Biofilm and flow regimes: developing a biological monitoring program for the Nymboida River, northern NSW

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Abstract

A well-designed monitoring program is critical for determining the extent of human impacts and the effectiveness of restoration activities in aquatic ecosystems. This project considers the Nymboida River, northern NSW, as a case study for developing a biological monitoring program. Water extraction from the Nymboida weir pool alters the flow regime to downstream habitats. Algal biofilms, which respond to local hydraulic conditions, are used in this project as biological indicators of response to the altered flow regime downstream of the weir. Longitudinal change on the Nymboida (the difference in biofilm assemblage attributes between upstream and downstream of the weir) was compared with longitudinal change on 'reference' rivers (rivers that do not have a weir and represent the desired condition for the Nymboida given current water and land-use constraints). This design allows us to determine if longitudinal change in biofilm on the Nymboida is greater than what we would generally find on equivalent rivers without a weir. The high variability in biofilm attributes (algal composition, biofilm mass, organic matter content and chlorophyll *a* at monitoring sites is assessed up-front in the design of the monitoring program so that optimum levels of sample replication can be determined. This will ensure that resources can be allocated efficiently while still providing enough information for managers to make informed decisions

Keywords

Biological Monitoring, flow regime, biofilm, variability, survey design, levels of change

Introduction

Increasing human demand on the world's water resources has led to the construction of dams and diversions that cause major alterations to natural flow regimes, with consequent impacts on aquatic biota and valuable ecosystem processes. While this has led to heightened interest and investment in the field of riverine restoration ecology, there remains a clear need for more intensive research to further both our theoretical and practical knowledge (Lake, 2005).

River restoration practices frequently include the prescription of 'environmental flows' that reinstate elements of the natural flow regime. In rivers, the flow regime influences physical habitats, lateral connectivity between the stream channel and floodplain and the life cycles of aquatic species and is therefore a key determinant of species distribution and abundance (Power *et al.*, 1995). Alterations to natural flow regimes can facilitate invasion and establishment of exotic species (Bunn & Arthington, 2002) and reduce the ecological integrity of flowing water systems (Poff *et al.*, 1997). While these impacts are increasingly recognised, there remains the need to better quantify biotic responses to altered flow regimes and assess the success of attempts at restoring natural flow regimes (Bunn & Davies, 2000).

On the Nymboida River, north-coast NSW, water has been extracted from the weir pool for hydro-electric power generation for more than 80 years. This alters the flow regime downstream of the weir by increasing the frequency and duration of low-flow conditions, potentially impacting ecological communities in downstream habitats. In 1997, flow protection rules were implemented to limit extraction during naturally 'low-flow' periods with the hope that restoring a more natural flow regime will improve the overall condition of the river. A biological monitoring program is being developed to assess the current condition of the Nymboida (i.e. whether or not it is different from the target/reference condition – see below) and any

future improvements under the new flow rules (i.e. is the Nymboida becoming more similar to the target/reference condition).

Biofilms are the assemblages of algae, bacteria, fungi, silt and detritus in submerged habitats (Burns & Ryder, 2001) and are being investigated in this project as a potential indicator of river condition. Through their substantial contribution to primary production in many river systems, algal biofilms can represent a major energy source in aquatic food webs (Lamberti, 1996). Local hydraulic conditions (e.g. water velocity) can alter the abundance and type of algae, the overall mass of biofilm and the amount of accumulated silt on the rock surface making biofilms a potentially valuable indicator of change in response to flow regime.

Important to the success of any restoration activity is a well-designed monitoring program that is capable of detecting directional change in river condition as environmental flow rules are implemented. Three of the key steps in designing an effective monitoring program are to i) set restoration targets against which condition of the Nymboida can be determined, ii) develop a survey design and statistical model that allow the data to be interpreted unambiguously and iii) assess natural variability in biological communities to ensure adequate sample replication (Downes *et al.*, 2002). This paper discusses these issues and provides examples from the Nymboida River project.

