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RESEARCH ARTICLE

Dynamics of pelagic mucilage produced by the invasive, cyclotelloid diatom, *Lindavia intermedia*, in oligotrophic lakes of New Zealand

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Abstract

Marine pelagic mucilages (e.g., marine snow) have been reported to a greater extent than their lacustrine counterparts. A pelagic mucilage primarily comprised of chitin secretions from the invasive centric diatom, *Lindavia intermedia*, has been reported since the early 2000s, primarily from large, oligotrophic pre-alpine lakes of the South Island of New Zealand. To better understand the factors related to mucilage abundance, we monitored its abundance as well as factors potentially related to mucilage production over time in four mucilage-afflicted lakes. Temporal mucilage dynamics were episodic, with peaks in abundance occurring during any season, but most often during summer and autumn. Chitin was confirmed to be an important component of the mucilage, but the chitin content varied between 1 and 12% of the mucilage dry mass in the lakes. An RT-qPCR assay for chitin synthase gene overexpression in *L. intermedia* showed that overexpression occurred in summer and autumn, often when peaks in mucilage abundance also occurred. A correlation between mucilage and phytoplankton abundance was only observed in one of the lakes. Both dissolved reactive and total phosphorus concentrations were often below analytical detection limits in these lakes. Nitrate concentrations were also low and showed negative correlations with mucilage abundance. This suggests either that the secrection of chitinous mucilage by *L. intermedia* significantly depleted the available N in the water column or that mucilage facilitated N uptake by *L. intermedia* and/or other microorganisms associated with the mucilage. Pelagic mucilage in New Zealand lakes shares many characteristics of other conspicuous mucilage phenomena, including lake snow and marine snow. While our correlational analyses revealed some relationships and associations with mucilage abundance, the strengths of these were quite variable, indicating that as yet unstudied mucilage loss processes in these lakes (e.g., sedimentation, disintegration, decomposition, assimilation) likely also play important roles in regulating mucilage abundance.

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Introduction

Reports of mucilaginous organic aggregates are common from marine environments, where macroscopic mucilages are often referred to as marine snow. Such mucilage can accrue to nuisance levels, occasionally forming conspicuous benthic and pelagic accumulations [e.g., 1, 2]. While pelagic mucilage appears to be less common in lakes than in the marine setting, it has been reported from several lakes, including Lakes Constance [3] and Kinneret [4]. However, reports of the smaller precursors of lake mucilage, known as extracellular polymeric substances (EPS) and transparent exopolymeric particles (TEP), are more common in the lake literature [e.g., 3, 5–7] than reports of conspicuous macroscopic mucilages.

The size spectrum of organic aggregates in aquatic environments spans from 1 μm to over 1 m [8]. Some confusion may exist regarding the varied nomenclature used to refer to aquatic organic aggregates. Here we consider lake snow and mucilage to be roughly synonymous, referring to conspicuous aggregated material suspended in the water column bound together by adhesive, extracellular, mucilaginous substances.

Pelagic mucilage was first reported in the South Island of New Zealand in 2004, in Lake Wānaka [9] by anglers. The initial complaints of the fouling of fishing lines and clogging of motor boat cooling systems soon grew to include the clogging of domestic water filters in Wānaka township, which obtains its municipal water from the lake. Subsequently, the phenomenon spread to other oligo- and mesotrophic lakes [10], also causing problems for hydroelectricity generation infrastructure and necessitating expensive upgrades to numerous municipal water supplies to remove the mucilage from their lake water intakes. In the Queenstown Lakes region, pelagic mucilage has also been reported to attach to boat hulls and to swimmers' bodies. While causing obvious problems for water users, the recent phenomenon of pelagic mucilage in these lakes will also have repercussions for lake food webs and lake functioning, as it does in marine systems [8].

The mucilage proliferations reported from South Island lakes have been attributed to secretions of β-chitin (β-(1→4)-linked *N*-acetylglucosamine) polymer from the invasive cyclotelloid diatom *Lindavia intermedia* [9, 11], which appears to have invaded New Zealand from the West Coast of North America [12]. Recently, molecular methods were developed to quantify *L. intermedia* cell densities in whole lake water and to quantify the expression of a chitin synthase gene in *L. intermedia* [11, 13]. In addition, two methods were developed to quantitatively sample the pelagic mucilage from lake water [13]. These methodological advances facilitate the study the temporal dynamics of lake mucilage biomass and production.

Numerous studies of marine snow formation from diatom polysaccharides have indicated that polysaccharide secretion increases under nutrient-limited conditions (e.g., [14–17]) and under high light illumination [18, 19]. In New Zealand, *L. intermedia* has almost exclusively been reported from nutrient-poor lakes, with TP <11 μg L^{-1} and TN < 300 μg L^{-1} [10]. Furthermore, episodic increases in polysaccharide production in diatoms have been shown to be associated with nutrient-limited or nutrient-depleted conditions [14–17]. Such findings have led some researchers to hypothesize that, because extracellular polysaccharides are derived from photosynthate, the condition of nutrient limitation in the presence of adequate illumination for photosynthesis results in the diversion of surplus photosynthate away from cell growth and reproduction and towards extracellular polysaccharide production [20]. Indeed, comparative study of multiple mucilage events in the Mediterranean Sea led Rinaldi *et al.* [21] to speculate whether algal mucilage production facilitates nutrient retention and/or harvesting, thereby enhancing growth and survival under nutrient-limited conditions. Lending credence to this hypothesis, the aggregation of diatoms into marine snow was reported to (1.) enhance the flow rate of high-molecular weight dyes to the diatoms [22], (2.) enhance the potential for anoxic microsites to develop within diatom aggregates, which can enhance nitrate storage within the aggregates [23], and (3.) to result in high enrichment factors for nitrate and phosphate in marine snow aggregates compared to the surrounding waters [8].

The persistent, complex questions of why and how mucilaginous aggregates benefit pelagic diatoms and what drives mucilage overproduction inspired our study of a pelagic mucilage phenomenon that recently manifested in a number of oligotrophic New Zealand lakes.

Specifically, we sought to elucidate (1.) seasonality in mucilage biomass and production, (2.) interannual variability and trends in mucilage biomass, (3.) the importance of chitin and chitin synthase gene expression (in *L. intermedia*) to mucilage biomass, (4.) associations between mucilage biomass and both *L. intermedia* and total phytoplankton biomass, and (5.) relationships between mucilage biomass and N and P availability.

Previous methods used to quantify mucilages and organic macroaggregates include underwater photography, sampling by SCUBA diver, syringes or water bottles and the use of sediment traps [8]. These methods were deemed inappropriate for the routine monitoring of pelagic mucilage in our lakes. Instead, we used recently developed methods for mucilage quantification [13] in two monitoring programmes which surveilled four low-nutrient-status lakes which had been reported to contain pelagic mucilage associated with *L. intermedia* [9].

Methods

Study lakes and sites

Four lakes were selected for study: Lakes Wānaka, Wakatipu, Hāwea and Moke Lake. These lakes exhibit a warm-monomictic mixing regime and generally low total phosphorus, nitrate and chlorophyll *a* concentrations (Table 1). The locations of the lakes and the sampling sites are shown in Fig 1.

