The green filamentous alga *Rhizoclonium* in Te Waikoropupū Springs

A molecular and ecological assessment

Prepared for Tasman District Council

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Executive summary

In November 2019, members of the public raised concerns about the appearance of unusual algal mats in parts of the margins of Te Waikoropupū Springs, near Takaka, Golden Bay. The alga was identified as a species of *Rhizoclonium* by Tasman District Council (TDC), NIWA and Manaaki Whenua-Landcare Research. Funding from an MBIE Envirolink Small Advice Grant and from TDC was secured to enable NIWA to carry out a literature search and summarise information about the species' distribution, environmental preferences/tolerances and potential to form nuisance growths, and for Landcare Research to use DNA analysis to identify the material to species level, if possible.

The DNA analysis and comparison with archived sequences placed the alga in the *Rhizoclonium riparium* complex. Members of this complex have a worldwide distribution and have been found throughout New Zealand since at least the 1940s. An identical archived DNA sequence was from a sample collected in Te Waikoropupū Springs in December 2004 indicating that *R. riparium* has been in the Springs for at least 15 years.

R. riparium is remarkable for its ability to inhabit waters across a very wide range of salinity (seawater to freshwater). The species' growth in fresh water (rather than sea water) appears to require hard water, as indicated by slightly alkaline pH (e.g., pH 8). Te Waikoropupū Springs water meets these requirements.

R. riparium typically grows as mats attached to beds of waterbodies but, like other types of filamentous algae, may detach from the bottom after rapid expansion and float to the surface.

Experimental work on *R. riparium* suggests that growth rates may be markedly enhanced by increasing temperature in the range up to 15 °C or higher. Water temperatures in Te Waikoropupū Springs were 11.2 °C to 13.3 °C in spot measurements up to 2015. Continuous measurements in the main part of the Springs from October 2017 to January 2018 suggested a constant temperature of around 11.7 °C. Water temperature data at the time the mats were observed in the Springs would be of interest.

Experimental studies have demonstrated that increasing light levels also stimulate *R. riparium* growth. Very high water clarity in Te Waikoropupū Springs would therefore favour expansion of *R. riparium* on the bottom.

The nutrient requirements of *R. riparium* are unclear except that waters with a high N:P ratio (which is the case in Te Waikoropupū Springs) may provide a competitive advantage for *Rhizoclonium* over other types of algae. Records from the literature and other New Zealand locations indicate that abundant *Rhizoclonium* (growing on the bottom or as floating mats) occurs in a wide range of dissolved N and P concentrations.

1 Background

Te Waikoropupū Springs, near Takaka, Golden Bay, are the largest freshwater springs in New Zealand. Their high discharge from multiple springs is among the clearest water ever measured (Davies-Colley and Smith 1995). The springs are a treasured local amenity and an important attraction for visitors to the area.

In November 2019, members of the public raised concerns with Tasman District Council and the news media about the appearance of algal mats in parts of the margins of Te Waikoropupū Springs. The mats appeared to be atypical and had not been observed in previous years (Figure 1-1). A sample sent to NIWA, Christchurch, on 19 November 2019 was confirmed by NIWA, and later by Manaaki Whenua-Landcare Research (MWLR), to consist mainly of a species of the green filamentous alga *Rhizoclonium*. *Rhizoclonium* includes many species, spanning freshwater, brackish and marine environments. No attempt was made at that time to identify the material to species level.

Figure 1-1: Algal mats in Te Waikoropupū Springs, November 2019. Photographs: Department of Conservation.

The genus *Rhizoclonium* is widespread and generally not problematic in New Zealand. However, a recent compilation of algal taxa recorded from Te Waikoropupū Springs (Matheson et al. 2018) did not include *Rhizoclonium*. Therefore, this algal genus was assumed at that time to be a new record for the Springs.

Tasman District Council sought advice from NIWA and MWLR on obtaining more information about the *Rhizoclonium* species that was forming the algal mats. An MBIE Envirolink Small Advice Grant was secured to enable NIWA "to carry out a literature search of *Rhizonclonium* species that fitted the description of the one found in the Springs to determine their distributions, environmental preferences/tolerances, potential to form nuisance growths, and any other relevant information". In addition, because definitive identification to species level is difficult from vegetative material, Tasman District Council provided separate funding to MWLR for additional analysis to sequence samples of the filaments' DNA, which could potentially enable species identification (by matching to existing sequences).

Here we report on both studies. A description of the material from microscope observations at NIWA and MWLR (Section 2) is followed by an account of the molecular analysis carried out by Landcare Research, which enabled placement of the specimens from Te Waikoropupū Springs in the *Rhizoclonium riparium* complex (Section 3). Section 4 presents the results of the literature search and review, focusing on *R. riparium*.

2 Description based on light microscope observations

Samples of the algal mats from Te Waikoropupū Springs were sent to NIWA on 21 November 2019. An informal description was made as follows:

> Filaments of cells usually about 12 µm wide, occasionally up to 25 µm, and up to 70 µm long (irregular lengths and widths). Filaments mainly unbranched but occasional branches at 90 degrees, with the branch starting as an extension of the cell wall. Some filaments had distinctive sharp bends (“elbows”). Cell wall mostly thin, but at intervals along filaments there were thickened cell walls either side of a cell dividing wall. Deep green narrow densely intertwined chloroplasts mostly filled the cells, with obvious pyrenoids scattered amongst the chloroplasts. A central nucleus was visible by changing the focus. Cell joins resembled those of *Cladophora*, but the form of the branches was different. Occasional sequences of empty cells were observed in the sample, with ruptured sides suggesting recent release of gametes (i.e., sexual reproduction).

