REFERENCE CONDITIONS AND EUTROPHICATION IMPACTS IN IRISH RIVERS: MEETING THE REQUIREMENTS OF THE WATER FRAMEWORK DIRECTIVE

FINAL REPORT

(Project 2000-FS-2-M1)

Prepared for the Environmental Protection Agency

Author:

Aisling Walsh

ENVIRONMENTAL PROTECTION AGENCY
An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co.Wexford, Ireland

DISCLAIMER

REFERENCE CONDITIONS AND EUTROPHICATION IMPACTS IN IRISH RIVERS: MEETING THE REQUIREMENTS OF THE WATER FRAMEWORK DIRECTIVE

FINAL REPORT

Published by the Environmental Protection Agency, Ireland

Printed on Recycled paper

ISBN:1-84095- to come 06/01/300
Price: €XX.XX

Abstract

An important objective of this project was to obtain an improved understanding of why ecological communities depart from reference conditions as pollution and eutrophication impacts on individual species and in particular indicator taxa such as *Ecdyonurus*. The effects of eutrophication on *Ecdyonurus* were studied using a novel split-stream experiment which involved artificially increasing the phosphorus concentrations in two oligotrophic rivers in the West of Ireland. Some of the nutrient manipulation experiments showed significant differences in algal biomass between the control and treated sections, but not all did so. Findings from this study appear to indicate that *Ecdyonurus* is not directly affected by the consequences of eutrophication.

The experiments did reveal surprising results showing the importance of N-limitation in the rivers studied. On analysing the N:P ratios in a number of rivers in the West of Ireland it was found that approximately 4% of the samples were N-limited with low MRP concentrations (<0.05mg/l P). This implies that a small proportion of high status rivers are N-limited rather than P-limited. Thus, in terms of the Water Framework Directive, there may be a case for introducing tighter regulations on the limits of nitrogen emitted to water bodies as well as the need to control phosphorus. Results from these studies highlight the complexity of the in-stream processes driving these N-limited and P-limited high status rivers.

It was hypothesised that the changes exhibited in the biomass in the split-stream experiment would be reflected in the gut contents of *Ecdyonurus venosus*, possibly showing a variation in algal taxa between both sections of the manipulation experiment. The split-stream experiment showed that *Ecdyonurus* did not demonstrate distinct preferences due to enrichment but neither were there any observed changes in the periphyton species community. On the basis of the findings from the study in the Castlebar River in 2003, *Ecdyonurus venosus* can be classified as both a herbivorous grazer and detritus feeder with a tendency towards opportunistic feeding.

The food preference of these larvae appears to be strongly dependent on the food available in the environment at a given time and they seem to feed on particles that are most abundant during a particular season or those that are easily accessible during a feeding episode. The *Ecydonurus* gut contents consisted mainly of epilithic algal tissue, plant particulate matter (detritus), biofilm matrix and inorganic debris (mineral material).

The life cycle of the Heptegeniidae was described for the first time in detail in five high status rivers in the West of Ireland. Of the three species of *Ecdyonurus* studied, *Ecdyonurus venosus* was the dominant species in all five high status rivers displaying a bivoltine life cycle with only a slight variation in emergence periods between sites. Findings show that one would expect to find this species when sampling during all seasons throughout the year. The life cycle of both *Ecdyonurus insignis* and *Ecdyonurus dispar* were found to be univoltine. The life cycle analyses in this study suggest that at least one species of *Ecdyonurus* should be present at all times of the year.

The life cycle of *Rhithrogena semicolorata* was substantially easier to interpret and clearly displayed a univoltine life cycle. The *Heptagenia* specimens were identified to genus level only and were therefore described as a genus group that appeared to adopt a univoltine life cycle. Findings from our studies support the hypothesis put forward that the various species of *Ecdyonurus* emerge in overlapping phases such that during the summer months larvae of at least one *Ecdyonurus* species will be present in the benthic riffle fauna of Irish rivers

Ecdyonurus is a good indicator of pollution and the water chemistry results appear to support the hypothesis that the presence of *Ecdyonurus* is associated with good water quality. The presence/absence of *Ecdyonurus* in the high status versus impacted sites supports its use as a significant bioindicator of water quality. A selection of indices and metrics were examined and the results revealed that the most significant differences between the high status and impacted sites were found using Margalef's index, total number of taxa and % EPT. Results from feeding and

microhabitat investigations suggest that as eutrophication and the impacts of organic pollution progress, a change in the feeding guilds and microhabitat preferences among the macroinvertebrate communities occurs.

