Didymosphenia geminata in the Waiau Arm and Lake Manapouri: a survey to investigate current distribution, and recommendations for ongoing monitoring

NIWA Client Report: CHC2005-037
May 2005

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Didymosphenia geminata in the Waiau Arm and Lake Manapouri: a survey to investigate current distribution, and recommendations for ongoing monitoring

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Prepared for

Meridian Energy

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Contents

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Executive Summary

- The introduced invasive alga *Didymosphenia geminata* has been proliferating in the Mararoa and lower Waiau rivers during the spring/summer of 2004/2005 and has been assigned "unwanted organism" status by Biosecurity New Zealand.

- Because this species is known to survive in lakes as well as rivers, concerns have been raised about the potential for an infestation in Lake Manapouri, via the Waiau Arm, which directly connects the Mararoa River and the lake.

- Under the present flow operating rules, flows in the Waiau Arm have often been away from the lake (the natural direction), e.g., for more than 60% of the time between 1997 and 2003. For the rest of the time, a proportion of the Mararoa River flow is diverted towards the lake as a result of regulation of flow through the Manapouri Lake Control (MLC) to the lower Waiau River.

- Following a request from Meridian Energy (subsequent to Meridian's discussions with Biosecurity New Zealand), a survey was undertaken on 6-7 April 2005 aimed at determining the distribution of *D. geminata* in the Waiau Arm, thereby providing input to the design of an ongoing monitoring programme.

- Samples were collected from 84 locations, mostly by boat. These comprised 50 from the margins of the Waiau Arm, 20 from the lake shore, and 14 drift samples from the central channel of the Waiau Arm.

- Twelve samples contained *D. geminata*. Of these, 10 were collected within 2 km of the MLC. The other two were collected from the north bank of the Waiau Arm, approximately 2.5 and 6.5 km from the lake entrance. *D. geminata* was very rare in the sample closest to the lake, and neither of the two samples was considered to indicate established colonies of the diatom.

- On the basis of the survey the sampling area is divided into three zones with progressively decreasing probabilities of finding *D. geminata*: Zone 1, the MLC area: Zone 2, Waiau Arm main reach; Zone 3, the lake entrance/lake shore.

- Hydrological conditions in the Arm in the year leading up to the surveys were examined. Flows were in a lakeward direction for almost 60% of that time, a higher proportion than the average for previous years. For much of the period of the *D. geminata* bloom (mid-July 204 to early March 2005) it was estimated that Mararoa water reached Lake Manapouri on almost 45% of days during this period.

- Hydrological conditions in 2004/05 appeared to provide ample opportunity for *D. geminata* to reach Lake Manapouri. That it was not found in greater abundance nearer to the lake may be attributed to the following:

    - *D. geminata* fragments appear not to be particularly buoyant. Therefore in the slow-flowing water of the Waiau Arm, they will tend to sink rather than float.

    - Slow-moving, often slightly turbid waters in the Waiau Arm, combined with mud/sand substrate, are not ideal habitat for *D. geminata*.

    - Rapid and frequent water-level fluctuations in both the Waiau Arm and Lake Manapouri will reduce opportunities for successful colonization by *D. geminata*.

    - The slight brown colour of Lake Manapouri water, combined with a relatively low pH, may also be unfavourable for the successful establishment of *D. geminata* in the lake itself.

- Nevertheless, Frazers Beach appears to provide potentially suitable physical habitat for *D. geminata*. If the species established here, even in low densities, the area would become another point from which the species could spread.

- An ongoing monitoring programme is suggested, based on regular sampling from Zones 1, 2 and 3, as defined from the survey. Since samples from Zone 1 are very likely to contain *D. geminata*, it is important to sample this area independently from the other two zones, to minimize the chance of transferring the alga back towards the lake. Monthly sampling is proposed, to be undertaken in conjunction with an existing water sample collection programme in the Waiau Arm. Approximate sampling points are listed, but GPS coordinates should be obtained for each to aid mapping. Sample collection procedures are described.

- The samples collected as part of the programme will be processed and mapped in the same way as the 84 samples collected for the current survey.

- If future surveys indicate that *D. geminata* is appearing more frequently in Zones 2 and 3, then it is expected Meridian will liaise with Biosecurity New Zealand and the other relevant agencies as to an appropriate response or further monitoring needs, at least until it can be established that the species will not grow in the lake due to unfavorable chemical or other conditions.

1. Introduction

The invasive alga *Didymosphenia geminata* was first identified from the Mararoa and lower Waiau Rivers, Southland, in October 2004. Both rivers experienced severe blooms of this diatom species over the summer of 2004-05, for the first time, although there is anecdotal evidence that *D. geminata* may have been present in the Mararoa River for the preceding 3 years. The species mainly inhabits rivers, however it is known to colonise stable substrates within the wave zone of lakes. Early taxonomic literature refers to *D. geminata* occurring in both lakes and rivers (Cleve 1894-96, Hustedt 1930), and there is at least one report of a massive bloom of the species along a lake-shore (see Kilroy 2004). For this reason, there is concern about the possibility of *D. geminata* entering Lake Manapouri via the Waiau Arm.

The Waiau Arm was formerly the main outlet from Lake Manapouri, carrying a mean flow of 450 m^3/s, which was discharged as the lower Waiau River (Jowett 1993). Since completion of the Manapouri Lake Control (MLC) in 1976, this part of the lower Waiau River has effectively become a 10-km extension of Lake Manapouri. Water flows in the Arm are generally slow and the flow often travels in its natural direction, away from the lake towards the MLC. However, under certain conditions, a proportion of the flow from the Mararoa River is directed towards the lake. Since prolific growth of *D. geminata* is present in the Mararoa River, these times of reverse flow in the Waiau Arm create the potential for *D. geminata* fragments to travel westward along the Arm and ultimately into the lake.

The hydrological characteristics of the Waiau Arm have been summarised in a report to Meridian Energy by Maunsell Ltd. (Maunsell 2003). It was calculated that water from the Mararoa River reached the lake on about 38 days per year at times when residence time of water in the Waiau Arm was at least 5 days (average from 1997 to 2003, Maunsell 2003). With a combination of higher flows and lower lake levels Mararoa water can also reach the lake in well under 2 days (Maunsell 2003). This implies that floating fragments of *D. geminata* could also travel through the lake relatively rapidly. However, whether *D. geminata* cells actually make it through to the lake will depend on several factors, including: the amount of sloughing of *D. geminata* taking place in the Mararoa River; the buoyancy of colony fragments; and whether the alga is able to establish colonies in the Arm itself. Further relevant factors are the residence time of Mararoa water in the Waiau Arm, the survivability of floating or settled fragments of *D. geminata* within the slow-moving, lake-like conditions, and the health and abundance of the diatom in the river, which could lead to different degrees of risk at different times of the year.

The work described in this report was requested by Meridian Energy following a request from Biosecurity New Zealand to set up a programme in the Waiau Arm to monitor the occurrence of *D. geminata* within the Waiau Arm and at the lake entrance. The project comprised three parts:

1. A survey of algal growth in the Waiau Arm, the lake entrance and the lake margins in the immediate vicinity, to ascertain whether or not the alga has already established colonies there.

2. A survey of drifting algae along the length of the Arm to determine the extent of floating *D. geminata* fragments.

3. Use of the results of the survey to determine suitable sites and methods for a regular monitoring programme aimed at the continued surveillance of *D. geminata* in the Waiau Arm and Lake Manapouri areas to minimize the risk of it entering and possibly establishing in Lake Manapouri.

The first two components of the work were undertaken by NIWA on 6-7 April 2005 and form the main focus of this report. Recommendations for an ongoing monitoring programme are presented.

2. Hydrological data

As mentioned above, under the current operating rules for the Manapouri Power Scheme, water flows in the Waiau Arm may travel in either direction, depending on flows in the lower Waiau and Mararoa Rivers, and on lake level. In general, if the Mararoa flow is more than that required to supply the minimum flow to the lower Waiau, then the surplus is directed towards the lake. The speed of lakeward flow depends on lake level – the lower the level, the faster the flow. Exceptions to the lakeward flow of surplus Mararoa water are:

- If the Mararoa water is turbid (e.g., in flood) its whole flow is released to the lower Waiau, to prevent dirty water entering the lake;

- If the lake level is near to its operating maximum, lake water may be spilled down the lower Waiau.

- If turbid water is detected in the Waiau Arm, then the flow through the MLC is increased until the turbidity is reduced to an acceptable level.

These operating rules, which were implemented in 1996, have resulted in a decrease in the amount of water diverted from the Mararoa River into Lake Manapouri, compared to the situation before 1996 (Maunsell 2003).

To characterize conditions leading up to the present survey we used daily mean flow records from the Mararoa at Cliffs (site 79737) and Waiau at Manapouri Structure TW (site 79719) to determine the net direction and volume of water flow within the Waiau Arm over a range of periods leading up to the sampling. Flow in the Waiau Arm was calculated as Mararoa flow minus lower Waiau flow. The flow record for the year leading up to the survey was examined and the following were calculated for selected time periods:

- Number of days the flow was in a lakeward direction;
- Number of days the flow was zero or flowing towards the MLC;
- % time the flow was lakewards;
- Mean overall flow;
- Median flow;
- Maximum flow in lakeward direction;
- Maximum flow towards the MLC.

The selected periods were arbitrary times leading up to the sampling (1 year, 1 month), the period between the two major lake spills in the year (July 2004 and March 2005), the period between the first major spill to the sampling time, and the period between the second major spill to the sampling time.