Two lake monitoring datasets were analysed (see Table 2):

1. Monthly monitoring data collected by the Otago Regional Council from 10 m depth at sites in the main basins of Lakes Wakatipu, Wānaka and Hāwea (see Fig 1 for site locations). The dataset spans the period September 2016 to June 2021. This dataset contains mucilage abundance (snow tow method—see below) as well as other physico-chemical parameters including nutrients and chlorophyll *a*.
2. Data obtained from Lakes Wakatipu (Frankton Arm), Wānaka (Stephenson's Arm) and Moke Lake from samples collected between January 2020 and March 2021 at approximately 6-weekly intervals from 15m depth in the first two lakes and 5 m depth from the shallower and more sheltered Moke Lake (Fig 1). Due to weather constraints, sampling occurred at

Table 1. Background information on the study lakes. TP is total phosphorus concentration. Water quality data are means (standard deviations).

Lake	Latitude	Longitude	Surface area (km^2)	Maximum depth (m)	Mucilage/*L. intermedia* first reported*	TP (μg L^{-1})	Nitrate-N (μg L^{-1})	Chlorophyll *a* (μg L^{-1})
Wānaka	44° 30' S	169° 00' E	192	311	2004/2005	1.5 (1.0)†	24 (11)†	0.8 (0.4)†
Wakatipu	45° 03' S	168° 30' E	291	380	2016/2015	1.5 (0.9)†	25 (9)†	0.6 (0.3)†
Hāwea	44° 30' S	169° 17' E	141	392	2016/2015	1.2 (0.7)†	8 (7)†	0.5 (0.2)†
Moke	45° 0.4' S	168° 34' E	0.90	41	2017/2008	2.5 (1.8)$	25 (25)$	2.3 (0.8)$

*from [11, 24].

†from this study. Otago Regional Council data from mid-lake sites at 10 m depth, sampled monthly from Sept. 2016 to June 2021.

$from this study. Nine samples from the western basin at 5m depth, sampled at approximately 6-weekly intervals.

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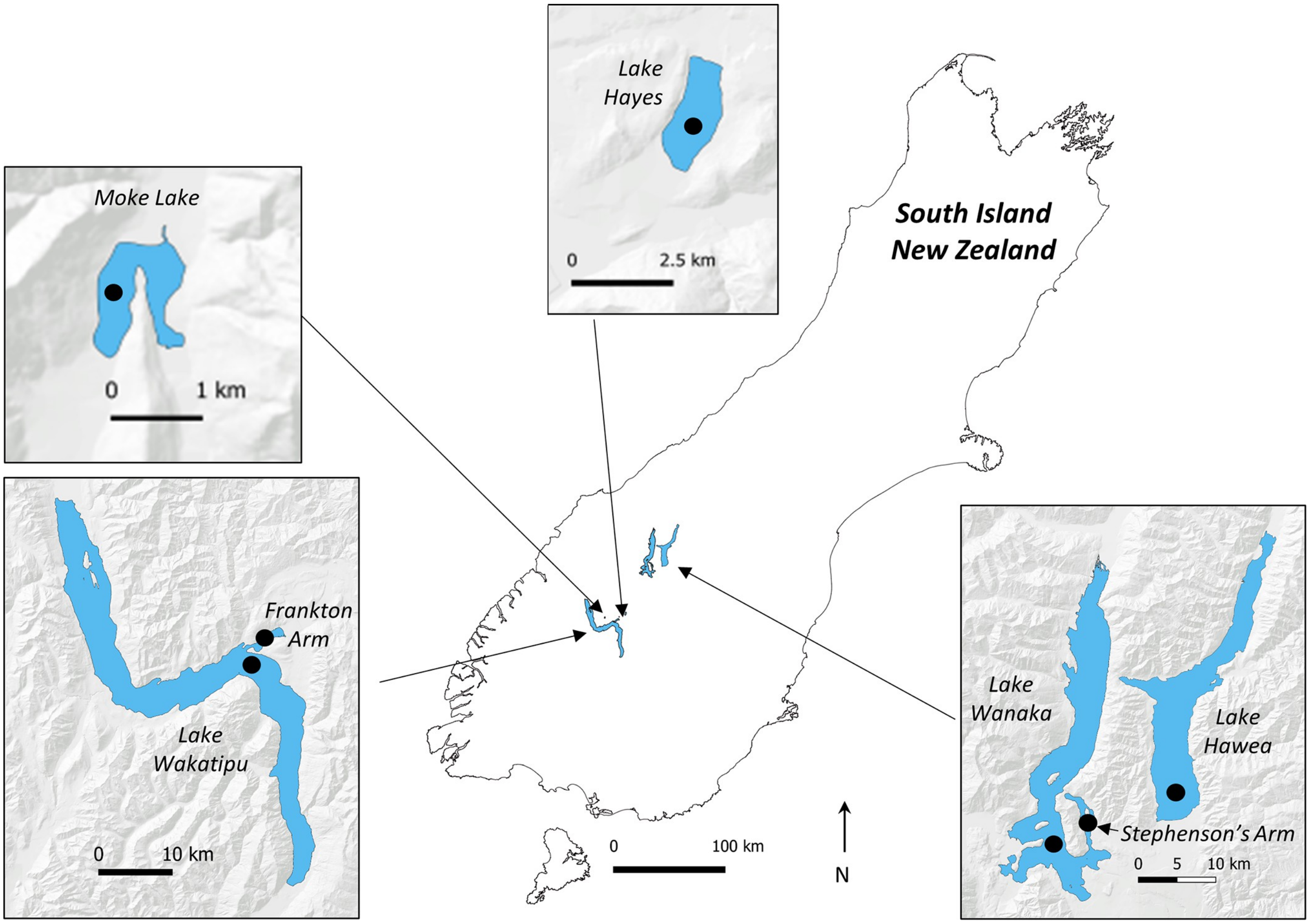

Fig 1. Locations of the study lakes and the sampling sites. Lakes Wakatipu and Wānaka have two sites each, reflecting conditions in the main lake basis and in embayments. Base map source data is from Land Information New Zealand (open source): https://data.linz.govt.nz/layer/50293-nz-lake-polygons-topo-150k/ and https://www.otago.ac.nz/surveying/research/geospatial/otago040574.html.

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between 5- and 8-weekly intervals except from February to June 2020, when sampling was interrupted due to Covid-19 restrictions. The dataset contains similar variables to dataset 1, with the addition of mucilage abundance measured by the snow pump method (see below) as well as estimates of *L. intermedia* cell density and chitin synthase and actin gene expression, both derived from qPCR methods (see below).

In Fig 1, the sites in the main lake basins are sites from dataset 1, whereas the sites in the shallow bays of Lakes Wakatipu and Wānaka (Frankton Arm and Stephenson's Arm, respectively) are from dataset 2.

Physico-chemical measurements

Van Dorn samplers were used to collect water from 10 m depth for dataset (1.) and 5 m and 15 m depths for dataset (2.). Moke Lake, being smaller and with a shallower thermocline, was sampled at 5 m, whereas the other lakes with deeper thermoclines were sampled at 15 m. Based on temperature profiles measured as part of the Otago Regional Council lake monitoring programme and from previous work undertaken on the lakes [25, 26], sampling depths

Table 2. Summary of sampling regimes used in the two datasets.

