Hindcasting cyanobacterial communities in Lake Okaro with germination experiments and genetic analyses

Susanna A. Wood [1, 2], Katrin Jentzsch [2], Andreas Rueckert [2], David P. Hamilton [2], S. Craig Cary [2, 3]

1. Cawthron Institute, Private Bag 2, Nelson, 7042, New Zealand. Ph: 64-3-5482319 Fax: 64-3-5469464. Email: susie.wood@cawthron.org.nz
2. Department of Biological Sciences, University of Waikato, Private Bag 3105, Hamilton, 3240, New Zealand.
3. University of Delaware, College of Marine and Earth Studies, Lewes, Delaware,, 19958, USA.

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Abstract

Cyanobacterial blooms are becoming increasingly prevalent worldwide. Sparse historic phytoplankton records often result in uncertainty as to whether bloom forming species have always been present and are proliferating in response to eutrophication or climate change, or if there has been a succession of new arrivals through recent history. This study evaluated the relative efficacies of germination experiments and automated rRNA intergenic spacer analysis (ARISA) assays in identifying cyanobacteria in a sediment core and thus reconstructing the historical composition of cyanobacterial communities. A core (360 mm in depth) was taken in the central, undisturbed basin of Lake Okaro, New Zealand, a lake with a rapid advance of eutrophication and increasing cyanobacteria populations. The core incorporated a tephra from an 1886 volcanic eruption that served to delineate recent sediment deposition. ARISA and germination experiments successfully detected akinete forming nostocaleans in sediment dating 120 years before present and showed little change in Nostocales species structure over this time scale. Species that had not previously been documented in the lake were identified including *Aphanizomenon issatschenkoi*, a potent anatoxin-a producer. The historic composition of Chrococcales and Oscillatoriales was more difficult to reconstruct, potentially due to the relatively rapid degradation of vegetative cells within sediment.

Introduction

Cyanobacteria are natural constituents of lentic and lotic waters, but they appear to have become increasingly prominent in recent decades, possibly in association with anthropogenic eutrophication (Mur *et al.,*, 1999) and climate change (Paerl & Huisman, 2008). In lentic systems cyanobacteria can proliferate rapidly in response to adequate nutrient supply and elevated water temperature, with stratification a key part of their surface accumulation (Oliver & Ganf, 2000). Cyanobacterial blooms are aesthetically unpleasant and can have serious environmental impacts (Paerl *et al.,* 2001).

During periods when conditions are unfavourable for planktonic growth, many cyanobacteria persist in lake sediments as resting stages, either as short filaments (hormogonia), akinetes (resting stages) or vegetative cells (Head *et al.,* 1998; Verspagen *et al*., 2004). Benthic populations that survive winter may provide significant inocula for the development of pelagic cyanobacterial populations (Preston *et al.*, 1980; Brunberg & Blomqvist, 2003; Kim *et al.*, 2005). It has been suggested that large overwintering populations are one reason why cyanobacteria with low specific growth rates (e.g., *Microcystis*) become dominant in summer phytoplankton communities (Reynolds, 1994). Analyses of surficial lake sediments can provide valuable forecasts of the potential species composition of pelagic blooms (Baker & Bellifemine, 2000; Faithfull & Burns. 2006), while sediment profiles may provide information on historical phytoplankton composition and abundance (e.g., Livingstone & Jaworski, 1980; Dickman & Glenwright, 1997; Tani *et al.*, 2002).

Various techniques have been used to analyse phytoplankton within sediment cores. Livingstone and Jaworski (1980) showed that akinetes from sediments deposited up to 64 years earlier could be germinated when incubated in culture media. Recently, molecular techniques have been successfully used to detect cyanobacteria (Innok *et al.*, 2005) and cysts of eukaryotic algae (Coyne & Cary, 2005) from sediments. Automated rRNA intergenic spacer analysis (ARISA) is a recently developed DNA finger-printing method (Fisher & Triplett, 1999) that exploits the length heterogeneity of the intergenic spacer region (ITS) between the 16S and 23S ribosomal genes. In this study we used both germination experiments and ARISA assays to investigate the cyanobacterial community composition in layers of a sediment core taken from a eutrophic lake of volcanic origin in the Rotorua district of New Zealand.