We also estimated the number of days during these periods on which flow from the Mararoa reached the lake. This was done using by examining the Waiau Arm flow record (daily means) and daily mean lake levels (Lake Manapouri at Supply Bay, site no. 79707), along with with Table 1 in Maunsell (2003). This table gives the residence time of water in the Waiau Arm at given lake levels and flows, and is based on calculated water volumes in the Waiau Arm over the range of lake levels. When the daily flow record showed that lakeward flows remained high enough for longer than the stated residence time (and were at the appropriate lake level), it was assumed that Mararoa water had entered the lake.

This information was expected to assist in determining the potential for *D. geminata* colonization or deposition in the Waiau Arm. The data were also used as a comparison with average conditions reported in Maunsell (2003) since the present operating rules came into effect in 1996.

3. Survey methods

3.1. Substrate sampling

Qualitative samples of periphyton were collected from sites along the margins of Lake Manapouri and the Waiau Arm on 6 and 7 April 2005. Because the lake level was relatively high on the day of sampling, we took samples from depths down to about 1 m, which was just below the minimum lake level since October 2004 (Figure 1). This ensured that our samples comprised algae and deposits accumulated over the past few months, not just growth during the high flows of the past few weeks. Most material was collected by wading or snorkel diving from a boat. A glass-bottomed underwater viewer enabled assessment of the macroscopic appearance of periphyton growth; observations were also made while snorkeling.

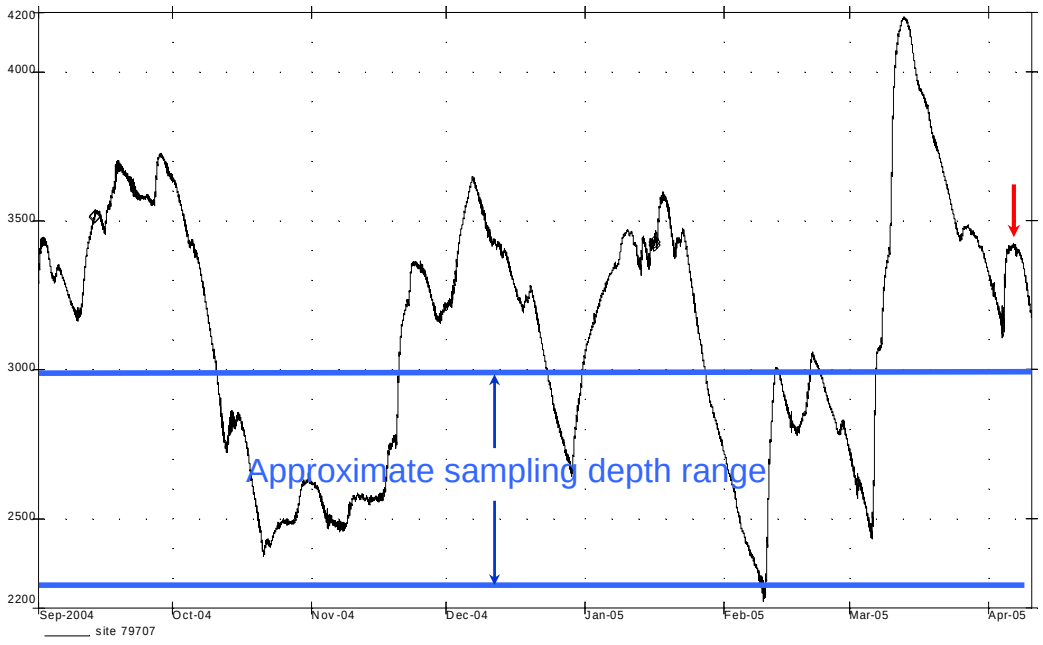

Figure 1: **Water level record, Lake Manapouri, from September 2004 to April 2005. Red arrow indicates approximate time of sampling. Samples were taken within the depth range shown. Add 174669 to value on y-axis to obtain lake level in mm above sea level.**

At each site, we collected periphyton from stable substrates (stones, submerged wood) by scraping or brushing. We also collected grab samples of epiphytic periphyton. Up to four subsamples were combined into a single container.

A hand-held GPS was used to record the coordinates of each collection point.

3.2. Drift sampling

Drift sampling was carried out on 6 April 2005. We adapted a drift net designed for collecting drifting invertebrates in flowing water to sample floating material. The apparatus comprises two 1-m long nets (300 μm mesh) mounted side-by-side on an aluminum frame. The net openings are 200-mm long plastic cylinders, diameter 140 mm, with about 100 mm separating the two openings. The nets terminate in detachable 600 ml containers, into which material is concentrated during deployment in flowing water. For use from a boat, a rope halter was made so that the net frame remained horizontal as it was towed. For each drift sample, the net was towed for 5 to 7 minutes at slow speed. The speed was determined by a combination of flow needed to keep the apparatus afloat just below the water surface, and drag on the rope, which had to be limited to prevent breakage. Each tow covered approximately 300 m. At the end of each tow, the two collecting containers were removed and the contents allowed to settle. Excess water was drained off, then all visible plant and algae fragments were picked out using a turkey baster. A subsample of the water was transferred (along with all the fragments) into a 120 ml sample container.

3.3. Microscope examination

Each sample was first examined for any macroscopic signs of *D. geminata* (e.g., small, whitish-brown colonies; pale, hairy-looking material which could possibly be *D. geminata* stalks).

Samples were then examined under a Leica inverted microscope at magnifications ranging from x100 to x400. Since *D. geminata* cells are so large, they are clearly visible even at the low magnification. At least 3 subsamples of ~2 ml were scanned from each sample. If necessary, the samples were homogenized before subsampling, using a hand-held electric blender.

In cases where *D. geminata* was found, a visual assessment of its relative abundance was carried out, based on the method described in Biggs & Kilroy 2000 (pp. 97-100). This assigns abundance scores on a scale of 1 to 8, relative to the abundance of the dominant taxon (score 8). In this case, we took a score of 8 to be abundance in typical samples from the lower Waiau River, which were dominated by this diatom. These samples were collected on 1-2 March 2005 during the annual survey of periphyton and invertebrates in the lower Waiau River (Kilroy et al. 2005), and were available for a direct comparison. The scoring system was adjusted slightly in order to indicate when only stalks and/or dead cells were found in a sample, as follows.

Score	Occurrence
0	no *D. geminata* (Dg) found in any subsample
1	Empty Dg frustules/stalks rare to occasional
2	Empty Dg frustules/stalks common
3	Live Dg, rare
4	Live Dg, occasional
5	Live Dg, occasional to common
6	Live Dg common in all subsamples
7	Live Dg abundant (but not the dominant taxon).
8	Live Dg dominant

Notes were made on other algal taxa present. This is relevant because periphyton community composition is indicative of habitat type. The presence of species such as *Gomphoneis minuta* var. *cassieae*, which has a similar growth form to *D. geminata*, and appears to inhabit the same kinds of environments, suggests that the habitat for that sample may also be suitable for *D. geminata*.

As an accuracy check on the microscope assessments, 10 randomly selected samples, in which *D. geminata* was not found initially, were re-checked (3 sub-samples each). Extra subsamples were checked for all samples in which *D. geminata* was found, to obtain a more accurate idea of abundance.

3.4. Precautions taken during sampling

The usual precautions recommended by Biosecurity New Zealand were adopted, to prevent transfer of the organism. A 1% solution of household bleach and/or thorough drying was used to sanitize all clothing and sampling equipment that had come in contact with the water and substrate, when moving from the Waiau Arm into the lake (which was necessary on one occasion) and at the end of the survey.

4. Results

4.1. Hydrology

Table 1 summarises the net flow of Mararoa water in the Waiau Arm on the 2 days of sampling, and for a range of earlier periods. Over the year from 6 April 2004, water from the Mararoa River flowed towards the lake for 58% of the time. However, mean flow over that time was 12.8 m^3/s in the *opposite* direction. This simply reflects the

Table 1: Summary of hydrology in the Waiau Arm for selected periods. (Refer to text for explanation of the choice of periods included.)

Period	No. days towards lake	No. days away from lake	% time in lakeward flow	Mean flow	Median flow	Max flow lakeward	Max flow away	No. days Mararoa flow reached lake
Year up to survey	208	152	58	12.8	-3	-26	324	108
7 April 04 – 6 Apr 05								
July 2004 spill to survey	174	88	66	0.76	-6	-26	243	101
13 Jul 04 – 6 Apr 05								
Period between spills	160	71	69	-4.04	-7	-26	72	102
13 Jul 04 – 6 Mar 05								
Month up to survey	14	17	55	36.5	1	-20	243	1
6 Mar 05 – 6 Apr 05								
Mar 2005 spill to survey	8	6	57	-1.71	-0.5	-8	4	0
20 Mar 05 – 6 Apr 05								
6 April 05	1	0	na	-4	na	-4	na	na
7 April 05	1	0	na	-7	na	-7	na	na
1997 – Sep 2003			39*	44*				n.d.

*data from Maunsell (2003)
n.d. = not determined
Negative flows indicate a flow direction towards the lake.

fact that when water is flowing from the lake, it tends to be a very large lake spillage event. Thus, the maximum flow towards the lake over the year was only 26 m^3/s, compared to a maximum of 324 m^3/s towards the MLC (Figure 2).

In the year being considered, the most recent large lake-spill events were in early July 2004, and mid-March 2005. In the period between these two events (13 July 04 to 6 March 05) flows were very stable, with lakeward flow almost 70% of the time, and a maximum flow towards the MLC of just 72 m^3/s. It is estimated that on about 101 days during this time (about 40% of the time) water from the Mararoa was able to travel through to the lake.

