

**MONITORING THE IMPACT OF WASTEWATER  
TREATMENT PLANT EFFLUENT ON THE WATER  
QUALITY AND BIOLOGICAL COMMUNITIES OF  
RECEIVING ENVIRONMENTS**



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# MONITORING THE IMPACT OF WWTP EFFLUENT ON THE WATER QUALITY AND BIOLOGICAL COMMUNITIES OF RECEIVING ENVIRONMENTS

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## ABSTRACT

Monitoring of aquatic macroinvertebrates has become a requirement of Victorian EPA treatment plant licences where point source discharges are released to streams, rivers and lakes. Macroinvertebrates such as mayflies and mudeyes are well known to anglers, are visible to the naked eye and are commonly found in rivers and streams. They are considered to be an excellent and cost-efficient indicator of river health.

Strict EPA protocols exist regarding the minimum effort required by treatment plant operators for in-stream monitoring and these are briefly introduced. These monitoring protocols outline appropriate sampling methodologies, site selection criteria and methods of data analysis such as SIGNAL and AUSRIVAS. Despite such directives it remains critical that studies are specifically designed to determine differences between macroinvertebrate communities upstream and downstream of treatment plants. Examples of such studies are given. The issues associated with the introduction of this new protocol from the project design to implementation and final feedback stage are outlined. The importance of baseline monitoring prior to treatment plant upgrade is also discussed.

## 1.0 INTRODUCTION

The Environment Protection Authority has developed clear protocols for biological monitoring of freshwater environments that receive wastewater effluent from treatment plants. These protocols are outlined in EPA Publication Number 441 (1995a). To summarise briefly, the protocol requires a minimum of 2 to 4 sites immediately upstream (control effect) and a minimum of 3-6 sites downstream of the treatment plant outfall (treatment effect) in the receiving waterway (EPA, 1995a). These sites represent “replicate” units, although, as will be discussed, there are statistical limitations on such an experimental design. The appropriate biological indicator should be selected for the study, which is dependent on factors such as individual features of the discharge (e.g. timing and amount), characteristics of stream morphology and whether there are existing or potential threats to the aquatic ecosystem (Environment Protection Authority 1998b).

In most cases the biological indicator used would either be macroinvertebrates, diatoms or a combination of the two<sup>1</sup>. Biological sampling in these studies conforms to the Rapid Bioassessment Methodology (RBA) outlined in EPA Publication 604 (Environment Protection Authority 1998a). This is a nationally adopted protocol that collects *qualitative* biological data, where the purpose is to sample the widest variety of community taxa permissible. In many circumstances there is a need to modify the RBA methodology to invoke a *quantitative* approach that relates abundance to other measures such as density or biomass such that parametric statistics may be used to identify significant effects on river health of treatment plant operation. Modifications to the RBA methodology are made by consultation between the researcher, the EPA and the treatment plant operator (water authority). Where possible a variety of habitats should be sampled (e.g. riffles, pools etc.) as community composition, and thus sensitivity, may vary between habitats.

Simultaneous physico-chemical sampling at all sites is also recommended in such studies. All physico-chemical water samples and *in-situ* measurements need to be undertaken monthly (when discharging). Stream and effluent discharge volume must be measured daily. Environmental parameters of importance include nutrients, suspended solids, *Escherichia coli* concentrations, dissolved oxygen, pH, flow velocity, electrical conductivity and biological oxygen demand (BOD). The *SEPP (Waters of Victoria)* (Government of Victoria 1988) sets water quality objectives for all general surface waters in the State, and provides a Statewide policy framework for several catchment-specific policies. Where numerical nutrient objectives are not provided in a SEPP, guideline concentrations from *Preliminary Nutrient Guidelines for Victorian Inland Streams* (Environment Protection Authority 1995b) will be used.

To conform with RBA methods, a minimum of two seasons (autumn and spring usually) of biological sampling is recommended to account for temporal variation in community composition, life history and stream discharge (Environment Protection Authority 1998a). In an ideal world it is recommended that biological monitoring commence two years prior to the implementation of a treatment plant. Where upgrades are made to existing treatment plants, biological monitoring should be undertaken at least one year prior to the upgrade and continue thereafter (Environment Protection Authority 1995a).

