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Algal communities in phytotelmata: A comparison of native *Collospermum* and exotic bromeliads (Monocotyledonae) in New Zealand.

Sarah A. Killick[1], Dan J. Blanchon[1], and Mark F. Large[1, 2]

[1]Biodiversity Management and Animal Welfare Research Group, Department of Natural Sciences,
Unitec Institute of Technology, Auckland, New Zealand
[2]Author for correspondence: mlarge@unitec.ac.nz

Abstract

Plants that grow epiphytically are often adapted to hold water in a central cup or in leaf axils to maintain hydration given that they cannot access soil water. These bodies of water, called phytotelmata, are miniature temporary aquatic ecosystems. This water frequently contains a variety of microorganisms. The New Zealand native *Collospermum hastatum* (Colenso) Skottsb. (Asteliaceae) is known to hold water within the leaf axils; however, an assessment of algal communities within this habitat has never been undertaken. To remedy this lack of knowledge, water samples were obtained from the leaf axils of *C. hastatum*, with exotic bromeliads for comparison. *Freycinetia banksii* A.Cunn. and *Astelia solandri* A.Cunn. were also investigated, but did not contain sufficient water for further investigation. Urban bromeliads investigated had a wide range of organisms within the phytotelmata, including four genera of diatoms. No desmids were identified. *Collospermum* and Forest-sourced bromeliad water held a comparatively less diverse range of biota, with a complete lack of algal presence. We propose three possible explanations for this; inadequate light levels for algal growth; unsuitable phytotelmic pH; and low background levels of algae for dispersal within native forest environments.

Introduction

A common adaptation in epiphytes is the ability to hold a body of rainwater, or phytotelmata, in leaf axils or a central cup (Richardson 1999). Bromeliads are a frequently studied group of phytotelmic plants (Brouard et al. 2011; Frank and Lounibos 2009; Jocque et al. 2010; Panizzo 2011). Many have leaves that grow in a characteristic rosette pattern, forming a central cup (Benzing 2000) and can hold large volumes of water (Brouard et al. 2011). Within some phytotelmic species of bromeliads, trichomes toward the leaf base are adapted to utilize phytotelmata for hydration (Horres et al. 2007; Takahashi and Mercier 2011). Some bromeliads excrete mucilage containing urease and proteases into the phytotelmata (Cambui et al. 2009; Carrias et al. 2001), altering acidity and promoting invertebrate and plant material decay. The host plant can then acquire the nutrients it requires, particularly nitrogen (Takahashi and Mercier 2011; Wittman 2000).

There is to date no published literature on the phytotelmic algae of New Zealand, and it is not known if water within New Zealand native epiphytes supports similar levels of algal diversity to that found in epiphytes elsewhere. Worldwide, only limited research into the algae of phytotelmata has been published. One study of bromeliad phytotelmata in Brazil recorded taxa of yellow-green algae (Xanthophyceae), diatoms (Bacillariophyceae), green algae (Chlorophyceae), Ulvophyceae, and desmids (Zygnematophyceae). Desmids were particularly common, with 58% of algae taxa within samples belonging to this order. These included species of the desmids *Cosmarium*, *Pleurotaenium*, *Staurastrum* and *Xanthidium*. Although diatoms (Bacillariophyceae) were less prevalent, there are records of species of *Aulacoseira* and taxa from the order Pennales (Sophia et al. 2004).

Water held within bromeliads provides a niche for both plant and animal communities. Invertebrates found within Amazonian bromeliads include *Diptera*, *Coleoptera*, *Hymenoptera*, *Hemiptera*, *Lepidoptera* (larvae), *Odonata*, *Dermaptera*, *Blattodea*, and *Isoptera* (Frank and Lounibos 2009; Torreias and Ferreira-Keppler 2011) that then support amphibia in both mature and immature states (Wittman 2000). Across Australia and Asia, *Turbellaria*, *Rotifera*, *Nematoda*, *Annelida*, *Crustacea*, *Odonata*, *Hemiptera*, *Orthoptera*, *Diptera*, *Coleoptera*, *Hymenoptera*, and Arachnids were present in phytotelmic habitats (Mogi 2004). This level of invertebrate diversity is dependent upon algae as a source of nutrition (Brouard et al. 2011).

