NIWA
Taihoro Nukurangi

Testing “Check, Clean, Dry” decontamination procedures

Trials on "lake snow" (*Lindavia intermedia*)

Prepared for Ministry for Primary Industries

June 2017

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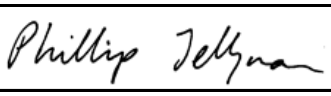
NIWA CLIENT REPORT No: 2017158CH
Report date: June 2017
NIWA Project: MPI16208

Quality Assurance Statement		
	Reviewed by:	Tracey Burton
	Formatting checked by:	America Holdene
	Approved for release by:	Phil Jellyman

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Executive summary

"Check, Clean, Dry" procedures currently recommended by Ministry for Primary Industries (MPI) to prevent or reduce the spread of nuisance aquatic organisms to new waterways are based on methods originally developed for the nuisance diatom *Didymosphenia geminata* (didymo). MPI has contracted NIWA to test the effectiveness of the current procedures on the freshwater diatom *Lindavia intermedia* (hereafter *Lindavia*) also known as lake snow or lake snot. *Lindavia* forms slimy accumulations in clean-water lakes, which block water intakes and adversely affect recreational activities. *Lindavia* is suspected to be a recent introduction into New Zealand, but this is uncertain. The species is known to be present in several lakes.

We tested the effect of four treatment methods recommended by MPI (dishwashing detergent, bleach, hot water, freezing), and two additional treatments (drying, salt solution), on the viability of *Lindavia* cells.The tests were carried out on samples of *Lindavia* collected from Lakes Wanaka and Wakatipu, within 48 h of collection. All tests were carried out at room temperature using material maintained at 4 – 10 °C. A Neutral Red (NR) staining method was used to assess cell viability following the treatments. In this method, live (viable) cells take up NR as a deep red stain in granules and vacuoles scattered throughout the cell. Non-viable cells do not take up the stain.

Freezing appeared to be the most effective treatment; no viable cells were observed following overnight freezing and thawing. All other treatments were not completely effective. Drying was largely effective, although a small proportion of cells took up NR. However, NR staining was not typical of viable cells, and the chloroplasts appeared degraded. There were similarly ambiguous staining results following the chemical (i.e., dishwashing detergent and bleach) and hot water treatments. Extending treatment times could achieve 100% effectiveness for all treatment methods, but this needs to be verified. Treatment with salt solution used at the recommended concentrations and exposure times was also not completely effective.

Summary results and recommendations are provided in the table below.

Treatment	Amount / level	Treatment time	Initial assessment	Recommendation
Dishwashing detergent	5% solution	1 minute	>90% effective	Extend treatment time
Bleach	2% solution	1 minute	>99% effective	Extend treatment time; check that bleach active ingredient is at least 40 g/L
Hot water	Above 60 °C	1 minute	Ambiguous result	Extending treatment time could achieve 100% effectiveness, but needs to be tested
Hot water	Above 45 °C	20 minutes	Ambiguous result	
Freezing	-20°C	Until solid	100% effective	Recommended
Drying	Room temp.	Dry to touch	>99% effective	Extend drying time (see below)
Drying	Room temp.	Dry to touch, then leave 48 h	Ambiguous result	Complete drying needs to be assured, in warm temperatures.
Salt	4% w/v	10 minutes	<90% effective	Not recommended without further testing
Salt	10% w/v	1 minutes	<80% effective	

1 Introduction

The “Check, Clean, Dry” message was first introduced by Biosecurity New Zealand (now MPI) in 2005, following the discovery of the bloom-forming, non-indigenous diatom *Didymosphenia geminata* (didymo, also known as rock snot) in a Southland river in 2004. At that stage, the methods underpinning the message were designed and tested specifically to help prevent the spread of didymo. The methods comprised simple procedures for decontaminating clothing and equipment that was likely to harbour live didymo cells and potentially spread them to other rivers. Development of the methods was described by Kilroy et al. (2007).

More recently MPI has expanded the scope of Check, Clean, Dry to include all aquatic nuisance organisms, not just didymo. MPI has therefore asked NIWA to test the effectiveness of the original decontamination procedures on other organisms (e.g., aquatic plants, introduced invertebrates). The focus of this study was the freshwater diatom *Lindavia intermedia* (hereafter *Lindavia*) also known as lake snow or lake snot.

Lake snow was first recognised as a problem in New Zealand in about 2004, after unusual algal slime began blocking intakes and filters in the water supply to Wanaka township, which is sourced from Lake Wanaka. The slime also adversely affects angling and other recreational activities. The dominant alga in the slime was the centric diatom *L. intermedia* (Novis et al. submitted). *Lindavia* is suspected to have been introduced to New Zealand around the same time as the arrival of didymo, but this is uncertain. Recent surveys have confirmed its presence in several other South Island lakes, and nuisance slime reported from Lake Waikaremoana in 2008 was recently confirmed as *Lindavia*. Nevertheless, *Lindavia* has not been detected in other recently surveyed lakes. Therefore inclusion of *Lindavia* as a target organism for Check, Clean, Dry still appears to be warranted.

