 21x31	-0.110	0.029	5476	-3.78	0.0002
Position 21	Position 22	-0.081	0.013	5476	-6.18	<.0001
Position 21	Position 23	-0.078	0.015	5476	-5.22	<.0001
Position 21	Position 24	-0.098	0.055	5476	-1.80	0.071
Position 21x31	Position 22	0.029	0.028	5476	1.03	0.303
Position 21x31	Position 23	0.032	0.029	5476	1.09	0.274
Position 21x31	Position 24	0.011	0.060	5476	0.19	0.852
Position 22	Position 23	0.003	0.013	5476	0.21	0.836
Position 22	Position 24	-0.018	0.054	5476	-0.33	0.742
Position 23	Position 24	-0.021	0.055	5476	-0.38	0.707

Table 4.9. Tests of pairwise differences in the effect of fertilisation on trap height at different trap-insertion positions. The effect of fertilisation at the basal position was significantly less than the effect of fertilisation at all other positions except 21x31 *(highlighted blue);* The effect at position 11 was significantly more than at the basal and 23 positions *(highlighted yellow - but the comparison with the basal position in blue;* The effect at position 21 was significantly more than the effect at the basal and 23 positions *(highlighted green - but the comparison with the basal position in blue).* Note: significance values are from unadjusted comparisons. Effects sizes in mm.

Comparison of Effects of Fertilisation						
Group 1	**Group 2**	**Difference in Size of Effect** (Group 2 – Group 1)	**SE**	**DF**	**t-value**	**p-value**
Basal	Position 11	0.101	0.026	5476	3.83	0.0001
Basal	Position 21	0.106	0.026	5476	4.01	0.0001
Basal	Position 21x31	0.047	0.041	5476	1.15	0.252
Basal	Position 22	0.076	0.023	5476	3.26	0.001
Basal	Position 23	0.051	0.026	5476	1.99	0.046
Position 0	Position 24	0.054	0.045	5476	1.22	0.223
Position 11	Position 21	0.004	0.026	5476	0.17	0.868
Position 11	Position 21x31	-0.054	0.041	5476	-1.32	0.187
Position 11	Position 22	-0.025	0.023	5476	-1.12	0.262
Position 11	Position 23	-0.050	0.025	5476	-1.99	0.046
Position 11	Position 24	-0.047	0.044	5476	-1.07	0.286
Position 21	Position 21x31	-0.058	0.041	5476	-1.43	0.154
Position 21	Position 22	-0.030	0.022	5476	-1.32	0.188
Position 21	Position 23	-0.054	0.025	5476	-2.17	0.030
Position 21	Position 24	-0.051	0.044	5476	-1.17	0.244
Position 21x31	Position 22	0.029	0.039	5476	0.74	0.461
Position 21x31	Position 23	0.004	0.040	5476	0.10	0.921
Position 21x31	Position 24	0.007	0.054	5476	0.13	0.898
Position 22	Position 23	-0.025	0.022	5476	-1.14	0.255
Position 22	Position 24	-0.022	0.042	5476	-0.51	0.607
Position 23	Position 24	0.003	0.043	5476	0.07	0.946

Table 4.10. Tests of pairwise differences in the effect of prey addition on lateral trap area at different trap-insertion positions. The effect of prey addition at position 11 was significantly more than all other positions except 21*(highlighted yellow;* The effect at position 21 was significantly more than all other positions except 11 *(highlighted green).* Note: significance values are from unadjusted comparisons. Effects sizes in mm^3.

Comparison of Effects of Feeding (prey addition)

Group 1	Group 2	Difference in Size of Effect (Group 2 – Group 1)	SE	DF	t-value	p-value
Position 11	Position 21	-0.058	0.035	4710	-1.67	0.096
Position 11	Position 21x31	-0.478	0.067	4710	-7.03	<.0001
Position 11	Position 22	-0.362	0.031	4710	-11.81	<.0001
Position 11	Position 23	-0.378	0.035	4710	-10.77	<.0001
Position 11	Position 24	-0.451	0.128	4710	-3.53	0.0004
Position 21	Position 21x31	-0.420	0.068	4170	-6.18	<.0001
Position 21	Position 22	-0.304	0.031	4170	-9.96	<.0001
Position 21	Position 23	-0.320	0.035	4170	-9.15	<.0001
Position 21	Position 24	-0.393	0.128	4170	-3.08	0.002
Position 21x31	Position 22	0.116	0.066	4710	1.76	0.079
Position 21x31	Position 23	0.100	0.068	4710	1.47	0.142
Position 21x31	Position 24	0.027	0.140	4710	0.19	0.848
Position 22	Position 23	-0.016	0.308	4710	-0.53	0.597
Position 22	Position 24	-0.089	0.127	4710	-0.70	0.483
Position 23	Position 24	-0.073	0.128	4710	-0.57	0.569

Table 4.11. Tests of pairwise differences in the effect of fertilisation on lateral trap area at different trap-insertion positions. The effect of fertilisation at position 11 was significantly more than all other positions except 21*(highlighted yellow;* The effect at position 21 was significantly more than all other positions except 11 *(highlighted green).* Note: significance values are from unadjusted comparisons. Effects sizes in mm^3.

Comparison of Effects of Fertilisation

Group 1	Group 2	Difference in Size of Effect (Group 2 – Group 1)	SE	DF	t-value	p-value
Position 11	Position 21	-0.009	0.034	4710	0.88	0.797
Position 11	Position 21x31	-0.243	0.053	4710	-4.55	<.0001
Position 11	Position 22	-0.185	0.029	4170	-6.27	<.0001
Position 11	Position 23	-0.234	0.032	4170	-7.14	<.0001
Position 11	Position 24	-0.243	0.058	4170	-2.45	<.0001
Position 21	Position 21x31	-0.234	0.053	4170	-4.39	<.0001
Position 21	Position 22	-0.176	0.029	4170	-6.01	<.0001
Position 21	Position 23	-0.225	0.033	4170	-6.91	<.0001
Position 21	Position 24	-0.234	0.058	4170	-4.06	<.0001
Position 21x31	Position 22	0.058	0.051	4710	1.14	0.253
Position 21x31	Position 23	0.009	0.053	4710	0.17	0.867
Position 21x31	Position 24	-0.000	0.070	4710	-0.00	0.9996
Position 22	Position 23	-0.049	0.028	4710	-1.74	0.082
Position 22	Position 24	-0.058	0.055	4710	-1.05	0.293
Position 23	Position 24	-0.009	0.057	4710	-0.16	0.876

Table 4.12. Means and ± SE of supplementary bristles and antenna branches of *Utricularia australis* traps grown with or without prey and with or without fertilisation over a 20-day greenhouse. Traps from an additional insertion position 24 were included in analysis but their means and errors are omitted here. Significance for main effects and interactions from F-tests (relative antenna length) and χ^2 tests (bristle and antenna branch counts) χ^2 tests on the reduction of residual deviance for final models: *** – P < 0.001; ** – P < 0.01; * – P < 0.05; ns – 0.1 > P > 0.05; **ns** – P > 0.1

		Supplementary lateral bristles						Antenna bristles						Antenna trunk:trap length ratio	
Trap-insertion position		Basal	11	21	22	23	21,31	Basal	1	21	22	23	21,31	1	21
Treatments															
- Prey	- fert	6.6±0.1	8.2±0.1	7.6±0.1	6.9±0.1	6.8±0.1	5.9±0.4	11.2±0.2	12.4±0.2	13.7±0.2	15.1±0.3	15.4±0.2	14.0±0.5	0.55±0.02	0.58±0.02
	+ fert	7.1±0.1	8.6±0.1	7.6±0.1	7.6±0.1	7.2±0.1	7.4±0.4	12.9±0.3	12.1±0.2	13.7±0.2	15.5±0.3	16.4±0.3	17.6±0.7	0.68±0.03	0.73±0.03
+ Prey	- fert	7.9±0.1	9.3±0.1	8.9±0.1	8.2±0.1	8.6±0.2	7.5±0.2	15.6±0.2	13.4±0.2	14.3±0.2	16.8±0.2	18.2±0.3	17.9±0.6	0.69±0.02	0.71±0.02
	+ fert	8.2±0.2	8.9±0.1	8.3±0.1	8.1±0.1	8.1±0.1	7.8±0.2	16.1±0.3	12.0±0.1	12.8±0.1	16.1±0.2	17.0±0.2	16.9±0.4	0.72±0.02	0.73±0.02
Prey		***						***						**ns**	
Fertilisation		**ns**						***							
Trap-insertion Position		***						***						*	
Prey x Fertilisation		***						***						**ns**	
Prey x Trap-insertion position		**						***							
Fertilisation x Trap-insertion position		**						***							

Table 4.13. χ^2 tests on the reduction of residual deviance for final models of the effect of feeding (prey addition), fertilisation and trap-insertion position on A) Supplementary bristles, B) Antenna branching.

A)

	DF	Deviance	Residual DF	Residual Deviance	P(>Chi)
Null			2716	863.51	
Feeding	1	52.461	2715	811.05	<.0001
Fertilisation	1	0.198	2714	810.85	0.385
Trap-insertion position	5	80.490	2709	730.36	<.0001
Prey by Fertilisation interaction	1	10.202	2708	720.16	<.0001
Prey by trap-insertion position interaction	5	5.039	2703	715.12	0.002
Fertilisation by trap-insertion position interaction	5	4.521	2698	710.60	0.004

B)

	DF	Deviance	Residual DF	Residual Deviance	P(>Chi)
Null			2716	1673.33	
Feeding	1	94.82	2715	1578.51	<.0001
Fertilisation	1	8.20	2714	1570.31	<.0001
Trap-insertion position	5	470.77	2709	1099.55	<.0001
Prey by Fertilisation interaction	1	19.74	2708	1079.81	<.0001
Prey by trap-insertion position interaction	5	65.00	2703	1014.81	<.0001
Fertilisation by trap-insertion position interaction	5	27.99	2698	986.82	<.0001

Table 4.14. The significance of feeding (prey addition), fertiliser addition on appendage expression at different trap-insertion positions. A) Supplementary bristles, B) Antenna branching. Note: significance values are from unadjusted comparisons. Tests were extracted by looking the main effect tests in the final logistic regression model, with each level of trap insertion set as the reference category. Note: effect sizes calculated from model estimates.