A range of New Zealand native plant species are known to hold water. Only *Collospermum hastatum* and *Phormium tenax* J.R.Forst. & G.Forst. are reported to hold water in their living leaf axils, based on studies of mosquito breeding habitat (Derraik 2005, 2009a,b; Derraik et al. 2005). A number of other monocotyledons with overlapping leaf bases exist within the New Zealand native flora (including thirteen endemic species of *Astelia*, (Moore and Edgar 1970) and two members of the Pandanaceae: the native *Freycinetia banksii* and the introduced *F. arborea* Gaudich. (de Lange et al. 2005). None of these have been investigated for the presence of phytotelmata.

This study investigated the phytotelmata of *Collospermum hastatum*, *Astelia solandri*, and *Freycinetia banksii*. Although bromeliads are not native to New Zealand, they are a common addition to gardens and several species have been included in this study for comparison.

Methods

Species examined: Three native monocotyledons had overlapping and clasping leaf bases, morphological structures suitable for holding water. *Collospermum hastatum* is a native epiphyte previously recorded as phytotelmic. In addition *Astelia solandri* and *Freycinetia banksii* were examined. Several species of exotic bromeliads (*Aechmea gamosepala* (Wittm.) L.B.Sm. & W.J.Kress; *Bilbergia vittata* Brongniart; *Neoregelia* sp. L.B.Smith; and *Vriesea hieroglyphica* (Carrière) E.Morren) were included for comparison.

Collection sites: Water samples were obtained from the native monocotyledons in forests in Clevedon, South Auckland (37°02'S 175° 01'E), Waitakere Ranges in West Auckland (36°53'S 174°32'E), and Maitaia, Kaipara Harbour in North-West Auckland (36°29'S 174°25'E). Further samples were later obtained from Great Barrier Island, in the Hauraki Gulf (36°10'S 175°28'E).

Cultivated bromeliads were investigated in managed conditions in Mount Albert (36°52'S 174°42'E), and native forest conditions (Waitakere Ranges (36°53'S 174°32'E), and Great Barrier Island (36°18'S 175°29'E) were also sampled.

Collection Method: Water samples were acquired using a modification of method of Derraik (2009b). The inner surface of the phytotelma were scraped using a single use, disposable 50mL pipette to loosen micro-epiphytes. A 50ml standard sample was extracted from leaf axils of the native species and a combined sample from the leaf axils and central cups of bromeliads. The extracted water was placed into separate bottles for in-laboratory investigation.

Morphological Identification: Settled liquid removed from the bottom of each sample was mixed with a drop of 100% glycerol in order to decrease protist movement. Samples were investigated visually using a Meiji Techno MTA4000H biological microscope, with a 5.0 megapixel Infinity 1–5C camera attachment. Photographs were taken of taxa present, and morphological identification to genus or species level was sought using appropriate sources (Fogg et al. 1973; Lee 2008; Novis and Moore 2014; Baker 2012).

pH measurement: Measurements of pH were taken with a Metler Toledo Seven Easy pH meter, calibrated to two decimal places.

Results

Water presence: *Native monocotyledons:* all plants of *Collospermum hastatum* investigated were found to hold water in the leaf axils (Fig. 1). Limited success was met in obtaining samples from *Freycinetia banksii*, although some plants with an upright habit, particularly during or soon after rain, held some water. Water within *Astelia solandri* leaf axils was rare. Neither *Freycinetia banksii* nor *Astelia solandri* contained sufficient water for further investigation.

