Manaaki Whenua
Landcare Research

Risk assessment for the development of mucilage events (lake snow) in Lakes Rotoiti and Rotoroa, Tasman District

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Risk assessment for the development of mucilage events (lake snow) in Lakes Rotoiti and Rotoroa, Tasman District

Contract Report: LC4004

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Summary

Project and Client

- This project on the abundance of the invasive diatom *Lindavia intermedia* in Nelson Lakes, and the consequent risk of lake snow, was commissioned by Tasman District Council with report writing supported by an Envirolink medium advice grant (2101-TSDC170) and support from the Department of Conservation to collect field samples.

Objectives

The research was commissioned in order to:

- determine abundances of *L. intermedia* over a 1-year period
- determine rates of chitin synthase gene expression – chitin is a main component of the lake snow matrix
- undertake basic water quality measurements of the lakes to obtain a baseline, since these lakes are not routinely monitored
- compare these data with those from the Central Otago Lakes, which contain lake snow and have been monitored over approximately the same interval, in order to assess the likelihood of lake snow appearing in Nelson Lakes.

Methods

- Samples were collected at approximately 6-weekly intervals between June 2020 and May 2021 at two sites in each of Lakes Rotoiti and Rotoroa.
- Samples/measurements were taken of *Lindavia intermedia* abundance and chitin synthase gene expression at 15 m depth, total nitrogen and phosphorus and chlorophyll *a* from depth-integrated samples, and Secchi distance.
- Snow tows using a weighted fishing line were performed at each sampling to check for the presence of lake snow.

Results

- Lake snow was not detected in either lake.
- *Lindavia intermedia* is present at low abundance in Lake Rotoroa, but was consistently more abundant in Rotoiti.
- The species reached much higher abundance in Lake Rotoiti in April/May 2021, and at the time of the last sampling had comparable cell densities to those recorded from Lake Wānaka where lake snow is persistent and problematic.
- Studies of mRNA pools showed no evidence of the overexpression of chitin synthase which has occurred coincident with lake snow in Otago lakes.
- The trophic states of Lakes Rotoiti and Rotoroa are comparable to the large lakes of Central Otago, which contain lake snow.

- There was no detectable relationship between trophic state attributes and *L. intermedia* abundance; there was also little difference in these metrics between sites and lakes.

Conclusions

- Lakes Rotoroa and especially Rotoiti are likely to develop lake snow; in the case of the latter this may occur as soon as summer 2021/22.
- We infer the current difference in *L. intermedia* abundance between the two lakes to be a result of earlier incursion of *L. intermedia* into Rotoiti.

Recommendations

- Ongoing monitoring of the situation in both lakes to determine the trajectory of *L. intermedia* populations and the likelihood of lake snow outbreaks in the coming summer.
- Testing of Rotomairewhenua/Blue Lake; if still negative, biosecurity information should be prominently displayed in nearby huts and facilities, given the positive status of Rotoroa.
- Monitoring of water quality in Lakes Rotoiti and Rotoroa, at least seasonally, to gauge long-term trends.

1 Introduction

Lindavia intermedia is an invasive freshwater diatom. Under certain conditions this eukaryotic alga exudes copious polysaccharide (chitinous) threads; at relatively high population densities the resulting macroaggregates cause a nuisance phenomenon known as 'lake snow', which transforms lake ecology, and coats lines and filters, compromising recreational use of lakes and council municipal operations when lake water use is required. To date, this has mainly been a problem in large pristine lakes in Otago and Canterbury, with isolated incidences elsewhere (such as Lake Waikaremoana in 2009; Novis et al. 2017). Although increasingly common in New Zealand (NZ) lakes, lake snow caused by *L. intermedia* appears very rare internationally: the same genotype of the same species is known to cause the same problem only in Lake Youngs (near Seattle, USA; Novis et al. 2017).

Lindavia intermedia often appears in rivers downstream of lakes that are subject to infestations. A recent survey of historical samples from the Buller River and the outlet of Lake Rotoroa taken during monitoring for *Didymosphenia geminata* in 2004 and 2005 failed to detect *L. intermedia* (Kilroy et al. 2018). However, the species appeared in subsequent samples from the Buller River downstream of Lake Rotoiti in late 2019, indicating its presence in the lake (Sivignon 2020),

Molecular methods to detect *L. intermedia* (using chloroplast DNA) and the transcription products (mRNA) of two of its genes, CHS2 (chitin synthase, which builds the chitin fibres that are the principle structural component of lake snow) and ACT1 (a reference gene), were developed and published recently (Novis et al. 2020). The DNA method was used to screen a large number of NZ lakes for *L. intermedia*, verifying the presence of the organism in both Lakes Rotoiti and Rotoroa. It was also detected in a third lake in the Nelson Region, the Maitai Reservoir above Nelson City, which is the subject of ongoing investigations with respect to this species (Novis & Schallenberg 2020). Despite occasional *L. intermedia* cell abundances comparable to those associated with lake snow in several Central Otago lakes, lake snow has not yet been detected in any lake in the Nelson area. In the case of the Maitai Reservoir this has been inferred to be due to its higher phosphorus concentrations relative to the lakes in Otago (Novis & Schallenberg 2020).

However, Lakes Rotoiti and Rotoroa, being large and pristine, more closely resemble the lakes of Central Otago where lake snow has been a persistent problem. The (possibly recent) development of populations in the Nelson Lakes are therefore concerning, and the current research was commissioned in order to:

- determine abundances of *L. intermedia* over a 1-year period
- determine rates of chitin synthase gene expression that might indicate the synthesis of a major component of lake snow
- undertake basic water quality measurements of the lakes to obtain a baseline, since these lakes are not routinely monitored
- compare these data with those from the Central Otago Lakes, which contain lake snow and have been monitored over approximately the same interval, in order to assess the likelihood of lake snow appearing in Nelson Lakes.

2 Methods

The qPCR methods used here are fully described by Novis et al. (2020). Samples were collected from two sites in each lake (Fig. 1) by Janet Newell and colleagues, Department of Conservation, St Arnaud, initially in June 2020 and then approximately every 6 weeks between September 2020 and May 2021. Sampling methods were chosen to align with the methods used in Central Otago. Briefly, samples were collected at 15-m depth at two sites in each lake using a Van Dorn sampler, and up to 2 L was filtered and preserved in RNALater for transport to Manaaki Whenua – Landcare Research, Lincoln, on ice, for freezer storage. Samples were processed in three batches (before and after the end of November 2020, and the last samples in June 2021). DNA and RNA were extracted from the samples, and the qPCR assays run with the necessary replication and controls on a Stratagene MX3000P real-time PCR machine. Copy numbers were calculated using recently prepared standards analysed on the same microplate, and cell densities estimated from a calibration published previously (Novis et al. 2020).