A)

	Feeding (prey addition)				**Fertilisation**			
Trap-insertion Position	**Size of effect[1] (num of bristles)**	**SE**	**t-value**	**p-value**	**Size of effect[2] (num of bristles)**	**SE**	**t-value**	**p-value**
basal	1.24	0.02	10.288	<.0001	1.12	0.02	5.324	<.0001
11	1.15	0.02	8.188	<.0001	1.06	0.02	3.458	<.0001
21	1.15	0.02	8.306	<.0001	1.04	0.02	2.475	0.013
21,1	1.20	0.04	4.146	<.0001	1.16	0.04	3.775	<.0001
22	1.18	0.02	7.977	<.0001	1.09	0.02	4.322	<.0001
23	1.24	0.02	10.133	<.0001	1.04	0.02	1.935	0.053

B)

	Feeding (prey addition)				**Fertilisation**			
Trap-insertion Position	**Size of effect[1] (num of bristles)**	**SE**	**t-value**	**p-value**	**Size of effect[2] (num of bristles)**	**SE**	**t-value**	**p-value**
basal	1.39	0.02	17.903	<.0001	1.15	0.02	7.722	<.0001
11	1.09	0.02	5.201	<.0001	0.98	0.02	-1.028	0.304
21	1.05	0.02	3.422	<.0001	0.99	0.02	-0.825	0.409
21,1	1.14	0.03	3.891	0.0001	1.10	0.03	3.003	0.003
22	1.13	0.02	7.232	<.0001	1.05	0.02	3.126	0.002
23	1.16	0.02	8.810	<.0001	1.05	0.02	2.664	0.008

[1]Mean difference in length between fed and unfed groups (yes – no)
[2]Mean difference in length between fertilised and unfertilised groups (yes – no)

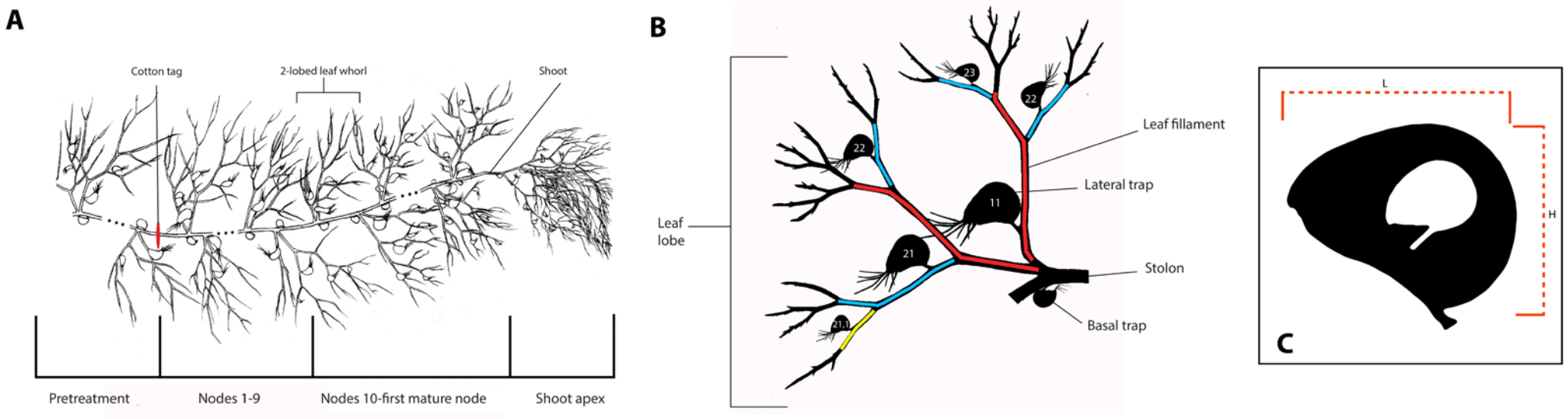

Fig. 4.1. Diagrams of *Utricularia australis* vegetative morphology. Labels show morphological units used in the study. **A**: Whole plant; red circle indicates the base of the shoot apex at the commencement of a 21 day growth experiment. Ellipses mark the position of additional nodes. **B**: Asymmetric 2-lobed Leaf. Appendages are not drawn to scale. Coloured lines indicate the order of branching within each lobe: red 1°, blue 2°, yellow 3° (after Friday, 1991). Numbers refer to trap insertion positions (see text for details). **C:** Silhouettes of a lateral (black) and basal (white) trap in lateral presentation illustrating shape differences. L= trap length, H= trap height. Appendages not shown.

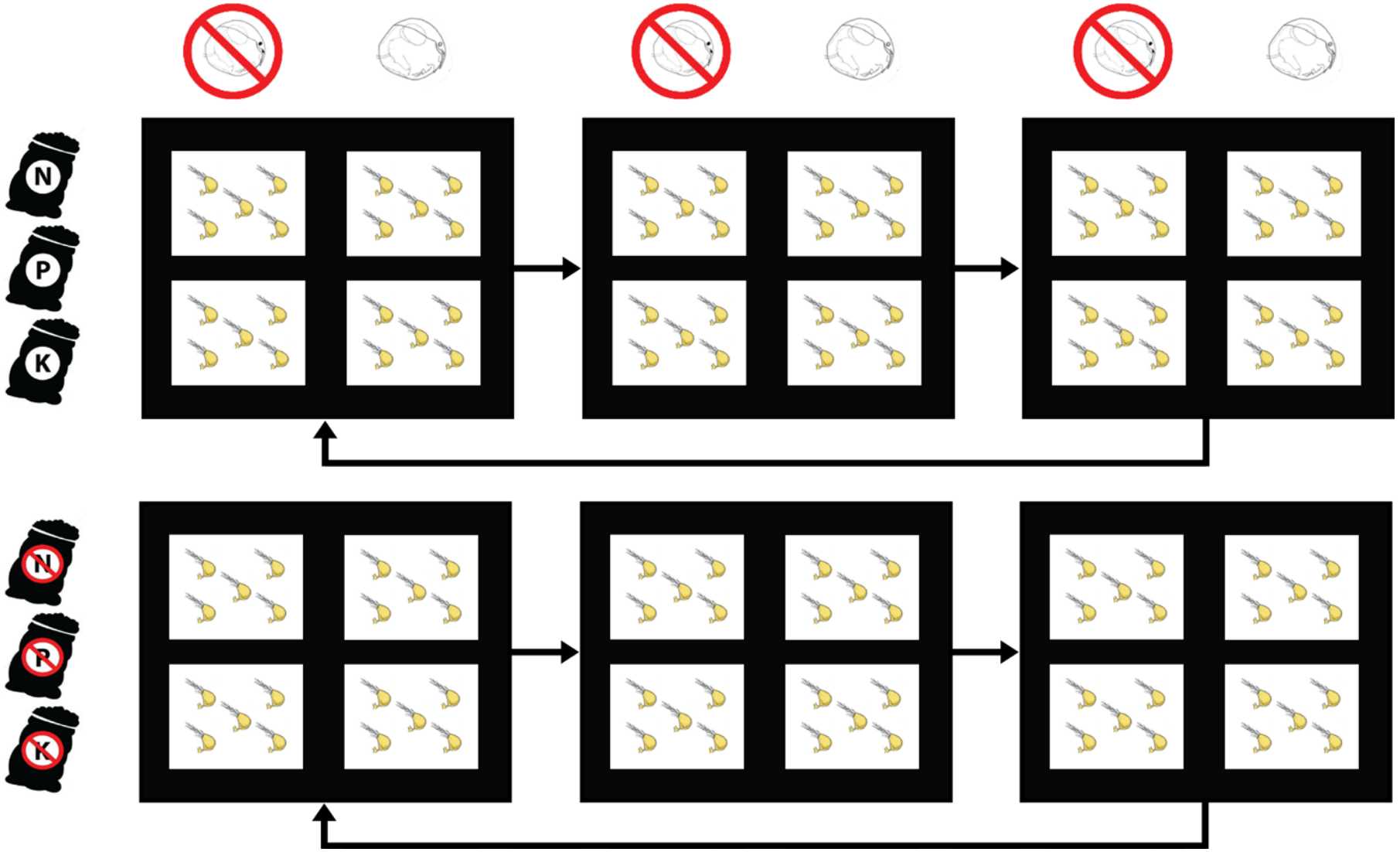

Fig. 4.2. Schematic diagram of a two factorial experiment testing the effects of Prey addition (top) and fertilisation (left) on the growth, trap expression (including appendages) and investment in carnivory of *Utricularia australis*. Receptacles used in the experiment are as follows, from outside in: trays (black) and tubs (white). Trap icons represent plants. Arrows indicate water circulation by pumps.

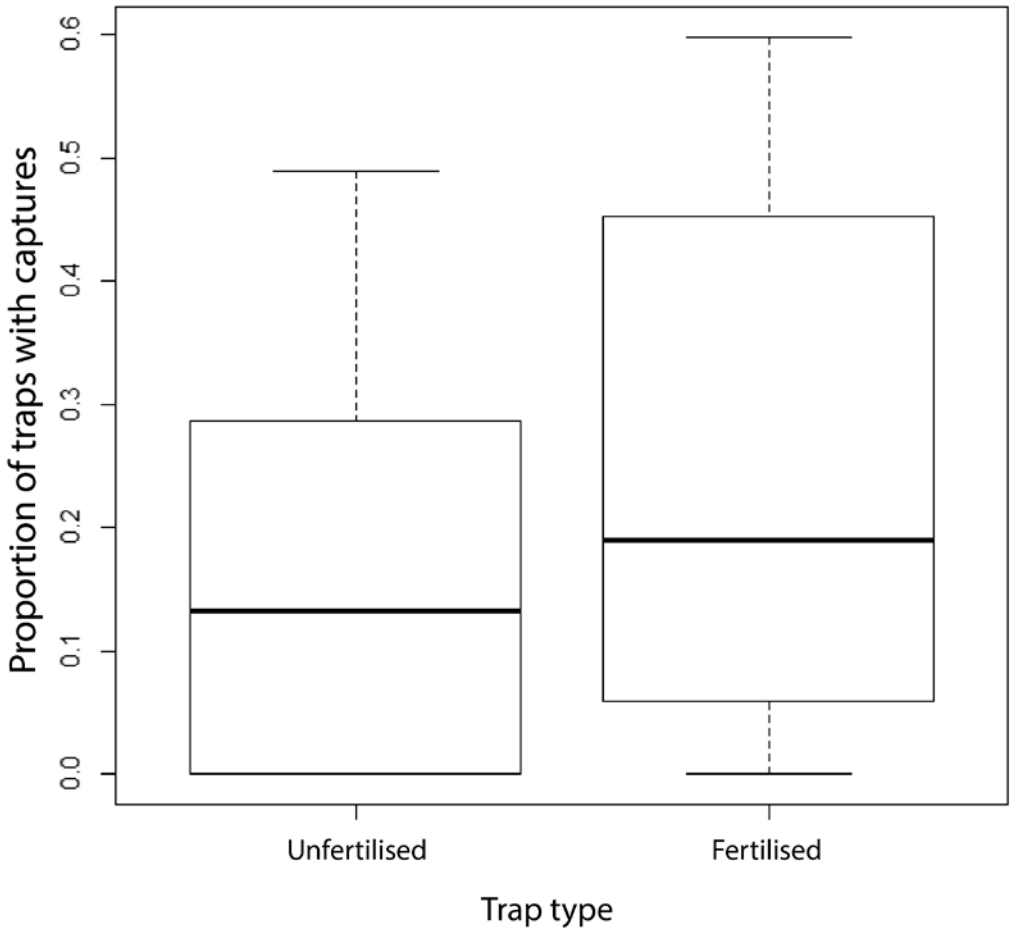

Fig. 4.3. Tukey boxplots showing proportion of traps with captures for *U. australis* plants at the termination of a 21 day growth experiment for fed (prey added) treatments at two levels of fertilsation: fertilised and unfertilised. n = 30 for each treatment. Bold lines show medium