A light-colored tephra deposited over the Rotorua district in the 1886 Tarawera volcanic eruption provides highly visual differentiation between lake sediments deposited before and after the Tarawera eruption (Nelson, 1983). Within this period many Rotorua lakes watersheds have been subject to European colonisation and changes in land use from native forest and scrub to pastoral farming and plantation forestry. Correspondingly, nutrient loads to many of the lakes have increased and, for some lakes, there is a well documented history of increasing trophic status, e.g., Lake Rotorua (White *et al.*, 1985) and Lake Rotoiti (Vincent *et al.*, 1984). However, there is little reliable historic information on phytoplankton species composition. Thus, it is difficult to ascertain if bloom forming species have always been present in the lakes and have proliferated

82 relatively recently, or if there has been a succession of cyanobacteria with new arrivals
83 through recent history.
84
85 Lake Okaro is a small, monomictic, eutrophic lake in the Rotorua district of central North
86 Island of New Zealand. It was formed as a hydrothermal explosion crater c. 900 years
87 before present (Healy, 1975). Pastoral farming proliferated rapidly in the Okaro
88 watershed in the 1950s (Jolly, 1968) and by 1970 around 95% of land use in the
89 watershed had been adapted for pastoral farming (McColl, 1972), similar to present day
90 land use. Compared with other New Zealand lakes, Lake Okaro has a long limnological
91 data record, dating back to the 1950s (e.g., Jolly, 1959; Fish, 1969; McColl, 1972; Flint,
92 1977; Dryden & Vincent, 1986; Forsyth *et al.*, 1988). This extended record includes a
93 period when the lake changed from a continuously oxygenated hypolimnion during the
94 eight-month seasonal stratification cycle, to being devoid of oxygen for all but one
95 month. Correspondingly, there have been large increases in nutrient concentrations,
96 increased relative abundance and biomass of cyanobacteria, and decline in diversity of
97 littoral benthos (Forsyth *et al.*, 1988). Since the 1970s the lake has had seasonally
98 recurrent cyanobacterial blooms (Dryden & Vincent, 1986). Gall and Downes (1997)
99 investigated fossil pigments in a sediment core from Lake Okaro. The pigments
100 myxoxanthophyll and canthaxanthin are specific to cyanobacteria. Neither of these
101 pigments was detected in the core in the period estimated from ^{210}Pb dating to be prior to
102 1900. A small increase in both myxoxanthophyll and canthaxanthin occurred between
103 1900 and 1950, followed by peaks of both pigments around 1965. The pigment analysis

104 unequivocally showed an increase in cyanobacterial concentrations in the lake, however,
105 it provided no information on possible changes in species composition.
106
107 The major objective of this study was to reconstruct the historical composition of
108 cyanobacterial communities in Lake Okaro in order to provide a sedimentary record of
109 their presence and long-term succession through a period of rapid progression of
110 eutrophication in the lake. A secondary objective was to evaluate the relative efficacies
111 of germination experiments and ARISA assays in reconstructing the assemblage of
112 cyanobacteria through the sediment profile.
113
114

115 **Methods**

116 *Sample site and core collection*

117 Lake Okaro is a small (0.33 km^2 surface area), shallow (max. depth 15 m) eutrophic lake
118 in central North Island (38°17'S, 176°23'E) of New Zealand (McColl, 1972). A sediment
119 core of length 360 mm was taken from the deepest, central part of the lake with a
120 cylindrical gravity corer (ø = 100 mm) on 22 May 2006. Visual inspection through the
121 acrylic barrel of the core indicated no disturbance of sediments into the overlying surface
122 waters. The sediment was extruded from the core barrel in discrete 20 mm sections
123 which were placed into sterile 50 mL Falcon tubes and stored at 4 °C in darkness.

124

125 *Age of core layers*

126 The Tarawera tephra was identifiable in the core as a discrete light-gray region
127 commencing 320 mm below the sediment surface and extending below 360 mm in our
128 core. Gall and Downes (1997) had previously identified the Tarawera tephra at a depth of
129 190 mm below the surface in a 1995 sediment core collected from the same central
130 location of Lake Okaro as part of a ^{210}Pb dating and sediment plant pigment study.
131 Alignment of the Tarawera tephra between cores allowed us to extrapolate their ^{210}Pb
132 dating data to our core. The average sedimentation rate between 1995 to the present day
133 is estimated to be 12 mm yr^{-1}, allowing approximate calculation of sediment age in the
134 upper section of the core.