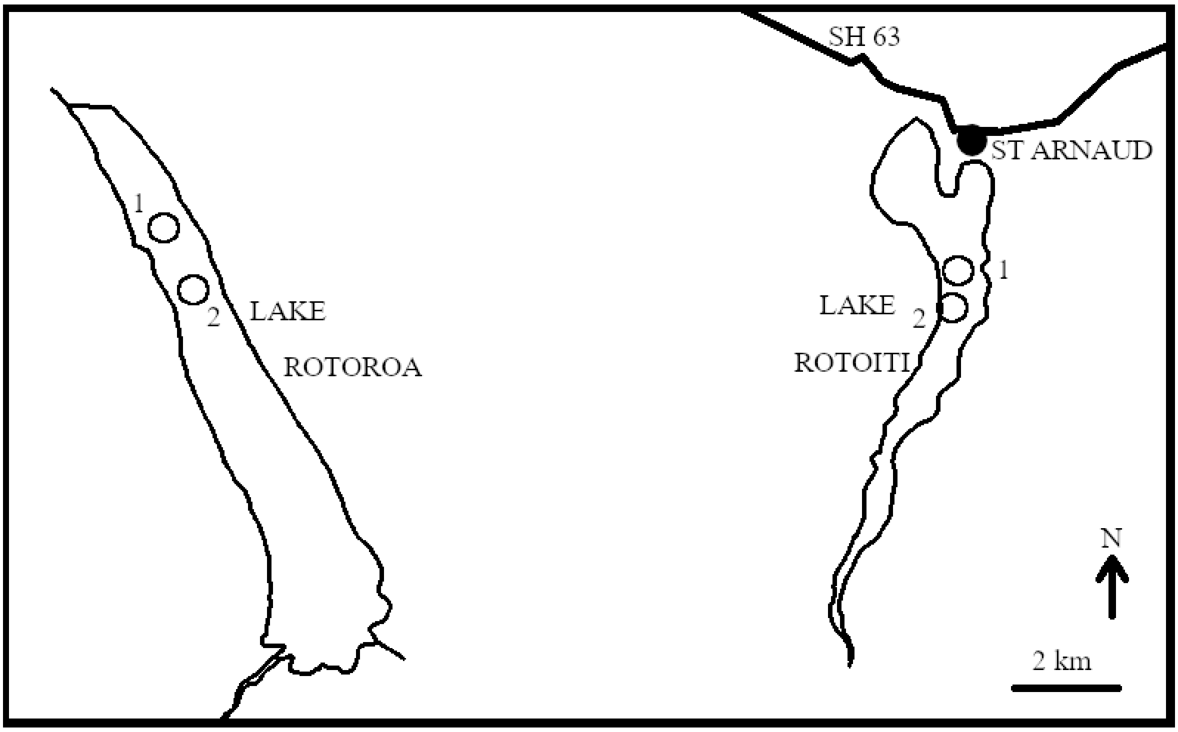

Figure 1. Location of sampling sites (open circles) on Lakes Rotoiti and Rotoroa.

The presence of lake snow at the sites was assessed using ‘snow tows’: a 30-m braided fishing line, weighted with a 900-g sinker, was towed at low speed for 1 km. Any material adhering to the line was stripped off with a gloved hand and collected in a container for weighing (Novis et al. 2020).

Secchi disk measurements of lake water clarity were taken using standard methods. An integrated nutrient sample was obtained by equally mixing Van Dorn samples from 0.5, 15, 30, and 45 m, and analyses of total nitrogen (TN) and total phosphorus (TP) carried out by Hill Laboratories using caustic persulphate digestion/alkaline persulphate digestion with automated Cd reduction/sulphanilamide colorimetry, and total phosphorus digestion with automated ascorbic acid colorimetry respectively. Chlorophyll *a* was measured from the same integrated samples by filtering three replicates of 250 mL through GF/F filters, which were then frozen and subject to the standard trichomatic spectrophotometric method with extraction in 90% alkaline acetone, measured in a 4 cm quartz cuvette (Wetzel & Likens 2002).

3 Results and Discussion

3.1 TLI calculation for Lakes Rotoiti and Rotoroa and comparison to other lakes of interest

Table 1 shows the TLI (trophic level index) estimates and their components for Lakes Rotoiti and Rotoroa based on the data collected during this study. Total nitrogen (TN) concentrations were considerably higher than those of the lakes studied in Central Otago, except for the enriched Lake Hayes. This may be due to the extensively forested catchments of Nelson Lakes, which, like that of the Maitai Reservoir, contribute nitrogen-containing dissolved organic matter that is not necessarily readily available to *L. intermedia*, although we note that TN of the forested Lakes Manapouri, Te Anau and Waikaremoana are approximately half that of Nelson Lakes (Environment Southland 2010; Howard-Williams et al. 2010). Other indicators of productivity, namely total phosphorus (TP), chlorophyll *a*, and Secchi disk readings, are comparable to the large, pristine Lakes Wānaka, Wakatipu, and Hawea. The overall trophic level index (TLI) scores of 2.4 for Nelson Lakes are slightly elevated relative to the large Otago lakes due to the higher TN concentrations (it should also be noted that we lacked a full set of August–September data for Lakes Rotoiti and Rotoroa due to the window of funding available). One value of TP, for Rotoiti site 1 on 27 January 2021, was supplied as 40 μg L^{-1}; we interpreted this as a typographic error as it is an order of magnitude higher than all other readings, and the value for site 2 on the same lake on that day was below detection. We therefore used the value of 4.0 μg L^{-1} instead. Where values were below detection, we used a value (1 μg L^{-1}) equal to half the detection limit for convenience. In summary, Table 1 shows the Nelson Lakes to be close in character (apart from TN concentrations) to the large pristine lakes of Central Otago, and certainly more so than the Maitai Reservoir.

Table 1. Trophic Level Index (TLI) data for selected lakes that are positive for *Lindavia intermedia* (although not necessarily for lake snow). Means and standard deviations for the components of the TLI are also shown. The Maitai Reservoir is included as the only other lake in the Nelson area known to be positive for *L. intermedia*

Lake	Metrics based on latest available year[a]					Lake snow status
	Total N ($\mu g\ L^{-1}$)	Total P ($\mu g\ L^{-1}$)	Chlorophyll *a* ($\mu g\ L^{-1}$)	Secchi disk (m)	TLI	
Hawea	78.8 ± 61	6.3 ± 3.6	0.77 ± 0.30	14.5 ± 2.5	2.0 (1.2)	+
Wānaka	106 ± 74	5.2 ± 1.5	0.98 ± 0.65	16.2 ± 3.2	2.1	+
Wakatipu	50 ± 42	4.0 ± 0	1.2 ± 1.0	16.4 ± 5.3	1.8	+
Dunstan	99 ± 58	5.8 ± 2.0	1.4 ± 0.8	Not available	2.0[b]	+
Moke	150 ± 10	6.0 ± 2	2.4 ± 0.4	6.3 ± 0.07	2.9	+
Hayes	253 ± 49	19.3 ± 7.4	6.4 ± 7.8	5.5 ± 2.8	3.7 (4.4)	-
Maitai	194 ± 46	40 ± 34	1.5 ± 0.84	3.6 ± 1.2	3.7	-
Rotoiti	256 ± 202	4.2 ± 2.5	0.63 ± 0.17	10.4 ± 2.3	2.4	-
Rotoroa	267 ± 127	3.4 ± 1.6	0.50 ± 0.25	11.0 ± 1.4	2.4	-

[a] As per original method of Burns et al. (2000); the only data available for Moke Lake are from January and February 2000, so the TLI is biased towards summer conditions. Latest available data for all lakes apart from Moke and Maitai are September 2016 to August 2017. Data used for Maitai encompass the period September 2018 to August 2019. Data for the two Nelson Lakes were only available between June 2020 and May 2021, so this range was used.