Dataset	Lakes	Sites	Sampling frequency (start and end dates)	Physico-chemistry	Mucilage	Molecular methods
(1)	Wakatipu	Main basin	Monthly (Sept. 2016 to June 2021)	• Nitrate-N • Dissolved reactive P • Total phosphorus • Chlorophyll *a*	Snow tow (dry mass)	
	Wānaka	Main basin				
	Hāwea	Main basin				
(2)	Wakatipu	Frankton Arm	6-weekly (Jan. 2020 to March 2021)	• Nitrate-N • Dissolved reactive P • Total phosphorus • Chlorophyll *a*	• Snow tow (dry mass) • Snow pump (dry mass, and chitin concentration)	• qPCR quantification of L. intermedia chloroplast DNA marker • qPCR quantification of chitin synthase gene expression
	Wānaka	Stephenson's Arm				
	Moke	Western basin				

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were selected to ensure that sampling occurred within the lower half of the mixed layers of the lakes through most of the stratified period. *In vivo* chlorophyll *a* fluorescence profiles in these clear water lakes often showed substantial reductions in fluorescence near the lake surface (e.g., [25]). Thus, we sought to sample the mixed layers while avoiding zones of reduced phytoplankton abundance at the lake surface.

Nitrate-N (NO_3-N), dissolved reactive phosphorus (DRP) and total phosphorus (TP) were measured from the van Dorn samples using standard colorimetric methods after pre-filtration through Whatman GF/F glass fibre filters (0.7 μm, nominal pore size). Samples for dataset 1 were kept on ice and in the dark until analysed by flow injection analyser at Hill's Labs (Hamilton, New Zealand), usually within 48h. Samples for dataset 2 were filtered onboard the boat and kept on ice until frozen for subsequent analysis by standard colorimetric analysis at the University of Otago, Department of Zoology. Total phosphorus was measured on unfiltered samples as DRP concentration after persulfate oxidation of the sample in an autoclave. The analytical detection limits for analytes in dataset 1 were 1.0 μg L^{-1} for the NO_3-N, TP, and DRP. Those for dataset 2 were: 1.0 μg L^{-1} for the NO_3-N and DRP and 2.0 μg L^{-1} for TP.

For dataset 1, chlorophyll *a* was measured spectrophotometrically after acetone extraction following the modified standard method 10200 H [27]. For dataset 2, chlorophyll *a* was measured by spectrophotometer (4 cm quartz cells) using the tri-chromatic method on filtered samples (Whatman GF/F glass fibre filters) after extraction with aqueous alkaline acetone [28].

Mucilage quantification

We quantitatively sampled mucilage abundance in three ways. In both datasets 1 and 2, the snow tow method was used, whereas in dataset 2 the snow pump method was also used to collect material for mucilage dry mass and chitin estimations. Details of these methods are described in Novis *et al.* [13], but briefly, the snow tow method involved towing a known length of braided fishing line to which an approx. 1 kg weight was attached for a known distance (usually 1 km) at a velocity of approx. 4 km h^{-1} (the total distance travelled was derived from velocity and time measurements for each sampling). After stopping the boat, the line was retrieved while the adherent mucilage was stripped from the line using a gloved hand (nitrile glove) and carefully transferred to a 15 mL screwcap vial using forceps, while retaining enough lake water to cover the mucilage sample. Samples were then stored in the dark on ice until processing (usually within 48 h). Sample dry weights were measured after dehydration in an oven (60˚C) for 24 h. Dry mass was standardised for the length of fishing line deployed (30 m) and for the distance travelled, resulting in a quantity of harvested mucilage expressed as milligrams dry mass collected in the upper 30 m of water per km travelled. Trials with the snow tow method showed that the mean difference between duplicated snow tow collections was 11.6% (N = 24; S1 Data). For logistical reasons were weren't able to undertake replicate sampling of snow tows on some sampling trips. When duplicates were taken, the mean of the duplicates is reported.

The snow pump method uses a submersible bilge pump to draw water through a 220 μm nylon sieve, as described in Novis *et al*. [13]. The pump was deployed at the same depths from which water sampling for nutrients and chlorophyll *a* was undertaken (Table 2). Pump rate and time of pumping were measured, allowing the quantification of material collected as dry mass per volume of whole water filtered. Large zooplankters were removed from the sieve in the field, before the remaining adhered material was stored in the dark and on ice before freeze-drying (usually within 48 h), which facilitated the complete removal of the material from the sieve. The dry mass of the sample was then measured.

Chitin quantification

The dry mass sample from the snow pump was then analysed for chitin content using the method described in Novis *et al*. [11]. Briefly, after acid hydrolysis of the sample and deacetylation of the polymeric β-1,4 linked N-acetylglucosamine sugars into glucosamine, the hexosamine was quantified using a colorimetric assay [29]. Chitin from shrimp shell (Sigma C7170) and a hydrolysis control containing no sample were also included with each set of analyses. The absorbance of the solutions was measured at 650 nm and the chitin (as anhydro 1,4 linked N-acetylglucosamine) content was determined from a glucosamine hydrochloride calibration curve.

L. intermedia chloroplast DNA marker estimates of cell density

Cell density of *L. intermedia* was determined using qPCR quantification of an *L. intermedia* chloroplast DNA marker (see Novis *et al*. [11] for full methods including tests of inhibition and limits of detection). This assay is based on the rps20-rpoB chloroplast intergenic spacer, which is identical in all lake-snow forming populations tested to date, and has shown specificity to *Lindavia* within the "bodanica" complex (*L. intermedia* being the only such species known from New Zealand to date). The assay is calibrated to cell concentrations measured microscopically over multiple occasions in the study lakes.

Chitin synthase and actin gene expression

Chitin synthase gene expression in *L. intermedia* was determined by qPCR quantification of chitin synthase (*chs2*) expression, using the expression of the actin gene (*act1*) in *L. intermedia* as a reference (see Novis *et al*. [11] for full methods, including tests of specificity and inhibition, and miqe checklist). The use of a reference gene that is constitutively expressed is fundamental to gene expression studies and the use of *act1* has been recommended as a reference gene for diatom metabolism [30, 31]. We therefore used *act1* expression to standardize *chs2* expression for changes in *chs2* expression due to variation in global cellular processes. Following Novis *et al*. [11, 13] we estimated the overexpression of chitin synthase as the difference in quantified mRNA between the two genes, rather than the ratio of the expression of the two genes. We did this in order to avoid the large relative errors associated with values measured near the limit of detection.

Statistical methods

Regression and correlation analyses were carried out in Microsoft Excel. Time series analyses (Kruskal-Wallis 1-way ANOVA by season and seasonal Kendall trend tests) were carried out in the statistical software, Time Trends [32]. Austral seasons were attributed as follows: summer is December to February, autumn is March to May, winter is June to August, and spring is September to November.

To test whether different lakes contained different abundances of chitin, a mixed model was constructed for the chitin abundance response variable that included a random effect for date sampled, using the lmer function in R package lme4 [33]. Statistical significance was assessed by creating Bayesian 95% highest probability density intervals using the HPDinterval function in the R package coda [34]. Estimated parameter ranges that excluded zero were judged to be statistically significant. Response data were log-transformed to overcome heteroscedasticity. Model fitting was assessed using standard diagnostics including residual plots. These analyses utilised data from June 2020 onwards, for which replicate samples were available.

Results

Temporal patterns of mucilage abundance

The monitoring data collected by the Otago Regional Council provided a 58-month (i.e., almost 5-year) time series of mucilage abundance, chlorophyll *a* and nutrient concentrations in the main basins of Lakes Wakatipu, Wānaka and Hāwea. During the monitoring period, chlorophyll *a* concentrations showed significant seasonal variation and increased significantly in all three lakes (Fig 2; Table 3). Mucilage abundance also showed significant seasonality in both Lakes Wakatipu and Wānaka, but not in Lake Hāwea. Mucilage only showed a significant trend over time in Lake Wānaka, but this was a negative trend, in contrast to the positive trend observed in chlorophyll *a* (Table 3).