Under the microscope we also observed that the cell walls became highly illuminated when viewed under polarised light. This feature is characteristic of algae in the order Cladophorales (such as *Cladophora*, *Chaetomorpha* or *Rhizoclonium*) and a few other algae, because the cell walls of these taxa comprise highly crystalline cellulose (Mihranyan 2011).

Features of the filaments observed under light microscopy are shown in Figure 2-1, Figure 2-2 and Figure 2-3.

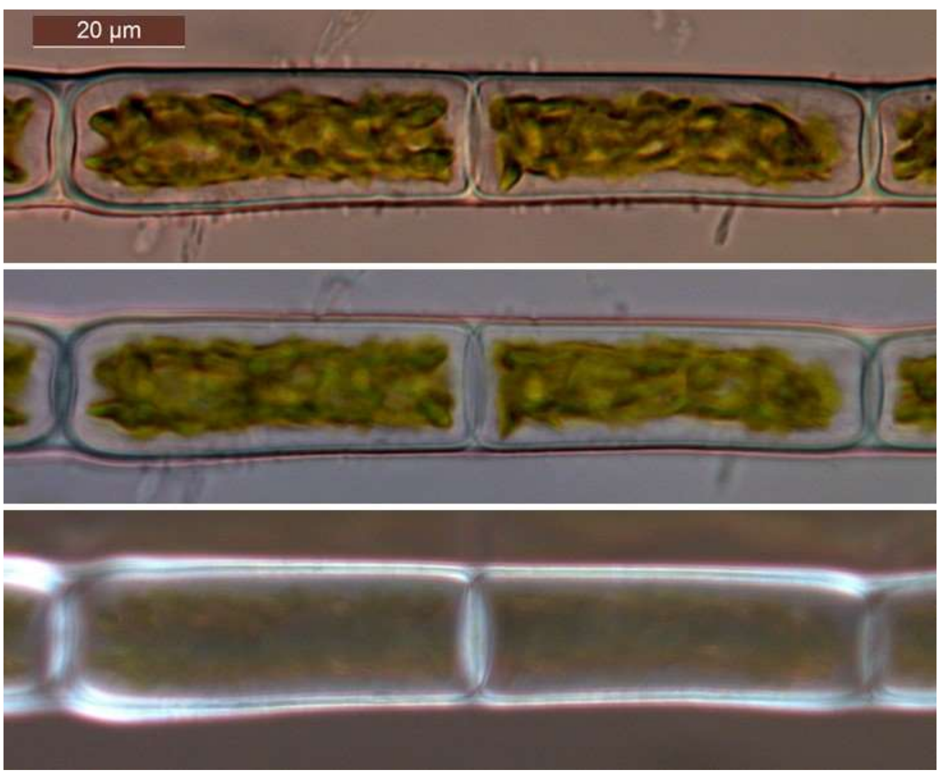

Figure 2-1: Light micrographs of *Rhizoclonium* filaments from Waikoropupū Springs under three types of illumination. Top: no filter, the focus highlights epiphytes on the outside of cell wall. Middle: daylight blue filter (creating more natural colours). Lower: polarized light, showing glowing cell walls. Photos: Phil Novis.

Figure 2-2: Light micrograph of a filament of *Rhizoclonium* from Te Waikoropupū Springs showing branching. Photo: Phil Novis.