[b] Due to the unavailability of Secchi data, the TLI figure for Lake Dunstan is directly from www.lawa.org.nz. Where calculated TLIs for the period September 2016 to August 2017 differ from those reported on lawa, the latter are shown in brackets.

3.2 Abundance of *Lindavia intermedia* in Nelson Lakes

Cell concentrations of *L. intermedia* inferred using the chloroplast DNA qPCR method for Nelson Lakes and selected comparison lakes over the study period are shown in Figure 2. The population in both lakes remained relatively low for most of the study period, although that of Lake Rotoiti was consistently somewhat higher than that in Lake Rotoroa. However, in April the concentrations of *L. intermedia* in Lake Rotoiti began to increase, and became comparable to values typically seen in Lake Wānaka (Fig. 3) in the final May samples from this lake. This late increase did not occur in Lake Rotoroa.

Because the values from the May 2021 Rotoiti samples are critical to interpretation, the DNA extracts were reanalysed by qPCR, giving a comparable result to the original assay (within the Wānaka 95% C.I. in Fig. 3). Furthermore, a smaller increase from previous values was seen in the April samples, supporting the notion that this is part of an upward trend that may still be continuing.

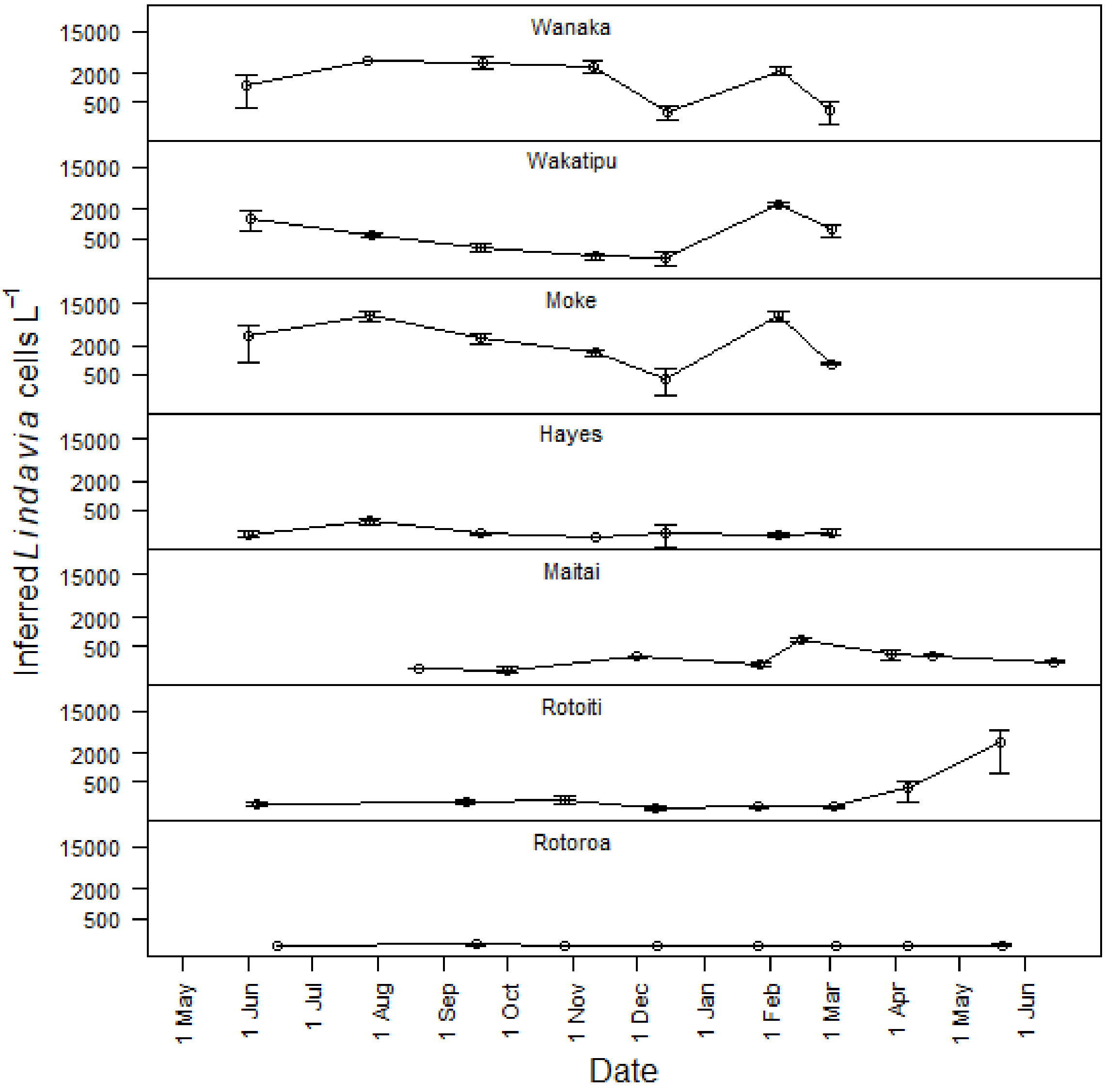

Figure 2. Densities of *Lindavia intermedia* in Nelson Lakes, compared with other lakes under the study period. Points represent means (ranges are shown; *n*=2). Note log vertical axis, used to resolve variation at lower densities.

