



**Effects of suspended
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Kakadu National Park**

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September 1997



**EFFECTS OF SUSPENDED SOLIDS
ON BENTHIC MACROINVERTEBRATE FAUNA
DOWNSTREAM OF A ROAD CROSSING,
JIM JIM CREEK, KAKADU NATIONAL PARK**

by

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**A thesis submitted in partial fulfilment of the requirements
for the degree of Honours in Applied Science.**

**University of Canberra
June 1997**

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ACKNOWLEDGEMENTS

I would especially like to thank the following people for their assistance with this project:

My supervisors, Chris Humphrey (*eriss*, Wetland Protection and Management Section) and Richard Norris (CRC for Freshwater Ecology, University of Canberra); for providing a constant source of guidance, advice and encouragement.

The Parks Australia (ANCA) Kakadu National Park staff for instigating and funding this project; and everyone at the Jim Jim Ranger Station, for their generous assistance and co-operation.

Rebecca Bennett, Stacey Braund, Ray Hall, Bill MacFarlane, Vicki Lee, Mal Merrett, Graham Loewenthal and Sam Scoofs assisted with field work. The *eriss* biomonitoring group of Ruth O'Connor, Lisa Thurtell, Barbara Klessa, Cate Lynch and David Norton provided a vital source of assistance and advice on everything from bug identification to data analysis. Bob Pidgeon and James Boyden of *eriss* complimented this study with fish surveys and assisted with the preparation of reports. Rebecca Bennett performed much of the water chemistry analysis; Peter Cusbert compiled and verified water chemistry results. Wayne Robinson, Patrick Driver and Phil Sloane at the CRCFE assisted with data analysis.

And finally, Mum and Dad for all their support and encouragement throughout my studies.

ABSTRACT

Tourist vehicle traffic using a seasonally-accessible, unformed road crossing on the upper reaches of Jim Jim Creek in Kakadu National Park, has caused a seasonal elevation in turbidity (as a result of suspended sediment) downstream of the road crossing in recent dry seasons. A modified BACIP experimental design was used to investigate the effects of this disturbance on the benthic macroinvertebrate fauna (at family-level) downstream of the road crossing. Paired sites in both Jim Jim Creek (upstream and downstream of the road crossing) and Twin Falls Creek (a control stream, with analogous but undisturbed upstream and downstream sites) were sampled for a period before and after the seasonal opening of the road crossing to public access. Turbidity levels peaked one month after the road opened, reaching an average maximum of 60 NTU (~100 mg/L suspended solids) 200 m downstream of the crossing and 30 NTU (~17 mg/L suspended solids) 1000 m downstream of the crossing. Although temporal autocorrelation of the BACIP data prevented statistical analysis by conventional inferential procedures, a technique of modelling the temporal trend in the data by introducing a covariate, stream discharge, was used. This enabled inferences to be drawn about impact-related effects in Jim Jim Creek after the onset of the disturbance.

Turbidity-related effects were evident in macroinvertebrate communities of rootmat samples collected 200 m downstream of the road crossing, whilst 1000 m downstream of the crossing, there was evidence of only some slight changes to communities. The changes observed were most evident in multivariate analyses, viz paired site dissimilarity measures and ordination. However, associated with these observed changes was a noticeable reduction in total macroinvertebrate abundance, attributed mostly to a decline in the abundance of Chironomidae at downstream Jim Jim Creek sites compared to control sites. Although the effects were limited in extent, they are cause for concern given the exceptional conservation value of the study area.

The modified approach to BACIP analysis, modelling a covariate of natural temporal change in dissimilarity data, was shown to be a valuable, cost-effective means of detecting impacts in a situation where the period of time available for monitoring was necessarily short.

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CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 Background

Kakadu National Park (KNP) located 120km east of Darwin (centroid 12.0667S 132.3667E) in the Northern Territory of Australia, covers an area of 1 980 400 ha, making it Australia's largest National Park. The Kakadu region has been World Heritage listed for its natural heritage and cultural value, possessing an ecosystem and landscape unique to wet-dry tropics of Northern Australia. For this reason, the region supports a large nature-based tourism industry, particularly during the dry season from May to October. As a result of the continuing escalation of the tourism industry, visitation to some areas has "...increased dramatically, placing unacceptable pressure on the environment and causing overcrowding." (ANCA, 1991).

The major tourist destinations, Jim Jim Falls and the adjacent Twin Falls, are centrally located within Stage 2 of KNP, where their respective creeks flow over the escarpment of the Arnhem Land plateau. Access to these sites is possible only in the dry season (May to October), by four wheel drive vehicle. The track leading to Twin Falls presently crosses Jim Jim Creek in an unformed road crossing approximately 4 km from the base of the escarpment. In recent tourist seasons, the frequency of use of the Jim Jim Creek road crossing has led to erosion and subsequent exposure of the clays that underlie the sand creek bed, resulting in highly noticeable turbidity for some distance downstream. Concern was raised by Park Managers and Traditional Owners that elevated levels of suspended solids, caused by vehicles using the crossing, may be having an adverse impact on the biota downstream of the crossing.

In order to assist with future management of the Jim Jim / Twin Falls region, this study was undertaken to assess the effects (if any) of disturbance (primarily suspended sediment), caused by vehicles, on macroinvertebrate biota downstream of the Jim Jim Creek road crossing. The macroinvertebrate study was complimented by fish surveys which were reported elsewhere (Stowar *et al.*, 1997).

1.2 Effects of Suspended Sediment on Aquatic Ecosystems

The amount of insoluble particulate matter suspended in water is frequently referred to as the concentration of 'suspended solids' in water chemistry analysis. This is usually quantified as the mass of filterable particulate matter in a given volume of water. Turbidity is often associated, and reported in conjunction, with suspended solids. Turbidity may be defined as "...the optical property of water which causes light to be scattered and absorbed rather than transmitted in straight lines through a sample..." (APHA, 1985). Turbidity and suspended solids, although related, quantify different properties of a water sample. In an 'ideal suspension', the relationship between turbidity and suspended solids is linear and is dependent on the extinction coefficient of the particles present (Gippel, 1988). However, in most water samples the presence of particles of different sizes and optical properties, as well as other non-particulate matter (such as coloured organic compounds which affect turbidity), may alter the theoretical relationship between suspended solids and turbidity markedly, to the extent that it may not necessarily be linear.

The turbidity of a water sample is much more readily quantified than the concentration of suspended solids, both in terms of cost and time. Thus, turbidity measurements are often used to infer elevated concentrations of suspended solids. It could be argued that every situation to which either measurement applies would be likely to have varied underlying physical characteristics (such as sediment particles sizes, particle mass, optical properties etc.). So, biologically, neither parameter is directly comparable, particularly between different studies.

Increased loads of suspended solids are a common effect of human activities on aquatic ecosystems, resulting from activities such as forestry, agriculture, mining and bank disturbance (Metzeling *et al.*, 1995). Elevated levels of suspended solids in waterways can be considered a 'pollutant' when raised above natural levels and can be viewed in a similar way to other toxicants in that both the concentration and period of exposure are important factors in determining the effects on aquatic biota (Hellawell, 1986). Additionally, sediment-related effects on biota may be caused by suspended, deposited or transported sediment. However unless the sediment concerned is particularly fine

(and hence remains suspended) or coarse (and is rapidly deposited), the precise cause of observed effects associated with elevated sediment input cannot be determined (Doeg and Milledge, 1991). The following review focuses on studies reporting elevated suspended sediment and turbidity levels; however in most cases it would be expected that there is some degree of sediment deposition also associated with the disturbance.

Numerous studies have been conducted of the effects of elevated levels of suspended sediment resulting from the above-mentioned activities, particularly based on ecosystems in the Northern Hemisphere and, to a lesser extent, temperate environments in Australia. There are, however, a lack of reported studies pertaining to tropical ecosystems. Even in well-studied environments, the specific effects on aquatic biota, of different levels of exposure to suspended sediment have not been well characterised. This is perhaps because of the overriding importance of site-specific factors such as sediment concentration, as well as duration of exposure and sediment characteristics (Newcombe and MacDonald, 1991).

1.2.1 Effects on Aquatic Plants

One of the most apparent effects of elevated levels of suspended sediments, and the resulting rise in turbidity, is the reduction in light penetration through the water column. This has been demonstrated to have adverse effects on the photosynthetic capability of algae and aquatic macrophytes (Lloyd, 1987). The extent to which sediments affect the primary productivity of streams is largely dependent on the level of turbidity. In extreme situations studied in Alaska, with turbidity levels of 1200 NTU, no primary production was observed, whilst levels of 170 NTU reduced primary production by 50 percent (Van Nieuwenhuysse and LaPerriere, 1986). Lloyd *et al.* (1987) found that increases in turbidity of only 5 NTU in shallow, clear-water streams may decrease productivity by up to 13 percent whilst an increase of 25 NTU may decrease primary production by 13-50 percent. In other studies, increases of turbidity as low as 9 NTU were shown to reduce algal biomass in streams by as much as 40 percent (Davies-Colley *et al.*, 1992).

Sediments may additionally affect aquatic plants (and hence productivity) by scouring algae from the stream-bed, hence reducing biomass. In other situations, nutrients or toxic compounds absorbed by suspended sediments may alter growth rates and biomass of aquatic plants (Newcombe and MacDonald, 1991).

1.2.2 Effects on Fish

Studies of the effects of suspended sediments on fish are numerous, particularly in relation to salmonid species in the Northern Hemisphere. A range of possible effects of increased suspended sediment concentrations on fish have been demonstrated.

Suspended sediment has been shown to directly affect fish clogging or coating gills. If levels of suspended sediment are sufficiently high, this alone can lead to direct mortality (Lloyd, 1987). Indirectly, turbid water may impair feeding behaviour, particularly of species using visual cues for foraging (Gardner, 1981); or alternatively, diets may be altered by changes in populations of prey species (Garman and Moring, 1993). Reproduction may also be affected, with sediment likely to cause impaired respiration and development of eggs, or in severe cases, smothering of eggs (Lloyd, 1987). Laboratory studies have indicated fish larvae may be particularly susceptible to the effects of suspended sediment, causing marked increases in mortality (Reynolds *et al.*, 1989). Fish may also display avoidance responses to turbid water (Bisson and Bilby, 1982), with the result that turbidity may create a barrier to migration or restrict the natural range of a species.

In general, the observed adverse effects of suspended sediment on fish may range from stress (resulting in increased incidence of disease and reduced growth rates) and mortality to behavioural responses (such as avoidance and altered feeding patterns) as well as deleterious effects on reproduction.

The difficulty in extrapolating effects of suspended sediment between geographical areas and amongst different fish species has been highlighted, with the biological effects of suspended sediment being dependent on many factors. These may include: the nature of the sediment, the degree of oxygenation of the water, water temperature, natural

levels of suspended sediment and the species of fish in question (Ryan, 1991). The widely-studied effects that are particularly well known for salmonid species may, therefore, be of little relevance to fish populations such as those in the tropical regions of northern Australia. Nevertheless the potential for suspended sediment to affect fish, at even low concentrations, has been frequently reported.

Wallen (1951) (cited by Lloyd, 1987) reported relatively high levels of turbidity required to kill warm-water fish, although much lower levels of turbidity were required to elicit acute sublethal responses. Adverse effects of turbidity and suspended sediment at various levels and duration of exposures have been widely reported (see reviews by Newcombe and MacDonald, 1991 and Lloyd, 1987). Such studies have indicated that even short pulses of relatively low turbidity water (10-25 NTU) may have deleterious effects on fish feeding and territorial behaviour, as has been demonstrated for the Coho salmon (Berg and Northcote, 1985).

Few studies have been reported for Australian ecosystems on the impacts of suspended sediment on fish populations. In one example, Richardson (1985) found that logging activities in south-eastern NSW resulted in reduced abundances of the common jollytail, *Galaxias maculatus*. Davies and Nelson (1994) reported decreased abundances of brown trout in a Tasmanian stream affected by logging. In these studies, it is difficult to disassociate the individual effects of suspended sediment, sedimentation and habitat alteration (eg. removed riparian vegetation, altered flow etc.). Sedimentation (and presumably associated suspended sediment) has been suggested to constitute a major factor threatening Australian native fish populations (Koehn and O'Connor, 1990), although there is little direct empirical evidence of this.

In one of the few laboratory studies that have been undertaken on Australian freshwater fish species, significantly increased mortalities of freshwater blackfish (*Gadopsis marmoratus*) and common galaxias (*Galaxias maculatus*) were reported when exposed to increased levels of suspended sediment (Koehn and O'Connor, unpublished data cited by Metzeling *et al.*, 1995).

As well as the direct effects on individuals, reproduction and behaviour, fish populations would undoubtedly be affected indirectly by any adverse effects of suspended sediment on plants and macroinvertebrates communities upon which they rely for food and shelter.

1.2.3 Effects on Macroinvertebrates

The benthic macroinvertebrate fauna of lotic systems display a wide variety of life histories and therefore sensitivities to environmental disturbance (Rosenberg and Resh, 1993). Thus the impacts of various forms of sediment-related pollution (eg. suspended sediment, deposited sediment or saltatively transported sediment) on macroinvertebrate communities may be many and varied.

Sediment particles are capable of affecting benthic macroinvertebrate biota in a number of ways. Direct physiological effects causing stress or mortality may include clogging by sediment particles of feeding apparatus in filter-feeding taxa (Newcombe and MacDonald, 1990). In similar fashion, the efficiency of diffusional respiratory structures may be affected by suspended or deposited sediment particles (Metzeling *et al.*, 1995).

Behavioural responses of aquatic invertebrates to elevated levels of suspended solids may also occur. Invertebrate drift, an avoidance response, has been demonstrated to occur as a result of increased levels of suspended sediment, both in impact assessment studies (eg. Richardson, 1985) and in field experiments (eg. Culp *et al.*, 1986; Doeg and Milledge, 1991).

Altered habitat characteristics by sediments affect the macroinvertebrate communities. For example deposited sediment may smother organisms inhabiting the stream-bed or fill substrate interstices, altering the habitat suitability for certain taxa. Another example of how sediments can affect habitat suitability is by affecting the availability of the clean surfaces for the attachment of macroinvertebrates, such as Simuliidae larvae, to their substrate (Williams, 1980). Sediment deposition may also affect detrital decomposition and availability, impacting on the food availability for many aquatic invertebrates (Metzeling *et al.*, 1995).

Yet another potential effect of sediment on macroinvertebrates is the scouring of the stream bed by saltating sediment particles, which may affect the habitability of the stream bed for many species (Chutter, 1969).

Many studies, both Australian and overseas, have investigated the effects of suspended sediment on aquatic biota. Studies conducted in New Zealand indicate that macroinvertebrate communities of that country may be more sensitive to suspended sediment than analogous communities in northern hemisphere streams (Quinn *et al.*, 1992). Accordingly, it has also been suggested that Australian macroinvertebrate communities, being perhaps in many instances similar, may also have greater sensitivity to suspended sediment than northern hemisphere communities (Metzeling *et al.*, 1995).

Changes in macroinvertebrate community structure have been commonly reported following elevated levels of suspended sediment in lotic systems. Often, field studies report the impacts of land-use activities such as forestry, mining, and runoff from agricultural and construction activities. Such disturbances are usually associated with a number of alterations to habitat eg. suspended sediment, deposited sediment, removal of riparian vegetation etc. Thus it is difficult to examine the specific effects of suspended sediment in isolation.

Case Studies from Forestry Activities

Studies of impacts of forestry on lotic environments commonly report elevated levels of suspended solids and turbidity. The literature pertaining to such studies has reported effects on macroinvertebrate communities ranging from reduced macroinvertebrate abundances to reduced taxa richness and altered community structure.

Overseas, the effects of suspended sediment and related disturbance resulting from forestry activities have been intensively studied. Gurtz and Wallace (1984) observed changes in community structure, including reduced densities of some taxa as well as increased densities of others, as a result of clear-felling in the Appalachian mountains. Lemly (1982) reported reduced densities of filter-feeding taxa as a result of logging activities near a North Carolina stream. In another study, Newbold *et al.* (1980) reported

increases in total macroinvertebrate fauna in streams but reduced taxa richness in streams near logged areas of northern California.

In an Australian study Richardson (1985) described changes in community structure, including the absence of apparently sensitive (such as filter feeding) species in logged areas. Davies and Nelson (1994) reported significant decreases in abundances of some taxa in logged areas of Tasmania. Species richness and taxa abundance were seen to decline in another Australian study relating to impacts of forestry and associated high loads of suspended solids, in south-western Australia (Growth and Davis, 1994).

Studies have indicated that the effects of forestry on macroinvertebrate communities may be long term, with effects still being detectable up to 40 years after the disturbance in one instance (Silsbee and Larson, 1983). In an Australian study, Growth and Davis (1991) reported community differences between streams in logged and undisturbed areas 8 years after the logging activity occurred.

Case Studies from Mining Activities

Studies of increased sediment loads to streams resulting from mining and associated activities frequently report adverse effects upon aquatic biota. Gammon (1970) observed decreased macroinvertebrate densities with elevated levels of suspended solids (and no appreciable sediment deposition), resulting from run-off from a stone quarry. Quinn *et al.* (1992) reported reduced invertebrate abundance and taxonomic richness downstream of an alluvial gold mine in New Zealand, which were significantly correlated with elevated turbidity. In this case, turbidity was seen to increase in the range of 23-154 NTU above a background of 1.3-8.2 NTU.

Studies of suction-dredge effluents, with attendant high suspended solids loads have reported a variety of results. In a Californian example, Harvey (1986) reported no apparent downstream effects on macroinvertebrate fauna, despite turbidity levels of up to 50 NTU against a background of less than 5 NTU. A similar result was gained in an experimental study of downstream effects of a suction dredge in Montana. In this case suspended sediments reached levels of 340 mg/L against an average background of

4.56 mg/L. In all these studies, the periods of suspended sediment elevation were short term and concentrations fluctuated widely.

Case Studies of Roads and Creek Crossings

In one of the few studies directly related to road activities, Barton (1977) reported for a stream in southern Ontario, a change in species composition, but no apparent changes in total abundance, associated with short-term elevation of suspended solids and sediment deposition. In a study of road construction activities in the Rocky Mountains of the USA, involving short term elevation in suspended sediments and little evidence of sediment deposition, Cline *et al.* (1982) observed reduced density, abundance, taxa richness, as well as altered taxonomic composition of the benthic macrofauna. The flow regime and duration of exposure were concluded to be important factors in the observed response. A study of the impacts of unpaved road crossings on streams in the Flinders Ranges in South Australia, reported some downstream changes in benthic macroinvertebrate community structure which were apparently associated with patches of sediment deposition (Wade, 1992).

Experimental Studies

Because of the non-specificity and range of factors involved in many of the disturbances described above, the importance of field and laboratory experiments becomes apparent, so that the specific effects of suspended solids on biota may be. Unfortunately, no such studies have been reported for Australian systems.

In a North American study, an experiment involving the addition of sediment to creeks at a rate of 1.7 g/L for 2 hours each week over a period of 6 weeks found no changes in total number of macroinvertebrates, number of species or calculated diversity indices, yet divergences were observed in the multivariate similarity of macroinvertebrate communities of the control and disturbed creeks (Fairchild *et al.*, 1987). In another experiment, Culp *et al.* (1986) added sediment to riffles in such a manner that sediment would either be deposited or transported. They reported no measurable impact on most taxa when sediment was deposited. In cases where sediment would be transported

saltatively, benthic invertebrate densities were reduced by greater than 50 percent in less than 24 hours, which they attributed to catastrophic drift.

In summary of published studies, it is clear that disturbances to biota arising from anthropogenically-derived, elevated sediment levels in streams are manifested in different ways, eg. from suspended sediment, deposited sediment and scouring by transported sediment, and the possibility of combinations of these. The degree of disturbance is also very specific to each situation and is dependent upon the level and duration of exposure to suspended solids. There is, however, a large body of evidence to conclude that sediment introduced into streams as a result of human activities has certain potential to cause changes to macroinvertebrate communities, even at relatively low levels.

1.3 Macroinvertebrates As Bioindicators

Macroinvertebrates have been widely used as general indicators of water quality. They have inherent properties which make them highly suited for this purpose, including a high abundance in most freshwater environments and a generally high taxonomic diversity ensures a comprehensive array of sensitivities to environmental stress (Rosenberg and Resh, 1993). Furthermore, because of the numerical abundance of organisms present, the data acquired are conducive to an array of statistical analysis techniques (Hellowell, 1986). The sedentary nature of these organisms means localised effects of pollution can be determined at various sites. Macroinvertebrates react quickly to stress but also have sufficiently long life-cycles that, in measurement of attributes of community structure, longer-term effects may be detected (Rosenberg and Resh, 1993). Spatial and temporal analysis of impacts is therefore possible (Hellowell, 1986). In addition, macroinvertebrates have a central role in aquatic food chains and processes such as nutrient cycling, decomposition and productivity (Reice and Wolhenberg, 1993).

The disadvantages of using macroinvertebrates as bioindicators may lie in the confounding effects of seasonal variation, as well as the potential for macroinvertebrate populations to be affected by factors other than pollution (Rosenberg and Resh, 1993). These potential pitfalls can often be circumvented by a rigorous experimental design.

The use of macroinvertebrates for biomonitoring is well established in the Kakadu region of Australia's Northern Territory (see Humphrey and Dostine, 1994), although the impact of suspended sediments on macroinvertebrate communities (and stream biota in general) on streams in the Wet-Dry tropics has not been previously studied.

1.4 BACI Study Designs and the Assessment of Unreplicated Disturbances

1.4.1 General Design Considerations in Biological Assessment

Sampling programs designed to assess the impacts of specific disturbances on ecosystems are constrained by the nature and type of disturbance, as well as the availability of potential study components such as disturbed sites and undisturbed control sites to facilitate replication. Impact assessment studies are often further constrained by limitations in space and time which exist for economic and practical reasons.

Regardless of the apparent constraints, a monitoring program will be of limited value unless it enables a rigorous and accurate assessment of any impacts that may occur. The challenge presented in undertaking such studies is to achieve such accurate assessment in a practical and cost-efficient manner, within the constraints that exist.

Despite the fact that many activities which potentially impact upon the environment are limited to a single source of disturbance, there is still the requirement of adequate replication and statistical independence in a sampling program designed to detect and assess impact. A common fault that besets many environmental impact assessments is inadvertent "pseudoreplication" in the study design. This constitutes "...the use of inferential statistics to test for treatment effects from experiments where treatments are not replicated (though samples may be) or replicates are not statistically independent." (Hurlbert, 1984). In streams, pseudoreplication is difficult to avoid, this issue being a

major concern in the design of assessment studies. Thus upstream control sites are not truly independent of downstream 'impact' sites (ie. 'treatment' effect cannot be randomly and independently assigned to 'replicates' because of geographical separation) and, moreover, these sites are *linked* by biological and physical processes (Hulbert, 1984; Cooper and Barmuta, 1993).

1.4.2 The Development of BACIP Designs

The simplest of approaches to examining the effects of a disturbance involves comparison of community differences on a single occasion 'Before' and 'After' an impact at a single 'Control' site as well as a single 'Impact' site, (perhaps with sample replication within sites)- hence a 'BACI' design (Green, 1979). An impact is inferred from interaction in an ANOVA design. This design is pseudoreplicated since treatment effects are not replicated. A major confounding factor in assessments based on this design is that differences observed between control and impact locations may be purely a product of spatial variation (Green, 1979). Incorporation of multiple control sites and even multiple impact sites in an attempt to quantify natural variation are an improvement on the design, although pre-existing spatial variation can never be discounted as a possible cause of the observed difference between locations (Keough and Mapstone, 1995).

To overcome the statistical problems of Green's BACI design, Stewart-Oaten *et al.* (1986) adapted the BACI design to incorporate *temporal* replication in sampling both before and after the onset of an impact. Furthermore, the sampling was *Paired* in time between the control and impact location, hence the development of the *BACIP* design (Stewart-Oaten *et al.*, 1992). This design was fundamentally different from the basic BACI design in that it was the change in the *difference* between control and impact locations that was measured and evaluated statistically. The set of difference values before the impact are compared with the set after the impact using a Student-t test or equivalent. In this design it was argued that spatial confounding could be removed as the *differences* in responses between sites would dampen absolute site values, these differences themselves constituting valid independent (temporal) replicates (Stewart-Oaten *et al.*, 1986, 1992).

1.4.3 Modifications to BACIP Designs

Incorporation of Additional Control Sites

The original BACIP design was devised by Stewart-Oaten *et al.* (1986) to assess the impact of a nuclear power generating facility in southern California on the nearby marine environment. Whilst initially two control locations were incorporated in the design, one location was lost during the study due to climatic events, bringing the study back to single control and impact locations. The advantages of multiple control locations in enabling better quantification of possible undisturbed conditions have been argued for by Underwood (1991, 1993, 1994) and various other subsequently. BACI-type designs involving multiple control sites have been termed MBACI (Keough and Mapstone, 1995) designs, 'M' signifying multiple control comparisons. In the following review, BACIP designs incorporating additional control sites have been termed MBACIP designs.

In order to satisfy the assumptions for the application of parametric statistics, additional control sites must be independent of one another, such that the effects on one site will not influence the others. For example biota may migrate upstream from downstream of a disturbance, thus upstream controls can not be considered truly independent. Selection of control sites is also obviously constrained to habitats that are similar to the impact sites both in terms of biota and environmental conditions, in the undisturbed state.

Provided suitable locations are available, additional controls can be utilised in a number of ways. The simplest approach is to compare each control site with disturbed sites in turn to give an indication of whether the observed state of the 'impacted' site is consistent with an undisturbed condition, where spatial variation is present. Such comparison with multiple controls may reduce the chance of making a type I error (detecting an 'impact' when not present) due to changes that may affect a single control site (Faith *et al.*, 1995). An alternative approach, and perhaps one that is preferable in that it improves the inferential power of statistical tests applied to MBACIP experiments, is that of the asymmetrical ANOVA (Underwood, 1993; Faith *et al.*, 1995).

This involves the simultaneous sampling of a single impact site and multiple control sites; or as suggested by Faith *et al* (1995), a pair of upstream-downstream sites in the impacted stream and similarly paired upstream-downstream sites in multiple control streams. A multi-factor ANOVA may be used to test for interaction between the impact location and control locations before and after the onset of the impact (figure 1.1).

The use of *paired* upstream-downstream sites in independent control streams, as opposed to single sites has been argued to be advantageous for a number of reasons. Firstly, the spatial gradient incorporated in sampling lessens the emphasis on localised variation, enabling stronger inference of impacts against the control condition (Faith *et al*, 1995). The second advantage of utilising paired upstream-downstream sites lies in the argument that impacts may be better evaluated within the regional context of environmental variability (Humphrey *et al.*, 1995), since impacted locations are assessed against other comparable systems, as opposed to individual sites which may vary widely in the natural condition.

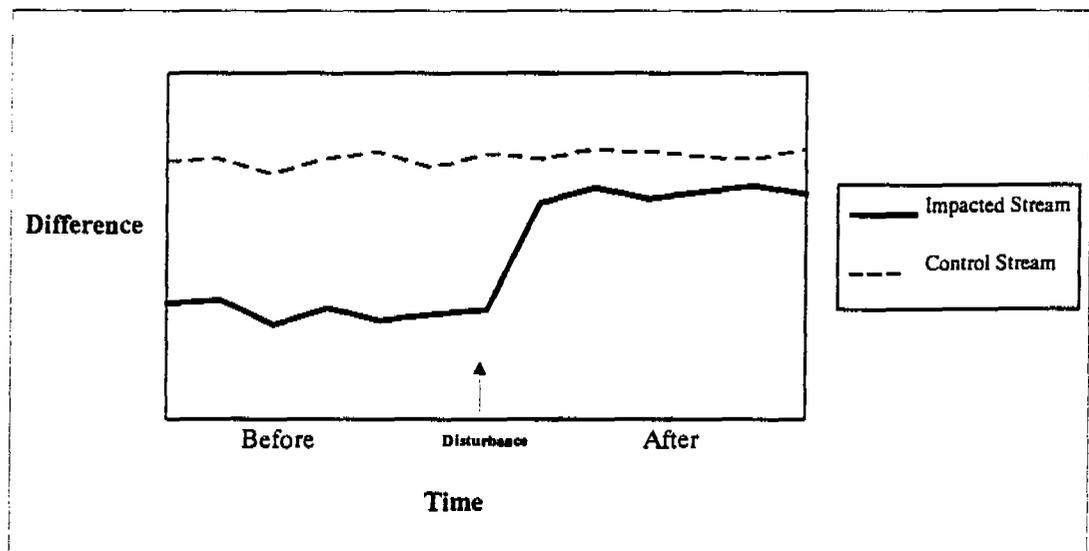


Figure 1.1 Idealised representation of a modified BACIP experiment, incorporating one additional control stream. The difference values are between paired upstream and downstream sites on (i) an 'impacted' stream where the downstream site is disturbed and (ii) a control stream where both upstream and downstream sites are undisturbed. The change in the difference values between upstream and downstream sites after the disturbance is detected statistically by way of ANOVA.