Following the most recent lake spill on 10-20 March 2005 there was a slight net flow towards the lake, with Mararoa water likely to have reached the lake on one day during the two weeks before the survey. On the two days of sampling again there was a slight flow towards the lake (Figure 2, Table 1). For comparison, from January 1997 to September 2003, there was a net lakeward flow in the Waiau for 39% of the time, and the mean flow over that period was 44 m^3/s, towards the MLC (Maunsell 2003).

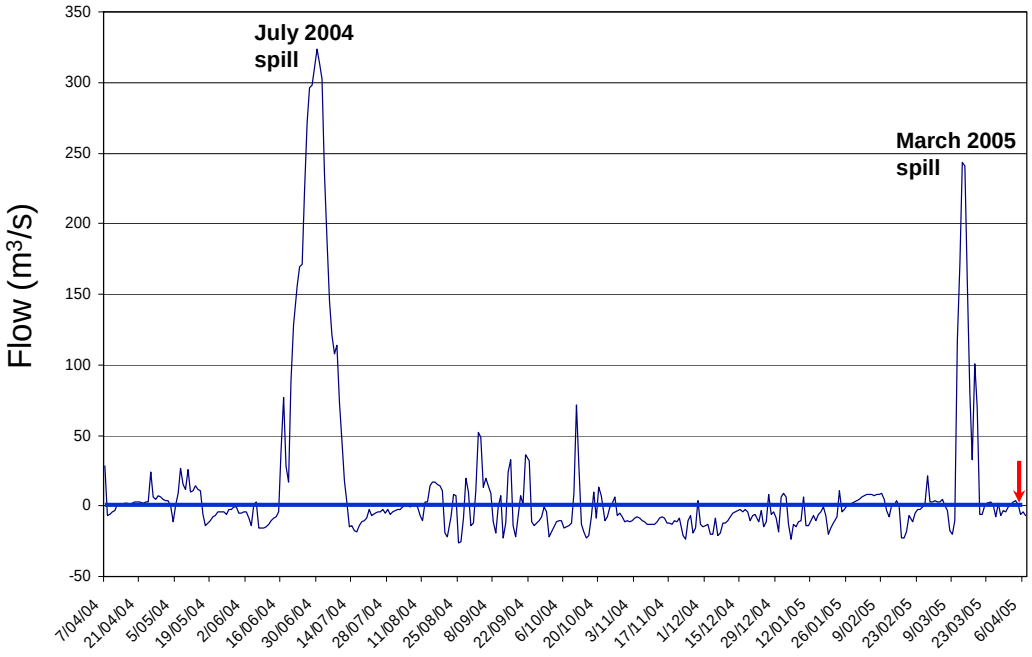

Figure 2: **Flows in the Waiau Arm from April 2004 to April 2005, calculated as Mararoa flow – lower Waiau flow. Flows below the blue line are towards the lake and above the blue line, towards the MLC. Red arrow indicates time of survey.**

4.2. Sample composition

A total of 84 samples was collected: 14 drift samples, 30 from the north side of the Arm, 20 from the south side and 20 from the lake edge. Site locations are shown in Figure 3. Details are given in Appendix 1, including observations of the main algal taxa observed in each sample and field notes about the sample type and habitat.

No samples showed obvious macroscopic signs of *D. geminata* growth. None of the typical colonies, small or large, were noted either in the field or when the samples were examined more closely in the laboratory. The three benthic samples collected closest to the MLC did have a slight "furry" appearance, which could possibly have been due to the presence of *D. geminata* stalks.

Microscopic examination showed that 12 of the 84 samples contained *D. geminata*, including one drift sample. All re-checked samples where *D. geminata* was not found gave the same (negative) result as on the first examination.

Locations of the 12 positive samples are shown on Figure 3, colour-coded according to their abundance scores. *D. geminata* was not the dominant alga (score 8) in any sample. Five of the positive samples, including the only positive drift sample, were

found within 1 km of the MLC. All these contained cells that appeared to be alive (red and blue circles). Five more positive samples found within the next km contained dead cells only (green circles). The two remaining positive samples were located much closer to the lake, sample 34 being only 2.5 km from the lake entrance.

No microscopic signs of *D. geminata* were found in any of the samples collected from around the lake entrance or from the lake shore.

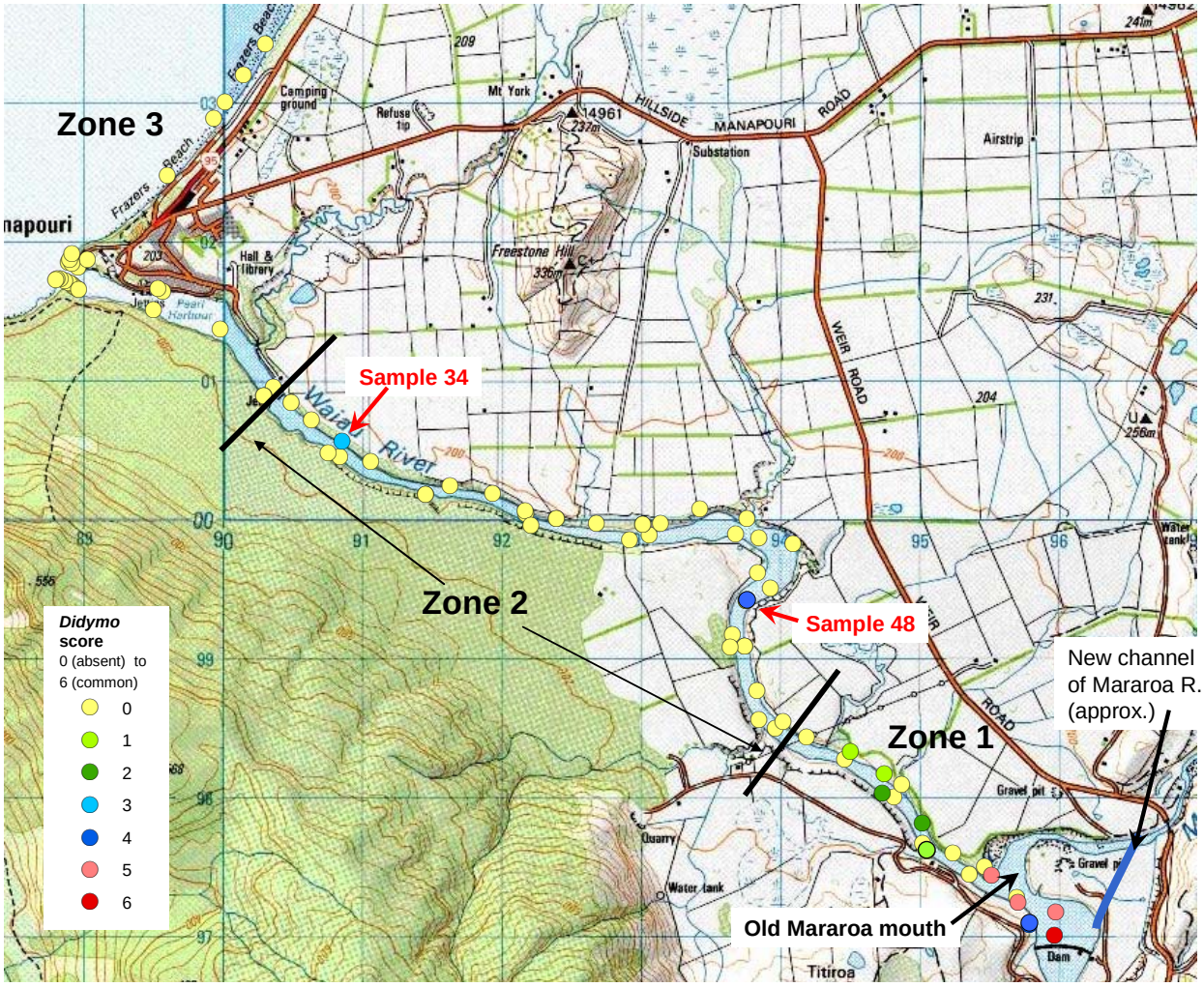

Figure 3: **Locations of sampling sites, colour-coded according for presence and abundance of *D. geminata* in samples. Refer to Section 3.3 for an explanation of the abundance scores, and to Section 5.1 for reference to Zones 1 to 3.**

5. Discussion

5.1. Distribution of *D. geminata*

As expected, we found most samples containing *D. geminata*, and the highest abundances, in the vicinity of the MLC. Water in this area is in constant direct contact with Mararoa River water, whether the flow is wholly directed down the lower Waiau, or partly diverted into the Waiau Arm. It was surprising that during the survey we saw no floating material that resembled *D. geminata*. The lack of macroscopic colonies on the substrate around the MLC was less surprising. The water here is essentially a large ponded area and poor habitat for *D. geminata* (Kilroy 2004, and see section 5.2 below). The distribution of *D. geminata* material near the MLC was predictable: apparently healthy cells still attached to stalks were in the area closest to the MLC, giving way to dead cells and stalk material in the first 2 km of the Waiau Arm itself.

Given the hydrological conditions over the period of the bloom of *D. geminata* in the Mararoa River (section 4.1), it was probably inevitable that some material would be found farther along the Waiau Arm and nearer to the lake. These were samples 34 and 48 (see Figure 3, and Appendix 1). Because of their closer proximity to the lake, a fuller description of these two samples is warranted.