Peaks in mucilage abundance were identified as periods when the abundances exceeded the threshold of one standard deviation above the mean of each time series. Thus, seven peaks in mucilage abundance were revealed over the 58-month period (Fig 3, α to π). Three peaks

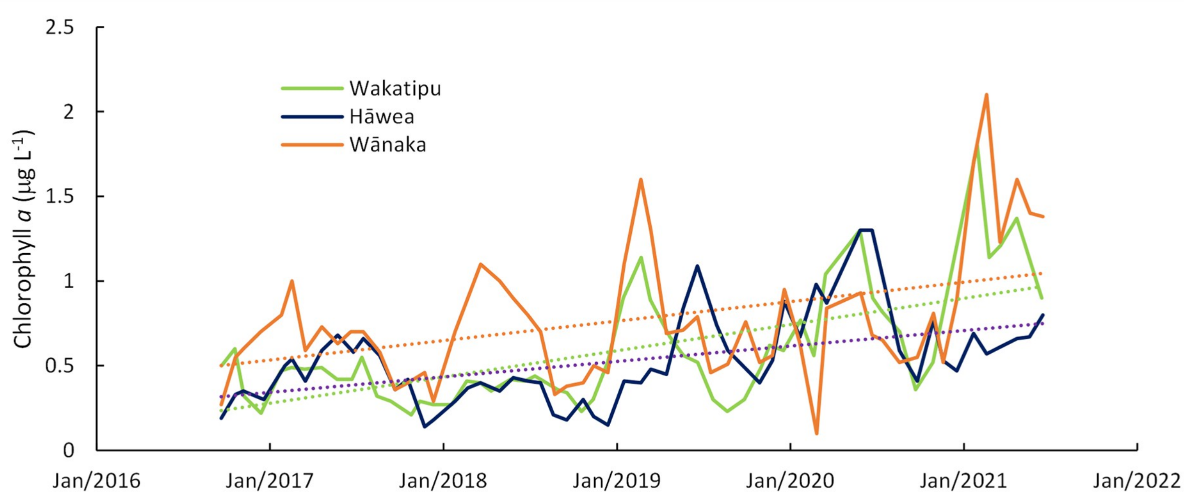

Fig 2. Time series of chlorophyll *a* concentrations from Lakes Wakatipu, Hāwea and Wānaka. Monthly samples were collected by the Otago Regional Council from September 2016 to June 2021. The dashed lines are linear regressions for each lake and are shown only to suggest trends. Appropriate statistical tests of the time trends are presented in Table 3.

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Table 3. Tests of seasonality and trend over time of chlorophyll *a* and mucilage in Lakes Wakatipu, Hāwea and Wānaka. The ranks of the mean values for each season are also shown. Data are monthly samples collected by the Otago Regional Council from September 2016 to June 2021 Austral seasons are: summer is December to February; autumn is March to May; winter is June to August; spring is September to November.

	Wakatipu		Hāwea		Wānaka	
Seasonality	***P*-value**[1]	**Rank of means**	***P*-value**[1]	**Rank of means**	***P*-value**[1]	**Rank of means**
Chlorophyll *a*	0.031	autumn > summer > winter > spring	0.006	winter > autumn > summer > spring	< 0.0001	autumn > summer > winter > spring
Mucilage	0.001	autumn > winter > summer > spring	ns	summer > winter > autumn > spring	0.005	summer > autumn > spring > winter
Trend	***P*-value**[2]	**Direction**	***P*-value**[2]	**Direction**	***P*-value**[2]	**Direction**
Chlorophyll *a*	< 0.0001	+ve	< 0.0001	+ve	< 0.0001	+ve
Mucilage	ns		ns		0.001	-ve

[1]Kruskal-Wallis non-parametric 1-way ANOVA by season

[2]Seasonal Kendal non-parametric trend test accounting for 4 seasons

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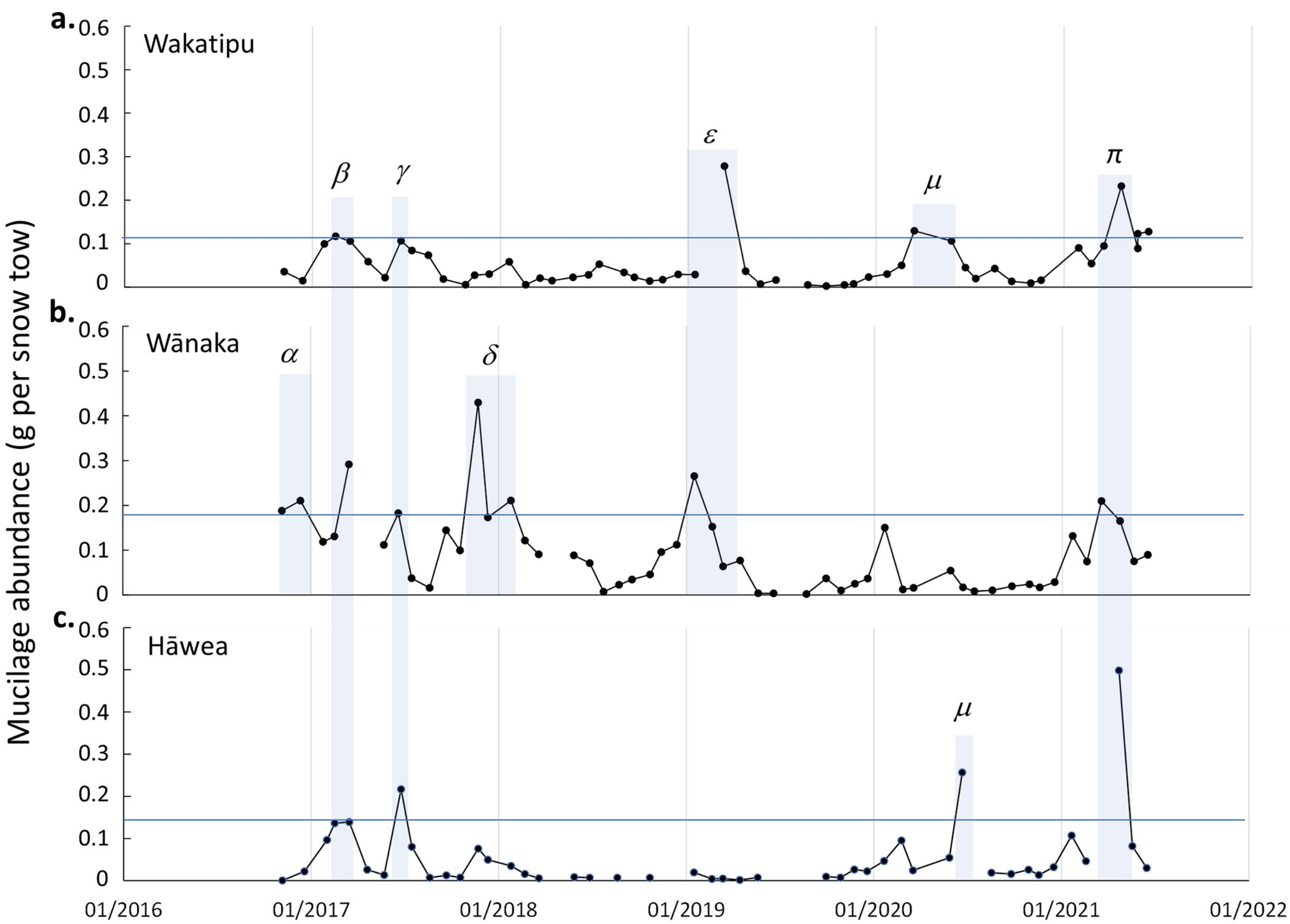

Fig 3. Interannual and seasonal variation in mucilage abundance in the main basins of Lakes Wakatipu (a.), Wānaka (b.) and Hāwea (c.). Vertical lines indicate January 1. Horizontal lines are peak thresholds defined as the mean + 1 standard deviation, that were used to define mucilage peaks and which are denoted by Greek letters.

