Testing "Check, Clean, Dry" decontamination procedures

New product tests on didymo and Lindavia

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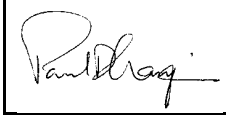	Approved for release by:	Paul Champion

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Check, Clean, Dry procedures currently recommended by the Ministry for Primary Industries (MPI) to prevent or reduce the spread of nuisance aquatic organisms include treatment with dishwashing detergent (1 minute contact to a 5% solution). The recommendation was based on cell viability tests on the nuisance diatom *Didymosphenia geminata* (didymo) using common brands of standard dishwashing detergent (Palmolive™, Sunlight™, Down-to-Earth™) carried out in 2006-07.

The Check, Clean, Dry message now includes all aquatic nuisance organisms, not just didymo. In 2017, to support the broader message, MPI commissioned further tests of the methods on other freshwater organisms including the nuisance freshwater diatom *Lindavia intermedia* (hereafter *Lindavia*). *Lindavia* forms slime accumulations in lakes, which block water intakes and interfere with recreation. The 2017 tests showed that *Lindavia* was slightly more resistant than didymo to decontamination using standard dishwashing liquid and it was recommended that an extended treatment time would likely be fully effective.

MPI now wish to provide further guidance to the public on which detergent products are effective because product selection may be based on their "environmentally friendly" claims. Initial tests on didymo in 2006-07 using selected environmentally friendly products indicated that these detergents were not as effective in killing didymo cells as standard detergents that make no environmental claims. MPI therefore requested further tests on both didymo and *Lindavia* using four detergent products currently in use:

- Eco-store detergent (sachets provided by MPI as part of Check, Clean, Dry campaign).
- Earthwise dishwashing liquid (Lemon and Aloe Vera).
- Jasco Citriclean (used by the Department of Conservation).
- Arnold lemon detergent dishwash liquid (used by some Regional Councils).

The Jasco and Arnold products did not claim specifically to be environmentally friendly, while the Eco-store and Earthwise products do make such claims.

All tests were carried out at room temperature using fresh didymo and *Lindavia* (within 48 hours of collection), maintained in the laboratory at 5 °C. An established 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. The four products were tested using the MPI recommended concentration (5%) and contact time (1 minute), except where we made up the Eco-store treatment solution following instructions on the sachets provided by MPI, which resulted in an 8% solution.

For didymo, the four products in order of effectiveness (mean percentage stained cells) were: Arnold Lemon detergent, Jasco Citriclean, Eco-store (MPI sachets) and Earthwise Lemon and Aloe Vera. The first three were largely effective. The Earthwise product was significantly less effective.

All four products were less effective on *Lindavia* than they were on didymo. The reason for lower effectiveness was likely that small *Lindavia* cells are partially protected by the mucilage (slime) around the cells. The four products in order of effectiveness were: Jasco Citriclean, Eco-store (MPI sachets) and Arnold Lemon detergent (moderately effective, with little difference between them), then Earthwise Lemon and Aloe Vera, which was generally ineffective.

An overall conclusion from the trials was that, for products that clearly make environmental claims, an 8% solution (as recommended by MPI on sachets of Eco-store detergent) is a more appropriate concentration for Check Clean Dry applications than the standard recommendation of 5%.

Summary results and recommendations are provided in the table below:

Treatment	Concen-tration	Contact time	Initial assessment (mean effectiveness)	Recommendation
Didymo				
Eco-store (MPI sachet)	8%	1 min	95% effective	Extend contact time
Earthwise Lemon and Aloe Vera	5%	1 min	<75% effective	Increase concentration and extend contact time
Jasco Citriclean	5%	1 min	99% effective	Extend contact time
Arnold Lemon detergent	5%	1 min	100% effective	Recommended
Lindavia				
Eco-store (MPI sachet)	8%	1 min	>80% effective	Extend contact time, but ideally re-test
Earthwise Lemon and Aloe Vera	5%	1 min	Not effective	Increase concentration and extend contact time, but ideally re-test
Jasco Citriclean	5%	1 min	>80% effective	Extend contact time, but ideally re-test
Arnold Lemon detergent	5%	1 min	>80% effective	Extend contact time, but ideally re-test

1 Introduction

The "Check, Clean, Dry" message was introduced by Biosecurity New Zealand (now part of the Ministry for Primary Industries, MPI) in 2005, following the discovery of the bloom-forming, non-indigenous diatom *Didymosphenia geminata* (didymo) in a Southland river in 2004. The methods underpinning the message were designed and tested specifically to help prevent the spread of didymo. The methods included the use of readily available household cleaning products (e.g., bleach, dishwashing detergent) for decontaminating clothing and equipment that was likely to harbour live didymo cells and potentially spread them to other rivers (Kilroy et al. 2007).

MPI has now expanded the scope of Check, Clean, Dry to include all aquatic nuisance organisms, not just didymo. To support the broader scope of the message, in 2017, MPI commissioned NIWA to test the effectiveness of the original decontamination procedures on other freshwater organisms. These included tests on aquatic plants and introduced invertebrates (Burton 2017), and on the freshwater diatom *Lindavia intermedia* (hereafter *Lindavia*) (Kilroy and Robinson 2017).

Lindavia, also known as lake snow or lake snot, was first recognised as a problem in about 2004, when algal slime began to block water intakes and filters in Lake Wanaka. The diatom species causing the slime was not confirmed until 2017, along with mounting evidence that *Lindavia* is a recent introduction to New Zealand (Novis et al. 2017, Kilroy et al. 2018).

Tests on the effectiveness of currently recommended Check, Clean, Dry methods (see http://www.mpi.govt.nz/travel-and-recreation/outdoor-activities/check-clean-dry/) concluded that *Lindavia* was slightly more resistant than didymo to decontamination with 2% bleach for 1 min, 5% detergent (dishwashing detergent) for 1 min, and hot water (60 °C for 1 min and 45 °C for 20 min). However, all methods were moderately effective and extending the contact time was suggested as a way to improve effectiveness. It was suggested that resistance to decontamination was caused by slime around the *Lindavia* cells, forming a protective layer. On the other hand, overnight freezing (-20 °C) was an effective method.