Sample 34

Location: approximately 2.5 km from the lake entrance, and about 600 m east of the Real Journeys slipway. Material was scraped from submerged wood at depths of 0.5 to 1 m. The substrate was firm sand/mud, sloping to a bed of *Elodea canadensis*, which started at about 1 m depth. A single fragment of *D. geminata* was located in the first subsample examined, comprising 6 cells containing chloroplasts, attached to a short length of stalk. Other algae present included the green filamentous alga *Oedogonium* (dominant), the stalked diatom *Gomphonema acuminatum* and *Cymbella* sp. Ten further subsamples were scanned; none showed any sign of *D. geminata*. Therefore it seems unlikely that this sample contained a thriving population. Other possibilities are:

1. This was a chance finding of a *D. geminata* fragment that had been washed down from the Mararoa very recently or possibly transported on a boat or during some other human activity, or via wildlife.

2. The sample had somehow become contaminated. This is unlikely as precautions were taken to avoid mixing samples, including thorough

cleaning of the microscope chamber between each sample and use of new pipetters for each sample. Particular care was taken for samples examined following a positive identification.

The first possibility seems the most likely, but it is impossible to determine what transported the fragment to that location. Regardless of how the fragment arrived there, it raises cause for concern because of its proximity to the lake. However, it should be stressed again that because of the rarity of *D. geminata* in the sample, it seems highly unlikely that this finding is evidence of colonization by *D. geminata* in this area.

Sample 48

Location: approximately 6.5 km from the lake entrance, northern margin, east of "Big Bend" and just east of a stand of large willow trees overhanging the water's edge. Samples were scraped from submerged logs and rocks at 0.5 to 1.0 m depth. *D. geminata* was found in 4 of 6 subsamples examined, a combination of cells with chloroplasts, attached to stalks, plus dead and unhealthy cells. *D. geminata* was "rare" or "occasional" in the positive subsamples (1 to 3 sightings in scans of the complete sample). Other algae present included *Synedra ulna* (dominant), *Pinnularia* sp., small naviculoid diatoms. Again, because of the rarity of the cells in the samples, this appears to be a chance finding of deposited fragments.

From the distribution of *D. geminata* found on 6-7 April 2005, we can divide the sampling area into three zones in terms of the chances of finding the species, as indicated on Figure 3:

1. MLC area, up to 2.3 km west of the MLC. *D. geminata* was found in ~55 % of samples (10 of 19).

2. Waiau Arm central reaches, from just east of the Real Journeys boat ramp. *D. geminata* found in ~5% of samples (2 of 37).

3. Pearl Harbour to Frazer's Beach. No *D. geminata* found in 28 samples.

Recommendations for an ongoing monitoring programme are based on these zones (see Section 6, and Appendix 2).

5.2. Habitat conditions in the Waiau Arm and Lake Manapouri

Conditions along the entire length of the Waiau Arm appear to be unfavourable for *D. geminata* colonization and growth, compared to the environment in the Mararoa and lower Waiau Rivers and given what we currently know about *D. geminata*'s environmental preferences (Kilroy 2004). Flow in the Waiau Arm at most times is imperceptible, and the margins appear to be sheltered from any wave action. Maunsell (2003) calculated that from 1997 to 2003 there were on average 11 events per year when the same water was residing in the Waiau Arm for more than 5 days. Such events lasted an average of 13 days. They also determined that the water residence time in the Arm can be up to 25 days or even longer (though such events are rare).

Light conditions and substrate type in the Waiau Arm also appear unsuitable for *D. geminata*. Except near the MLC and at the old Mararoa entrance (Figure 3), the channel is steep-sided and deep. Water clarity is often poor in the central reaches, though this improves towards the lake entrance (personal observations). In most of the channel, the substrate is firm mud or sand, with extensive macrophyte beds, mostly *Elodea canadensis*, but incuding some stands of the indigenous macrophytes *Potamogeton ochreatus* and *Myriophyllum triphyllum*. Stony substrate is restricted to the area around the MLC, the lake entrance and part of Frazer's Beach.

In both the lower Waiau and Mararoa Rivers, *D. geminata* has been frequently observed growing attached to plants as well as to rocks. During the present survey, prolific growth of algal epiphytes was observed, mostly along the north bank from the western end of Big Bend to the old Mararoa mouth. These growths were mostly on *P. ochreatus*, *M. triphyllum* or short mixed macrophyte communities, rather than on *Elodea*. They comprised mainly *Cymbella* sp. in a mixed assemblage of other diatoms; no *D. geminata* was found. Although the growth was quite thick in places (Figure 4), its texture was loose and slimy – entirely different from that of *D. geminata*.

In the lake itself, conditions in some locations may be more favourable for colonization by *D. geminata*. The most suitable areas for colonization will have large cobbles or boulders on the bed and be exposed to wave action. This provides the stable substrate and constant water movement that *D. geminata* apparently requires to establish and maintain thriving colonies. The cobble-lined substrate off Frazers Beach may be especially favourable as it faces the prevailing wind direction and will frequently be affected by waves. The presence of *Gomphoneis minuta* var. *cassieae* in some of the samples taken off Frazers Beach also suggests that the physical habitat that may be suitable for *D. geminata*. *G. minuta* var. *cassieae* is a stalked diatom, like *D. geminata*, and has been observed in habitats likely to be suitable for *D. geminata*, such as hill-country streams, especially after periods of low flows (Biggs & Kilroy 2004).

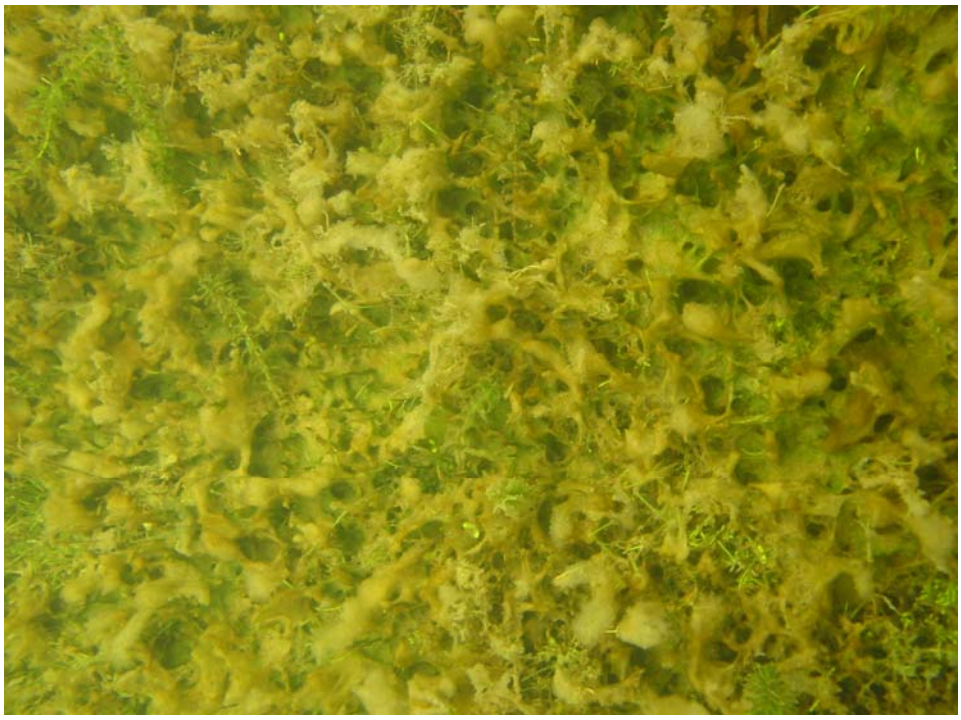

Figure 4: **Typical appearance of epiphytic diatoms growing on aquatic plants in the Waiau Arm. These algal growths were dominated by *Cymbella* spp., mixed with a variety of diatom species and other algae.**

Other features of Lake Manapouri appear less favourable. First, water level fluctuations can be large. As Figure 5 shows, the difference between maximum and minimum lake levels in from 1997 to date is well over 1.5 m, and the fluctuations can be relatively rapid. Thus unless *D. geminata* colonies establish at minimum water levels, there is a high chance that at some stage the colonies will be exposed to desiccation. Tests have already shown that *D. geminata* will survive up to 2.5 days desiccation in the shade, less in full sunlight (Kilroy 2005). The same water level fluctuations will, of course, apply in the Waiau Arm.

A second possibly less favourable feature of Lake Manapouri is its water chemistry. Although there is little conclusive information about the water chemistry requirements of *D. geminata*, overseas observations suggest that high water clarity is a requirement (Sherbot & Bothwell 1993). As indicated, Waiau Arm water tends to be turbid. On the other hand, Lake Manapouri water appears clear. However, it is slightly brown-coloured with humic material, and this may also affect its light climate (Rae et al. 2001). Lake Manapouri water is also slightly acidic (~6.5, Reid et al. 1999), which may not be ideal for *D. geminata*. Evidence for a preferred pH range is again inconclusive, but populations are generally reported from waters with pH >7 (Kawecka and Sanecki 2003, Noga 2003, Martyn Kelly, UK, pers. comm.) The mean pH for the lower Waiau River is 7.73 (NIWA data).