A further development of MBACI designs proposed by Underwood (1991,1993, 1994) has been termed the Beyond BACI design. This complex design, as well as incorporating multiple control locations, involves hierarchical sampling on multiple spatial and temporal scales. This approach has been promoted for its ability to detect impacts that may be occurring on different spatial or temporal scales. Such an approach may also be useful when the most appropriate scale of sampling to detect impacts is unknown (Underwood, 1991). This approaches requires extensive monitoring effort before and after the onset of the impact, and even so, the precise sampling regime chosen will affect the ability to detect different types of impact (Keough and Mapstone, 1995).

An alternative approach to the incorporation of multiple control sites, suggested by Green (1989), involves sampling of many control sites and deriving difference values between each control site and the potentially impacted site at *one* point in time before and after the onset of the disturbance. Thus, the inclusion of many site comparisons provides a form of *spatial* replication (cf. *temporal* replication of differences between the impact site and control site(s), as in the conventional BACIP design). This approach relies on the availability of numerous independent control sites to provide replication. Suitable control sites are frequently not available in such numbers.

Multivariate Measures of Site Difference

The actual measure of difference between samples collected according to BACIP principles is another area where various adaptations of the BACIP design have been made. Stewart-Oaten *et al.* (1986) used univariate measures, namely differences in taxa abundance to evaluate site differences. An alternative approach involves the use of multivariate dissimilarity measures, which are based on community data. Multivariate measures may be preferable to univariate measures, particularly when sampling of an entire community, such as benthic macroinvertebrates, is undertaken.

Multivariate methods provide a means of detecting and describing subtle patterns of difference across many variables (such as different taxa) (Green, 1980). With reference to macroinvertebrate communities, many responses to disturbance are observed as

changes in community composition and / or the community balance of the array of taxa present (Friedrich *et al.*, 1992). Multivariate measures are sensitive to both of these types of community change. Furthermore, Smith *et al.* (1988) suggest that different species act as 'replicates' of one another, thus observation of the overall community may provide an intensified response relative to observations of individual taxa. Multivariate measures may be particularly appropriate for assessing impacts on entire communities (Faith *et al.*, 1995), which would be an objective if the conservation of entire communities- or 'ecological integrity' (itself a topic of much debate; see Karr, 1991; 1993) is concerned.

Various multivariate measures of similarity are available (see Washington, 1984). Faith *et al.* (1995) evaluated a number of multivariate measures of dissimilarity in a BACIP experiment. It was observed that the Bray-Curtis measure of dissimilarity was the most robust measure to different disturbance patterns, suggesting it provided the greatest statistical power of the different measures tested- some of which displayed variable consistency.

1.4.4 Statistical Analysis of BACIP Data

One of the benefits of BACIP and related experimental designs is that temporal replication provides independent measures of location difference. These can be analysed using parametric statistical tests (for example a student's t test or multi-factor ANOVA), which are generally considered among the most powerful statistical tests of two means. There are, however a number of assumptions that must be satisfied by the dissimilarity data to enable application of these tests (Keough and Mapstone, 1995), namely:

1. The difference values between sites for each sampling occasion must be independent of the values on other sampling occasions.
2. There must be no trend in difference values through time, particularly in the 'before' period.
3. The data to which the t test is applied must satisfy t-test assumptions (normal distribution, equal variances etc.) (see Sokal and Rohlf, 1981).

Clearly there will be instances where the above-mentioned assumptions are not met and parametric statistics cannot be applied. Various data transformations may be attempted to satisfy the assumptions, however, in some circumstances factors violating the assumptions will persist. Abandoning the BACIP approach all together may result in the loss of valuable information. Alternative analysis options may be attempted before such a decision is made.

Trends in the difference values through time present a particular challenge, as they often only become apparent after the data has been collected. In cases where trends are apparent in the data, one possible analytical approach may be to make allowance for additional perturbing influences by introducing covariates (Stewart-Oaten *et al.*, 1986; Humphrey *et al.*, 1995). Trends in the difference values between locations or treatments and may be modelled using regression incorporating the covariates. Tests for treatment effects may be possible by analysis of covariance (comparing the elevation of regression lines) or comparing the slopes of regression lines (using methods such as those suggested by Snedecor and Cochran, 1980).

Keough and Mapstone (1995) suggest a possible solution to temporal correlation in difference values in modelling and analysing the trends themselves (cf. introducing covariates). By regarding the samples for the 'before' and 'after' not as random measurements, but as separate ordered series, trends may be analysed as functions which estimate the change in difference values in the disturbed or undisturbed condition. In this case it may be possible to apply time series analysis techniques to test for treatment effects (see Chatfield, 1984).

1.5 Scope and Objectives of this Study

This study was primarily undertaken to assess the effects (if any) of vehicles using the Jim Jim Creek road crossing on the benthic macroinvertebrate biota downstream of the crossing. The nature of the disturbance, as observed in previous tourist seasons, has been most apparent as increased turbidity resulting from elevated levels of suspended solids.

The scope of this study is limited to the evaluation of the effects of the disturbance on macroinvertebrate communities in Jim Jim Creek for a distance of 1km downstream of the road crossing. The ability to extrapolate to other situations is curtailed by the limited spatial extent of the study and the site-specific nature of the disturbance being studied.

The specific objectives of this study are:

1. To employ a modified BACIP sampling design to the assessment of the effects of vehicle induced disturbance on macroinvertebrate communities downstream of the Jim Jim Creek road crossing during the 1996 tourist season.
2. To evaluate any observed impacts in relation to their consequences for the natural heritage value of the area.
3. To make an appraisal of the application of a modified BACIP approach to the assessment of this moderately-short term disturbance downstream of the road crossing on Jim Jim Creek .

CHAPTER 2**MATERIALS AND METHODS**

CHAPTER 2: MATERIALS AND METHODS

2.1 Site Description

Jim Jim Falls and Twin Falls are centrally located within Kakadu National Park in the Northern Territory of Australia. Spaced approximately 10 km apart (in a straight line), these two large waterfalls are situated where Jim Jim Creek and Twin Falls Creek, respectively, flow over the Arnhem Land escarpment. Approximately 11 km downstream of Jim Jim Falls, Twin Falls Creek flows into Jim Jim Creek. Jim Jim Creek then flows over extensive flat lowlands, before becoming a major tributary of the South Alligator River, discharging into Van Diemen Gulf (figure 2.1).

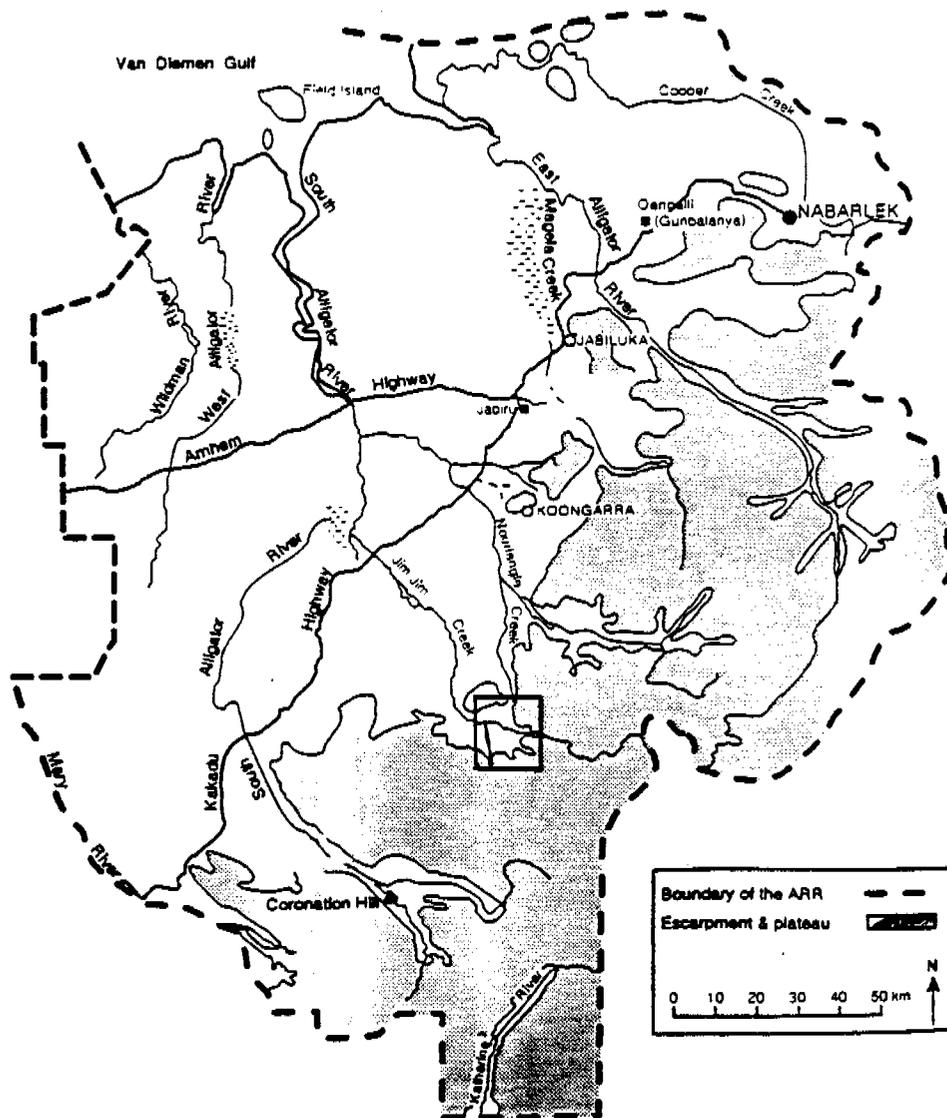


Figure 2.1. Map of the Alligator Rivers Region, encompassing Kakadu National Park. The boxed area shows the study location (see Figure 2.2).

The Kakadu region, in which Jim Jim Falls and Twin Falls are located, has a monsoonal climate, characterised by distinct 'wet' and 'dry' seasons, linked by short transitional periods (Humphrey and Dostine, 1994). The region's rainfall (averaging 1480 mm annually at Jabiru) falls almost entirely in the 'wet' season, which extends between the months of November and March. In marked contrast, there is little (if any) rainfall during the 'dry' season, between the months of May and September.

The extensive Arnhem Land sandstone plateau, over which Jim Jim and Twin Falls creeks flow, rises 200-300 m above sea level. The landform from the base of the Arnhem Land escarpment to the South Alligator River floodplain comprises flat lowlands of mostly tropical savanna-woodland. Major creek-lines are defined by more luxuriant riparian vegetation consisting mostly of *Pandanus aquaticus*, *Melaleuca* spp. and *Syzygium* spp.

The basal escarpment reaches of both Jim Jim and Twin Falls creeks consist of anastomosed sandy creek channels with numerous deep pools separated by shallow runs. As a consequence of the seasonal rainfall, the flow regime of both Jim Jim and Twin Falls creeks is also highly seasonal, with wet season rains resulting in high velocity flows of large volumes of water, which scour the creek-bed. Flow is recessional during the dry season (May to September), receding to a minimum in the late dry season, by which time the creeks may stop flowing. At the cessation of flow a series of interconnected pools is formed. Water quality in the basal escarpment reaches of both streams during the dry season would be expected to be consistent within creek systems whilst creek flow persists. Once flow ceases, however, water quality among remaining pools of water may be expected to vary widely (Humphrey *et al.*, 1990). Throughout the dry season, the upper reaches of Jim Jim and Twin Falls creeks are characterised by consistently high water clarity, a result of there being no surface runoff during this period.

Jim Jim Falls and Twin Falls are popular dry season tourist destinations, accessible to the public by four-wheel drive vehicle usually between the months of June and October. The precise timing of public accessibility is determined by road conditions, which are directly affected by the onset, intensity and cessation of the wet season.

Approximately 4 km downstream of Jim Jim Falls, the tourist access track leading to Twin Falls crosses Jim Jim Creek. The road crossing is approximately 15 m long and has no engineered road structures to stabilise the creek-bed and facilitate vehicle movement. As a result of vehicle-induced erosion, the Jim Jim Creek road crossing has, in recent years, been a point source of suspended solids pollution during the dry season, when the road crossing is in use by private and commercial tour vehicles. Although the levels of suspended solids or turbidity downstream of the road crossing have never been quantified and compared with levels upstream of the crossing, park rangers have reported a highly noticeable reduction in water clarity in Jim Jim Creek downstream of the road crossing, during its use in the dry season.

The actual disturbance caused by elevated levels of suspended solids is limited in duration due to the fact that the Jim Jim Creek road crossing is closed to all vehicles with the onset of the wet season. The ensuing high flows result in flushing of the creek system, re-sorting of creek-bed sediments and scouring of the creek channel; essentially restoring an unimpacted condition until the road crossing is opened in the following dry season.

The nearby Twin Falls Creek, although accessible at the base of the waterfall, does not have any human activity associated with it that would be expected to result in significant sediment input to the creek system. Being similar in nature to Jim Jim Creek and located nearby, Twin Falls Creek offers an ideal independent control location for the assessment of the impacts of the Jim Jim Creek road crossing.

The following methods describe the sampling that was undertaken to assess the impact of suspended solids (and other possible impacts associated with vehicles using the crossing) on the macroinvertebrate biota downstream of the Jim Jim Creek road crossing.

All sampling was undertaken within 1200 m sections of Jim Jim Creek and Twin Falls Creek respectively. In both creeks, these sections extended from approximately 3.8 km to 5 km from the creek's respective waterfalls, at the base of the Arnhem Land escarpment.

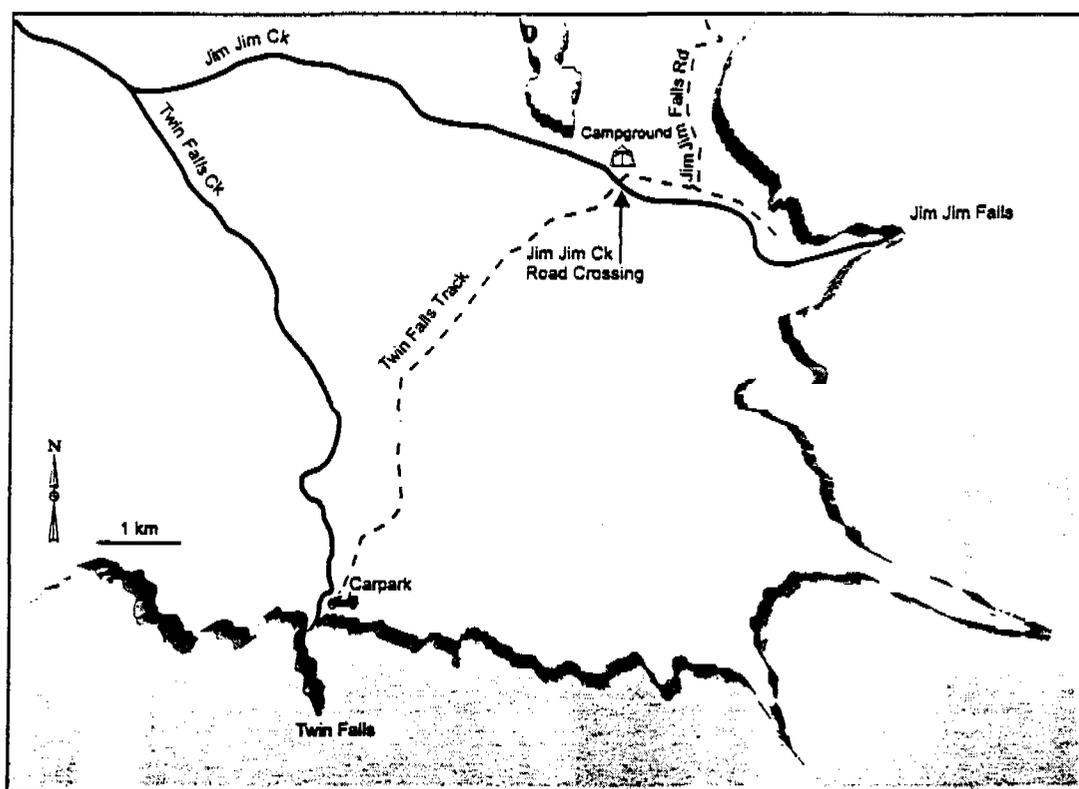


Figure 2.2. Map showing the Jim Jim Falls and Twin Falls locality, including the Jim Jim Creek road crossing adjacent to the campground.

2.2 Experimental Design

The ability to conduct sampling prior to the opening of the Jim Jim Creek road crossing, before any downstream disturbance was experienced, enabled an experimental design based on modified BACIP (*Before-After, Control-Impact, Paired differences*) principles (Stewart-Oaten *et al.*, 1986).

As outlined in section 1.4, BACIP designs involve the measurement of *difference* between control and impact sites. The advantage therein is that temporally replicated difference values, between a single control and impact site, may provide independent replication and hence enable the use of inferential statistics to test for treatment effects. Furthermore, the use of 'difference' between sites enables pre-existing local variation between 'control' and 'impact' sites to be accounted for and effectively 'cancelled out'.

Two modifications to the conventional BACIP (Stewart-Oaten *et al.*, 1986) design were incorporated into this study to increase the inferential power of the design, namely:

- (i) Multivariate measures of dissimilarity were used as the measure of site difference (Faith *et al.*, 1995).
- (ii) Additional control sites in a nearby 'independent' stream were incorporated in the design (Underwood, 1991).

In Jim Jim Creek, simultaneous sampling was conducted of an upstream (of the road crossing) 'control' site and two downstream 'impact' sites on a number of occasions both prior to, and after, the opening of the Jim Jim Creek road crossing to the public (hence defining the 'before' and 'after' impact periods). The two downstream sites were located at different distances from the road crossing, to incorporate a spatial gradient downstream of the road crossing. This downstream spatial gradient may enable determination of thresholds of effects or the spatial extent of any impact, to some extent. Analogous upstream and downstream sites in the additional control stream, Twin Falls Creek, were also sampled simultaneously, over the same period as the sampling of Jim Jim Creek sites.

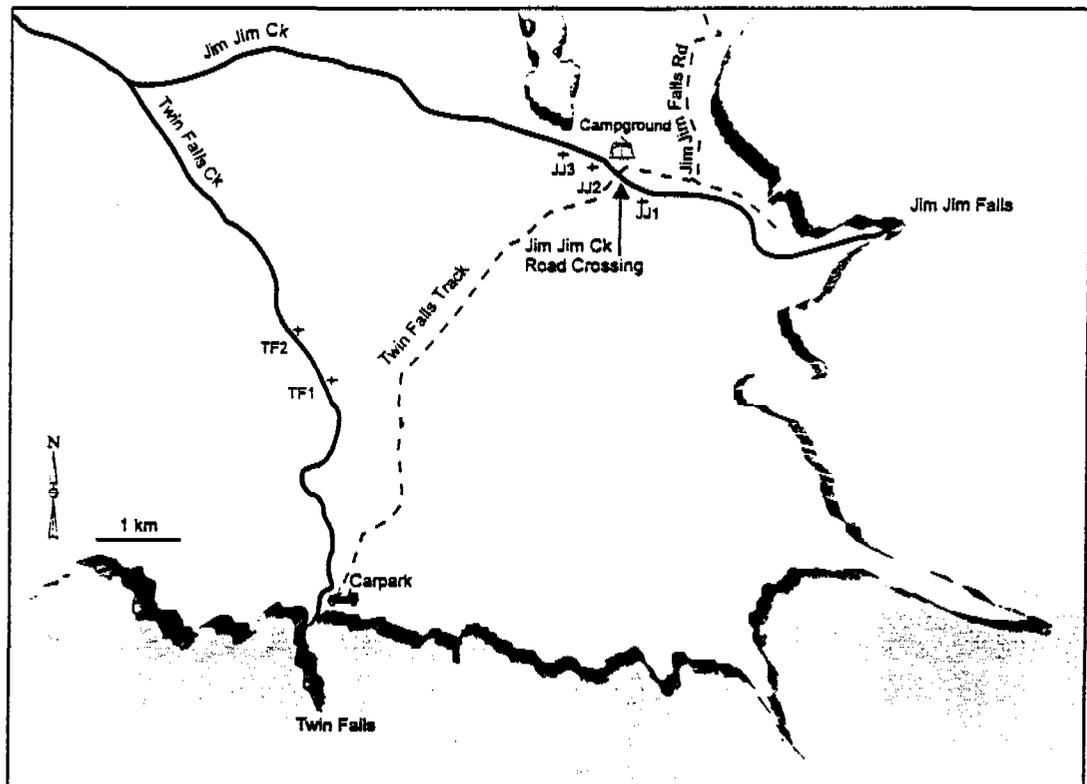
Difference values were derived between *pairs* of upstream-downstream sites in each creek. Thus, in the analysis, a series of dissimilarity values were calculated for the potentially impacted Jim Jim Creek (at two spatial scales, separately) and for the Twin Falls Creek control. Provided appropriate assumptions are met, a two-way ANOVA can be applied to the data, testing for interaction between locations (Jim Jim and Twin Falls Creek) and time (before impact/ after impact) (Humphrey *et al.*, 1995).

2.2.1 Sampling Sites

Three sites were selected on Jim Jim Creek, one 200 m upstream of the road crossing (JJ1), one 200 m downstream of the Jim Jim Creek road crossing (JJ2), and another 1000 m downstream of the road crossing (JJ3) (Table 2.1 and Figure 2.3). Two sites were selected on the undisturbed Twin Falls Creek, incorporating the same spatial separation as the furthest upstream and downstream sites on Jim Jim Creek. Thus, the upstream Twin Falls Creek site (TF1) and downstream Twin Falls Creek site (TF2) were selected to correspond with the Jim Jim Creek sites JJ1 and JJ3 respectively (being equidistant, along their respective creeklines, from the Arnhem Land escarpment) (figure 2.3). At all sampling locations in both streams, the substrate, depth, width of the creek, as well as the available macroinvertebrate habitats for sampling, were very similar.

Table 2.1. Location and GPS coordinates (WGS 84) of sampling sites.

Site code	Location	Longitude	Latitude
JJ1	200 m upstream from road crossing	132.81603688	13.27098484
JJ2	200 m downstream from road crossing	132.80972625	13.26690914
JJ3	1000 m downstream from road crossing	132.80219371	13.26435003
TF1	3800 m downstream from Twin Falls	132.77873185	13.29604692
TF2	1200 m downstream from TF1	132.77921687	13.28533337

**Figure 2.3.** Map showing the location of sampling sites on Jim Jim Creek (JJ1, JJ2, JJ3) and Twin Falls Creek (TF1, TF2), in relation to the source of disturbance, the Jim Jim Creek road crossing.

2.2.2 Sampling Regime

A 'before' impact period of monitoring was available prior to the opening of the Jim Jim Creek Road road crossing to the public. To maximise the period of monitoring of the 'before' impact condition, sampling commenced as soon as access to sites was possible, in late April 1996. At this time, both Jim Jim and Twin Falls Creeks were flowing strongly and the sand creek-bed had been scoured by wet season flows. Because of the uncertainty of when the Jim Jim Creek road crossing would be opened to the public (and hence onset of the disturbance), a relatively high frequency of sampling was conducted in the 'before' period- on a fortnightly basis at all sites. Twin Falls Creek sites were sampled on alternate weeks to Jim Jim Creek as concurrent sampling was logistically impossible. The Jim Jim road crossing was opened to the public on 24th June 1995, delineating the commencement of the potential impact ('after') period.

Sampling was scaled back to a monthly basis after the opening of the road crossing and was conducted until mid September when the flow of Jim Jim and Twin Falls creeks had receded to a minimum and the main tourist season had ended. The lower frequency of sampling in the 'after' period (monthly) enabled Jim Jim and Twin Falls creeks to be sampled simultaneously.

2.3 Chemical and Physical Environmental Variables

2.3.1 General Water Chemistry

Water samples were collected on a monthly basis, concurrently with macroinvertebrate sampling, for determination of pH, electrical conductivity, alkalinity, bicarbonate, orthophosphate, total phosphorus, total organic carbon, alkali metals (Na, K, Ca, Mg), heavy metals (Al, Fe, Mn, Cu, Pb, Zn, U, Ni, Cd, Cr - total unfiltered) and other major ions and nutrients (Cl^- , NO_3^- , SO_4^{2-} , NH_4^+). Techniques used for analysis are outlined in Table 2.2 (also refer to appendix 1). All samples, with the exception of ICPMS and ICPAES analysis of metals, were analysed by the ERISS analytical chemistry laboratory, a NATA-registered facility. ICPMS and ICPAES analysis was performed by ANSTO at Lucas Heights, NSW.

2.3.2 Turbidity and Suspended Solids

Turbidity

Field measurements of nephelometric turbidity were made at each site, on each sampling occasion, using an Activon Portable Turbidimeter. Three measurements were made and the turbidity value averaged. Calibration was maintained and checked using formazin turbidity standard solutions, with measurement consistency verified against measurements made in the laboratory using a Hach ratio/XR model 43900 turbidimeter (procedure specified by ERISS Analytical Chemistry Methods, code number 1- refer to appendix 1). In addition, turbidity immediately downstream of the Jim Jim road crossing was measured continuously (every hour) by a Hydrolab Datasonde 3 Data Logger which was permanently maintained at site JJ2, making hourly measurements for the duration of the study.

Suspended Solids

Samples were collected on a monthly basis, concurrently with macroinvertebrate sampling, for determination of total suspended solids. A gravimetric technique was employed involving filtration of a water sample of either 1000 mL or 500 mL (depending on the amount of suspended sediment) through Sartorius SM 11106 cellulose acetate membrane filters (pore size 0.45 μ m, 47mm diam.). The organic and inorganic components of total suspended solids were determined by a gravimetric technique involving ashing the filtered solids at 400° C. The procedures followed for both total suspended solids and organic and inorganic components is specified in detail by ERISS Analytical Chemistry Methods, code numbers 12 and 16, respectively (refer to appendix 1).

Suspended Solids – Turbidity Relationship

Additional water samples were collected periodically in Jim Jim Creek, throughout the study period, for determination of turbidity and total suspended solids (using the methods outlined above). These measurements were plotted with against one another to establish a predictive relationship between the two parameters, which could possibly be used in future monitoring based on the easily of turbidity. Simple linear regression was performed using the SAS statistical package (SAS, 1995).

Table 2.2. Water Chemistry Methods

Parameter	Technique	ERISS Analytical Chemistry Methods code number (Refer to appendix 1)
pH	Electrometric	8
Alkalinity	Acidimetric titration	1
Conductivity	Electrometric	5
Turbidity	Nephelometric	1
Total Suspended Solids	Gravimetric	12
Inorganic and Organic Solids Residue	Gravimetric	16
Orthophosphate	Spectrophotometric	29
Total Phosphorus	Acid digestion and spectrophotometric	37
Na ⁺ , K ⁺ , NH ₄ ⁺	HPLC	106
SO ₄ ²⁻ , Cl ⁻ , NO ₃ ⁻ , Ca ²⁺ , Mg ²⁺	HPLC	108
Total Organic Carbon / Dissolved Organic Carbon	Acidification, persulfate oxidation	4
Al, Mn, Fe	ICP-AES	External
Cu, Pb, Zn, U, Ni, Cd, Cr	ICP-MS	External

2.3.3 Chlorophyll Analysis

Water samples were collected at each sampling site on a monthly basis for determination of chlorophyll a, b and c. Samples of 500 mL of creek water were filtered on site and the retained sample stored on ice and then frozen until processed. Samples were emulsified in 10 mL of 90 percent acetone, then filtered. Concentrations of extracted chlorophyll in the filtrate were determined by optical densities measured at 750 nm, 664 nm and 645 nm and 630 nm with a spectrophotometer, the measurement at 750nm being a correction for turbidity. Detailed explanation of procedures and calculations are outlined by Camilleri *et al.* (unpubl. Laboratory Manual, ERISS).