Use of dishwashing detergents for carrying out Check Clean Dry procedures has proved popular because these products are inexpensive, readily available, easy to use in the field, and, unlike bleach for example, gentle on other materials and safe to handle. Previous tests on didymo using dishwashing detergent used the widely available products Palmolive™, Sunlight™ and Down-to-Earth™ (Kilroy et al. 2007), and tests on *Lindavia* used Sunlight™ (Kilroy and Robinson 2017). These brands make no environmental claims.

With wide uptake of the Check, Clean, Dry message, MPI wish to provide further guidance to the public on which detergent products are effective. Environmental concerns have encouraged stakeholders to select products based on their "environmentally friendly" claims. However, initial tests on didymo in 2006-07 using selected environmentally friendly products indicated that these detergents were not as effective in killing didymo cells as detergents that make no environmental claims (Kilroy et al. 2007). MPI therefore requested further tests on both didymo and *Lindavia* using four additional detergent products that are currently in use.

In this report we describe tests carried out on didymo and *Lindavia* using the four selected products. At MPI's request, we followed currently recommended Check, Clean, Dry methods (i.e., exposure to a 5% v/v solution of the product for 1 minute). The scope of this project did not include identifying effective exposure times and/or concentrations if the current methods were not effective.

2 Methods

2.1 Products tested

MPI requested tests on the following four brands of dishwashing detergent. Abbreviations used in the text and tables are shown in square brackets.

- Eco-store brand detergent (provided by MPI as part of Check, Clean, Dry campaign; sachets were provided by MPI for these tests) [Eco-store (MPI sachet), Eco-store].
- Earthwise dishwashing liquid (Lemon and Aloe Vera type; purchased from supermarket) [Earthwise Lemon and Aloe Vera, Earthwise].
- Jasco Citriclean (used by the Department of Conservation; a sample for testing was provided by Brenda Lawson of DoC) [Jasco citriclean, Jasco].
- 20 L Lemon detergent dishwash liquid from Arnold Products Limited (used by some Regional Councils; purchased from https://www.arnoldproducts.co.nz/product/20l-lemon-detergent-dishwash-liquid-4883.htmx). [Arnold lemon detergent, Arnold].

Refer to Appendix A for more information on each product, from the brands' websites. Note that the marketing information on Jasco Citriclean and Arnold lemon detergent does not appear to make any specific claims that these products are environmentally friendly.

Test solutions of Eco-store brand were prepared following the instructions on the sachets using distilled water, which produced an 8% solution (20 ml made up to 250 ml). We rinsed all product from the sachet into the solution as it was being mixed.

We prepared 5% solutions of Earthwise, Jasco and Arnold by making up 10 ml of the product to 200 ml using distilled water.

2.2 Laboratory facilities and permissions

The tests described below were carried out in the MPI-approved PC2 containment facility in the NIWA, Christchurch, laboratory (Facility #569). Didymo is classed as an "unwanted organism" and NIWA holds permission from MPI to carry out experiments using live didymo cells (dated 6 May 2010) in the PC2 laboratory, with microscope scans carried out in an adjacent PC1-rated equipment room, following specified protocols.

Lindavia is not classified as an unwanted organism, but the tests were carried out using the same protocols as used for didymo.

2.3 Sourcing and maintaining live material for testing

2.3.1 Didymo

Samples of didymo were collected at around 11 am on 20 January 2020 from the Opuha River, Canterbury. We collected whole rocks, each with several developing didymo colonies, and transported them direct to the PC2 laboratory.

Each rock was transferred to a large glass beaker and covered with river water. The beakers were placed on a shaker table in a controlled temperature cabinet at 5 °C and in a 12:12 light: dark cycle. Gentle shaking (100 rpm) ensured water movement around the didymo colonies, to simulate a river

environment. Sub-samples were tested for viability in the afternoon of 20 January. The main tests (including controls) were conducted on 21 January, and additional replicates (including controls) were tested on 22 January 2020.

2.3.2 *Lindavia*

A sample of *Lindavia* (as lake snow slime) was collected from Lake Wanaka during a routine monthly water quality monitoring run on 22 January by Otago Regional Council staff (contact: Nathan Manning, Environmental Officer, Otago Regional Council). The sample was collected by towing a line through *L. intermedia* "patches", scraping the snagged material (slime) into a 1 litre container and topping up with lake water. The sample was couriered on ice to NIWA in Christchurch and arrived at around 10 am on 23 January 2020.

The *Lindavia* sample was transferred to two glass beakers which were maintained under the same conditions as didymo, except that the shaker table was not required. Sub-samples were tested for viability within 2 h of the sample arriving. Tests on all four products, with controls, were carried out on the same day (23 January). Additional replicates (including controls) were tested on 24 January 2020.

2.4 General method for 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 and used successfully on *Lindavia* by Kilroy and Robinson (2017). Both didymo and *Lindavia* are diatoms, and we have already observed that NR staining is an effective technique for examining the viability of diatom taxa other than didymo.

Full details about NR staining are provided in Appendix 1 of Kilroy et al. (2007). In summary, 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. Viewed under a microscope at 200× or 400×, 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 cells respond in various ways 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. A treatment is judged to be 100% effective when *either* no stained cells are observed in complete microscope scans of at least three subsamples, *or* stained cells are seen, but the staining is abnormal or very faint and there are other signs of cell deterioration present, such as discoloration or shrinkage of chloroplasts.

For all tests we started with a stock solution of NR made up by dissolving 200 mg of the dye (a black metallic powder) in 200 mL of distilled-grade water (0.1% w/v solution). This was further diluted with filtered river or distilled-grade water to a working solution of about 4% v/v for use with didymo or *Lindavia* respectively.

All microscope scans were carried out using a Leica DMLB compound microscope.