In this report we describe tests on *Lindavia* using the four Check, Clean, Dry methods currently recommended on the MPI website (http://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/). We also trialled two additional procedures that scored highly in the original didymo-based decontamination methodology (Table 4 in Kilroy et al. 2007). Note that the scope of this project was to test the currently recommended methods. The scope did not include identifying effective exposure times and/or concentrations if the current methods were not effective.

2 Methods

2.1 Decontamination methods

The six methods tested in this study are listed Table 2-1.

Table 2-1: Decontamination methods tested on *Lindavia intermedia*. The first four methods are those recommended by MPI on the current Check, Clean, Dry page of the MPI website. Salt solution and drying were highly ranked methods in Kilroy et al. (2007). Treatment times are the minimum recommended time; MPI suggests allowing longer times for absorbent items.

Option	Amount	Treatment time
Methods recommended by MPI		
Dishwashing detergent or nappy cleaner	5% solution (500 mL diluted to 10 litres in water)	Soak or spray all surfaces for at least 1 minute
Bleach	2% solution (200 mL diluted to 10 litres in water)	Soak or spray all surfaces for at least 1 minute
Hot water[1]	Above 60°C Above 45°C	Soak for at least 1 minute Soak for at least 20 minutes
Freezing		Until solid
Additional tests		
Drying[2]	[at room temperature]	Dry until dry to touch, then leave for at least 48 h before using in another waterway
Salt[3]	4% w/v solution	Soak for at least 10 minutes
Salt	10% w/v solution	Soak for at least 1 minutes

[1] 60°C – hotter than most tap water; 45°C – uncomfortable to touch.
[2] As recommended in MPI's Check, Clean, Dry pocket guide.
[3] See Table 4 in Kilroy et al. (2007)

2.2 Determining cell viability

Cell viability after each treatment assay was determined using the Neutral Red (NR) staining technique developed for determining the viability of didymo cells. Both organisms are diatoms, and we have already observed that NR staining is an effective technique for examining the viability of diatom taxa other than didymo. Details about NR staining are provided in Appendix 1 of Kilroy et al. (2007). Briefly, NR stain is taken up by cells when they are alive, but not when they are dead. Live cells take up the stain in vacuoles in the cell. The acidic contents of the vacuoles in healthy cells cause NR to retain its red colour and also prevent the stain from leaching back into the cell. The vacuoles appear as deep crimson-purple spots or granules scattered throughout the cell. In more neutral conditions (such as when internal membranes are damaged), NR turns straw-coloured. Uptake of NR by cells does not always produce clear results because the cells respond differently when they are compromised in some way. Therefore, in some cases, some interpretation is required when assessing the effectiveness of methods to kill cells. Observations on the shape, colour and arrangement of the chloroplasts also contribute to the assessments.

We conducted preliminary trials on *Lindavia* sourced from Lakes Hawea, Wanaka and Wakatipu in March and April 2017 to (A) confirm that the NR staining of *Lindavia* would be clear enough to distinguish viable from non-viable cells, and (B) determine a standard staining time for NR.

In relation to (A), we found that live *Lindavia* cells took up the stain in the same way as didymo with dark-red stained spheres of varying sizes scattered throughout the cell (Figure 2-1a,b). Cells that had been heat killed (exposure to 100 °C water) did not take up the stain (Figure 2-1c). The only difference in the procedure from that used for didymo was that microscope examination at 1000 x was necessary, rather than 400 x. *Lindavia* cells are typically about 25 µm in diameter (range 15–40 µm). In contrast, didymo cells are about 100 µm long and 40 µm wide, on average.

In relation to (B), determining a standard staining time, we found that exposure to NR for at least three minutes was enough time for *Lindavia* cells to take up the stain. We used 10-15 minutes staining time for didymo, but noted that didymo cells exposed to NR for longer than 15 min could start to die as a result of the stain. Subsequent research on didymo showed that the cells took up NR almost immediately after immersion in the stain solution.

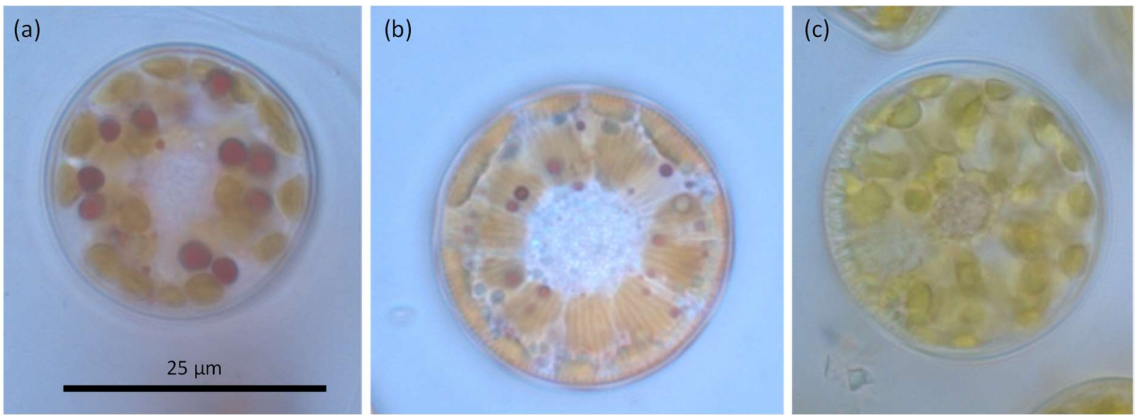

Figure 2-1: ***Lindavia intermedia*** **cells after staining with Neutral Red .** Live, healthy cells took on a deep red colour in granules or vacuoles throughout the cell, but not in the centre (a, b). Heat-killed cells (100 °C) did not take up the stain except for a slight pink tinge in the central area (c).