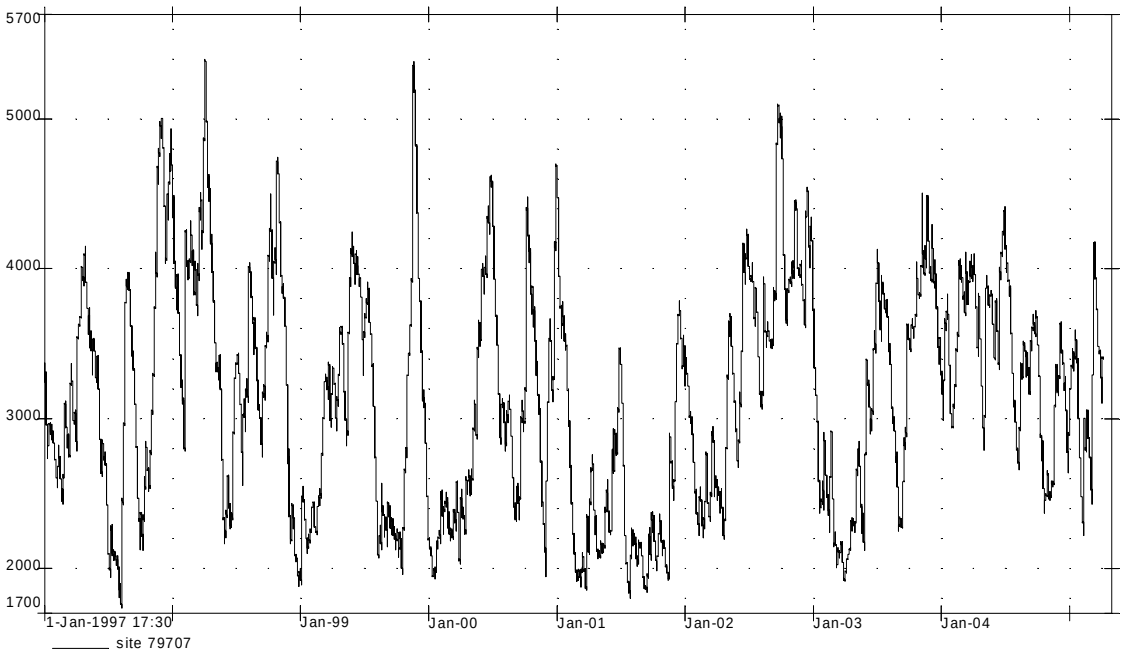

Figure 5: Lake Manapouri water level from 1997 to 2005. Add 174669 to value on y-axis to obtain lake level in mm above sea level.

At this stage it seems prudent to assume that this part of Lake Manapouri may provide favourable habitat for *D. geminata*, until it can be proved otherwise. While the presence of the diatom in low densities in this area may not cause a problem *per se*, it will provide another location from which the species can be spread, especially through human contact. Its presence in the area at any density is therefore highly undesirable.

5.3. Potential for *D. geminata* to reach Lake Manapouri

As Table 1 shows, in the year leading up to the survey described in this report, the amount of time the flow direction in the Waiau Arm was towards the lake was almost 50% greater than the mean percentage calculated for 1997 and 2003 (Maunsell 2003). Over the spring/summer period of 2004/5, the proportion of time in lakeward flow was even greater. Additionally we calculated that for about 40% of that time, Mararoa water was actually reaching the lake, sometimes in as little as 2 days or less. This means that during the time that *D. geminata* has been blooming in the Mararoa River, there would appear to have been better than average opportunities for the diatom to reach the lake. Given these flow conditions, it is not surprising that that there were positive identifications of *D. geminata* some considerable distance from the MLC.

The moderately large lake spill just over 2 weeks before the survey probably reduced the number of sightings of *D. geminata* in Arm on the day of the survey. Future

monitoring following different hydrological conditions should help to determine the effects of different flows.

If future surveys indicate that *D. geminata* is appearing more frequently in Zones 2 and 3, then it is expected Meridian will liaise with Biosecurity New Zealand and the other relevant agencies as to an appropriate response or further monitoring needs, at least until it can be established that the species will not grow in the lake due to unfavorable chemical or other conditions. At that stage it would be worthwhile undertaking tests to determine whether the species can actually survive in the lake, or whether water chemistry and/or other conditions are in fact unsuitable.

Nevertheless, as mentioned, Mararoa water reaching the lake is only one of many factors that influence the movement of *D. geminata*. Perhaps the most relevant question is "Does *D. geminata* float?" Fragments of *D. geminata* sloughed from live colonies in the Mararoa River clearly reach the MLC. Presumably transport of colonies in flowing water is how the species spread into the lower Waiau. In this case, the water turbulence would keep small fragments of material afloat. However, unless the colonies are buoyant in still water, any material trapped in the ponded water behind the MLC will gradually sink out of the water column. The appearance of many *D. geminata* fragments in samples taken close to the MLC, and the absence of macroscopic colonies, suggests that this is what happens. *D. geminata* was found in only one of our drift samples, the last sample collected before reaching the MLC. In that case *D. geminata* was not obvious macroscopically as the sample was mainly a mixture of the green filamentous algae *Zygnema* and *Spirogyra*. It appears that the *Didymosphenia* cells had become entangled with the green algae, and hence remained afloat.

In the laboratory, it has been observed that both small fragments and larger colonies of *D. geminata* generally sink when placed in river water (personal observations). However, this has not been formally tested.

A further concern is that, during the containment phase of the present incursion of *D. geminata* in New Zealand, wildlife, as well as human activities, may spread the organism. This was mentioned as a possible means by which a *D. geminata* fragment could have reached sampling site 34 in the present survey (Section 5.1). In New Zealand, waterfowl may be the most likely vectors. There is evidence from the literature that a species of planktonic diatom is cabable of surviving passage through the guts of ducks (Atkinson 1980). Research is needed to establish whether birds or animals are capable of spreading live *D. geminata*.

6. Ongoing surveys

Using the results of this initial sampling, the sampled area can be divided into three zones, each with a different probability of finding *D. geminata* (see Figure 3 and Section 5.1). No *D. geminata* was found in Zone 3, however, at this stage, the sample size of 28 is too small to make any statement at this stage that the chances of *D. geminata* being in that area are negligible.

Therefore, the issue now is to increase our confidence that no *D. geminata* has reached Zone 3, and to check that the occurrence in Zone 2 does not increase. This can be achieved by ongoing sampling in those zones. In Zone 1, proximity to the Mararoa River means that there will always be a high chance of finding *D. geminata* in that area as long as it is growing upstream. Ongoing sampling is proposed in this area in order to establish whether *D. geminata* colonies are actually growing and expanding, or whether the sightings there are accumulated deposited fragments (as appears to be the case at the moment).

A detailed monitoring protocol is given in Appendix 2, and summarised here.

- Sampling will be approximately monthly (may coincide with ongoing water sampling in the Waiau Arm).

- A total of 13 samples are to be collected on each sampling occasion, four each from Zones 1 and 2 and five from Zone 3. A further 2 drift samples are to be taken on Zone 2. Most samples can be taken by boat.

- Because *D. geminata* is known to be common in Zone 1, it is important to sample this area independently of the other two. Zones 3 and 2 should be sampled (in that order) using a boat from Pearl Harbour. Zone 1 should be sampled using a boat put in and taken out at the MLC, which can be appropriately treated following the survey.

- Except for the drift samples, each sample will comprise 3 to 5 subsamples of algal growth from submerged wood or stones, or epiphytes (algae growing amongst underwater plants).

- Sampling sites are marked on the map provided, and a brief description is provided. To assist with mapping, GPS-coordinates (7-figure Eastings and Northings) should be taken at each site. These and will also pinpoint sampling site locations that have no clear landmarks.

- It will be important to sample from the appropriate range of lake depths. Ideally, sampling would be undertaken when the lake level is low. If this is not possible, then samples must be taken down to depths equivalent to the lowest lake level over summer 2004/05.

- For detailed instructions and a map of sampling sites, refer to Appendix 1.

7. Conclusions

In the survey undertaken on 6 – 7 April 2005, 12 of 84 benthic and drift samples taken from the Waiau Arm and Lake Manapouri margins contained *Didymosphenia geminata*. Although the discovery of *D. geminata* fragments far from the MLC and only 2.5 km from the lake entrance is of concern, we consider it encouraging that more positive identifications were not made, in view of the hydrological conditions during the time of the *D. geminata* bloom in the Mararoa River. Between September 2004 and March 2005 Mararoa water flowed towards the lake for almost 70% of the time, and reached the lake about 45% of the time. According to previous records, this is atypical: under average conditions, lakeward flows would be much less frequent. Nevertheless, no sign of *D. geminata* was found in samples collected within 2 km of the lake, or on the lake shore. We found no evidence of growing colonies of *D. geminata* within the Waiau Arm.

Habitat conditions in the Waiau Arm appear to be unsuitable for *D. geminata* to thrive, however, the wave-washed, cobble substrate off Frazers Beach may well be suitable, as long as water chemistry is not limiting and if colonies are not subject to desiccation

As a result of the present survey, we defined three zones in the Waiau Arm. In Zone 1, near the MLC, the chances of finding *D. geminata* in a sample are >50%; in the main reaches of the Waiau Arm, Zone 2, the chances are lower, at about 5-6%. In Zone 3, the wharf area to the lake entrance and nearby areas of the lake shore, the probability of finding the alga is less than 5% (from this survey), as none was found in 28 samples.

A protocol for a regular sampling programme within all three Zones is presented. The aims are:

- to check *D. geminata* distribution following different hydrological conditions;

- in Zone 1, to keep track of whether *D. geminata* is establishing and growing in that area.