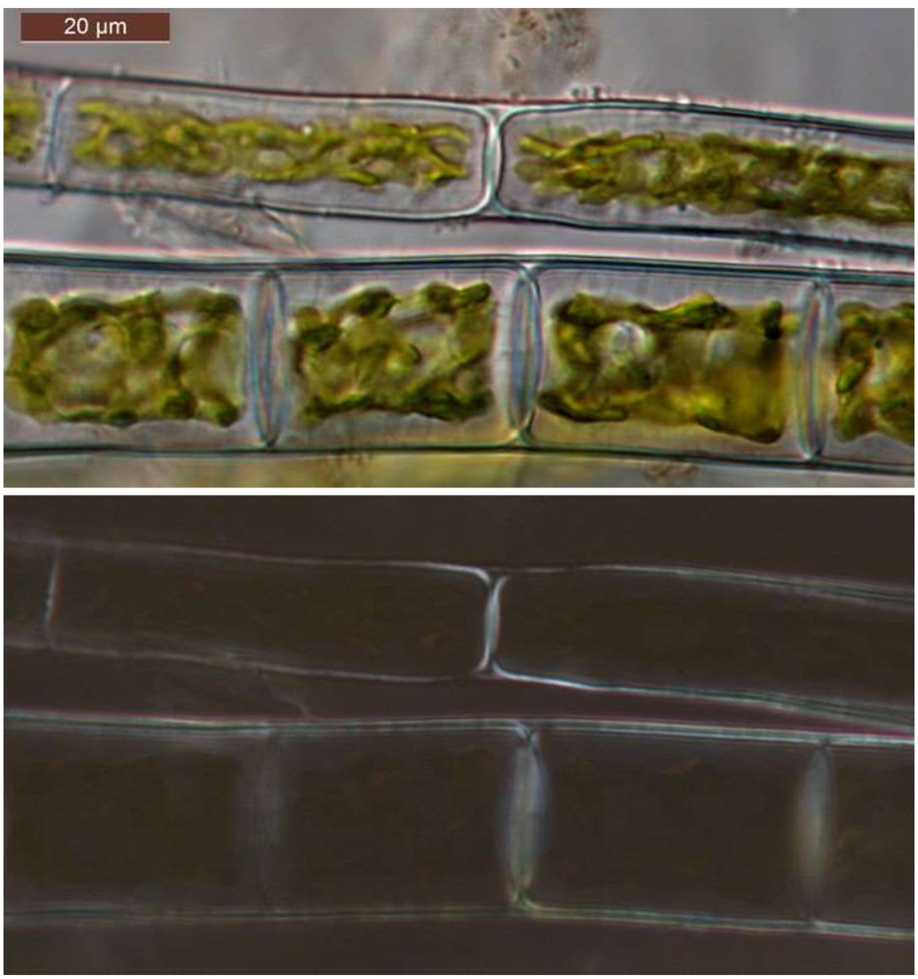

Figure 2-3: Light micrographs of two *Rhizonclonium* filaments of different diameter. The lower photograph shows that the cell walls of both filaments glowed under polarized light. This, along with the similar form of the chloroplasts suggested that both filaments were *Rhizoclonium*. Photos: Phil Novis.

The microscope observations confirmed the suggestion by Rob Smith (Tasman District Council) that the alga was a species of *Rhizoclonium*. Comparison of the microscope observations with accounts of the common freshwater species *Rhizoclonium hieroglyphicum* (e.g., Parodi and Caceres 1993) suggested strong similarities with this species, although cell dimensions reported for *R. hieroglyphicum* were larger than those observed in the material from Te Waikoropupū Springs. The uncertainty about the identity of the alga at species level led to the suggestion that molecular analysis of the material might be informative.

3 Systematics and molecular analysis

3.1 A brief synopsis of the recent systematics of *Rhizoclonium*

Rhizoclonium is a genus of filamentous green algae in the class Ulvophyceae, most members of which are marine. *Rhizoclonium* itself is found in both freshwater and marine localities. It is placed in the order Cladophorales and has traditionally been distinguished from the closely related genus *Cladophora* by lacking branches, which *Cladophora* typically displays in abundance (e.g., Leliaert & Boedeker 2007). Recent years have seen drastic taxonomic revision in Cladophorales, and a common question has been whether branching or lack thereof is a synapomorphy[1] distinguishing *Rhizoclonium* from *Cladophora*. Some analyses tend to support the traditional separation based on this character (e.g., Zhao et al. 2016), but others with better taxon sampling appear to show that branching and non-branching specimens are intermixed, with branching having been lost or gained multiple times in this group. Realisation that branching was not a consistently inherited trait led to the recognition of the new genus *Pseudorhizoclonium* (Boedeker et al. 2016), yet recent work on Cladophorales from Lake Baikal, Russia, suggests that branching and non-branching forms can be sister taxa within several cladophoralean genera (Boedeker et al. 2018). Without molecular sequencing, it may be difficult to determine the genus of a specimen in the modern system, let alone its species. Although new species of *Rhizoclonium* continue to be described and named (Zhao et al. 2016, 2018) the vast majority of available molecular sequences are not yet attached to a species name. Nonetheless, useful information can often be concluded about the identity of a specimen and the distribution of taxa with the help of DNA information, and this has been attempted here.

3.2 Methods

Two samples of *Rhizoclonium* sp. from Te Waikoropupū Springs, identified to genus level microscopically, were received at the Allan Herbarium, Lincoln, in November 2019 and stored at -20°C. DNA extraction, primers, and PCR conditions for the SSU and LSU rDNA genes[2] were as given by Zhao et al. (2016).

Sequencing was undertaken by MWLR in Auckland, New Zealand. Sequences were obtained from three replicate extractions, and both DNA strands were sequenced in each case, to generate an assembled consensus sequence using the alignment tool in MEGA X (Kumar et al. 2018). Datasets for each gene were compiled using sequences available in Genbank and were principally composed of sequences used by Boedeker et al. (2016), Saber et al. (2017), Zhao et al. (2018), and Sherwood et al. (2019). Accession numbers are provided on the phylogenies. Sequences were aligned using the ClustalW algorithm implemented in MEGA X and checked by eye. The SSU dataset contained 45 sequences and 1681 base positions (134 variable, 102 parsimony-informative[3]). The LSU dataset contained 92 sequences and 583 base positions (178 variable, 164 parsimony-informative).

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[1] A synapomorphy is a trait or feature seen in two or more organisms that is there because it has been inherited from a common ancestor. *Cladophora* and *Rhizoclonium* share multiple features but have traditionally been separated by possessing branched and unbranched filaments, respectively. The question is whether branched filaments are characteristic of all species in *Cladophora* (because all species are derived from a branched common ancestor) and similarly for unbranched filaments in *Rhizoclonium.* Alternatively, branching could be an incidental feature that is unrelated to any ancestor.

[2] Small subunit (SSU) and large subunit (LSU) sequences of ribosomal DNA (rDNA) are typically used to determine phylogenetic relationships.

[3] Parsimony-informative sites group at least two taxa against at least two others (so they are informative about clades). Variable sites (in which at least one sequence is different from the others at that position in the alignment) still provide information; parsimony-informative sites are a subset of variable sites.

Because the goal was to establish which sequences were closely related to those from other studies, rather than to resolve questions of deeper phylogeny, the datasets were analysed using the distance-based Neighbour-Joining method (Saitou and Nei 1987). Distances were computed using the Maximum Composite Likelihood metric (Tamura et al. 2004), and the analyses conducted in MEGA X with the pairwise deletion option and 1,000 bootstrap replicates (Felsenstein 1985).