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appeared simultaneously in all three lakes (β, γ, π), two peaks occurred simultaneously in two lakes (ε, μ), and two peaks occurred in only one of the lakes at a time (α, δ). Thus, there was a moderate degree of temporal coherence in mucilage peaks among the three lakes. Where seasonal differences in mucilage abundance were statistically significant, the rankings of the mean mucilage abundances by season were autumn > winter > summer > spring for Lake Wakatipu and summer > autumn > spring > winter for Lake Wānaka (Table 3). In contrast, chlorophyll *a* seasonality was much stronger and more consistent among the lakes, with phytoplankton biomass being consistently lowest in spring time in all three lakes.

Thus, seasonal factors (e.g., temperature, solar radiation, thermal stratification, wind energy) appeared not to have as strong an influence on mucilage abundance as on phytoplankton biomass. Furthermore, significant interannual increases in phytoplankton abundance were not reflected in trends in mucilage abundance.

The importance of chitin and chitin synthase gene expression in pelagic mucilage

Both the snow tow and snow pump methods were used to measure mucilage abundance in Moke Lake and in Lakes Wānaka (Stephenson's Arm) and Wakatipu (Frankton Arm). The importance of chitin in the mucilage was assessed by measuring the chitin content of the mucilage as well as the overexpression of the *chs2* gene, both quantified from the snow pump samples. The chitin content of the snow pump samples (by dry mass) ranged from 2 to 12% in Moke Lake (mean = 5.2%), 2 to 9% in Lake Wānaka (mean = 3.9%) and 1 to 6% in Lake Wakatipu (mean 2.4%). Mixed modelling, accounting for the random effect of sampling date, indicated that both Moke Lake and Lake Wānaka contained significantly higher concentrations of chitin than Lake Wakatipu overall, although chitin concentrations in Moke Lake and Lake Wānaka were not significantly different from each other. Full details of the statistical modelling are shown in the S1 Text.

While chitin measured in the snow pump samples undoubtedly contributed to mucilage, chitin from other cell constituents is also included in the chitin content estimates. Thus, to further explore the importance of chitin to mucilage dynamics, we examined relationships between mucilage abundance and chitin concentrations in the lakes (Table 4). Indeed, chitin concentrations generally correlated well with mucilage dry mass collected both by the snow pump and snow tow methods. Only in Lake Wānaka were the correlations between chitin and mucilage weak or non-significant.

Chitin synthase gene (*chs2*) overexpression was not correlated with either estimate of mucilage abundance in any of the lakes (Table 4). However, chitin synthase overexpression is more likely a measure of chitin production as opposed to chitin standing stock. Thus, the lack of correlation between production and standing stock does not necessarily mean that *chs2* overexpression does not underpin chitin production by *L. intermedia* in the lakes. This is because we

Table 4. Pearson correlation coefficients between two measures of mucilage abundance vs chitin concentration and chitin synthase (*chs2*) overexpression in three lakes and in the combined lake dataset. Numbers in parentheses are the number of data points (*N*). * indicates $0.05 > P > 0.01$. ** indicates $0.01 > P > 0.001$. *** indicates $0.001 > P > 0.0001$. **** indicates $P < 0.0001$. Non-significant correlations ($P > 0.05$) are indicated by "ns".

	Moke		Wānaka		Wakatipu		Combined lakes	
	Snow tow (6)	Snow pump (9)	Snow tow (9)	Snow pump (9)	Snow tow (9)	Snow pump (9)	Snow tow (24)	Snow pump (27)
Chitin concentration (μg L^{-1})	0.94**	0.98****	ns	0.87*	0.97****	0.92**	0.67***	0.97****
Chitin synthase overexpression (copy number)	ns	ns	ns	ns	ns	ns	ns	ns

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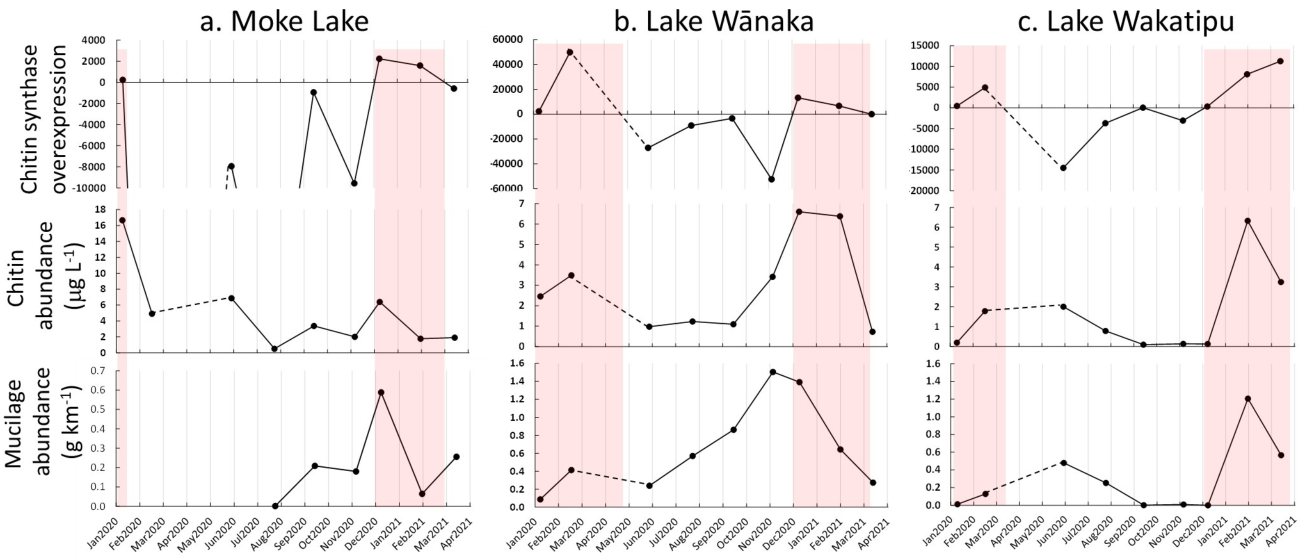

Fig 4. Time series of chitin synthase (*chs2*) overexpression, chitin concentration from the snow pump method, and mucilage abundance estimated from the snow tow method for three lakes over an 18-month period. Periods of *chs2* overexpression are highlighted by red shading. The dashed line indicates the 15-week sampling interval, which had to be extended due to Covid-19 restrictions. The unit for *chs2* overexpression is the copy number (e.g., *chs2* minus *act1* copies). In Moke Lake, quantitative determinations of mucilage abundance commenced in July 2020 (4a.). Extreme negative values of *chs2* overexpression in Moke Lake (4a.) were truncated in the graph to facilitate examination of the periods when *chs2* overexpression values were positive. The dates shown on the x-axis indicate the beginning of the indicated months.

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have not quantified loss rates, which likely contribute to net chitin and mucilage standing stocks. Therefore, to further explore the potential influence of *chs2* overexpression on mucilage and chitin abundance, we present time series of these variables, which allow the examination of temporal patterns of mucilage and chitin abundance specifically in relation to periods of *chs2* overexpression (Fig 4, shaded periods).