135

136 *Germination experiments*

137 To assess the viability of akinetes and vegetative cells in the sectioned core, 1 g aliquots
138 were taken from each Falcon tube which contained the 20 mm layer of the core, and re-
139 suspended in Erlenmeyer flasks (100 mL) containing 75 mL of MLA medium (Bolch &
140 Blackburn, 1996). The flasks were incubated in a growth cabinet under a light regime of
141 100 μmol m^{-2} s^{-1} with a 12:12 h light:dark cycle, at a temperature of 18 ± 1 °C. All
142 germination experiments were undertaken in duplicate. An aliquot (5 mL) of the medium
143 was collected from each flask every four days over a duration of 20 days and preserved
144 with Lugol's Iodine.
145
146 *Cyanobacterial identification from germination experiments and ARISA profiles*
147 Identification of cyanobacteria was carried out using an inverted Olympus microscope
148 (IMT-2) and Utermöhl settling chambers (Utermöhl, 1958). Identification to species level
149 was made with taxonomic guides of; Baker (1991; 1992), Baker and Fabbro (2002),
150 McGregor and Fabbro (2001), Wood *et al.*, (2004) and McGregor (2007).
151
152 Rueckert *et al.*, (2007) and S.A Wood (unpub. data) established a New Zealand specific
153 cyanobacterial ITS library allowing phylogenetic information (16S rRNA gene
154 sequences) to be assigned to peaks in ARISA profiles. During germination experiments
155 several species were observed for which no 16S rRNA gene or ITS sequence information
156 was available. In these instances 2 mL aliquots were collected, frozen and pelleted by
157 centrifugation (18 000 g, 10 min). The supernatant was removed by sterile pipeting. DNA
158 was extracted from the remaining pellets using the Invitrogen Purelink™ Genomic DNA
159 Kit (Invitrogen, New Zealand) according to the gram negative bacteria extraction

protocol supplied by the manufacturer. The 16S rRNA gene and ITS sequences were determined by cloning and sequencing of PCR products as described in Rueckert *et al.*, (2007). Sequences generated during this work were deposited in NCBI Genbank database under accession numbers **EU402396 – 7.**

Isolation of DNA from sediment

Sub-samples of sediment taken from each 20 mm layer of the core were centrifuged (5 000 g, 5 min) to remove excess water. The supernatant was removed using sterile pipeting and DNA was extracted from *c.* 0.25 g of sediment using the MoBio Power Soil™ kit (Carlsbad, United States of America) according to the manufacturer's protocol.

ARISA fingerprinting and analysis

ARISA PCR reactions were carried out using cyanobacterial specific primers as described previously (Wood *et al.,* 2008). Amplicons were diluted 1 in 10 with sterile water, and 2 µL of product mixed with 0.25 µL of ROX-labelled genotyping internal size standard ETR900R (GE Healthcare, Auckland, New Zealand). The sample was made up to 10 µL with 0.2 v/v Tween-20 in sterile water. Intergenic spacer lengths were determined by electrophoresis using the MegaBACE system (Amersham Pharmacia Biotech). Run conditions were 44 °C separation temperature, 10 kV voltage and 120 min separation time.

ARISA fragment lengths (AFLs) were analysed by Genetic Profiler V.2 (GE Healthcare, Auckland, New Zealand) and data transferred to Microsoft Excel for further processing.

183 All AFL information was transposed to presence/absence data for further analysis. To
184 account for occasional small shifts in AFL between analyses and ensure that species
185 diversity was not over estimated, AFL that differed ≤ 2 bp were considered identical. If
186 multiple AFL fell within this range then only the AFL with the highest fluorescence was
187 maintained (Wood *et al.*, 2008). AFL falling below a threshold of 250 Fluorescence
188 Units were considered 'background noise'. AFL of less than 300 bp were considered to
189 be too short for the ITS to be valid (Wood *et al.*, 2008) and were removed from further
190 analysis.
191
192 *Anatoxin-a analysis*
193 To investigate potential anatoxin-a production by one of the species identified in the
194 germination experiments, *Aphanizomenon issatschenkoi*, aliquots (5 mL) of two cultures
195 (from sediment depths 140-160 mm and 160-180 mm) were collected at day 20 and
196 frozen (-20 °C). These samples were selected as they contained the greatest abundance of
197 *Aph. issatschenkoi* at this time during the germination experiment. The samples were
198 subsequently thawed and an equal volume of acetonitrile and formic acid added to 0.1%
199 v/v, then extracted using sonication for 10 min. Following centrifugation (3 500 g, 10
200 min) an aliquot of the supernatant was analysed directly for anatoxin-a using liquid
201 chromatography-mass spectrometry (LC-MS) as described in Wood *et al.*, (2007*a*).
202