3.3 Results and discussion

The SSU gene proved to be relatively sparsely sampled in this group of *Rhizoclonium*, with the result that only a single sequence was a close match (identical) to the material from Te Waikoropupū Springs (Figure 3-1). The identical specimen, H17, was collected from a coastal marine habitat in California, USA.

The LSU gene was much more revealing. In this phylogeny specimen H17 was joined by a further nine specimens that were identical or nearly identical to the specimen from Te Waikoropupū Springs. The nine specimens are highlighted by the bold vertical black line in Figure 3-2. Significantly, one of these (C13, second from the top of the highlighted group) was collected previously from Te Waikoropupū Springs on 31 December 2004. This would indicate that the recent proliferation of *Rhizoclonium* in the Springs is not due to a recent invasion, but due to (temporarily) favourable habitat conditions.

Specimen C13 was accession A033197 from the collection in the Museum of New Zealand Te Papa Tongarewa Herbarium (WELT). The details of this and other WELT specimens indicated in Figure 3-2 are given in Table 3-1. A collection date was provided for A033197 only and the sample was collected by C. Boedecker.

Due to the ongoing state of flux of systematics in this group of algae (see Section 3.1), it is difficult to apply a species name at present. Nonetheless, the sequence falls into a group that has been described elsewhere as the "*Rhizoclonium riparium* complex" (Boedeker et al. 2016), and this name can be used informally. Boedeker et al. noted that the *riparium* complex possesses "stunning osmoregulation capacities", with the same genotypes found in marine and freshwater environments. The genotype found in Te Waikoropupū Springs would seem to exemplify this capability for tolerance of freshwater environments in a typically marine species.

Table 3-1: Details of previously collected specimens which are genetically identical or near-identical to the recent collection of *Rhizoclonium* from Te Waikoropupū Springs.

WELT accession number	Original identification	Locality	Date collected
A033197	*Rhizoclonium* sp.	Te Waikoropupū Springs, NZ	31 Dec 2004
A033240	*Rhizoclonium* sp	Ythan Estuary, Scotland	
A033283	*Rhizoclonium* sp	Meije, South Holland, Netherlands	
A033686	*Rhizoclonium* sp	Coastal marine site, Iceland	
A033725	*Rhizoclonium* sp	Coastal marine site, Australia	
OR 1833	*Rhizoclonium* implexum	Coastal marine site, USA	
OR 1867	*Rhizoclonium* implexum	Coastal marine site, USA	
OR 2070	*Rhizoclonium* cf. *lubricum*	Heceta Head cliffs, Oregon, USA	
OR 2132	*Rhizoclonium* cf. *riparium*	South Slough, Coos Bay, Oregon, USA	