Setting restoration targets

Undisturbed reference sites can provide a target condition for restoration efforts when historical information is lacking (Palmer *et al.*, 2005). Ideally, reference sites are chosen to represent the state of the river in the absence of human interference (Downes *et al.*, 2002). However, such undisturbed sites are often unavailable and target conditions may need to be determined by other means. In this scenario, 'reference' rivers may be selected to represent a particular desired state (which may not necessarily be representative of what was before) and/or consultation with stakeholders and interest groups may be used to identify public values and help guide the restoration targets (Downes *et al.*, 2002).

The Nymboida River is situated on the lower slopes of the ranges on the north-coast of NSW. Cattle-grazing is the main land-use in the Nymboida valley and in nearby valleys across the region. Accordingly, no reference rivers are available that might represent the natural state of the Nymboida in the absence of human impacts. Therefore, we have worked in consultation with a River Monitoring Committee (a committee consisting of representatives from local industries (including our industry partner North Coast Water), government agencies, local landholders and other interest groups) to select three rivers from the NSW north coast to represent realistic targets for the Nymboida River, given regional constraints of land-use. Although not pristine, these 'reference' rivers were selected to be representative of the desired future condition of the Nymboida.

By monitoring biofilm variables over time, we will be able to determine if the Nymboida River is becoming more similar to the 'reference' rivers for biofilm variables (but see Downes *et al.*, 2002 for a discussion of the statistical/inferential difficulties associated with assessing restoration 'success'). We will also be able to determine if any changes in biofilm assemblages on the Nymboida occur concurrently with flow restoration. It should be noted, however, as suitable 'control' rivers do not exist (i.e., similar rivers with a weir and extraction, but without flow restoration measures), we will not be able to draw firm conclusions on the relationship between flow restoration and biofilm response.

Is the Nymboida similar to reference rivers?

Preliminary surveys have been conducted to assess whether the Nymboida River is similar to the reference rivers for several biofilm variables. Biofilm assemblages at sites downstream of the weir were assessed against sites upstream of the weir, where the flow regime is unaltered. To discriminate between the 'potential' effects of flow modification and natural longitudinal change (Downes *et al.*, 2002), comparisons were made between the Nymboida and the 3 nearby reference rivers, also with upstream-downstream reaches but without any major alterations to the natural flow regime. All reference river catchments were located on the NSW north coast in close proximity to the Nymboida so that rainfall events and other climatic conditions are similar among catchments. No major tributaries enter the rivers between the most upstream and most downstream sites, ensuring a consistent flow regime between reaches. The length of reaches and distance

between reaches was as similar as possible for each river with all reaches being approximately 5-10km long the distance between reaches being 8-13km on all rivers.

In designing this survey we have attempted to detect any changes in biofilm assemblages that correlate with changes in flow regime between upstream and downstream of the weir. However, we refer to 'potential' effects of flow modification because, at this stage, we are unable to determine conclusively if flow modification *causes* differences in biofilm assemblages between upstream and downstream of the weir, or if changes in biofilm assemblages are *co-incident* with changes in flow regime. While there are no other obvious environmental shifts between upstream and downstream of the weir, we can not rule out the possibility that something other than the change in flow regime is driving biofilm response.

