Ciliate diversity in stream biofilms revealed by group-specific PCR primers

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Introduction

The ciliates are a diverse protozoan phylum, thought to be of considerable ecological importance in stream ecosystems, including organisms which are abundant and important consumers of bacteria, algae and other protozoa. Understanding of ciliate diversity and ecology is limited, however, particularly in benthic habitats such as stream biofilms.

In this study, phylum-specific PCR primers were used in combination with cloning, sequencing and terminal restriction fragment length polymorphism (T-RFLP) analysis to investigate ciliate communities in stream biofilms.

Research Objectives

1. To characterise ciliate diversity associated with stream biofilms using phylum-specific PCR primers and associated molecular techniques.

2. To determine whether the community structure of biofilm-associated ciliates, as determined by molecular analyses, differs between seasons and between streams subject to differing levels of human impact.

Methods

1. Sampling

Biofilm samples were collected from each of four streams in Auckland, New Zealand. These represent different levels of anthropogenic impact based on surrounding catchment use, and range from a minimally-impacted native forest stream to a highly impacted urban stream (figures 1-4).

Biofilm material was collected from streambed rocks (or the concrete substrate in the case of highly impacted Pakuranga Stream), during 2005 and 2006, by scrubbing with Speci-Sponges®, which were placed in sterile Whirl-Pac® bags. DNA was extracted using a bead-beating method.

Figure 1: Cascade Stream. Very low impact. Located within an extensive native forest reserve, with walking tracks in the area.

Figure 2: Stoney Creek. Low impact, with native forest, low density housing and roads in the catchment. A tributary of Opanuku Stream.

Figure 3: Opanuku Stream. Medium impact, with native forest, low density housing, roads and agricultural development within the catchment.

Figure 4: Pakuranga Stream. High impact. Located within a densely populated urban catchment. Runs down an artificial concrete channel.

2. Cloning and sequencing analysis

1. 18S sequences amplified using newly-developed ciliate-specific PCR primers 384F and 1147R [1]
2. Ciliate 18S rRNA gene clone library construction
3. Clone libraries screened by HaeIII RFLP profiles, and differing RFLP profiles sequenced
4. 18S sequences identified by matching to GenBank sequences. Sequences differing by over 97 % considered to represent different OTUs

3. T-RFLP analysis

1. 18S sequences amplified using ciliate-specific PCR primers 384F and 1147R respectively labelled with HEX and FAM fluorophores
2. PCR products purified, concentration measured and standardised
3. PCR products digested overnight with HaeIII and RsaI
4. Terminal restriction fragments detected and quantified using GeneMapper 4.0 (Applied Biosystems)
5. HEX and FAM peak area data combined and normalised for each sample
6. T-RFLP data compared between different seasons and different streams using ANOSIM and non-metric multidimensional scaling (Primer v6.1.6)

Acknowledgements

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References

1. Dopheide, A., Lear, G., Stott, R. & Lewis, G. (2008), Molecular Characterization of Ciliate Diversity in Stream Biofilms. *Applied and Environmental Microbiology* 74 (6): 1740-1747.

www.streambiofilm.org.nz

Results

1. Cloning and sequencing

A total of 240 clones were investigated among four clone libraries, of which 176 (73 %) were determined to be of probable ciliate origin. Within these putative ciliate clones 54 different taxonomic units, representing seven ciliate classes, were identified (Figure 5). The range of ciliate sequences detected in the different streams showed little overlap. Fewest sequences were detected in the very low impact stream, in which sequences matching the sessile peritrich *Zoothamnium* sp. were most common. The high impact stream was distinguished by a high frequency of clones matching *Mesanophrys* sp. and *Entorhipidium* sp., and several different litostomatean sequences. Interestingly, clones matching sessile peritrich sequences (*Epistylis* spp., *Vorticella* sp. and *Zoothamnium* spp.) were common in samples from all three stony-bottomed streams, but were not detected in samples from the high impact, channelised stream. A limited number of potential non-ciliate sequences were detected, showing that primer specificity is imperfect. Most of these GenBank matches were fragmented or of poor quality, however, and interpretation of these possible non-ciliate sequences remains uncertain.