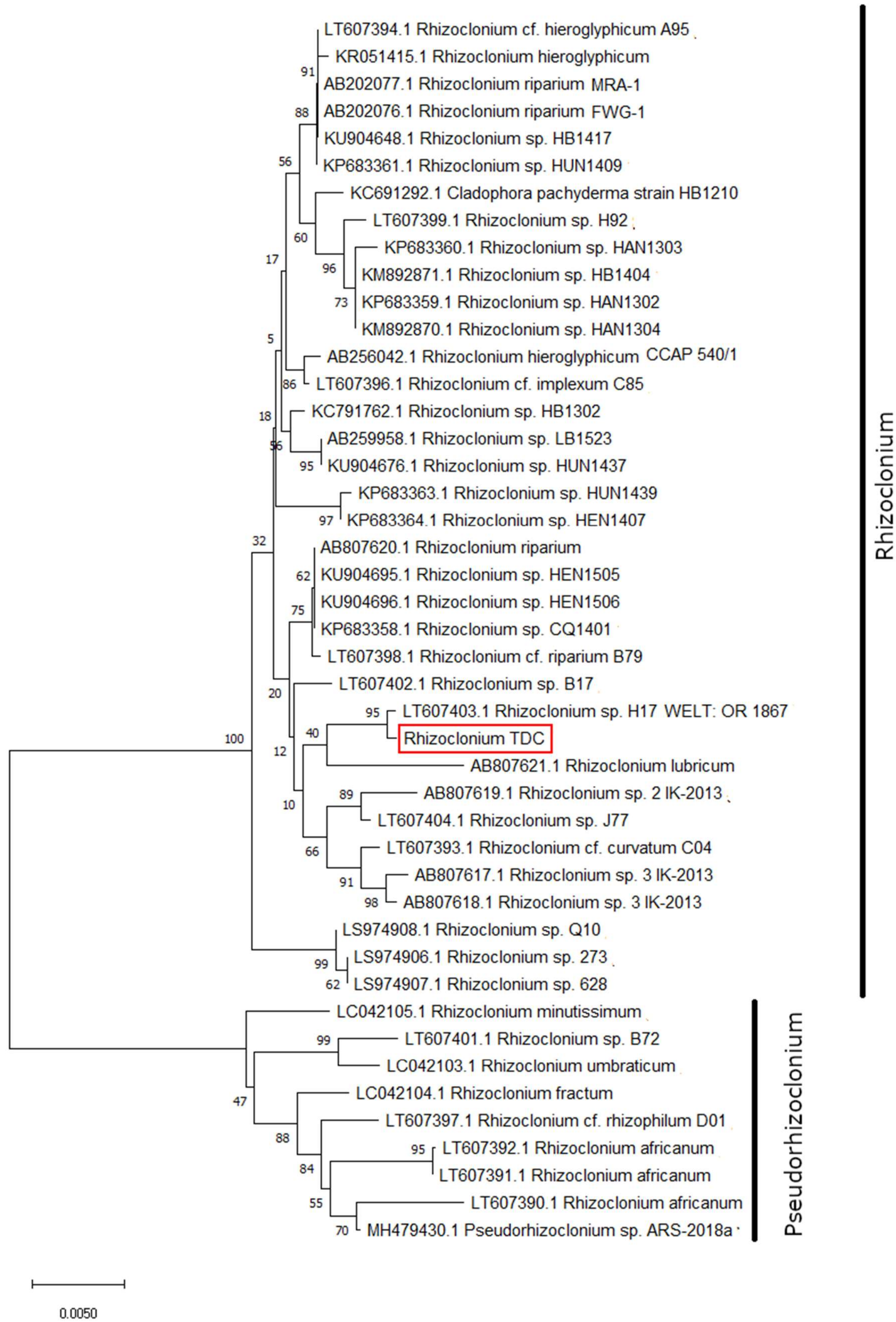

Figure 3-1: Neighbour-joining phylogeny of the SSU gene for *Rhizoclonium*, showing the position of the specimen from Te Waikoropupū Springs (red box).

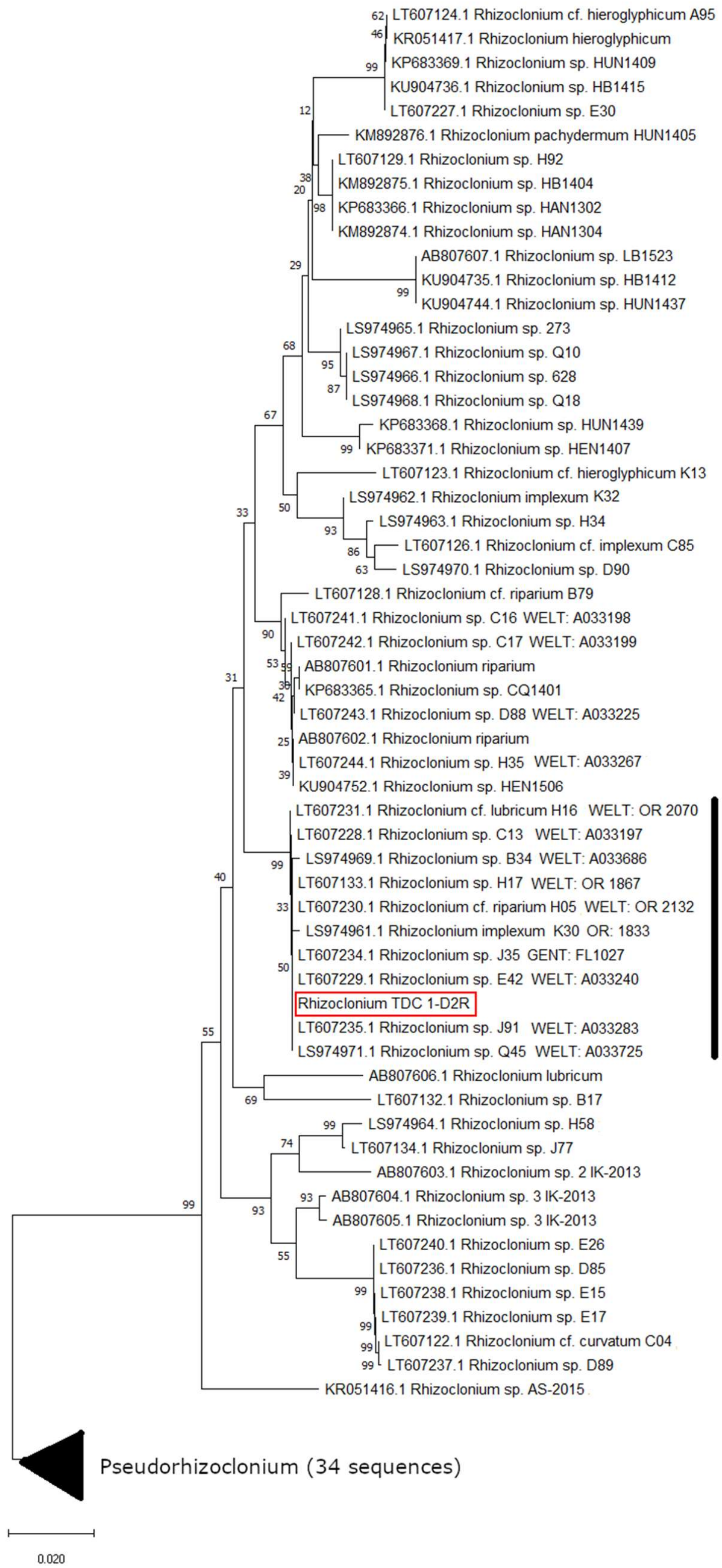

Figure 3-2: Neighbour-joining phylogeny of the LSU gene for *Rhizoclonium*, showing the position of the specimen from Te Waikoropupū Springs specimen (red box). The bold vertical black line on the right indicates the sequences discussed in the text.

4 Distribution and ecology of *Rhizoclonium riparium*

This section addresses the main purpose of the Small Advice Grant which was to carry out a literature search relating to *Rhizoclonium riparium* (Roth) Harvey 1849 to determine its distribution, environmental preferences/tolerances, potential to form nuisance growths, and any other relevant information.

4.1 *Rhizoclonium* and *R. riparium* in New Zealand

Rhizoclonium has been informally identified to genus level from various freshwater locations around New Zealand. For example, *Rhizoclonium* sp. was observed at 24 of 153 river sites throughout New Zealand, surveyed for filamentous algae during low flow conditions in 1983 (Biggs and Price 1987). Records since then include observations from Northland (Kilroy and Lambert 2019). The genus is also included in MWLR's freshwater algae online guide (https://www.landcareresearch.co.nz/resources/identification/ algae/identification-guide/interpretation/indicator-taxa/poor-streams/rhizoclonium). All these identifications have been to genus level only.