The results of future surveys will be incorporated into the dataset collected on 6-7 April, and will serve to increase confidence that *D. geminata* is not spreading further into Zones 2 and 3 – as long as no findings are made in Zone 3, and findings in Zone 2 remain at 5% or less, on average (calculated cumulatively). If the species starts to appear more frequently in Zone 2, or in Zone 3, then consideration may have to be given to stopping Mararoa water entering the lake, at least until it can be established that conditions in the lake will preclude the growth of *D. geminata*.

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Appendix 1

Site coordinates, sample details, taxonomic notes and locality/habitat details of samples taken during the survey of the Waiau Arm and Lake Manapouri, 6-7 April 2005. Refer to section 3.3 for an explanation of the scoring system for *D. geminata*. Coordinates listed for the drift samples are at the start of the drift.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
1	2088958	5501625	wood	south bank	0	Zygnema, Mougeotia, Phormidium, Cymbella, Tabellaria, Eunotia, Navicula.	south bank by yellow buoy, wood, fallen trees
2	2088936	5501645	rock scrape/wood	lake margin	0	Zygnema, Cymbella, Cymbella Navicula, Brachysira, Navicula, Encyonema, Tabellaria, Pinnularia, Gomphonema acuminatum, Bulbochaete, Spirogyra	algae on all rocks and wood.
3	2088935	5501649	rock scrape	lake margin	0	Cymbella, Navicula, Gomphonema, Tabellaria, Coleodesmium, Zygnema, Phormidium	big rock scraped, small stone sampled.
4	2088917	5501665	rock scrape	lake margin	0	Dichothrix, Phormidium, Cymbella, Tabellaria, Pinnularia, Encyonema, Eunotia, Melosira varians, Zygnema.	opposite northern edge, a bit downstream.
5	2088902	5501671	rock scrape/wood	lake margin	0	Phormidium, Oedogonium, Cymbella, Tabellaria, Pinnularia, Melosira varians, Ulothrix, Aulacoseira, Fragilaria, Surirella,	just below manuka grove. Thick algal growth, blue green some moss.
6	2088872	5501684	rock scrape/wood	lake margin	0	Zygnema, Cymbella, Tabellaria, Dichothrix, Oedogonium, Spirogyra, Synedra, Aulacoseira, cf. Coleodesmium on moss.	just below manuka grove. Thick algal growth, blue green some moss.
7	2088841	5501699	rock scrape	lake margin	0	Moss- Navicula, Brachysira. Silt - Zygnema, Dichothrix, Tabellaria,Cymbella, Navicula., Eunotia, Dichothrix.	grass/reeds at edge, stony bottom, and moss.
8	2088815	5501699	rock scrape	lake margin	0	Zygnema, Cymbella, Tabellaria, Brachysira, Navicula, Nitzschia, Netrium, Eunotia, Pinnularia, Epithemia.	grass/reeds at edge, stony substrate
9	2088806	5501689	rock scrape	lake margin	0	Tabellaria, Navicula, Cymbella spp. Phormidium, Eunotia, Brachysira, Oedogonium, Aulacoseira, Mougeotia, lots of silt.	grass/reeds at edge, silty substrate, soft and muddy
10	2088972	5501620	wood	south bank	0	Phormidium, Tabellaria, Zygnema, naviculoids, Cymbella, nematodes, Synedra.	downstream of mooring beach. Sandy bottom, sampled from submerged wood.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
11	2089581	5501610	drift	centre	0	Synedra ulna, Eunotia, Navicula, Diatoma vulgare, Tabellaria,	5min drift from Pearl Harbour east.
12	2089994	5501332	drift	centre	0	Oedogonium, Navicula, Eunotia, Synedra.	
13	2090857	5500410	drift	centre	0	Oedogonium, Navicula, Cymbella	
14	2092203	5499998	drift	centre	0	no algae found	no algae seen
15	2093094	5499843	drift	centre	0	Navicula, Sellaphora, zooplankton	
16	2093886	5499825	drift	centre	0	Elodea fragments. Navicula spp.	
17	2093694	5499127	drift	centre	0	zooplankton, naviculoids, Staurastrum, Anabaena	
18	2094003	5498443	drift	centre	0	zooplankton, Navicula, Cymbella, Brachysira	
19	2094505	5498220	drift	centre	0	zooplankton, naviculoids.	
20	2094860	5497948	drift	centre	0	Tabellaria, Eunotia, Asterionella, Dichothrix.	
21	2095067	5497613	drift	centre	0	Navicula, Tabellaria	
22	2095404	5497390	drift	centre	0	Navicula, Tabellaria	Approaching weir. Willow leaves. Mite/fleas
23	2095747	5497221	drift	centre	0	Zygnema, Bulbochaete, Oedogonium with epiphytic naviculoids, Achnanthidium. Spirogyra	near weir, floating green algae.
24	2096027	5497116	drift	centre	5	**Didymosphenia** (live cells with stalks), Zygnema, and Spirogyra.	just inside weir.
25	2088966	5501786	rock scrape	north bank	0	Dichothrix, Aulacoseira, Tabellaria, Eunotia, Encyonema, Navicula, Gomphonema, Fragilaria, desmids, Rhopalodia.	Frazers Beach. Rocks with lots of silt.
26	2089035	5501840	rock scrape	north bank	0	Cymbella, Fragilaria, Tabellaria, naviculoids, Oedogonium, Zygnema, Phormidium, Aulacoseira, Synedra, Vaucheria.	Frazers Beach. Rocks with lots of silt.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
27	2088900	5501817	rock scrape	lake margin	0	Dichothrix, Navicula, Cymbella, Tabellaria	in lake itself. Brown flecks in sediment on rocks.
28	2088924	5501808	rock scrape	lake margin	0	Dichothrix (on mossy stuff), Zygnema, Navicula, Phormidium/oscillatoria, Epithemia, Gomphonema	in lake itself. Brown flecks in sediment on rocks.
29	2088909	5501835	rock scrape	lake margin	0	Blue green, cf. Coleodesmium, Encyonema	small bay, small brown flecks of algae on rocks.
30			rock scrape	lake margin	0	Dichothrix, Tabellaria, Cymbella, Eunotia	small bay, small brown flecks of algae on rocks.
31	2090378	5500916	rock scrape/wood	north bank	0	Tabellaria, Klebsomidium, Synedra, Gomphonema acuminatum, Spirogyra, Melosira varians, Oedogonium, naviculoids, Gonatozygon.	2 stones, obvious algae.
32	2090508	5500805	rock scrape/wood	north bank	0	Eunotia, Navicula, Cymbella, Rhopalodia musculus, Synedra, Melosira varians.	2 stones and wood.
33	2090654	5500678	wood	north bank	0	Encyonema/Cymbella, big Surirella, Navicula, Tabellaria, Pinnularia	margins with hardish sand bottom, with elodea bed starting at about 1m.
34	2090875	5500527	wood	north bank	3	One sprig of **Didymosphenia** (6 cells, with chloroplasts). Oedogonium, G acuminatum, big Cymbella (on plants). 11 subsamples checked, Didymo in one only.	margins with hardish sand bottom, with elodea bed starting at about 1m.
35	2091083	5500378	plant	north bank	0	Many dead diatoms, Phormidium, Synedra, Pinnularia, big Nitzschia, Surirella, Gonatozygon	bark/plants. Many dead diatoms
36	2091655	5500204	plant	north bank	0	Cocconeis, Eunotia, Nitzschia, Gomphonema. Lots of silt.	stones, bark, plant. No algae on plants.
37	2091963	5500143	rock scrape/wood	north bank	0	Synedra, Phormidium, Eunotia, Oedogonium, G. acuminatum (stalked), Tabellaria, Navicula.	algae loosely attached to plants.
38	2092197	5500018	rock scrape/wood	north bank	0	Gyrosigma, Frustulia, Pinnularia, Melosira varians, Eunotia, Surirella, Closterium.	algae loosely attached to plants.
39	2092423	5499963	rock scrape/plant	north bank	0	Navicula, Synedra, small diatoms, Phormidium, Dichothrix, Pinnularia	rocks