2.3 Sampling of *Lindavia*

For the main tests, we used samples of *Lindavia* collected by Otago Regional Council on 23 – 24 May 2017, from Lakes Wanaka and Wakatipu. A further sample collected from Lake Wakatipu on 1 June was used for the tests on salt solutions. The samples were collected by towing 30 m of weighted line for 1000 m at 5 knots/h, and then scraping off the algae caught on the line into a one litre container and topping up to 90% full with lake water. The samples each comprised 40–50 mL wet material (estimated) and this was sufficient for multiple tests. Samples were couriered to NIWA, on ice, on 24 May and 1 June 2017.

2.4 Experimental procedure

The main set of tests was carried out on 25 May and the salt tests on 2 June 2017. All tests were conducted at room temperature with the sample material and treatment solutions held at about 5 – 8 °C. All tests were carried out on a minimum of three subsamples of material from each of the two lakes. Control samples were tested at the start of the trials and after every 6-8 tests. The methods were tested in random order so that there could be no bias in the results caused by laboratory conditions.

A stock solution of NR was made up by dissolving 200 mg of the dye powder in 200 mL of distilled water (0.1% w/v solution). This was further diluted to 5-7% v/v for use with *Lindavia.*

2.4.1 Chemical treatments

Stock solutions were made of the three aqueous treatment methods (dishwashing liquid, bleach,[1] and salt). For the treatments, small samples of *Lindavia* (about the size of a mustard seed) were separated from the massed sample and transferred to about 10 mL of the test solution in a 35 mL vial. During the treatment, the vial was shaken gently to simulate expected movement through scrubbing or brushing. At the end of the designated exposure time (see Table 2-1), the samples were removed from the treatment using fine forceps and immediately rinsed in three successive rinses of distilled-grade water. The rinsed samples were then transferred to 5-10 mL of NR in a 35 mL vial, for staining. During staining the vial was shaken to aid penetration of the stain to all parts of the sample. The whole sample was then transferred to a glass slide, and covered with a coverslip, for counting. Control samples were subjected to the same procedure except that the treatment step was omitted.

2.4.2 Heat treatments

Glass petri dishes of lake water were heated to 45 and 60 °C in temperature-controlled ovens. Once up to heat, samples were transferred to the dishes, as for the chemical treatments, and left at that temperature for the designated treatment time. Staining and slide preparation were carried out as for the chemical treatments.

2.4.3 Drying and freezing

For the drying treatment, drops of lake water containing *Lindavia* were transferred to Petri dishes and left exposed to air overnight, until they were just dry. Samples were stained and counted at that stage, and again 48 h later. Temperature was logged during the trials on 25 May and ranged between 15.8 and 20.5 °C for the first 24 h and between 11.5 and 18.4 °C for the next 48 h.

For the freezing treatment, samples in about 10 mL of water were placed in a -20 °C freezer and left overnight. The samples were thawed at room temperature. Staining and slide preparation were carried out as for the chemical treatments.

2.5 Cell counts

All counts were performed within 15 minutes of preparation of the slide. Prepared slides were first scanned at low power (200 x) under a Leica DMLB microscope to confirm that the NR stain had reached all of the sample. Slides were then scanned at a magnification of 1000 x (oil immersion).

If stained cells were observed, counts were made of stained and unstained cells up to a total of at least 100 cells and used to calculate the percentage of stained (live) cells in the subsample. Counts were made on transects covering different parts of the subsample, to ensure that the cell count was representative of the entire slide. Previous trials had shown that, using this method, a count of 100 cells was sufficient to obtain a consistent estimate of the percentage of stained cells (see Appendix 2 in Kilroy et al. 2007).

Microphotographs were taken during the counts to record of the appearance of *Lindavia* cells following the different treatments.

[1] We used Sunlight™ dishwashing liquid and Janola extra™ bleach.

3 Results and discussion

Control samples of the material collected on 23–24 April comprised on average 77% live (i.e., stained) cells. There was no difference between the two lakes. All raw cell counts are presented in Appendix A and summary results are in Table 3-1.

Table 3-1: Summary results of the viability tests for each of the treatments. For all tests, *n* = 5 or more.

Treatment	Amount / level	Treatment time	Result (% viable, ± std. dev.)
Controls	Room temp.	n/a	77 ± 8.9
Dishwashing detergent	5% solution	1 minute	6.3 ± 9.9
Bleach	2% solution	1 minute	0.4 ± 1.1
Hot water	Above 60 °C	1 minute	14.5 ± 16.7
Hot water	Above 45 °C	20 minutes	11.0 ± 8.8
Freezing	-20°C	Until solid	0.0 ± 0.0
Drying	Room temp.	Dry to touch	0.4 ± 0.5
Drying	Room temp.	48 h later	2.6 ± 2.1[1]
Controls (for salt tests only)	Room temp.	n/a	56.5 ± 7.4
Salt	4% w/v	10 minutes	10.1 ± 5.1
Salt	10% w/v	1 minute	22.8 ± 21.2[1]

[1] Note that these percentages included cells that stained abnormally (around the perimeters only) and the chloroplasts also appeared degraded. We have taken a conservative approach and assumed that these cells are potentially viable.