The survey design is illustrated in Figure 1. An upstream and downstream reach was sampled on each river. Three sites (riffles) were sampled in each reach and 20 cobbles were sampled from each of 3 habitats (edge, riffle and glide) in each site for biofilm variables including total dry mass. Biofilm samples were collected from the upper surface of 20 cobbles per habitat per site by scrubbing the upper surface of each cobble with a soft toothbrush into 200ml of distilled water. The upper surface of each cobble was wrapped in foil and excess foil cut away. The relationship between foil weight and area was developed to calculate the upper surface area of each cobble. Each biofilm sample was analysed for total dry mass by drying the sample at 80°C to constant mass and then weighing it.
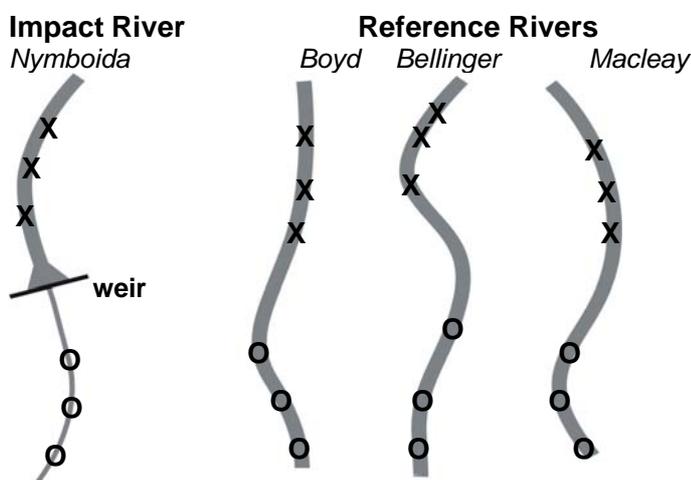

Figure 1. Survey design for assessing longitudinal change on the Nymboida River (with a weir) compared with longitudinal change on reference rivers (without a weir) (see text for more details). X, upstream sites; O, downstream sites.

An asymmetrical Analysis of Variance (ANOVA) model (Underwood, 1993) and a sequence of statistical tests can be applied to the above design to determine if longitudinal change on the Nymboida is greater than what we would generally find on the reference rivers. Figure 2 and Table 1 show results from the first sampling event in August 2005 as an example of the type of results we might expect. These results are for biofilm mass in the shallow edge habitat. There are overall differences among rivers in the total mass of biofilm, however, the pattern *within* a river is inconsistent (Figure 2). That is, the effect of 'Reach' (being an upstream or downstream reach) depends on which river we look at, indicated by the significant interaction term 'Reach x River' in the ANOVA (Table 1). More specifically, there is no difference between upstream and downstream reaches on the reference rivers, but a significant difference between reaches on the Nymboida (as shown by further breaking down the 'Reach x River' term in the analysis using *a priori* contrasts). In other words, we found a statistically significant increase in the mass of biofilm downstream of the weir on the Nymboida, but no difference between upstream and downstream reaches on the reference rivers.

The increase in biofilm mass downstream of the weir on the Nymboida correlates with an increase in the frequency and duration of low-flow conditions (flows <80th percentile flows) downstream of the weir. This pattern is consistent with other studies (e.g. Clausen & Biggs, 1997) that have found stable flows and low velocities to support higher average biofilm mass compared with those that fluctuate greatly. These data support the model that the altered flow regime on the Nymboida has affected biofilm assemblages and long-

term monitoring should help us determine if this condition is persistent or improving with time (i.e. becoming more similar to the reference rivers).
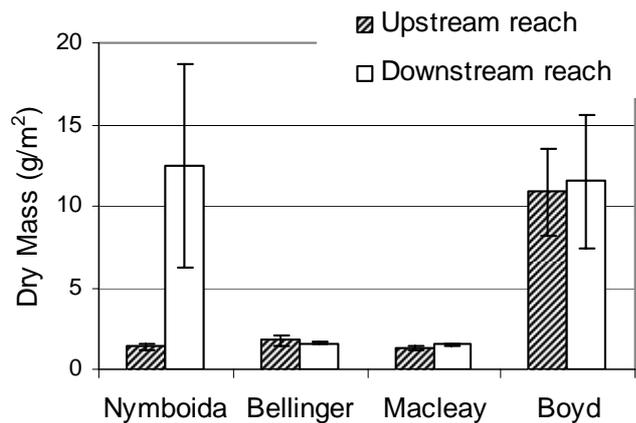

Figure 2. Total biofilm dry mass (gm^{-2}± S.E., n=3 sites per reach) on cobbles in the shallow edge habitat in upstream and downstream reaches of the Nymboida River and 3 reference rivers.