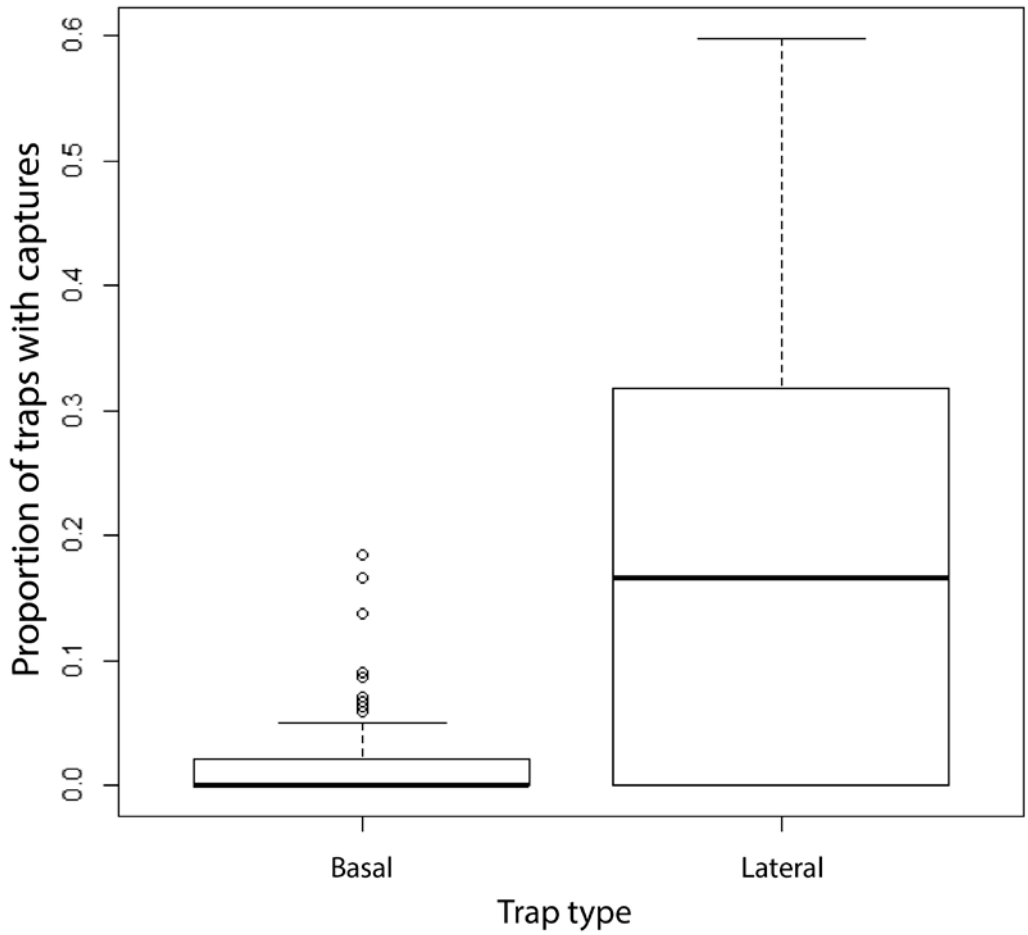

Fig. 4.4. Tukey boxplots showing proportions of basal and lateral traps plant^{-1} with captures for *U. australis* plants at the termination of a 21 day growth experiment. n = 60 for each trap type.

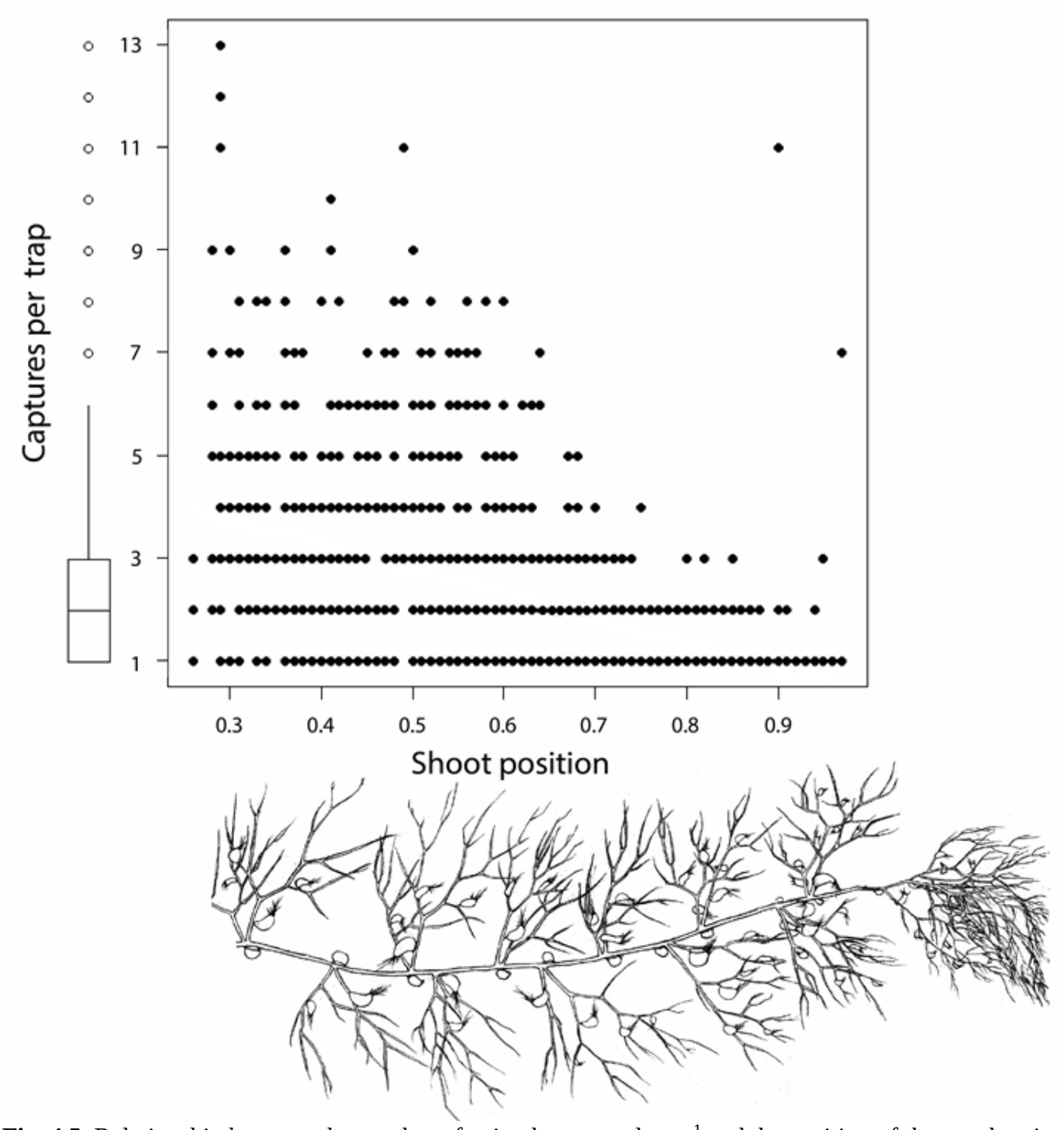

Fig. 4.5. Relationship between the number of animals captured trap^{-1} and the position of the trap-bearing leaf on *Utricularia australis* shoots (see text for details). The box plot beside the y-axis shows the distribution of captures trap^{-1}.

5. General Discussion

Even among botanical carnivores, *Utricularia* is in many respects an unusual genus of plants. Members are characterised by their plastic growth and fuzzy morphology that frequently defies traditional classification (Rutishauser and Brugger, 1992; Rutishauser and Isler, 2001) and has permitted adaptive radiation into a wide range of habitats (Juniper *et al.*, 1989; Taylor, 1989; Reut and Jobson, 2010). The unique trapping organs of the genus are highly derived, being without clear analogues in other carnivorous plant groups (Lloyd, 2008).

The question still remains, how developed were *Utricularia* traps before they began to function as carnivorous organs? From the outset it is counterintuitive enough that trap activation, dependant on a negative pressure gradient and therefore encapsulation in water, should have evolved within an ancestral-line having terrestrial lifestyles, and radiated later into aquatic habitats (Jobson *et al.*, 2003; Müller and Borsch, 2005). There is ample evidence showing *Utricularia* can survive and reproduce on vegetarian diets of planktonic algae and detritus (Richards, 2001; Peroutka *et al.*, 2008; Koller-Peroutka *et al.*, 2014) which are conveniently replenished through the action of spontaneous firing (Adamec, 2011a; Vincent *et al.*, 2011b). Traps are also host to a living community of microorganisms who may provide nutritional benefit to plants under certain circumstances (Mette *et al.*, 2000; Richards, 2001; Gordon and Pacheco, 2007; Peroutka *et al.*, 2008; Alkhalaf *et al.*, 2009; Sirová *et al.*, 2009). It is therefore possible that traps could have evolved as a kind of subterranean vacuum cleaner, to draw in detritus from the ambient water, and were then exapted into the trapping of animal prey (Adamec, 2011a). Characters specialised for the capture of meiofauna seem to be comparatively minor innovations, such as trigger hairs which appear to serve no other role than to facilitate trap activation (Cameron *et al.*, 2002; Fleischmann, 2012b), or aquatic appendage morphology that functions as a prey-attractant Utricularia (Darwin, 1875; Meyers and Strickler, 1979) - at least in the case of particular prey (See **Chapter 2**).

5.1 Aquatic appendages and prey attraction

Utricularia are not generally recognised as having adopted the visual, scent and reward based attraction strategies of other carnivorous clades (Guisande et al., 2007). The isolation of trapping structures by soil and/or water has provided no opportunity for prey-attraction strategies employed Nepenthaceae and Sarraceniaceae such as convergence with pollinator attraction mechanisms (Moran and Clarke, 2010) or flower mimicry (Di Giusto *et al.*, 2010). Some tentative evidence to the contray is provided by Jobson and Morris (2001) who show prey are attracted in greater numbers to untreated traps in comparison to boiled traps and similar sized particles of inert substrate. They did not, however, determine any particular mechanism causing the effect. Nevertheless, *Utricularia* do exhibit non-random prey capture (Harms, 1999; Harms and Johansson, 2000; Mette *et al.*, 2000; Jobson and Morris, 2001; Richards, 2001; Guiral and Rougier, 2007), which makes it likely they have evolved some form of prey attraction (Ellison and

Gotelli, 2001). The only mechanism elucidated so far is the action of the appendages of aquatic species from the section Utricularia (Darwin, 1875; Meyers and Strickler, 1979): paired, dorsal, dendriform structures called antennae supplemented by clusters of simple setae or 'bristle sets' (Taylor, 1989). These structures have been shown to increase the capture rate of a filament-feeding, cladoceran prey species (Meyers and Strickler, 1979; **Chapter 2**) by acting as lures. Aquatic *Utricularia* appear to be the only carnivorous plant group that employ this strategy, with the possible exception of *Nepenthes albomarginata* that also exploits the feeding behaviour of (termite) prey with a thatch of white trichomes at the trap entrance (Merbach *et al.*, 2002). As with the trichomes of *N. albomarginata*, aquatic appendages do provide a legitimate reward, and while the feeding preferences of prey are being exploited to increase capture rates, not every animal that utilises the lure is trapped. However, unlike *N. albomarginata* that offers its own tissue (Merbach *et al.*, 2002), aquatic *Utricularia* require their antennae to be baited with periphyton in order to attract prey (Guiral and Rougier, 2007).