2.3.4 Physical Environmental Variables

Sampling sites were similar in stream width, depth, substrate composition, and riparian vegetation. For each sampling occasion, physical habitat variables were measured as outlined in Table 2.3.

Accompanying the monthly sampling of invertebrates at each site, measurements were made for calculation of instantaneous stream discharge. For this, a transect was placed across the creek and water velocity measured at 1.0 m intervals on the cross-section; each measurement was made at a depth of 0.6 x total water depth. Water velocity was measured using a miniature current meter (Hydrological Services, Model OSS PC1). At the laboratory, cross-sectional area was determined graphically using water depth measurements made at the same (0.5 m) intervals across the section. Discharge values were derived from the product of average water velocity along the transect and the cross-sectional area of the river.

Table 2.3. Methods for measurement of physical habitat variables at each sampling site.

Parameter	Method	Frequency of Measurement
Flow	Timing a standard cork over a 2 m transect, parallel to creek flow.	Each sample
Average Depth	Measuring stick, 3 measurements (ends and mid point) averaged along sample transect.	Each sample
Water Temperature	Mercury thermometer.	Each sampling site, for each occasion.
Creek Discharge*	Flow meter, measuring water velocity and depth at 1 m intervals along a transect placed perpendicular to the creek (see text).	Monthly, each sampling site

2.4 Macroinvertebrate Sampling

The predominant macroinvertebrate habitats identified in Jim Jim and Twin Falls Creeks were sand, rootmat edge and macrophyte edge. Sand is the predominant creekbed substrate in both creeks, consisting of a medium-grained, quartz sand, upon which an organic floc forms wherever there is an absence of strong stream currents. Once these organic flocs have developed, they are quickly colonised by macroinvertebrates. The second habitat termed 'rootmat' in this study was found in sections of both creeks where the roots of *Pandanus aquaticus* and *Melaleuca* spp. growing in the riparian zone provide a fibrous root habitat along the edge of the creek channel. The third habitat consisted of edge macrophytes including *Xyris* sp., *Eleocharis* spp., *Blyxia* sp. and *Eriocaulon* sp., present at irregular intervals along both Jim Jim and Twin Falls creeks.

Initially, sampling of all 3 major natural substrates (sand, rootmat and edge macrophytes) was undertaken. However, sampling of the macrophyte edge habitat ceased by July, due to receding creek levels and as a consequence, results for this habitat are not reported.

2.4.1 Sampling Procedures

Sand Habitat

Sampling of the sand habitat was performed by lightly drawing a 250 μm kick net (basal width of 25 cm) across a pre-marked 5 m transect of the sand. The creek-bed surface immediately in front of the net was agitated by hand to suspend any organic matter and invertebrates, this material then being swept into the net. Only sand upon which an organic floc had formed (as opposed to clean-scoured sand in areas of stronger stream flow) was sampled. Transects of suitable habitat were selected at random and sampled in a direction parallel to, and against, the direction of flow of the creek; so that any suspended matter was washed downstream into the net.

The contents of the net were transferred into a 20 L bucket half-filled with clean creek water, on the creek bank. Macroinvertebrates and organic matter were elutriated and separated by vigorous stirring by hand of the contents of the bucket, followed by pouring off of organic material into a 250 μm sieve. This process of elutriation was conducted three times with each sample. The sample retained by the sieve was preserved immediately in 70 percent ethanol for transport back to the laboratory.

At each of the sampling sites and on each sampling occasion, three replicate sand samples were collected. Each replicate represented a total sampling area of $\sim 1.25 \text{ m}^2$ of sand habitat.

Rootmat Habitat

Replicate two-metre transects of this habitat were sampled at random in a similar manner to the sand substrate, ie. by lightly drawing a 250 μm kick net (basal width of 25 cm) along the substrate, against the flow of the creek whilst vigorously agitating the substrate by hand. Again the macroinvertebrates and organic matter were elutriated from the sample by washing three times in half-buckets of creek water, pouring off the sample into a 250 μm sieve. Samples were preserved in 70 percent ethanol for transport back to the laboratory.

At each of the sampling sites and on each sampling occasion, three replicate rootmat samples were collected. Each replicate represented a total sampling area of $\sim 0.5 \text{ m}^2$ of rootmat habitat.

2.4.2 Subsampling

A sub-sample containing approximately 150 macroinvertebrates was obtained from whole samples using a 'riffler' (geological splitting device). To achieve this, samples were suspended in a 4 L jug of tap water by vigorous stirring with a large spatula. The entire sample was then poured evenly across the 'riffler', the two halves of the sample separating and collecting in receptor trays at the base of the device. This process was repeated with successive halves to attain a fraction containing the desired sample size. The final subsamples were strained through a 250 μm sieve and replaced in 70 percent ethanol for sorting.

2.4.3 Sample Processing and Identification

Macroinvertebrates were sorted from the organic residue under a stereomicroscope using a channelled plastic sorting tray. Benthic macroinvertebrate fauna were identified to family level, with the exception of order Acarina and class Oligochaeta, which were not identified below this level. Macroinvertebrates were identified using the keys of Williams (1980) and Hawking (1995), unpublished keys developed for the Kakadu region by the Environmental Research Institute of the Supervising Scientist (ERISS), together with the macroinvertebrate voucher collection held by ERISS.

2.5 Data Analysis

2.5.1 Calculation of Dissimilarity and Statistical Analysis of BACIP Data

According to multivariate MBACIP principles, statistical comparisons are made between independent control and potentially impacted streams by way of dissimilarity values between paired upstream and downstream sites in each creek. Thus to make such comparisons, dissimilarity values were derived for Twin Falls Creek, TF1 vs TF2 and Jim Jim Creek sites at two different spatial scales, JJ1 vs JJ2 and JJ1 vs JJ3, for each sampling occasion.

Multivariate dissimilarity values were calculated using the Bray-Curtis measure from the multivariate statistical analysis package, PATN (Belbin, 1993a). Separate multivariate comparisons of site data were made using raw (untransformed) data, transformed ($\log_{10}(x + 1)$) data, rank order abundance data and binary (taxa presence-absence) data. In addition, a univariate measure of site difference, using total macroinvertebrate abundance, was examined. Independence of temporal replicates ('differences') is a critical BACIP assumption. Thus, in each case, observation of the paired site dissimilarities or differences plotted against time were used to evaluate which data transformation best met this criterion or assisted otherwise in data interpretation.

Because of serial correlation in the BACIP dissimilarity values in both streams over time, ANOVA of derived dissimilarity data was not appropriate for use in a statistical test of impact. Instead, the potential for data analysis by way of ANalysis of COVariance (ANCOVA) was examined. The principle of this approach is to test for differences in dissimilarity between the control and impact stream whilst keeping constant an explanatory environmental correlate (or covariate) of the variation in dissimilarity. In this case, stream discharge was the obvious covariate.

In the event, ANCOVA was not required for data analysis and instead a simple regression approach was applied - as explained and presented in the results. Regression analysis was performed on dissimilarity (dependent variable) and creek discharge data (independent variable) using the statistical package SAS (SAS Institute, 1995). For each dissimilarity value, a corresponding creek discharge value was derived by averaging the two discharge values of the upstream-downstream creek sites in question. Regression was performed on all data from the undisturbed condition (all TF1/TF2 data, and pre-impact JJ1/JJ2 and JJ1/JJ3 data). These data were plotted, along with derived 95 percent confidence intervals for the regression relationship, and the 'after' impact values (for JJ1/JJ2 and JJ1/JJ3, after the opening of the road crossing) then superimposed upon these data in the same plot.

2.5.2 Multivariate Ordination

Ordination using both untransformed and $\log_{10}(x+1)$ transformed abundance data was performed on individual replicate data (3 per sampling occasion). Samples from the 'before' and 'after' impact periods were analysed separately to eliminate some of the temporal effects that might have obscured impact-related changes. The ordination analysis was performed with the statistical package PATN (Belbin, 1993a) using the Semi-Strong-Hybrid Multi-dimensional Scaling (SSH) option based on the Bray-Curtis dissimilarity index. Three dimensions were required to reduce the 'stress' value for the ordination below an acceptable level of 0.2 (Belbin, 1993b).

Significant taxa and environmental parameters correlating with the ordination space were determined using Principle Axis Correlation (PCC) and Monte-Carlo significance testing with 100 'random starts' (Belbin, 1993a). Taxa and environmental parameters reported to be significantly correlated were those with a probability (p) of less than 0.01 in the Monte-Carlo analysis.

2.5.3 Taxa Abundance Comparisons

Comparisons were made among sites and over time, of total taxa abundance and abundances of major taxa individually to provide an insight into the biological basis of any macroinvertebrate community changes being observed.

CHAPTER 3

RESULTS

CHAPTER 3: RESULTS

3.1 Water Quality

Results of physico-chemical analyses of water samples from Jim Jim and Twin Falls creeks are presented in Tables 3.1, 3.2 and 3.3, together with the values or ranges of the constituents that are recommended not to be exceeded in the ANZECC (1992) water quality guidelines. Water quality in both creeks, prior to the opening of the road crossing, was typical of waters draining the sandstone portions of the Arnhem Land plateau (as compared with data presented in Humphrey *et al.* (1990)). After the opening of the Jim Jim Creek road crossing on June 24th 1996, downstream water quality was seen to be degraded with regard to the markedly increased levels of turbidity and suspended solids (Figures 3.1 and 3.2). The measured levels of some metals, notably iron and aluminium, were also seen to rise, as a consequence of the suspended particulate matter in the samples. In other respects, however, water quality in both creek systems was seen to be consistent throughout the study period among all sites, both undisturbed and disturbed.

3.1.1 General Water Chemistry Parameters

Values of pH, bicarbonate, alkalinity, electrical conductivity and total organic carbon for surface waters of the five creek sites throughout the study period are shown in Table 3.1. The waters of Jim Jim and Twin Falls creeks were slightly acidic throughout the dry season, with a pH range across all sites and sampling occasions of 5.7 - 6.5. The waters were also extremely soft (range across all sites and sampling occasions: 0.6 - 4 mg/L HCO₃) with very low buffering capacity (alkalinity) (range across all sites and sampling occasions: 0.5 - 3.3 mg/L CaCO₃). A slight increase was observed over the study period in these parameters at all sampling sites. Conductivity was also extremely low and again displayed a temporal increase (range across all sites and sampling occasions 8.1 - 16 μ S/cm). Almost all of the suspended organic carbon measured from surface waters of the two streams was in dissolved form and hence only dissolved organic carbon data are presented here. Dissolved organic carbon was low at all sites with no distinct temporal trend evident. Significantly, there was no apparent anomaly in any of the above-mentioned water chemistry variables downstream of the road crossing on Jim Jim Creek subsequent to its opening to traffic in late June (Table 3.1).

Table 3.1 General water quality characteristics of monthly water samples from Jim Jim and Twin Falls creeks, throughout the study period. See Table 2.1 for details of site locations.

Variable ¹	Site	Sampling Date						ANZECC guidelines
		25-Apr-96	24-May-96	26-Jun-96	24-Jul-96	23-Aug-96	20-Sept-96	
EC ($\mu\text{S}/\text{cm}$)	JJ1	8.1	10	12	14	15	16	-
	JJ2	9	12	13	12	12	12	
	JJ3	9.1	12	13	12	12	12	
	TF1	9.9	12	12	12	12	14	
	TF2	9.9	12	12	12	12	14	
pH	JJ1	5.7	6.0	6.1	6.1	6.2	6.3	6.5 - 9.0
	JJ2	6.0	6.1	6.3	6.2	6.2	6.4	
	JJ3	6.1	6.2	6.4	6.3	6.4	6.4	
	TF1	6.3	6.3	6.4	6.2	6.4	6.4	
	TF2	6.3	6.3	6.1	6.2	6.5	6.5	
Alkalinity ($\text{mg}/\text{L CaCO}_3$)	JJ1	0.5	0.9	1.8	2.7	3.2	3.3	-
	JJ2	1.0	1.8	2.6	2.5	2.7	2.8	
	JJ3	1.1	1.7	2.6	2.9	2.7	2.5	
	TF1	1.4	1.7	2.4	2.0	1.7	2.6	
	TF2	1.3	2.0	2.1	1.6	1.8	2.3	
Bicarbonate ($\text{mg}/\text{L HCO}_3$)	JJ1	0.6	1.1	2.2	3.3	3.9	4.0	-
	JJ2	1.2	2.2	3.2	3.1	3.3	3.4	
	JJ3	1.3	2.0	3.2	3.6	3.2	3.1	
	TF1	1.7	2.1	2.9	2.4	2.1	3.1	
	TF2	1.6	2.5	2.6	2.0	2.2	2.8	
DOC ¹ ($\mu\text{g}/\text{L}$)	JJ1	1100	900	900	1900	300	1000	-
	JJ2	1000	1100	900	300	100	300	
	JJ3	1000	1100	1000	100	200	200	
	TF1	1100	1200	900	1600	<100	100	
	TF2	1200	1000	900	100	<100	100	
TSS ¹ ($\mu\text{g}/\text{L}$)	JJ1	2200	1300	3100	2800	6900	9300	<10% change in seasonal mean
	JJ2	3500	2500	3100	12000	100000	22000	
	JJ3	3500	1000	4300	7500	17000	15000	
	TF1	2500	2300	1000	1900	1700	4200	
	TF2	1700	1000	5300	1000	4100	5000	
Turbidity* (NTU)	JJ1	0.97	0.95	2.35	2.05	2.13	2.37	<10% change in seasonal mean
	JJ2	1.25	2.04	5.67	18.06	59.8	38.92	
	JJ3	1.89	2.54	7.58	16.45	28.97	14.56	
	TF1	1.96	0.64	2.45	1.13	2.18	1.26	
	TF2	0.87	1.24	1.26	2.38	2.46	0.93	

¹ EC = Electrical Conductivity; DOC = Dissolved Organic Carbon; TSS = Total Suspended Solids.

* Mean Monthly

The low ionic content of creek waters indicated by the conductivity measurements was reflected in the very low range in concentrations of the alkali metals, sodium (range across all sites and sampling occasions: 900 - 1600 $\mu\text{g/L}$), potassium (range: <50 - 180 $\mu\text{g/L}$), magnesium (range: 250 - 660 $\mu\text{g/L}$) and calcium (range: 90 - 440 $\mu\text{g/L}$), and the anions, sulfate (range: 30 - 600 $\mu\text{g/L}$) and chloride (range: 1500 - 2400 $\mu\text{g/L}$) (Table 3.2). As indicated by the range of measurements, there was some degree of variability among the measurements of these parameters, although no temporal or site-specific trends were evident. The levels of orthophosphate and total phosphorus, as well as ammonium and nitrate, were extremely low (Table 3.2).

The above-mentioned water quality characteristics are typical of waters derived from sandstone catchments of the Arnhem Land plateau, being acidic, low in dissolved solids, poorly buffered and low in nutrients (Humphrey, 1990).

Table 3.2 Major ions and nutrients in monthly water samples from Jim Jim and Twin Falls creeks throughout the study period. See Table 2.1 for details of site locations.

Variable ¹	Site	Sampling Date						ANZECC guidelines
		25-Apr-96	24-May-96	26-Jun-96	24-Jul-96	23-Aug-96	20-Sept-96	
Ortho - P (µg/L)	JJ1	8	<2	<2	<2	5	<2	-
	JJ2	9	<2	<2	<2	7	5	
	JJ3	2	<2	<2	3	9	4	
	TF1	3	<2	<2	<2	7	2	
	TF2	<2	<2	<2	3	3	5	
Total P (µg/L)	JJ1	9	<5	12	<5	<5	20	<10
	JJ2	9	<5	10	<5	22	19	
	JJ3	39	<5	47	11	45	16	
	TF1	NR	<5	72	<5	<5	<5	
	TF2	13	<5	19	18	24	10	
Ammonium (µg/L)	JJ1	NR	30	30	30	30	30	20 - 30
	JJ2	30	30	30	30	30	30	
	JJ3	30	30	30	30	30	30	
	TF1	30	30	30	30	30	30	
	TF2	30	30	30	30	30	30	
Nitrate (µg/L)	JJ1	NR	10	10	10	10	10	<100
	JJ2	10	10	10	10	10	10	
	JJ3	10	10	10	10	10	10	
	TF1	10	10	10	10	10	10	
	TF2	10	10	10	10	10	10	
Calcium (µg/L)	JJ1	NR	120	290	440	380	350	-
	JJ2	120	150	100	160	120	100	
	JJ3	120	120	150	130	150	130	
	TF1	130	130	140	170	140	190	
	TF2	90	170	NR	180	110	130	
Potassium (µg/L)	JJ1	NR	50	90	50	60	70	-
	JJ2	50	50	60	60	70	70	
	JJ3	50	50	50	70	60	80	
	TF1	50	50	50	130	100	140	
	TF2	50	50	80	50	110	180	
Sodium (µg/L)	JJ1	NR	1200	1300	1400	1300	1200	5000 *
	JJ2	1000	1300	1100	1100	1000	1000	
	JJ3	1100	1100	1200	1400	900	1000	
	TF1	1100	1200	1200	2000	1200	1300	
	TF2	1200	1400	1200	1600	1200	1500	
Magnesium (µg/L)	JJ1	NR	250	460	510	620	660	-
	JJ2	300	430	650	560	590	580	
	JJ3	320	430	550	560	570	560	
	TF1	340	390	430	440	400	470	
	TF2	350	410	440	490	420	470	
Chloride (µg/L)	JJ1	NR	1900	2400	2100	2100	1900	-
	JJ2	1700	1900	1800	1600	1600	1300	
	JJ3	1700	1800	1800	1500	1500	1200	
	TF1	1800	1900	1700	1900	1900	1900	
	TF2	1800	1900	1900	2000	2000	2100	
Sulphate (µg/L)	JJ1	NR	240	200	200	30	30	-
	JJ2	550	280	30	200	30	30	
	JJ3	270	140	200	400	30	30	
	TF1	350	170	30	400	200	200	
	TF2	140	500	30	600	200	30	

¹ Ortho - P = Orthophosphate; Total P = Total phosphorous.

* Interim guide only.

3.1.2 Turbidity and Suspended Solids

Turbidity

The consistently high water clarity typical of the upper reaches of Jim Jim and Twin Falls creeks was confirmed by the extremely low levels of turbidity measured at the undisturbed sample sites. Throughout the entire season, these sites had mean turbidity values (\pm SE.) of 2.04 ± 0.13 NTU for JJ1, 1.63 ± 0.35 NTU for TF1 and 1.88 ± 0.19 NTU for TF2. Similarly, low levels of turbidity were observed downstream of the Jim Jim Creek road crossing prior to its opening to public vehicle access, with average turbidity of 1.65 ± 0.20 NTU and 2.22 ± 0.31 NTU for JJ2 and JJ3 respectively between April and early June.

Elevated levels of turbidity were observed downstream of the Jim Jim Creek road crossing subsequent to its opening to public in late June 1996, contrasting markedly with the low turbidity that persisted at control sites throughout the dry season. Downstream of the Jim Jim road crossing, turbidity began to rise immediately after the opening of the road crossing, peaking at both downstream sites in August when average turbidity for the month was 59.80 ± 14.43 NTU at site JJ2 and 28.97 ± 3.31 NTU at site JJ3. Turbidity at both downstream sites declined to some extent after the month of August, but remained substantially elevated above the levels observed concurrently in undisturbed sites up to the conclusion of the study (and tourist season) in October (Figure 3.1). As indicated by the different levels of turbidity measured at the two downstream sites, spaced 800m apart, there was a consistent decline in turbidity with increasing distance downstream of the road crossing. The turbidity levels measured at both downstream sites were, nevertheless, substantially elevated above background levels.

It is noteworthy that for the entire period at which the Jim Jim Creek road crossing was opened to the public, the elevated levels of turbidity at both downstream sites were well in excess of the ANZECC (1992) water quality guidelines, which recommend that mean nephelometric turbidity should not change by more than 10 percent. The changes observed in this study represent elevations of up to 1200 and 600 percent at sites JJ2 (200m downstream) and JJ3 (1000m downstream) respectively.

Suspended Solids

The concentration of total suspended solids measured at undisturbed sites (JJ1, TF1 and TF2, as well as JJ2 and JJ3 prior to the opening of the road crossing) were extremely low (generally less than 5 mg/L) (Figure 3.2). These measurements illustrate the extremely low natural levels of suspended solids characteristic of this system throughout the dry season.

Concentrations of total suspended solids increased slightly downstream of the Jim Jim Creek road crossing in July and then rose sharply to a marked peak in August, with levels 200 m downstream of the Jim Jim Creek road crossing (Site JJ2) reaching 100 mg/L. The measurements taken concurrently 1000m downstream of the road crossing (site JJ3) similarly displayed a peak in suspended solids concentrations in August, although the level was considerably lower (17 mg/L) than immediately downstream of the road crossing (Figure 3.2).

As was shown in the turbidity data, suspended solids concentrations at downstream Jim Jim Creek sites receded to some extent after August, with a particularly notable decline occurring immediately downstream of the road crossing at site JJ2. Nonetheless, the concentrations remained substantially elevated above background levels for the duration of the study.

The constituents of total suspended solids were found to be almost entirely inorganic, with the organic fraction consistently being below detection limits (less than 50 µg/L) for the procedure employed to measure the organic residue (see methods).

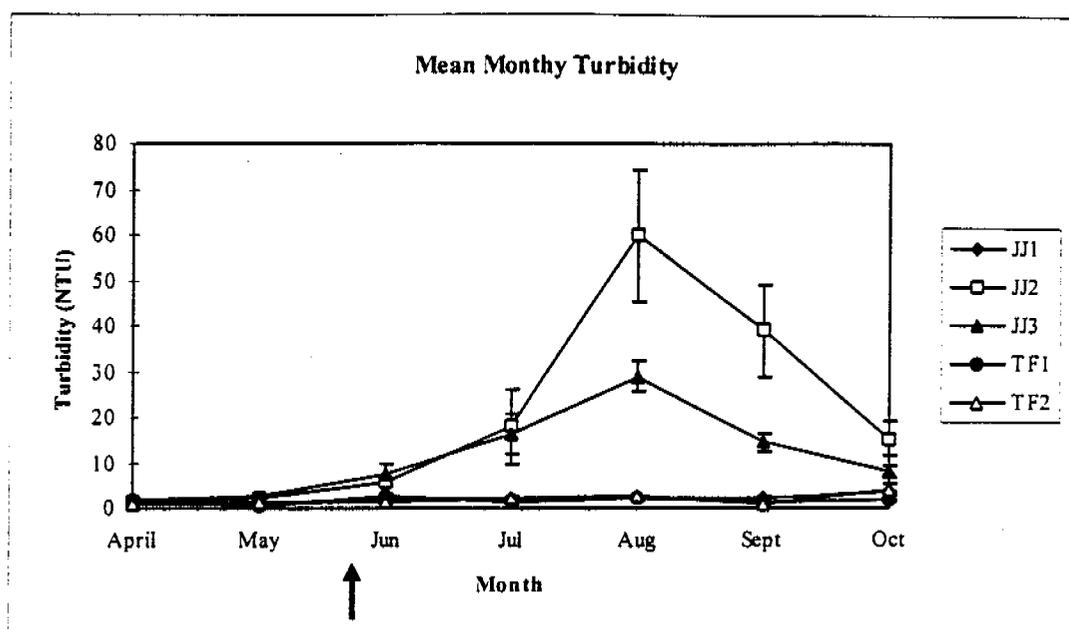


Figure 3.1. Mean monthly turbidity at each sampling site throughout the study period. Error bars indicate SE. The arrow indicates the opening of the Jim Jim Creek road crossing to the general public.

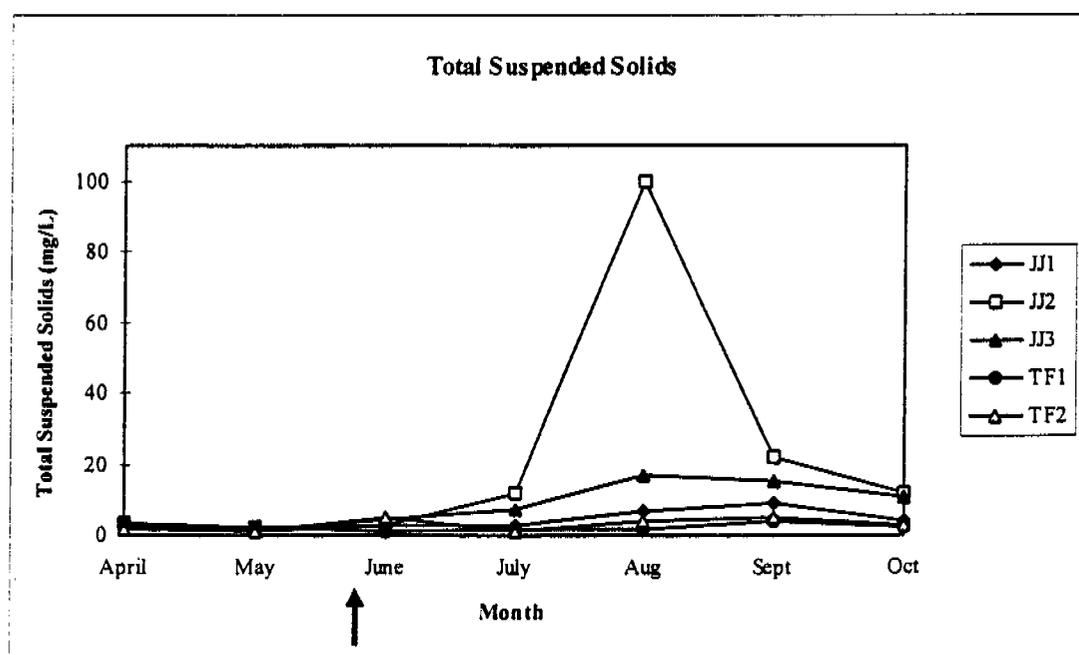


Figure 3.2. Monthly measurements of total suspended solids at each sampling site. The arrow indicates the opening of the Jim Jim Creek road crossing to the general public.

Relationship between Turbidity and Suspended Solids

There was a significant regression relationship found between turbidity measurements and the concentration of suspended solids in the water sample ($R^2 = 0.8419$, $p < 0.0001$) (Figure 3.3). Such a relationship was seen to be approximately linear as expected by the theoretical relationship between optical turbidity and concentration of suspended particles in an 'ideal' suspension. In this study there is some uncertainty associated with this relationship at higher turbidity levels - a consequence of the limited number of data points at high levels of suspended solids. A breakdown of the linear relationship may arise in cases where the size distribution of suspended particles varies with turbidity levels (Gippel, 1988).