Periods of chitin overproduction (December to March or April) occurred only in the austral summer (December to February) and autumn (March to May). These seasons also corresponded to seasons of elevated mucilage/chitin abundance in Moke Lake and Lake Wānaka (Table 3; Fig 4). However, the magnitudes of *chs2* overexpression were not reflected in the abundances of mucilage/chitin in the lakes. For example, in Lake Wānaka, *chs2* overexpression was highest in February 2020, when there were only relatively small peaks of mucilage/chitin, whereas *chs2* overexpression was lower in January and February 2021, when substantial peaks of mucilage and chitin abundance occurred (Fig 4). While seasonality in *chs2* overexpression was generally associated with seasons of higher mucilage and chitin abundance, some discrepancies in the synchrony and magnitudes of chitin synthase gene expression vs mucilage and chitin abundance exist in these lakes.

Mucilage association with phytoplankton and nutrients

As the chitinous fibrils produced by the diatom, *L. intermedia*, are a key component of the mucilage found in the lakes, we investigated whether variation in pelagic mucilage abundance in the lakes is correlated with phytoplankton biomass, *L. intermedia* abundance and nutrient availability. The demonstration of strong relationships would imply the importance of drivers such as phytoplankton biomass, productivity and nutrient availability to mucilage abundance.

Examination of the 58-month Otago Regional Council dataset for Lakes Wakatipu, Hāwea and Wānaka showed that chlorophyll *a* concentration only correlated to mucilage abundance estimates from snow tows in Lake Wakatipu, but not in the other two lakes (Fig 5A).

The Otago Regional Council also collected nitrate and dissolved reactive phosphorus data, but, unfortunately, the phosphorus concentrations were almost always below analytical detection limits, preventing the examination of the relationship between phosphorus availability and mucilage abundance. However, mucilage abundance was significantly negatively correlated to nitrate-N concentrations in Lakes Wakatipu and Wānaka suggesting an interaction between mucilage and nitrate in these lakes (Fig 5B). The relationship for Lake Hāwea also showed a negative tendency, but was only borderline significant ($P = 0.06$).

L. intermedia concentrations were not available for the 58-month dataset, but estimates of *L. intermedia* concentration by qPCR were conducted in the shorter dataset. Again, Lake Wakatipu showed the strongest relationships between *L. intermedia* concentrations and the two estimates of mucilage abundance (Fig 6). No significant relationships between *L. intermedia* concentration and mucilage abundance were observed in the other lakes.

Discussion

Temporal mucilage dynamics and associations with phytoplankton biomass

Pelagic mucilage in our study lakes has been reported to be associated with the secretion of chitinous fibrils by the centric diatom, *Lindavia intermedia* [9, 11, 12]. The abundance of lake snow mucilage elsewhere, in Lakes Constance (Germany/Switzerland/Austria) and Kinneret (Israel), has also been associated with phytoplankton standing stocks [3, 4]. The secretion of extracellular polysaccharides by diatoms has been shown to be light-dependent [18, 20] and, therefore, we investigated whether pelagic mucilage abundance in our study lakes was related to phytoplankton and *L. intermedia* biomass and whether temporal patterns of mucilage abundance tracked the strong seasonality of phytoplankton biomass in the study lakes.

Seasonality in phytoplankton biomass and productivity is typical of the deep, oligotrophic lakes that *L. intermedia* has successfully colonised, where strong seasonal patterns of solar radiation, water temperature, wind energy and thermal stratification play important roles in structuring the annual cycle of productivity (e.g., [25, 35, 36]). A study of the phytoplankton of Lake Wānaka post-invasion by *L. intermedia* showed that this invasive diatom contributed strongly to the seasonal phytoplankton biomass development in this lake [25]. Therefore, we tested whether the temporal pattern of mucilage abundance in the lakes was also seasonal and related to variation in phytoplankton and *L. intermedia* biomass in the lakes. If mucilage production were coupled to phytoplankton biomass, we would also expect to see strong seasonality in mucilage biomass and production. Our analysis of the almost 5-year time series of mucilage abundance from three lakes showed significant seasonality in mucilage abundance in Lakes Wānaka and Wakatipu, but not in Lake Hāwea. In general, periods of relatively high mucilage abundance tended to occur in summer and autumn, but mucilage peaks did not occur only in these seasons, nor were they consistently observed in these seasons.

Our analyses also indicated that peaks in mucilage abundance showed some degree of coherence among the three lakes, although occasional peaks occurred in one or two lakes without manifesting in other lakes. This suggests that regional, climatic drivers and related phenomena such as thermal stratification dynamics likely influence mucilage abundance, but that some lake-specific, factors probably also play an important role in determining the timing of mucilage peaks in the lakes.

Significant correlations between mucilage abundance and phytoplankton concentration were found only in Lake Wakatipu. Furthermore, significant increases in chlorophyll *a* over the study period in all three lakes were not mirrored by increases in mucilage abundances,