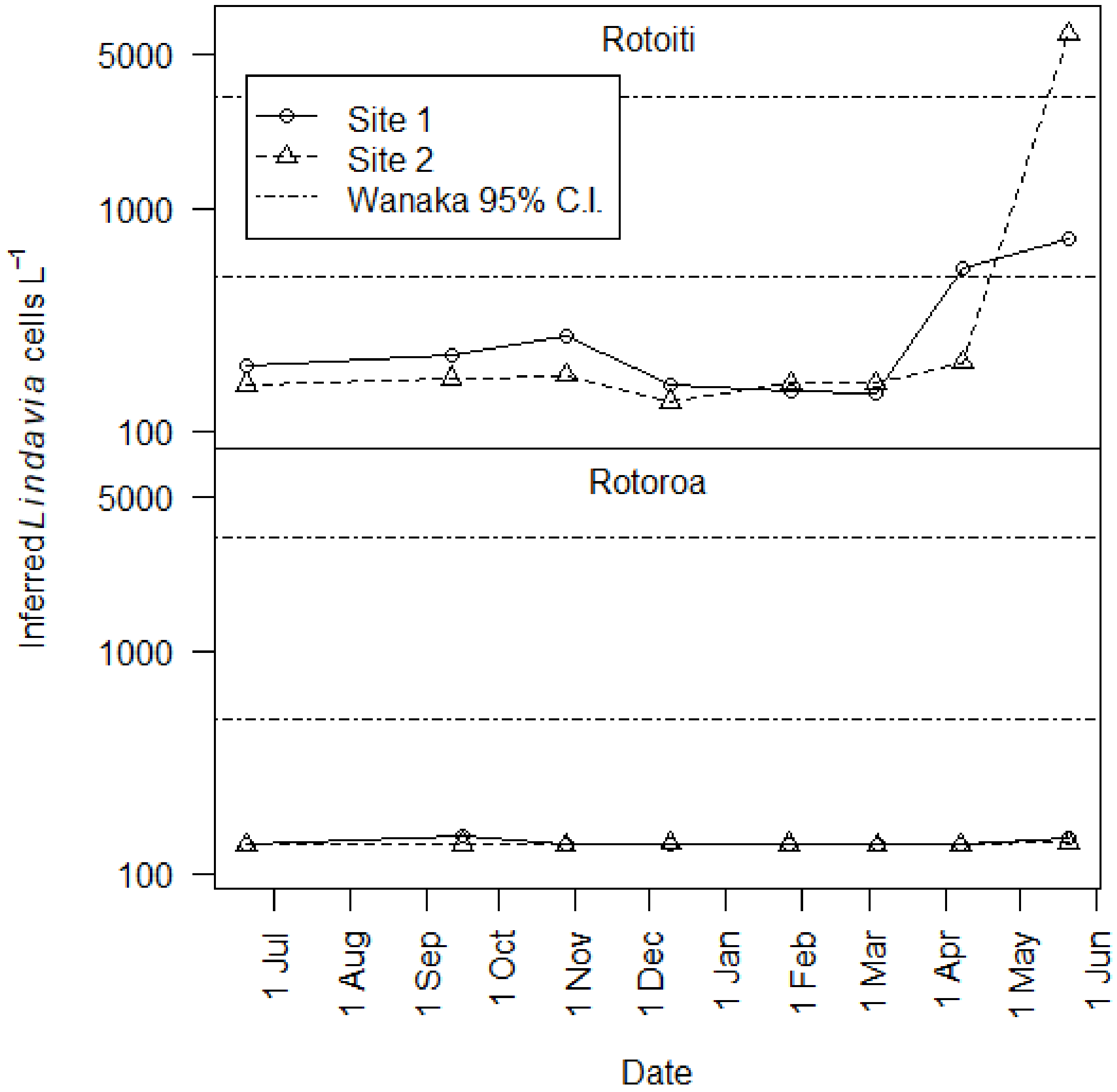

Figure 3. Densities of *Lindavia intermedia* over time in the individual sampling sites in Lakes Rotoiti and Rotoroa, with the 95% confidence interval of the mean *L. intermedia* density in Lake Wānaka over all summer samples (December to February inclusive; shown by the two horizontal dashed lines). Note that lake snow can be found in Lake Wānaka year-round, but appears to be produced mostly in summer to autumn (manuscript in preparation).

3.3 Lake snow status – snow tows and relative RNA concentrations

Interestingly, despite the higher inferred cell densities in Lake Rotoiti during May, the snow tow method detected no mucilage events at any time, and this supports other data (not shown; manuscript in preparation) suggesting that lake snow is mostly generated in summer and autumn. A comparison of CHS2-ACT1 mRNA balance (Fig. 4) suggests that the former gene, thought to be responsible for the production of chitin in lake snow fibres, and the upregulation of which tends to coincide with lake snow development (Fig. 5), is more active in *L. intermedia* from the Central Otago lakes compared with Nelson Lakes, including on a per cell basis (Fig. 6).

The formation of lake snow seems to depend on two factors: 1) the potential of cells within the population to form lake snow; and 2) the population being of adequate size for aggregates of lake snow to form at a detectable or nuisance level. As shown in Figures 3–6, Lakes Wānaka, Wakatipu, and Moke often satisfy both these criteria, whereas Lake Hayes satisfies (1) only, Lake Rotoiti satisfies (2) only, and Lake Rotoroa currently satisfies neither.

Although lake snow is poorly understood, the related phenomenon of marine snow has been the subject of much research. Studies have often found that aggregation in marine flocculating species of diatoms (and other organisms) is triggered by stresses, such as nutrient depletion at the end of a bloom, often with the aid of physical processes such as turbulence (Calleja 1984; Smetacek 1985; Riebesell 1991a, b; Monti et al. 1995; Passow et al. 2012). These stresses in Nelson Lakes may not occur until summer; thus a continued high cell density in Lake Rotoiti may lead to lake snow. However, the temporal occurrence of lake snow is not straightforward to predict. Even lakes of relatively similar nature, such as Wakatipu and Wānaka, often differ in the timing of lake snow production. For instance over summer 2020/2021 lake snow abundance in the latter peaked in December, compared to February in the latter (Fig. 5). There is also some evidence that lakes in heavily forested catchments respond more sporadically than those in more open catchments: a lake snow event in Lake Waikaremoana in 2009 has apparently not been repeated, even though sampling confirmed that *L. intermedia* has persisted in the lake (Novis et al. 2017).