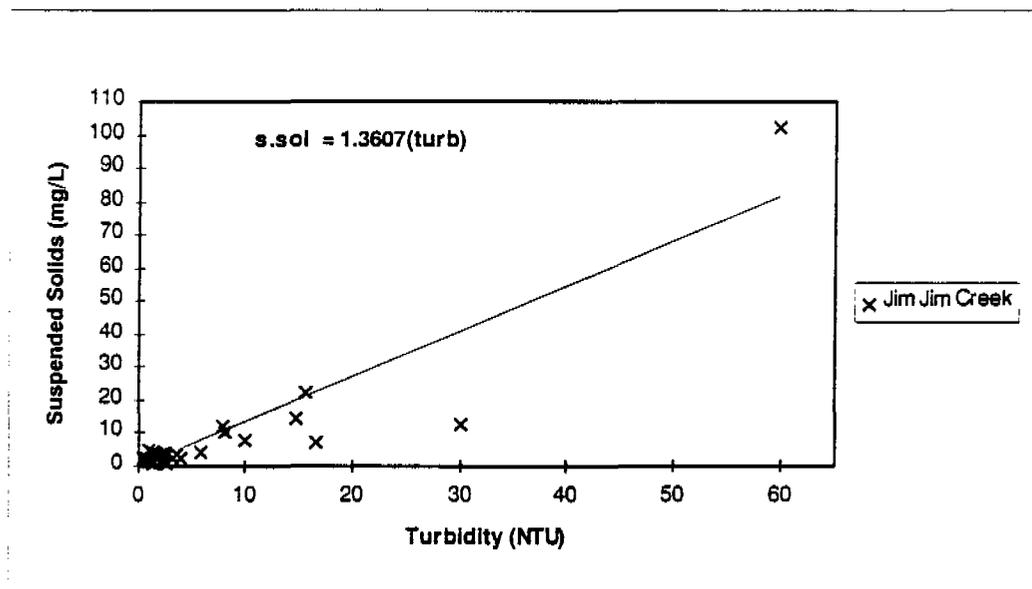


Figure 3.3. Relationship between turbidity and total suspended solids in samples collected from Jim Jim Creek.

3.1.3 Heavy Metals

There was considerable variation in the measured levels of total iron and aluminium among surface waters of the undisturbed sampling sites (JJ1, TF1 and TF2) over the study period. For example, the range of measurements observed throughout the study period among undisturbed sites (JJ1, TF1, TF2), was 10 - 810 µg/L for iron and 11 - 49 µg/L for aluminium. However, the concentrations of these metals observed downstream of the road crossing were clearly elevated in relation to this background (Table 3.3). These values, representing total concentrations of the respective metals in *unfiltered* samples, are simply a reflection of the mineralogy of suspended clay particles present in the water samples. The near-neutral pH confirms that these metals would be predominantly in particulate (non-ionic), and hence non-chemically toxic, form (Dr C. LeGras, pers. comm., ERISS).

The levels of trace metals (Cu, Pb, U, Zn, Cd, Mn, Cr) at control sites were negligible, being below detection limits in most cases. Slight elevations of Zn, U, Pb, Cu, and Mn were seen downstream of the Jim Jim Creek road crossing (although all levels were well within ANZECC (1992) guidelines). This may again be attributed to the insoluble clay particulate matter present in the samples collected downstream of the road crossing. Notably, the concentrations of these metals began to show elevation, to a small extent, prior to the opening of the road crossing to the general public, when use of the road crossing was limited to occasional crossings by park management vehicles. This indicates that there was some downstream elevation in particulate matter early in the study period, which was not detected by measurements of turbidity or suspended solids.

3.1.4 Chlorophyll Analysis

The phytoplanktonic biomass, as measured by of chlorophyll a, b and c, observed at all sites in Jim Jim and Twin Falls creeks was negligible (below detection limits in most samples).

Table 3.3 Heavy metals in monthly water samples from Jim Jim and Twin Falls creeks throughout the study period. See Table 2.1 for details of site locations.

Variable ¹	Site	Month						ANZECC guidelines	
		25-Apr-96*	24-May-96	26-Jun-96	24-Jul-96	23-Aug-96	20-Sept-96		
Manganese (µg/L)	JJ1	3	5	5	5	4	10		
	JJ2	4	5	3	7	5	12		
	JJ3	5	5	2	5	7	11		
	TF1	4	5	4	10	12	6		
	TF2	4	4	5	9	6	6		
Iron (µg/L)	JJ1	10	190	410	400	420	490		<1000
	JJ2	370	530	670	110	980	1300		
	JJ3	360	540	660	760	1100	1400		
	TF1	200	220	280	360	810	230		
	TF2	240	180	390	390	290	210		
Aluminium (µg/L)	JJ1	17	22	49	38	24	42		
	JJ2	33	48	23	40	760	270		
	JJ3	32	42	86	14	980	420		
	TF1	16	12	12	24	25	12		
	TF2	16	12	21	23	49	11		
Chromium (µg/L)	JJ1	NR	<0.5	<0.5	<0.5	<0.5	<0.5	10	
	JJ2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	JJ3	NR	<0.5	<0.5	<0.5	<0.5	1.4		
	TF1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
Copper (µg/L)	JJ1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		2 - 5
	JJ2	NR	<0.5	<0.5	1	1.2	0.7		
	JJ3	NR	<0.5	<0.5	<0.5	0.6	1		
	TF1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
Nickel (µg/L)	JJ1	NR	<1	<1	<1	<1	<1		
	JJ2	NR	<1	<1	<1	<1	<1		
	JJ3	NR	<1	<1	<1	<1	<1		
	TF1	NR	<1	<1	<1	<1	<1		
	TF2	NR	<1	<1	<1	<1	<1		
Lead (µg/L)	JJ1	NR	<0.5	<0.5	<0.5	<0.5	<0.5	1 - 5	
	JJ2	NR	<0.5	<0.5	0.6	0.8	<0.5		
	JJ3	NR	<0.5	<0.5	<0.5	<0.5	0.6		
	TF1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
Uranium (µg/L)	JJ1	NR	<0.02	<0.02	<0.02	<0.02	<0.02		<5
	JJ2	NR	<0.02	0.03	0.07	0.09	0.08		
	JJ3	NR	<0.02	<0.02	0.05	0.03	0.08		
	TF1	NR	<0.02	<0.02	<0.02	<0.02	<0.02		
	TF2	NR	<0.02	<0.02	<0.02	<0.02	<0.02		
Zinc (µg/L)	JJ1	NR	<0.5	0.8	<0.5	<0.5	<0.5		
	JJ2	NR	<0.5	<0.5	0.7	1.4	<0.5		
	JJ3	NR	<0.5	<0.5	0.8	<0.5	<0.5		
	TF1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
Cadmium (µg/L)	JJ1	NR	<0.5	<0.5	<0.5	<0.5	<0.5	0.2 - 2	
	JJ2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	JJ3	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF1	NR	<0.5	<0.5	<0.5	<0.5	<0.5		
	TF2	NR	<0.5	<0.5	<0.5	<0.5	<0.5		

* NR = No result

3.2 Statistical Analysis of BACIP Data

Statistical inference in ascribing turbidity-related impacts upon macroinvertebrate communities in this study were, potentially, best provided by BACIP site dissimilarity comparisons.

Multivariate dissimilarity values for both sand and rootmat habitats, calculated using raw (untransformed) data, showed a large degree of variation among sampling occasions. Furthermore, strong temporal trends in dissimilarity, particularly for rootmat habitat, in the 'before' and 'after' periods violated the assumption of independence required of temporal replicates in (M)BACIP designs, thereby invalidating the application of the usual parametric tests (student's t test or ANOVA) (Stewart-Oaten *et al*, 1986; Keough and Mapstone, 1995) (Figures 3.4 and 3.5).

As suggested by Keough and Mapstone (1995), various data transformations may be performed in an attempt to remove temporal trends in difference values, although this may result in the loss of some biological information.

A number of data transformations were applied to the data in an attempt to eliminate the temporal trends in the dissimilarity values, and hence enable the application of the usual parametric statistical tests. These transformations included $\log_{10}(x+1)$, rank-order of abundance and presence-absence of taxa (at the family level). As well, the univariate measure of site difference, total taxa abundance, was examined. In all cases, parametric statistical analysis still was not possible because of persistent temporal trends in the dissimilarity or difference values. In the case of presence-absence data, a general lack of inter-site and temporal variability - ie. no apparent differences in macroinvertebrate response amongst sites - indicated the loss of valuable biological information.

Although temporal trends were present in the dissimilarity values calculated using $\log_{10}(x+1)$ transformed data, this transformation reduced the variability of the dissimilarity values to a large extent (Figures 3.6 and 3.9). Thus, the $\log_{10}(x+1)$

transformation was deemed to be most appropriate for interpretation and analysis of community levels changes.

It is noteworthy, however, that the $\log_{10}(x+1)$ data transformation, as well as reducing variability of the dissimilarity values, altered some of the trends *and* relative positions of site comparisons from the different streams. This suggests that the transformation of the data resulted in a different measure of community attributes, rather than simple dampening of the extreme variability that arises from raw taxa abundance data .

Because the application of conventional BACIP statistical tests could not be applied to the data, alternative trend analysis approaches were used to infer impact, as outlined below.

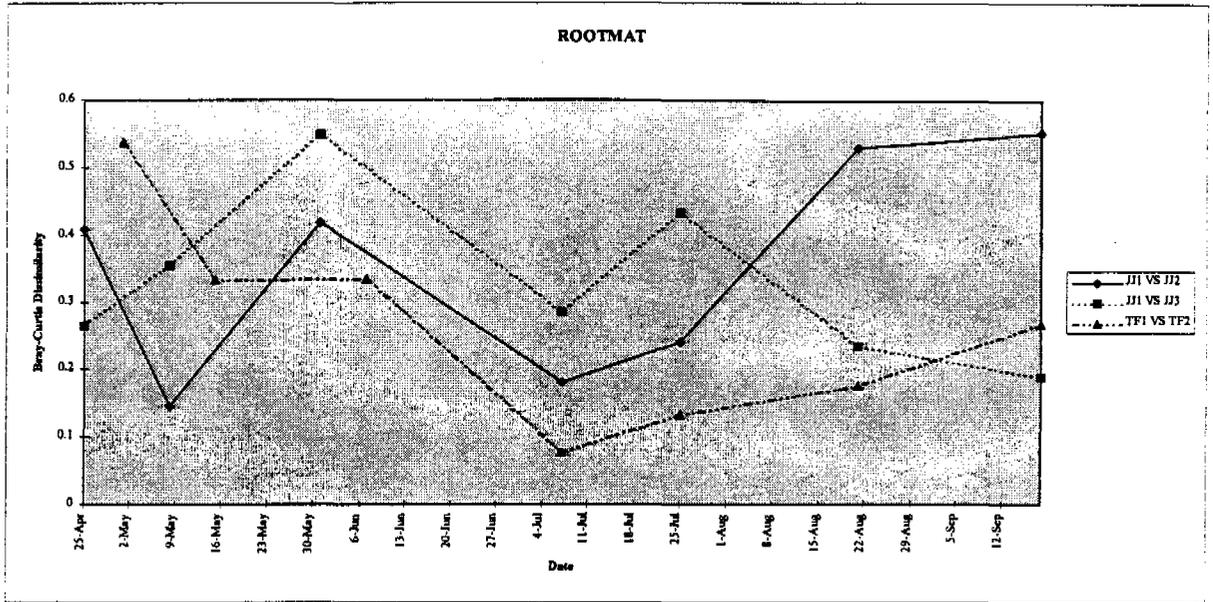


Figure 3.4. Multivariate dissimilarity values between paired upstream / downstream sites for the macroinvertebrate communities sampled from the *rootmat* habitat, based on untransformed data.

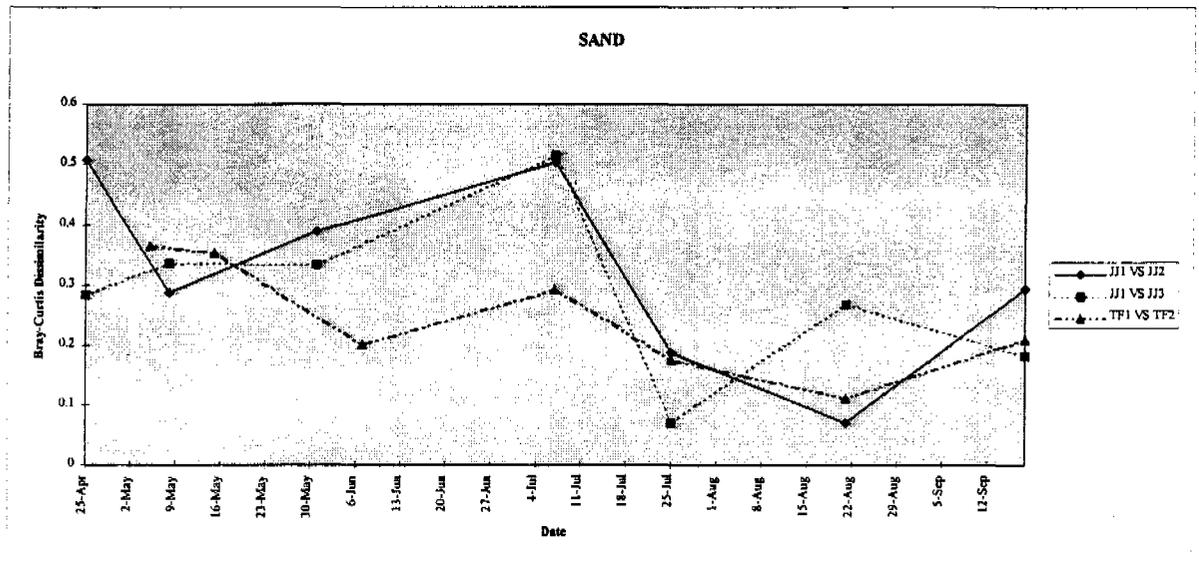


Figure 3.5. Multivariate dissimilarity values between paired upstream / downstream sites for the macroinvertebrate communities sampled from the *sand* habitat, based on untransformed data.

Rootmat Habitat

The control stream (TF1/TF2) displayed a temporal trend of 'decreasing dissimilarity' between paired sites throughout the dry season. The decrease in dissimilarity was particularly rapid early in the study period, levelling out to a consistent decline from early June onwards (Figure 3.6). Overall, this signifies that the macroinvertebrate communities between upstream-downstream sites in the control stream were becoming more similar throughout the dry season.

In the early part of the study period (before any disturbance), the dissimilarity in community structure between upstream and downstream sites in the potentially-impacted stream, Jim Jim Creek (JJ1/JJ2 and JJ1/JJ3), was considerably less than in the control stream (TF1/TF2). Moreover, the dissimilarity values between the paired sites at the two levels of site comparison (JJ1/JJ2 and JJ1/JJ3) were very similar. By early June, the dissimilarity between the paired sites on Twin Falls Creek had declined to values similar to those of the (as yet unimpacted) Jim Jim Creek site pairs (Figure 3.6).

The Jim Jim Creek road crossing was opened to the general public on in late June 1996, marking the commencement of the 'after' period, in which impact-related changes to the macroinvertebrate communities of Jim Jim Creek were observed. Thus, after the opening of the road crossing, the dissimilarity values for the Twin Falls Creek paired control sites were seen to continue the trend of 'decreasing dissimilarity' until the end of the study period in mid-September (Figure 3.6). In contrast, the site dissimilarity values for Jim Jim Creek paired sites in the latter part of the study period showed a distinct divergence from the trend displayed by the Twin Falls Creek control. This divergence was seen as a marked *increase* in the dissimilarity values between paired sites, at both levels of spatial separation (JJ1/JJ2 and JJ1/JJ3). The divergence was most pronounced in the dissimilarities for JJ1/JJ2, incorporating the site located 200 m downstream of the road crossing. In this case, the divergence relative to the control stream commenced in late July, approximately one month after the opening of the Jim Jim Creek road crossing. The dissimilarity comparison for JJ1/JJ3, incorporating the site 1000 m downstream of the road crossing, began to show a departure from the trend displayed by

the control stream only in late August. Furthermore, the degree of divergence at this spatial scale was less than the dissimilarities involving site JJ2 (Figure 3.6).

In summary, a trend of 'decreasing dissimilarity' was observed in the control stream throughout the study period. The observations in Jim Jim Creek are in accord with this trend until late in the study period, when at both spatial scales (200 m and 1000 m downstream of the road crossing), Jim Jim Creek paired sites diverged from the trend displayed for the undisturbed condition. This suggests a likelihood of an impact-related community change downstream of the road crossing, coinciding with its use by vehicles and associated elevated levels of suspended solids and turbidity. The divergence of the values based on the site 200m downstream of the Jim Jim Creek road crossing is greater than that for the values based on the site 1000m downstream of the crossing. This indicates the possible impact-related community changes 200m downstream of the road crossing represent a greater divergence from the control condition than 1000m downstream.

It is noteworthy that the departure of the dissimilarity values involving the site JJ2 was *less* pronounced using transformed data (Figure 3.6) than in the dissimilarity values based on untransformed data (Figure 3.4). However, the reduced variability in the dissimilarity values based on transformed data enables stronger inference of trends against the control condition. In contrast, the trend in dissimilarity values involving the site JJ3 relative to the control stream is unclear on the basis of untransformed data.

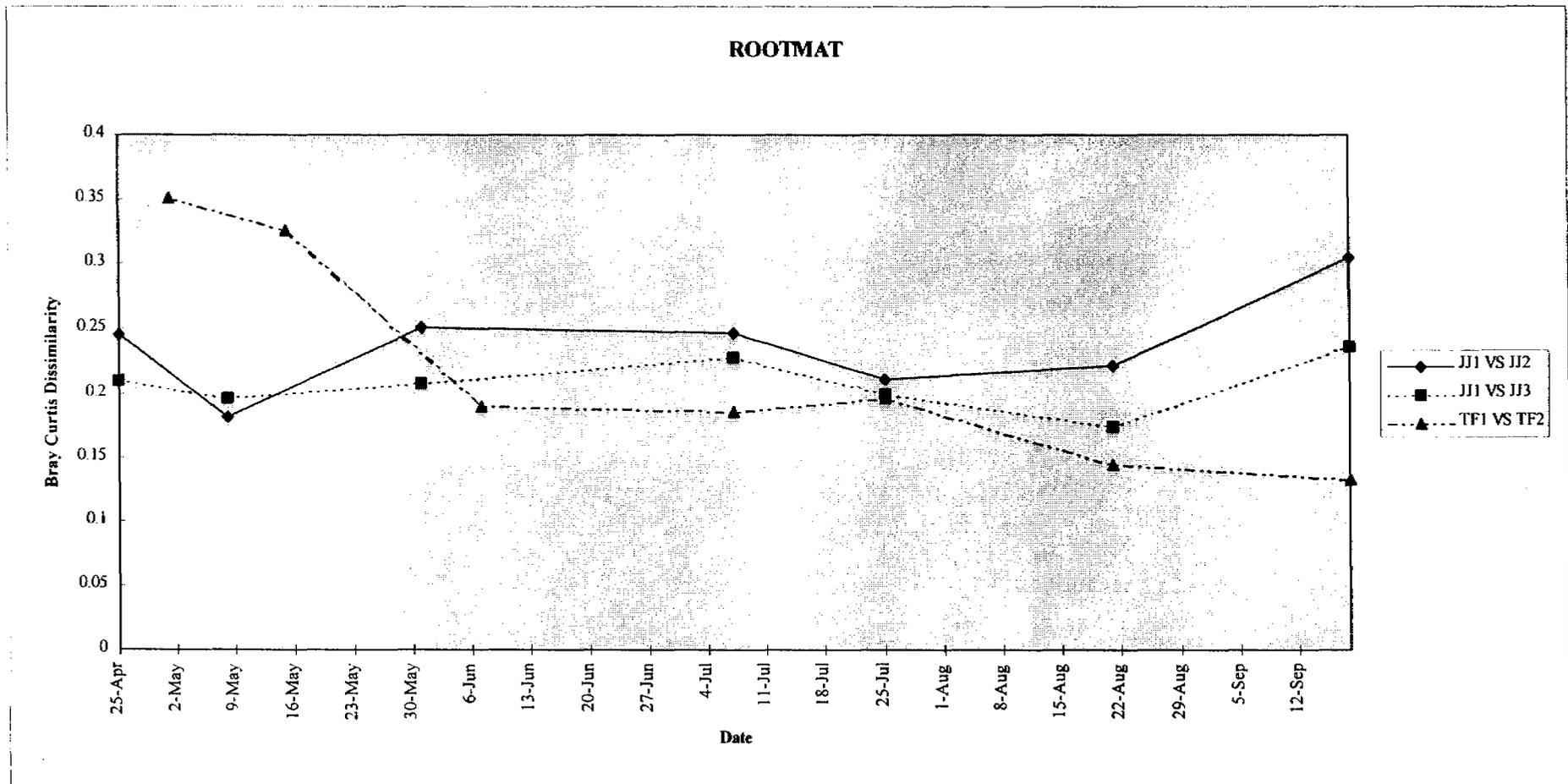


Figure 3.6. Multivariate dissimilarity values between paired upstream / downstream sites based on samples collected from the *rootmat* habitat, using $\log_{10}(x+1)$ transformed data.

In circumstances where the statistical assumptions appropriate for parametric analysis of data from a MBACIP design of the type used here *are* met (see section 1.4.4), the mean dissimilarity for each stream in the 'before' and 'after' period would be compared using a two-way ANOVA, testing for interaction between creeks (control and impacted) and time ('before' and 'after'). Because of lack of independence of the temporal replicates (dissimilarities), other data analysis options were explored. The observed trend in community dissimilarity is perhaps not unexpected considering the strong temporal change occurring in environmental conditions (with receding stream flow) within the single dry season study period.

Statistical tests on non-independent data may be possible by introducing an explanatory environmental correlate (or covariate) of the trend in dissimilarity. Where necessary ANCOVA may be used to test the relationship between the control and impact streams (see section 1.4.6). In this study, stream discharge, which showed a decline throughout the study period, was used as an explanatory variable of the decline in paired site dissimilarity observed in the control or unimpacted stream.. This is corroborated by a previous study of an undisturbed stream in the Kakadu region where the same positive association between the dissimilarity of pairs of upstream/downstream sites and the discharge status of the stream has been observed (C.Humphrey, unpublished data) (Figure 3.7).

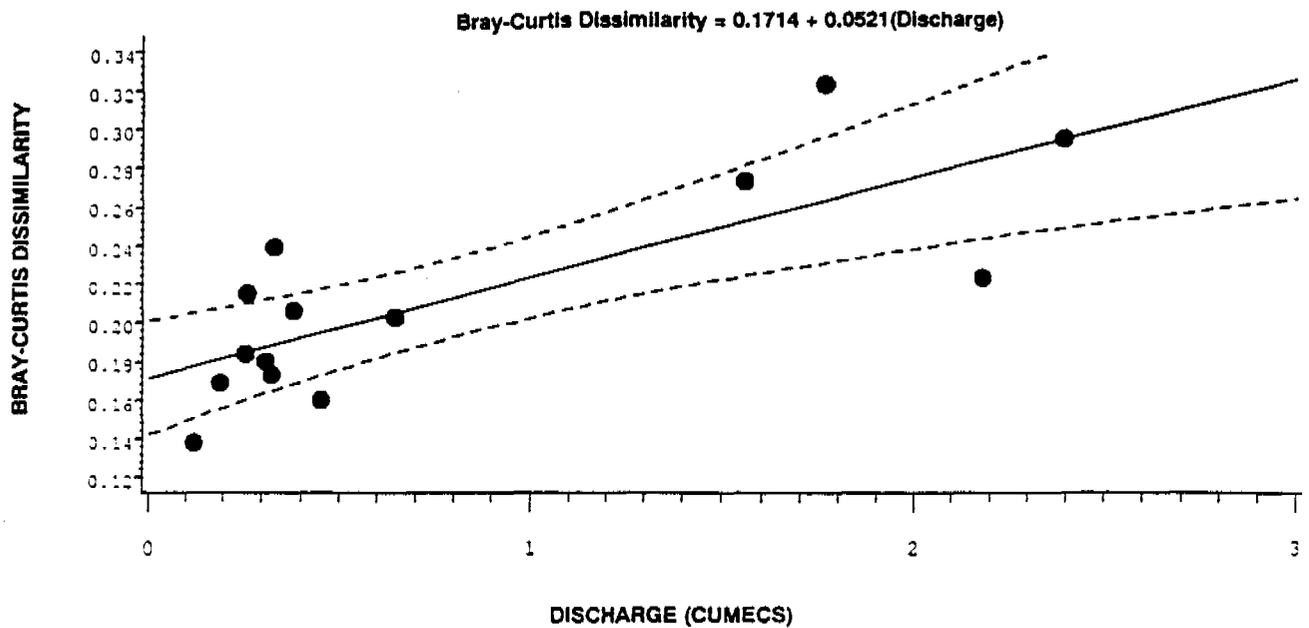


Figure 3.7. Association of dissimilarity between paired upstream and downstream sites (separated by 800m), and creek discharge in the undisturbed upper reaches of the South Alligator River; showing regression line and 95 percent confidence interval ($R^2 = 0.600$; $F=17.997$; $d.f.=1,12$; $p=0.0011$). Dissimilarity values are based on $\log_{10}(x+1)$ transformed data (C.Humphrey, unpublished data).

In this study, linear regression of the dependent variable, dissimilarity, and the independent variable, instantaneous creek discharge (averaged between the paired sites) was conducted using paired upstream-downstream site data in the unimpacted condition (data from both creeks combined). A highly significant association between dissimilarity and discharge was found ($R^2 = 0.8036$; $F = 45.013$; $df = 1,11$; $p = 0.0001$) (Figure 3.8). Few of the data points lie outside the 95 percent confidence interval for this regression relationship. Further, the strength of this linear regression ($R^2 = 0.8036$) illustrates the homogeneity of the relationship for data points derived from both Jim Jim and Twin Falls creeks. The regression relationship also indicates that the observed difference in dissimilarity values between Jim Jim and Twin Falls creeks for sampling occasions early in the study period (Figure 3.6) is a result of significant differences in discharge of the two creeks at this time.

Given the dissimilarity - discharge association, it may be expected that impacted sites, as a result of the additional perturbing influence of the disturbance, may show a departure from the relationship in the undisturbed condition when these data are

superimposed upon the same plot for unimpacted sites. When the values for Jim Jim Creek *after* the onset of the disturbance are plotted on the same regression line derived for the undisturbed state, they are seen to consistently fall outside the 95 percent confidence limits for the regression line of the undisturbed condition (Figure 3.8). The fact that these points are clear outliers, being inconsistent with both the trend of the 'impact' stream in the 'before' period, as well as the control stream throughout the study period, is strong evidence that an impact related community change has occurred downstream of the road crossing.

The degree of departure of the impacted sites is greatest at the lowest discharge values, corresponding with the last sampling occasion (indicating the increasing divergence of the macroinvertebrate communities in the latter part of the study period). Furthermore, the degree of divergence from the unimpacted condition is perhaps greater for the site 200m downstream (JJ1/JJ2) relative to 1000m downstream (JJ1/JJ3), indicating that the likely community change is greater at the JJ2 site than the JJ3. These observations reinforce the indications provided by the plots of site dissimilarity through time.

Whilst the spatial separation between JJ1/JJ2 (= 400 m) differed from that of TF1/TF2 and JJ1/JJ3 (=1200 m), this factor was found not to contribute significantly to the regression relationship of paired-site dissimilarity and discharge. Thus, with step-wise regression using the variables of paired site distances and creek discharge at the time of sampling, the partial coefficient of the distance variable was not significant ($R^2 = 0.0049$; $F = 0.2577$; $df = 2,10$; $p = 0.6227$).

No significant regression relationship could be derived for discharge and dissimilarity for the Jim Jim Creek paired site data, all sampling occasions combined ($F = 0.579$; $df = 1,12$; $p = 0.4614$). Furthermore, the graphical approach (Figure 3.8) indicated that the impacted sites clearly lay outside the relationship established for the undisturbed condition. Thus a formal test of homogeneity of line elevations (ANCOVA) or slopes (see methods in Snedecor and Cochran, 1980) between the 'impacted' and 'control' condition was not required.