Exotic bromeliads*:* species of *Neoregelia*, *Vriesia*, *Aechmea*, and *Bilbergia* were all found to hold water (Fig. 2).

pH level: Summary measurements of pH are presented in Table 1

Fig. 1. Close up view of the epiphytic *Collospermum hastatum* (Colenso) Skottsb., showing the water containing leaves of the rosette. Waiatarua, Auckland, New Zealand.

Phytotelmata content

Collospermum hastatum

None of the *Collospermum hastatum* samples investigated had algae present; however, the cyanobacterium *Chroococcus* sp. ciliates, pollen grains, fungal and fern spores were noted (Table 2, Figs 1, 3).

Urban bromeliad species

Diatoms from four genera were present, with *Pinnularia* being the most common. The diatom genera *Navicula, Eunotia,* and *Cymbella* were also identified. The colonial alga *Dictyosphaerium* sp. was present in both *Neoregelia* and *Vriesia* urban populations, but was more prevalent in the latter (Table 2, Fig. 3).

Other organisms and biological material present included the protozoa *Vorticella* sp., the euglenoid *Lepocinclis acus* (O.F.Müller) Marin & Melkonian and several ciliates including *Paramecium* spp. as well as fungi, pollen grains, and trichomes from *Solanum mauritianum* Scop.

Forest-sourced bromeliad species

Bromeliad water sourced from areas of native forest did not contain algae. However, the cyanobacterium *Chroococcus* sp was present in small numbers, as were non-photosynthetic microorganisms, such as *Paramecium* sp. and fern spores and pollen grains.

Discussion

Although common in urban Bromeliads, diatoms were absent from all *Collospermum* plants investigated in this study. We consider three possible explanations for this. First, that light levels in *Collospermum* phytotelmata may be insufficient for algal growth. Second, that the pH of *Collospermum* phytotelmata may be unsuitable