3.1 Dishwashing detergent

The first sample tested returned 26% live (stained) cells. All subsequent samples returned 6% stained cells or less, and in two samples we detected no stained cells. The stained cells tended to occur in clumps and were lightly stained (just a few granules) (Figure 3-1).

The chloroplasts of *Lindavia* have been described as: "… brown, arranged around the outside of the cell in healthy material, numerous, plate-like and evenly spaced, distributed against the valve peripheral zone … but usually not the central area" (Novis et al., submitted). Following treatment with dishwashing detergent, the chloroplasts often appeared to be reduced in number, or dense and misshapen (Figure 3-1). The stained samples were deemed to be viable, chloroplasts' appearance suggested that with longer exposure to the detergent, no cells would remain viable.

Figure 3-1: Examples of the few lightly stained cells observed in samples treated with dishwashing detergent. Cells such as these were classed as viable. For scale, see Figure 2-1.

3.2 Bleach

Stained cells were observed in one of the six samples tested, in very low numbers. All of the staining was pale pink rather than dark red, suggesting that some change was underway. The samples changed colour from brown to green when added to the bleach treatment. The chloroplasts in many cells became paler and scattered throughout the cell (Figure 3-2). We determined that the bleach treatment was an effective method of killing all cells under the conditions of the trial. However, we used a bleach with a relatively high content of the active ingredient (Janola extra™, 42 g/L sodium hypochlorite). In an earlier preliminary trial using a 2% solution of a product with 21 g/L active ingredient, 26% stained cells were observed (compared to 66% in the control sample in that test). Therefore the recommended 2% concentration may not be strong enough for some products (e.g., Budget™ brand).

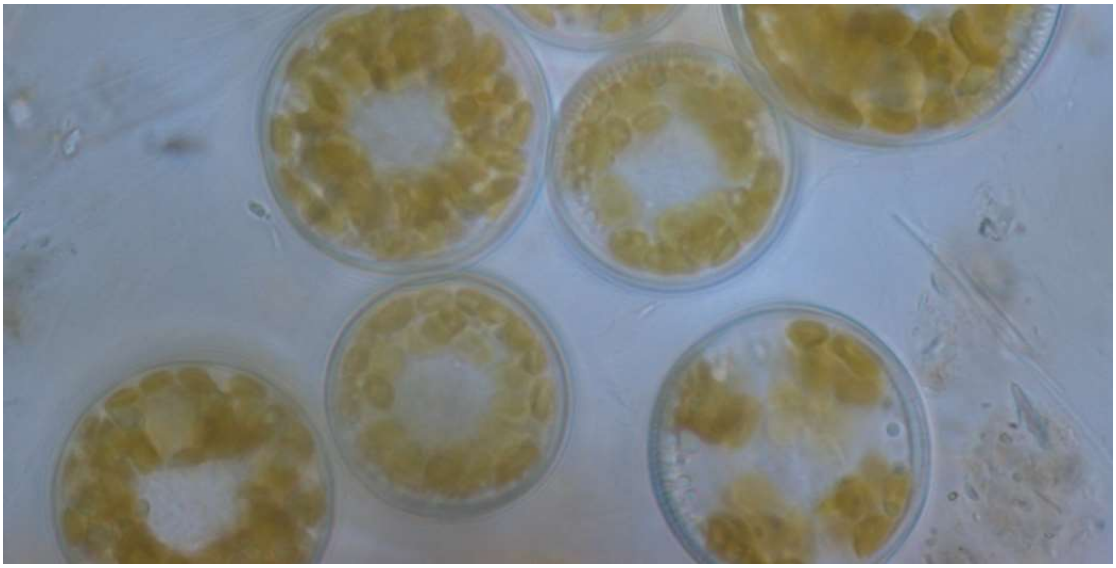

Figure 3-2: *Lindavia* cells after treatment with 2% bleach followed by staining with Neutral Red. These cells failed to stain and were classed as non-viable. The chloroplasts were also misshapen in many cases.

3.3 Hot water

Exposure to 45 °C water for 20 minutes was slightly more effective than exposure to 60 °C water for 1 minute (Table 3-1). In both cases, many of the stained and unstained cells also had pink staining in the central area of the cell (Figure 3-3). Pink stained cells with dark granules were classed as stained (i.e., potentially viable) and pink-stained cells with no or only very faint granules were classed as partially stained (i.e., probably not viable). If the central pink stain (which was also observed after freezing and salt treatments) represents some disturbance to the cell nucleus then it may also be lethal. However, this is uncertain.

We noted that many unstained cells in these treatments were a greenish colour, with misshapen chloroplasts. As with the bleach treatment, this suggested that longer exposure to heat might have produced a more definitive result.