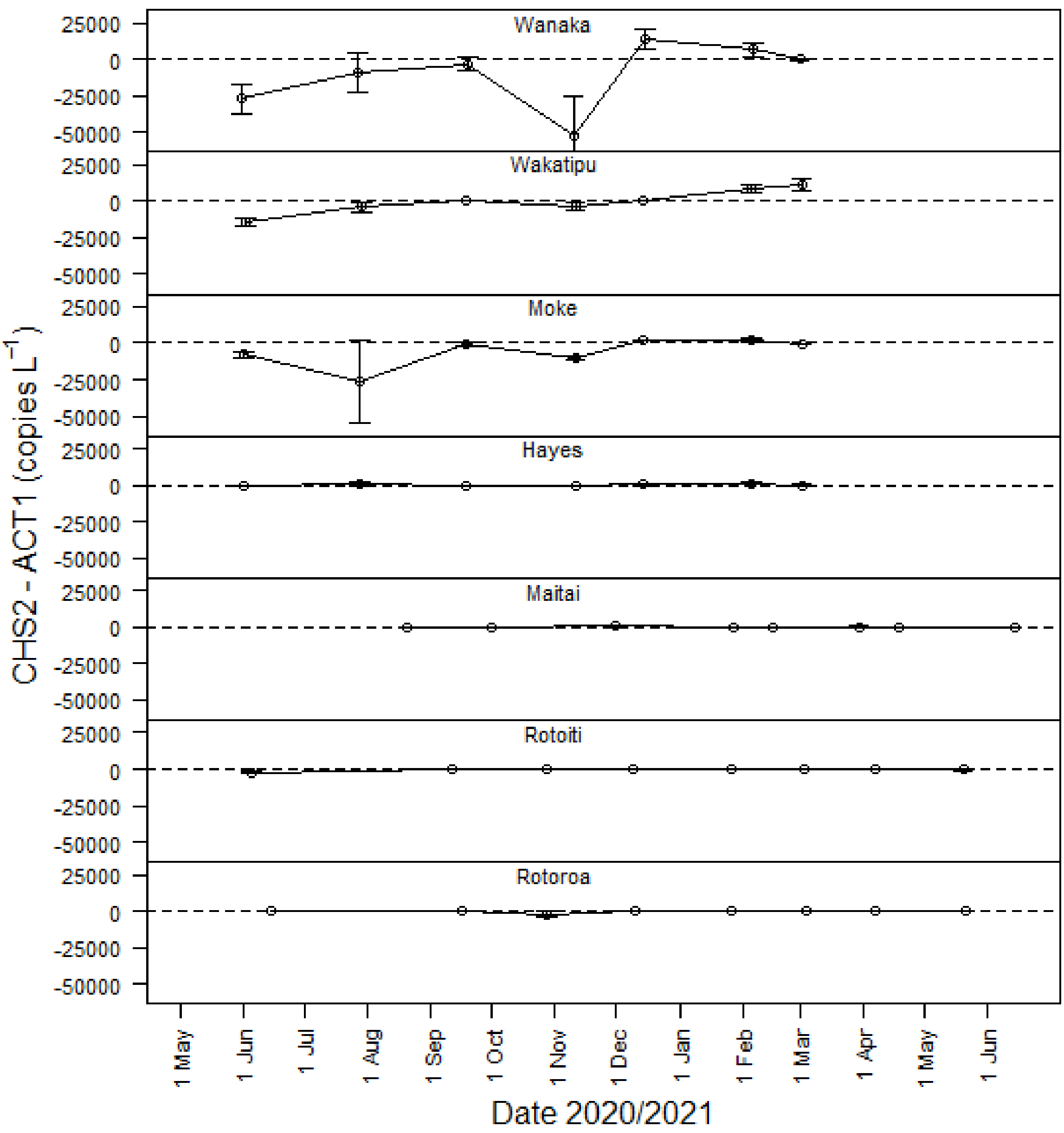

Figure 4. Differences in absolute copy numbers of CHS2 and ACT1 mRNA per unit volume. The CHS2 gene encodes chitin synthase, involved in the production of chitin, which is the structural component of lake snow fibres. ACT1 is a reference gene expected to represent background gene expression; thus data above zero (the dashed horizontal line) indicate overexpression of chitin synthase. Overexpression coincides with lake snow increases, especially in Lakes Wānaka and Wakatipu (see Fig. 5).

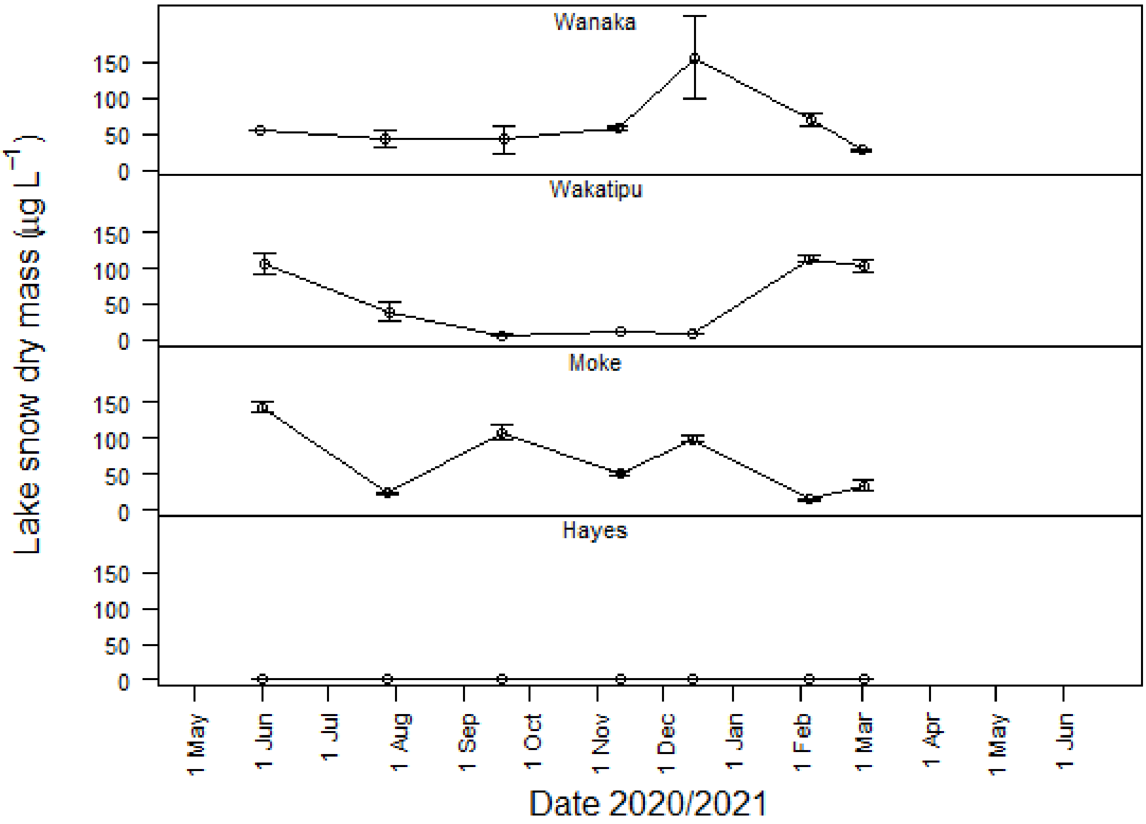

Figure 5. Lake snow dry weight over time in four lakes monitored with a submersible pump method (Novis & Schallenberg 2020). Note the relationship between chitin synthase overexpression in Figure 4 with the summer increases in lake snow in Lakes Wānaka and Wakatipu. Lake snow has not been detected in the eutrophic Lake Hayes.