ACKNOWLEDGEMENTS

This report has been prepared as part of the Environmental Research Technological Development and Innovation Programme under the Productive Sector Operational Programme 2000-2006. The programme is financed by the Irish Government under the National Development Plan 2000-2006. It is administered on behalf of the Department of the Environment and Local Government by the Environmental Protection Agency which has the statutory function of co-ordinating and promoting environmental research.

My sincere thanks to my supervisor Dr. Mary Kelly-Quinn for her advice and guidance throughout the course of this study. I would like to thank Professors John Bracken and Tom Bolger for the use of departmental facilities, and my committee members Dr. Bret Danilowicz and Dr. Tom Hayden for their helpful suggestions and comments.

This project was funded by the Irish Environmental Protection Agency under the ERDTI programme 2000-2006, their sponsorship is gratefully appreciated. I wish to convey a special thanks to Mr. Martin McGarrigle, EPA, Castlebar for his knowledge, assistance and advice during the course of the last five years.

I would like to thank the chemistry laboratory in the EPA Regional Inspectorate in Castlebar for processing the water samples, to Hugh McGinley and Pat Durkin from the EPA Hydrometric Office, Castlebar, for the help with constructing the split-stream experiment and to Noriana Kennedy and Eli Mulvey for assistance with the laboratory and fieldwork. Many thanks to Berine Ni Chathain for providing invaluable assistance with diatom identifications and to Dr. Robin Raine in the Martin Ryan Marine Institute in NUI, Galway for the use of a fluorescent microscope.

I would like to thank the staff and postgraduates in the Limnology unit in UCDs' Zoology Department, especially Wayne Trodd. The help provided

by Fiona Kelly for assistance with the sediment analysis is gratefully acknowledged.

To my colleagues in the EPA in Castlebar, particularly Karol Donnelly and Ruth Little for their help, good humour and encouragement over the years.

TABLE OF CONTENTS

Chapter 1

Introduction

1.1 Introduction

A fundamental goal of ecology is to synthesise information about the natural world and then to predict how the structure and function of species, populations, communities and/or ecosystems respond to change. There is particular interest among lotic ecologists to find ways of predicting the effects of change on freshwater systems, not only because streams are naturally variable, but also because streams and rivers are vulnerable to anthropogenic disturbances (Power *et al*., 1988). Water, once a seemingly unlimited renewable resource, is in direct need of protection and conservation as aquatic environments are now among our most endangered habitats. The restoration and maintenance of healthy river ecosystems have become important objectives of river management and monitoring programmes (Gore, 1985; Karr, 1991; Rapport, 1991). The overall aim of monitoring programmes is to assess ecological communities and to determine the environmental factors that are responsible for altering them. The ecological community, however, is among the most difficult entities to measure, define and understand. So, while the concept is attractive, its complexity confounds attempts of unique and concise measurements that account for the environmental factors or disturbances directly responsible for deleterious alterations (Moog, 1995).

This problem can be overcome by adapting some species level concepts. Many physical characteristics of the environment have direct influence on and to a great degree determine the distribution, abundance and behaviour of individual organisms and the populations to which they belong. The study of how an individual species responds to its environment, or

moreover, specific characteristics of the environment, is termed autecology (Moog, 1995). Environmental pressures of anthropogenic origin can disrupt the ecological integrity of a system causing a change in autecological relations.

The complexity of these interactions in lotic ecosystems is not yet fully understood. Factors that are particularly important include the influence of local physical conditions on organisms, life histories and the effects of species interactions on lotic communities and ecosystems. The relative importance of local and contemporary versus biogeographic or historical factors in determining species distribution and the response in lotic communities to major environmental changes, needs further (Power *et al*., 1988).

Mechanisms, responses and interactions between organisms and the environment are so numerous that it is impractical to measure but a few. Bioindicators play a very important role in measuring the effects of environmental stress in aquatic habitats. Bioindicators are species that interact with key environmental factors resulting in observable and characteristic responses that alter both the makeup and the ecological function of a particular community. The use of species as opposed to higher taxonomic groups, is emphasised by Moog (1995) in his studies, and he and his co-workers strongly agree that most higher taxonomic groups are either too heterogeneous in their response to environmental stress or lack the useful sensitivity that individual species have to environmental factors.