Table 1. Analysis of Variance with *a priori* contrasts within the ReachxRiver term showing: [1.] **the relationship between upstream and downstream reaches depends on the river being considered;** [2.] **reference rivers were consistent in the relationship between upstream and downstream reaches for biofilm mass;** [3.] **the relationship between upstream and downstream reaches on the Nymboida was different from the reference rivers.**

Source of Variation	Df	Mean Squares	*F*-ratio	*P*
River	3	9.72	15.84	
Reach(upstream vs downstream)	1	2.70	4.40	
Reach x River [1]	3	2.58	4.20	*
among reference rivers [2]	2	0.04	0.07	ns
Nymboida vs reference rivers [3]	1	7.65	12.47	**
Site(Reach x River)	16	0.61	15.76	**
Residual	450	0.04		

Df, degrees of freedom; *P,* probability; ns non-significant, *p<0.05, **p<0.01

Biofilm variability, effect sizes and replication

As with many ecological communities, biofilm assemblage composition can vary considerably in space and fluctuate in time. At the cobble scale, biofilm composition can be influenced by local hydraulic conditions and grazer densities while within a riffle or pool, shading, localised nutrient inputs and substrate size/stability can alter assemblages (Burns & Ryder, 2001). Variability is important in maintaining the complexity and integrity of aquatic ecosystems (Poff *et al.*, 1997; Power *et al.*, 1995) and reinstating this dynamic condition should be incorporated into restoration targets (Palmer *et al.*, 2005). However, high variability can decrease our ability to detect changes in the system and therefore has important implications for the monitoring program design, particularly regarding levels of replication for sample collection and processing. Before finalising the sampling design for a monitoring program, it is important to consider effect size or % change in the system that we are aiming to detect (e.g. 20% increase in biofilm dry mass), which also influences levels of replication. The following sections provide a discussion of the relationship between variability, effect sizes and replication using data from the Nymboida River. In the context of the monitoring program, these data help determine the precision with which we can estimate biofilm characteristics at each site.

Quantifying algal community composition
Algal biofilms often show high taxonomic richness relative to other aquatic groups (Lowe & Pan, 1996) and measures of taxonomic richness show potential as indicators of a change in river condition (Chessman *et al.*, 1999). High variability in taxonomic composition can be an intrinsic characteristic of the population as well as an artefact of the methods we use to quantify composition. By assessing this variability up-front, adequate replication and appropriate quantification techniques can be ensured. To illustrate this, we sampled

algae from 10 cobbles (samples) from a shallow pool on the Nymboida River. Cumulative algal richness (to genus level) was recorded for counts of 100, 250, 500, 750 and 1000 algal cells of each sample (Figure 3). These data show that the way in which we quantify taxonomic richness (e.g. how many algal cells we count and from how many samples) can have a considerable effect on the interpretation of monitoring data. While there seems to be little benefit in counting large numbers of algal cells per sample (>500 cells), counting too few cells per sample could grossly underestimate taxonomic richness, particularly for small sample sizes. Note, in this example we used a single sub-sample from each biofilm sample, but a similar process could be used to determine the required number of sub-samples per sample for a given level of precision.
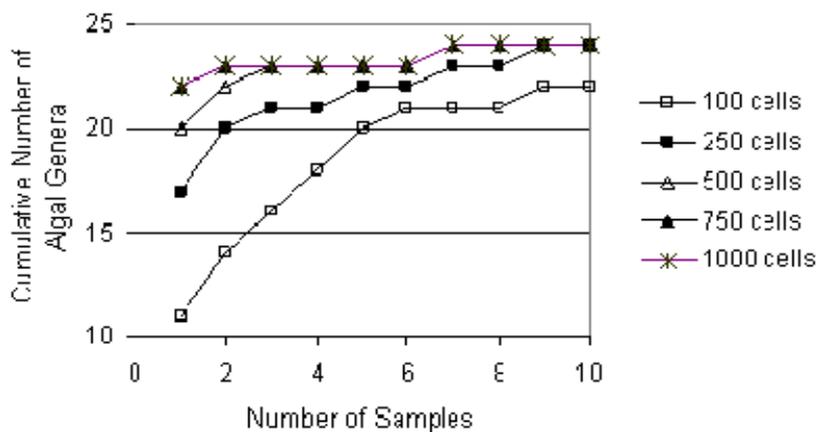

