The Stream Biofilm Project Newsletter 2009

What started out as a study of slime for The Stream Biofilm Project Team gets more fascinating and complex by the day! Our work in the last few months has begun to show fascinating ecological insights into the occurrence and behaviour of bacteria in biofilms, and to yield some real practical tools for measuring ecological impact in streams and piped water systems. We also now have a comprehensive biodiversity description for our sites that span bacteria, protozoa and macrobenthic invertebrates. You'll find this on our stream biofilm website.

Associate Professor Gillian Lewis- Project Leader

Bacterial indicators of stream health

Could bacterial communities replace macroinvertebrate communities as the standard biological indicator of stream health and function? We recently evaluated the reliability of biofilm bacteria as an indicator of freshwater ecological health within a range of Auckland streams. Between streams, differences in the structure of bacterial communities showed similar trends to those observed for macroinvertebrate communities (the current indicator of choice for assessment of stream health). The use of bacterial biofilm communities as a complementary indicator of freshwater ecological health has many advantages as:

(i) Only a very small amount of biofilm is required for bacterial analysis, so many samples can be obtained.
(ii) Biofilm bacteria are remarkably easy to sample.
(iii) Bacterial samples can be removed with minimal site disturbance, allowing repeated sampling at the same location.
(iv) Diverse populations of bacteria are typically present within even the most impacted sites, allowing analysis under conditions where most invertebrate indicators of ecological health are effectively extinct.
(v) Hundreds of samples may be analysed at relatively low cost, within just days. This compares very favourably to high-throughput invertebrate methods.

Fig. 1: Both bacteria (left) and caddisfly larvae (right) are important components of most stream ecosystems.

Are some *E. coli* long term residents of the environment?

Escherichia coli is commonly used in water quality monitoring as an indicator of faecal contamination. While *E. coli* is widely believed to only grow in the gut of vertebrate organisms, some *E. coli* can grow and multiply in water and soils. We are investigating whether a natural environmental population of *E. coli* exists in New Zealand which does not derive from faecal sources and which has evolved without recent contact with a host. Using DNA sequencing, we are monitoring the genetic differences in the core genome of *E. coli* isolates extracted from a wetland, biofilms and from animals. If we find that a natural population of *E.coli* exists outside the gut, more questions will be raised about the implications of these organisms for water quality monitoring.

The effect of heavy metals on biofilm organisms

Stormwater associated metals accumulate quickly in biofilms and impact the bacterial communities. In light of these findings, we have recently investigated potential field applications and been involved in two projects. The first project looked at 20 streams in the Auckland region and the levels of metals associated with sediments and biofilm to assess the correlations between metal levels and microbial (protozoa and bacterial) communities. The second project, run in partnership with the Auckland Regional Council, aimed at testing the efficacy of a stormwater treatment train. These studies show that heavy metals including copper, zinc, cadmium and nickel accumulate to very high levels in biofilms thereby making the biofilm a potentially useful integrated indicator of metal contamination.

The three dimensional structure of stream biofilms.

The three dimensional structure of stream biofilms from the four Auckland streams: Cascades Stream, Stoney Creek Stream, Opanuku Stream and Pakuranga Stream has been assessed by confocal scanning electron microscopy of fluorescently stained preparations. Biofilm was analysed *in situ* on chips of rock or concrete. Rock chips were used so that the biofilm could be viewed in its natural organisation and arrangement. Opanuku Stream biofilm (Figure 2) shows different size clusters of organisms growing in a pillar like formation in dense patches across the rock surface (see 2A,B & C). Similar clusters were also present at Stoney Creek and Cascades Stream. Pakuranga Stream (Figure 3) concrete is covered with dense, more evenly sized clusters of biofilm across the very uneven surface (Figure 3A). These images show that the biofilm in both sites has a complex, non uniform, structure even in winter months.

The biofilm below was stained with the fluorescent dye SYBR green and imaged on a Leica SP2 confocal laser scanning microscope. Image A is looking down on top of the biofilm. Image B and C are slices through vertical and horizontal transects (as shown by grey lines), respectively.

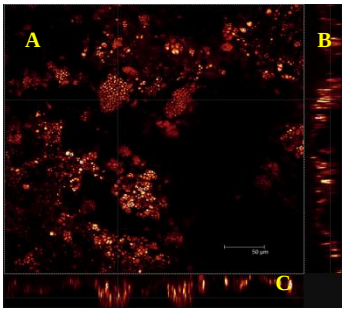

Figure 2: Opanuku Stream biofilm May 2006.

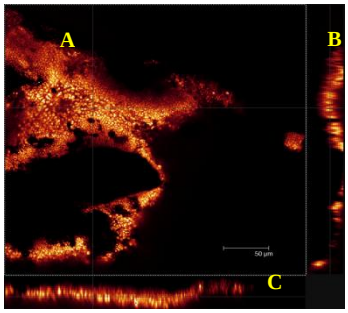

Figure 3: Pakuranga Stream biofilm May 2006.

Feeding time in the biofilm…….