Results

Germination experiments

A total of eleven different species of cyanobacteria were identified through microscopic examination. The species were from three different orders; Chroococcales (3), Oscillatoriles (3) and Nostocales (5; Table 1). Cyanobacteria were identified in all sediment layers except the deepest (340-360 mm), which overlapped with the Tarawera tephra, commencing around 320 mm. The highest diversity occurred in the two surface layers (0-20 and 20-40 mm) where all eleven species were found. *Aphanizomenon issatschenkoi* was the most common species through the depth profile, found in 15 of the 18 sediment layers (Table 1).

Eight days into the germination experiment akinetes were visible in *Aph. issatschenkoi*, allowing definitive identification of this species (Figure 1A, 1B). No akinetes were observed in other Nostocales species. However, distinctive features (i.e., terminal cell shape, spiral breadth) allowed the identification of *Anabaena circinalis* and *Aph. gracile*. A second *Anabaena* species was tentatively identified as *Anabaena* sp. *Nova* (Figure 1C), as described in Baker and Fabbro (2002). Observations of specimens of this species conformed to the following morphological description; trichomes solitary and regularly spiralled, coils of 20-35 μm width and closely compacted; vegetative cells spherical, slightly compacted at the poles, 6-7 μm breadth and with gas vesicles; heterocytes spherical and 7–8 μm breadth. A third much smaller *Anabaena* sp. was also identified in 14 of 18 sediment layers. The specimens observed conformed to the following

morphological description; trichomes irregularly coiled and occasionally entangled; vegetative cells barrel-shaped, 3.5–5 μm in length and 2.5–3 μm in breadth (Figure 1D).

Four of the species identified in our germination experiment (*Aphanothece* sp., *Geitlerinema* sp., *Anabaena* c.f. sp. *Nova* and *Aph. issatschenkoi*) had not previously been documented amongst the Lake Okaro phytoplankton community (Dryden & Vincent, 1986; Bay of Plenty Regional Council, unpub. data).

Cyanobacterial ITS library

Two 16S rRNA gene and ITS sequences that were not in the New Zealand cyanobacterial ITS library (Rueckert *et al.*, 2007; SA Wood unpub. data) were obtained from the clone libraries. The 16S rRNA gene segment sequences were submitted to BlastN (Altschul *et al.*, 1997) to identify other highly homologous sequences. The partial 16S rRNA sequence (~1200bp) from clone Okaro10 (Genbank EU402396) matched at greater than 99% sequence homology to *Pseudanabaena* sp. 1tu24s9 and PCC7408 (Genbank AM259269 and AB039020). The partial 16S rRNA sequence (~1200bp) from clone Okaro9 (Genbank EU402397) matched at greater than 99% sequence homology to *Anabaena sigmoidea* 0tu36s7 and 0tu38s4 (Genbank AJ630434 and AJ630435). The sequence-derived ARISA fragment lengths (AFL) for these species were; 688 bp and 450 bp.

ARISA analysis

247 Analysis of ARISA data for all samples produced a total of 19 distinct AFL. The
248 number of AFL in each sample ranged from one in the deepest layer (340-360 mm) to
249 nine (60-80 mm; Table 1). Unlike the germination experiment the diversity did not
250 decrease with depth. Of the 19 AFL, ten could be attributed to known planktonic
251 cyanobacterial ITS lengths based on our current ITS library. The most commonly
252 detected AFL (460 bp), identified in 16 samples, was attributed to *A. circinalis.* This was
253 closely followed by the 421 bp AFL of *Aph. issatschenkoi,* detected in 15 samples
254 (Table 1).
255
256 *Anatoxin-a detection*
257 The two sub-samples collected from the germination experiment culture (layers 140-160
258 mm and 160-180 mm) tested positive for anatoxin-a using LC-MS.
259
260