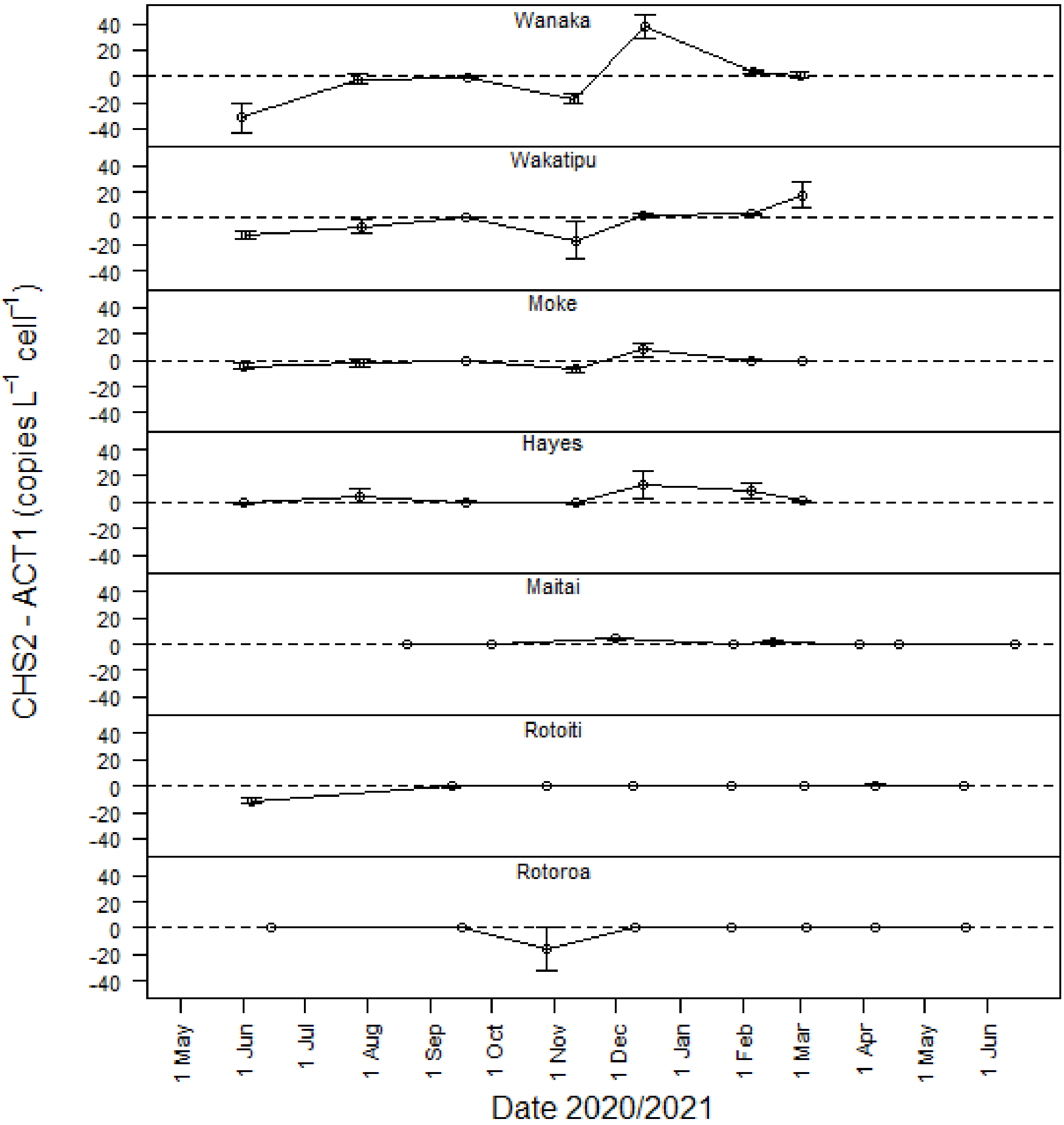

Figure 6. Differences in copy numbers of CHS2 and ACT1 mRNA per unit volume of lake water (i.e. the data from Fig. 4) scaled by cell densities. Note that although *L. intermedia* in Lake Hayes appears to overexpress chitin synthase on the cellular level, the population seems to be too small (Fig, 2) for lake snow to develop (Fig. 5). Conversely, although cell densities in Lake Rotoiti appear to be sufficient for lake snow (Figs 2,3), the cells do not currently appear to synthesise chitin at a sufficient rate. This is likely to change in summer if the population remains high.

3.4 Relationship between *L. intermedia* populations and water quality metrics

Time series plots of TN, TP, and chlorophyll *a* for Lakes Rotoiti and Rotoroa are provided in the Appendix as supplementary data (Figs A1-2). Variation in these metrics within and between lakes was generally greater than the variation in *L. intermedia* abundance, especially when the last two samplings are discounted. In the latter case, when *L. intermedia* began to increase in Lake Rotoiti (Fig. 2), the decrease in TN in the same lake (Fig. A1) could be related (i.e. nitrogenous compounds could have been consumed during algal growth). However, an earlier difference in TN between the lakes could not be attributed to any difference in cell densities of *L. intermedia*, and chlorophyll *a*, a measure of the abundance of the overall algal community, stayed fairly static over the later period. The latter observation suggests that growth dynamics of *L. intermedia* are not closely coupled to those of other algal species in the water column.

Concentrations of TP were consistently low (except for one very high reading, see 3.1) and close to (or below) the detection limit. It is difficult to draw any conclusions from this other than to observe that the lakes remain in pristine condition. Secchi disk readings also fell within a fairly consistent range (6–12 m).

The overall data suggest that the two lakes, and sites within each lake, are similar to each other (Fig. A2). Nothing in the data from Lake Rotoiti seems to explain why the population of *L. intermedia* is consistently more abundant and developing faster there than in Lake Rotoroa (Fig. 3). We therefore propose that this is attributable to a founder effect – the population in Rotoiti arrived in the lake sooner and has consequently had more time to establish and increase. Lake Rotoiti is more accessible and more popular than Lake Rotoroa, with a state highway and the settlement of St Arnaud located close to the lake shore; recent research has found that humans are very important vectors of *L. intermedia*, and road access to lakes is a strong predictor of occurrence of the species (Kilroy et al. 2021).

The lower elevation areas in the catchments of Lakes Rotoiti and Rotoroa are mostly forested, and this is a point of difference in comparison with the large Otago lakes, which produce lake snow. Forested catchments likely contribute more dissolved organic matter such as humic and fulvic acids to lake waters, which can affect water chemistry and microbial ecology. We infer from reference samples and historical anecdotal information that *L. intermedia* in Lake Waikaremoana (Bay or Plenty/Hawke's Bay) produced a transient lake snow event in 2008/9 (Novis et al. 2017). *L. intermedia* has also been reported to produce lake snow in Lake Sumner (Canterbury), which has a largely forested catchment (Kilroy et al. 2018). Lakes Manapouri and Gunn (Southland) have *L. intermedia* (LAWA 2020), but so far lake snow has not been confirmed from these lakes. *L. intermedia* is present in the Maitai Reservoir (Nelson City), which has a largely forested catchment, but again, lake snow has not been reported from this water body (Novis & Schallenberg 2020). From this information, it appears that *L. intermedia* can readily colonise and proliferate in lakes with forested catchments, but it is unclear whether aspects of water chemistry related to dissolved organic matter in these lakes partially or wholly inhibits the production and/or formation of lake snow. More research is needed on this topic.

3.5 Assessment of changes in lake water quality

Water chemistry of Lakes Rotoiti and Rotoroa has been very sporadically investigated. Taylor (1971) reported values for inorganic and organic nitrogen at multiple stations on both lakes between 1969 and 1971. Summing these components provides values theoretically comparable to the TN measurements reported by us. For Lake Rotoiti, this gives a range of values of 97–152 µg L^{-1} TN, and 3–137 µg L^{-1} TN for Lake Rotoroa. These ranges overlap those reported by us (Table 1) but are noticeably lower than our mean values. On the other hand, Taylor (1971) reported soluble phosphorus as high as 43 and 22 µg L^{-1} in Lakes Rotoiti and Rotoroa respectively, which are much higher than our values. However, it is unlikely that soluble phosphorus analytical methods used by Taylor would have accurately measured the low P levels that are measured in these lakes today.

Burns and Rutherford (1998) assessed the trophic state of Lake Rotoiti from February 1992 to June 1994. They reported a mean TN from monthly samples from two sites in the lake to be 59 µg L^{-1} – much lower than the mean TN concentration for the lake that we report here in Table 1. Again, chlorophyll *a*, TP, and Secchi depths reported in Burns and Rutherford were similar to the values reported here for Lake Rotoiti in Table 1.