Freshwater macroinvertebrates are a group of animals visible to the naked eye which reside in aquatic environments and include insects, worms, leeches, crustaceans and snails. Macroinvertebrates are a cost-efficient indicator of river health, and an ideal biological monitoring tool in that:

- ◆ they have differential susceptibility to different pollutants,
- ◆ they have short life cycles, and therefore many life stages (e.g. larvae, pupae, adults) may be studied in a short period of time,
- ◆ they are relatively immobile, and are therefore unable to escape the effects of instream pollution stresses,
- ◆ they are relatively easily sampled, and
- ◆ they usually occur in great diversity and numbers (Davey, 1980).

Perhaps the greatest advantage in using freshwater macroinvertebrate communities in the assessment of river health is that these fauna are extremely sensitive to changes in water quality, and ecological responses to environmental stresses may be seen over a very short time-scale (Cairns & Dickson 1971). Macroinvertebrate communities also provide a continuous record of environmental degradation, as opposed to snap-shot physico-chemical sampling of surface waters.

Macroinvertebrates are also biologically important in that they are a major component of freshwater environments and are important food resources for fish, amphibians and waterfowl (Cairns & Dickson 1971). Macroinvertebrate communities also have important functional roles within streams with respect to nutrient cycling (Vannote et al. 1980) and organic matter processing (Wallace et al. 1977, Ward 1989).

The following discussion will outline a number of the methods used by qualified aquatic ecologists to identify the effects of wastewater from treatment plants on river health, including the use of biological indicators and univariate/multivariate statistical tools. Examples of results will be presented where appropriate.

## 2.0 DISCUSSION

The purpose of such studies is to compare macroinvertebrate community structure and water quality between upstream (control) and downstream (impacted) sites to evaluate a *treatment* effect. All analyses take into consideration habitat variations between sites and seasonal variation, including flows. Therefore selection of suitable sites must be made that reduce confounding variables such as the presence of incoming storm water drains or stream tributaries, and differences in stream morphology such as stream substrate composition and riparian stream cover. Interpretation of the data is assisted by numerical analyses, including parametric univariate analyses such as Analysis of Variance and BACI designs, exploratory multivariate statistics such as classification and ordination, and also by indices including SIGNAL (Stream Invertebrate Grade Number Average Level, Chessman 1995) and by predictive models, for example, AUSRIVAS (AUSTRALIAN RIVER Assessment System, Simpson et al. 1997). These are discussed briefly with some examples below. Interpretation must assess results against ecological (e.g. waterways in the Yarra River Catchment, Environment Protection Authority 1999) and water quality objectives (SEPP guidelines, Victorian Government 1988).

### ***SIGNAL Score***

The SIGNAL score is a biotic index which uses all community data to produce a score between 0 and 10 that reflects the degree of water pollution determined by the presence or absence of macroinvertebrate families of a known tolerance or intolerance to pollutants. This is an easily used tool for water managers. SIGNAL scores below 4 indicate probable severe pollution, scores of 4-5 indicate probable moderate pollution, scores of 5-6 indicate doubtful quality, possible mild pollution and scores greater than 6 suggest clean water status (Chessman 1995). Specific SIGNAL scores have been developed for the Yarra River catchment (Environment Protection Authority 1999) and, in draft form, for the Western Port catchment (Environment Protection Authority 2000).

### ***AUSRIVAS Model***

AUSRIVAS is a community modelling tool which predicts which macroinvertebrate families should be present in specific stream habitats under reference conditions. It does so by comparing test data (in this case from upstream and downstream sites of the treatment plant) with a group of reference sites which are as free as possible of environmental impacts but with similar morphological characteristics. A ratio is calculated which expresses the observed number of families found at a test site against the expected number of families found under reference conditions, and this is known as the O/E Index. O/E scores may be compared to bands representing different levels of biological condition, as recommended under the NRHP (Barmuta et al. 1997). Given the confounding environmental effects associated with urban streams, such an approach would best suit regional waterways with treatment plants.