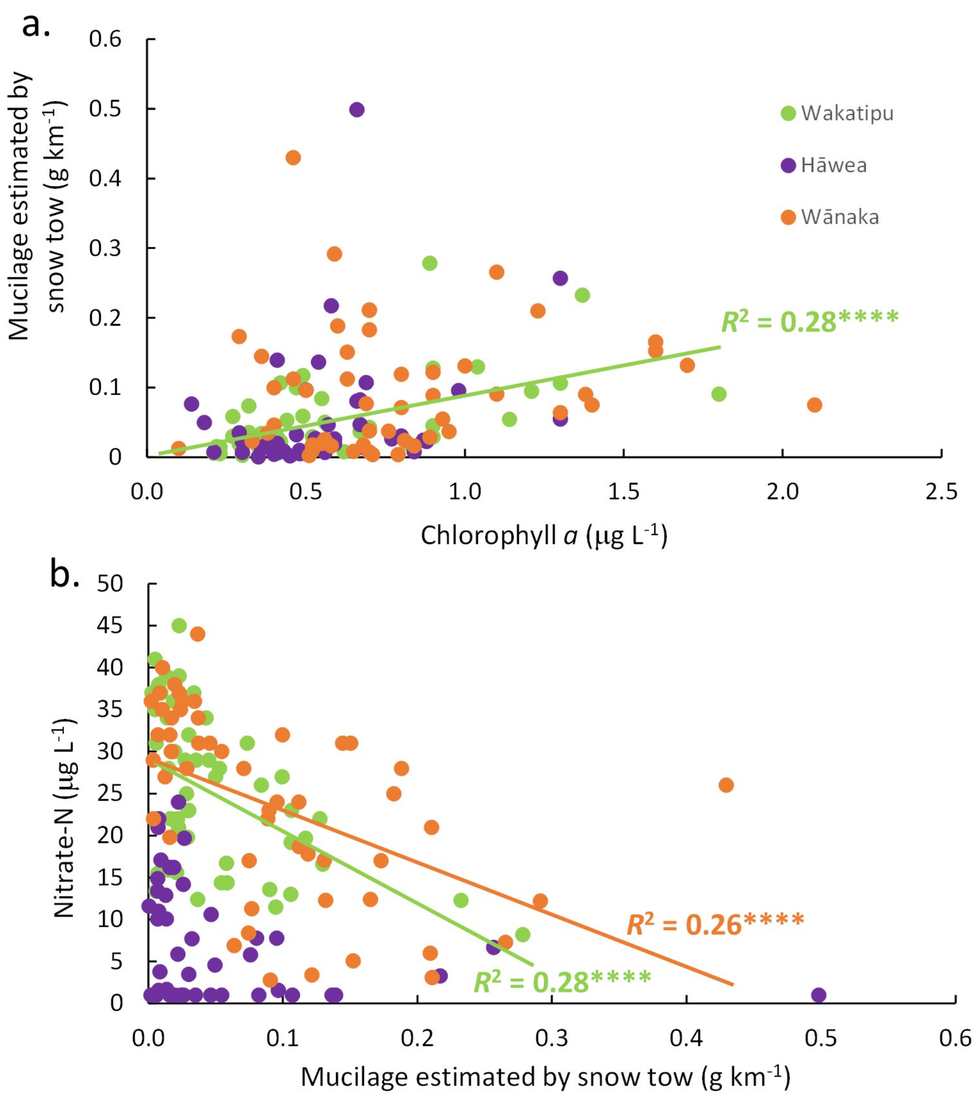

Fig 5. Within- and among-lake correlations between mucilage abundance estimated by snow tows and both chlorophyll *a* and nitrate-N concentrations. Monthly data were collected by the Otago Regional Council for 58 months between September 2016 and June 2021. Mucilage is the dependent variable in the relationship with chlorophyll *a* (a.) and the independent variable in the relationship with nitrate-N concentration (b.). Only statistically significant linear relationships are shown. **** indicates $P < 0.0001$. The *P*-value for the nitrate-N relationship for Lake Hāwea was 0.06 and is therefore not indicated as statistically significant.

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which showed no trends in Lakes Wakatipu and Hāwea and a significant negative trend in Lake Wānaka over the sampling period.

Mucilage abundance in Lake Wakatipu was seasonal and correlated with both phytoplankton biomass and *L. intermedia* concentrations, but mucilage abundance did not correlate with either phytoplankton biomass nor *L. intermedia* concentrations in Lakes Wānaka, Hāwea or in Moke Lake. The strong association between mucilage abundance and phytoplankton and *L. intermedia* in Lake Wakatipu suggests that phytoplankton dynamics do play a role in mucilage abundance, but the lack of statistical evidence for the same linkage in the other lakes suggests that other factors mediate the relationship between *L. intermedia* and mucilage abundance in those lakes.

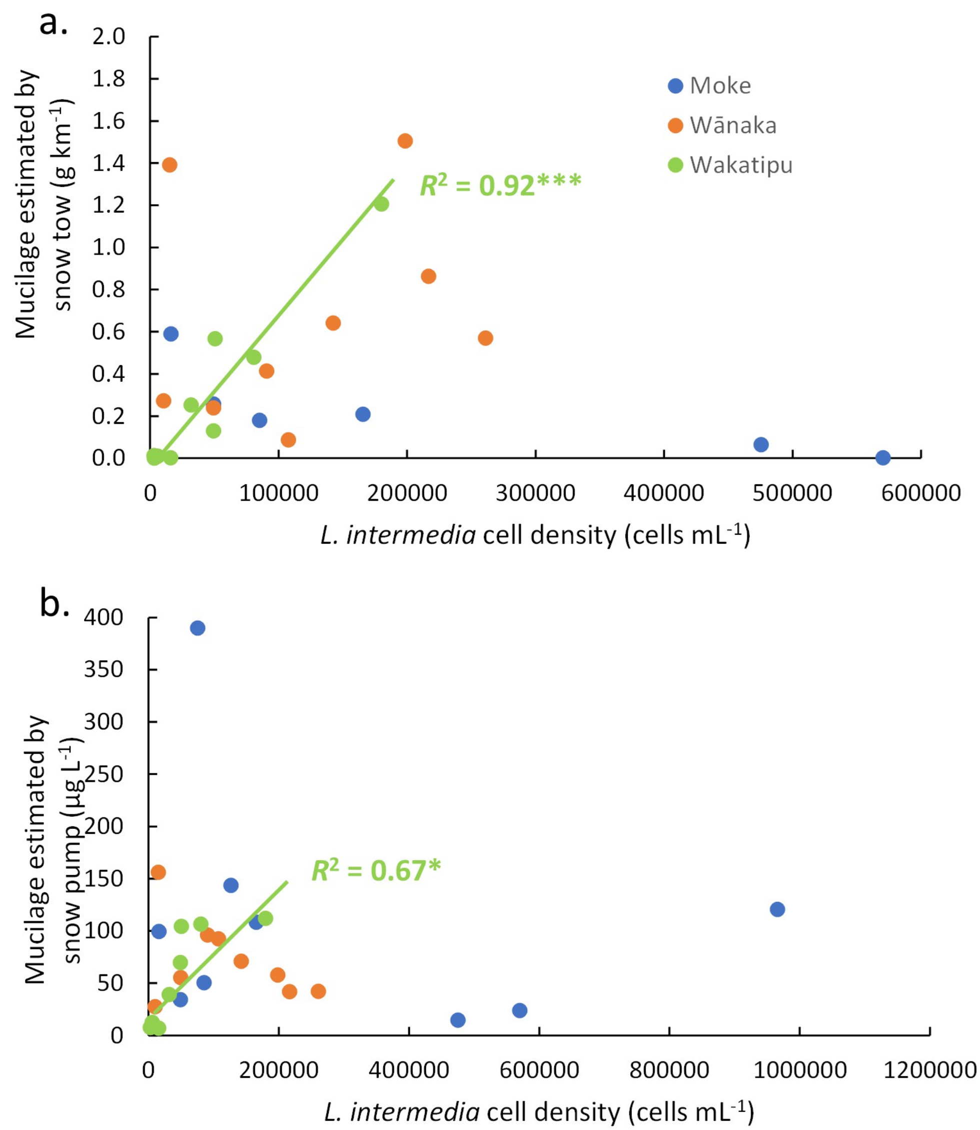

Fig 6. Within- and among-lake correlations between *Lindavia intermedia* concentrations and mucilage abundance estimated by the snow tow (a.) and snow pump methods (b.). Data were collected at approximately 6-weekly intervals from January 2020 and March 2021. Only significant linear regressions are shown. ** indicates P < 0.01.

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Mucilage abundance is a function of both mucilage production and mucilage loss processes. The presence of even very weak density stratification can cause sedimenting mucilage [37] to accumulate at pycnoclines [38], where mucilage could subsequently be redistributed into the mixed layer by deep mixing due to wind-induced turbulence and/or shear at the thermocline. Periods of wind induced turbulence and shear at the thermocline have been shown to be associated with lake snow abundance in Lakes Constance and Kinneret [3, 4]. Larger lake snow aggregates can sink rapidly and accumulate at the thermocline, where wind events can then entrain them back into the mixed layer [3] and such dynamics may influence the abundance of mucilage collected by our sampling methods. Other mucilage loss processes potentially affecting mucilage standing stocks in our lakes include disaggregation [39], decomposition [40] and grazing by zooplankton [41] and fish [42]. Further study is required to elucidate which of these factors could significantly influence mucilage standing stock and its relationship to mucilage production rate in our study lakes.

The importance of chitin as a constituent of mucilage

The mucilage abundance estimated by the snow tow method correlated strongly with the chitin abundance in both Lake Wakatipu and Moke Lake, but the correlation in Lake Wānaka was non-significant. Based on these results, the snow tow method and the snow pump method of estimating chitin concentration both seem to quantify the abundance of nuisance mucilage in the lakes across a wide range of mucilage abundances. However, our observations of variation in the color and cohesiveness of the mucilages collected in our study indicates that there are some temporal and lake-specific differences in the mucilage material in these lakes. This is confirmed by the variable chitin content of the mucilage, which ranged from 1 to 12% of mucilage dry mass.