Rhizoclonium riparium is one of 11 species of *Rhizoclonium* listed as being present in New Zealand, although three of the species were only tentatively assigned to the genus (Broady et al. 2012). The three tentative species and two others are listed as freshwater species, and the remaining six (including *R. riparium*) are listed as marine species. The species *R. riparium* has an entry in the New Zealand Plant Conservation Network website, where it is assigned to the flora category "non-vascular – Native" https://www.nzpcn.org.nz/flora/species/rhizoclonium-riparium/). In addition, *R. riparium* was included in an account of the marine algae of New Zealand published in the 1950s (Chapman 1956). This latter record was based on collections made by V. W. Lindauer in the 1930s and 1940s (Cassie 1971). The species was distributed from Northland to Fiordland, suggesting it is a native species.

Major algal floras (online and hard copy) generally list *R. riparium* as a cosmopolitan species (i.e., worldwide distribution) (e.g., www.algaebase.org).

4.2 Literature search

An initial search for literature relating to the genus *Rhizoclonium* included a search on Web of Science with "*Rhizonclonium*" as a search term. This search was refined by (a) adding the search term "*riparium*" and (b) adding the terms "nuisance" OR "proliferat*" OR "bloom" OR "degrad*" to capture studies on *Rhizoclonium* as a problematic algal species. In addition to the Web of Science search we retrieved selected literature that was referred to in the published material, including theses.

The initial Web of Science search returned 155 hits on 9 June 2020. The earliest publication was in 1921, but almost 45% of the references found were published since 2010. Thirty publications were returned when "riparium" was included in the search, and *R. riparium* was mentioned in at least one further publication. Including the terms intended to capture research on potential problems with *Rhizoclonium* (but excluding riparium) returned only 13 publications.

The 155 publications covered a wide range of topics, with almost 45% covering applications of *Rhizoclonium* and related algae (Table 4-1). The use of *R. riparium* in applications such as aquaculture (as a food source) and biofuel production highlights the ability of the species to produce high biomass under favourable conditions. Only two studies focussed specifically on *Rhizoclonium* as a nuisance alga, although the genus is occasionally listed as an alga capable of nuisance proliferations in rivers (e.g., Biggs and Price 1987, Stevenson et al. 2012).

Table 4-1: Summary of studies on *Rhizoclonium* identified from a literature search. A total of 69 studies focussed on applications and 86 studies on various aspects of biology. N (a) shows the total number of studies in each topic, and N (b) the number that focussed on *R. riparium*.

Group	Topic	N (a)	Notes	N (b)
Applications	Active compounds	7	Extraction of pharmaceutical and other compounds from *Rhizoclonium* and related taxa	3
	Aquaculture	12	Growth and use of *Rhizoclonium* as a food source in aquaculture	8
	Biofuels	12	Includes reviews mentioning *Rhizoclonium*, specific studies on biomass accumulation, and ethanol extraction	2
	Disease spread	9	Studies on role of *Rhizonclonium* as a substrate ("carrier") of the cholera bacterium, 1988 - 1999, with review in 2008.	0
	Metal accumulation	16	Studies on adsorption, bioaccumulation and removal of various metals	2
	Wastewater remediation	5	Use of *Rhizoclonium* for nutrient removal in wastewater finishing ponds	0
	Other applications	8	Miscellaneous applications (e.g., human nutrition, cosmetics, fertilizer, paper-making)	1
Biology	Biogeography / taxonomy	21	Regional distribution of *Rhizoclonium* and related species, including molecular studies	3
	Cell chemistry	7	Chemical analysis of cell contents	0
	Cytology	7	Studies on internal cell structure(s) (all published 1980 - 1994).	0
	Ecology	44	Effects on physico-chemical variables, and effects on aquatic foodwebs and fish. Earliest (1921) to most recent (2019) paper	7
	Other	2	Gene expression, DNA extraction	1
	Morphology	3	Growth form of filaments	0
	Nuisance growth	2	Effect on hydro-power canals, biofouling of boats	0
	Parasites	2	Effect of parasites on *Rhizoclonium*	0
	Taxonomy	9	Descriptions of new species, and more general systematic studies, including phylogenetic studies	3

4.3 Growth form

Rhizoclonium riparium is normally described as a benthic species that forms thick mats along the water's edge in estuaries or rivers, or grows in tufts attached to hard substrata (e.g., Pomeroy and Stockner 1973). However, formation of floating mats at the water surface (as seen in Te Waikoropupū Springs) is not uncommon (e.g., Snook 1987). Formation of floating mats at the surface of slow flowing waters is typical of benthic green filamentous algae in general during times of rapid growth and, once at the surface, the algae continue to grow (Biggs 1996).

4.4 Environmental preferences/tolerances of *R. riparium*

The literature search yielded eight publications that covered ecological aspects of *R. riparium* (Table 4-1), only three of which included relevant information (Imai et al. 1997, Sommer 1996, Pescheck et al. 2014). A further study (Hall and Walmsley 1991) duplicated results from a Master of Science thesis (Snook 1987) and we used the latter reference (which was not included in the 155 publications from the literature search). Snook (1987) was especially relevant as it focused on environmental conditions associated with *R. riparium* mats similar to those observed in Te Waikoropupū Springs, although the site was a wastewater treatment plant maturation pond. Useful information was also obtained from an additional study that focussed on growth conditions for aquaculture (Chao et al. 2005).