### ***Univariate Statistics***

Analysis of Variance (ANOVA) models may be used to evaluate treatment effects of wastewater release. Typically all environmental parameters and summary biological parameters such as total numbers of individuals, SIGNAL score total numbers of families and numbers of key macroinvertebrate families (as identified by SIGNAL) are used as the independent variable in these analyses. The statistical test is conducted to evaluate whether any of these parameters is statistically significant between upstream and downstream sites, and given an adequate experimental design, these significant differences may be attributed to the wastewater release. The example below (Table 1) includes some hypothetical environmental data from the Goulburn River catchment.

It may be seen that both the tributary which receives wastewater, and the main waterway this tributary discharges into have been monitored. This may be recommended in the experimental design if the treatment plant is situated on a small tributary in close proximity to a major waterway. In this case multiple sites upstream and downstream of the tributary on the Goulburn River were also sampled to evaluate whether the treatment plant was also having an effect on the major waterway. The ANOVA results include statistical tests conducted on total nitrogen, total phosphorous and dissolved oxygen concentrations. It may be seen that on Hughes Creek there were significant effects (at a confidence limit of 95%) for all parameters with respect to treatment (control vs. impacted sites) and season (spring vs. summer), with no significant interaction effect between season and treatment.

To summarise this result, we could say total N, total P and dissolved oxygen were:

- ◆ significantly affected by the treatment plant,
- ◆ significantly affected by seasonal variation, and
- ◆ effects did not vary between seasons and remained consistent.

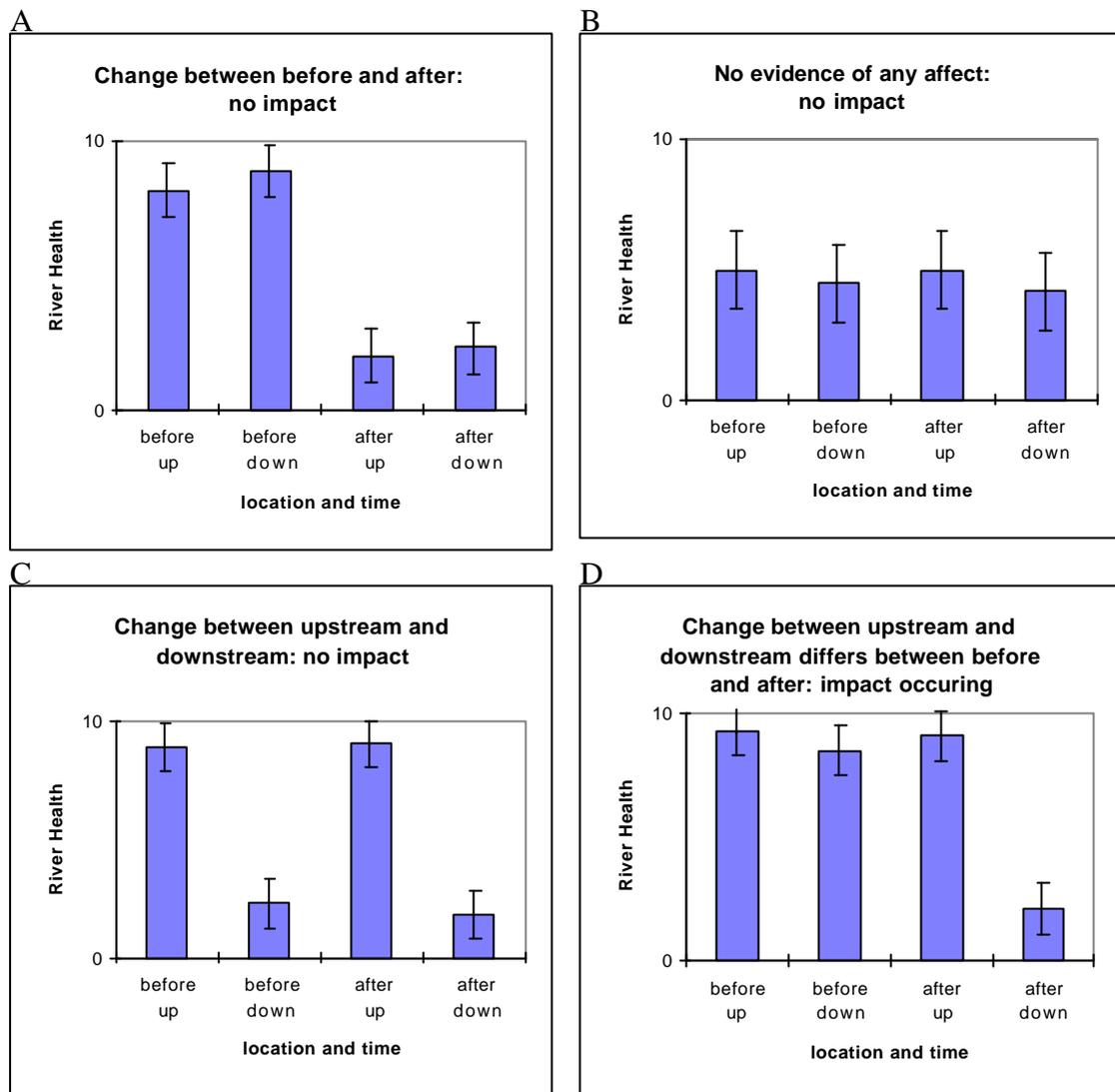
**Table 1:** *Hypothetical ANOVA Results*