2.5 Experimental procedure and cell counts

The tests were conducted at room temperature (18°C) with the sample material and treatment solutions held at about 5°C. All tests were carried out on a minimum of three subsamples of material. Control samples were tested at the start of the trials and after every 6-8 tests. The products were tested in a random order unknown to the microscope operator to avoid any involuntary bias in the counts.

2.5.1 Procedure for didymo

The tests consisted of the following steps:

1. A small piece of mat (approx. 5 mm x 5mm) was snipped from the top of a didymo colony. We selected pieces that appeared to be healthy and with plenty of cells (i.e., a pink to dark brown colour (not yellow or green), with individual cells just visible to the naked eye as dark specks). Each replicate was taken from a different colony or different rock.

2. At least three replicates were tested. If the first three results were variable, we tested two further replicates.

3. The test piece was dropped into about 20 ml of the product solution in a 35 ml vial for exactly 1 minute.

4. The test piece was removed using forceps and rinsed by swirling the sample in two successive vials of clean, filtered river water (double layer 40 µm filter, which should exclude all didymo cells).

5. Rinsed test pieces were briefly blotted dry and then transferred to a vial of about 20 ml NR working solution. We allowed a staining time of 4 minutes.[1]

6. Subsamples of stained test pieces were transferred immediately to a glass microscope slide, teased apart so that as many cells as possible were in contact with the glass, and topped with a cover slip.

7. Microscope counts were conducted immediately using a Leica DMLB microscope. Slides were first scanned at 200 x. If stained cells were observed, counts were made on random fields of view at 400 x, working systematically across and down the cover slip so that no areas were viewed more than once. At least 100 cells were counted on each slide, and assessed as either stained or not stained, following Appendix 2 in Kilroy et al. (2007). We did not include empty cells or broken cells in the counts.

8. Control pieces were treated in the same way as treatment pieces except that the "treatment" was filtered river water.

9. Except for the initial controls, slides were numbered rather than labelled so that the microscope operator had no expectation of what the result should be.

10. Notes were made during the counts on the appearance of the cells, the appearance of the stain, any features of the stalks, and whether other algae were taking up or not taking up the stain.

[1] Note that Kilroy et al. (2007) specified a staining time of 15-20 minutes. Subsequent trials showed that didymo cells took up NR almost immediately after immersion in the stain solution.

2.5.2 Procedure for *Lindavia*

The treatment and staining method for *Lindavia* was similar to that for didymo with the following variations:

1. Each *Lindavia* sample comprised a very small amount of slime material (e.g., 1 mm x 2 mm), which typically contained thousands of cells.
2. Treatment and staining were carried out in small Petri dishes (30 mm diameter), placed on a white tray so that the test pieces could be seen more clearly.
3. The rinsing and staining solutions were made up with distilled-grade water rather than filtered lake water because of the difficulty in filtering water containing so much slime.
4. Staining time was extended to 5 minutes because preliminary tests showed that *Lindavia* cells took up stain more slowly than didymo cells. Samples were gently agitated and teased apart in the treatment and stain solutions to aid penetration of the solutions to as many cells as possible.
5. The microscope procedure was similar to that for didymo except that slides were first scanned at low power (40 x) to determine whether any portions of the sample had escaped staining. Stained parts of the sample typically had a pinkish background, whereas unstained parts (usually in the middle of dense areas of cells) were golden-brown. These unstained areas were relatively easy to see in controls, but less obvious in treatment samples (see comments in Results below). We did not count cells in the unstained areas in control samples.

3 Results

3.1 Didymo

Control samples tested on 21 and 22 January returned an average of 74 ± 7 % viable cells (mean and standard deviation), as indicated by NR staining as expected for healthy cells (Table 3-1, Figure 3-1a, Table B-1).

The four products in order of effectiveness (mean percentage stained cells) were: Arnold Lemon detergent, Jasco Citriclean, Eco-store (MPI sachets) and Earthwise Lemon and Aloe Vera (Table 3-1).

Figure 3-1: Typical appearance of didymo cells. (a) A live cell after staining. Note golden brown chloroplast with well-defined edges. (b) Dead cells after Jasco treatment and staining. Note slight pink tinge around cell perimeter, greenish colour of chloroplasts and diffuse appearance along the left-hand edge of the chloroplasts.

Table 3-1: Summary results of tests on didymo assessing effectiveness of four detergent products for decontamination . Controls were run at the start of the trial and periodically throughout the trials.

Treatment	No. tests	Percentages of stained cells			
		Mean	Std Dev	Minimum	Maximum
Eco-store (MPI sachet)	5	5	6	0	14
Earthwise Lemon and Aloe Vera	5	26	17	10	55
Jasco Citriclean	4	1	1	0	3
Arnold Lemon detergent	3	0	0	0	0
Control (no treatment)	7	74	7	62	82

3.1.1 Eco-store (MPI sachet)

The Eco-Store treatment was generally effective on didymo. A small proportion of cells appeared to stain normally. However, most cells that took up stain were not typical of healthy, viable cells (see notes in Table B-1).

3.1.2 Earthwise Lemon and Aloe Vera

The Earthwise product was less effective than the other products, with statistically significant differences (two-sample t-tests, $P < 0.05$). We counted cells that appeared to have stained normally (indicating healthy cells) in all five replicates, although in other cells the staining was pale (see Table B-1). Following treatment with Earthwise dishwashing liquid, we observed that a high proportion of didymo cells were detached from their stalks, although this effect was not quantified.

Mean % stained (viable) cells (26 ± 17%) viable cells was significantly lower than in the controls (two-sample t-test, $P < 0.005$), suggesting that there was some effect on didymo. On the other hand, small diatoms appeared to be staining normally.

3.1.3 Jasco citriclean

Jasco citriclean was the second most effective product tested. Few stained cells were observed in three replicate tests. Unstained cells were visibly affected by exposure to the product solution, with misshapen chloroplasts that were green in colour rather than the golden-brown of healthy cells (Figure 3-1b). The few stained cells had green-brownish chloroplasts and were counted as viable.