Comparing the data in Table 1 with these sparse historical data, the TN concentration in these lakes increased substantially since the 1990s. Our more complete data from the Frankton Arm of Lake Wakatipu also indicate > 2-fold increase in TN in that lake, and this increase has happened since 2017. Further investigation is required to elucidate the changes in TN observed in these lakes, but these changes probably have had little influence on *L. intermedia* dynamics and the production of lake snow in these lakes because the Frankton Arm produced lake snow even when the mean TN concentration averaged around 50 µg L^{-1}.

4 Conclusions

Cell concentrations of *Lindavia intermedia* have (very recently) reached a level in Lake Rotoiti that puts the lake at significant risk of developing lake snow in the near future, possibly in summer 2021/22. We base this on current data for the lakes compared against other New Zealand lakes of similar nature that now contain lake snow. If lake snow develops in the Nelson Lakes it is possible it will be more sporadic than the persistent occurrences observed in the more open lakes further south, but there are currently little data on *L. intermedia* from lakes in heavily forested catchments to enable a confident prediction.

If lake snow develops in Lake Rotoiti, we regard it as extremely likely that the same will occur in Lake Rotoroa. The lakes are very similar in nature and the best explanation for the current lower densities in Rotoroa is a later date of colonisation of the lake by this invasive species.

5 Recommendations

We recommend:

- Ongoing monitoring of the situation in both lakes (especially Rotoiti, given its current state) to determine the trajectory of *L. intermedia* populations and the likelihood of lake snow outbreaks in the coming summer; this would also provide much-needed data on lakes in forested catchments that could be useful in future incursions by the species.
- Sampling of Rotomairewhenua/Blue Lake (with very careful regard to biosecurity practices) to assess the presence of *L. intermedia*. This lake is world-renowned for its clarity and would likely be vulnerable to infestation, given that Lake Rotoroa is positive. If still negative, biosecurity information should be prominently displayed in Blue Lake Hut and huts either side (Sabine, West Sabine, George Lyon, Waiau, and Caroline Creek).
- Monitoring of water quality in Lakes Rotoiti and Rotoroa, at least seasonally, to gauge long-term trends.

6 Acknowledgements

Analysis and report writing was funded by an Envirolink medium advice grant (2101-TSDC170). We thank the Department of Conservation, St Arnaud, for collecting field samples, and Tasman District Council for funding the laboratory analyses. We thank Nelson City Council for permission to include data from the Maitai Reservoir in this report.

7 References

Burns N, Bryers G, Bowman E 2000. Protocol for monitoring trophic levels of New Zealand lakes and reservoirs. Lakes Consulting Client Report 99/2, prepared for Ministry for the Environment.

Burns NM, Rutherford JC 1998. Results of monitoring New Zealand lakes, 1992–1996. Volume 3 – Data and Results. NIWA Client Report MFE80216/3.

Calleja GB 1984. Microbial aggregation. Boca Raton, FL: CRC Press.

Environment Southland 2010. Our Ecosystems: Southland Water 2010: Part 2. Invercargill: Environment Southland. https://www.es.govt.nz/repository/libraries/id:26gi9ayo517q9stt81sd/hierarchy/environment/water/our-ecosystems.pdf [accessed 24 June 2021].

Howard-Williams C, Law K, Vincent CA, Davies J, Vincent WF 1986. The limnology of Lake Waikaremoana with special reference to littoral and pelagic primary producers. New Zealand Journal of Marine and Freshwater Research 20: 583–597.

Kilroy C, Lambert P, Novis P 2018. *Lindavia intermedia* in New Zealand's fresh waters: a search of archived samples. NIWA Client Report 2018101CH prepared for Otago Regional Council.

Kilroy C, Whitehead AL, Wood SA, Vandergoes MJ, Lambert P, Novis PM 2021. Predicting the potential distribution of the invasive freshwater diatom *Lindavia intermedia* in New Zealand lakes. Aquatic Invasions 16 in press.

LAWA 2020. Lake snow found in Mavora Lakes. https://www.lawa.org.nz/get-involved/news-and-stories/environment-southland/2020/january/lake-snow-found-in-mavora-lakes/ [accessed 24 June 2021].

Monti M, Welker C, Dellavalle G, Casaretto L, Umani SF 1995. Mucous aggregates under natural and laboratory conditions: a review. Science of the Total Environment 165: 145–154.

Novis PM, Schallenberg M 2020. *Lindavia intermedia* in the Maitai Reservoir, Nelson, and the risk of lake snow formation. Manaaki Whenua-Landcare Research Contract Report LC3739, prepared for Nelson City Council.

Novis P, Schallenberg M, Saulnier-Talbot É, Kilroy C, Reid M 2017. The diatom *Lindavia intermedia* identified as the producer of nuisance pelagic mucilage in lakes. New Zealand Journal of Botany 55: 479–95.

Novis PM, Sales RE, Gordon K, Manning N, Duleba M, Ács É, Dressler M, Schallenberg M 2020. Lindavia intermedia (Bacillariophyceae) and nuisance lake snow in New Zealand: chitin content and quantitative PCR methods to estimate cell concentrations and expression of chitin synthase. Journal of Phycology 56: 1232–1244.

Passow U, Ziervogel K, Asper V, Diercks A 2012. Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environmental Research Letters 7: 035301.

Riebesell U 1991a. Particle aggregation during a diatom bloom. I. Physical aspects. Marine Ecology Progress Series 69: 273–280.

Riebesell U 1991b. Particle aggregation during a diatom bloom. II. Biological aspects. Marine Ecology Progress Series 69: 281–291.

Sivignon C 2020. Lake snow algae found in Buller River, fears for Lake Rotoiti. https://www.stuff.co.nz/environment/118904296/lake-snow-algae-found-in-buller-river-fears-for-lake-rotoiti [accessed 15 June 2021].

Smetacek VS 1985. Role of sinking diatom life-history cycles: ecological, evolutionary and geological significance. Marine Biology 84: 239–251.

Taylor ME 1971. Unpublished report on a biological survey of Lakes Rotoroa and Rotoiti, to the Nelson Lakes National Park Board by the Cawthron Institute, Nelson. 118 p. Held in the Cawthron Institute Library.

Wetzel RG, Likens GE 2002. Limnological analyses. 2nd edn. New York: Springer-Verlag. 391 p.