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
39a	2092423	5499963	rock scrape/plant	north bank	0	Eunotia, Tabellaria, Closterium (not much).	plants
40	2092711	5499931	wood	north bank	0	Phormidium/Oscillatoria, Pinnularia, Navicula, Gomphonema, small diatoms,	before big bend. Flax and grass on bank.
41	2093047	5499920	plant	north bank	0	Cymbella (slime), Pinnularia (large species), Spirogyra, Synedra, Tabellaria, Navicula, Phormidium/Oscillatoria, Vaucheria.	algae on macrophytes, very thick slimy coating.
42	2093047	5499920	bark	north bank	0	Navicula, big Pinnularia, Cymbella, Frustulia	
43	2093173	5499931	wood	north bank	0	Pinnularia, G. acuminatum, Phormidium/Oscillatoria, Eunotia, Surrirella, Tabellaria, Fragilaria, Synedra, Cymbella	logs/sticks with brown algae. Elodea with no epiphytes. Not much algae.
44	2093459	5500033	plant	north bank	0	Tabellaria (many chains, dominant), Melosira, Synedra, Oedogonium, Spirogyra,	macrophytes Potamogeton ochreatus, elodea and myriophyllum. Epiphytes on Myriophyllum, not elodea. Clean macrophytes, bare rocks.
45	2093800	5499964	plant/epiphytes	north bank	0	Melosira (dominant), Vaucheria, Tribonema, Microspora	Amongst willows. Epiphytes on P. ochreatus, approx 20 m u/s.
46	2094131	5499781	wood	north bank	0	"Leptolyngbya", small naviculoids, Eunotia, brown organic stuff, Pinnularia, Fragilaria.	amongst willows, on big bend, manapouri side. Muddy substrate potamgeton ochreatus. Not much sign of epiphytes.
47	2093967	5499458	rock scrape/plant	north bank	0	Rhopalodia cf. musculus, Melosira varians, Navicula, Vaucheria, Achnanthidium Synedra/Fragilaria, Oedogonium	willows, big bend. D/s water sample buoy. Macrophytes potamogeton, short mixed macrophyte community, mud, not much algae.
48	2093794	5499371	rock scrape/wood	north bank	4	Dead and live **Didymosphenia** plus stalks. Dead diatoms. Live Synedra, small naviculoids (Brachysira?), Pinnularia	out of willows, end of big bend. Algae covered logs.
49	2093794	5499371	plant	north bank	0	Oedogonium, Synedra, Navicula, Cymbella, Pinnularia, Klebsomidium, Tabellaria, G. acuminatum, Oscillatoria.	out of willows, end of big bend. Algae covered logs.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
50	2093783	5499039	plant	north bank	0	Oedogonium, Tabellaria, Synedra, Navicula, Cymbella, G. acuminatum, Pinnularia, Spirogyra, Phormidium.	flax, sandy substrate, macrophytes at 1m. Fallen trees. Epiphytes.
51	2093783	5499039	rock scrape/bark scrape	north bank	0	Synedra, Tabellaria, Oedogonium, Bulbochaete, Nitzschia/Hantschia, G. acuminatum, wavy Oedogonium.	flax, sandy substrate, macrophytes at 1m. Fallen trees. Bark scrape.
52	2093871	5498718	plant	north bank	0	Navicula, Tabellaria, big Hantzschia, Synedra, Phormidium/oscillatoria, Gomphonema, Oedogonium.	epiphytes and bark scrape. Manuka, rushes. Short macrophytes plus epiphytes. Green bobbles on bark.
53	2094061	5498495	plant/wood	north bank	0	Synedra, Navicula, Tabellaria, Pinnularia, Melosira	manuka, flax; some branches with algae. Muddy substrate, elodea, short plants, some wood.
54	2094228	5498383	plant	north bank	0	Synedra, Gomphonema, Navicula, Oedogonium	epiphytes and bark scrape. Flax, manuka, plus willow. Green bobbles on wood.
55	2094545	5498275	plant	north bank	1	Synedra, Oedogonium, G. acuminatum, Tabellaria, Cymbella aspera. **Didymosphenia** (empty frustule).	epiphyte/bark scrape. Mud substrate. Elodea plants very green. Elodea starts ~1m depth.
56	2094788	5498114	wood	north bank	1	Synedra, G. acuminatum, Pinnularia, Encyonema/Cymbella, Phormidium, Oedogonium, **Didymosphenia**, (empty frustules, 6 samples checked)	scrub, young willow, flax, manuka. Not much algae. Elodea closer in. Sample of Potamogeton.
57	2094915	5498041	plant/epiphytes	north bank	0	Synedra, Navicula, Oedogonium, G. acuminatum, M. varians.	creek entrance 800 m from weir. Macrophytes (Myriophyullm) with thick epiphytes.
58	2095063	5497761	plant/bark scrape	north bank	2	Synedra, Tabellaria, G. acuminatum, dead **Didymosphenia**, plus stalks	flax and ferns 200m u/s of tributary, scrub. Very little algae.
59	2095288	5497538	rock scrape	north bank	0	Oedogonium, Navicula, Synedra, Gomphonema spp.	2 stones, plus I bit of bark. Stones at side of steep drop off. Elodea. Not much algae, green film.
60	2095515	5497442	wood/epiphytes	north bank	0	Small naviculoids, Nitzschia, Eunotia, big Pinnularia, Bulbochaete, Gloeocystis.	inlet of Mararoa mouth. Myriophyllum beds, very clean. Some epiphytes.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
61	2095564	5497379	rock scrape	north bank	5	Synedra, Oedogonium, stigeoclonium, **Didymosphenia** (common, but mostly dead looking).	just before channel, shallow area, stony substrate. Uniform myriophyllum.
62	2095838	5497024	rock scrape	south bank	4	Synedra, **Didymosphenia** (couple of cells and stalks)	adjacent to control gates ~500m at side of island. Stony bottom thin, slime, uniform.
63	2096017	5496946	rock scrape	south bank	6	Synedra, **Didymosphenia** (mostly detached cells, with chloroplasts)	200m u/s of gates. Rocky substrate, steep drop off.
64	2095758	5497186	rock scrape	south bank	5	**Didymosphenia** (common), Synedra, Zygnema.	end of rocky bit, opp Mararoa river mouth.
65	2095100	5497554	rock scrape	south bank	1	Oedogonium, Zygnema, Synedra, Cymbella aspera, G. acuminatum, Pinnularia. 2 **Didymosphenia** (dead).	water level/turbidity site. Rocky substrate around recorder.
66	2094776	5497977	plant/sticks	south bank	2	Gomphonema acuminatum, Navicula, Eunotia, **Didymosphenia** (empty cells).	u/s of recorder/ steep drop off to Elodea bed. Green bobbles on wood.
67	2093885	5498504	plant	south bank	0	Few live algae, a few Navicula.	entrance to small creek on south side. Dirty water, much less clear than main arm/ colder. Hard mud substrate. Silt.
68	2093678	5499036	wood	south bank	0	Nitzschia, Encyonema, small Navicula, Phormidium.	before big bend (d/s). Manuka, steep bank.
69	2093875	5499572	plant/epiphytes	south bank	0	Cymbella (slime), Navicula, Pinnularia, Oscillatoria	Big bend. Shallow bar, short macrophytes covered in epiphytes.
70	2093717	5499851	wood	south bank	0	Cymbella, Navicula, Gomphonema, Oscillatoria, Achnanthidium.	west of big bend. Ferns, willows; solid substrate, steep. Algae on branches only.
71	2092952	5499813	plant/wood	south bank	0	Coleodesmium, Frustulia, Cymbella aspera, Pinnularia.	log scrape. Willows 100m before outlet. Deep, steep drop off.
72	2092239	5499916	rock scrape	south bank	0	Cymbella, M varians, naviculoids, Surirella, Fragilaria, Nitzschia.	beech forest, steep drop off. Silty.
73	2091477	5500137	plant	south bank	0	Navicula, Oscillatoria/Phormidium, Oedogonium, Pinnularia, Cymbella/Encyonema, small filaments (like ulothrix)	opposite Cabots boat mooring. Epiphytes.

Sample	E	N	Sample type	Location	Didymo score	Taxonomic notes	Locality/habitat notes
74	2091477	5500137	plant	south bank	0	Navicula, Cymbella, Nitzschia, Frustulia, Oedogonium, Fragilaria, Encyonema, Navicula, Pinnularia	opposite Cabots boat mooring. Bark scrape.
75	2090774	5500436	wood	south bank	0	Phormidium/Oscillatoria, Pinnularia, Cymbella, Gomphonema, Tabellaria, Achnanthidium, Surirella.	600m d/s Pearl Harbour. Steep drop off. Muddy substrate to Elodea bed.
76	2090311	5500854	wood	south bank	0	Synedra, Oedogonium, Spirogyra, G. acuminatum, (slimy mass), Navicula, Phormidium, Bulbochaete, Achnanthidium	epiphytes. Steep drop off. Opposite Real Journeys shed. Muddy substrate to Elodea bed.
77	2090310	5500850	wood	south bank	0	Navicula, Phormidium, Frustulia, Synedra, Pinnularia.	bark scrape.
78	2089546	5501632	rock scrape/wood	north bank	0	Fragilaria, Oedogonium, Cymbella cistula, Tabellaria, Synedra, Hydra wirth chloroplasts?, Bulbochaete. (took photo)	Pearl Harbour wharf.
79	2089511	5501474	plant/rock scrape	south bank	0	Cymbella (dominant), Tabellaria, Gomphonema (some stalked), Zygnema, big Surirella, Phormidium, Gyrosigma, Aulacoseira	Opposite Pearl Harbour, u/s of boat landing, slimy logs, loaded with brown algae.
80	2090322	5503401	rock scrape	lake margin	0	Cymbella, Encyonema, Gomphonema (some stalked), naviculoids, Phormidium, Frustulia, Neidium? Netrium?	Frazers Beach.
81	2090162	5503173	rock scrape	lake margin	0	Oedogonium, Cymbella, (some stalked), Gomphonema (some stalked), Ulothrix, Tabellaria, Spirogyra, Achnanthidium, Gyrosigma, Netrium, Gomphoneis, Synedra.	Frazers Beach. 4 rocks, not much algae. Charophytes on beach.
82	2090032	5502978	rock scrape	lake margin	0	Oedogonium, Bulbochaete, Cymbella, Encyonema, naviculoids, Synedra, stalked Gomphonema, Gomphoneis, Tabellaria, Dichothrix, Stigeoclonium.	Frazers beach, rocks.
83	2089948	5502856	rock scrape	lake margin	0	Oedogonium, Cymbella, Dichothrix, Gomphoneis, Fragilaria, Eunotia, Stigeoclonium, Fragilaria.	Frazers beach middle, lots of sediment
84	2089614	5502449	rock scrape	lake margin	0	No algae.	Frazers beach south, gravel/sand.

Appendix 2

Suggested procedures for surveillance of the Waiau Arm and entrance to Lake Manapouri for presence/absence of *Didymosphenia geminata*

The following is a suggested procedure for a continuing monitoring programme in the Waiau Arm and Lake Manapouri in the vicinity of the entrance to the Arm.