3.1.4 Arnold lemon detergent

The Arnold lemon detergent was the most effective product on didymo. Although a small proportion of cells took up some stain, the staining in these cells was abnormal, and concentrated at the perimeter of the cells. These cells were assumed to be non-viable. Furthermore, green filamentous algae in the sample also did not stain.

3.2 *Lindavia*

The proportion of viable cells determined after NR staining in the controls was variable and also uncertain because the appearance of the cell masses under the microscope at low power indicated that the stain had not penetrated to all the cells. Areas of dense cells were golden-brown with no staining (Figure 3-2a), while parts of the mass were pink, with stained cells (Figure 3-2b). We extended the staining time and also tried to tease apart the cells mass in the stain to reduce this effect, but we suspect that some clumps of cells always escaped staining. Counts of stained and unstained cells in the pink-coloured areas indicated 70 ± 11% viable cells. We assumed that this percentage was consistent throughout the sample.

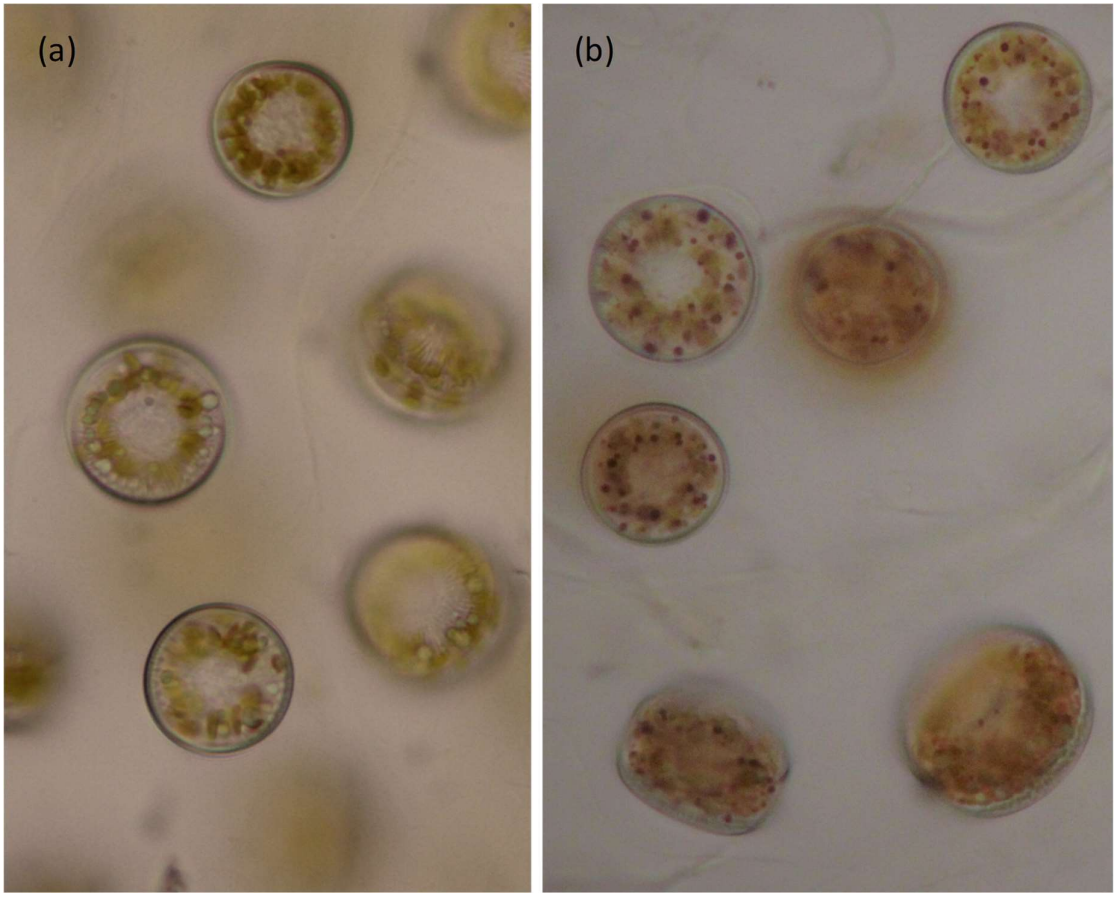

Figure 3-2: Typical appearance of live *Lindavia* cells. (a) unstained cells; (b) stained cells. Note the prominent dark red-coloured spots (vacuoles) in stained cells.

The four products in order of effectiveness (mean percentage viable cells) were: Jasco Citriclean, Eco-store (MPI sachets), Arnold Lemon detergent and Earthwise Lemon and Aloe Vera (Table 3-2). Mean percentages of stained cells were not statistically significantly different among the first three products (ANOVA post-hoc comparisons, $P > 0.15$).

Table 3-2: Summary results of tests on *Lindavia* assessing effectiveness of four detergent products for decontamination.

Treatment	No. tests	Percentages of live cells			
		Mean	Std Dev	Minimum	Maximum
Eco-store (MPI sachet)	5	15	9	0	21
Earthwise Lemon and Aloe Vera	5	56	29	22	90
Jasco Citriclean	5	8	6	1	16
Arnold Lemon detergent	5	17	17	1	36
Control (no treatment)	8	70	11	47	79

3.2.1 Eco-store (MPI sachet)

The Eco-Store treatment was moderately effective, although percentages of live cells were variable (Table 3-2). In one replicate, no stained cells were observed and the cells were a greenish colour. However stained and possibly viable cells were observed in the other four replicates. The staining was often light (i.e., small numbers of very small granules compared to those in the controls, Figure 3-2b). Stained cells tended to occur in clumps, suggesting that the treatment had not reached the cells in those areas, but the stain did (see notes in Table B-2).

3.2.2 Earthwise Lemon and Aloe Vera

As for didymo, the Earthwise product was less effective than the other products (two-sample t-tests, $P < 0.05$) (Table 3-2). We counted cells that appeared to have stained normally (indicating healthy cells) in all five replicates, although in other cells the staining was pale (see Table B-1). The counted percentages of live cells were not statistically different from those in the controls (two-sample t-test, $P > 0.35$).