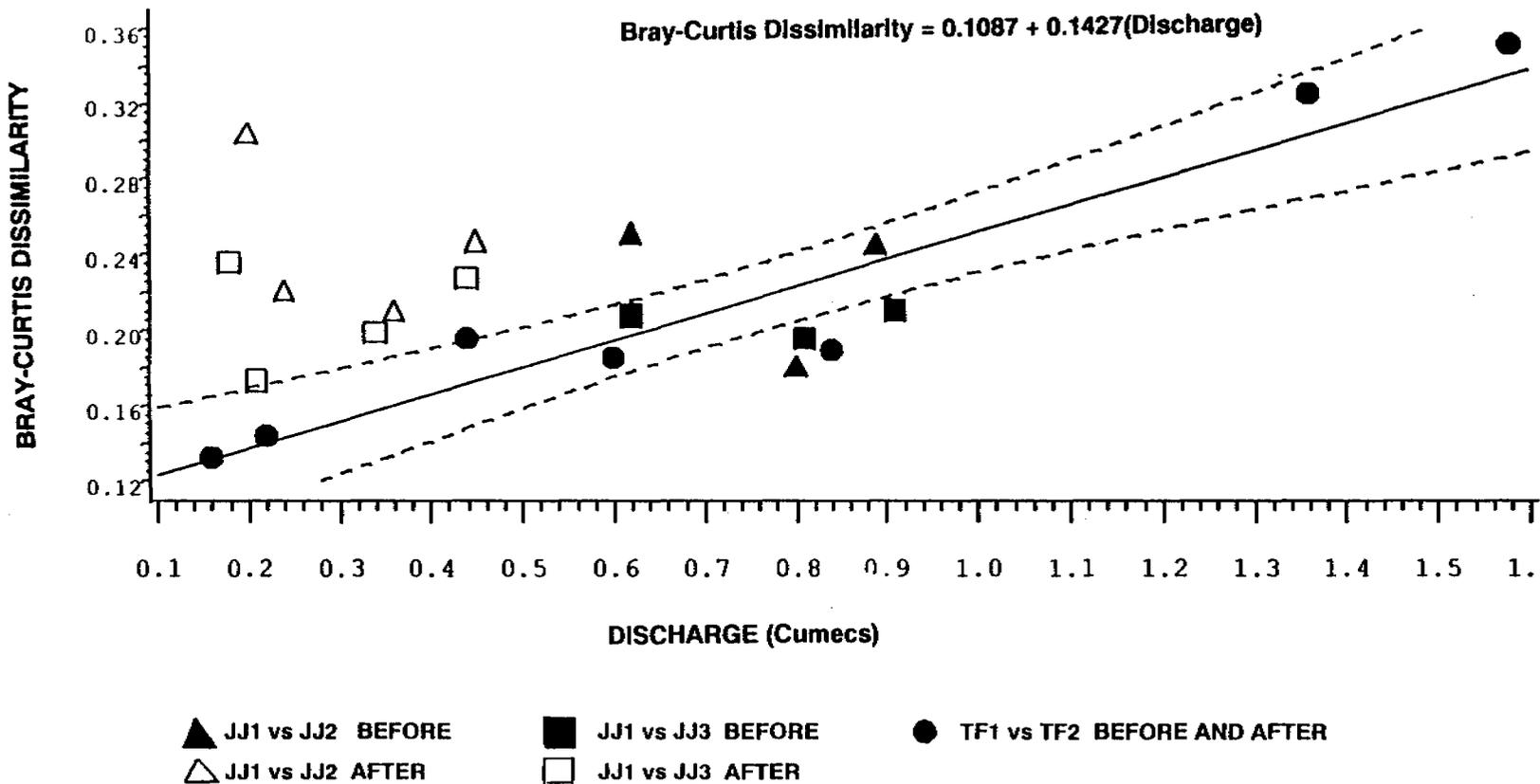


Figure 3.8. Regression relationship between dissimilarity of paired upstream and downstream sites, and instantaneous creek discharge at the time of sampling for *rootmat* habitat in the Jim Jim Creek study. The regression line and 95 percent confidence interval is shown for the undisturbed condition in both Jim Jim and Twin Falls creeks (shaded symbols). The values for the Jim Jim Creek after the opening of the road crossing (impacted) are also plotted (open symbols). Dissimilarity values are based on $\log_{10}(x+1)$ transformed data.

Sand Habitat

The dissimilarity values for the sand habitat are particularly 'noisy', with a large amount of temporal variability. Using $\log_{10}(x+1)$ transformed data, the Jim Jim Creek dissimilarities do not display any consistent departures from the control stream in the early part of the study period, with considerable interaction between the two streams throughout the study period (Figure 3.9). In August there was a slight divergence of the dissimilarity values for the 200 m downstream site comparison (JJ1/JJ2) in relation to the other two comparisons (TF1/TF2 and JJ1/JJ3) (Figure 3.9). However this could not be considered strongly indicative of any impact related community change given the variability displayed among locations and sampling occasions.

In contrast to the rootmat, regression of dissimilarity and discharge in the unimpacted condition showed no significant relationship for sand habitat ($F = 0.565$; $df = 1,11$; $p = 0.4681$). Thus the option of covariate trend analysis was not available.

The temporal variability confounding the effects of any distinct trends of pairs of sites, relative to one another throughout the study period, is also seen in the untransformed data (Figure 3.5).

In summary, there are no distinct trends evident, outside the temporal variability observed at all sites, that would indicate any conclusive change in the macroinvertebrate communities of Jim Jim Creek after vehicle access to the crossing.

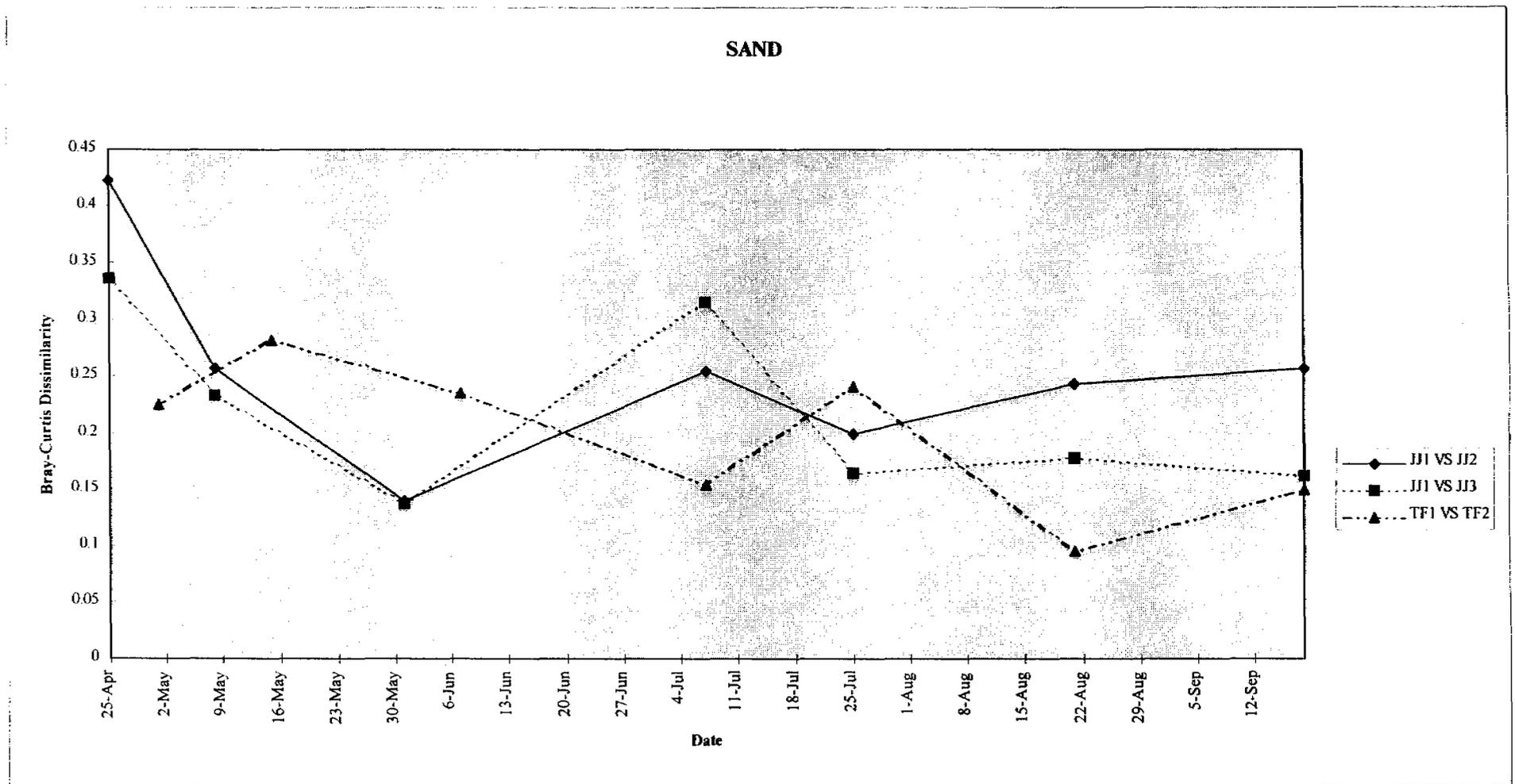


Figure 3.9. Multivariate dissimilarity values between paired upstream / downstream sites based on samples collected from the *sand* habitat, using $\log_{10}(x+1)$ transformed data.

3.3 Multivariate Ordination

A complimentary approach to the comparison of community changes amongst sites is multivariate ordination, whereby samples are represented in ordination space on the basis of their multivariate similarity. To eliminate some of the temporal influences associated with declining creek discharge throughout the dry season, ordination was performed on sample data for the 'before' period and 'after' period separately. For this analysis, samples collected in early July were included in the 'before' period since there was no apparent elevation in suspended solids levels at this time. Ordinations were performed on raw (untransformed) data as well as $\log_{10}(x+1)$ transformed data to give an indication as to what extent the necessity of transforming the data in the BACIP analysis (section 3.2) may have had in influencing the observed results. Both untransformed and transformed data ordinations are presented herein. Ordinations were performed in three dimensions, which was necessary to reduce the 'stress' below the suggested maximum of 0.2 (Belbin, 1993b).

Rootmat Habitat

The predominant characteristic of the ordination of rootmat samples in the 'before' impact period was the relative homogeneity among samples for all sites. This was indicated by the interspersion of samples from all sites in ordination space. Both the ordination based on untransformed data (Figure 3.10), and the transformed data (Figure 3.13) show this interspersion. Whilst only axes 1 and 2 out of the three axes of the 3-dimensional ordination are plotted in Figures 3.10 and 3.13, plots of the third axis against the other two axes (data not shown here) displayed similar interspersion among sites. Hence, the macroinvertebrate communities sampled in the rootmat habitat show no indication of being consistently 'different' at any particular sampling site(s) in the 'before' impact period.

In contrast to the samples collected in the 'before' period, the rootmat sample ordination in the 'after' period shows a clear separation of the JJ2 samples (200 m downstream) from the samples collected from other sites, particularly the six points which represent replicate samples from the last two (August and September) sampling occasions.

Examination of plots of axes 1 and 2 against axis 3 for the 'after' period samples (data not shown here) gave no additional interpretative information to the plots shown in Figures 3.11 and 3.14. Whilst this separation of the JJ2 samples is greater in the ordination based on untransformed data (Figure 3.11), the separation is still evident in the transformed data (Figure 3.14). In the ordination based on untransformed data, two JJ3 replicate samples for the last sampling occasion also lie in a similar position in ordination space to the JJ2 samples (Figure 3.11). The separation of these sites was not apparent in the ordination based on transformed data (Figure 3.14). The undisturbed sites (JJ1, TF1 and TF2), as well as most replicates from site JJ3, constitute a separate cluster (from the above-mentioned outliers) and are generally interspersed with one another in ordination space; in ordinations based on both transformed and untransformed data (Figures 3.11 and 3.14 respectively).

Principle axis correlation was performed on the 'after' period sample ordinations to assist in explaining the outliers from the above mentioned sites.

In the ordination based on untransformed data, principle axis correlation revealed that turbidity and suspended solids were both significantly ($p < 0.01$) correlated with the ordination in the direction of the JJ2 site separation (Figure 3.12a). Alkalinity and bicarbonate were also significantly correlated ($P < 0.01$) with the ordination in the 'after' period, but ran in a direction perpendicular to the JJ2 site separation (Figure 3.12a). There was also no separation of any particular sites along these gradients in alkalinity and bicarbonate, indicating that their correlation with the macroinvertebrate sample ordination was a product of temporal trends in these parameters as well as the macroinvertebrate communities at all sites.

In the transformed ordination, the environmental parameters of turbidity, conductivity, alkalinity and bicarbonate were correlated with the ordination (Figure 3.15a). Again, the correlation of turbidity was seen to run in a direction similar to the separation of the JJ2 sites. Notably, suspended solids were not significantly ($p < 0.05$) correlated with the ordination based on transformed data. This is possibly a consequence of impact-related community changes occurring subsequent to the peaking of suspended solids concentrations. The other environmental correlates (conductivity, alkalinity and bicarbonate) ran in similar directions to one another, but close to perpendicular to that of turbidity and the JJ2 site separation (Figure 3.15a). As stated above, these non-disturbance-related correlates are most likely a product of temporal change, across all sites, in these parameters and also the macroinvertebrate communities.

Taxa significantly correlated ($P < 0.01$) with the ordination based on untransformed data in the 'after' period included Chironomidae, Ceratopogonidae, Baetidae, Caenidae and Acarina. All of these taxa were negatively correlated in directions aligned with some degree of JJ2 site separation (ie. reduced abundances at site JJ2). Of these taxa, Chironomidae had a particularly strong correlation with the ordination ($R = 0.89$), suggesting that this taxa, in particular, was adversely affected by the downstream elevation of suspended solids in Jim Jim Creek (Figure 3.12b).

Taxa significantly correlated with the transformed ordination included Chironomidae, Elmidae, Caenidae and Baetidae (Figure 3.15b). Of these, Chironomidae was seen to be correlated in a similar but opposite direction to the separation of the JJ2 sites, denoting again, adverse effects of the disturbance upon this taxon. Elmidae, Baetidae and Caenidae were all negatively correlated in directions that may explain some of the JJ2 site separation, indicating their apparently lower abundances in the ordination space occupied by the JJ2 outliers.

The ordination of samples collected in the 'after period' indicates the general similarity of sites that remained undisturbed (JJ1, TF1, TF2) and the apparently unimpacted state of most samples from the JJ3 downstream site. The JJ2 samples, and the two JJ3 replicates that constitute outliers from the 'cluster' of the undisturbed-site samples in

ordination space are likely to have experienced impact-related community changes. This was indicated by their change from being interspersed with undisturbed samples, to being outliers, between the 'before' and 'after' impact periods. Furthermore, principle axis correlation revealed that abundance of several major taxa were negatively correlated in the direction of impacted site separation indicating their lower abundance at these sites relative to other unimpacted sites.

The ordination results in the 'after' period are in accord with the observations made of the BACIP site dissimilarities, in that the greatest degree of multivariate divergence was greater with untransformed data. The transformed data indicated similar effects but of lesser magnitude in the analysis. This is suggestive that the disturbance is affecting the 'common' (highly abundant) taxa, as $\log_{10}(x+1)$ transformation has the general effect of down-weighting the influence of abundant taxa .

Overall, the ordination based on rootmat samples reinforces the BACIP observation of a definite multivariate community change at site JJ2, relative to the other undisturbed locations, after the opening of the Jim Jim Creek road crossing to traffic. The observation of several impacted JJ3 replicate samples from late in the dry season further supports the BACIP results, in which slight changes to macroinvertebrate communities were detected at site JJ3, late in the study period.

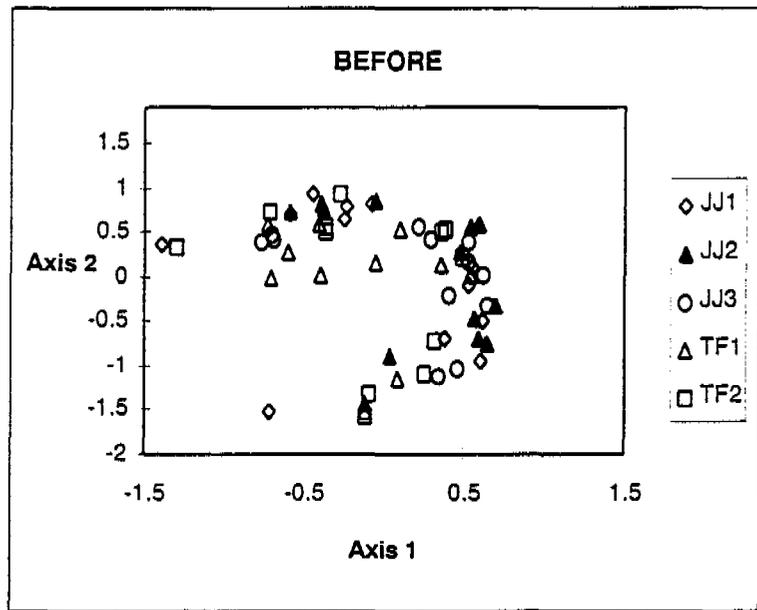


Figure 3.10. Multivariate ordination of rootmat samples based on untransformed data for all sites before the onset of the disturbance(stress = 0.11) (using SSHMDS ordination in 3 dimensions).

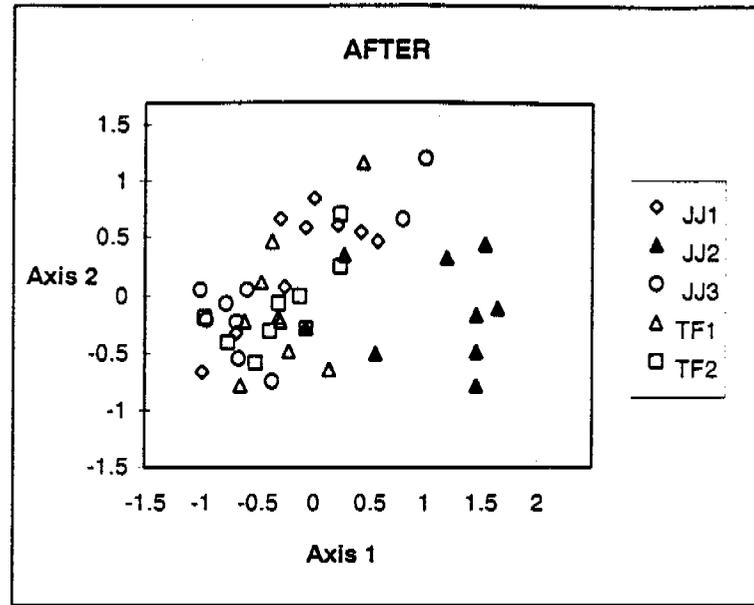


Figure 3.11. Multivariate ordination of rootmat samples based on untransformed data for all sites after the onset of the disturbance (stress = 0.12) (using SSHMDS ordination in 3 dimensions).

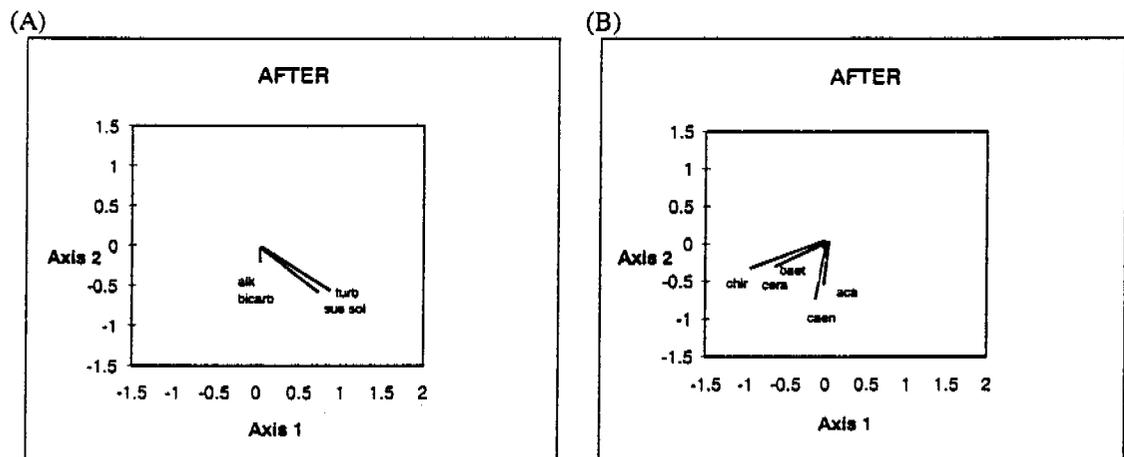


Figure 3.12. Environmental variables (A) and macroinvertebrate taxa (B), significantly correlated ($p < 0.01$) with the 'after' period ordination shown above in Figure 3.11.

[Codes for the correlates and their associated R values are as follows: (A) turb = turbidity (0.79); sus sol = total suspended solids (0.64); alk = alkalinity (0.61); bicarb = bicarbonate (0.58); (B) chir = Chironomidae (0.89); cera = Ceratopogonidae (0.57); baet = Baetidae (0.72); aca = Acarina (0.69); caen = Caenidae (0.77)].

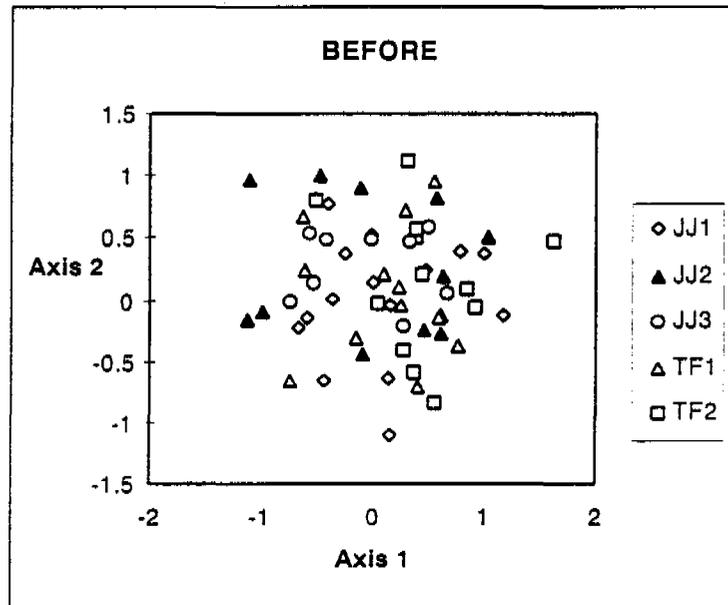


Figure 3.13. Multivariate ordination of rootmat samples based on $\log_{10}(x+1)$ transformed data for all sites before the disturbance (stress = 0.19) (using SSHMDS ordination in 3 dimensions).

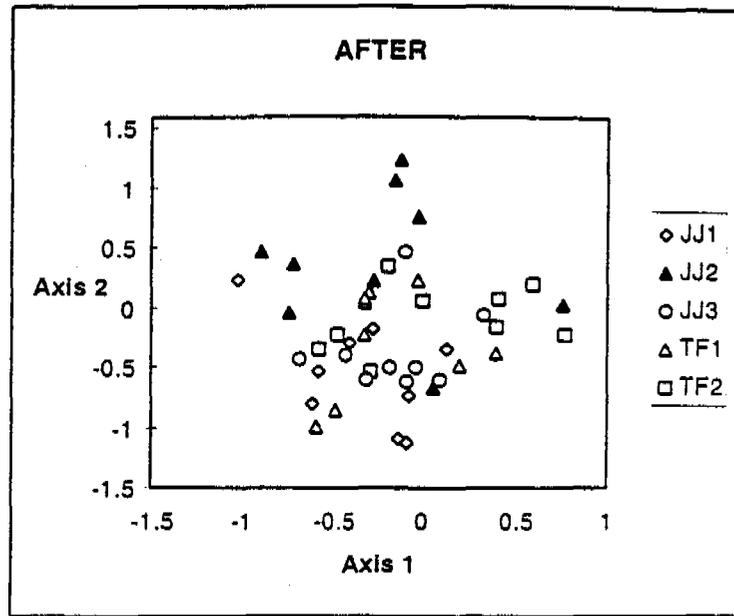


Figure 3.14 Multivariate ordination of rootmat samples based on $\log_{10}(x+1)$ transformed data for all sites after the onset of the disturbance (stress = 0.21) (using SSHMDS ordination in 3 dimensions).

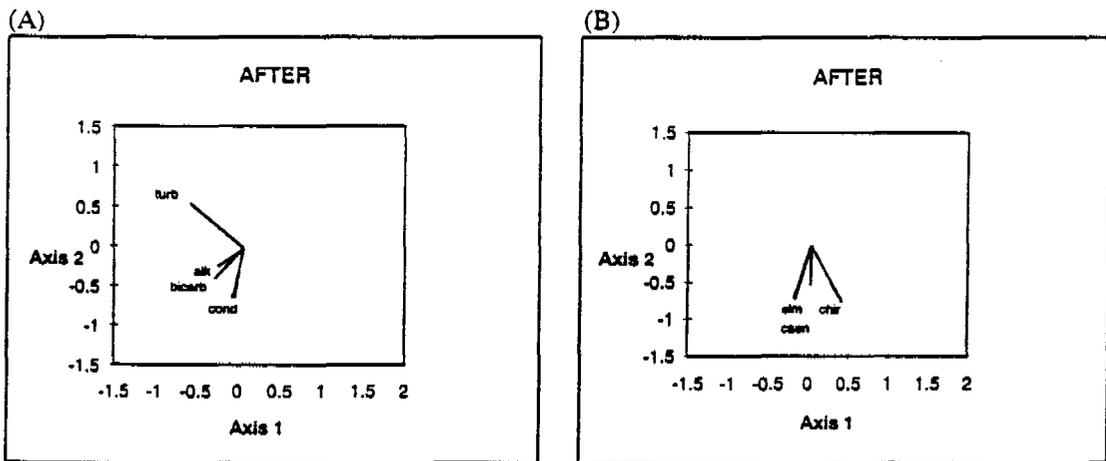


Figure 3.15. Environmental variables (A) and macroinvertebrate taxa (B), significantly correlated ($p < 0.01$) with the 'after' period ordination shown above in Figure 3.14.

[Codes for the correlates and their associated R values are as follows: (A) turb = turbidity (0.71); cond = conductivity (0.59); alk = alkalinity (0.63); bicarb = bicarbonate (0.54); (B) chir = Chironomidae (0.66); elm = Elmidae (0.57); caen = Caenidae (0.64)].

Sand Habitat

The ordinations based on the sand samples, collected in the 'before' period show a general interspersion of points corresponding with replicates from different sites, on the basis of both untransformed and transformed data (Figures 3.16 and 3.18). A similar interspersion (in observation of all three dimensions) of all sites was seen in the ordination of samples collected in the 'after' period. Plots of axes 1 and 2 are given in Figures 3.16 and 3.18, being characteristic of the interspersion seen in all three dimensions. The interspersion of points in the 'before' period ordination indicates their similarity in the pre-impact (undisturbed) condition.

A similar interspersion was observed among sampling sites in the 'after' period in ordinations based on both transformed and untransformed data (Figures 3.17 and 3.19). Examination of the other dimensions (plots of axes 1 and 2 against axis 3) confirmed that there was no grouping or separation of samples.

The consistent interspersion of samples from downstream sites with undisturbed sites, in both 'before' and 'after' periods indicates an overall similarity in macroinvertebrate community structure at these sites in both periods. Thus, there is a general absence of apparent impact-related changes to the macroinvertebrate communities in this habitat downstream of the road crossing

Results from the multivariate ordination reinforce the observation, made in comparison of site dissimilarity values, of no apparent impacts in the sand habitat samples resulting from vehicular usage of the Jim Jim Creek road crossing.