Discussion

Historic changes in cyanobacterial composition

A series of limnological observations in Lake Okaro extending back to the 1950s provide quantitative evidence of a progressive decline in water quality to the present time. The data include dissolved oxygen profiles, which show some oxygen remaining in the hypolimnion throughout seasonal stratification (1955-56; Jolly, 1968) but declining to the point where sometime between 1961-64, the hypolimnion was anoxic by the end of stratification. By 2005-6, the hypolimnion was anoxic for all but one month of the seasonal stratification cycle (Paul *et al.*, 2008). The phytoplankton community in Lake Okaro has been studied at irregular intervals over the past five decades and the data clearly show a shift in species composition. The most conspicuous of these shifts was the appearance of cyanobacteria. Jolly (1959) observed no colonial cyanobacteria in samples collected from Lake Okaro during 1955-56. The earliest reports of bloom forming cyanobacteria in Lake Okaro were in the 1960s when *Anabaena* was the dominant species (Fish, 1968). This observation corresponds with the findings of Gall and Downes (1997), who measured concentrations of myxoxanthophyll and canthaxanthin, pigments specific to cyanobacteria, in a sediment core taken from the center of the lake. They found a marked increase in these pigments during the 1960s. In the 1970s and 1980s, *A. spiroides*, *A. flos-aquae* and *Microcystis aeruginosa* were all reported at various times amongst the species contributing to persistent blooms (McColl, 1972; Flint, 1977; Dryden & Vincent, 1986). Monthly monitoring of Lake Okaro since the 1990s has shown that blooms of *Anabaena* spp. and *Microcystis* spp. occur regularly in spring and summer (Bay of Plenty Regional Council unpubl. data; Paul *et al.*, 2008).

The results of the ARISA and germination experiments clearly show that cyanobacteria have been a component of the phytoplankton community in Lake Okaro since the beginning of the century. Cyanobacteria were detected using both methods in the 320-340 mm layer, just above the Tarawera tephra, dating from 1886. The ARISA assay showed greater diversity throughout the sediment layers. One consideration when using molecular techniques such as ARISA, is that these methods detect the presence of genes (or gene fragments) and this does not necessarily correspond to viability (Coyne & Cary, 2005). A further consideration when interpreting the data from both methods is that different species vary in their tolerance to, and persistence in the sediments. Vegetative cells are not preserved well in lake sediments (Räsänen *et al.*, 2006) and this may explain the paucity of Chroococcales and Oscillatoriales in deeper sediment layers. This was particularly apparent in the germination experiments, with the majority of species from these orders were not observed below 120 mm. Therefore, using analysis of sediment cores to document chronological changes for all species may be misleading. *Microcystis* spp., for example, were not detectable (via either method) below 120 mm, which equates to an approximate sediment date of 1995. However, historic records (Dryden & Vincent, 1986) show that this species had already formed blooms in Lake Okaro by 1979.

Certain cyanobacteria from the Nostocales and Stigonematales orders produce akinetes, i.e., resting spores. These cells have thicker walls making them more resistant to decomposition (Räsänen *et al.*, 2006). In this study akinetes were germinated from

306 sediments that had been deposited *c.*120 years before present, indicating that the viability
307 of akinetes persists for long periods of time.
308
309 The identification of *Aph. issatschenkoi* in almost all sediment layers and to sediment
310 depths of 340 mm (120 years old), using both ARISA and germination experiments was
311 unexpected. This species has only recently been identified in New Zealand (Wood *et al.*,
312 2007*a*) and has never been identified in the phytoplankton community of Lake Okaro
313 (Fish, 1968; McColl, 1972; Flint, 1977; Dryden & Vincent, 1986; Bay of Plenty Regional
314 Council unpubl. data). A possible reason for the abundance of *Aph. issatschenkoi* in the
315 sediment core is the production of multiple akinetes by this species. In the germination
316 experiments this species produced multiple akinetes (up to seven akinetes per filament;
317 Figure 1B). It is plausible that conditions in Lake Okaro are never optimal for akinete
318 germination, thus populations may have only ever occurred at low concentrations.
319 Various studies (e.g., Baker & Bellifemine, 2000; van Dok & Hart, 1997) have shown
320 that germination depends on the occurrence of a relatively narrow range of conditions
321 occurring in both the sediment and water column.
322
323 Wood *et al.*, (2007*a*) detected the potent neurotoxin, anatoxin-a, in a culture of *Aph.*
324 *issatschenkoi* isolated from Lake Hakanoa, Waikato, New Zealand. Anatoxin-a has been
325 responsible for multiple animal deaths in New Zealand (Wood *et al.*, 2007*b*) and
326 worldwide (e.g., Gugger *et al.*, 2005). Anatoxin-a was detected in the two samples
327 collected from the germination experiments. *Aphanizomenon issatschenkoi* could
328 therefore become a significant health risk and careful examination of phytoplankton

samples from Lake Okaro should be undertaken to document both potential risk to lake users as well as any changes in its abundance as water quality restoration is attempted (Paul *et al.*, 2008).