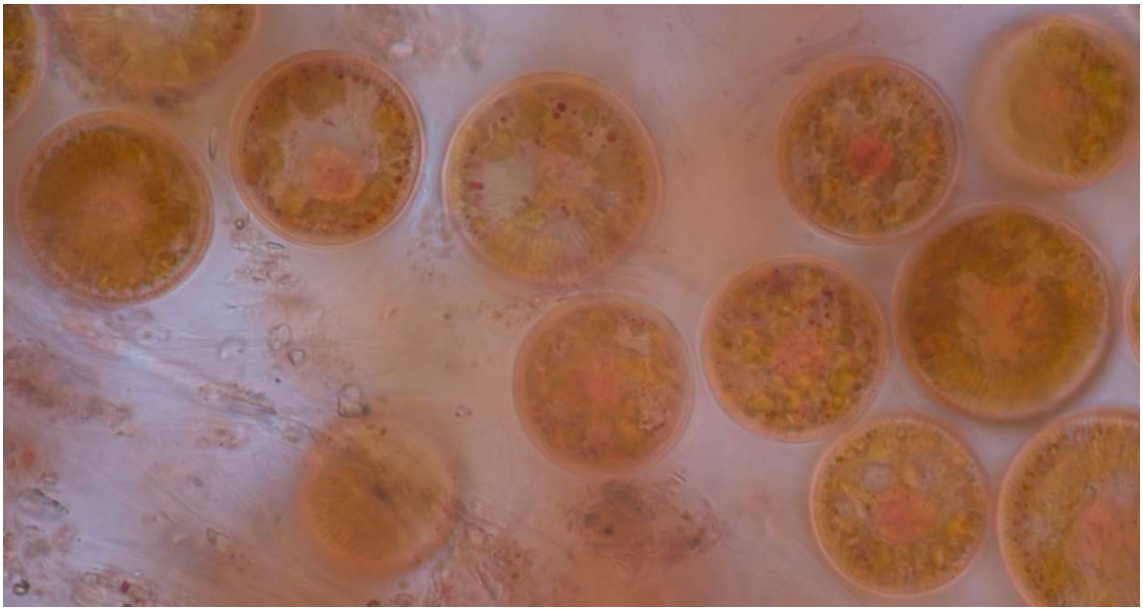

Figure 3-3: Pink centres and greenish chloroplasts in *Lindavia* cells following hot water treatment and Neutral Red staining. The reason for the central pink stain is unknown, but the cells stained more or less normally in the rest of the cell and were therefore classed as viable.

3.4 Freezing

No stained cells were observed in any of the six samples that were frozen overnight. A variable proportion of cells had pink-stained central areas, but none of these also had the dark granules typical of (assumed) viable cells (Figure 3-4).

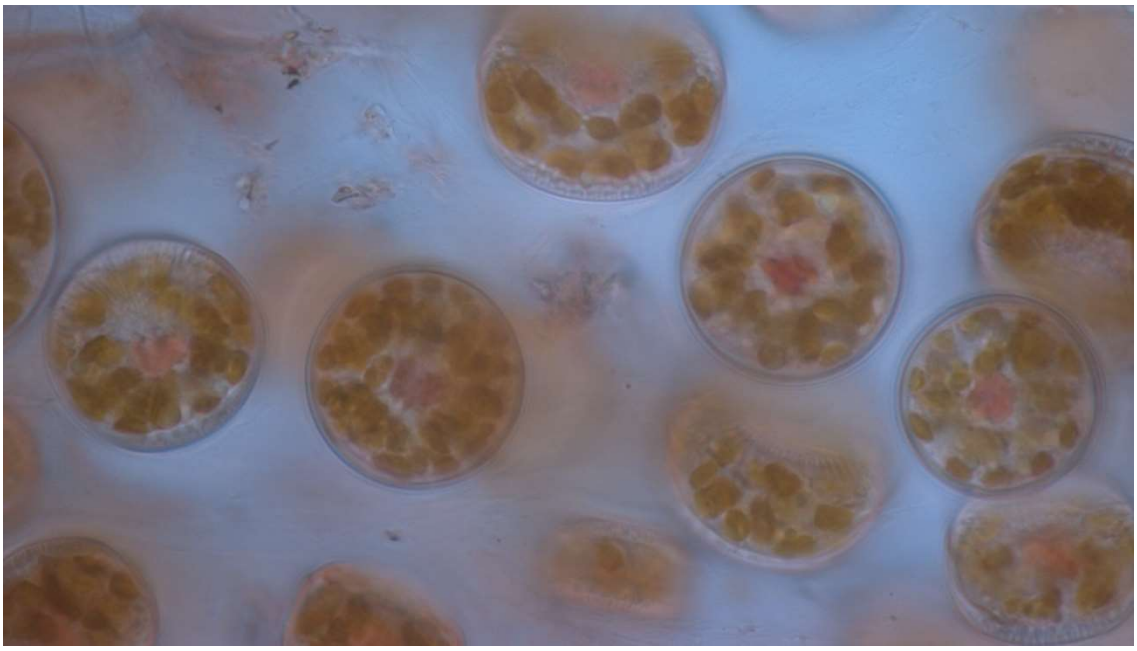

Figure 3-4: *Lindavia* cells following overnight freezing, thawing and Neutral Red staining. Note the stained central area in these cells. The reason for the staining is unknown, but the cells were classed as non-viable because no "normal" staining was apparent in the rest of the cell.