In *Insectivorous Plants* (1875) Darwin hypothesised that antennae and bristles may also function collectively as a drift net to steer potential prey, moving through the plant matrix, towards an encounter with the trap door. I failed to find any evidence that the bristles and antennae of section Utricularia species functioned as a funnel in the wider sense Darwin (1875) proposed. Of the four ubiquitously distributed micro-crustacean species tested (**Chapter 2**), appendages only positively affected the capture rates of the cladoceran *Chydorus sphaericus*, confirming previous results by Meyers and Strickler (1979). The capture rates of three other phytophilous species, an ostracod *Cypridopsis vidua* and two copepods, *Acanthocyclops robustus* and *Mesocyclops* cf. *leuckarti* were unaffected by appendage removal.

The presence of appendages decreased the capture rate of a pelagic cladoceran species, the daphnid *Scapholebris kingi*. Hegner (1926 as cited in Skutch, 1928) also observed appendages hindering rather than guiding potential prey (*Paramecium* spp.). Given that filament feeding is currently the only behaviour successfully exploited to enhance capture rates, filament feeders should be over represented in traps relative to their ambient densities in prey assemblages containing a variety of feeding behaviours. Mette *et al* (2000) found *U. australis* trapped *C. sphaericus* in preference to other animals, including cyclopoid copepods, in keeping with this prediction. Conversely, I found a section Utricularia species (*U. australis)* selected cyclopoid copepods over cladocerans (**4.3.3**) in keeping with the field surveys by Harms and Johansson (2000), Richards (2001; no estimate of ambient density provided), and Guiral and Rougier (2007), and a prey selection experiment by Harms and Johansson (2000)

It is unlikely we will ever have an exact picture of what the prey assemblages surrounding species of aquatic *Utricularia* were like during the evolution of dendriform antennae. Assuming antennae and

bristles did evolve to enhance prey capture, the comparatively low capture probability of *S. kingi* compared to *C. sphaericus* (see caveats in section **2.4**) and other observations of plant preference for phytophilous over planktonic taxa (Harms, 1999), suggest that the negative impact of these appendages on the capture rates of pelagic (as opposed to phytophilous) animals would be unlikely to offset any benefits; unless pelagic meiofauna were the dominant prey taxa available during the evolution of aquatic appendages. Conversely, would the increase in capture rates of phytophilous filament feeding animals conferred by filiform appendages be strong enough to result in their selection on this basis if such animals did not dominate? Contemporary *Utricularia* populations suggest not. Firstly, appendages are not obligatory, as the naked trapped *U. purpurea* and *U. myriocista,* and reduced appendage forms of species such as *U. aurea* and *U. vulgaris* are still effective predators (Skutch, 1928; Richards, 2001). If antennae and bristles provided such a strong advantage as to be a basis for selection, then why is such polymorphic appendage expression maintained in species like *U. aurea* and *U. vulgaris*? Additionally, these species also occur in contemporary sympatric populations with other members of section Utricularia exhibiting fully expressed appendages (Taylor, 1989). For example, *U. aurea* was found growing with *U. corneliana, U. gibba* and *U. stellaris* (Jobson, 2012b). If antennae and bristles are sufficiently advantageous to be under positive selection, then why are species or forms with reduced appendages not locally extirpated by competitive exclusion? Of the five *Utricularia* species abundant in the shallow water aquatic beds of lake Rohunta, Massachusetts, *U. gibba, U. intermedia, U. purpurea, U. radiata,* and *U vulgaris,* it is not species with full appendage expression such as *U. radiata* or the rapacious *U. gibba* that dominate, but exappendiculate *U. purpurea* (Hickler *et al.*, 2000).

5.2 Investment in carnivory and appendage plasticity

Like other carnivorous plants, *Utricularia* alter their structural investment in carnivory (IIC) in response to environmental variation. IIC is now commonly defined as the proportion of total plant dried weight (DW) made up of traps, or the ratio of trap number to the DW of the vegetative organs (Knight and Frost, 1991; Guisande *et al.*, 2004; Adamec, 2007a; Adamec, 2008b; Adamec *et al.*, 2010; Adamec, 2015, in press). Current research points to an upregulation of IIC by declining shoot N and P content, superseded under optimal photosynthetic conditions where the IIC is upregulated by surplus photosynthates (Adamec, 2015, in press).

Thus far attempts to study the effect of prey capture (feeding), on plant growth and the IIC have been unable to separate the effects of prey-derived mineral nutrition from ambient mineral nutrition (Englund and Harms, 2003) or have attempted to eliminate the effect of ambient nutrition entirely (Adamec, 2008a; Adamec, 2015, in press). In a two-factor experiment crossing fertilisation of the ambient water with prey capture, I found that prey capture had the greatest positive effect on a range of plant growth parameters, including biomass gain (**Chapter 4**). Prey addition, but not fertilisation, also increased all three measures

of trap size (length, height and area), but the extent of this effect varied depending on the insertion position of the trap within a leaf. Feeding therefore changed the size distribution of traps within a leaf. The IIC was little affected by either form of nutrition. However, I subjected plants in this growth experiment to near optimal photosynthetic conditions by providing photosynthetically active radiation (PAR) at ~ 317 µmol m^{-2} s^{-1} and two supplementary sources of free CO_2; under such conditions shoot N and P content (and therefore mineral nutrition, from whichever source) may not play a dominant role in the investment in carnivory. Additionally, methodological problems associated with competition between replicates for a finite pool of ambient nutrition may have exacerbated the relative effect of prey capture. Therefore, future experimentation will be required to confirm these results. In addition to more extensive replication (avoiding the necessity for a high level of pseudo replication with treatments), future studies could include CO_2 concentration and/or PAR as additional factors.

If antennae and bristle sets have evolved to enhance prey capture, then their expression can also be considered an investment in carnivory. Manjarrés-Hernández *et al.* (2006) found the length of the antennae of *U. foliosa* (sect. Utricularia) relative to the trap bladder was significantly higher in field sites with low NO_3^-. This suggests that appendage expression may be regulated to offset nutrient limitation, potentially by the same mechanisms as the IIC. I found no experimental evidence to support this hypothesis; neither ambient nutrition, nor feeding had a significant effect on the antenna/bladder length ratio, for lateral traps of *U. australis*. Prey addition did cause a numerical increase in the relative antennae length, in the absence of fertilisation. If feeding leads to a corresponding increase in shoot N and P content, this result contradicts the expectation that as an investment in carnivory, antennae length would be downregulated, unless the photosynthetic regulation dominating the IIC also effects antenna expression. The relative antennae length was significantly different between traps inserted at different positions on the leaf. Larger traps closer to the leaf base had relatively shorter antennae (only lateral traps from two insertion positions were compared). Friday (1991) compared the relative antennae length of the midline and peripheral lateral traps of *U. vulgaris* and found that much smaller peripheral traps had larger antenna length to trap length ratios. They noted that this difference probably has little functional significance when the absolute sampling area of the appendages is taken into account. Although traps of the smaller size class have relatively longer antennae than larger ones, the larger traps still have longer antennae and sample over a greater area.

I also quantified appendage expression in a novel way, by counting the number of bristles in bristle sets, and the number of branches (also setae, identical to bristles) on antennae. The amount of setae dictates the reticulum of the net formed by the appendages and could influence the size of prey guided towards the valve. Bristling and branching were affected by both water level and nutrition, isometrically scaling

with trap size. The direction of the response differed between the two environmental factors. When the amphibious *U. gibba* grew in a terrestrial phase (treatments without a water column; **Chapter 3)**, the degree of bristling and branching was reduced in comparison to those grown as affixed aquatics, resembling the truncated, recurved, awl-shaped appendages of congeners in section Lecticula. This pattern is not congruous with the appendage expression of contemporary affixed aquatics such as *U. stygia*, whose subterranean traps are extremely dendriform (Taylor, 1989). However, shoot-dimorphic species such as *U. stygia*, have plentiful access to CO_2 courtesy of their water-borne leaves specialised for photosynthesis. The bladder sizes of (independently sampled) traps in the terrestrial phase were smaller than those in the aquatic treatments (cf. the comparatively large traps of *U. stygia*; **Table 1.2**) suggesting isometric scaling between appendage expression and bladder size. It is therefore possible to conclude the comparatively reduced appendages were the result of less structural carbon available for investment.

The availability of mineral nutrition produced the opposite effect in traps of *U. australis* (**Chapter 4)**. Antennae and bristle counts increased in treatments with the addition of nutrition, as did trap size; feeding had a stronger effect than fertilisation on appendages (the latter having a significant effect only in the case of antennae branching), and the magnitude of the responses were dependant on the insertion position of the trap. The effect sizes (model estimated differences between the numbers of bristles and antenna branching induced by treatment) were very small (less than two setae) and therefore, as with antennae length, of questionable functional significance.

Of particular interest is the discovery of opposing supplementary bristle and antenna-branch expression patterns within leafs of independently growing *U. australis* fragments (**Chapter 4)**. While traps closer to the base of the leaf had more numerous lateral bristles, it was the traps near the apex of the leaf that had more extensively branched antennae. This pattern was independent of environment. Fewer antennae branches results in a larger reticulum for these appendages. This would give smaller prey animals, whose capture is less energetically defensible, opportunities to escape the net created by the antennae and may help explain the capture of disproportionately larger prey by larger traps, when smaller size classes of prey are also available to them (Friday, 1991). Nonetheless, in the context of persistent, spontaneous trap firing, where the energetic costs of resetting the trap are not offset by any prey derived nutrition (Adamec, 2011a; Vincent *et al.*, 2011b), the probability of trap activation by small prey, and therefore prey density would have to be high for the "size-class filtering" effect to function as an evolutionary driver. Also, the trapdoor can be approached from a variety of directions and the lateral bristles also provide a potential barrier to incoming prey traversing the leaf matrix. If appendages function as size filters then there is no immediately obvious reason why supplementary bristling would have an opposing pattern of expression

to antenna branching. Nor is there an apparent reason for opposing expression patterns if both types of appendages evolved in concert as a 'net' to funnel prey capture. Regarding the luring of filament feeders, although I was not able to show an effect of the bristle sets or antennae operating independently, Meyers and Strickler (1979) did manage to distimguish between the efficacy of each; although antennae played a greater role in enhancing captures rates, bristles were also effective in luring prey. Therefore more bristles should be every bit as advantageous as more antennae branches if each bristle/branch is functioning as an indiviual lure.