Relationships between biotic and abiotic factors and the composition and structure of lotic macroinvertebrate communities have received considerable attention (Clenaghan *et al*., 1998). Temporal changes in macroinvertebrate composition have been related to life history patterns in the community (Egglishaw and Mackay, 1967; Armitage *et al*., 1975), which have been hypothesised to have evolved in response to food availability (e.g. Vannote *et al*., 1980) and seasonal changes in physico-chemical factors (e.g. Bunn *et al*., 1986). The temporal and spatial distribution of an organism is tightly connected to its physiological responses to varying levels of environmental

factors. If these responses are known, then the distribution of an organism can be used to indicate the magnitude of these environmental factors. The key biotic and abiotic factors that influence aquatic organisms are water temperature and oxygen balance (Horner and Welch, 1981), food (nutrient) composition and availability (McCormick and Stevenson, 1991; Mullholland *et al*., 1991; Hill, Boston and Steinman, 1992), light intensity (Steinman *et al*., 1989), current-substrate relation (Richards *et al*., 1993) and habitat structure or cover (Hawkins *et al*., 1982). Autecological relations, that is, the interactions between individuals and these environmental factors are obviously the fundamental elements in discovering the causes of community disturbance.

1.2 Thesis outline

This investigation focuses on an autecological study of the genus *Ecdyonurus* in relation to some of the key biotic and abiotic factors controlling its distribution, in five high status and five impacted rivers in the West of Ireland. The project was divided into four key areas, each investigating a particular aspect of the overall ecology of *Ecdyonurus*.

Chapter 2 investigates the effect of artificial eutrophication on an oligotrophic system. Little is know about the effects of eutrophication on *Ecdyonurus*. The aim was to artificially increase the concentration of phosphorus and observe the response of *Ecdyonurus* using a novel split-stream devised specifically for this project.

To date, there have been no investigations in Ireland into the diet of *Ecdyonurus* and this project includes a study of the feeding ecology of this macroinvertebrate. Chapter 3 examines and describes the gut contents of *Ecdyonurus venosus* (Fabricius) in order to establish the preferred diet of this mayfly. The larvae were taken from the Castlebar River, the same river that was used for the split-stream experiment outlined in Chapter 2. A preliminary investigation was carried out on *Ecdyonurus* specimens taken from a number of the high status rivers.

More detailed knowledge of the life history of *Ecdyonurus* is required in order to improve the sensitivity of the EPA Q-value System and also to interpret the impact of pressures on the ecological status of rivers. Chapter 4 outlines the detailed investigation into the life cycle of the four species of *Ecdyonurus* and other Heptageniidae species studied in the five high status rivers selected for this project.

At the high status sites, *Ecdyonurus* are able to flourish and maintain sustainable populations while at the impacted sites these taxa once survived but for reasons unknown, no longer exist. Chapter 5 outlines the comprehensive sampling programme undertaken in the five high status and five impacted rivers over a 16-18-month period which included monthly

macroinvertebrate and river water sampling. The riverbed substrates were also sampled in order to establish the sediment fraction sizes in each of the ten sites. A range of potential biotic interactions between *Ecdyonurus* and other members of the faunal community was also investigated, comparing the faunal communities at the impacted sites with those at the high status sites. The purpose of this investigation was to increase our knowledge of the changes that occur during the eutrophication process by studying and comparing the differences in the chemical, physical and biotic factors between the high status and impacted sites.

1.3 Project context

The genus *Ecdyonurus* is a key water quality bioindicator and its presence or absence has been used as an important element of the EPA's biological River Quality System (Q-value System) since 1970 for the ecological assessment of Irish rivers (Flanagan and Toner, 1972; McGarrigle *et al*., 2002). This system has proved to be very successful in defining water quality in rivers. Table 1.1 illustrates the relationship between the Irish Q-value System and traditional chemical measures of organic pollution and eutrophication (adapted from McGarrigle, 2001). As Q-value improves the concentrations of biochemical oxygen demand (BOD), ammonia, nitrate, and phosphate decline significantly. The Q-value System is therefore clearly linked to standard chemical measures of water quality.

Table 1.1 'Typical' physico-chemical values for Q-value categories: median, mean and standard deviation of the nationally reported values for all sites within each Q-value band. (Adapted from McGarrigle, 2001).