3.5 Drying

A small number of stained cells were observed in the samples allowed to dry until they were no longer obviously wet (24 h), but most did not take up the stain (Figure 3-5). A small proportion of cells in samples left exposed to air at room temperature for a further 48 h (hereafter the 72 h treatment) also stained. In this case the staining pattern was always atypical, and the chloroplasts appeared degraded (Figure 3-6). We consider that these cells were very likely non-viable, but cannot be certain.

For the first 24 h of drying, mean room temperature was 17.2 °C, and for the next 48 h mean temperature was 15.3 °C. Thus the overall temperature for the whole 72 h period was relatively low and may have led to incomplete drying. The higher percentage of stained cells in the 72 h treatment, than in the first 24 h, may have arisen because the first samples were taken from the outer (thinner) edge of the dried material, which dried thoroughly in the first 24 h. Subsequent samples from the thicker part of the dried mass could have stayed slightly damp in the cooler temperatures.

Figure 3-5: *Lindavia* cells following exposure to air until no longer wet, and Neutral Red staining. All these cells were classed as non-viable, as they failed to stain. The chloroplasts were also misshapen in many specimens.

Figure 3-6: *Lindavia* cells following exposure to air until no longer wet, then further exposure for 48 h, and Neutral Red staining. A small proportion of cells (shown here) took up stain, but in an atypical pattern. The appearance of the chloroplasts suggests that the cells are likely to be non-viable, but we took a conservative approach and classed the stained cells as viable.

3.6 Salt

The tests on salt were carried out on material from a single sample collected from Lake Wakatipu on 1 June 2017. Five controls from this sample returned lower percentages of stained cells than in both of the samples collected earlier (Table 3-1).

Counting all stained cells as viable returned higher viability (23%) following the 1 minute treatment with 10% salt solution, than the 10 minute treatment with a 4% solution (11%) (Table 3-1, and see Appendix A). Some of the cells stained normally (i.e., similar to that in the controls), while some stained lightly (see top panel in Figure 3-7). In a proportion of cells in some samples (especially after the 10% treatment) stained granules were sometimes concentrated around the cell perimeter (similar to that seen following drying, see Figure 3-6). These cells were classed as partly stained (Appendix A) to distinguish them from cells that stained normally. They generally had degraded chloroplasts, but we took a conservative approach (as for the drying treatment) and counted them as viable.

Many of the stained and unstained cells in both treatments contained large vacuoles not apparent in normal cells (compare cells in Figure 2-1 with those in Figure 3-7).