While the experiments in this thesis do not provide a definitive refutation of prey-capture enhancement driving selection for aquatic appendage evolution, a number of my experimental findings run contrary to predictions generated by this hypothesis and thereby challenge its veracity. It is possible aquatic appendage morphology may not have evolved as an investment in carnivory at all. The Filiform nature of aquatic appendages may be linked to the morphological expression of the leaves and shoots that bear them. Aquatic plants have converged upon filamentous, dissected bauplans in response to the CO_2 exchange problem presented by the low diffusivity of CO_2 in water and the Prandtl boundary layer of the leaf (Sculthorpe, 1971). Thin, elongated modules increase the surface to volume ratio and decrease the thickness of the unstirred water surrounding the plant. It is conceivable that the same selective pressure acting on the stolons and leaf-like organs of sect. Utricularia aquatics also droves the evolution of dendriform antenna and bristle sets from hypothetical, awl-like, dorsal appendages of a terrestrial ancestor. The exploitation of filament feeding behaviour to enhance capture rates may be an exaption, like the predatory nature of the traps themselves, or simply an incidental effect of a morphological trait that has never been under selection for prey capture enhancement.

Alternatively, filiform appendages could reduce the rate of trap door clogging in aquatic *Utricularia* in a fashion similar to that proposed for terrestrial species by Lloyd, (1933). Paired dorsal appendages are a common feature of *Utricularia* species from a variety infra-generic sections, with a variety of lifestyles (Taylor, 1989). Parsimonious interpretation (by inspection) of the phylogeny presented in **Fig. 1.2** (modified from Jobson *et al.*, 2003) suggests that the dendriform antennae of section Utricularia evolved from thicker, more awl-shaped, dorsal appendages that persist in sub-aquatic species such as *U. resupinata* (sec. Lecticula, trap forms with supplementary bristles) and *U. limosa* (sec. Nelipus, no supplementary bristle sets). Recurved appendages may help maintain the trap viability of terrestrial species by reducing the clogging of the trap door with overly large soil particles and other particulate matter (Lloyd, 1933; pers. obs.).

While thickened, recurved, awl-shaped appendages may be good at deterring particulate matter such as grains of sand or peat, more elongate and delicate forms may be better optimised for the task in a more fluid environment where the particulate matter is finer and has a greater tendency to aggregate. A pilot-experiment involving the ventral-wing and dorsal-appendage ablation of *U. dichotoma* traps (Gardiner, unpublished data) found no evidence to suggest that terrestrial appendages do actually prevent trap door blockage, at least in this species. Even if terrestrial appendages do not function to reduce blockage, this does not in itself rule out the possibility that selection for 'silt fencing' may have begun once *Utricularia* underwent radiation into aquatic habitats.

However, if the silt fence hypothesis were true, it is curious that appendages of section Utricularia affixed aquatic species still retain their highly branched elongate forms. The affixed aquatic species from section Utricularia (including *U. ochroleuca*, *U. stygia* and *U. intermedia*) have dimorphic shoots that partition carnivory and photosynthesis. Trapping organs arise on 'anchoring' shoots with reduced leaves that are buried in the substrate, much like terrestrial *Utricularia*. The shoots in the water column are divided into filamentous leaf-like organs characteristic of aquatic angiosperms (Sculthorpe, 1971) and seldom bear traps. By a combination of the appendage prey-attraction and affixed aquatics as evolutionary intermediates hypotheses, the traps of these species should have a morphology similar to the sub aquatics *U. limosa* and *U. resupinata*, with antennae reduced and recurved in comparison to the suspended aquatics of sec. Utricularia. This is not the case. Taylor (1989) describes the antennae of *U. ochroleuca* and *U. intermedia* as being "long, much-branched, setiform" with both having lateral bristle sets: "a few lateral simple setae". At the time of Taylor's monograph *U. stygia*, pictured in **Chapter 1**, was held in synonymy with *U. ochroleuca*. Finally, the caveat of exappendiculate, suspended-aquatic species flourishing in sympatry with fully appendiculate congeners (see **5.1**) also applies here. It would be interesting to compare door blockage rates, and the rate at which bladders fill with detritus, among these species.

5.3 Future directions

Prey capture

If the filamentous appendage form found in section Utricularia has been selected for by prey capture enhancement, the same pattern should present in other independently derived, aquatic *Utricularia* species. Section Pleiochasia contains not only independently derived semi aquatics but also the only independently derived suspended aquatic outside of section Utricularia, *U. tubulata* (Jobson et al., 2003; Reut and Jobson, 2010). While simplified in comparison to the terrestrial members of the section, these species have appendages that are remarkably more filamentous. There is also a high preponderance of appendage polymorphy among semi-aquatic Pleiochasia species, with individual plants also displaying

variation in the number of filiform traps and their degree (Taylor, 1989; Reut and Jobson, 2010). Pleiochasia species provide opportunities for additional testing of the aquatic prey capture hypothesis with ablations. Additionally, the observed within-plant appendage polymorphy of these Pleiochasia semi-aquatics mean Before-After Controlled-Impact (BACI) designs, involving water level variation, could be used to for environmentally mediated changes in appendage form. The traps of *U. purpurea* and *U. myriocista* from sect. Vesiculina may be without appendages (Taylor, 1989), but ablations could be performed on the elongated fascicle of glands projecting from their trap doors to test if they also lure filament feeders or assist in capture by acting as outsized trigger hairs.

Simple ablation experiments could be extended by using microsurgical techniques to switch and reattach appendage sets among species to look the influence of size differences while preserving shape, and to control for possible effects on prey capture rates associated with between-species variation in trap bladder characters, such as trapdoor to bladder size ratios and bladder orientation in relation to trap stalk. Ablation experiments could also be conducted to test other appendage function hypotheses; successive removing of terrestrial and epiphytic appendage sets to test whether they prevent trap blockage and assist in water retention around the trap door area. Further prey-capture studies on sect. Utricularia species could also include rotifers and creeping animals such as annelids as the effect of appendages on their capture rates has not been studied, yet these animals have been recorded in trap contents surveys (Harms, 1999; Mette *et al.*, 2000; Richards, 2001; Guiral and Rougier, 2007). Additionally, in all of the above cases experiments could be extended so prey species are not tested in isolation, but as part of assemblages or two or more species to test for an effect of behavioural interactions between microcrustaceans on appendage efficacy. Such assemblages would involve cultivating prey animals from a variety of trophic levels, collected from field sites where *Utricularia* is present.

Dietary (trap contents) comparisons between sympatric species with varying appendage expression could also help test the filamentous appendage prey-capture enhancement hypothesis (as well as many other hypotheses relating to niche partitioning). These analyses have thus been hampered by difficulties with morphological identification of prey species to a high taxonomic resolution (Guiral & Rougier, 2007) and sampling bias towards of hard over soft prey (Jobson, 2001). Molecular ecological methods, while bringing their own sets of problems, could be used to address these issues. Even in the absence of pre-existing sequences to provide accurate taxonomic information, next generation sequence can produce a prey spectrum for each trap, based on operational taxonomic units (OTUs); these can then be used to compare trap contents between levels of the factor of interest.

Appendage expression

The appendages of *Utricularia* traps are complicated three dimensional structures; basic linear morphometric methods are therefore not entirely suitable for making detailed comparisons among appendage sets to test hypotheses about their expression. For example, the dendriform nature of antennae from sect. Utricularia species is not the only key difference between these aquatics and their close terrestrial congeners. A change in curvature of dorsal structures in relation to the trap door is also a response to the aquatic lifestyle; from being recurved over the valve area, to reflexed back towards the dorsal surface of the bladder. These spatial relationships cannot be clearly visualised or quantified using two-dimensional orthographic projections. Advances in 3-dimensional (3D) morphometrics have already permitted comparative shape analyses of biological structures (Dercksen *et al.*, 2008), including initial attempts at evolutionary analysis of plant modules (Van der Niet *et al.*, 2010; Van Der Niet *et al.*, 2011).

With the range of imaging techniques, such as optical projection tomography (Sharpe, 2009; see **Appendix 2**) becoming increasingly cost-effective, and the advent of tools for 3D shape analysis (Shen *et al.*, 2009), measures of appendage and trap expression such bristle and branching numbers, antenna:bladder length and bladder height:length ratios could be replaced with more robust quantification such as surface areas or volumes. Both 2D and 3D morphometric methods could also be used to look for indirect evidence for appendage-shape evolution in response to the prandtl boundary layer problem. Co-ordinates of transformation can be obtained for shape transformations between the leaves of terrestrial species and those of their closest aquatic (of various habits) congeners. These same co-ordinates of transformation could then be applied to traps and particularly trap appendages. The resulting morphologies would then in turn be assessed for similarity with traps and appendages belonging to the same aquatic species.

Even in the absence of analysis tools for use in 3-D morphometrics, 3D imaging can be creatively combined with other conventional analyses. Weight ratios are common tool for assessing differences in structural investment used frequently in *Utricularia* research (Friday, 1992; Englund and Harms, 2003; Porembski et al., 2006; Kibriya and Jones, 2007; Adamec, 2008b; Adamec, 2011e). Using these methods directly to compare investment in different components (e.g. appendages, trigger hairs, bladders) of *Utricularia* traps is extremely difficult due to the microscopic sizes of these structures and limits on the readability of easily accessible micro-balances. The rise of affordable 3D printing, however, opens up new possibilities. Traps can be imaged and printed subject to the same scaling factor to maintain relative size differences and thicknesses. These approximations can then be dissected and the resulting components weighed, with allowance if needs be for any differences between the plant and 3D-printing materials in density to volume ratios.

6. References

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Appendices

Appendix 1. Output from statistical tests

1. Output from statistical tests for Chapter Three

1.1 Experiment One: ***U. gibba* and *U. australis* with *C. sphaericus***

1.1.1 Logistic regression output for final model of rate of prey captured.

	Estimate	Std Error	z-value	p-value
(Intercept)	-0.887	0.201	-4.418	<.0001
Plant species:				
U. gibba	1.169	0.285	4.105	<.0001
Appendages:				
(reference: None)				
Antenna only	0.653	0.272	2.398	0.017
Bristles only	0.377	0.276	1.367	0.172
All	0.687	0.272	2.524	0.012
Plant species-by-appendages interaction:				
U. gibba, Antenna only	0.736	0.421	1.747	0.081
U. gibba, Bristles only	1.012	0.423	2.391	0.017
U. gibba, All	0.945	0.423	2.237	0.025
AIC=286.02, Null deviance = 344.82 on 47 DF, residual variance=133.74 on 40 DF				

1.1.2 χ^2 test based on the reduction of residual deviance for final model of number of rate of prey captured.

	DF	Deviance	Residual DF	Residual deviance	P (>Chi)
NULL			47	344.83	
Plant species	1	167.986	46	176.83	<.0001
Appendages	3	35.670	43	141.16	<.0001
Plant species-by-appendages interaction	3	7.419	40	133.74	0.060

1.1.3 Simultaneous tests for general linear hypothesis for interaction between levels of appendage ablation for *U. australis*.

	Estimate	Std Error	z-value	p-value
Bristles only- No appendages	0.376	0.275	1.367	0.520
Antenna only – No appendages	0.653	0.272	2.398	0.077
All appendages – No appendages	0.687	0.272	2.524	0.056
Antenna only – bristles only	0.276	0.263	1.050	0.720
All appendages – bristles only	0.310	0.263	1.179	0.640
All appendages – antenna only	0.034	0.260	0.130	0.999

1.1.4 Simultaneous tests for general linear hypothesis for interaction between levels of appendage ablation for *U. gibba*.