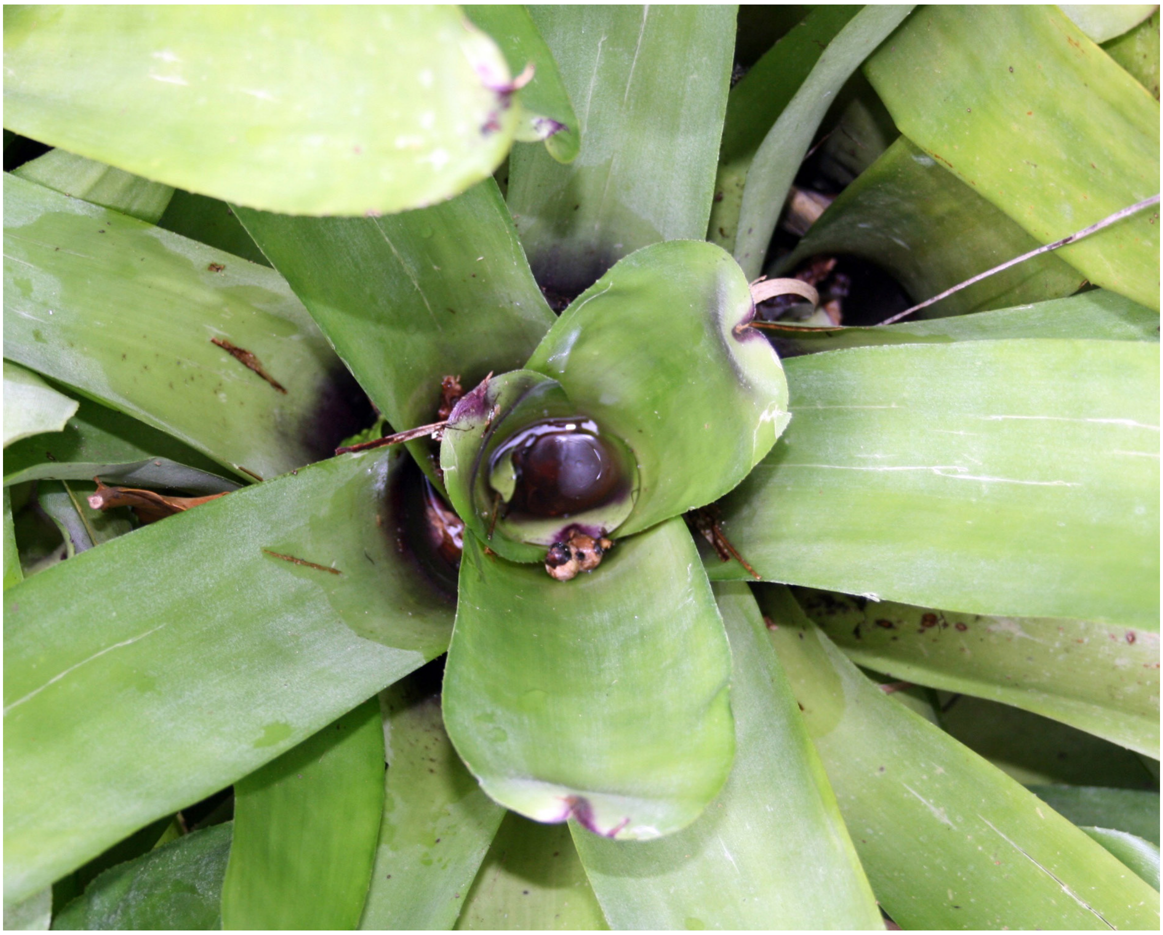

Fig. 2. Close up view of *Aechmea gamosepala* Wittm., showing the leaf rosette and water reservoir. The species is an endemic of Southern Brazil here grown under native forest at Waiataurua, Auckland, New Zealand.

for the growth and reproduction of diatoms and/or desmids. Third, diatoms and desmids may be unable to disperse to *Collospermum* phytotelmata in native forest sites.

Insufficient light levels, particularly in the basal section of *Collospermum* may inhibit algal growth. Tank bromeliads, such as the species examined in this study, are morphologically suited to hold water due to their rosette leaf pattern with overlapping and clasping leaves, which has not only a central cup but also openly spread leaf axils. This leaf pattern may allow higher levels of light to be transmitted into the phytotelma, providing sufficient light for photosynthetic microorganisms (see Fig. 2). In contrast, *Collospermum hastatum* has no central cup and holds water only in the bases of closely overlapping and clasping leaves (Fig. 1). In addition, the basal section of *C. hastatum* has black pigmentation, which appears to reduce light transmission. These morphological features that allow water retention, especially the closeness of the leaves, may decrease light in the basal section. Low light levels would not provide a suitable environment for algae to thrive in, a factor explored in previous phytotelma investigations which found bromeliads in more shaded areas to have much lower, or in some cases no, algal presence (Sophia et al. 2004).

Alternatively, the phytotelma pH in *Collospermum* may be unsuitable for algal survival and growth, with algae failing to reach visually detectable levels. Although we were not able to find research on the effect phytotelmic pH has on algal assemblages, freshwater algal communities have been noted as pH-sensitive within New Zealand lakes (Stephens 2011) and streams (Schowe et al. 2013). Some species of the diatom genera *Eunotia* and *Pinnularia* have been found to be acidobiontic (Hill et al. 2001; Chen et al. 2014), while species of *Cymbella* and *Navicula* have been reported as being intolerant of low pH (Hill et al. 2001). Of the other genera found in this study, some species of *Chroococcus* (Findlay and Kasian 1986), *Dictyosphaerium* (Pereira et al. 2013), *Lepocinclis* (Hrdinka et al. 2013), *Paramecium* (Heydarnejad 2008) and *Vorticella* (Green and Kramadibrata 1988) are known to be tolerant of acid conditions. Acidic phytotelma is common in bromeliads, primarily because of organic debris and microorganisms within the tank, as well as chemical output of the plant itself (Jocque and Kolby 2012). An investigation of bromeliad acidity by Lopez (2014) found that washed bromeliad tanks, filled with near-neutral solution experienced a decrease in pH within six days.