Protozoa eat a lot of bacteria, algae, and other protozoa, and are therefore an important link in stream food webs. How are biofilm communities affected by these feeding interactions? We have been feeding fluorescent biofilm bacteria to ciliate protozoans, to see if the ciliates show selective feeding preferences. Our results suggest that ciliates can be fussy eaters, preferring to eat certain flavours of bacteria over others. Two different types of ciliate—one an active swimmer, the other a crawler—seem to have very different ways of locating bacteria, and different feeding preferences. Bacterial biofilms exposed to grazing by these ciliates respond by developing clumps and channels and increasing in biomass. These observations show that protozoan feeding habits may influence biofilm community composition and structure.

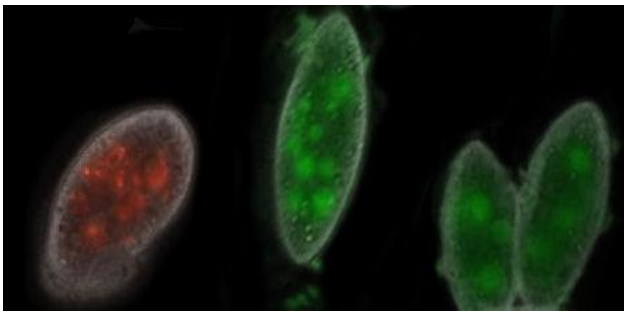

Figure 4: Ciliates feeding upon biofilm bacteria. These bacteria produce red or green fluorescent proteins, which allow us to see how much the ciliates have eaten – the ciliates can squeeze in a lot of bacteria!

Biofilm neighbours: friends or enemies?

The stream biofilm can be likened to a city of microbes where diverse community members survive on limited space and nutrients. Our observations show that there are bacteria which mix well with others but there are also those who do not get on at all! Two such pairs of biofilm bacteria were identified and the interactions between the component bacteria characterized using a novel metabolomic approach based on the analyses of small molecules which are produced and secreted into the growth environment. Analysis of each type of mix showed a different set of chemicals were produced when compared to each bacteria grown on its own. Moreover, novel molecules were produced in the antagonistic pair when compared to the more sociable pair. This observation suggests that molecules produced by the pair were responsible for the reaction observed between the organisms (fig 5). This work suggests that bacteria in the biofilm interact chemically with their neighbours and that these interactions are important to the structure and community composition of the stream biofilm.

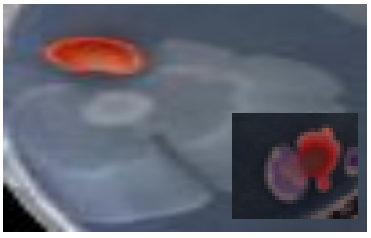

Figure 5:
Biofilm bacteria in pairs showing an antagonistic response and a more sociable reaction (inset)

A biofilm bacteria that builds a home out of slime and manganese!

A newly discovered New Zealand bacterium temporarily named JOSHI_001 deposits manganese and iron oxides in unusual ring structures called anelli and lives inside stream biofilms. A study of 10 urban and 2 pristine streams in Auckland found manganese containing anelli in biofilms from all sites. Anelli were more common in biofilms from streams with more developed catchments, and were the dominant biofilm structure in many of the impacted sites. This suggests that anellus forming bacteria have a competitive advantage within impacted streams. This unusual bacteria appears to have an ecological role in depositing metals from water onto rock surfaces, and potential biotechnological applications in water treatment, bioremediation, and microbial fuel cells. Current work includes investigation of the protein responsible for manganese oxidation and ecological studies. We will also be able- with a grant from the Joint Genome Institute, to sequence the entire genome of JOSHI_001 to provide more insight into this novel, kiwi bacteria!

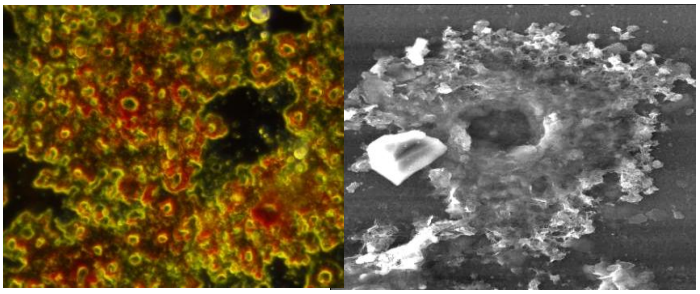

Figure 6: Colonies of JOSHI_001-
Left x 400 Right X 10, 000 magnification.

Conferences and Publications

This has been a **very** productive year for the group with 8 peer reviewed publications and a published conference paper describing our research. We have also completed a study and commissioned report to the Auckland Regional Council using our bacterial methods to study stormwater treatment. We have presented research at 5 international and 4 local conferences with 3 more international 2 national conferences before year end. Most notable was the International Society for Microbial Ecology Conference in Cairns in 2008 where all our students and technicians had a chance to present their work and meet with luminaries in the field. Gillian Lewis has also had several invitations to speak at American Universities and Councils on the study of stream biofilms and there uses in stream restoration and management.

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Want to know more?

If you want to know more about our research, please visit our website: www.streambiofilm.org.nz. If you have any questions, comments or suggestions please contact us at:

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