	Estimate	**Std Error**	**z-value**	**p-value**
Bristles only- No appendages	1.389	0.322	4.320	<.0001
Antenna only – No appendages	1.389	0.322	4.320	<.0001
All appendages – No appendages	1.632	0.323	5.047	<.0001
Antenna only – bristles only	<.0001	0.354	0.000	1.000
All appendages – bristles only	0.243	0.355	0.684	0.903
All appendages – antenna only	0.430	0.355	0.648	0.903

1.2 Experiment two (i): ***U. gibba* and *U. australis* with *C. sphaericus, C. vidua* and *S. kingi.***

1.2.1 Logistic regression output for final model of rate of prey captured.

	Estimate	**Std Error**	**z-value**	**p-value**
(Intercept)	-1.402	0.174	-8.064	<.0001
Plant species:				
U. gibba	1.140	0.198	5.767	<.0001
Appendages:				
None	-0.710	0.194	-3.665	0.0003
Prey species: (reference: C. sphaericus)				
C. vidua	-1.374	0.358	-3.837	0.0001
S. king	-3.337	0.543	-6.147	<.0001
Plant species-by-prey species interaction:				
U. gibba, C. vidua	0.198	0.381	0.520	0.603
U. gibba, S. kingi	1.163	0.525	2.216	0.027
Prey species-by-appendages interaction:				
C. vidua, none	0.747	0.353	2.118	0.034
S. kingi, none	0.945	0.423	2.237	<.0001
AIC=319.62, Null deviance = 349.45 on 71 DF, residual variance=149.26 on 63 DF				

1.2.2 χ^2 test based on the reduction of residual deviance for final model of number of rate of prey captured.

	DF	**Deviance**	**Residual DF**	**Residual deviance**	**P (>Chi)**
NULL			71	349.45	
Plant species	1	85.014	70	264.44	<.0001
Prey species	2	88.837	68	175.60	<.0001
Appendages	1	2.577	67	173.02	0.108
Plant species-by-prey species interaction	2	5.780	65	167.24	0.056
Prey species-by-appendages interaction	3	17.986	63	149.26	0.0001

1.2.3 Simultaneous tests for general linear hypothesis for interaction between prey species and appendages for *U. australis*.

	Estimate	**Std Error**	**z-value**	**p-value**
C. vidua: with-without	-0.980	0.607	-1.615	0.286
C. sphaericus: with-without	0.711	0.320	2.222	0.077
S. kingi: with-without	-1.440	1.123	-1.282	0.488

1.2.4 Simultaneous tests for general linear hypothesis for interaction between prey species and appendages for *U. gibba*.

	Estimate	**Std Error**	**z-value**	**p-value**
C. vidua: with-without	0.321	0.352	0.912	0.740
C. sphaericus: with-without	0.712	0.244	2.915	0.011
S. kingi: with-without	-0.823	0.376	-2.190	0.083

1.3 Experiment two (ii): ***U. gibba* and *U. australis* with *S. kingi.***

1.3.1 Logistic regression output for final model of proportion of prey captured**.**

	Estimate	**Std Error**	**z-value**	**p-value**
(Intercept)	-1.561	0.143	-10.899	<.0001
Appendages:				
With	-1.354	0.281	-4.815	<.0001
AIC=123.07, Null deviance = 72.620 on 35 DF, Residual variance=45.515 on 34 DF				

1.3.2 ANOVA output on final model of proportion of prey captured**.**

	DF	**Deviance**	**Residual DF**	**Residual deviance**	**P (>Chi)**
NULL			35	72.620	
Appendages	1	27.105	34	45.515	<.0001

1.4 Experiment three: ***U. australis* with cyclopoid copepod.**

1.4.1 Logistic regression output for final model of proportion of prey captured.

	Estimate	**Std Error**	**z-value**	**p-value**
(Intercept)	0.876	0.204	4.291	<.0001
Appendages:				
With	-0.364	0.234	-1.558	0.119
Leaf:				
Entire	-0.054	0.234	-0.230	0.818
AIC=96.567, Null deviance = 23.716 on 23 DF, residual variance=21.248 on 21 DF				

1.4.2 χ^2 test based on the reduction of residual deviance for final model of proportion of prey captured.

	DF	**Deviance**	**Residual DF**	**Residual deviance**	**P (>Chi)**
NULL			23	23.716	
Appendages	1	2.415	22	21.301	0.120
Leaf	1	0.053	21	21.248	0.818

2. Output from statistical tests for Chapter Three

2.1 Trap length

2.1.1 Regression output for final model of trap length.

Random effects:

	Intercept	**Residual**
StdDev:	0.049	0.149

0.049 ^2/(0.049 ^2 + 0.149^2) = 0.097

After taking out the variance (of trap body length) attributable to environment (water and light level combination), clone and the environment-by-clone interaction, of the remaining residual variance in length 10% is attributable to differences between treatment blocks. The rest is attributable to the difference between individual plant fragments).

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	1.058	0.037	540	28.591	<.0001
Environment: *(reference: High water & high light)*					
High water & low light	-0.012	0.026	540	-0.481	0.631
Low water & high light	-0.088	0.022	540	-4.018	0.0001
Clone:	0.225	0.018	540	12.439	<.0001
Environment-by-clone interaction:					
High water & low light, Clone 2	-0.017	0.033	540	-0.515	0.607
Low water & high light, Clone2	-0.083	0.031	540	-2.692	0.007

AIC=-475.475; Shapiro-Wilk normality test: W = 0.99653, p-value = 0.2442 for residuals

2.1.2 ANOVA for final model of trap length.

	numDF	**denDF**	**F-value**	**p-value**
Intercept	1	540	1043.389	<.0001
Environment	2	540	42.449	<.0001
Clone	1	540	236.316	<.0001
Environment-by-clone interaction	2	540	3.689	0.026

2.1.3 Tukey contrasts for pairwise comparison of clone within environment for final model of trap length.

	Estimate	**Std Error**	**z-value**	**p-value**
High water & low light: Clone 2-Clone 1	0.208	0.027	7.560	<.0001
High water & high light: Clone 2-Clone 1	0.225	0.018	12.439	<.0001
Low water & high light: Clone 2-Clone 1	0.141	0.025	5.625	<.0001

2.1.4 Tukey contrasts for pairwise comparison of environment within clone for the final model of trap length.

	Estimate	**Std Error**	**z-value**	**p-value**
Clone 2: High water & low light - High water & high light	0.029	0.021	-1.419	0.550
Clone 1: High water & low light - High water & high light	-0.012	0.026	-0.481	0.986
Clone 2: Low water & high light - High water & high light	-0.172	0.022	-7.751	<.0001
Clone 1: Low water & high light - High water & high light	-0.088	0.022	-4.018	0.0003
Clone 2: Low water & high light - High water & low light	-0.142	0.025	-5.792	<.0001
Clone 1: Low water & high light - High water & low light	-0.076	0.028	-2.717	0.035

2.2 Trap height

2.2.1 Regression output for final model of trap height.

Random effects:

	Intercept	**Residual**
StdDev:	0.038	0.107

0.038 ^2/(0.038 ^2 + 0.107^2) = 0.112

After taking out the variance (of trap body height) attributable to environment (water and light level combination), clone and the environment-by-clone interaction, of the remaining residual variance in length 11% is attributable to differences between treatment blocks. The rest is attributable to the difference between individual plant fragments).

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	0.752	0.029	540	26.342	<.0001
Environment: (reference: High water & high light)					
High water & low light	-0.012	0.018	540	-0.635	0.526
Low water & high light	-0.042	0.016	540	-2.629	0.009
Clone:	0.195	0.013	540	15.029	<.0001
Environment-by-clone interaction:					
High water & low light, Clone 2	-0.023	0.024	540	-0.980	0.328
Low water & high light, Clone2	-0.058	0.022	540	-2.592	0.010

AIC=-832.7614; Shapiro-Wilk normality test: W = 0.99105, p-value = 0.002 for residuals

2.2.2 ANOVA for final model of trap height.

	numDF	denDF	F-value	p-value
Intercept	1	540	922.960	<.0001
Environment	2	540	24.290	<.0001
Clone	1	540	353.150	<.0001
Environment-by-clone interaction	2	540	3.371	0.035

2.2.3 Tukey contrasts for pairwise comparison of clone within environment for the final model of trap height.

	Estimate	Std Error	z-value	p-value
High water & low light: Clone 2-Clone 1	0.172	0.020	8.707	<.0001
High water & high light: Clone 2-Clone 1	0.195	0.013	15.029	<.0001
Low water & high light: Clone 2-Clone 1	0.137	0.018	7.611	<.0001

2.2.4 Tukey contrasts for pairwise comparison of environment within clone for the final model of trap height.

	Estimate	Std Error	z-value	p-value
Clone 2: High water & low light - High water & high light	-0.035	0.015	-2.351	0.095
Clone 1: High water & low light - High water & high light	-0.012	0.018	-0.635	0.960
Clone 2: Low water & high light - High water & high light	-0.099	0.016	-6.231	<.0001
Clone 1: Low water & high light - High water & high light	-0.042	0.016	-2.629	0.045
Clone 2: Low water & high light - High water & low light	-0.064	0.018	-3.642	0.002
Clone 1: Low water & high light - High water & low light	-0.030	0.020	-1.485	0.504

2.3 Antenna

2.3.1 Logistic regression output for final model of number of antenna branches.

	Estimate	Std Error	t-value	p-value
(Intercept)	3.155	0.013	240.721	<.0001
Environment:				
(reference: High water & high light)				
High water & low light	-0.146	0.023	-6.286	<.0001
Low water & high light	0.032	0.019	1.644	0.101
Clone:	0.233	0.017	13.460	<.0001
Environment-by-clone interaction:				
High water & low light, Clone 2	0.048	0.030	1.616	0.107
Low water & high light, Clone2	-0.107	0.027	-3.957	<.0001

AIC=NA, Null deviance = 321.12 on 448 DF, residual variance=166.92 on 443 DF, quasipoisson dispersion parameter 0.3746

2.3.2 χ^2 test based on the reduction of residual deviance for final model of number of antenna branches.

	DF	Deviance	Residual DF	Residual deviance	P (>Chi)
NULL			448	321.12	
Environment	2	19.164	446	301.96	<.0001
Clone	1	124.950	445	177.01	<.0001
Environment-by-clone interaction	2	10.089	443	166.92	<.0001

2.3.3 Tukey contrasts for pairwise comparison of clone within environment for the final model of number of antenna branches.

	Estimate	Std Error	z-value	p-value
High water & low light: Clone 2-Clone 1	0.280	0.024	11.712	<.0001
High water & high light: Clone 2-Clone 1	0.233	0.017	13.460	<.0001
Low water & high light: Clone 2-Clone 1	0.126	0.021	6.091	<.0001

2.3.4 Tukey contrasts for pairwise comparison of levels of environment factor within clone for the final model of number of antenna branches.