3.2.3 Jasco citriclean

The effect of Jasco Citriclean on *Lindavia* cells was similar to that observed for didymo in that the chloroplasts in treated cells were often a definite green colour rather than the golden-brown of healthy cells (Figure 3-3) and mean percentage of stained (potentially viable) cells was lowest (Table 3-2). However, stained cells were present, with staining ranging from red granules around the perimeter, to pale granules, to normal staining. Clusters of unstained cells with golden (i.e., potentially normal) chloroplasts were also observed. We assumed that these cells were viable (but unstained) and did not include them in the counts. We counted only cells in areas with a pinkish background indicating that the NR stain was present.

3.2.4 Arnold lemon detergent

The effect of the Arnold lemon detergent was inconsistent across replicates. In the first replicate scanned only one stained cell was observed. However, in the next two replicates, about one third of cells counted were stained (Table B-2). The staining was relatively normal for viable cells. The final two replicates had fewer stained cells, but it was difficult to gauge their viability status because the chloroplasts were often not clearly different from those in live cells. We noted a lot of mucilage in the last two samples (Figure 3-3).

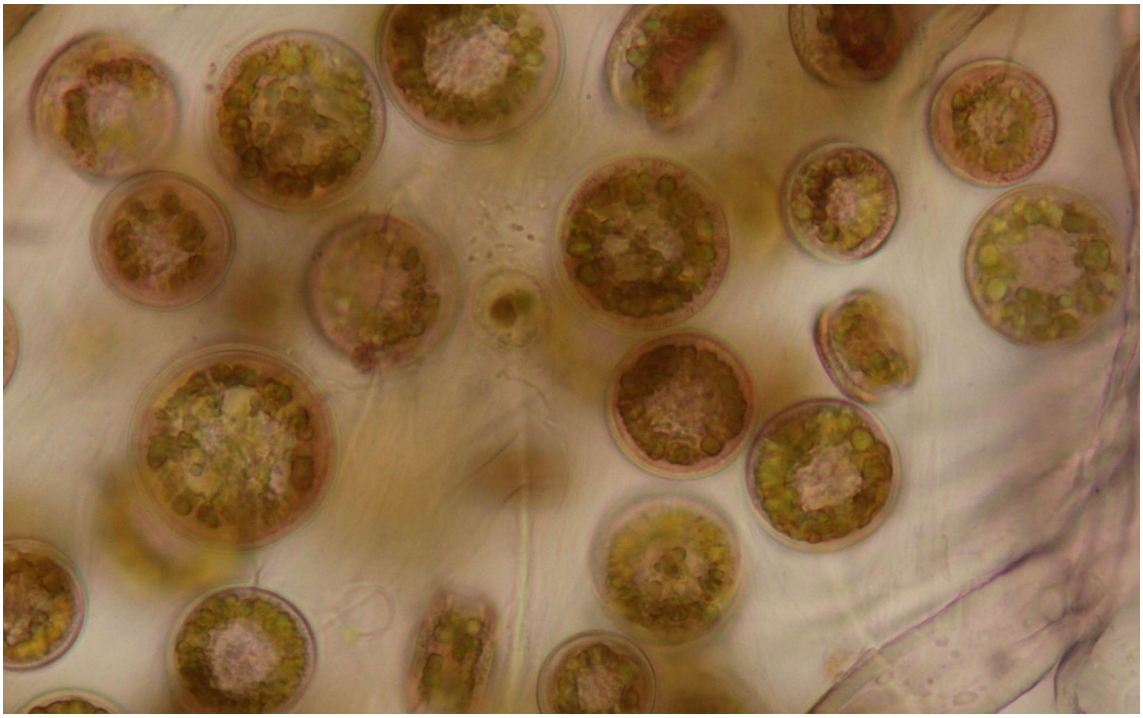

Figure 3-3: ***Lindavia*** **cells after treatment with Jasco followed by staining.** Note the green colour of the chloroplasts compared to that in Figure 3.1(a). Note also the mucilage surrounding the cells.

4 Discussion

4.1 Didymo

Three of the four products (Eco-store, Jasco and Arnold) were largely effective for deactivating didymo cells after exposure for 1 minute to a solution of the strength recommended by MPI (i.e., 8% for the Eco-store product in sachets, and 5% for the other products). The low percentages of cells counted as stained (i.e., potentially viable) after the Eco-store and Arnold treatments suggested that extending the contact time would likely result in no potentially viable cells. Unstained cells or abnormally stained cells (counted as non-viable) also had abnormal chloroplasts (i.e., shrunken or misshapen, with diffuse edges).

The Earthwise product was less effective at deactivating didymo cells than the Eco-store, Jasco and Arnold products. However, significantly lower percentages of stained (potentially viable) cells than in the control suggested that a better result might be obtained by increasing the concentration and/or extending the contact time. For example, application of an 8% solution may have produced a similar effect to that of the Eco-store product. The two products make similar environmental claims (see Appendix A). For products that clearly make environmental claims, an 8% solution (as recommended by MPI on their sachets of Eco-store detergent) appeared to be a more appropriate concentration than the standard 5% recommendation.

From the information in Appendix A, we note that no strong environmental claims are made for the Jasco or Arnold products. Consistent with that, their performance for decontaminating for didymo was similar to that of the regular brands of dishwashing liquid tested by Kilroy et al. (2007).

4.2 *Lindavia*

As noted by Kilroy and Robinson (2017), we found that conducting viability tests on *Lindavia* was more challenging than on didymo for at least three reasons:

1. *Lindavia* cells are smaller than didymo cells (20–40 µm in diameter in the sample from Lake Wanaka, compared to 100–120 µm long for didymo cells from the Opuha River). Detection of staining required careful scanning at a magnification of 400 x.

2. In *Lindavia*, healthy cells have multiple small plate-like chloroplasts arranged in a wide band around the cell perimeter, but not usually in the centre (Novis et al. 2017). It was difficult to recognise changes in the form of these small chloroplasts unless there was a definite colour change. In contrast, didymo has a single large chloroplast in which shrinkage and deformation are easy to recognise.