Q-value	**Q1**	**Q1-2**	**Q2**	**Q2-3**	**Q3**	**Q3-4**	**Q4**	**Q4-5**	**Q5**
Max No. Sites	**10**	**5**	**20**	**27**	**81**	**154**	**351**	**156**	**105**
Parameter **Median BOD** (mg O_2/l)									
Median	4.00	4.50	2.70	2.70	2.10	1.70	1.60	1.50	1.30
Mean	8.35	4.28	3.04	2.87	2.31	1.95	1.67	1.49	1.41
SD	10.23	1.36	1.60	1.41	1.05	0.92	0.42	0.45	0.37
Parameter **Median Unfiltered Molybdate Reactive Phosphorus** (mg P/l)									
Median	0.209	0.153	0.135	0.130	0.070	0.043	0.030	0.020	0.015
Mean	0.681	0.159	0.184	0.187	0.116	0.063	0.047	0.027	0.022
SD	1.240	0.033	0.174	0.195	0.145	0.076	0.054	0.024	0.019
Parameter **Median Oxidised Nitrogen** (mg N/l)									
Median	1.85	1.19	1.67	1.79	1.70	1.50	1.20	0.88	0.54
Mean	2.33	1.44	1.68	2.36	2.02	1.62	1.55	1.29	0.76
SD	1.62	0.83	0.91	2.37	1.53	1.21	1.31	1.14	0.77
Parameter **Median Ammonia** (total) mg N/l									
Median	0.380	0.380	0.220	0.190	0.080	0.050	0.040	0.030	0.030
Mean	1.012	0.307	0.376	0.385	0.152	0.082	0.062	0.046	0.049
SD	1.220	0.197	0.538	0.497	0.265	0.104	0.077	0.048	0.073

The National Biological Survey of Rivers, in progress since 1971, has provided a unique time series and a useful set of historical conditions and trends in Irish rivers. In many cases the historical survey results can be used to provide reference conditions for the purposes of the EU's Water Framework Directive.

The EPA's river biologists have provided a range of high status and impacted sites for this project based on their experience of Irish rivers. There is a need for an in-depth analysis of the impacts of eutrophication on river ecosystems and in particular, there is a need to be able to quantify the changes as a river ecosystem departs from its high status or reference condition as it is termed in the EU Water Framework Directive. The sites selected and described below form a basis for undertaking such an analysis and this forms a major part of the current study.

Serious organic pollution has declined steadily in Irish rivers since the 1970s and now accounts for approximately one percent of polluted river channel. In parallel with this, however, a second trend of increasing slight and moderate levels of pollution has occurred resulting in some 30% of surveyed river channel now being classed as slight or moderately polluted (Clabby *et al*., 1992; McGarrigle *et al*., 2002).

Eutrophication is regarded as the main reason for this decline in water quality in Irish rivers with a notable loss of species and a widespread change in trophic status over a 30-year period (McGarrigle, 2001). It is vital, therefore, in establishing reference conditions to understand the detailed mechanisms by which eutrophication affects river ecology. Thus, in setting reference conditions for rivers, it is important to have a more precise understanding of the driving forces behind the long-term changes that have occurred already in Irish rivers and in macroinvertebrate communities particularly as they are the most important element defining Q-values in the Irish Quality Rating System. The high status sites chosen for this project had ideal conditions for the most sensitive taxa. Some of the eutrophic sites contained these sensitive indicators but only sporadically during the study programme while other eutrophic sites did not have any of these taxa at all.

National statistics and long term trends are largely based on the EPA Q-values. The Q-values in turn are based on the response of macroinvertebrates especially but also of phytobenthos and macrophytes in rivers to pollutants such as phosphorus, and inputs of organic matter or toxic

substances such as ammonia, heavy metals and pesticides. Similarly, they respond to physical factors such as substratum, flow velocity, siltation channel alteration, erosion and temperature effects. Finally, the influence of biotic factors such as predation and inter-specific competition will also play a role in defining the final ecological community of inhabiting a river stretch. Because the Water Framework Directive requires the definition of reference conditions for different ecological types and regions, it is particularly important to be able to understand and define the reasons for departure from reference conditions causing loss of sensitive indicator species such as *Ecdyonurus*.

1.4 The Water Framework Directive

1.4.1 Background

As part of a substantial restructuring of EU water policy and legislation, a Directive establishing a new framework for community action in the field of water policy (2000/60/EC; European Commission 2000) was agreed by the European Parliament and Council in September 2000 and came into force on 22nd December 2000. The Directive, generally known as the Water Framework Directive (WFD), rationalises and updates existing water legislations and provides for water management on the basis of River Basin Districts (RBD's). It outlines a legal structure for the assessment of all types of water bodies in Europe.

The purpose of this Directive is to establish a Framework for the protection of inland surface waters, transitional waters, coastal and groundwaters. A focus of the assessment systems demanded by the WFD is the use of biotic indicators (macrobenthic fauna, fish fauna and aquatic flora). It is potentially the most significant piece of legislation ever to be enacted in the interests of conservation of freshwater and saline ecosystems. It seeks to incorporate existing legislation into the new framework with bioassessment as the key approach. The Directive uses biological measures to a greater extent than previous directives such as the Freshwater Fish Directive, where water quality was based predominately on chemical determinands. There is quite a distinction between water quality and ecological status and systems used currently to establish the former are only a small part of those needed for the latter (Moss *et al*., 2001).