Three other species; *Aphanothece* sp., *Geitlerinema* sp. and *Anabaena* c.f. sp. *Nova*, were observed in the sediment core, and had not previously been documented in the lake. *Aphanothece* sp. and *Geitlerinema* sp. were only recorded in the upper layers of the core. These are small species and may have been overlooked in routine monitoring programmes where low (200×) magnification was used. *Anabaena* c.f. sp. *Nova* was observed in multiple sediment layers in both germination and ARISA experiments. This species is very similar morphologically to *A. spiroides*. Given the abundance of *A. spiroides* in historic records (Dryden & Vincent, 1986) it seems probable that this species has been misidentified. The 16S rRNA gene sequence for the Lake Okaro strain showed a very high homology to *A. sigmoidea* (Rajaniemi *et al.*, 2005) suggesting that the current taxonomic classification of this species may need revision.

There were multiple peaks observed in the ARISA profiles that could not be assigned to species observed in germination experiments. ITS information was not available for all species (e.g., *Aphanothece* sp., *Geitlerinema* sp.) therefore it is likely these species would account for some of the AFL. Additionally, it has been shown that interoperonic differences in spacer length occur within the genomes of microorganisms (Nagpal *et al.*, 1998), thus a single species may contribute more than one peak to an ARISA profile. Previous studies (e.g., Gugger *et al.*, 2002; Wood *et al.*, 2008; Table 2) indicate that

352 species of the order Nostocales commonly have two types of intergenic spacer regions
353 (i.e., two AFL), whereas Chroococcales and Oscillatoriales have only one. Only one ITS
354 length has been identified for some of the species (e.g., *Anabanea* c.f. sp. *Nova*). The yet
355 to be determined second ITS may account for some of the unidentified AFL.
356 Phylogenetically unrelated species can have identical ITS lengths, therefore ARISA may
357 underestimate species diversity or possibly the wrong species may be assigned to an
358 AFL. For example, we assigned *A. lemmermannii* to the AFL of 471, however, this
359 species was not present in the germination experiments. Some of the unassigned peaks
360 could also be due to artefacts produced during DNA extraction and PCR (Taton *et al.*,
361 2006).
362
363 *Surface sediments*
364 There has been debate on the importance of the role of benthic cyanobacterial in re-
365 inoculation of pelagic populations. Some studies have suggested germination of akinetes
366 or recruitment of hormogonia or vegetative cells from the surface sediment plays a
367 critical role in bloom initiation (e.g., Rother & Fay, 1977; Brunberg & Blomqvist, 2003;
368 Kim *et al.*, 2005). Conversely, Reynolds (1975), Karlsson-Elfgren (2003) and Verspagen
369 *et al.*, (2005) found that recruitment of akinetes and vegetative cells from surface
370 sediments had little influence on summer pelagic cyanobacterial populations.
371
372 The result from the Lake Okaro sediment core suggests that the importance of sediment
373 recruitment varies between species. One of the surprising findings was the complete
374 absence of *A. planktonica* from the sediment surface layers. *Anabaena planktonica* was