Sampling areas

Samples are to be collected from Zones 1, 2 and 3 as identified in the survey on 6-7 April.

- Zone 1: MLC extending west for 2.5 km, to where the power lines cross the channel.

- Zone 2: Waiau Arm, extending west from where the power lines cross the channel (about 1.5 km east of Big Bend) to approximately 100 m east of the Real Journeys slipway.

- Zone 3: Waiau Arm westwards to the lake entrance from the Real Journeys slipway, to approx 2 km north along Frazers Beach and 300-400 m along the lake shore from the south side of the entrance to the Waiau Arm.

Sampling sites are listed in the table below, with a description. Locations are shown on the map. It's hard to pinpoint an exact place for each because there are few many landmarks in the Arm. As long as the samples are taken more or less in these locations that will be fine. The idea is to space them out over the whole length of the channel. GPS coordinates should be taken on each sampling occasion so that the points can be mapped. The same locations should be sampled each time, if possible.

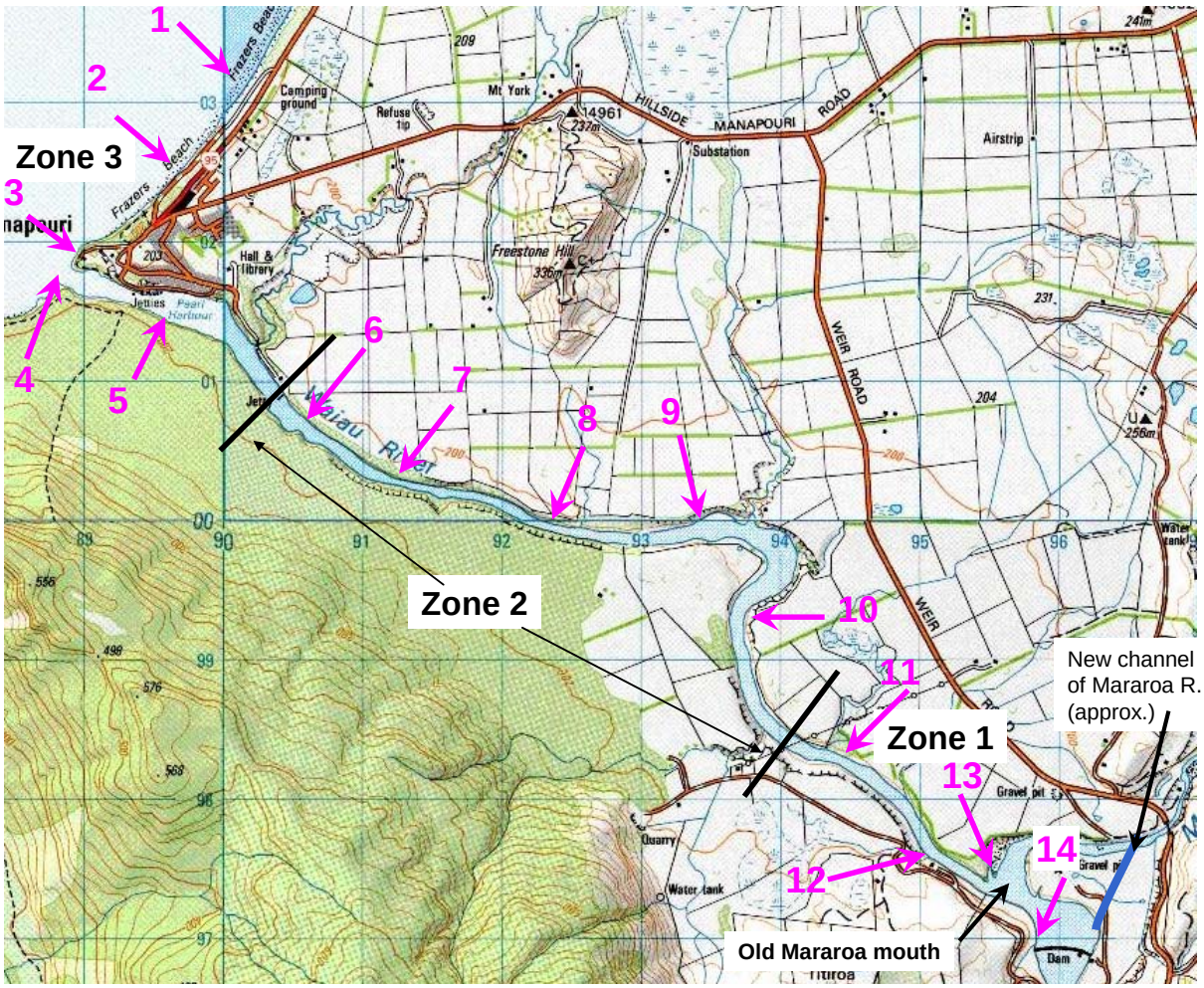
To prevent transfer of *D. geminata* back into the lake it is important to take the samples in the order listed (i.e., start with Zone 3, then do Zone 2). Zone 1 should be done separately using a boat put in and taken out at the MLC, and appropriately treated following the sampling (thorough drying for at least 48 hours until every part of the boat is completely dry, or wash down with 1% bleach).

Sampling locations should be in areas of relatively shallow water near the channel margins and should either have a cobble/rock substrate or submerged wood.

Zone	Sample	Description
3	1	Frazers Beach north, cobble substrate
	2	Frazers Beach south, cobble substrate
	3	Lake shore just around from entrance (small rocky bay just across from backpackers place)
	4	Lake entrance, southern margin (area with rock substrate – sample rocks and wood)
	5	Arm, southern margin, opposite Pearl Harbour
2	6	Arm, northern margin, about 300 m east of Real Journeys slipway
	7	About 200 m west of Cabots boat mooring
	8	Northern margin, about 100 m west of small stream
	9	Northern margin, just before Big Bend (west of stream entrance)
	10	Northern margin, east of Big Bend, just after the end of the overhanging willows
2	D1	Drift sample from sample 10 site back around Big Bend,
	D2	Drift sample, from about start of beech forest on south bank

Take Zone 1 samples in a separate trip, as there is plenty of Didymo in this area

1	11	Northern margin, about 100 m after a small creek entrance
	12	Southern margin, turbidity recorder. Take scrapings from 2-3 rocks.
	13	In small inlet beside old Mararoa River mouth
	14	Cobble substrate adjacent to MLC



Sampling procedure

Equipment

- Sample containers (120 ml screw-top, stackable cups, white lids) [supply left with Fred Inder on 7 April]
- Larger containers for holding rocks when scraping/scrubbing (e.g., ice-cream containers)
- Penknife or small kitchen knife for scraping rocks and wood
- Toothbrush/nail brush for scrubbing fine algal layers
- Drift net, including 600 ml collecting jars
- Strong rope for towing drift net
- Chilly bin for holding samples after collection
- Clipboard, data sheets, pencil(s)
- GPS

Sample in Zone 3 first, then Zone 2 (Didymo is more likely to turn up in Zone 2, so this is to minimize any chance of transferring the organism to the lake.)

Sample Zone 1 from the MLC (take the boat in and out at the MLC).

- All sampling can be done by boat, except that the samples around the lake entrance, northern side, and Frazers Beach, may be easier to collect on foot.
- **At each sampling site, record the location using a GPS – 7-digit eastings and northings. This is important to enable us to map the locations accurately. (For drift samples, record the location at the start and finish of each tow.) Record on sampling sheet.**
- Samples must be collected from appropriate depths, i.e., down to the equivalent to the minimum lake level, and slightly deeper, over the summer of 2004-05. Samples may also be collected from shallower depths if the lake level has been higher for more than 3 weeks. Try to collect from a range of depths at each site. (NB Minimum lake level over the summer of 2204-2005 was approx. 177.00 m.)

- At each sampling location, retrieve 3 or 4 cobbles of pieces of submerged wood from a range of depths, bearing in mind the position of minimum water level over summer 2004/5.

    - Using a knife, scrape algal material from each rock/wood piece, and place in a 120 ml sampling container.

    - Pool the material from each site into the same container.

    - If the site has submerged plants with algae growing amongst the plants, also take a sample of that (include the plants if it's easiest).

    - It is better to get more material than less – put plenty in the container and top up with water.

- For the drift samples, tow the drift net for about 10 mins at slow speed. At the end of the tow, drain the nets, then remove the sampling containers. Allow material to settle for a few minutes before pouring off surplus water. Pick out all visible pieces of algae, etc., in the sample and transfer to a 120 ml container (a turkey baster works well for this). The samples may contain water fleas etc, swimming around – don't worry about getting these. Top up the 120 ml container with water from the sample, then discard the rest.

- Bill Jarvie, Southland Fish & Game, Te Anau, should have two drift nets + 600 ml containers belonging to NIWA.

- Label containers with date and sampling number. Labeling can be done in advance. (Use waterproof marker pens.).

- Fill in sampling sheets (see next page). NIWA will provide these on waterproof paper.

- Keep the samples cool (e.g., transfer to a chilly bin as they are collected). Courier as soon as possible to: NIWA, 10 Kyle St, Christchurch, attn. Cathy Kilroy/Karen Robinson (ph 03 348 8987).

Note: Just in case Didymo is present in the area sampled please make sure that all equipment is dried thoroughly (48 h) or scrubbed/soaked (5 min) in a 1% solution of household bleach at the end of the survey. This applies *especially* to boots and waders, if it was necessary to get out of the boat to collect the samples.