Transformation Test	Total N		Total P		DO	
	None		Log 10		Log 10	
	F	P	F	P	F	P
<b><i>Hughes Creek</i></b>						
Season	10.352	0.006	129.831	0.000	18.191	0.001
Treatment	13.762	0.000	177.918	0.000	8.677	0.004
Season * Treatment	0.199	0.822	1.922	0.183	0.657	0.534
<b><i>Goulburn River</i></b>						
Season	19.691	0.004	1.345	0.290	74.360	0.000
Treatment	0.491	0.510	0.413	0.544	1.118	0.331
Season * Treatment	0.055	0.823	0.000	0.992	3.017	0.133

### ***BACI Designs***

Whilst this design will produce results that can be used by the ecologist, there is a very important experimental design violation inherent in this study which is prevalent in all such studies. The design is “pseudoreplicated” (Hurlburt 1984) because we have no real way of knowing this result is not the effect of simple longitudinal variations along the waterway of these parameters as we only have one experimental unit (the creek). In an ideal world the design would incorporate a number of morphologically similar creeks in the immediate geographic area, each with treatment plants. Such a design is obviously not possible in the real world, so this violation is often overlooked. There are, however, ANOVA methods which may be invoked to overcome pseudoreplication (or lack of spatial independence) if studies are concerned with treatment plants that will only come online in the future. BACI (Before-After-Control-Impact) designs (Underwood 1993) use monitoring data from upstream and downstream sites collected prior to treatment plant construction and discharge, and then compare these differences with upstream and downstream sites monitored after discharges have commenced. In this example the interaction term (time \* treatment) is the important test statistic. If this term is significant it may be inferred that spatial effects between upstream and downstream only occurred once discharge had been initiated, and thus treatment effects are attributed to the treatment plant. Hypothetical data is presented in Figure 1 which illustrates the possible outcomes of a BACI design on river health before and after discharge has commenced.

**Figure 1:** *A Set Of Hypothetical Results From A BACI Design. Note: Only in Graph D is there evidence that an impact is occurring.*