Within this study, all water samples from bromeliad genera from both urban and forest-source populations were found to be acidic ($\bar{x}$=pH 5.70, σ=0.9), with those of *Collospermum* phytotelmata being slightly lower ($\bar{x}$=pH 5.08, σ=0.6); (local rain water having a pH 6.20). The cause of the pH decrease in phytotelmic water pH in *Collospermum hastatum* is unknown and it is not known if the trichomes in *Collospermum* leaf axils secrete mucilage.

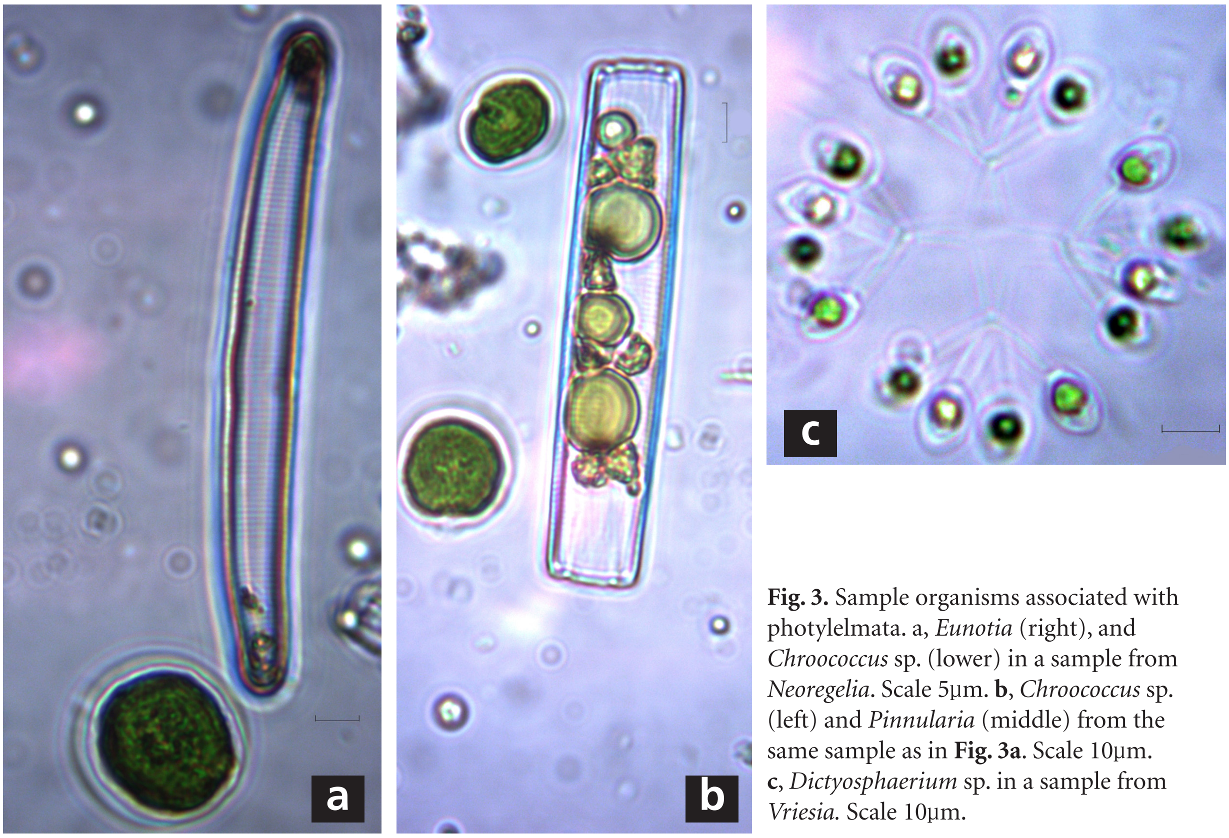

Fig. 3. Sample organisms associated with photylelmata. a, *Eunotia* (right), and *Chroococcus* sp. (lower) in a sample from *Neoregelia*. Scale 5µm. **b**, *Chroococcus* sp. (left) and *Pinnularia* (middle) from the same sample as in **Fig. 3a**. Scale 10µm. **c**, *Dictyosphaerium* sp. in a sample from *Vriesia*. Scale 10µm.