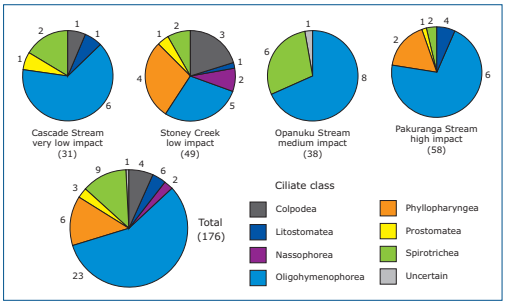

Figure 5: Proportion of clones belonging to different ciliate classes, with the number of different sequences detected in each class and stream shown (values in parentheses are total numbers of ciliate clones detected)

2. T-RFLP analysis

T-RFLP analysis provided clear evidence of differences in ciliate assemblages between different streams and seasons. The fewest T-RFLP peaks were usually detected in profiles from the very low impact stream, while the most peaks were variously detected in profiles from low, medium or high impact streams (figures 6 and 7). In total, 197 differently HEX-labelled terminal fragments and 201 different FAM-labelled terminal fragments were detected among all samples, indicating a high level of ciliate diversity.

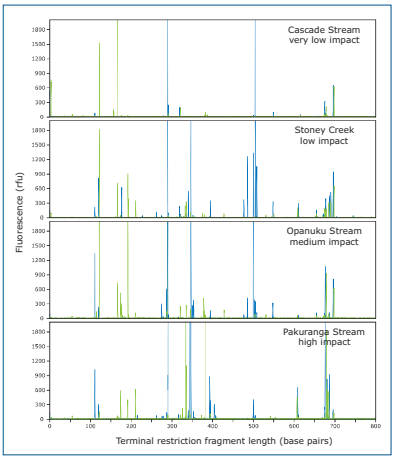

Figure 6: T-RFLP profiles derived from November 2005 (spring) stream biofilm samples using ciliate-specific PCR profiles. Size of green and blue peaks respectively indicates abundance of HEX-labelled and FAM-labelled terminal restriction fragments.

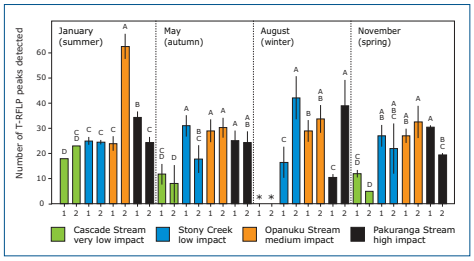

Figure 7: Number of terminal fragments (average of HEX and FAM-labelled fragments) detected in profiles derived from stream biofilm samples using ciliate-specific PCR primers. Numbers 1 and 2 denote samples from two different positions within streams. Significant differences between samples from each month are indicated by levels A-D (student's t-test, $p < 0.05$). *insufficient biomass for analysis.

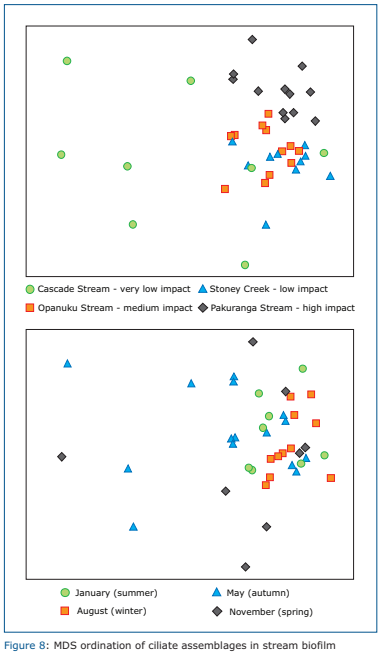

Figure 8: MDS ordination of ciliate assemblages in stream biofilm samples based on T-RFLP profiles, labelled by stream and season. Samples which are more alike cluster together more closely. (2-d stress = 0.19)

Many of the most common peaks were among the largest detected, and were present in a significant proportion of samples from all months. These results suggest the existence of common and resilient ciliates which are present in different seasons and streams, plus less common ciliates with more restricted distributions.

Multidimensional scaling shows evidence of significant differences between ciliate assemblages present in different seasons and different streams (figure 8), confirmed by ANOSIM results (data not shown). Particularly clear differences are seen between samples from the most impacted site compared with those from the less degraded streams. These differences can be linked to environmental parameters characteristic of degraded stream environments, suggesting ciliate communities in the highly impacted stream are skewed towards organisms tolerant of elevated light and temperatures, lowered dissolved oxygen, and increased nutrient and contaminant levels.

Conclusions

Ciliate-specific PCR primers 348F and 1147R were shown to be effective for detection of a broad range of ciliate diversity, and showed the existence of diverse ciliate communities in stream biofilms.

T-RFLP analysis revealed significant differences in biofilm-associated ciliate communities between different seasons and between streams subject to different levels of human impact.

T-RFLP data was an effective method for tracking shifts in ciliate communities and linking differences to environmental parameters.

These methods can be applied to other protozoan phyla, with the potential to contribute to improved understanding of the role of protozoa in aquatic ecosystems.