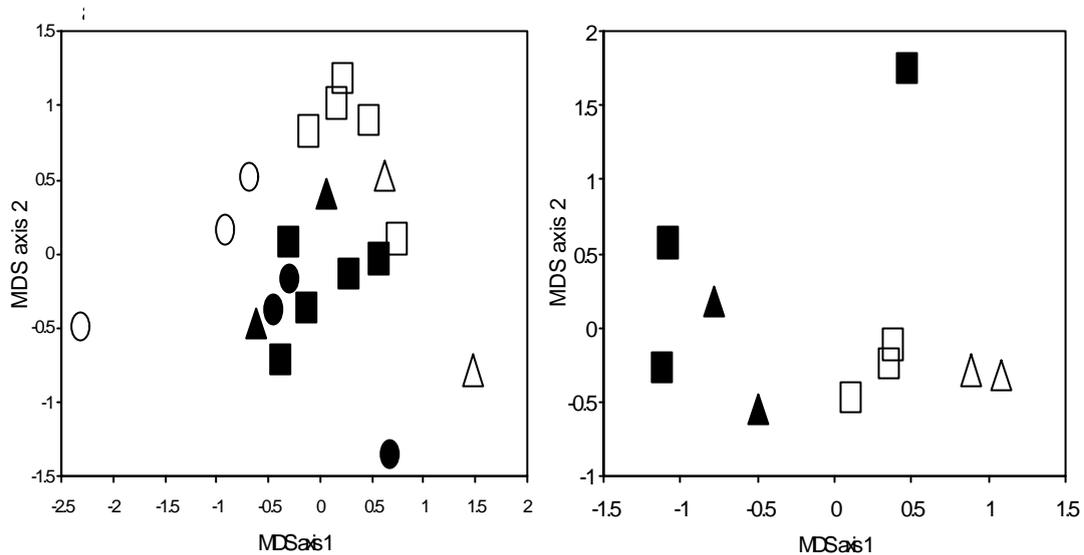


### ***Multivariate Analysis***

Exploratory data techniques such as clustering and ordination may be used to find spatial patterns between biological community data and environmental data. Whilst these techniques are not as statistically reliable and capable of producing discrete results as univariate techniques, they are capable of summarising large sets of data and are useful for catchment level interpretation in particular. Results from an ordination are presented in Figure 2. The ordination technique calculates what is known as a dissimilarity matrix which summarises multivariate data as distances between samples. These distances may then be plotted as axis scores on an ordination graph and this is the equivalent of expressing these distances as would be done on a spatial map. Spatial patterns may then be discerned, for example, in the ordination to the right in Figure 2 there is a clear separation of seasons on Axis 1, whilst upstream and downstream sites during each season are separated secondarily on Axis 1 as well. In some cases the axes reflect different patterns, for example Axis 1 may discriminate seasonal differences while Axis 2 may discriminate between upstream and downstream sites.

Although not presented in the example in Figure 2, environmental data may also be fitted to the ordination space, and through statistical simulations it is possible to identify significant environmental parameters which are responsible for the spatial patterns in the biological data. These may be fitted as vectors to the ordination graph. This multivariate approach becomes a more interpretative method than univariate analysis and is dependent on the ecologists ability to make sense of environmental and biological patterns with respect to the operations of the treatment plant.

**Figure 2:** *An example of biological ordination results using hypothetical community data.*



Each symbol represents one sampling event per season, the shapes represent upstream, downstream or recovery sites, and filled or unfilled shapes represent season.

Other techniques such as Analysis of Similarity (ANOSIM) are multivariate equivalents of the univariate ANOVA models which may identify treatment effects. Key macroinvertebrate indicator families may also be identified by SIMPER analysis which determines which taxa are responsible for observed differences between upstream and downstream sites (Clarke & Warwick 1994).

### 3.0 CONCLUSIONS

The main “take-home” messages for treatment plant operators from this paper are:

- ◆ Biological monitoring is required for all streams receiving wastewater discharges from treatment plants in Victoria, as stipulated by the Environment Protection Authority (1995a)
- ◆ The Environment Protection Authority (1995a) has identified a strict protocol for the minimum sampling effort, methodology and analysis required where treatment plants are discharging. In many cases these may be varied to account for local factors such as proximity to major waterways or if treatment plants are situated in estuarine or intermittent stream systems. In all cases it is critical that studies are specifically designed to evaluate treated effluent effects.

- ◆ The most common biological indicators used in such studies are macroinvertebrates and diatoms, which are relatively easy and inexpensive to sample.
- ◆ A wide range of ecological and statistical tools are available to identify where significant effects of treatment plant operations occur. Where these occur management decisions must be adopted to minimise environmental stress, which may include disposal to drainage basins or plantations, or alternate timing of effluent disposal to take into account biological community factors and flow discharge patterns.

#### 4.0 END NOTE

<sup>1</sup> For the purpose of this paper the discussion will be restricted to focus on macroinvertebrates only. Diatoms, however, are particularly suited to assessing nutrient effects as their species distributions react directly to nutrient concentrations (Environment Protection Authority, 1998b)

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