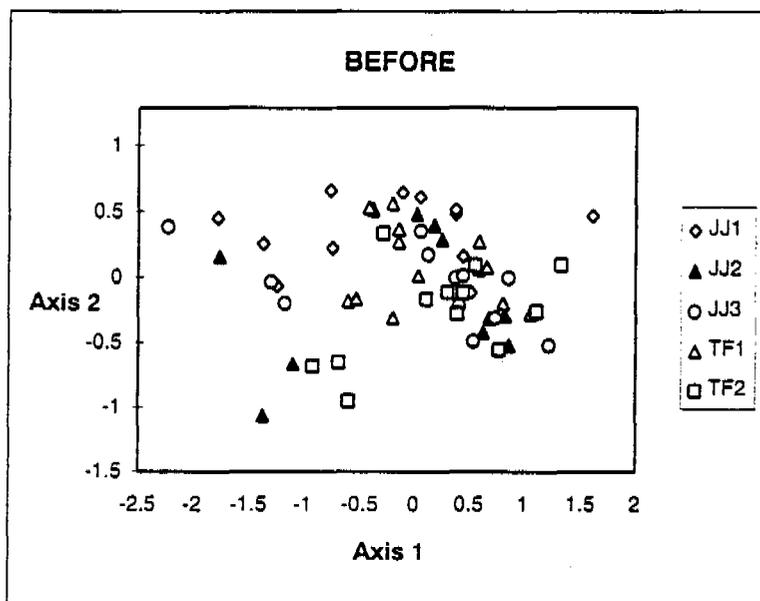


Figure 3.16. Multivariate ordination of sand samples based on untransformed data for all sites before the onset of the disturbance (stress = 0.11) (Using SSHMDS ordination in 3 dimensions).

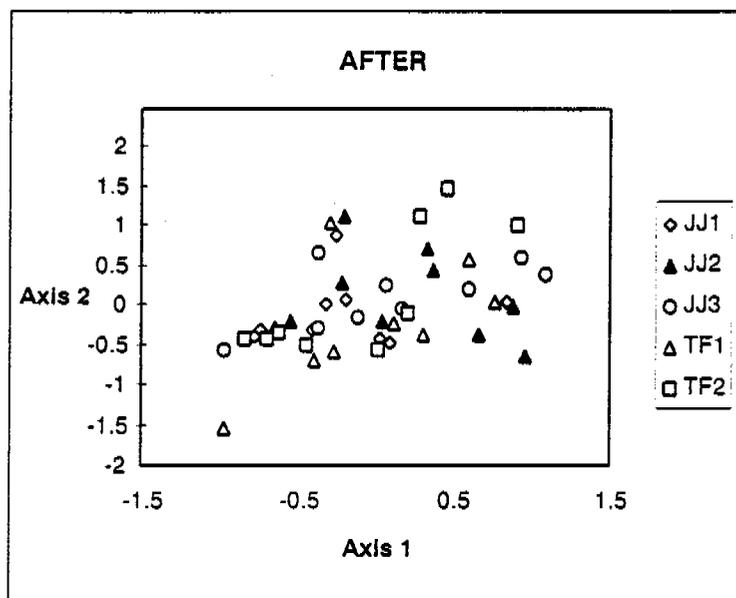


Figure 3.17. Multivariate ordination of sand samples based on untransformed data for all sites after the onset of the disturbance (stress = 0.096) (Using SSHMDS ordination in 3 dimensions).

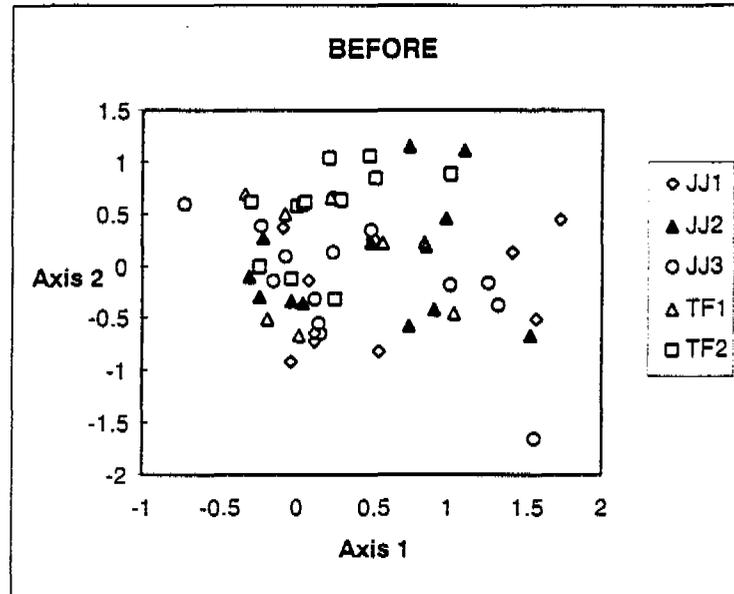


Figure 3.18. Multivariate ordination of sand samples based on $\log_{10}(x+1)$ transformed data for all sites before the onset of the disturbance (stress = 0.18) (Using SSHMDS ordination in 3 dimensions).

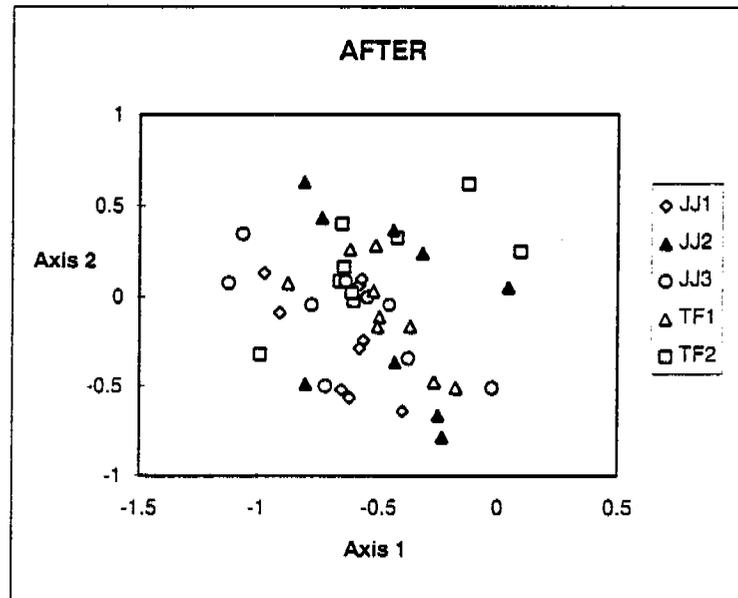


Figure 3.19. Multivariate ordination of sand samples based on $\log_{10}(x+1)$ transformed data for all sites after the onset of the disturbance (stress = 0.19) (Using SSHMDS)

3.4 Taxa Abundance Comparisons

Twenty-six macroinvertebrate families, as well as order Acarina and class Oligochaeta, were represented amongst rootmat and sand habitat samples collected during the study. In both the sand and rootmat habitat, the families Chironomidae, Elmidae, Caenidae, Baetidae Ceratopogonidae and order Acarina accounted for the majority of the benthic invertebrate fauna (appendices 2A and 2B). At the family level, the taxonomic composition of the rootmat and sand habitat was very similar. Only five low-abundance taxa were not represented in both habitats. Of the taxa present, Chironomidae represented the largest proportion of the fauna recovered from most samples. As described in the following sections, the rootmat and sand habitat displayed distinctly different temporal trends with regard to taxa abundance.

The following comparisons are based on the mean abundance of the specified taxon across the three replicate samples, for each site and sampling occasion.

Rootmat Habitat

In the rootmat habitat, mean total taxa abundance increased at all sites up until the early July sampling occasion, when the mean taxa abundance in samples tended to plateau. There was a general similarity of mean taxa abundance (which is directly related to macroinvertebrate density) among the sampling sites on each sampling occasion. The exceptions were exceedingly high macroinvertebrate abundances at Twin Falls Creek sites relative to other sites for the early July sampling occasion; and significantly, lower mean taxa abundance (relative to other sampling sites) at site JJ2 on the last two sampling occasions (August and September) (Figure 3.20).

The mean abundance of Chironomidae throughout the study period was seen to closely match the mean total taxa abundance, a consequence of the high representation of Chironomidae in all samples. The abundance of Chironomidae was seen to be markedly reduced at site JJ2 relative to other study sites late in the dry season (August and September), as was also reflected in mean total taxa abundance (Figure 3.21).

Low abundances of elmids were observed at all sites early in the study period (Figure 3.22). Subsequently they became relatively abundant on certain sampling occasions, but showed considerable variability among sampling sites throughout the study period. The high degree of temporal and spatial variability in Elmidae abundance makes it difficult to discern whether low abundances at the downstream site JJ2, in the final sampling occasions (Figure 3.22), are the result of turbidity effects. The ephemeropteran families, Caenidae and Baetidae, also demonstrated a high degree of temporal and spatial patchiness among sampling sites. It is notable, however, that with regard to these two families, site JJ2 had consistently low abundances relative to the other sampling sites, on sampling occasions from late July onwards (Figures 3.23 and 3.24). High variability was also observed in the abundance of Ceratopogonidae among sites, although the abundance at site JJ2 was reduced relative to other sampling sites on the final sampling occasion (September) (Figure 3.25). Abundance of Acarina was relatively consistent among all sampling sites on the different sampling occasions throughout the study period (Figure 3.26).

In summary, there was a high degree of inter-site variability in the mean abundance of the major taxa represented in samples collected throughout the study period. There was, however, a clear decline in chironomid abundance in the latter part of the study period at site JJ2. Further, it was apparent that site JJ2 did not display the same temporal variability in the abundance of several other taxa (Baetidae, Caenidae, Ceratopogonidae) that was observed at other sampling sites, but rather had consistently low abundances of these taxa.

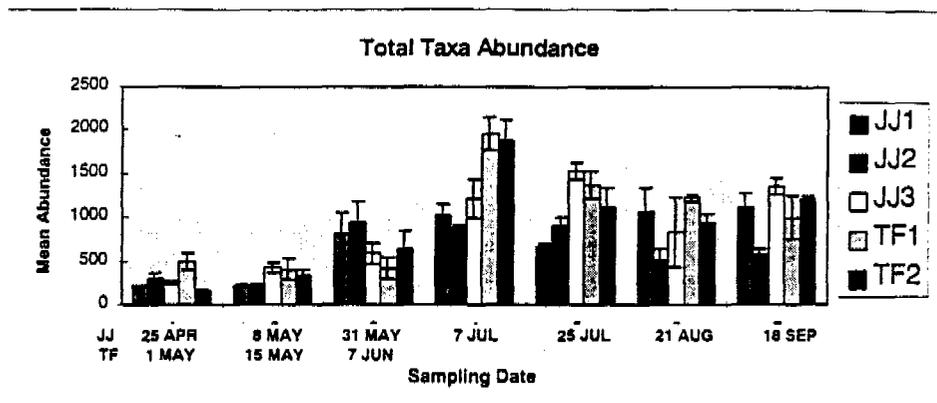


Figure 3.20. Mean total taxa abundance in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

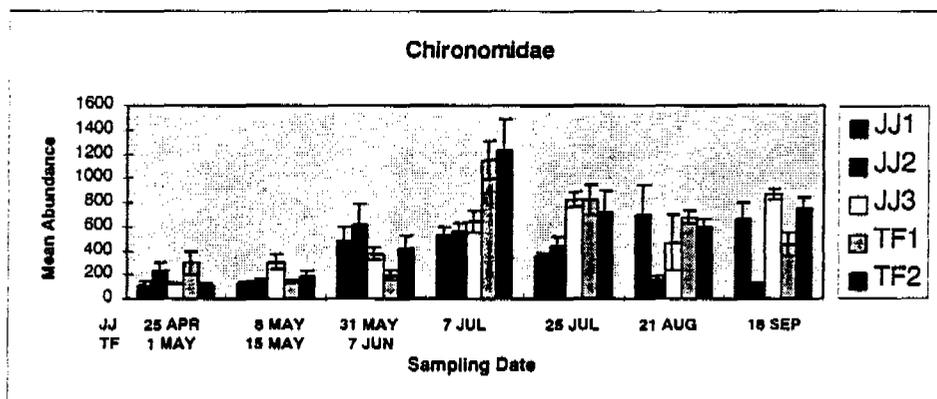


Figure 3.21. Mean abundance of Chironomidae in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

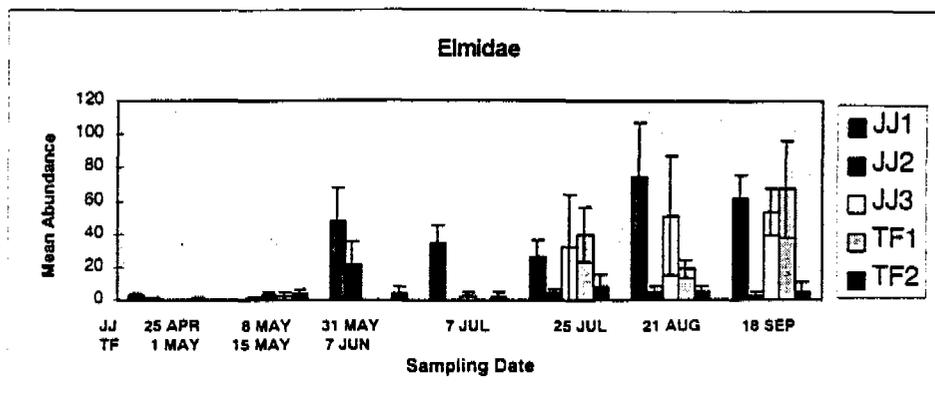


Figure 3.22. Mean abundance of Elmidae in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

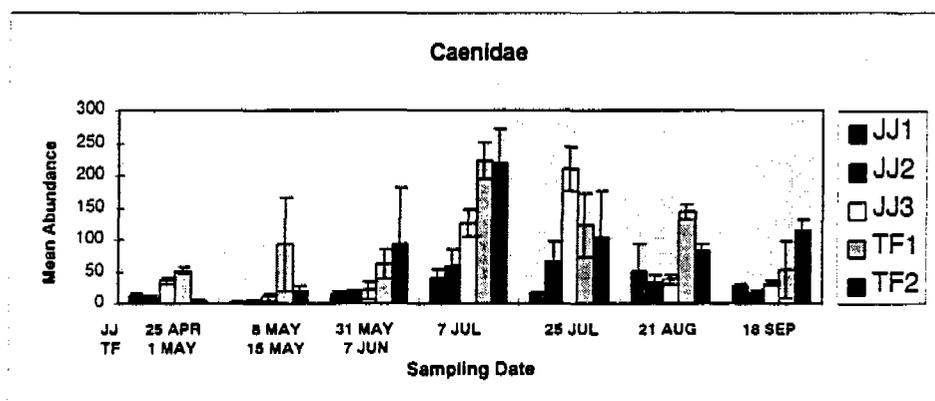


Figure 3.23. Mean abundance of Caenidae in rootmat samples for five sampling sites, on each sampling occasion throughout the study period.

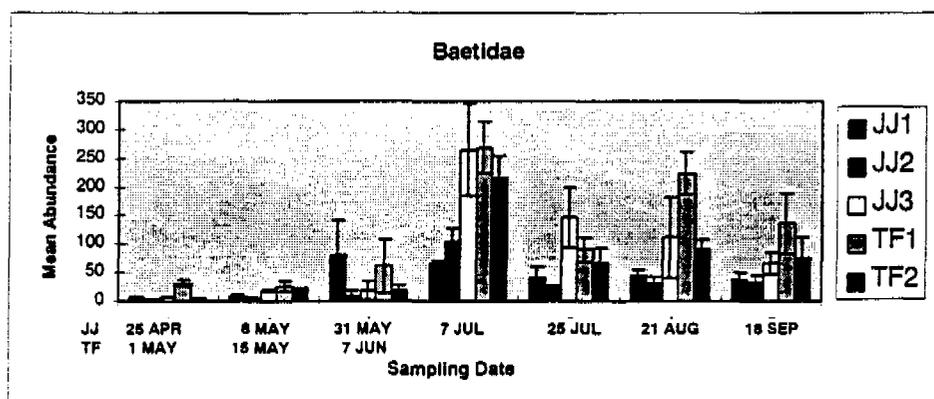


Figure 3.24. Mean abundance of Baetidae in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

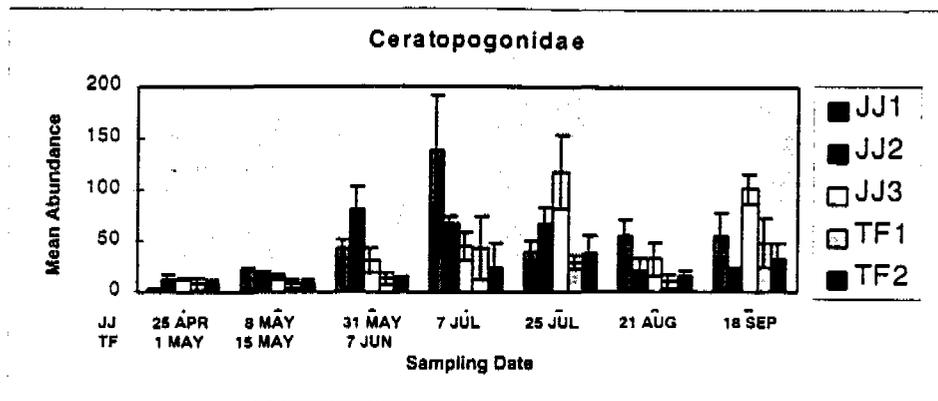


Figure 3.25. Mean abundance of Ceratopogonidae in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

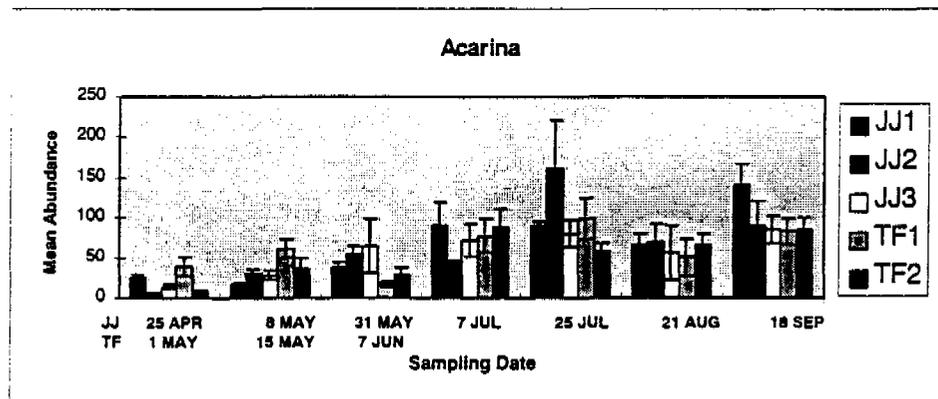


Figure 3.26. Mean abundance of Acarina in rootmat samples for the five sampling sites, on each sampling occasion throughout the study period.

Sand Habitat

In the sand habitat, total taxa abundance showed a steady increase throughout the study period, reaching a maximum at the conclusion of the sampling in late September. Notably, the mean taxa abundance at site JJ2 was considerably lower than all other sampling sites on the last sampling occasion (Figure 3.27). Mean chironomid abundance similarly showed a general increase throughout the study period. Within this trend, however, Chironomid abundance was seen to be very variable among sites and sampling occasions (Figure 3.28). Elmidae became a particularly abundant taxon in sand habitat samples from late July onwards. For the last sampling occasion, site JJ2 had considerably lower abundances of Elmidae than all other sites (Figure 3.29). The mean abundance of Caenidae and Baetidae was generally consistent among all sample sites, although the abundance of Caenidae at site JJ2 was seen to be less than other sampling sites on the last sampling occasion (September) (Figures 3.30 and 3.31). The abundance of Ceratopogonidae in the sand habitat showed extreme variability amongst replicate samples at each site, as indicated by the large values of the standard error about the mean (Figure 3.32). Considering this variability, there was a general consistency among all sampling sites in the abundance of Ceratopogonidae on each sampling occasion. A large amount of temporal and inter-site variability was observed in the abundance of Acarina. However within this variability, the abundance at site JJ2 was considerably less than the other sampling sites on the last two sampling occasions (Figure 3.33).

Overall, the abundance of individual taxa displayed a general consistency among all sampling sites throughout the study period. However there was some indication of reduced abundance of a number of the major taxa at site JJ2, relative to other sites, on the last sampling occasion (September) only. The significance of this observation as a possible impact-related change to the community is uncertain considering there was no evidence in the BACIP analysis or multivariate ordination for community changes at site JJ2 on the last sampling occasion.

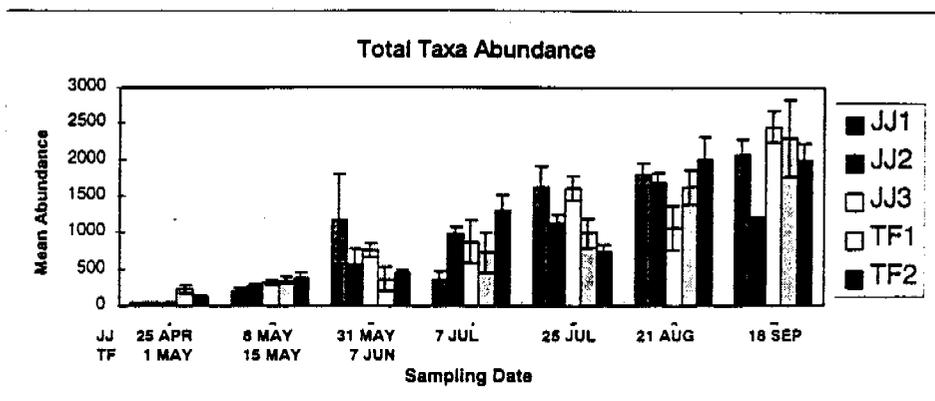


Figure 3.27. Mean total taxa abundance in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

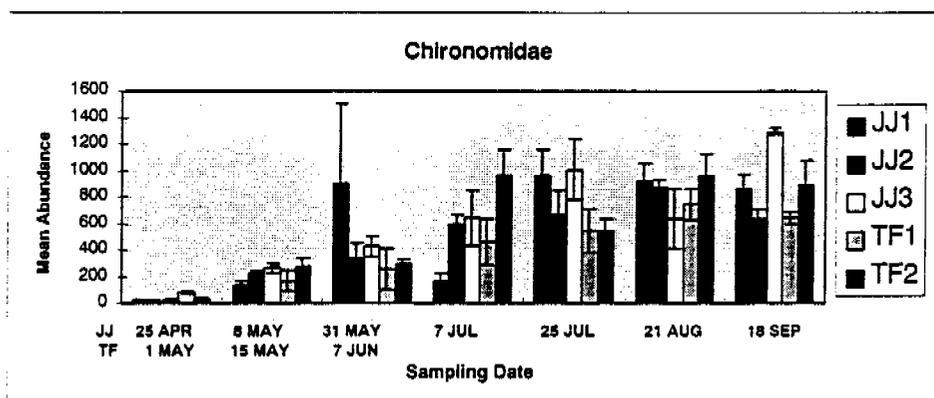


Figure 3.28. Mean abundance of Chironomidae in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

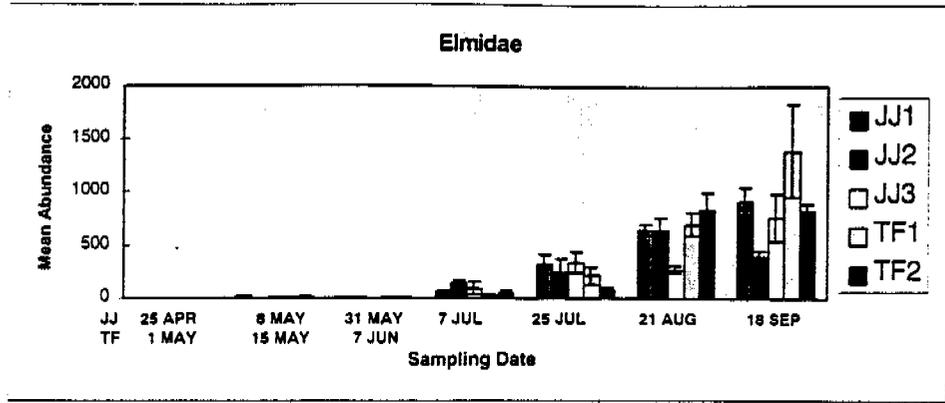


Figure 3.29. Mean abundance of Elmidae in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

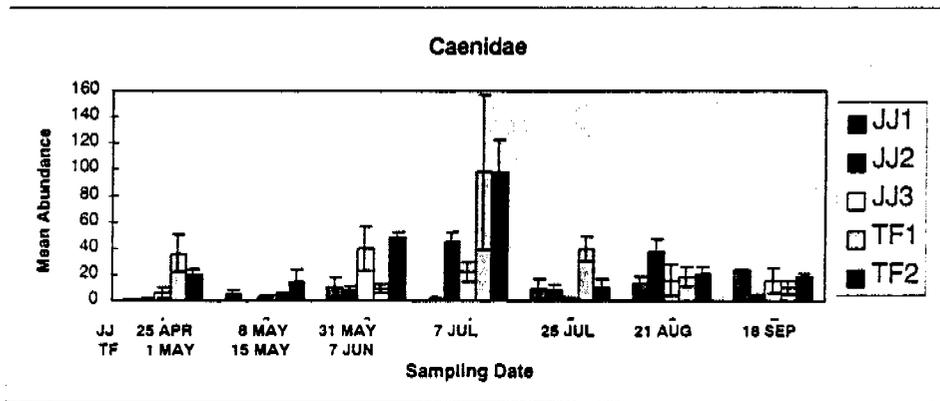


Figure 3.30. Mean abundance of Caenidae in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

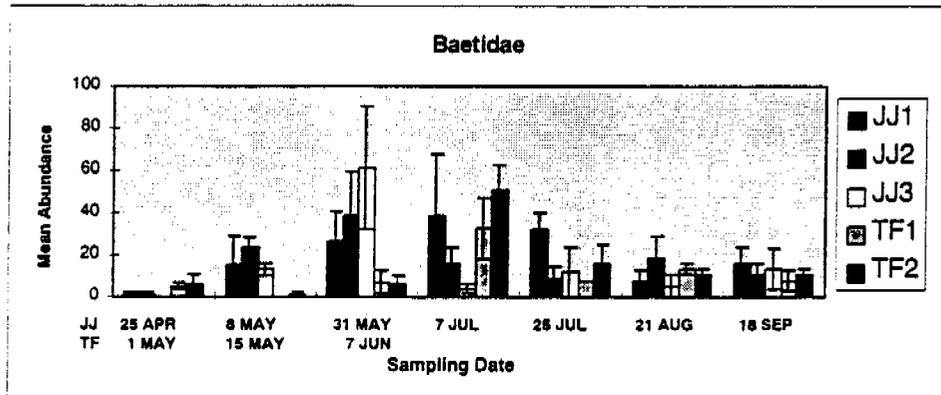


Figure 3.31. Mean abundance of Baetidae in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

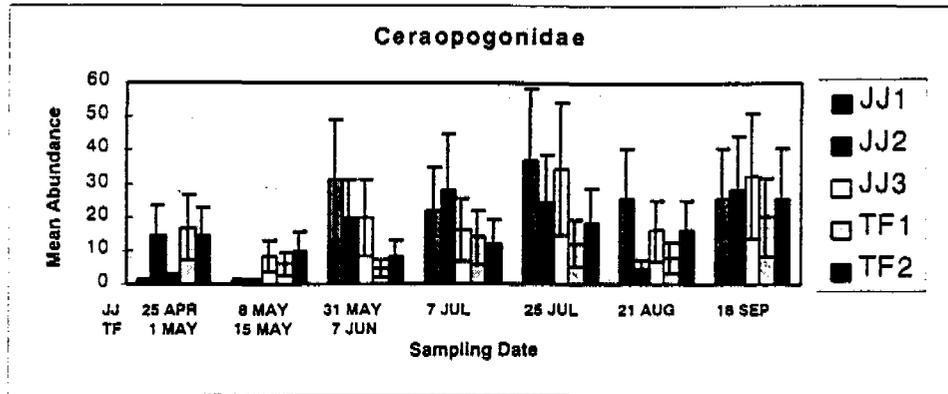


Figure 3.32. Mean abundance of Ceratopogonidae in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

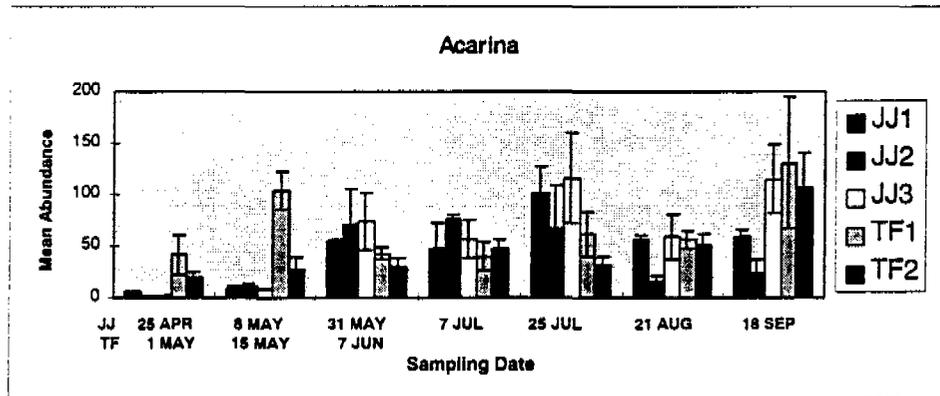


Figure 3.33. Mean abundance of Acarina in sand samples for the five sampling sites, on each sampling occasion throughout the study period.