The third and final possibility considered is that diatoms and desmids may be unable to disperse to *Collospermum* phytotelmata in native forest sites. While we were unable to find literature on the presence of diatoms and/or desmids specifically in the New Zealand forest environment, there is extensive literature on their presence in New Zealand freshwater lakes, streams, and wetland environments (Stephens 2011; Croasdale and Flint 1986; Kilroy and Sorrell 2013). Within Lake Pupuke, Auckland, Cassie (1989) recorded 96 taxa of algae, including 30 diatom taxa. Furthermore, a survey by the National Institute of Water and Atmospheric Research (NIWA 2014) recorded the presence of diatoms, including *Eunotia* spp. and *Pinnularia* spp. within West Auckland (Te Henga) sediment. Algae and their resting spores are potentially known to become airborne (Novis et al. 2008), and cultured aerobiological samples are reported from the Indian subcontinent, to produce complex algal colonies (Sharma and Singh 2010). Various authors have also discussed the likely passive dispersal of diatoms and other algae by insects (Milliger et al. 1971; Revill et al. 1967). It is known that mosquitoes in particular use *Collospermum* and bromeliads for the larval stage of their lifecycle (Derraik and Heath 2005). In a survey in the Auckland region, Derraik et al. (2005) observed native mosquito species within West Auckland forest environments, and introduced species in all sampled urban areas. These observations suggest that possible modes of dispersal (including wind) should be available in the environment where *Collospermum* exists. We found that bromeliads cultivated in forested environments lacked diatoms and desmids. Given that bromeliads grown in relatively shaded urban conditions such as those also covered in this study do possess an extensive algal assemblage, this may imply that the dispersal/penetration of diatom and desmid propagules is low or negligible in native forest sites.

Despite the lack of desmids and diatoms in the phytotelmata of *Collospermum*, the presence of cyanobacteria, ciliates and fungi indicates that a distinct community exists. Similarly, a Brazilian study of bromeliads in shaded environments found an abundance of aquatic fungi, but rarely any algae (Sophia et al., 2004). These organisms are not easily identified by traditional methods. Consequently we are in the process of investigating the same phytotelmata using high throughput DNA sequencing.

Taxon	**pH**			
	Mean	**σ**	**Range**	**n**
Collospermum hastatum	5.08	0.55	4.26 – 6.00	11
Aechmea gamosepala	5.78			1
Bilbergia vittata	6.07	0.52	5.71–6.44	2
Neoregelia sp	6.18	1.06	5.61–6.98	6
Vriesea hieroglyphica	5.34	1.15	4.53–6.16	2

Table 1 The variation of the pH of water from *Collospermum hastatum*, *Aechmea gamosepala*; *Bilbergia vittata*; *Neoregelia* sp.; and *Vriesea hieroglyphica*. σ = variance; n = sample size

Summary of organisms and other biological material present in phytotelmata

Host plants	**Algae**	**Other**
Bromeliaceae		
Neoregelia spp *Aechmea* spp. *Bilbergia* spp. *Vriesia sp*	*Pinnularia* sp. *Navicula* sp. *Eunotia* sp. *Cymbella* sp. *Dictyosphaerium* sp.	*Chroococcus* sp *Lepocinclis acus* *Paramecium* sp. *Vorticella* sp. Trichomes (*Solanum muritianum)*
Asteliaceae		
Collospermum hastatum		*Chroococcus* sp. *Alternaria* sp. Unidentified fungi Fern spores (*Dicksonia squarrosa, Cyathea* spp.) Pollen grains (*Dacrydium*)

Table 2. General summary of organisms and other biological material extracted from cultivated Bromeliads and the NZ native *Collospermum hastatum*. A full list of material based on a host species is available from the authors Ornagisms.

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