Figure 3. Effect of cumulative sample size and cell counts per sample on measures of algal genus richness.

Biofilm assemblage structure
In addition to algal community composition, structural attributes such as biofilm mass, percent organic matter and algal biomass (chlorophyll *a*) are commonly used as coarse measures of biofilm composition. The sample size required to detect a given effect size will depend on the biofilm attribute being considered and, therefore, required sample sizes are seldom the same for each attribute. Calculations for sample sizes should also be based on sites with the highest variability (assuming data from all sites are normally distributed) so that sample sizes for a given effect size are calculated conservatively, allowing data to be interpreted with greater confidence. Figure 4 is based on data from biofilm samples from cobbles in slow-flowing riffles (water velocity 0.25-0.55m/s) on the Nymboida and shows the relationship between detectable effect size versus the number of samples per site. Two biofilm attributes (chlorophyll *a* and total dry mass) and two sites on the Nymboida River (one site with high variability in these attributes and one with low) are shown. While the relationship between detectable effect size and number of samples (shape of the curve) appears similar for the two attributes/sites, the actual number of samples required to detect an effect size of, for example, 50% varies from 6 to 20, depending on the site and attribute considered.
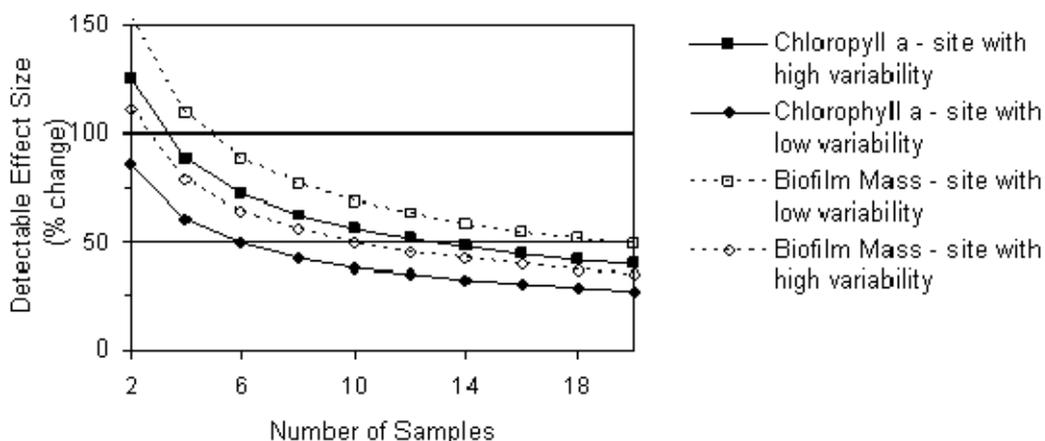

Figure 4. Relationship between detectable effect size and the number of samples collected per site for chlorophyll a (at one site with high variability and one with low variability) and biofilm mass (also at one site with high variability and one with low variability). For this example, α=0.05 and β=0.20.

Wilson, A.L., Dehaan, R.L., Watts, R.J., Page, K.J., Bowmer, K.H., & Curtis, A. (2007). Proceedings of the 5th Australian Stream Management Conference. Australian rivers: making a difference. Charles Sturt University, Thurgoona, New South Wales.