375 first detected in New Zealand in 2000 and has rapidly spread throughout the North Island
376 (Wood *et al.*, 2004). Dense populations (at times > 80 000 cells mL^{-1}) of *A. planktonica*
377 have been recorded in Lake Okaro during the past four summers (Bay of Plenty Regional
378 Council unpubl. data). The absence of this species in the surface sediments suggests it is
379 able to survive in a pelagic vegetative state throughout the winter. Other *Anabaena*
380 species have been observed to overwinter in a pelagic vegetative state. Head *et al.*, (1999)
381 found *A. flos-aquae* filaments overwintered in the water column, and surmised that this
382 pelagic population was the primary source of subsequent cyanobacterial growth. In the
383 Okaro core, the highest cyanobacterial diversity was observed in the surface layers (0 -
384 40 mm), indicating that overwintering in either a vegetative state or as akinetes is an
385 important adaptive strategy for many species, not including *A. planktonica*. Further
386 research involving sediment traps and year-round phytoplankton monitoring is required
387 to elucidate the importance of sediment overwintering and determine variables that
388 trigger recruitment in Lake Okaro.
389
390 *Conclusions*
391 Sediment germination experiments and/or molecular techniques can be used to
392 successfully monitor chronological changes in community structure in most akinete
393 producing species over long time scales. For Nostocales, the results of this study indicate
394 that there has not been a dramatic change in cyanobacterial species composition in Lake
395 Okaro in the past 100 years. The methods used in this study do not measure quantitative
396 changes, but other studies (e.g., Flint, 1977; Dryden & Vincent, 1986) indicate an
397 increase in planktonic cyanobacterial abundance in response to nutrient enrichment. The

absence of *A. planktonica* in the sediment, demonstrates that overwintering on the sediment is not an important survival strategy for all cyanobacterial species. Analysis of the sediment core revealed the presence of previously unreported cyanobacterial species. Of particular concern is anatoxin-a producing *Aph. issatschenkoi*. This species has recently become dominant in other New Zealand lakes and because of its potential threat to human health, its abundance should be closely monitored.

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Table 1. Cyanobacterial species identified in 20 mm sections of a sediment core from Lake Okaro; Germination (x), ARISA (ITS 1 •, ITS 2 +). Germination experiments were undertaken in duplicate and samples collected every four days for 20 days, this table shows combined result for each layer.

Sediment Depth (cm)	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	20-21	22-23	24-25	26-27	28-39	30-31	32-33	34-35
Est. sediment date[a]	2006	2004	2002	2001	1999	1997	1995	1987	1976	1965	1954	1943	1932	1921	1911	1900	1886	>1886
Chroococcales																		
Aphanocapsa sp.	x	x																
Aphanothece sp.	x	x			x													
Microcystis spp. (531)[b]	x •	x •	x	x •	x •	x •												
Synechocystis sp. (565)												•	•	•			•	•
Oscillatoriales																		
Geitlerinema sp.	x	x	x	x	x													
Pseudanabaena limnetica (688)	x	x		x	x •				•	x	x							
Nostocales																		
Anabaena circinalis (460, 672)	x •	x	x • +	x • +	x • +	x • +	•	x • +	x • +	x •	•	• +	•	•	• +	x •	• +	
An. lemmermannii (471)	•	•	•	•	•			•	•	•	•	•	•	•	•			
Anabaena sp. A	x	x	x	x		x	x	x	x	x	x	x	x	x				
Anabanea c.f. sp. *Nova* (450)	x	x •	x •	x •	x •	x	x	x •	x •	x	x •	x •	x	•	•	x •		
Aphanizomenon gracile (440)	x •	x •	x •	x	x •	•	•	•		•	•	•	•	•				
Aph.issatschenkoi (421, 646)	x •	x • +	x •	• +	x • +	x		x • +	x • +	x • +	x • +	x • +	x •	x • +	x • +	x • +	x • +	
Unassigned AFL[c]																		
440	•	•	•	•					•	•								
463	•	•																
482					•			•	•						•			
585				•														
591	•							•		•			•	•	•	•		
625																•	•	
679					•					•	•	•	•	•				
701								•		•								
943												•	•					
Total number of species via germination	**10**	**10**	**7**	**7**	**8**	**5**	**2**	**4**	**4**	**5**	**4**	**3**	**3**	**2**	**1**	**3**	**1**	**0**
Total number of AFL	**8**	**7**	**6**	**7**	**9**	**3**	**2**	**8**	**7**	**8**	**6**	**8**	**8**	**8**	**6**	**5**	**4**	**1**

[a] Sediment date estimated by alignment with ^{210}Pb dated core of Gall and Downes (1997).
[b] AFL given in brackets.
[c] AFL, ARISA fragment lengths

Figure 1. Light photomicrographs of a selection of cyanobacterial species identified in 20 mm sections of a sediment core from Lake Okaro via germination experiments. (A, B) *Aphanizomenon issatschenkoi*, (C) *Anabaena* sp. *Nova*, (D) *Anabaena* sp. a, akinete; h, heterocyte. Scale bars = 10 μm.

Figure 1.