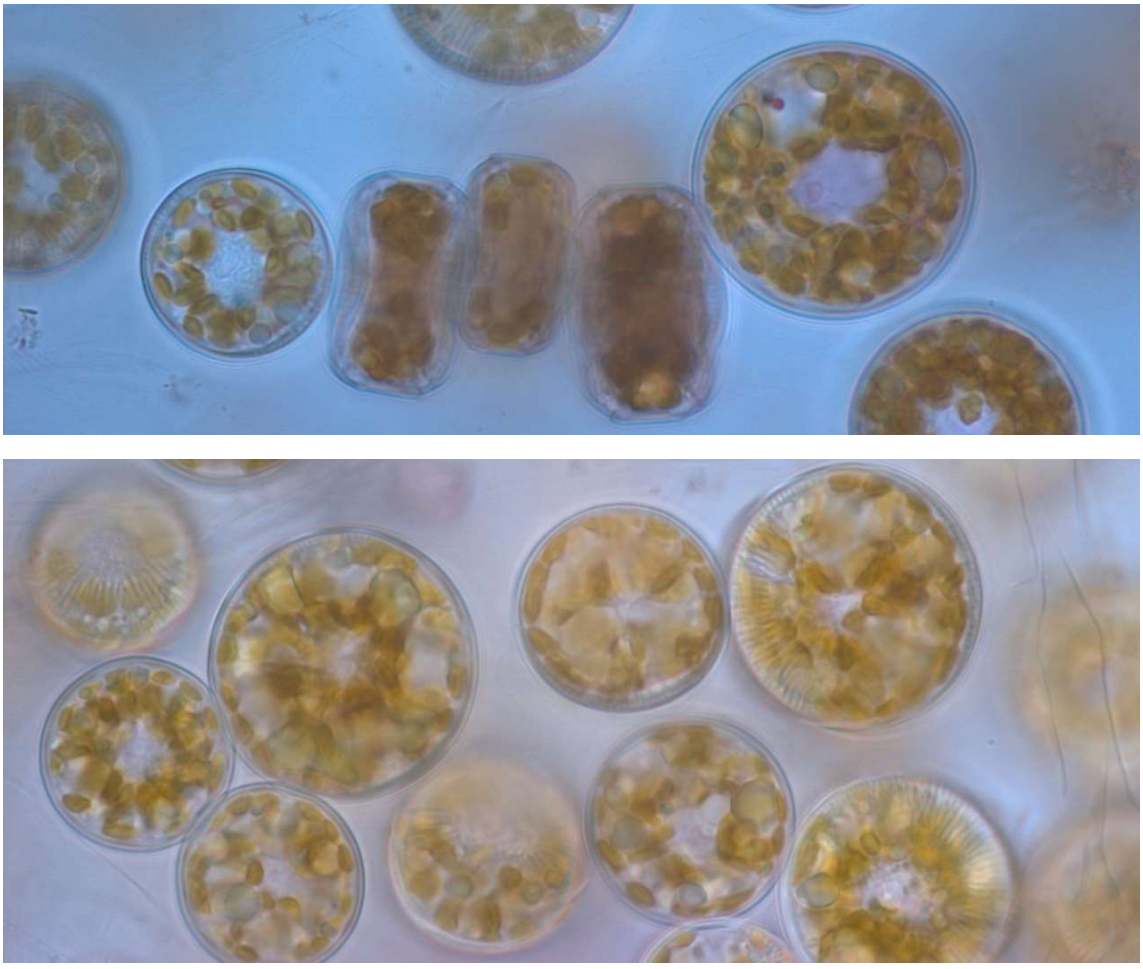

Figure 3-7: *Lindavia* cells following immersion in salt solution followed by NR staining. The top panel is an example of cells following 10 min immersion a 4% w/v solution. Note light staining in some cells. The bottom panel shows cells after a 1 min treatment in 10% w/v salt solution. Note large vacuoles in some cells.

3.7 Commentary on results

Check, Clean, Dry methods need to be effective over a short period, reflecting the time that river and lake users may be prepared to spend on cleaning clothing and equipment before moving to another waterway. This particularly applies to the chemical treatments, which may damage clothing and equipment after longer exposure times.

The overall outcome of the trials was that the currently recommended rapid Check, Clean, Dry methods were not quite as effective on *Lindavia* as they were on didymo at the time the methods were developed. Only freezing was 100% effective, based on the NR test. The results of the dishwashing liquid and bleach tests indicated that longer exposure (e.g., at least 2 minutes) would likely be completely effective. This remains to be tested.

Hot water treatment is a relatively benign decontamination method in terms of damage to other materials. However the NR tests yielded ambiguous results in that the significance to viability of a pink-stained central area (not seen in control treatments) was not certain. It is likely that longer treatment at both temperatures would have produced a more definitive result.

Drying was also largely effective, though some cells continued to stain even after a further 48 h exposure following a dry appearance. The degraded appearance of chloroplasts and atypical staining pattern suggested that the cells were unlikely to be viable. Drying at a higher room temperature (than 15.3 °C) may have produced a clearer result.

Neither salt solution was completely effective at the recommended exposure times.

The difference in effectiveness of chemical treatments between didymo and *Lindavia* may relate to their different life forms. Both species produce large amounts of mucilage in suitable conditions. In didymo cells mucilage is concentrated in the stalks, and the cells are directly exposed to the water column[2]. In contrast, *Lindavia* cells are distributed within their mucilage strands, and may be more resistant to decontaminants simply because it takes longer for the chemical to penetrate the mucilage and reach the cells.

We recognised that mucilage might also have prevented NR from penetrating to all cells. This may partly explain the relatively low percentages of stained cells in some of the control samples. In treated samples, the issue of poor NR penetration was not considered to be a major problem, because the stain clearly affected large parts of all samples (as seen by either stained or partly stained cells, or signs of red-staining in other material in the sample). The extent of stain penetration could be seen in a low-power scan of the sample, but it is possible that some pockets of sample escaped staining.

[2] Didymo cells, like all diatoms, are enclosed by a layer of mucilage, but this is likely to be relatively thin.

4 Summary and recommendations

One of the four recommended Check, Clean, Dry treatments recommended by MPI (http://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/) – **freezing** overnight – was unambiguously effective in killing 100% of *Lindavia* cells.

We determined that treatment with **2% bleach** was highly likely to be 100% effective, but suggest that a slightly longer exposure time would assure effectiveness. Also, use of a bleach brand with at least 40 mg/L sodium hypochlorite is recommended.

Some stained (i.e., potentially viable) cells remained after treatment with **5% dishwashing detergent** for 1 minute. However, chloroplasts were visibly affected and again we suggest that a longer exposure time might complete the treatment.

The results after **hot water treatment** (both 60 °C for 1 min and 45 °C for 20 min) were ambiguous in that many stained cells remained after treatment, but in many cases the central area of the cell stained pink, and this was rarely observed in control treatments. The significance of this pink central stain is uncertain. The samples always included clumps of cells that were discoloured and dead; therefore we assume that the pink-stained cells were a stage before that, and longer exposure might be effective.

Of the two additional methods trialled, **drying** was largely effective, although some cells took up NR stain even after 72 h drying. The combination of atypical staining and degraded chloroplasts suggested that the cells were non-viable.

Finally, treatment with **4% w/v salt for 10 min** or **10% w/v salt for 1 min** could not be considered effective because normal stained cells remained in both cases. However exposure to salt clearly affected many cells. Salt treatment may be an effective decontamination method with longer treatment times, but this needs to be tested.

The overall results and recommendations are summarised in Table 4-1.

Table 4-1: Summary of outcomes of tests, and recommendations for Check, Clean, Dry procedures for prevention of the spread of *Lindavia intermedia*.