The findings are summarised below in three sections (salinity/conductivity, temperature and light, and nutrients). A brief evaluation of the relevance to conditions in Te Waikoropupū Springs is provided at the end of each section.

4.4.1 Salinity / conductivity

Rhizoclonium riparium has been described as a "holeuryhaline alga" (Nienhuis 1974), meaning that it occurs across the whole spectrum of seawater to estuarine / brackish water to freshwater. Such wide salinity tolerance is unusual in algae. For example, Imai et al. (1997) stated: "A few genera are known to include both marine and freshwater species, but no algal species has been found to be distributed as widely from freshwater to marine environments as *R. riparium*." See also comments by Boedeker et al. (2016) (Section 3.3).

Laboratory studies showed that *R. riparium* grew best in salinity of 13.6 ppt (approximately 40% seawater by volume) and slowest at 0.1 ppt (equivalent to freshwater) (Imai et al. 1997). However, when the pH of the 0.1 ppt medium was adjusted to 8.1 with bicarbonate buffer (HCO_3^-), photosynthetic rates increased to match those in the more saline water (Imai et al. 1997). These authors concluded that "this alga can perform photosynthesis well in a wide range of salinities when supplied with HCO_3^-." This result implies that the freshwater habitats of *R. riparium* are expected to have high hardness (leading to high conductivity) and slightly elevated pH. Consistent with this, *Rhizoclonium* species are typically encountered in rivers with high conductivity (e.g., mean of 300 µS/cm, Biggs and Price 1987).

Conductivity in Te Waikoropupū Springs was measured as 520 – 710 µS/cm in the 1990s (Kim and Hunter 1997). More recent measurements (October 2017 to January 2018) indicated conductivity varying between about 500 and 800 µS/cm over that period (Gall 2018, Gall and Milne 2018). Furthermore, the Springs waters have relatively high concentrations of calcium carbonate (i.e., high hardness, 190 mg $CaCO_3$/L) (Young et al. 2017), reflecting their karst geological setting. Measurements of pH in the Springs have ranged from 6.9 to 8.4 since 1970, and over the full record there has been an increase in pH of about 0.5 pH units (or 0.1% per year) (Young et al. 2017). In the 2017-2018 study, pH ranged from 7.6 to 7.9 (Gall 2018). The combination of high conductivity and hardness and slightly alkaline pH in the Springs therefore currently appears to be in the range that would favour *R. riparium* growth, based on the experiments by Imai et al. (1997).

4.4.2 Water temperature and light

The optimum growth temperature for *R. riparium* has been estimated to be around 15 °C (water temperature), based on laboratory experiments (Snook 1986). There was a 3 – 4-fold increase in growth rates between 5 and 15 °C, but growth rates were lower at 20 °C. A parallel study on the effect of temperature on germination of *R. riparium* (i.e., sexual reproduction) identified highest growthrates at around 15 – 20 °C. Germination occurred only after first stressing the algae by exposure to a high temperature (40 °C) followed by a change of the medium in which they were being grown (Snook 1986). A higher temperature (25 °C) was identified for optimum growth of *R. riparium* in another experiment (Chao et al. 2005). However, Chao et al. (2005) carried out the trials in seawater (20 ppt, a little over half strength, and >130 x more saline than Te Waikoropupū Springs) whereas the waters studied by Snook (1987) had salinity more similar to the waters of Te Waikoropupū Springs (~5 x more saline).

Snook (1986) observed that at 15 °C, growth rates increased with light intensity (up to 275 $\mu E/m^2/s$) and showed no signs of leveling off (i.e., even higher light could have increased growth rates further). The highest light level tested (275 $\mu E/m^2/s$) is equivalent to relatively muted light conditions outdoors. For example, on a sunny day in mid-summer, ambient light would exceed 2000 $\mu E/m^2/s$ at mid-day. At this intensity, photoinhibition of growth would be expected. However, *R. riparium* has been shown to possess effective screening from UVB radiation (which is a one cause of photoinhibition of algal growth) by photoprotective pigments (Pescheck et al. 2014). Snook (1987) detected some photoinhibition in *R. riparium*, especially at water temperatures exceeding 25 °C, but also noted photoadaptation. Therefore, the growth-inhibiting effects of extremely high light levels may be less pronounced on *R. riparium* than on some other algae.

From the combination of laboratory experiments and field observations Snook (1986) concluded that "mat expansion in the pond appeared to be controlled primarily by water temperature but could be limited by light supply when surface mats shaded a significant area of the pond." Snook (1986) studied material from both surface mats and the benthos and identified the species in both locations. In unshaded areas the clear water of the pond enabled *R. riparium* "to proliferate from the benthic habit[at] due to favourable light, temperature and nutrient conditions" (see below for nutrient concentrations).

Water temperatures measured in Te Waikoropupū Springs between 1994 and 2015 ranged from 11.2 °C to 13.3 °C (Young et al. 2016), with little seasonal variability (Kim and Hunter 1997). These readings are spot temperatures often taken at the edge of the Main Spring, and so may be relevant to *Rhizoclonium* growth at the edges. Recent continuous measurements from an instrument deployed close to the deepest part of the Springs (at an unknown depth below the surface) confirmed constant temperatures of 11.65 to 11.73 °C from October 2017 to January 2018 (Gall 2018). Further spot temperature measurements between February 2016 and February 2018 were consistent with the continuous record (11.6 to 11.8 °C) (Gall and Milne 2018). These typical temperatures may be too low to support optimum *R. riparium* growth, but small temperature increases at the margins could enhance growth. Water temperatures measured during the time the mats were observed would be of interest.