Conclusions

This study demonstrated an increase in biofilm mass on the Nymboida River downstream of the weir, which was greater than longitudinal change on reference rivers. This pattern correlates with an increased frequency and longer duration of low-flow conditions downstream of the weir and is consistent with other studies relating biofilm mass and flow regime (e.g., Clausen & Biggs, 1997). While there is growing interest in applying restoration techniques to reduce or reverse the human-induced degradation of aquatic ecosystems, scientific knowledge on how best to achieve this is still extremely limited (Palmer *et al.*, 2005). The Nymboida River project provides an opportunity to firstly assess the current condition of the river after 80 years of water extraction, and secondly, to monitor change in the river under the current flow protection rules. The use of reference rivers in the design of the monitoring program ensures that we have a clear image of a target condition for the Nymboida. Assessing variability and determining adequate replication up-front, will ensure the monitoring program will lead to reliable, or at least unbiased, interpretation and management decisions with known risks (Downes *et al.*, 2002). It will also ensure that resources can be allocated efficiently while still providing enough information for managers to make informed decisions.

References

Bunn, S. E., & Arthington, A. H. (2002). Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. *Environmental Management, 30*(4), 492-507.

Bunn, S. E., & Davies, P. M. (2000). Biological processes in running waters and their implications for the assessment of ecological integrity. *Hydrobiologia, 422/423*, 61-70.

Burns, A., & Ryder, D. S. (2001). The potential for biofilms as biological indicators in Australian riverine systems. *Ecological Management and Restoration, 2*(1), 53-63.

Chessman, B. C., Growns, I., Currey, J., & Plunkett-Cole, N. (1999). Predicting diatom communities at the genus level for the rapid biological assessment of rivers. *Freshwater Biology, 41*, 317-331.

Clausen, B., & Biggs, B. J. F. (1997). Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology, 39*, 327-342.

Downes, B. J., Barmuta, L. A., Fairweather, P. G., Faith, D. P., Keough, M. J., Lake, P. S., Mapstone, B. D., & Quinn, G. P. (2002). *Monitoring ecological impacts: concepts and practice in flowing waters.* Cambridge: Cambridge University Press.

Lake, P. S. (2005). Perturbation, restoration and seeking ecological sustainability in Australian flowing waters. *Hydrobiologia, 552*, 109-120.

Lamberti, G. A. (1996). The role of periphyton in benthic food webs. In R. J. Stevenson, M. L. Bothwell & R.L. Lowe (Eds.), *Algal ecology: freshwater benthic ecosystems* (pp.533-572).San Diego:Academic Press.

Lowe, R. L., & Pan, Y. (1996). Benthic algal communities as biological monitors. In R. J. Stevenson, M. L. Bothwell & R. L. Lowe (Eds.), *Algal ecology: freshwater benthic ecosystems* (pp. 705-733). San Diego: Academic Press.

Palmer, M. A., Bernhardt, E. S., Allan, J. D., Lake, P. S., Alexander, G., Brooks, S., Carr, J., Clayton, S., Dahm, C. N., Follstad Shah, J., Galat, D. L., Gloss, S., Goodwin, P., Hart, D. D., Hassett, B., Jenkinson, R., Kondolf, G. M., Lave, R., Meyer, J. L., O'Donnell, T. K., Pagano, L., & Sudduth, E. (2005). Standards for ecologically successful river restoration. *Journal of Applied Ecology, 42*, 208-217.

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., Sparks, R. E., & Stromberg, J. C. (1997). The natural flow regime: a paradigm for river conservation and restoration. *BioScience, 47*, 769-783.

Power, M. E., Sun, A., Parker, G., Dietrich, W. E., & Wootton, J. T. (1995). Hydraulic food-chain models: an approach to the study of food-web dynamics in large rivers. *BioScience, 45*(3), 159-167.

Underwood, A. J. (1993). The mechanics of spatially replicated sampling programmes to detect environmental impacts in a variable world. *Australian Journal of Ecology, 18*, 99-116.