However, bulk chitin measurements (such as ours) measure not just chitin in secreted mucilage, but also chitin produced at multiple sites within the cell and on the cell surface [43]. While microfibrils secreted by *L. intermedia* are composed of β-chitin [11], chitin is also associated with a variety of cell functions in diatoms including in the construction and reinforcement of cell walls and other structures [44–46]. In addition, although larger zooplankton were manually removed from the snow pump samples, chitin associated with microzooplankton will have contributed to chitin measurements. Unfortunately, no method currently exists to separate these other components from the microfibrils extruded from *L. intermedia* cells.

On the other hand, microscopic analysis of material collected by the snow tow method indicates that the material collected is predominantly composed of *L. intermedia* and its mucilaginous matrix, with no evidence of zooplankton in the samples and few other algal taxa apparent.

Novis *et al.* [13] speculated that the characteristic adhesiveness of the nuisance mucilage (*L. intermedia* macroaggregates) in the study lakes could be due to secondary polymers produced by *L. intermedia* or associated microbes. However, studies of similar diatom-derived marine mucilages in the Adriatic Sea indicate that mucilage can simply result from the self-organisation of polysaccharide diatom fibrils into supramolecular structures resembling "gel networks" via physical bonds due to intermolecular forces [1]. This raises the possibility that the chitin mucilage produced by *L. intermedia* in our lakes could also form extensive gel networks, but further work is required to confirm this.

These issues highlight the challenges in attempting to quantify the essential constituents of pelagic mucilage in water bodies [8].

Chitin synthase overexpression

Given the challenges in using bulk chitin measurements to study pelagic mucilage dynamics highlighted above, we further studied mucilage dynamics utilizing the chitin synthase *chs*2 gene overexpression methodology developed by Novis *et al.* [11, 14] to examine how *chs2* gene expression in *L. intermedia* compares with chitin and mucilage dynamics in our study lakes.

While *chs2* overexpression was not correlated with mucilage abundance in our datasets (either within or among lakes), *chs2* overexpression was limited to the summer in autumn seasons in the study lakes, generally corresponding to times when mucilage abundance (snow tow dry mass) and chitin concentrations were elevated in the lakes. However, elevated levels of these substances also occasionally occurred in winter (e.g., Fig 4, Lake Wakatipu, mucilage and chitin abundance), when *chs2* was not overexpressed. Thus, the mucilage was sometimes abundant when *L. intermedia* was apparently not overexpressing *chs2*.

Chitin production is related to diverse functions in diatom cells and has been attributed to chitin synthase encoded by different genes [44]. *Chs2* expression may be upregulated or downregulated in response to particular stimuli, independent of physiological conditions that may affect global cellular processes. For example, the condition of nutrient limitation has been reported to stimulate the upregulation of chitin synthesis [43, 44]. Since the location of action in a cell cannot be deduced by the gene sequence, a possible concern with our method is that the assay utilises a gene that is not involved in fibril production. However, the observed general synchrony of periods of *chs2* overexpression with periods of elevated chitin/mucilage abundance is consistent with the notion that *chs2* overexpression relates, at least to some degree, to chitin fibril biomass.

Relationships between nitrate and mucilage abundance

Numerous studies have hypothesised and speculated on how mucilage production and aggregate formation in pelagic diatoms could confer a competitive advantage under conditions of nutrient stress (e.g., [14–17, 21–23]). In New Zealand lakes, *L. intermedia* has conspicuously invaded nutrient poor lakes, and it has been suggested that conditions of low phosphorus availability (e.g., total phosphorus $\leq$ 11 μg L^{-1}) favours the successful colonisation of New Zealand lakes by *L. intermedia* [10]. By simultaneously monitoring nutrient availability and mucilage abundance in our study lakes, we have been able to examine relationships between mucilage production and nutrient availability in our lakes.

Previous nutrient enhancement bioassay studies in our lakes reported that phytoplankton productivity (i.e. carbon fixation rates) in Lake Wānaka was often P-limited, whereas in Lake Wakatipu it could be limited by N and/or P [25]. Unfortunately, both dissolved reactive phosphorus and total phosphorus concentrations in our lakes were often below analytical detection limits (e.g., total phosphorus was $\leq$ 1.0 μg L^{-1} in 101 out of 138 samples), limiting our ability to make inferences about the relationship between phosphorus availability and mucilage production. However, the very low phosphorus availability in our lakes supports findings of other studies on diatom mucilages and aggregations that suggested a link between mucilage/aggregate production and nutrient limitation (e.g., [14–17, 21–23]).

Chitin contains 7% nitrogen by mass and, therefore, the extracellular secretion of mucilaginous chitin fibrils by *L. intermedia* requires that N supplies exceed requirements for cell growth and maintenance. Therefore, it is unlikely that mucilage production by *L. intermedia* would be favoured under conditions of growth limitation by N availability. Nevertheless, while nitrate levels were generally low in our study lakes (mean NO_3-N = 19 μg L^{-1}, max. = 45 μg L^{-1}), we observed significant negative relationships between nitrate concentrations and mucilage abundance in Lakes Wānaka and Wakatipu (the negative relationship for Lake Hāwea was almost significant; $P = 0.06$). These negative relationships show that ambient nitrate concentrations decreased with increasing mucilage abundance in the lakes. This is consistent with a simple hypothesis that the production of chitinous mucilage containing N by *L. intermedia* exerts a demand for N that draws down ambient nitrate concentrations in lake water. However, an alternative hypothesis, that the presence of mucilage enhances nitrate uptake into biomass [22, 23], is also consistent with the observed correlations. Regardless of the actual mechanism underpinning these negative relationships, our results indicate that theabundance of mucilage in our nutrient-poor study lakes correlates negatively with N availability.

Conclusions

Accurate quantification of pelagic mucilage is a challenging undertaking and a variety of methods have previously been used, none of which were deemed satisfactory for the routine monitoring of pelagic mucilage in large, oligotrophic New Zealand lakes. Both the newly developed snow tow and snow pump methods revealed that temporal mucilage dynamics were less seasonally regular and more episodic than phytoplankton dynamics. This is consistent with highly episodic mucilage dynamics reported elsewhere [e.g., 21].

The pelagic mucilage phenomenon in our study lakes shares many characteristics of conspicuous mucilage phenomena reported elsewhere, including other types of lake snow and marine snow. While our correlational analyses showed some strong relationships between mucilage abundance, *L. intermedia* cell density, *chs2* gene overexpression and nitrate concentrations, the strengths of the associations were variable both over time and among lakes, indicating that, in addition to drivers of mucilage production, mucilage loss processes should also be considered when assessing the factors related to pelagic mucilage abundance in aquatic ecosystems.

Supporting information

S1 Data. Duplicate snow tow samples collected from three lakes at different depths.
(XLSX)

S1 Text. Mixed model output. Chitin abundance (μg L^{-1}) response variable with a random effect for date sampled was undertaken using the lmer function in R package lme4 [33]. Bayesian 95% highest probability density intervals were calculated using the HPDinterval function in the R package coda [34]. Response data were log-transformed. Data used were only those for which replicate samples were available (n = 42).
(DOCX)

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Funding acquisition: Marc Schallenberg, Phil M. Novis.

Investigation: Marc Schallenberg, Phil M. Novis.

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Project administration: Phil M. Novis.

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Writing – original draft: Marc Schallenberg, Phil M. Novis.

Writing – review & editing: Marc Schallenberg, Hugo Borges, Tracey J. Bell, Simon F. R. Hinkley, Phil M. Novis.

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