Comparison of Rootmat and Sand Substrates

There were clear differences in the temporal trend in total invertebrate abundance in the sand and rootmat habitats during the study. In the rootmat, mean macroinvertebrate abundance in samples (and hence density) showed a plateau mid-way through the study period. In contrast, invertebrate density in the sand habitat continued to increase throughout the entire study period. In both habitats, Chironomidae formed the predominant taxa, although in the sand habitat Elmidae were also abundant, particularly in the latter half of the study period.

Chironomid abundance showed a clear decline in the rootmat habitat at site JJ2 for the August and September sampling occasions. There was also some evidence for a reduction at this site in Ceratopogonidae and the two ephemeropteran families, Baetidae and Caenidae; although the latter two taxa were also characterised by extreme variability in abundances among the undisturbed sites. In the sand habitat, there was only limited evidence for changes at site JJ2 in the abundance of major taxa. Lower chironomid, elmid and Acarina abundance were observed at site JJ2 on the last sampling occasion. However, there was also a high degree of variability in the abundance of these taxa among sampling sites on previous sampling occasions.

3.5 Summary of Results

Rootmat Habitat

There was strong evidence in the rootmat habitat samples for an impact-related change in macroinvertebrate communities at site JJ2 after the opening of the Jim Jim Creek road crossing to traffic. The divergence in community structure at this site relative to the unimpacted sites became most apparent on the last two sampling occasions (August and September). This result was indicated by both the BACIP data and multivariate ordination. The BACIP approach provided some evidence for impact-related community changes at site JJ3 on the last sampling occasion, although this was not as evident in the ordination analysis. The two different multivariate approaches (BACIP analysis and ordination) both demonstrated a community response to disturbance in Jim Jim Creek. Taxa abundance data across sites and sampling occasions complimented the multivariate approaches in explaining

which taxa were responsible for the observed changes in downstream macroinvertebrate communities. A distinct reduction was observed in the mean overall taxa abundance in samples from the rootmat habitat collected on the last two sampling occasions from site JJ2, relative to other sampling sites. Observation of the abundances of major taxa revealed that the reduction in overall abundance was largely due to reduced abundance of chironomids. This observation was strengthened by principle axis correlation which demonstrated that chironomid abundance was negatively correlated with the ordination space in the direction of JJ2 samples, whilst turbidity and suspended solids were positively correlated in a similar direction.

Sand Habitat

Most evidence pointed to the absence of impact-related community changes in the sand habitat. The BACIP data obtained for the sand habitat showed that the community changes occurring in Jim Jim Creek were similar to those observed in the undisturbed Twin Falls Creek throughout the study period. This was supported by multivariate ordination which showed the downstream Jim Jim Creek sites to be consistent with the undisturbed sites, even after the onset of the disturbance. Observation of taxa abundance data indicated that mean taxa abundance at site JJ2 was less than other sampling sites for the last sampling occasion. However, this was in a context of high variability in taxa abundance among sites throughout the study period. Considering the lack of any indication of an impact using multivariate analysis techniques, and that there was no *consistent* trend of reduced taxa abundance in the after-impact period the single observation of reduced abundance; the likelihood of this representing a impact-related change, is difficult to infer.

CHAPTER 4

DISCUSSION

CHAPTER 4: DISCUSSION

Approaches to the effective monitoring and assessment of environmental impacts have been become one of the foremost issues in applied science. This study highlighted some of the primary issues related to the monitoring of such impacts, such as the practicality of implementing a study within the constraints imposed by locality, the time available to gather data and the characteristics of the disturbance. The necessity for adaptability in analytical approaches was illustrated by the need in this study to employ a modified experimental design and analysis whilst preserving as much valuable biological information collected as possible. This study also illustrates the importance of interpretation and evaluation of observations in the context of *all* available evidence *and* the situation being assessed, in this case the study area being of particularly high conservation value.

4.1 The Physical Disturbance in Jim Jim Creek

The maximum levels of turbidity (~60 NTU) and suspended solids (~100 mg/L) observed downstream of the Jim Jim Creek road crossing represents a significant alteration of conditions occurring naturally in this system during the dry season. It is worth noting that the levels of optical turbidity and suspended solids observed for a distance of 1000 m downstream of the Jim Jim Creek road crossing substantially exceed the guidelines set for Australian waters (ANZECC 1992). These guidelines recommend that seasonal mean turbidity of a waterway should not change by more than 10 percent (when measured nephelometrically, as in this study), whereas increases of up to 1200 and 600 percent were observed 200 m and 1000 m downstream of the road crossing, respectively.

The elevated levels of suspended sediment measured in this study would be well in excess of any natural levels experienced by this system, particularly during the dry season. At other times of the year, notably during flood events in the wet season, brief pulses (1 to 2 days) of elevated suspended sediment (eg. 48 mg/L) may be experienced, as has been reported for Magela Creek, another major creek system within Kakadu National Park (Hart *et al.*, 1982). Thus it may be assumed that the disturbance is of concern in the light of the one of the objectives of managing the area, namely "...to protect Park resources from the undesirable effects of...erosion, pollution and other activities of people." (ANCA, 1991).

The short delay in the rise and subsequent peak of turbidity levels after the opening of the crossing may be attributed to the time taken for the relatively clean sand deposited on the creek-bed during the Wet season to be eroded away from sections of the crossing to expose the finer clay sediment that underlies the sand. The decline in turbidity levels in the latter part of the tourist season may be due to the decline in stream flow throughout the study period, with lower water velocities reducing the amount of sediment transported from the road crossing and perhaps also the distance suspended particles would be transported. The frequency of vehicular usage of the crossing also declined towards the end of the tourist season (Stowar *et al.*, 1997).

Associated with the increased suspended solids load arising in Jim Jim Creek downstream of the crossing and after the road opening, was a marked elevation in the measured levels of total iron and aluminium. However, the near-neutral pH of the water would mean these metals are in predominantly in an insoluble hence non-toxic form. Thus, the measured levels of these metals are merely reflecting the chemical composition of the clay particles in the water.

Direct comparison of suspended solids pollution between different studies is hampered by differing levels of exposure and duration of disturbance effects, which have an important influence on the biological effects of such disturbances (Newcombe and MacDonald, 1991). Nevertheless, the observed levels of turbidity and suspended solids in this study represent levels well in excess of thresholds which have previously been demonstrated to have significant effects on macroinvertebrate communities, which may be as low as 10-30 mg/L in a high water clarity background (Newcombe and Macdonald, 1991; also see section 4.3.3).

4.2 Detection of Macroinvertebrate Community Changes using BACIP designs

4.2.1 BACIP Designs and Analysis Options

BACI-type designs with their modifications for multivariate data and addition of spatial controls (described in Chapter 1) have proven to be a powerful means of monitoring environmental disturbances and detecting impacts (Stewart-Oaten *et al.*, 1986; Faith *et al.*, 1995; Chapman *et al.*, 1995; Keough and Mapstone, 1995).

Criticism of BACI-type designs (assuming the addition of multiple independent controls) relates primarily from the confounding effects of natural environmental variability on the ability to detect impacts. Osenberg *et al.* (1994) described a situation where the power of a BACIP design was limited by a large amount of natural temporal variability. In the same context, it has been suggested that statistically significant community changes may only be detected after relatively long periods of monitoring (Bunn, 1995). Even with such long periods of monitoring it has been suggested that natural changes occurring over long periods may make inferences of impact-related community changes difficult (Smith *et al.*, 1993). Much of the development of BACI designs, for example the incorporation of multiple control locations (Underwood, 1993), has been aimed at circumventing such potential problems and maximising statistical power. In most circumstances, a well-designed BACIP sampling design with appropriate modifications, is widely recognised as one of the best available approaches to detecting and assessing the degree of impacts arising from unreplicated disturbances.

To avoid the confounding influences of seasonal factors and chance events, most BACIP experiments must be run over an extended period (Thrush *et al.*, 1994). Indeed, many potential impact-causing activities demand such long-term monitoring - for example the ongoing biological monitoring programme for mining activities in the Kakadu Region combine inter-annual data (Humphrey and Dostine, 1994).

In certain circumstances, such as in the present study, a short-term BACIP design may be utilised, particularly when demanded by the nature of the disturbance. In the region where this study was conducted, most climatic anomalies are associated with the monsoon season, after the cessation of which, environmental conditions may be considered highly consistent for the rest of the dry season. As the disturbance being investigated in this study was confined to a dry season, advantage could be made of the predicability of environmental conditions during this period. Thus a high sampling frequency, 'within season' BACIP approach, was considered legitimate for this study, provided the assumption of non-dependence of the macroinvertebrate community among sampling occasions was met. A fortnightly to monthly frequency of sampling was considered possible given the results of previous studies which have indicated a substantial turnover of stream macroinvertebrate communities on such a scale in this tropical region (Marchant, 1982; Paltridge, 1992).

As outlined in chapter 1, certain assumptions must be met in the BACIP data before the conventional statistical methods (eg. ANOVA) can be used to infer significant impact-related changes in the macroinvertebrate communities. In the case of violations of some assumptions, such as non-normality of the temporally-replicated difference values, alternative statistical tests may be available (Carpenter *et al.*, 1989; but see criticisms of these approaches charged by Stewart-Oaten *et al.*, 1992). Temporal trends in 'difference' data gathered according to a BACIP design present a particular problem as there may be few simple solutions for adjusting the data so observations are independent of one another.

Trends in data may be caused by sampling at too frequent intervals, sampling the same cohort of organisms (especially long-lived organisms) or if random occurrences (such as climatic events) have long lasting effects at only one of the sites (Stewart-Oaten, 1993). In the current study, trends were present in the data which could be attributed to the very strong, *unidirectional* environmental influence related to receding creek flow throughout the dry season.

Dealing with trends in data gathered according to a BACIP study design has received little attention in the literature. Various data transformations may be attempted to remove such trends (Keough and Mapstone, 1995). However when trends persist, abandoning the BACIP approach all together may result in the loss of valuable biological information and thus statistical power. A possible solution is to treat the individual dissimilarity values as ordered series and analyse the trends themselves (Keough and Mapstone, 1995). An alternative approach to trend analysis is to treat perturbing influences as covariates in regression analysis. Then, regression relationships for the impacted and unimpacted condition may be compared by ANCOVA (comparing the elevations of regression lines) (implied in Stewart-Oaten *et al.*, 1986).

4.2.2 The Use of Covariates and Trend Analysis in BACIP Experiments

In this study, the trend in site dissimilarity values was found to be well accounted for by the creek discharge at the time of sampling. Thus, regression analysis was possible, accounting for the natural trend in dissimilarity by way of creek discharge averaged between paired sites. However, statistical analysis of covariance was not necessary in the current study because no significant discharge-dissimilarity relationship was found for collective Jim Jim Creek data in the 'after' period. Moreover, The disturbance effect *increased* dissimilarity of the 'after' data at a time when *decreasing* dissimilarity would be expected - as assessed using data for two independent undisturbed streams in the Kakadu region. The analysis was reduced, simply to showing in a graphical manner that samples collected after the onset of the disturbance lay well outside the trend (and its statistical confidence boundary) observed for the unimpacted condition. Such an observation strongly suggesting biological impairment of the downstream Jim Jim Creek sites.

The covariance approach used here is an adaptation of that suggested in passing by Stewart-Oaten *et al.* (1986). The original suggestion for this form of analysis was based on the conventional BACIP design (paired difference values but unpaired *sites* and a single control location), where (coincidentally discharge was used as an example), difference in volume of flow at the control and impact *sites* was the issue (ie. discharge events not matched at both sites). Furthermore in such cases (and for that matter in many environments), the discharge values were assumed to be independent events in time, extending across the full time series of 'before' and 'after' data. Thus, in the ensuing analysis, separate regression lines may be derived for each site separately and for each time period ('before' and 'after'), each time period overlapping in ranges of discharge. Provided there is a linear relationship in both the impacted and unimpacted condition, the regression lines can readily be tested for differences in elevations (using ANCOVA) or slopes (using tests such as those outlined by Snedecor and Cochran, 1980).

The situation, and thus approach to analysis pursued in this study, is somewhat different from that described above for number of reasons. Firstly, discharge was consistently recessionary throughout the study period, there being no overlap in the range of discharge values for 'before' and 'after' periods for each stream (c.f. if discharge varied in either sampling period).

A second feature of the covariance approach used in this study is that the discharge-dissimilarity regression described the relationship between the dissimilarity of a *pair* of upstream / downstream sites (not individual sites) and the discharge status of the *stream* at the time of sampling. Thus, the dissimilarity-discharge relationship is *not* a product of differing discharge between sites, which in this study was found not to differ significantly between upstream and downstream sites in the same stream. Finally, the present approach differed from the scenario provided by Stewart-Oaten *et al.* (1986) in that independent control data were available in both phases of impact assessment, 'before' and 'after' impact. This strengthened inferences drawn about impact.

The covariance approach may be particularly suited to an environment such as that reported in this study, where the relationship between discharge and dissimilarity may be strong and highly predictable. In environments with less seasonality in flow, the relationship between dissimilarity and an environmental variable such as instantaneous creek discharge may be confounded by the extreme variability in discharge regimes in less seasonal environments.

Considering there were no apparent changes in taxonomic composition (at the family level) throughout the study period (appendices 2a and 2b), it would appear that the observed decrease in dissimilarity between paired sites with recessionary creek discharge may be the result of a decrease in patchiness among sites with regard to taxa richness and relative taxa abundance. Greater patchiness at higher creek discharge could possibly arise as a result of greater variability in environmental conditions. For example, Lancaster and Hildrew (1993) reported changes in the microdistribution of macroinvertebrate taxa in relation to patches experiencing different flow rates. Similarly, Cobb *et al.* (1992) observed a strong association between discharge (and substrate stability) and density of invertebrate fauna in a stream in Manitoba, Canada. At higher flow rates, it might be expected that there would be greater intra-site environmental variation and hence patchiness of macroinvertebrate community structure - as a consequence of increased bank width, habitat exposure and microhabitat differences in flow (eddies, backwaters, etc.). Colonisation of habitat by drifting organisms may also be variable with microhabitat variation in stream flow.

The inferences that can be drawn in analysing the data in the present study by way of regression approaches are more limited than had the data been analysed with no serial correlation found in the time series of data. Use of environmental correlates of variation of the dependent variable in this case implies a sound ecological basis as to why such a relationship would hold. Assumptions about relationships between biotic and abiotic factors in ecological applications may often be tenuous. The possible explanations for the dissimilarity-discharge relationship observed in this study and provided above, nevertheless, would appear intuitively appealing.

The second major limitation is that there is a strong reliance on the additional control creek in drawing inferences about the relationship between discharge and dissimilarity at low discharges. Thus the incorporation of additional control streams becomes imperative. Ideally, if such an analysis were to be pursued, a number of additional control streams would be incorporated in a design and perhaps data for the impacted stream in an entire dry season without any disturbance (although this is clearly impossible for this study). In the present study, there is no way of completely ruling out a natural difference between the two creeks in the dissimilarity / discharge relationship between sites at low levels of creek discharge (hence the benefits in investigating other methods of data analysis as well, such as ordination). However, in this study, corroborative data from an additional stream in the Kakadu region (upper South Alligator River) was able to be used to support the finding of decreasing dissimilarity with recessional flow in seasonally-flowing streams of the region.

Despite the limitations of the approach in its current form, modelling the variation in dissimilarity in the unimpacted condition by way of covariates, and comparing with the relationship for disturbed sites, may offer a potential avenue for analysis of BACIP data that possess strong temporal trends. The covariance approach could possibly be refined to facilitate statistical detection of impact-related changes in monitoring programs by incorporation of data from *within* entire seasons (c.f. longer term studies) in the undisturbed condition and also incorporation of multiple control streams. With or without provision of an extended period of monitoring in the impacted condition, statistical comparison between the trends in the impacted and unimpacted condition should allow inferences to be drawn.

4.3. Suspended Sediment-related Impacts in Jim Jim Creek

In the current study, inferences about possible impact-related community changes were drawn from the above-mentioned BACIP analysis, complemented with multivariate ordination and observation of taxa abundance. In totality, the analyses provided strong evidence for macroinvertebrate community changes occurring immediately downstream of the Jim Jim Creek Road crossing late in the study period. Some marginal effects were also suggested 1000 m downstream of the road crossing. In evaluating biological impairment due to the disturbance in question, the importance of drawing upon all available evidence to ensure accurate interpretations are made regarding impacts, has been stressed on numerous occasions (eg. Underwood and Peterson, 1988).

Multivariate analysis indicated a divergence of the macroinvertebrate community structure in the rootmat habitat downstream of the Jim Jim Creek road crossing compared with that observed in control sites. This was most strongly indicated at the site 200m downstream, with some evidence for community-level changes 1000m downstream. Although this was best indicated as a multivariate response, the impact appeared to be associated with reduced abundances of macroinvertebrates downstream of the road crossing, particularly of the family Chironomidae (consistently the most numerically abundant taxon at all sites, impacted and control). In contrast, no changes, outside that explained by natural variability, were observed in the sand habitat, despite the taxonomic similarity (at family level) of the rootmat and sand habitat.

4.3.1 Habitat Sensitivity

Differences in 'habitat sensitivity' are not uncommon in studies of macroinvertebrate studies (Kerans *et al.*, 1992). There are a number of possible explanations for observed differences in the sensitivity of different habitat for suspended solids-related impacts.

The rootmat habitat may be expected to be more exposed to current velocities than the sand habitat. This is due to the fact that the areas of sand habitat sampled were, by necessity, those in lower flow areas which had developed an organic floc, whilst the rootmat habitat was mainly present along high-flow erosional edge sections of the creek channel. Thus, it is likely that rootmat communities would experience greater exposure to the abrasive effects of

suspended solids than the sand communities. Abrasion by sediment particles can have direct effects on macroinvertebrates, particularly those with delicate filter-feeding or respiratory structures (Newcombe and MacDonald, 1991).

Different habitat characteristics of sand and rootmat may have affected the ability to detect impact-related changes in the communities of each substrate. The sand habitat showed a continuing increase in macroinvertebrate density throughout the study period, whilst in the rootmat habitat, the macroinvertebrate density plateaued. A probable explanation for this may be the continually increasing build-up of detrital material on the sand bed with the progression of the dry season (which has in other studies been demonstrated to be positively correlated with invertebrate density (eg. Drake, 1984)). In the rootmat, invertebrate density was perhaps primarily governed by pre-existing habitat structure, and only limited initially by scouring effects of high flows. The natural changes occurring within the sand habitat community may have prevented the detection of subtle disturbance related changes, with the dynamic nature of this community being a far greater influence on community change than the effects of suspended sediment.

It is also possible that the differences in habitat sensitivity may be a consequence of taxonomic differences between macroinvertebrates inhabiting the sand and rootmat habitat that were not revealed by this study. The relatively high level of taxonomic identification employed in this study (family level) limits the ability to draw conclusions about the taxonomic similarity between the two habitats. It is worth noting, however, that sand habitats are considered relatively depauperate habitats (Hynes, 1970) and as a consequence of the lower taxa richness it may be that they display a less diverse array of sensitivities than other more complex habitats.

4.3.2 Delayed Onset of Impact

There was a delay of several weeks after the peak of turbidity before any impact-related macroinvertebrate community changes were detected. Moreover, the macroinvertebrate response tended to intensify whilst turbidity subsided to some extent later in the dry season. This delay could be attributable to a number of factors. Firstly, previous studies of suspended solids have indicated that the duration of exposure is an important factor in determining

biological effects (Newcombe & MacDonald, 1991). Thus it is likely that many invertebrates would withstand a single or brief pulse of suspended sediment without any adverse effects. In contrast, prolonged exposure to suspended sediment, with its associated adverse physiological effects and alteration of habitat characteristics, will often result in mortality or emigration of aquatic invertebrates.

A second factor possibly explaining the observed delay in biological response may be effects of suspended sediment upon reproduction or recruitment rather than through direct mortality of the resident macroinvertebrate community. In these circumstances, community changes may only be detected after there has been sufficient time for natural 'turnover' of the macroinvertebrate community.

4.3.3 Interpretation of Impact-related Macroinvertebrate Community Changes

The most evident community change occurring downstream of the Jim Jim Creek road crossing, measured by univariate means, was reduced macroinvertebrate abundance. Within this was a noticeable reduction in chironomid (non-biting midge) abundance, which was consistently the most abundant taxon at all sites, control and impacted.

Although no previous studies of suspended sediment have been reported relating directly to creek environments in the Wet-Dry tropics, numerous studies have reported impacts on macroinvertebrates (including reductions in macroinvertebrate density) in relation to similar levels of suspended solids disturbance. Table 4.1 provides a summary of the results in the context of similar studies reported elsewhere.

Table 4.1. Summary of observations reported by selected studies on the effects of suspended sediment on stream macroinvertebrate communities.

Location	Nature of Disturbance	Exposure		Observed Effects on Benthic Macroinvertebrates	Reference
		Max. Turb. or S.sol. (Background)	Duration		
Jim Jim Creek, Kakadu NP, NT.	Elevated turbidity downstream of a road crossing	60 NTU/100 mg/L (<5 NTU/10 mg/L)	3 months	macroinvertebrate community changes; probably reduced taxa abundance, primarily Chironomidae.	N/A
Australia (SE NSW)	Elevated turbidity and sedimentation resulting from forestry activities	1800 NTU, 30.1 NTU ave (1.3 NTU)	8 months	Reduced abundances of selected taxa; increased invertebrate drift.	Richardson (1985)
Australia (VIC.)	Elevated suspended sediment plus sedimentation associated with dam construction	480 NTU (generally low, but range 1-110)	pulses over 3 years	Reduced abundances of a range of species.	Chesman <i>et al.</i> (1987)
Australia (SW WA)	Suspended inorganic solids associated with forestry.	60 mg/L (<10 mg/L)	4 months	Mean species richness decreased, mean total taxa abundance decreased, though not statistically significant.	Growns and Davis (1994)
Australia (ACT)	Elevated suspended solids following storms, resulting from urban development.	560 mg/L (generally < 5 mg/L)	brief pulses over several years prior to study	Reduced species richness and macroinvertebrate density.	Hogg and Norris (1991)
New Zealand	Clay discharges from mining activities	154 NTU (< 8.2 NTU)	Mining for 2-8 years prior to study	Reduced invertebrate densities downstream (by 9-45%).	Quinn (1992)
U.S.A (North Carolina)	Elevated suspended sediment and sedimentation identified associated with runoff from logged and residential development areas.	2360 mg/L (generally < 5 mg/L)	Not Specified	Reduced species richness, abundance and biomass of filter feeding taxa.	Lemly (1982)
USA (Wyoming)	20-fold increase in suspended sediment, no appreciable sediment deposition.	>300 mg/L (<20 mg/L)	2 months	Densities of some taxa decreased (including chironomids); some increased (eg oligochaetes), others unchanged.	Gray and Ward (1982)
U.S.A (Colorado)	Elevation of suspended solids associated with road construction activities.	range of max levels among sites: 70-500 mg/L (<10 mg/L)	4 months	Reduced density, abundance and diversity of macroinvertebrate the community.	Cline <i>et al.</i> (1982)
Canada (Ontario)	Short term elevation of suspended solids in association with highway construction	1390 mg/L (<5 mg/L)	8 months	Altered species composition, no change in total abundance or species richness.	Barton (1977)

Many studies, in an effort to elucidate the underlying biological changes caused by suspended sediment, have investigated taxa or life-history specific responses. Filter-feeding taxa have been frequently shown to be adversely affected by suspended sediment (eg. Lemly, 1982; Mayak and Waterhouse, 1983; Gurtz and Wallace, 1984). The impact on these taxa can be attributed to suspended sediments directly affecting the organism physiology.

Other studies have attributed macroinvertebrate community changes to the indirect effects of suspended sediment, for example environmental alteration causing an avoidance response (eg. as demonstrated by Culp *et al.*, 1986), reduced epilithic productivity limiting the available food resources for invertebrates (Quinn *et al.*, 1992) or, in cases of significant amounts of sediment deposition, reduced detrital availability (Bunn, 1988).

In the present study, the level of taxonomic resolution employed (family level) was insufficient to infer effects in relation to the life histories of the taxa (Resh and Unzicker, 1975). The family Chironomidae, which was reduced in abundance in this study is a very diverse group of invertebrates; it displays a wide range of life history strategies and thus sensitivities to different forms of pollution (Lindegaard, 1995). Because of this, it is impossible to draw inferences about the mechanisms by which this group was affected in the present study. As might be expected from such a diverse group, observed impacts on chironomid communities in relation to elevated levels of suspended solids have reported a variety of results. Gray and Ward (1982) observed reduced abundance of Chironomidae in streams following sediment releases from a dam; they speculated that this was due to interference of larval feeding or of respiratory processes by scouring or clogging of the silk tube structures of tubiferous species. Extence (1978) reported slight reductions in the density of the Chironomidae larvae in response to elevated levels of suspended sediment following construction of a highway, although in that study chironomids proved to be one of the least sensitive taxa.

The importance of lower levels of taxonomic identification in determining actual responses and sensitivities within the Chironomidae family has been demonstrated by many studies. Edwards *et al.* (1972) reported a disproportionate number of the Chironomidae subfamily Orthoclaadiinae relative to Chironominae in a stream affected by coal particles. A possible cause for this is the high number of filter feeders and detritivores in the Chironominae subfamily, which may be adversely affected by particulate suspended and deposited particulate matter (Hellowell, 1986). Reports of sensitivity to suspended sediment of selected chironomid species are common (eg. Blythe *et al.*, 1984; Richardson, 1985; Culp *et al.*, 1986; Quinn *et al.*, 1992).

Increases in chironomid abundance have also been reported in association with elevated levels of suspended sediment. For example Newbold *et al.* (1980) reported significant increases in total taxa abundance, which they attributed to increased Chironomidae abundance in streams affected by runoff from logged areas in Canada.

In general, the diversity of life history strategies within the Chironomidae have led them to be considered to be "ubiquitous exploiters" (Hellowell, 1986). Thus, in many situations, macroinvertebrate community changes observed at the family level may not reflect specific sensitivities to disturbance, hence the variety in responses reported on the Chironomidae family.

As a result of the diversity of life history strategies held within each family of macroinvertebrate communities, proposed mechanisms for the community changes observed in this study are only speculative. Taxa may have been affected either directly or indirectly by the suspended sediment downstream of the road crossing. Direct effects would be expected on taxa that are sensitive to the presence of suspended sediment particles. As outlined previously various members of the family Chironomidae may be considered sensitive.

Alternatively, the observed community changes may have arisen through indirect effects on the natural habitat within Jim Jim Creek. Chlorophyll analysis showed phytoplanktonic productivity to be negligible in Jim Jim Creek and thus unaffected by turbidity; however, other aspects of productivity, particularly that of the periphyton and epiphyton, which were present in Jim Jim Creek (pers. obs.) may have been affected by elevated levels of turbidity. Arunachalam *et al.* (1991) reported the importance of algal biomass as a determinant of macroinvertebrate density in a monsoonal system. They reported a seasonal cycle of algal biomass, with productivity being greatest in the pre-monsoonal period and lowest in the post monsoon. Although purely speculative, if such a cycle existed in the Jim Jim Creek study area, it would be expected that turbidity may interfere with this through adverse changes to algal productivity. Further evidence for this possibility lies in studies reporting chironomid densities to be strongly correlated with the standing stock of such algae (eg. Rasmussen, 1984; Cattaneo, 1983), as well as many reports of benthic algae as primary food source for numerous chironomid species (eg. Soluk, 1985; Johnson *et al.*, 1989; Mason and Bryant, 1975). This speculation, however, could only be resolved by experimental studies.