3. As noted in the Methods section above and in Kilroy and Robinson (2017), the sticky mucilage that encloses *Lindavia* cells apparently inhibited penetration of the NR stain to all cells and may also have inhibited treatment. Patchy penetration of both the stain and the treatment solutions meant that we had to make calls on whether or not to include some unstained areas of the subsample in our counts (see Results). These areas could have been viable cells that the stain did not reach, or non-viable cells that did not take up the stain. As noted above, chloroplast changes were not always clear in *Lindavia*. Consequently, we tried to exclude these unstained patches from our counts.

We believe that the counts of stained vs, unstained cells in the *Lindavia* samples were likely representative of the proportions of potentially viable and likely non-viable cells in the samples because we restricted counts to areas that appeared to have been penetrated by the stain.

The overall result was that all four products were less effective on *Lindavia* than they were on didymo. The reason for lower effectiveness was likely that small *Lindavia* cells are partially protected by the copious amounts of mucilage (slime) around the cells.

All live diatoms secrete mucilage, which has a variety of functions (Hoagland et al. 1993). In didymo, mucilage is primarily secreted into the stalks, and partly around the cell, the latter to facilitate movement in cells detached from their stalks. In planktonic diatoms like *Lindavia*, mucilage strands secreted from around the cells aid buoyancy and access to light in the open waters of lakes. Excessive mucilage production by *Lindavia* also makes it a nuisance from a human perspective.

In treated samples, we observed that stained *Lindavia* cells sometimes occurred in clumps or groups (refer to notes in Table B-2). This could indicate that the treatment affected the mucilage enough to allow subsequent stain penetration, but not enough to deactivate the cell. In that case, a longer exposure to the detergents may improve effectiveness. Further tests at a longer contact time would be required to confirm if that was the case.

In the meantime, we suggest that a precautionary approach for more effective deactivation of *Lindavia* cells is longer contact with detergents than the recommended 1 minute, plus combination with one of the methods trialled in Kilroy and Robinson (2017), such as drying or hot water. In addition, as for didymo, when using Earthwise or Eco-store detergents, an 8% solution (as obtained when following the instructions on the MPI-provided sachets) is recommended.

We note that freezing was 100% effective on *Lindavia* (Kilroy and Robinson 2017) and is therefore the preferred decontamination method.

5 Conclusions and recommendations

In this study we assessed the effectiveness of four detergents (dishwashing liquids) for decontamination of two nuisance freshwater diatoms, *Didymosphenia geminata* (didymo) and *Lindavia intermedia* (*Lindavia*) following MPI's Check, Clean, Dry procedures. We trialled three products using MPI's recommended concentrations (5%) and contact times (1 minute). The fourth product (an Eco-Store product) was provided in by MPI in 20 ml sachets and was made up to 250 ml, as instructed. A summary of results and recommendations is provided in Table 5-1.

Based on the tests, **Arnold Lemon detergent** was 100% effective and **Jasco Citriclean** was 99% effective for deactivating **didymo** cells. These two products made no strong environmental claims in their online marketing information (Appendix B). Their performance was similar to that of the regular brands of dishwashing liquid tested in earlier trials. Extending contact time for Jasco Citriclean would provide more certainty of complete effectiveness. The **Eco-store detergent** (provided by MPI in sachets) was almost as effective as the above two products on didymo, but we note that the solution made up following directions on the sachet was 8%, i.e., stronger than the recommended 5%. **Earthwise Lemon and Aloe Vera** was only 75% effective on didymo, on average. Increasing both the concentration of the solution (e.g., to 8%) and contact time could improve effectiveness.

None of the products was 100% effective on ***Lindavia***. **Jasco Citriclean, Arnold Lemon detergent** and the **Eco-store** detergent had equivalent performance. Longer contact time might improve effectiveness, but this needs to be tested. **Earthwise Lemon and Aloe Vera** was ineffective on average. Increasing both the concentration of the solution (e.g., to 8%) and contact time could improve effectiveness, but this needs to be tested.

An overall conclusion from the trials was that for products that clearly make environmental claims, an 8% solution (as recommended by MPI on sachets of Eco-store detergent) is a more appropriate concentration for Check Clean Dry applications than the standard recommendation of 5%.

Table 5-1: Summary of outcomes of viability tests on didymo and *Lindavia* using four detergents, with recommendations for future use of products in the Check Clean Dry procedures. *Solution prepared following instructions on MPI sachets. Arnold Lemon detergent (red type) was the only product that was 100% effective in the tests.

Treatment	Concen-tration	Contact time	Initial assessment	Recommendation
Didymo				
Eco-store (MPI sachet)	8%*	1 min	95% effective	Extend contact time
Earthwise Lemon and Aloe Vera	5%	1 min	<75% effective	Increase concentration to 8%, extend contact time
Jasco Citriclean	5%	1 min	99% effective	Extend contact time
Arnold Lemon detergent	5%	1 min	100% effective	Recommended
Lindavia				
Eco-store (MPI sachet)	8%*	1 min	>80% effective	Extend contact time, but ideally re-test
Earthwise Lemon and Aloe Vera	5%	1 min	Not effective	Increase concentration to 8% and extend contact time, but ideally re-test
Jasco Citriclean	5%	1 min	>80% effective	Extend contact time, but ideally re-test
Arnold Lemon detergent	5%	1 min	>80% effective	Extend contact time, but ideally re-test

6 Acknowledgements

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7 References

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Appendix A Product information

The following information on each of the tested products was copied directly from the brands' websites.

Eco-store (MPI sachet)

https://ecostore.com/nz/lemon-dish-liquid-389/

ECO-STORE Lemon Dish Liquid

This powerfully concentrated plant and mineral-based formulation gets dishes and glassware sparkling clean and grease-free with just a squirt, while being kinder to hands.

Made from plant and mineral-based ingredients.

No SLES, DEA, synthetic dyes or perfumes.

pH balanced to be gentle on hands.

Suitable for septic tanks.