	Estimate	**Std Error**	**z-value**	**p-value**
Clone 2: High water & low light - High water & high light	-0.099	0.018	-5.435	<.0001
Clone 1: High water & low light - High water & high light	-0.146	0.023	-6.286	<.0001
Clone 2: Low water & high light - High water & high light	-0.075	0.019	-3.994	.0004
Clone 1: Low water & high light - High water & high light	0.032	0.019	1.644	0.401
Clone 2: Low water & high light - High water & low light	0.024	0.021	1.156	0.728
Clone 1: Low water & high light - High water & low light	0.178	0.024	7.439	<.0001

2.4 Dorsal bristles

2.4.1 Logistic regression output for final model of dorsal bristle number.

	Estimate	**Std Error**	**t-value**	**p-value**
(Intercept)	0.615	0.030	20.340	<.0001
Environment: (reference: High water & high light)				
High water & low light	-0.330	0.037	-8.999	<.0001
Low water & high light	-0.346	0.036	-9.537	<.0001
Clone:	0.736	0.032	22.964	<.0001
AIC=NA, Null deviance = 275.98 on 448 DF, residual variance=108.20on 445 DF, quasipoisson dispersion parameter 0.2365				

2.4.2 χ^2 test based on the reduction of residual deviance for final model of dorsal bristle number.

	DF	**Deviance**	**Residual DF**	**Residual deviance**	**P (>Chi)**
NULL			448	275.98	
Environment	2	33.78	446	242.20	<.0001
Clone	1	134.00	445	108.20	<.0001

2.4.3 Tukey contrasts for pairwise comparison of clone within environment for the final model of dorsal bristle number.

	Estimate	**Std Error**	**z-value**	**p-value**
High water & low light: Clone 2-Clone 1	0.766	0.070	10.98	<.0001
High water & high light: Clone 2-Clone 1	0.753	0.045	16.81	<.0001
Low water & high light: Clone 2-Clone 1	0.679	0.061	11.08	<.0001

2.4.4 Tukey contrasts for pairwise comparison of levels of environment factor within clone for the final model of dorsal bristle number.

	Estimate	**Std Error**	**z-value**	**p-value**
Clone 2: High water & low light - High water & high light	-0.327	0.043	-7.633	<.0001
Clone 1: High water & low light - High water & high light	-0.341	0.071	-4.802	<.0001
Clone 2: Low water & high light - High water & high light	-0.373	0.045	-8.209	<.0001
Clone 1: Low water & high light - High water & high light	-0.299	0.061	-4.923	<.0001
Clone 2: Low water & high light - High water & low light	-0.046	0.052	-0.883	0.877
Clone 1: Low water & high light - High water & low light	0.047	0.077	0.540	0.978

2.5 Lateral bristles

2.5.1 Logistic regression output for final model of lateral bristle number.

	Estimate	**Std Error**	**t-value**	**p-value**
(Intercept)	2.056	0.020	102.649	<.0001
Environment:				
(reference: High water & high light)				
High water & low light	-0.088	0.035	-2.518	0.012
Low water & high light	-0.052	0.030	-1.716	0.087
Clone:	0.286	0.026	10.972	<.0001
Environment-by-clone interaction:				
High water & low light, Clone 2	-0.112	0.045	-2.516	0.012
Low water & high light, Clone2	-0.110	0.042	-2.639	0.009

AIC=NA, Null deviance = 200.69 on 448 DF, residual variance=132.50 on 443 DF, quasipoisson dispersion parameter 0.292

2.5.2 χ^2 test based on the reduction of residual deviance for final model of lateral bristle number.

	DF	**Deviance**	**Residual DF**	**Residual deviance**	**P (>Chi)**
NULL			448	200.69	
Environment	2	17.197	446	183.50	<.0001
Clone	1	48.153	445	135.34	<.0001
Environment-by-clone interaction	2	2.844	443	132.50	0.008

2.5.3 Tukey contrasts for pairwise comparison of clone within environment for the final model of lateral bristle number.

	Estimate	Std Error	z-value	p-value
High water & low light: Clone 2-Clone 1	0.174	0.036	4.808	<.0001
High water & high light: Clone 2-Clone 1	0.286	0.026	10.972	<.0001
Low water & high light: Clone 2-Clone 1	0.176	0.033	5.424	<.0001

2.5.4 Tukey contrasts for pairwise comparison of levels of environment factor within clone for the final model of lateral bristle number.

	Estimate	Std Error	z-value	p-value
Clone 2: High water & low light - High water & high light	-0.327	0.043	-7.633	<.0001
Clone 1: High water & low light - High water & high light	-0.341	0.071	-4.802	<.0001
Clone 2: Low water & high light - High water & high light	-0.373	0.045	-8.209	<.0001
Clone 1: Low water & high light - High water & high light	-0.299	0.061	-4.923	<.0001
Clone 2: Low water & high light - High water & low light	-0.046	0.052	-0.883	0.877
Clone 1: Low water & high light - High water & low light	0.042	0.077	0.540	0.978

3. Output from statistical tests for Chapter Four

3.1 Prey capture and selectivity

3.1.1 Logistic regression output for final model of prey capture counts: Trap type (lateral and basal)

	Estimate	Std Error	z-value	p-value
(Intercept)	-4.935	0.673	-7.33	<.0001
Trap type:	2.461	0.209	11.77	<.0001
AIC=450.4075, 6 trays (variance <.0001, Std Dev =<.0001), 12 tubs within trays (variance=4.681, Std Dev =2.164)				

3.1.2 Logistic regression output for final model of prey capture counts of *U. australis* plants grown under different levels of feeding.

	Estimate	Std Error	z-value	p-value
(Intercept)	-4.099	0.430	-9.522	<.0001
Feeding:	3.276	0.505	6.486	<.0001
AIC=706.0, 6 trays (variance 0.142, Std Dev =0.376), 12 tubs within trays (variance=0.555, Std Dev =0.745)				

3.1.3 Logistic regression output for final model of prey capture counts of *U. australis* plants for fed treatments grown under different levels of fertilisation.

	Estimate	**Std Error**	**z-value**	**p-value**
(Intercept)	-1.024	0.230	-4.449	<.0001
Fertilisation:	0.414	0.325	1.275	0.202

AIC=574.4, 6 trays (variance 0.097, Std Dev =0.311), 12 tubs within trays (variance=0.053, Std Dev =0.232)

3.1.4 Negative binomial regression output for final model of counts of four prey species *C. sphaericus, S.* cf. *vetulus,* and *A. robustus* and *M. leuckarti* (pooled as cyclopoid copepods), in two locations, within traps and with tubs

Random effects:

	Intercept	**Residual**
StdDev:	**0.496**	**0.847**

0.496^2/(0.496^2 + 0.847^2) = 0.255

after taking out the variance (of counts of plankton) attributable to species, location (plant or tub), and the species-by-location interaction, of the remaining residual variance, 26% of variance in counts attributable to differences between tubs (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	4.305	0.262	97	16.445	<.0001
Prey species: *(reference:* Cyclopoid copepods*)*					
C. sphaericus	-1.452	0.227	97	-6.398	<.0001
S. cf. *vetulus*	-3.907	0.260	97	-15.036	<.0001
Location: (*reference*: within traps)					
Tub	-0.073	0.389	97	-0.189	.851
Prey species-by-location interaction:					
C. sphaericus, tub	2.345	0.550	97	4.267	<.0001
S. cf. *vetulus*, tub	3.680	0.565	97	6.509	<.0001

N=4108, 60 plants within 12 trays (variance=0.496), residual variance=0.847, θ= 1.023

3.2 Growth responses

3.2.1 Logistic regression output for final model of proportion of nodes with side shoots in nodes of *U. australis* plants grown under different levels of fertilisation and feeding.

	Estimate	**Std Error**	**t-value**	**p-value**
(Intercept)	-2.518	0.272	-9.263	<.0001
Feeding:				
(reference: unfed*)*				
Fed	0.506	0.269	1.883	0.060
Fertilisation:				
(*reference*: unfertilised)				
Fertilised	0.6210	0.2615	2.375	.0176
AIC=720.3218, 6 trays (variance <.0001, Std Dev =<.0001), 12 tubs within trays (variance=0.0703, Std Dev =0.2651)				

3.2.2 Regression output for final model of side shoots dry weight as percentage of total dry weight from of *U. australis* plants grown under different levels of fertilisation and feeding.

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	-1.183	1.940	44	-0.610	0.545
Feeding:					
(reference: unfed*)*					
Fed	5.628	2.488	9	2.262	0.050
Fertilisation:					
(*reference*: unfertilised)					
Fertilised	3.568	2.557	4	1.395	0.235
AIC=370.9332, 6 trays (variance 0.725), 12 tubs within trays (variance=4.083, residual =4.479)					

3.2.3 Poisson regression output for number of mature nodes of *U. australis* plants grown under different levels of fertilisation and feeding

	Estimate	**Std Error**	**t-value**	**p-value**
(Intercept)	2.940	0.055	53.12	<.0001
Feeding:				
(reference: unfed*)*				
Fed	0.347	0.060	5.80	<.0001
Fertilisation:				
(*reference*: unfertilised)				
Fertilised	0.078	0.061	1.28	0.199
AIC=339.9090, 6 trays (variance 0.0002, Std Dev =0.158), 12 tubs within trays (variance=0.002, Std Dev =0.045)				

3.2.4 Regression output for final model of stolon dry weight from *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**93.653**	**122.634**

93.653^2/(79.093^2 + 122.634^2) = 0.412

After taking out the variance (of dry stolon weight) attributable to feeding and fertilisation, of the remaining residual variance, 41% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	145.167	60.877	48	2.385	0.021
Feeding: *(reference:* unfed*)*					
Fed	258.333	62.660	5	4.123	0.009
Fertilisation: (*reference*: unfertilised)					
Fertilised	66.333	2.557	4	0.899	0.420

AIC=745.116

3.2.5 Regression output for final model of leaf dry weight from *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**177.620**	**390.755**

177.620^2/(177.620^2 + 390.755^2) = 0.171

After taking out the variance (of leaf dry weight) attributable to feeding and fertilisation, of the remaining residual variance, 17% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	358.167	159.785	48	2.242	0.030
Feeding: *(reference:* unfed*)*					
Fed	693.667	143.860	5	4.822	0.005
Fertilisation: (*reference*: unfertilised)					
Fertilised	-17.667	201.779	4	-0.088	0.934

AIC= 872.723

3.2.6 Regression output for final model of lateral trap dry weight from *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**320.944**	**439.373**

320.944^2/(320.944^2 + 439.373^2) = 0.348

After taking out the variance (of lateral trap dry weight) attributable to feeding and fertilisation, of the remaining residual variance, 35% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	256.333	197.283	48	1.299	0.200
Feeding: *(reference:* unfed*)*					
Fed	899.333	217.267	5	4.139	0.009
Fertilisation: (*reference*: unfertilised)					
Fertilised	220.000	232.893	4	0.945	0.398

AIC= 889.251

3.2.7 Regression output for final model of basal trap dry weight from *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**25.456**	**23.805**