4.4 Evaluation of Impacts from a Conservation Perspective

4.4.1 Spatial and Temporal Extent of Impact

Impacts on macroinvertebrate communities were detected 200 m downstream of the road crossing, with only slight disturbance related impact 1000 m downstream of the crossing by the conclusion of the macroinvertebrate sampling (and main period of usage of the road crossing) in mid September. Thus it would appear that the effects of suspended sediment on the benthic macroinvertebrate communities are quite localised. However, consideration must also be made of the fact that this disturbance constitutes a barrier to the continuity of the escarpment reaches of Jim Jim Creek, possibly impinging on the use of this area of the creek by other fauna (eg. the natural range of migrating fish).

Studies of the effects of suspended sediment on stream ecosystems have often reported relatively rapid recovery of macroinvertebrate communities after the cessation of sediment input and flushing of the system by periods of increased discharge (eg. Cline *et al.*, 1982; Barton, 1977). Thus, it would be expected that in Jim Jim Creek, seasonal closure of the road crossing, high wet season flows flushing the turbid water and the natural turnover of macroinvertebrates, would restore the creek to an undisturbed condition each year. This assumption is reinforced by observations made prior to the opening of the Jim Jim road crossing, when the downstream sites were observed to be biologically similar to undisturbed sites. Thus the impacts on macroinvertebrate fauna in Jim Jim Creek are most likely to be confined annually to the period between commencement of the road crossing usage (with perhaps a lag of several weeks as was detected in this study) and the recommencement of flows in following the wet season.

Some studies have indicated the occurrence of long-term macroinvertebrate community changes associated with suspended sediment (eg. Silsbee and Larson, 1983; Campbell and Doeg, 1989). However these studies have also reported associated long term habitat changes, such as removal of vegetation in catchment areas, or significant deposition of sediment (Moring, 1982). In the case of Jim Jim Creek, the high flows experienced in the Wet season and the resulting 're-sorting' of creek-bed sediments would negate such long-term alteration to a large extent.

Thus it is likely that the detected macroinvertebrate impact is limited to the late Dry season and then, only for a short section of the creek downstream of Jim Jim Creek road crossing. It is important to recognise, however, that macroinvertebrates are *indicators* of biological impairment - one of their inherent advantages for this purpose is their sensitivity and responsiveness to short-term environmental changes (Hellowell, 1986). Other aspects of the ecological impairment they indicate, for example the impacts on populations of higher consumers such as fish, may be longer term.

4.4.2 Other Biological Evidence

The value of biological monitoring programs based on a range of organisms was reinforced by observations of fish communities made in conjunction with this study. These observations showed that there was a 90 percent decline, downstream of the Jim Jim Creek road crossing, in the numbers of Mariana's hardyhead (*Craterocephalus marianae*) (Stowar *et al.*, 1997). This species' range is confined to the west Arnhem Land region (Larson and Martin, 1990), highlighting the significant conservation value of the area in which this study was based.

4.4.3 Water Quality Criteria in Areas of High Conservation Value

The issue of water quality assessment criteria for areas of high conservation value, such as Jim Jim Creek is a complex one and detailed discussions about this are beyond the scope of this study. However, a number of issues relating to the 'acceptable' level of impact are raised in this study. Guidelines such as those provided by ANZECC (1992) should be viewed in the light of the situation being assessed and their recommendations perhaps viewed with caution given the conservation value of the area being studied (although in this case, the disturbance was seen to greatly exceed ANZECC guidelines). It could be argued, that an objective of 'no observable effects upon organisms included in biological monitoring' as has been adopted for the monitoring of uranium mining in the Kakadu region (Humphrey *et al.*, 1995), should be extended to this situation, in which case the effects detected in this study should be cause for concern and appropriate action if at all possible.

One final consideration with regard to the assessment of impacts of turbidity on Jim Jim Creek is that of aesthetics. In this study, water downstream of the Jim Jim Creek crossing was noticeably discoloured. It has been suggested that a 20 to 50 percent reduction in water clarity may be the detectable threshold for the human eye with regard colour changes associated with suspended sediment (Davies-Colley, 1988; cited by Ryan, 1991). Such visually detectable change may occur well before any biological effects are detected. The inaccessibility to the general public of the section of Jim Jim Creek downstream of the road crossing may somewhat negate such considerations of the aesthetic impact.

4.5 Limitations of this Study

This study was solely aimed at assessing the effects of vehicle-induced disturbance downstream of the Jim Jim Creek road crossing. Whilst this study might have application to other high-clarity lotic sites in the Wet-Dry tropics of northern Australia in the dry season, caution is required in extrapolating, with any confidence, the effects to other situations. As discussed above, turbidity-related effects may depend upon season, exposure, nature of the sediment being transported, amongst other factors.

Temporal confounding of BACIP replicates negated the use of the most rigorous analytical procedures for inferring impact (in this case, ANOVA). This resulted in the need to draw on a full spectrum of other analysis techniques (including modelling of the confounding variable), as well as results of other studies, supporting conclusions about effects caused by suspended solids.

4.6 Conclusions

There were strong indications that the macroinvertebrate communities 200 m downstream of the Jim Jim Creek road crossing were affected by sediment originating from the crossing. Furthermore, the fact that there was only a marginal degree of impact (if any) at the site monitored 1000 m downstream, indicates a threshold of effects between the two sites. Such thresholds are difficult to quantify in absolute terms, because both suspended sediment concentration and duration of exposure are determinants of the biological effects. Nevertheless, based on family-level data, the suspended solids concentrations of up to 17 mg/L at site JJ3 were insufficient to instigate a detectable response, whilst the levels of up to 100 mg/L at JJ2 had a marked impact on the macroinvertebrate fauna in the rootmat habitat.

The data gathered according to a BACIP design, although found to be unsuitable for conventional BACIP statistical analyses, nevertheless provided a clear indication of the impact-related community changes. The incorporation of an additional control stream was imperative in being able to infer such changes. This highlights the benefits in incorporating such independent controls in conventional BACIP approaches, including

for situations where recourse to alternative data analysis procedures may be required, such as in this study.

Whilst regression approaches are not optimum in BACIP data analysis, they are shown here to be of value in enabling an assessment of disturbance-effects in an intensive but short period of baseline and post-impact data collection. A long time-series has conventionally been a prerequisite for BACIP designs, imposing a limitation on the situations to which they can be applied.

In this study, based in an area of World Heritage conservation value, the use of multivariate statistical approaches – including multivariate dissimilarity values in BACIP analysis - may be particularly appropriate. In this respect, there are two potential advantages of the multivariate approach (compared with univariate, population approaches) for areas of high conservation value. These are apparent from Humphrey *et al* (1995) and Faith *et al* (1995) and include: (i) use of data pertaining to *communities* of organisms, which best provide an assessment of ecosystem-level changes, and a useful surrogate measure of biodiversity changes; and (ii) proven statistically-sensitive (powerful) responses, and therefore indicators of *subtle* change.

Overall, the approach taken in this study had its limitations, although it was clearly adequate to infer (- in combination with other analysis techniques, as well as observation of other taxa eg. fish communities -) biological impacts and hence the need to take appropriate measures to alleviate the problem in the interests of the area's conservation value. Thus, although this approach may represent a compromise to longer-term, more comprehensive, biological assessment, it indicates that short-term monitoring is both feasible and practical. With increasing time and resource constraints imposed upon impact assessment studies, novel analytical methods, other than the most statistically rigorous ones, will need to be sought in determining and assessing the degree of human-related disturbance. For point-source disturbances in streams, this study perhaps provides a model for the way in which such investigations might be designed to achieve the objectives required for water quality management.

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APPENDICES

APPENDIX 1: ERISS Analytical chemistry methods.

Method Code No.	Analyte	Technique	Reference
1	Alkalinity	Acidimetric titration	APHA* 2320B
4	Carbon (total and dissolved)	Acidification, persulphate oxidation	APHA* 5310D
5	Conductivity	Electrometric	APHA* 2510D
8	pH	Electrometric	APHA* 4500-H ⁺
12	Total suspended solids	Gravimetric	APHA* 2540D
16	Inorganic and Organic Residue in waters	Gravimetric	ERISS† (internal report IR5)
14	Turbidity	Nephelometric	APHA* 2130B
29	Filterable available phosphorus	Spectrophotometric	APHA* 4500PE
37	Total Phosphorus	Acid digestion and molybdenum blue spectrophotometric method	APHA* 4500PE
106	Cations: sodium, potassium and ammonium-N	HPLC	ERISS†
108	Anions, Cations; (chloride, nitrate-N, sulphate, magnesium and calcium EDTA solvent system	HPLC	ERISS†
External	Cu, Zn, Pb, Cr, Ni, U, Cd (total, unfiltered)	Inductively coupled plasma mass spectroscopy (ICPMS)	ANSTO‡
External	Al, Fe, Mn (total, unfiltered)	Inductively coupled plasma atomic emission spectroscopy (ICPAES)	ANSTO‡

*APHA- methods as specified in APHA (1995)

APHA (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Edition. American Public Health Association, Washington.

†ERISS- unpublished 'in-house' developed method

Environmental Institute of the Supervising Scientist analytical chemistry laboratory, Jabiru NT.

‡ANSTO- Analysed externally by ANSTO

Australian Nuclear Science and Technology Organisation laboratories, Lucas Heights NSW.

APPENDIX 2A: Macroinvertebrate taxa collected in rootmat samples from Jim Jim and Twin Falls Creeks.

SITE/SAMP. OCCASION	JJ1/1			JJ2/1			JJ3/1			TF1/1			TF2/1		
	25 April 96			25 April 96			25 April 96			1 May 96			1 May 96		
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	1309	1310	1311	1318	1319	1320	1326	1327	1328	1349	1350	1351	1361	1362	1363
ACARINA (INDET) (X)	30	28	16	6	6	8	14	10	18	36	20	60	4	10	11
ANISOPTERA (INDET) (L)	0	0	0	0	1	0	0	0	0	4	0	0	0	0	0
BAETIDAE (N)	12	6	0	5	1	0	8	8	8	28	44	24	3	2	6
CAENIDAE (N)	8	22	6	1	7	18	44	34	28	44	64	48	4	6	5
CERATOPOGONIDAE (L)	2	2	4	15	19	0	14	12	12	0	4	20	8	16	3
CHIRONOMIDAE (L)	132	48	156	113	293	308	132	136	118	424	104	372	68	156	108
CHIRONOMIDAE (P)	6	2	0	2	5	0	6	0	0	4	16	4	3	0	0
COENAGRIONIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
CORIXIDAE (N)	0	0	0	1	1	4	0	0	0	0	0	4	2	2	0
CULICIDAE (L)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
CULICIDAE (P)	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
DYTISCIDAE (L)	0	0	0	0	0	0	0	0	0	4	0	12	0	0	0
DYTISCIDAE (A)	0	0	0	0	0	0	0	0	0	0	4	4	0	0	0
ECNOMIDAE (L)	2	0	0	0	2	0	0	2	0	8	8	0	0	0	0
ELMIDAE (L)	2	4	4	2	0	2	0	0	0	0	0	0	0	0	3
ELMIDAE (A)	4	70	28	2	0	0	6	8	2	8	0	16	4	6	0
GOMPHIDAE (L)	4	2	2	2	8	6	10	4	6	0	16	12	6	0	0
HYDROPSYCHIDAE (L)	0	12	0	1	0	2	4	0	0	4	12	0	0	0	0
HYDROPTILIDAE (L)	2	2	4	2	1	4	2	4	0	12	8	8	2	6	1
LEPTOCERIDAE (L)	10	14	4	1	18	22	20	42	16	12	0	12	6	2	2
LEPTOPHEBIIDAE (N)	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	8	6	2	7	2	0	2	8	2	0	4	0	0	0	0
PALAEMONIDAE (X)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PROTONEURIDAE (L)	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0
PYRALIDAE (L)	2	2	4	0	0	0	4	2	0	0	0	0	0	0	0
TABANIDAE (L)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0

APPENDIX 2A: (cont.)

SITE/SAMP. OCCASION	JJ1/2			JJ2/2			JJ3/2			TF1/2			TF2/2		
	8	May	96	8	May	96	8	May	96	15	May	96	15	May	96
DATE															
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	1410	1411	1412	1419	1420	1421	1428	1429	1430	1702	1703	1704	1711	1712	1713
ACARINA (INDET) (X)	16	20	14	34	24	36	18	38	28	40	64	80	24	20	64
ANISOPTERA (INDET) (L)	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
BAETIDAE (N)	4	16	6	8	6	2	14	22	22	40	8	32	24	24	24
CAENIDAE (N)	6	2	0	4	8	2	18	12	0	32	8	240	32	0	24
CERATOPOGONIDAE (L)	22	22	24	16	16	22	12	18	14	0	8	16	16	0	8
CHIRONOMIDAE (L)	142	108	160	138	134	182	422	286	216	160	128	152	288	120	168
CHIRONOMIDAE (P)	0	4	0	0	0	0	0	0	2	8	0	0	16	0	0
COENAGRIONIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORIXIDAE (N)	0	0	0	0	0	0	8	2	2	0	0	0	32	0	0
CULICIDAE (L)	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
CULICIDAE (P)	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
DYTISCIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DYTISCIDAE (A)	0	4	0	2	2	2	0	0	4	0	0	0	0	0	0
ECNOMIDAE (L)	16	16	18	2	6	6	8	10	22	0	0	0	0	8	56
ELMIDAE (L)	0	0	0	0	0	2	4	0	6	8	0	0	0	4	8
ELMIDAE (A)	2	0	0	0	0	4	2	8	0	8	16	24	0	0	16
GOMPHIDAE (L)	0	0	0	0	2	0	2	0	0	0	0	24	0	0	0
HYDROPSYCHIDAE (L)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	2	4	6	4	4	2	0	8	6	0	0	0	0	0	8
LEPTOCERIDAE (L)	8	6	6	2	4	2	6	12	0	16	24	48	0	0	0
LEPTOPHLEBIIDAE (N)	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	0	4	0	0	0	0	2	0	2	0	0	8	0	0	0
PALAEMONIDAE (X)	4	2	4	0	4	0	2	2	2	0	0	8	0	0	0
PROTONEURIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PYRALIDAE (L)	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE (L)	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0

APPENDIX 2A: (cont.)

SITE/SAMP. OCCASION	JJ1/4			JJ2/4			JJ3/4			TF1/4			TF2/4		
	7	July	96												
DATE															
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	2169	2170	2171	2175	2176	2177	2181	2182	2183	2187	2188	2189	2193	2194	2195
ACARINA (INDET) (X)	96	136	40	40	40	48	96	88	32	48	64	120	128	88	48
ANISOPTERA (INDET) (L)	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0
BAETIDAE (N)	72	56	72	72	104	144	328	360	104	224	224	360	216	280	152
CAENIDAE (N)	32	24	64	32	32	112	160	128	88	248	168	256	280	112	264
CERATOPOGONIDAE (L)	240	64	112	56	64	80	40	72	24	24	104	0	0	72	0
CHIRONOMIDAE (L)	656	400	528	688	568	432	784	680	464	856	1392	1208	1096	856	1736
CHIRONOMIDAE (P)	0	8	0	8	0	0	8	40	16	16	16	32	0	0	0
COENAGRIONIDAE (L)	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORIXIDAE (N)	0	8	0	0	0	0	0	0	8	0	8	48	16	0	0
CULICIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
CULICIDAE (P)	0	8	0	0	0	0	0	0	0	0	8	0	8	0	0
DYTISCIDAE (L)	8	0	0	0	0	0	0	0	0	0	0	0	16	0	0
DYTISCIDAE (A)	0	0	8	0	0	8	0	0	0	8	0	0	0	0	0
ECNOMIDAE (L)	40	32	40	8	0	8	16	24	24	72	80	48	48	32	72
ELMIDAE (L)	56	24	24	0	0	0	8	0	0	0	0	0	0	0	8
ELMIDAE (A)	56	24	24	0	8	0	0	0	8	0	0	0	0	24	24
GOMPHIDAE (L)	0	16	0	0	0	0	8	8	0	0	0	0	0	0	0
HYDROPSYCHIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	0	8	8	0	0	8	0	0	0	24	16	8	0	16	0
LEPTOCERIDAE (L)	0	8	8	0	16	8	0	0	0	56	48	56	0	24	24
LEPTOPHEBIIDAE (N)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	8	0	16	24	0	0	0	0	0	0	0	0	0	0	0
PALAEMONIDAE (X)	0	8	0	0	0	0	8	0	0	0	0	8	8	0	0
PROTONEURIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PYRALIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE (L)	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTEA (INDET.) (L)	8	0	0	0	0	0	0	0	8	0	0	0	0	0	0

APPENDIX 2A: (cont.)

SITE/SAMP. OCCASION	J1/5			J2/5			J3/5			TF1/5			TF2/5		
	25	July	96	25	July	96	25	July	96	25	July	96	25	July	96
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	2257	2258	2259	2263	2264	2265	2269	2270	2271	2275	2276	2277	2281	2282	2283
ACARINA (INDET) (X)	96	76	96	248	188	48	104	48	88	56	144	96	80	44	48
ANISOPTERA (INDET) (L)	0	0	0	0	12	0	0	0	0	0	0	0	0	0	8
BAETIDAE (N)	80	28	16	32	24	24	120	248	72	88	128	56	112	72	16
CAENIDAE (N)	16	16	16	128	40	32	272	152	208	24	168	176	248	16	48
CERATOPOGONIDAE (L)	56	40	24	40	96	64	136	168	48	40	16	32	16	72	24
CHIRONOMIDAE (L)	348	348	400	336	584	412	712	864	904	728	680	1072	784	384	992
CHIRONOMIDAE (P)	0	0	8	0	0	16	24	8	8	0	8	16	8	4	8
COENAGRIONIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0
CORIXIDAE (N)	8	0	0	0	0	20	24	0	24	16	24	8	16	12	0
CULICIDAE (L)	0	0	0	0	0	0	24	8	0	0	0	0	0	12	0
CULICIDAE (P)	0	0	8	0	0	0	0	0	0	0	0	8	0	0	0
DYTISCIDAE (L)	0	0	0	0	0	0	8	0	0	0	8	0	16	0	0
DYTISCIDAE (A)	0	0	8	16	0	0	8	0	0	0	0	0	0	0	0
ECNOMIDAE (L)	16	16	8	8	4	40	24	24	0	32	8	40	48	12	56
ELMIDAE (L)	32	8	40	0	8	4	0	96	0	48	64	8	0	24	0
ELMIDAE (A)	24	28	48	16	4	8	0	8	0	8	0	8	0	0	0
GOMPHIDAE (L)	4	4	0	0	8	4	0	24	0	8	8	8	0	0	0
HYDROPSYCHIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	8	16	8	0	0	4	24	24	8	32	40	48	32	8	8
LEPTOCERIDAE (L)	12	28	8	64	56	16	0	16	16	16	32	56	32	24	24
LEPTOPHLEBIIDAE (N)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	0	0	8	0	0	4	0	8	0	0	0	0	0	0	8
PALAEONIDAE (X)	0	4	0	24	8	4	8	8	0	0	8	8	0	0	8
PROTONEURIDAE (L)	0	0	0	8	0	4	0	0	0	0	0	8	0	0	0
PYRALIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	8	12	0
TABANIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TIPULIDAE (L)	4	0	0	0	8	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	4	0	0	12	16	0	8	0	0	0	16	0	0	8

APPENDIX 2A: (cont.)

SITE/SAMP. OCCASION	TF1/6			TF2/6			JJ1/6			JJ2/6			JJ3/6		
	21	Aug	96												
DATE															
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	2345	2346	2347	2351	2352	2353	2357	2358	2359	2363	2364	2365	2369	2370	2371
ACARINA (INDET) (X)	96	32	24	40	72	88	72	88	40	112	56	40	40	8	120
ANISOPTERA (INDET) (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE (N)	208	168	296	64	88	120	32	64	40	48	40	8	80	8	248
CAENIDAE (N)	136	128	168	104	80	64	0	16	136	24	56	24	40	24	48
CERATOPOGONIDAE (L)	0	8	24	16	8	24	32	56	80	40	24	0	16	16	64
CHIRONOMIDAE (L)	656	776	608	456	632	688	528	392	1192	168	216	96	240	224	928
CHIRONOMIDAE (P)	0	16	40	0	0	24	0	0	8	0	16	0	0	0	8
COENAGRIONIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
CORIXIDAE (N)	8	8	16	0	16	16	0	16	0	152	48	32	0	0	8
CULICIDAE (L)	0	0	0	0	8	8	0	8	0	0	0	0	0	8	16
CULICIDAE (P)	0	0	0	8	8	0	0	0	0	0	0	0	0	0	8
DYTISCIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	8	16
DYTISCIDAE (A)	0	0	0	0	0	0	8	0	8	80	16	16	0	0	8
ECNOMIDAE (L)	8	8	48	16	8	16	0	0	32	8	8	8	16	32	16
ELMIDAE (L)	24	8	24	8	8	0	80	128	16	8	0	8	32	0	120
ELMIDAE (A)	8	16	8	8	16	8	32	8	8	24	0	0	8	16	0
GOMPHIDAE (L)	0	0	16	8	0	16	0	0	0	0	8	0	0	0	0
HYDROPSYCHIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	8	16	8	8	8	0	0	0	16	0	0	0	24	0	0
LEPTOCERIDAE (L)	8	0	0	8	24	8	8	8	32	56	16	24	16	8	8
LEPTOPHLEBIIDAE (N)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	0	8	8	0	0	0	0	8	0	0	0	8	0	0	0
PALAEMONIDAE (X)	8	0	16	8	0	0	0	0	0	16	0	8	0	0	0
PROTONEURIDAE (L)	0	0	0	0	0	0	0	16	0	0	0	8	0	0	0
PYRALIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	0	0	0	0	0	0	0	0	0	0	0	8	8	0

APPENDIX 2A: (cont.)

SITE/SAMP. OCCASION	J11/7			J12/7			J13/7			TF1/7			TF2/7		
	18	Sept	96												
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	2461	2462	2463	2466	2467	2468	2472	2473	2474	2478	2479	2480	2484	2485	2486
ACARINA (INDET) (X)	184	144	96	56	64	152	64	120	72	56	80	112	56	104	96
ANISOPTERA (INDET) (L)	0	0	8	0	0	0	0	0	0	0	0	8	0	0	0
BAETIDAE (N)	16	32	64	32	56	16	104	56	40	32	176	200	128	8	96
CAENIDAE (N)	32	16	32	8	16	24	24	40	32	0	16	144	112	88	144
CERATOPOGONIDAE (L)	24	96	48	16	24	24	112	120	72	24	24	96	16	64	16
CHIRONOMIDAE (L)	432	904	656	112	120	136	904	928	776	264	576	528	728	928	624
CHIRONOMIDAE (P)	0	16	0	8	0	24	0	24	40	0	8	40	8	24	0
COENAGRIONIDAE (L)	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CORDULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
CORIXIDAE (N)	0	0	0	40	80	144	8	24	0	16	32	16	8	0	16
CULICIDAE (L)	8	0	8	0	0	0	8	0	0	8	8	16	0	0	16
CULICIDAE (P)	8	0	0	8	0	0	0	0	0	0	0	0	0	0	0
DYTISCIDAE (L)	0	0	0	0	8	8	0	8	16	0	16	0	0	0	8
DYTISCIDAE (A)	0	0	0	88	0	0	0	8	8	0	16	40	16	0	0
ECNOMIDAE (L)	8	0	40	8	8	16	24	24	48	0	0	56	24	16	24
ELMIDAE (L)	88	40	56	0	8	0	32	80	48	104	88	8	0	0	16
ELMIDAE (A)	16	0	56	0	40	72	8	8	8	8	8	0	0	32	8
GOMPHIDAE (L)	0	24	8	16	0	8	0	0	0	0	0	16	0	0	8
HYDROPSYCHIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	8	32	16	0	8	8	16	16	16	8	16	8	24	16	8
LEPTOCERIDAE (L)	0	56	32	40	48	40	24	48	16	8	0	80	32	0	40
LEPTOPHEBIIDAE (N)	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
LIBELLULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OLIGOCHAETE (X)	8	0	0	0	0	0	0	8	0	8	0	0	16	0	8
PALAEMONIDAE (X)	0	0	0	8	48	8	0	0	8	0	0	8	8	8	8
PROTONEURIDAE (L)	0	0	8	0	24	8	0	0	0	0	0	0	0	0	0
PYRALIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TABANIDAE (L)	0	16	0	0	0	0	0	0	0	0	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	0	0	0	0	16	8	0	0	0	0	8	0	0	16

APPENDIX 2B: (cont.)

SITE/SAMP. OCCASION	J1/3			J2/3			J3/3			TF1/3			TF2/3		
	DATE	31	May	96	31	May	96	31	May	96	7	June	96	7	June
REPLICATE NO.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
ERISS SAMPLE NUMBER	1819	1820	1821	1825	1826	1827	1834	1835	1836	2072	2073	2074	2078	2079	2080
ACARINA (INDET) (X)	52	56	56	76	128	11	40	52	128	44	52	32	20	28	44
ANISOPTERA (INDET) (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BAETIDAE (N)	48	32	0	44	72	0	96	84	4	18	0	4	12	0	8
CAENIDAE (N)	0	8	24	12	12	1	64	48	8	16	8	4	52	52	40
CERATOPOGONIDAE (L)	44	60	0	48	36	9	8	28	48	8	0	0	32	36	20
CHIRONOMIDAE (L)	304	284	2112	516	360	127	552	296	432	560	108	96	352	232	308
CHIRONOMIDAE (P)	4	0	0	12	0	2	0	16	4	12	0	0	0	4	0
COENAGRIONIDAE (L)	4	0	0	0	0	0	0	4	0	0	0	0	0	0	0
CORIXIDAE (N)	0	4	128	32	0	0	0	0	8	2	0	0	16	4	4
CULICIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CULICIDAE (P)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DYTISCIDAE (A)	8	0	0	0	0	0	8	4	0	0	0	0	0	0	0
DYTISCIDAE (L)	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
ECNOMIDAE (L)	32	32	8	40	44	5	56	48	0	24	28	12	24	0	8
ELMIDAE (A)	0	8	16	4	8	0	16	12	0	0	12	16	0	0	0
ELMIDAE (L)	8	12	24	20	8	4	0	8	0	6	16	0	12	0	0
GOMPHIDAE (L)	0	12	8	0	4	0	24	0	0	2	0	0	0	0	0
HEBRIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPHILIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HYDROPTILIDAE (L)	12	8	32	8	12	0	48	8	4	0	0	4	0	0	0
LEPTOCERIDAE (L)	36	44	16	24	28	7	32	24	24	4	0	0	0	8	4
OLIGOCHAETE (X)	12	4	0	8	0	0	8	4	0	0	0	12	16	0	8
PALAEMONIDAE (X)	0	4	0	0	4	0	0	4	0	0	0	0	0	0	0
PHILOPOTAMIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PYRALIDAE (L)	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
SCIRTIDAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SIMULIDAE (L)	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0
TABANIDAE (L)	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0
TIPULIDAE (L)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ZYGOPTERA (INDET.) (L)	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0

APPENDIX 3: Creek discharge data.

Date	JJ1/JJ2	JJ1/JJ3	TF1/TF2
25-Apr	0.89	0.91	
1-May			1.58
8-May	0.80	0.81	
15-May			1.36
31-May	0.62	0.62	
7-Jun			0.84
7-Jul	0.45	0.44	0.60
25-Jul	0.36	0.34	0.44
21-Aug	0.24	0.21	0.22
18-Sep	0.20	0.18	0.16

(All discharge values are the average between the two sites, in Cumecs).