Treatment	Amount / level	Treatment time	Initial assessment	Recommendation
Dishwashing detergent	5% solution	1 minute	>90% effective	Extend treatment time
Bleach	2% solution	1 minute	>99% effective	Extend treatment time; check that bleach active ingredient is at least 40 g/L
Hot water	Above 60 °C	1 minute	Ambiguous result	Extending treatment time could achieve 100% effectiveness, but needs to be tested
Hot water	Above 45 °C	20 minutes	Ambiguous result	
Freezing	-20 °C	Until solid	100% effective	Recommended
Drying	Room temp.	Dry to touch	>99% effective	Extend drying time (see below)
Drying	Room temp.	Dry to touch, then leave 48 h	Ambiguous result	Complete drying needs to be assured, in warm temperatures.
Salt	4% w/v	10 minutes	Not clearly effective	Not recommended without further testing
Salt	10% w/v	1 minutes	<80% effective	

5 Acknowledgements

This study was funded by the Ministry for Primary Industries. We thank Jeff Donaldson (MPI) for coordinating the collection of samples for testing and for providing samples for preliminary trials. Thanks also to Nathan Manning (Otago Regional Council) for collecting the samples used for the bulk of the testing. We thank Tracey Burton and Phil Jellyman for their helpful reviews of the report.

6 References

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Novis, P., Schallenberg, M., Saulnier-Talbot, E., Kilroy, C., Reid, M. (submitted). "Lake snow", the result of large scale mucilage production by microalgae in clean New Zealand lakes, is caused by the centric diatom Lindavia intermedia. *New Zealand Journal of Botany*.

Appendix A Complete results of all trials

Results of cell counts of all replicates of *Lindavia* following exposure to the Check, Clean, Dry treatments, and staining with the vital stain, Neutral Red (NR).

The first treatment in the table was a heat control on two samples, to confirm that cells known to be non-viable did not take up NR.

Under 'Cell counts', stained cells took up NR as dark crimson granules or spheres throughout the cell, and were assumed to be viable. Partially stained cells included cells in which the stain was apparent only at the centre of the cell as a pink colour, or (rarely) as pale-pink-stained granules or spheres. These cells were deemed to be non-viable, as were cells that did not take up any NR. In the salt treatments, cells classed as partly stained had small granules around the edges; these were distinguished from normally staining cells but all were classed as potentially viable.

				Cell counts				
Treatment	**Conc. or level**	**Exposure time**	**Sample origin**	**Stained**	**Partly stained**	**Un-stained**	**% Stained (i.e. viable)**	**Notes**
Heat control	100 °C	50 min	Wakatipu	0	0	100	0	Test to confirm that non-viable cells do not take up NR
			Wanaka	0	0	100	0	
Control	NA	NA	Wakatipu	105	0	9	92	At start and finish of trials and after every 6–8 individual tests on different treatments.
				101	1	25	80	
				101	5	28	75	
				87	10	24	72	
				93	2	34	72	
			Wanaka	101	0	16	86	
				102	1	23	81	
				96	0	64	60	
				104	4	18	83	
				97	2	22	80	
				109	2	48	69	
Dishwashing detergent	5%	1 min	Wakatipu	34	2	96	26	
				1	0	103	1	
				5	0	101	5	
			Wanaka	0	0	109	0	
				0	0	107	0	
				7	0	103	6	
Bleach	2%	1 min	Wakatipu	0	0	127	0	
				0	0	102	0	
				0	0	116	0	
			Wanaka	3	3	109	3	

				0	0	104	0	
				0	0	108	0	
Heat	45 °C	20 min	Wakatipu	33	62	29	27	
(moderate)				0	60	43	0	Cells classed as partly stained had a pink-stained central area (see text) (non-viable). Stained cells often also had pink-stained centres (viable).
				11	56	54	9	
			Wanaka	15	31	71	13	
				10	57	56	8	
				11	32	76	9	
Heat	60 °C	1 min	Wakatipu	54	7	57	46	
(hot)				26	36	76	19	Cells classed as partly stained had a pink-stained central area (see text) (non-viable). Stained cells often also had pink-stained centres (viable).
				14	18	98	11	
			Wanaka	0	16	98	0	
				11	9	100	9	
				3	11	106	3	
Freezing	-20 °C	24 h	Wakatipu	0	28	95	0	
				0	28	94	0	Cells classed as partly stained had a pink-stained central area, and these were counted as non-viable (see text)
				0	66	54	0	
			Wanaka	0	10	104	0	
				0	13	110	0	
				0	31	2	0	
Drying	Room	24 h	Wakatipu	0	0	118	0	
	Temp.			0	0	123	0	
				0	0	145	0	
	17.2 °C							
			Wanaka	1	0	104	1	
				1	0	110	1	
				0	1	125	0	
	15.3 °C	+ 48 h	Wakatipu	8	0	160	5	
				0	0	200	0	
				8	0	188	4	Stained cells had dark red granules, but staining pattern was atypical (very fine granules around the edges)
				9	0	239	4	
			Wanaka	6	0	194	3	
				0	0	100	0	
				0	0	100	0	
Salt	4%	10 min	Wakatipu	10	0	119	8	Cells classed as partly stained had stain concentrated around edges, and degraded
	(w/v)			17	0	117	13	
				7	0	104	6	
				8	6	118	11	

				22	5	96	22	chloroplasts. Partly stained cells were counted as viable.
	10%	1 min	Wakatipu	15	18	104	24	
	(w/v)			6	4	118	8	
				2	1	136	2	
				33	45	60	57	
				8	25	108	23	
Control				98	0	37	73	
(salt tests)				117	0	62	65	
				80	0	66	55	
				92	3	53	62	
				73	0	51	59	