The light climate of Te Waikoropupū Springs is exceptional, suggesting that *R. riparium* growth would occur even if the species was located on the benthos.

4.4.3 Nutrients

Nutrient concentrations in wastewater ponds studied by Snook (1986) were very high (i.e., dissolved inorganic N (DIN) typically > 25 mg/L and dissolved reactive phosphorus (DRP) typically > 10 mg/L)) and met the usual definition of eutrophic (Dodds 2007). However, we found little information in the literature checked that either agreed or disagreed with a comment in Snook (1986) that *R. riparium* is "not usually associated with eutrophic systems". In a recent survey of sites in Northland, *Rhizoclonium* sp. was abundant at sites with a range of median N and P (DIN from 0.14 to 0.39 mg/L, and DRP from 0.001 to 0.09 mg/L) (Kilroy and Lambert 2019).

Stevenson et al. (2012) included *Rhizoclonium* sp. in the category of nuisance filamentous green algae that tended to occur only when total phosphorus concentrations exceeded 0.027 mg/L, in waterways in which N : P ratios indicated limitation of algal growth by insufficient P. A somewhat consistent result with Stevenson et al. (2012), for *R. riparium*, was obtained in laboratory experiments on inter-algal competition (Sommer 1996). Sommer (1996) demonstrated that *R. riparium* (and also *Cladophora pygmaea*) dominated periphyton communities only when P was limiting (i.e., a surplus of N compared to P) and also only when concentrations of silica (Si) were low (~0.07 mg/L), and experimental light levels were highest (100 μE/m^2/s).

Mean nitrate-nitrogen concentrations in Te Waikoropupū Springs are currently around 0.43 mg/L (Gall and Milne 2018). The 47-year monitoring record presented in Young et al. (2017) shows that nitrate-N concentrations have ranged between 0.29 and 0.66 mg/L (excluding outliers) since 1995. Grab data collected by the Friends of Golden Bay indicated that, in early 2020, concentrations exceeded 0.5 mg/L (Friends of Golden Bay 2020).

Dissolved reactive phosphorus (DRP) concentrations in the Springs have ranged from 0.001 to 0.010 mg/L since 2015 (Young et al. 2017), with a current median of 0.005 mg/L (Gall and Milne 2018). These numbers represent very low DRP concentrations (close to the laboratory detection limit) to moderately low concentrations. The ratio of nitrate to DRP (at least 40 : 1 by weight) suggests an environment in which algal growth is strongly limited by P, based on accepted ratios (e.g., McDowell et al. 2009).

Based on the observations by Sommer (1996) and Stevenson et al. (2014), the current ratio of nitrate to DRP concentrations in the Springs is favourable for *R. riparium*. However, measurements of silica indicate relatively high concentrations (4.5 mg/L, Kim and Hunter 1997[4]), which should favour diatom growth rather than R*. riparium*, at all light levels, according to the results of the Sommer (1996) experiments. However, it should be noted that the results of controlled experiments in the laboratory are not necessarily always reflected in the “real” world, where multiple unknown environmental influences can lead to unexpected outcomes (Carpenter 1996).

[4] Converted to 4.5 mg/L from 160 μmol/L.

5 Conclusions

We found:

- DNA analysis and comparison with other molecular sequences indicated that the *Rhizoclonium* species forming the algal mats in Te Waikoropupū Springs in November 2019 was part of the *R. riparium* complex.
- One of the identical DNA sequences used in the comparison was from a sample collected in Te Waikoropupū Springs in December 2004. Therefore, *R. riparium* has been in the Springs for at least 15 years and was not new to the Springs in 2019.
- *Rhizoclonium riparium* is known from locations all over the world. Records show that it has been known in New Zealand from at least the 1940s and, given its relatively widespread geographic distribution, it is assumed to be native.
- *R. riparium* is remarkable for its ability to inhabit waters across a very wide range of salinity (seawater to freshwater).
- Recent literature on *R. riparium* and other *Rhizoclonium* species in applications such as aquaculture (as a food source) and biofuel production highlights the ability of species in this genus to produce high biomass under favourable conditions.
- Although *R. riparium* typically grows on the beds of slow-flowing streams and still waters, formation of floating mats is not unusual. Many other green filamentous algae also form floating mats.
- Growth of *R. riparium* in fresh water (rather than sea water) appears to require hard water with high pH (i.e., approaching pH 8). Te Waikoropupū Springs water meets both requirements.
- *R. riparium* growth rates may be markedly enhanced by increasing water temperature in the range up to 15 °C or higher. Water temperatures in Te Waikoropupū Springs ranged from 11.2 °C to 13.3 °C up to 2015. More recent continuously monitored (October 2017 to January 2018) and spot temperature data (2016 – 2018) confirmed consistent temperatures of around 11.7 °C in the main part of the Springs. Further water temperature data, including from the margins at the time the mats were observed, would be of interest.
- Experimental studies indicate that increasing light levels also stimulate *R. riparium* growth. Therefore, the very high water clarity of Te Waikoropupū Springs would favour growth of *R. riparium* on the bottom, where rapid expansion could subsequently lead to detachment of floating mats.
- The nutrient requirements of *R. riparium* are unclear except that waters with a high N:P ratio (which is the case in Te Waikoropupū Springs) may provide a competitive advantage over other types of algae. Records from the literature and other New Zealand locations indicate that abundant *Rhizoclonium* (growing on the bottom or as floating mats) occurs in a wide range of dissolved N and P concentrations.

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