25.456^2/(25.456^2 + 23.805^2) = 0.533

After taking out the variance (of basal trap dry weight) attributable to feeding and fertilisation, of the remaining residual variance, 53% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	55.00	18.717	48	2.938	0.005
Feeding: *(reference:* unfed*)*					
Fed	67.333	15.930	5	4.227	0.008
Fertilisation: (*reference*: unfertilised)					
Fertilised	5.333	23.954	4	0.223	0.835

AIC= 565.042

3.2.8 Regression output for final model of ration of stolon dry weight to leaf dry weight from of *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**3.521**	**2.905**

3.521^2/(3.521^2 + 2.905^2) = 0.595

After taking out the variance (of stolon dry weight/leaf dry weight) attributable to feeding and fertilisation, of the remaining residual variance, 60% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	31.465	2.010	48	15.651	<.0001
Feeding: *(reference:* unfed*)*					
Fed	-4.657	2.167	5	-2.149	0.084
Fertilisation: (*reference*: unfertilised)					
Fertilised	7.177	2.395	4	2.996	0.040

AIC= 324.743

3.2.9 Regression output for final model of biomass gain of *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**96.371**	**131.111**

96.371^2/(96.371^2 + 131.111^2) = 0.351

After taking out the variance (of dry weight biomass gain) attributable to feeding and fertilisation, of the remaining residual variance, 35% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	140.617	56.403	48	2.493	0.016
Feeding: *(reference:* unfed*)*					
Fed	207.167	65.129	5	3.181	0.025
Fertilisation: (*reference*: unfertilised)					
Fertilised	56.033	65.129	4	0.860	0.438

AIC= 750.917

3.3 Investment in carnivory

3.3.1 Regression output for final model of structural investment in carnivory (IIC) of *U. australis* plants grown under different levels of fertilisation and feeding.

Random effects:

	Intercept	**Residual**
StdDev:	**304.527**	**316.785**

304.527^2/(304.527^2 + 316.785^2) = 0.480

After taking out the variance (of IIC) attributable to feeding and fertilisation, of the remaining residual variance, 48% of variance in weight is attributable to differences between tubs nested within trays (and the rest is attributable to the differences between individual plant fragments)

Fixed effects:

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	41.876	1.874	48	22.343	<.0001
Feeding: *(reference:* unfed*)*					
Fed	3.636	2.164	5	1.680	0.154
Fertilisation: (*reference*: unfertilised)					
Fertilised	5.471	2.164	4	2.528	0.069
AIC= 304.527					

3.3.2 Regression output for final model of the ration of antenna length to trap length ratio (IIC) of *U. australis* plants grown under different levels of fertilisation and feeding

	Value	**Std Error**	**DF**	**t-value**	**p-value**
(Intercept)	0.593	0.049	169	12.003	<.0001
Feeding: *(reference:* unfed*)*					
Fed	0.071	0.050	5	1.409	0.218
Fertilisation: (*reference*: unfertilised)					
Fertilised	0.068	0.049	4	1.374	0.241
Insertion position: *(reference:* 11*)*					
Position 21	0.028	0.049	169	1.994	0.048
AIC= -284.026					

Appendix 2. Laser-scanning confocal method for trap imaging

Below is the methodology used to produce the images of traps and prey-animals presented in **Chapters 2 and 3** respectively. These images are an incidental by-product of a larger methodology I developed to produce three-dimensional (3D) models of traps for morphometric analysis; work that was subsequently abandoned. This methodology, which utilises confocal scanning technology to create optical sections, was created as an alternative to micro-computed tomography (Micro CT). Micro CT is an X-ray based 3D-imaging technology that was unsuitable for the soft, desiccation-prone, and undersized *Utricularia* traps.

Over the course of my experimental work (2011-2014) this confocal methodology has by-in-large been rendered obsolete due to the increased availability and application of cost-effective optical projection tomography (OPT), an imaging technology which combines aspects of both confocal imaging and Micro CT. OPT is a vastly superior tool for the 3D visualisation of plant modules.

2.1 Trap Preparation

Staining

Traps of all species were fixed using either 4% formalin or > 50% ethanol (EtOH) for a minimum of 48 hours. Traps were then rinsed in deionised water (H_2O) then subjected to a graded ethanol series (25%, 50%, 75%, and 95%) for at least 30 minutes at each concentration. Traps were left in 95% EtOH for a minimum of 24 hours to remove residual chlorophyll, then subject to a decreasing EtOH series (95%, 75%, 50% and 25%) for at least 30 minutes at each concentration.

Traps were then transferred in liquid by a 3ml graded Pasteur pipette (Raylab) from 25% EtOH to a 0.1% aqueous solution of Congo Red (disodium 4-amino-3-[4-[4-(1-amino-4-sulfonato-naphthalen-2-yl)diazenylphenyl]phenyl]diazenyl-naphthalene-1-sulfonate) (Ajax Chemicals, Sydney, Australia. Batch #004165); Congo red solution was prepared with deionised H_2O. Traps remained in the Congo red solution for at least 24 hours after which they were rinsed by gradually pipetting away the stain and replacing it with deionised water. This process was repeated over a 24-hour period until all excess stain was removed. Traps were immersed in liquid at all times from fixation onwards.

Mounting

After staining, traps were mounted sandwich-style between two cover slips, by one of the two following methods:

1. A nitrile-rubber toric joint (O-ring) with an internal diameter of 7.65 mm and thickness of 1780 μm (Moody & Winter Sales PTY Ltd.) was fixed to a 22x22 mm, thickness No 0 (80 – 130 μm)

glass coverslip (Electron Microscopy Sciences.) under 6x magnification on a stereoscope (Wild M3C, Leica). The O-ring was laid flat and the upper edge coated with a thin film of cyanoacrylate (Holdfast NZ Ltd.). The coverslip was then lowered on top and gentle pressure applied. After drying, the coverslip was turned over and the well formed by the O-ring partially filled with cool, 1% Ultra-low gelling Temperature Agarose (Type IX-A, Sigma-Aldrich. A2576-5G. Lot #SLBC0856V) using a micropipette (P200, Gilson) with 200µl barrier tips (Neptune).

A stained trap was then pipetted into the well within a droplet of H_2O using a 3ml graded Pasteur pipette (Raylab) and agarose solution either removed or added until the well was filled to just below the brim. The mount was then removed to the stage of a second Wild M3C stereoscope within a 5°C cool room, where the trap was positioned prior to the agarose setting using microslide tools (BioQuip). In almost all cases the traps were positioned ventral side up then were gently dragged posteriorly in order allow their appendages settle naturalistically. An attempt was always made to centre the trap within all three dimensions of the well although buoyancy often resulted in slight upward displacement within the z- axis. Mounts were then covered and left to set for a minimum of two hours on the stereoscope stage. They were then refrigerated at 5°C overnight within a 60x15mm plastic tissue culture dish (greiner bio-one) containing a small piece of damp sponge.

After 24 hours mounts were removed from the refrigerator and the wells were filled by micropipette to overtopping with additional 1% Ultra-low gelling temperature Agarose. Next, a second 22x22 mm, No. 0 glass coverslip was gently lowered from one side onto the upper surface of the O-ring and squared with the first coverslip beneath the O-ring. Gentle pressure was applied until excess agarose was displaced and this upper coverslip contacted firmly with the O-ring. The sealed well therefore remained free of air bubbles. A small weight was placed on top of the mount to maintain close contact between the upper coverslip and the O-ring, and the mount removed to a cell culture dish containing a piece of dampened sponge and refrigerated overnight at 5°C degrees. The following day the mount was once again removed from refrigeration and nitrocellulose in the form of clear nail lacquer (QVS) pipetted between the coverslips on the outside of the O-ring to form an additional seal between the inside surfaces of the coverslips and the O-ring. Finished mounts had a depth of 1940-2040 µm. Cover slip surfaces were kept clean and free from damage throughout the process by using Grade 105 lens cleaning tissue (Whatman International Ltd.) as an intermediate to all points of contact.

2. The second type of mount was created for small specimens to reduce depth. It was constructed after a similar fashion to that detailed above, except four polyurethane elastomeric radio opaque separators (Orthoclasic) were substituted for a single O-ring and adhered to the corners of the first coverslip. A large drop of agarose was place on top of the coverslip between, but not touching the four bands and the trap suspended and positioned within it. After setting, additional agarose was added until the depth of the drop exceeded the height of the separators. A thin film of cyanoacrylate was spread on the upper surfaces of the separators and a second cover slip lowered into position and adhered. Care was taken to ensure no cyanoacrylate came into contact the agarose. No nitrocellulose was used to form an additional seal. As the agarose in these mounts was not contained within an air tight seal, they were more prone to desiccation than those created with O-rings and did not keep as long.

2.2 Microscopy

Specimens were imaged as using laser scanning confocal microscopy on an inverted confocal microscope (ZEISS LSM710, Carl Ziess, Jena, Germany) with an AxioObserver microscope stand, a Plan-Apochromat 10x/0.45 M27 lens and a DPSS 561-10 laser with 561 laser lines. The system was running on ZEN 2010 (Carl Ziess, Jena, Germany).

Traps were imaged in two halves. The mounts were placed directly onto the microscope stage and imaged to a depth at which the returning signal grew too faint (approximately 600 μm, although this varied between specimens) to delineate the external morphology. Mounts were then flipped and the second half of the trap imaged. The stacks were intentionally created to overlap in the middle of the specimen. This was to provide a range of possibilities for combination during post-processing.

While the interval was kept consistent between both image stacks, to reduce time (and therefore cost) the number of tiles varied if one half of the specimen could be imaged within a smaller field without cropping.

Post-processing

Because specimens were imaged in two halves these image stacks had to be combined before model generation. Firstly, the image stacks for each specimen were decomposed into a full resolution series of individual Tagged Image Files (TIFs) using the export function in ZE- lite 2011 (Carl Zeiss, Jena, Germany). File names for these individual TIFs consisted of an unchanging alpha-numeric referring to the image stack, suffixed by a numeric that indicated the original position of the TIF within the stack. The stack with the largest number of slices was exported first. The resulting sequence of TIFs is hereafter arbitrarily referred to as the “upper sequence”. The alpha-numeric of the second, smaller image stack was altered upon export, resulting in a sequence of TIFs (“lower sequence”) appended to after those of

upper sequence. As the specimens had been flipped 180 degrees during imaging, the lower sequence of TIFs was reversed by altering the numerical suffix. Due to the overlap of the original images stacks, a point of transition between the upper and lower sequences was chosen by comparing pairs of TIFs from each sequence. A point of transition was chosen that maximised representation of the specimen (by way of luminance) in both sequences and extraneous TIFs were discarded.

Finally, if each half of a specimen had been imaged using different tile settings, then the dimensions of the image files within the completed sequence had to be standardised. This was accomplished by up-sizing the smaller sequence. Using Adobe Photoshop (CS3 Extended, Adobe Systems Inc., Version 10.0), each TIF to be resized was copied and pasted into a new, black back grounded image file of the correct dimensions that was subsequently flattened and saved as a TIF. Each specimen was now represented by a single, unbroken, correctly ordered sequence of image files with matching dimensions.

I then generated orthographic projections of the traps in AMIRA or Zen 2010.