Earthwise Lemon and Aloe Vera

https://earthwise.co.nz/dish-liquid-lemon-aloe-vera/

PLANT POWERED DISH LIQUID: LEMON & ALOE VERA

Earthwise Dish Wash Liquid is a low suds, gentle plant based formula which will leave your dishes squeaky clean and streak-free. It's grey water and septic tank safe, and contains biodegradable surfactants.

FREE FROM SLS, Cocamide DEA.

AVAILABLE IN: 400ml, 750ml

Contains: Aqua (Water), Sodium Lauryl Ether Sulphate (Plant Derived Surfactant), Sodium Chloride (Sea Salt), Cocamidopropyl Betaine (Plant Derived Surfactants), Caprylyl/Capryl Glucoside, Dehydroacetic Acid, Benzyl Alcohol, Aloe Barbadensis (Aloe Vera) Leaf Extract, Fragrance (Parfum), Citric Acid (Naturally Fermented pH Adjuster).

Jasco citriclean

https://www.jascodist.co.nz/product/jasco-citrus-multi-citrus-cleaner-degreaser-20ltr-8256.htmx

JASCO CITRUS-MULTI CITRUS CLEANER DEGREASER 20Ltr[2]

Product Code: JBCIT20

[2] We assume that this is the product used by the Department of Conservation from the product colour (a reddish brown).

U.O.M: 20L Citrus Multi is a powerful D-Limonene based natural universal cleaner derived from citrus fruits. Formulated with a blend of biodegradable surfactants for the effective removal of organic based soiling, grease, fats and other dirt and grime. Because of the high dilution rate, Citrus Multi can also be used for heavy duty degreasing and removal of other soils including oils, waxes, body fats, soap scum and carbons. It can also be used selectively for carpet and laundry spotting. MPI Approved C31 (all animal product except dairy)

Arnold lemon detergent

https://www.arnoldproducts.co.nz/product/20l-lemon-detergent-dishwash-liquid-4883.htmx

LEMON DETERGENT DISHWASH LIQUID (Arnold Products Limited.)

Manual dish washing liquid (not for use in dishwashers)

Technical Notes

PRODUCT DESCRIPTION

Lemon dishwashing detergent is an economical general purpose detergent suitable for a variety of uses including dish washing, car wash, floors and windows etc.

Is ok in septic tanks. We recommend tipping unused buckets of product on the drive or ground which will break down in a couple of days.

DIRECTIONS

Hand Dishwashing: 1 to 2 teaspoons per 10 litres HOT water.

Windows: 1 to 2 teaspoons warm water.

PRECAUTIONS

- Store in original container.
- Do not mix with other chemicals.
- Rinse container when finished, recycle where possible.
- Keep out of reach of children.

WARNINGS

- All cleaning products should be considered harmful if ingested.

FIRST AID

- Ingestion: drink 1-2 glasses of water. Seek medical advice. Do not Induce vomiting. Do not give anything by mouth to an unconscious or convulsing patient.
- Skin Contact: rinse with cold water for 15 minutes and seek medical advice if irritation persists.

- Eye contact: flush with cold water for 15 minutes and seek medical advice if irritation persists.
- Inhalation: remove from further exposure. Seek medical advice if discomfort continues.
- Spillage: contain spillage with absorbent and dispose in accordance with local regulations.

Appendix B Complete results of microscope scans on all individual samples tested

Table B-1: Results of all individual trials on didymo. Samples are listed in the order in which they were tested. All four products were tested on 21 January with three replicates. Additional replicates of the Eco-store, Earthwise and Jasco products were tested on 22 January. The control tested on 22 January indicated that the samples were still in good condition. *The Eco-store product was made up to an 8% solution, following the directions on the MPI sachets provided.

Order	Date	Slide No.	Treatment (product)	Concen-tration	Contact time	Rep	Stained count	Unstained count	total	% live	Notes
1	21-Jan-20		Control			1	122	33	155	78.7	
2	21-Jan-20		Control			2	114	40	154	74.0	
3	21-Jan-20		Control			3	123	54	177	69.5	photos 12-15.
4	21-Jan-20		Eco-store	8%*	1 min	1	16	99	115	13.9	Very light staining, cells appeared to be dying as counting progressed.
5	21-Jan-20		Eco-store	8%*	1 min	2	2	106	108	1.9	Stained around edges only.
6	21-Jan-20		Control			4	124	35	159	78.0	
7	21-Jan-20	1	Eco-Store	8%*	1 min	3	10	120	130	7.7	Chloroplasts normal colour, large cells stained. Most cells small and stubby. Some staining looked 'normal'.
8	21-Jan-20	2	Jasco	5%	1 min	1	1	145	146	0.7	Chloroplasts greenish, the stained cells also had greenish colour.
9	21-Jan-20	3	Jasco	5%	1 min	2	0	121	121	0.0	Photo #16. stalks stained, green chloroplast.
10	21-Jan-20	4	Jasco	5%	1 min	3	0	113	113	0.0	Stalks stained, green-coloured chloroplasts.
11	21-Jan-20	9	Control			5	93	57	150	62.0	Small diatoms also took up stain.
12	21-Jan-20	11	Earthwise	5%	1 min	1	31	100	131	23.7	Many cells detached from stalks, most stained cells pale. Some normal looking.
13	21-Jan-20	12	Earthwise	5%	1 min	2	26	92	118	22.0	Cells detached. Small diatoms staining. Pale staining, some normal-looking.
14	21-Jan-20	13	Control			6	108	37	145	74.5	Cells mostly attached..