Appendix 1 – Supplementary data

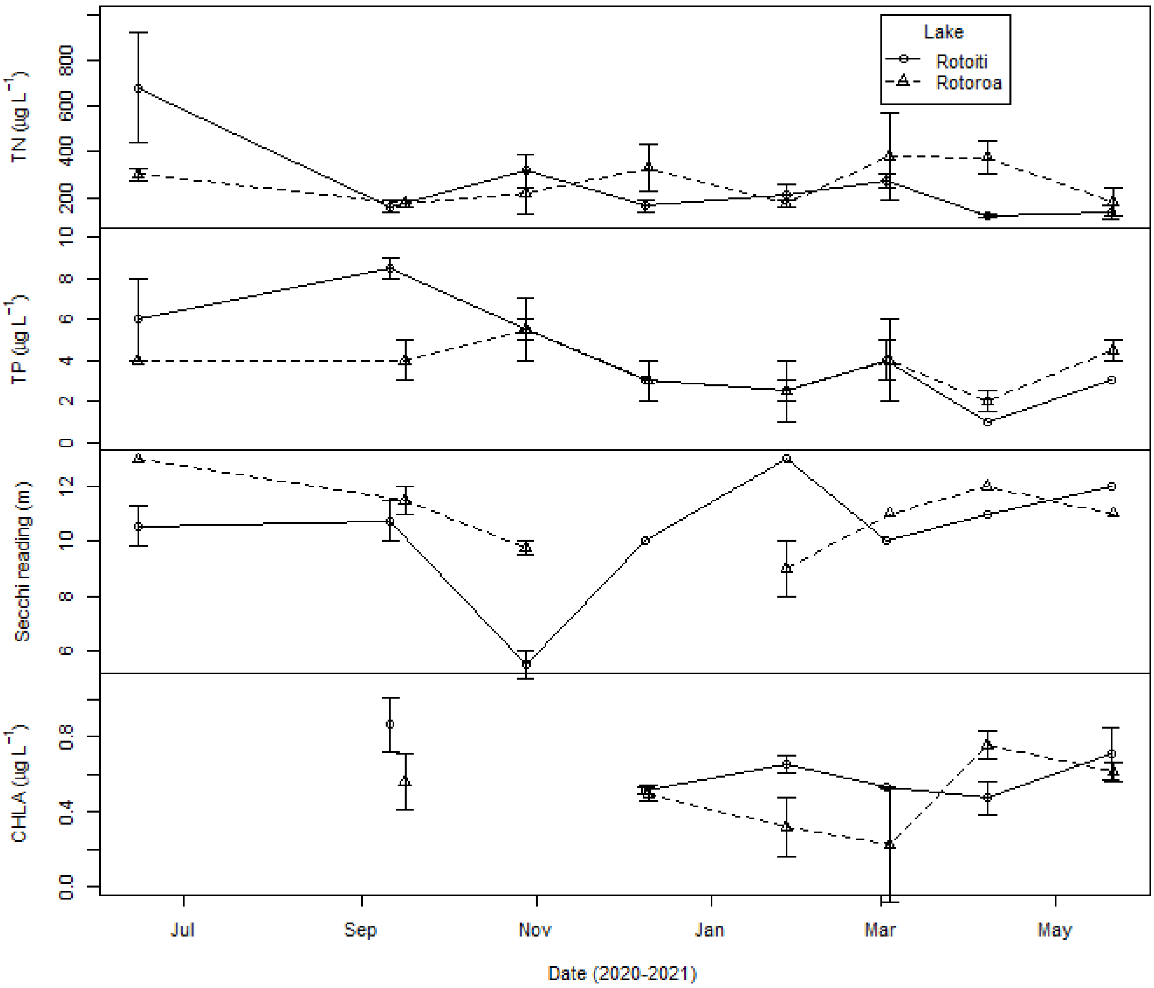

Figure A1. Time series plots of trophic state metrics for Lakes Rotoiti and Rotoroa. Data represent means (ranges are shown; *n*=2). Discontinuous lines in the chlorophyll and Secchi plots indicate missing data.

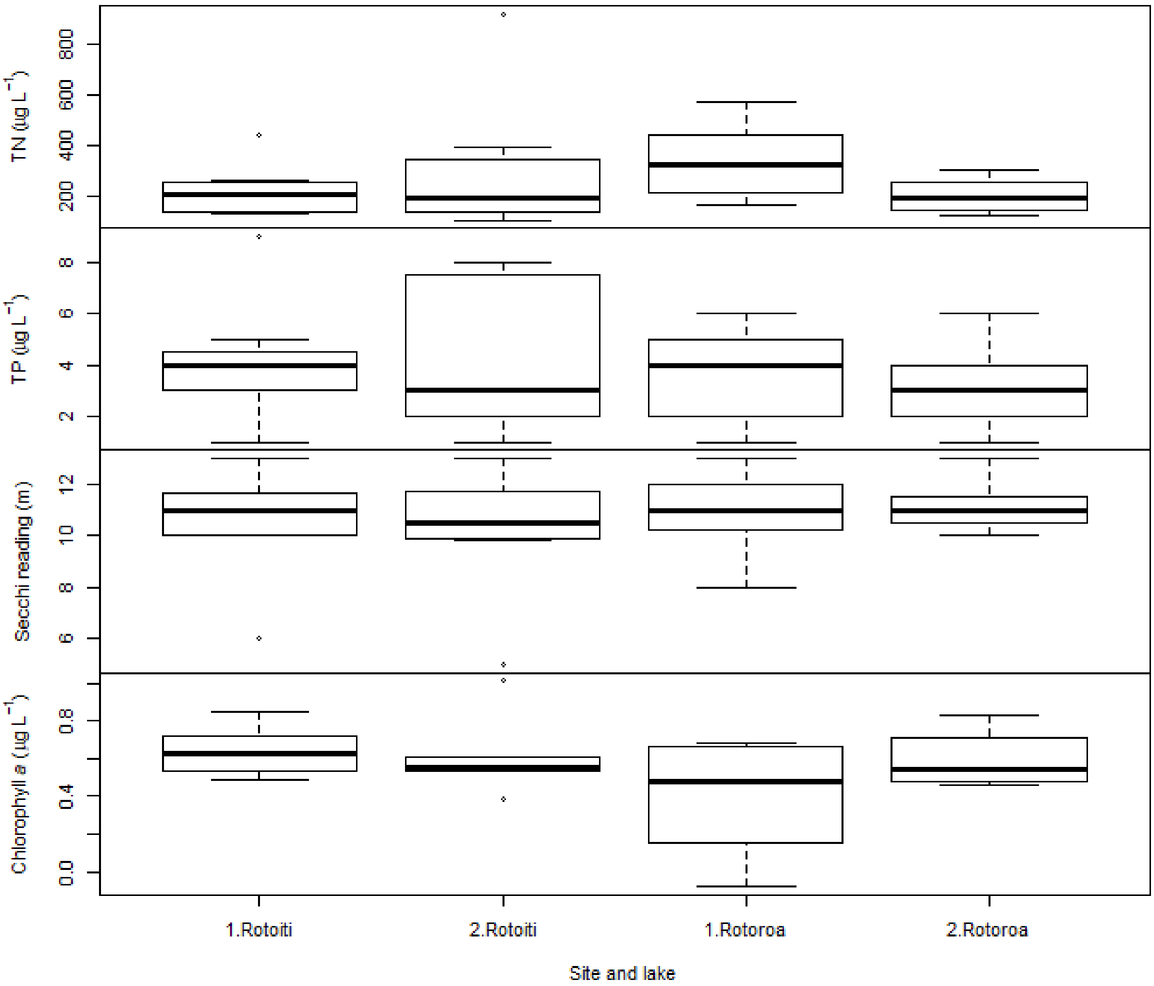

Figure A2. Box-and-whisker plots of lake productivity metrics at each site over all sampling times for Lakes Rotoiti and Rotoroa. Dark bars represent medians, the boxes encompass lower to upper quartiles, whiskers and points are range and outliers according to the default formula in base R.