Order	Date	Slide No.	Treatment (product)	Concen-tration	Contact time	Rep	Stained count	Unstained count	total	% live	Notes
15	21-Jan-20	14	Earthwise	5%	1 min	3	12	108	120	10.0	Cells detaching, pale staining
16	21-Jan-20	15	Arnold	5%	1 min	1	0	120	120	0.0	Stained cells not normal. Stain concentrated at membrane, counted as dead, not in granules. Green filamentous algae in sample also did not stain.
17	21-Jan-20	16	Arnold	5%	1 min	2	0	139	139	0.0	Stained cells not normal. Stain concentrated at membrane, not in granules, counted as dead. Green filamentous algae not stained.
18	21-Jan-20	17	Arnold	5%	1 min	3	0	120	120	0.0	Scanned whole slide. As above.
19	22-Jan-20	18	Control			7	120	27	147	81.6	Staining looks good, lots of dividing cells.
20	22-Jan-20	20	Eco-store		1 min	4	0	111	111	0.0	A couple of cells lightly stained but not in granules, on and off stalks. Some tiny diatoms stained.
21	22-Jan-20	21	Eco-store		1 min	5	0	108	108	0.0	A couple of cells lightly stained but not in granules, on and off stalks. Some tiny diatoms stained. Stalked 35, not stalked 38, so 50:50.
22	22-Jan-20	23	Earthwise	5%	1 min	4	18	82	100	18.0	small diatoms stained, spirogyra stained, some cells on stalks stained, lightly. Not all of them.
23	22-Jan-20	24	Earthwise	5%	1 min	5	60	50	110	54.6	Most unstained cells were detached. Some attached cells stained very lightly but had normal healthy chloroplasts. Other diatoms stained.
24	22-Jan-20	25	Jasco	5%	1 min	4	3	105	108	2. 8	Greenish chloroplasts. A couple lightly stained but still greenish.

Table B-2: Results of all individual trials on *Lindavia*. Samples are listed in the order in which they were tested. All four products were tested on 23 January with three replicates. Two additional replicates of all four products were tested on 24 January. The controls tested on 24 January indicated that the samples were in moderate to good condition. *The Eco-store product was made up to an 8% solution, following the directions on the MPI sachets provided.

order2	Date	Slide No.	Treatment (product)	Concen-tration	Contact time	Rep	Stained count	Unstained count	total	% live	Notes
1	23-Jan-20	1	control			1	94	25	119	79.0	Clumps of unstained cells within mucilage. Dense cells not stained, and not counted.
2	23-Jan-20	2	control			2	112	37	149	75.2	as above.
3	23-Jan-20	3	control			3	107	32	139	77.0	as above.
4	23-Jan-20	4	Jasco	5%	1 min	1	8	148	156	5.1	Cells greenish. Cell mass more spread out than in controls. A few stained.
5	23-Jan-20	5	Jasco	5%	1 min	2	1	146	147	0.7	As above. One dense patch checked. Cells stained a little on the outside of the patch.
6	23-Jan-20	6	Jasco	5%	1 min	3	15	118	133	11.3	Greenish. Stained cells had granules around the outside edge of the cell, in a part circle.
7	23-Jan-20	7	MPI	8%*	1 min	1	25	120	145	17.2	Light staining, a few dark spots, dense patches.
8	23-Jan-20	8	MPI	8%*	1 min	2	24	108	132	18.2	Light staining in clumps.
9	23-Jan-20	9	MPI	8%*	1 min	3	31	117	148	21.0	Light staining in clumps. Unstained cells not as green as #4-6.
10	23-Jan-20	10	control			4	147	43	190	77.4	Colony of dense cells with defined edge. Clumps of healthy cells not stained.
11	23-Jan-20	11	control			5	99	37	136	72.8	Colony of dense cells with defined edge. Clumps of healthy cells not stained.
12	23-Jan-20	12	control			6	48	55	103	46.6	Note: time delay in counting. Cells with golden colour, dense clusters.
13	23-Jan-20	13	Earthwise	5%	1 min	1	83	47	130	63.9	Plenty stained, some normally.
14	23-Jan-20	14	Earthwise	5%	1 min	2	105	35	140	75.0	Colony slightly spread out, generally “normal” staining.
15	23-Jan-20	15	Earthwise	5%	1 min	3	120	13	133	90.2	Cells seem more spread out, no clumps. Definite stain in cells, sometimes pale, often dark. Brown colour (not green).
16	23-Jan-20	16	Arnold lemon	5%	1 min	1	1	121	122	0.8	Dense clump. Shrunken chloroplasts.

order2	Date	Slide No.	Treatment (product)	Concen-tration	Contact time	Rep	Stained count	Unstained count	total	% live	Notes
17	23-Jan-20	17	Arnold lemon	5%	1 min	2	52	93	145	35.9	Many stained throughout.
18	23-Jan-20	18	Arnold lemon	5%	1 min	3	50	103	153	32.7	Many stained throughout.
19	24-Jan-20	1	control			7	83	37	120	69.2	V. light staining in most cases (small but dark granules), stained/unstained mixed up.
20	24-Jan-20	2	control			8	88	59	147	59.9	Clumps of unstained cells (diffuse slime, not as compact as yesterday's samples).
21	24-Jan-20	3	MPI	8%*	1 min	4	0	152	152	0.0	No stained cells seen. Slightly greenish-gold.
22	24-Jan-20	4	MPI	8%*	1 min	5	28	111	139	20.1	Clumps of stained cells in mucilage, looked normal. Otherwise greenish-gold.
23	24-Jan-20	5	Earthwise	5%	1 min	4	31	108	139	22.3	Golden brown / green. Light staining in clumps of cells.
24	24-Jan-20	6	Earthwise	5%	1 min	5	36	85	121	29.8	Diffuse, stringy appearance under low power. Golden brown / green chloroplasts.
25	24-Jan-20	7	Arnold lemon	5%	1 min	4	19	141	160	11.9	A lot of mucilage, stringy, multiple golden brown chloroplasts. Occasional stained cells amongst unstained. Whole slip scanned and no big patches of stained cells seen.
26	24-Jan-20	8	Arnold lemon	5%	1 min	5	3	129	132	2.3	Multiple golden brown chloroplasts, but very few stained.
27	24-Jan-20	9	Jasco	5%	1 min	4	12	113	125	9.6	Definite greenish chloroplasts in most cells, but some with stained in background or pale stained granules. Golden area (could be protected?)
28	24-Jan-20	10	Jasco	5%	1 min	5	20	108	128	15.6	Greenish colour common, but large parts of sample golden with a few stained cells. A lot of mucilage.