1.4.2 Ecological boundaries

The Directive requires catchments to be managed in a holistic manner whose ultimate effectiveness will be reflected in the degree to which aquatic habitats are restored to 'good' ecological status (Moss *et al*., 2001). Maintaining 'high' ecological status, where it already exists, or achieving 'good ecological status' is the ultimate goal. One of the first requirements

of the Directive is the development of a typology among flowing, standing, transitional and coastal waters. Within each of these groups, ecotypes must be defined and the pristine (reference) characteristics of each type defined. On establishing individual ecotypes, each is classified into a system according to deviations from this reference condition (high quality). Furthermore, the ecological status of a water body is defined by comparing the biological community composition present with the near-natural reference condition (Hering *et al*., 2004). Reference conditions for Irish rivers will be included in the forthcoming characterisation report for Ireland to be completed under Article 5 of the WFD by 22nd March 2005.

The classification system includes 'good', 'moderate', 'poor', and 'bad' quality and must be established by comparing biological elements with their reference condition and taking into account supporting chemical and morphological characteristics. As mentioned above, WFD includes the concept of "no deterioration" in ecological quality but the main aim is to generate plans for the gradual improvement of the ecological quality of all surface waters until they achieve "good" status. Within the WFD, the aim to provide a more sustainable water system is based on the view that good ecological quality is more "natural" and therefore more sustainable (Logan, 2001). Methods for WFD compliant classification of ecological status of each of the biotic elements listed in Annex V of the WFD are currently under development in Ireland and elsewhere across Europe in preparation for the Directive's monitoring programmes, which must commence in 2006.

1.4.3 The Reference condition

A central part of the EU WFD is the identification of expected background reference conditions with no or minimal anthropogenic stress. According to the Directive, Member states are required to identify reference conditions for the purpose of defining a reference biological community (European Commission, 2000; REFCOND, 2003). There have been a number of definitions of "reference" conditions put forward to date:

- Expected background (i.e. reference) conditions with no or minimal anthropogenic stress and satisfying the following criteria: (1) they should reflect totally, or nearly, undisturbed conditions for hydromorphological elements, general physicochemical elements, and biological quality elements, (2) concentrations of synthetic pollutants should be close to zero or below the limit of detection of the most advanced analytical techniques in general use, and (3) concentrations of specific non-synthetic pollutants, should remain within the range normally associated with background levels (European Commission, 2000; REFCOND, 2003)
- Representing important aspects of 'natural' or pre-Columbian conditions and at the same time, politically palatable and reasonable (Hughes, 1995)
- The condition that is representative of a group of minimally disturbed sites organised by selected physical, chemical and biological characteristics, (Reynoldson *et al*., 1997)
- Regionally-representative sites that are indicative of expected background conditions in the absence of anthropogenic stress (Johnson, 1999)

The idea of a reference condition is a critical element in approaches now being developed for biomonitoring and bioassessment of aquatic system. In America for instance, the reference condition is central to currently accepted ideas of 'biocriteria' being developed by the US Environmental Protection Agency (EPA) (Davis and Simon, 1995). A similar approach has been used in the UK for river classification and water-quality assessment (Wright, 1995). It is also being used in Canada to develop sediment guidelines for the Great Lakes (Reynoldson *et al*., 1995) and is the basis for the National River Health Program in Australia (Parsons and Norris, 1996). Ideally, a river is assessed and its ecological status is compared with the reference state (pristine state). The assumption being that the reference state is in itself sustainable because anthropogenic impacts are "minimal" or as maybe the case, they are simply more sustainable than an impacted situation (Logan, 2001).

1.4.4 Classifying ecological status

Due to anthropogenic impacts on the environment it can often be quite difficult to find pristine rivers of high ecological status. The definition of ecological status itself relates to the observed status of the site when compared with a reference status. In describing reference conditions, water-quality monitoring is required based on a pre-established criteria that exist at a wide range of sites rather than relying on information from one or a few control sites. These reference conditions are based on spatial, temporal or ecologically modelled conditions where human impacts are minimal and the reference conditions then serve as the control against which test-site conditions are compared. The idea of the reference condition is really one of the best available condition and it is represented by information from numerous test sites (Reynoldson *et al.*, 1997). Many countries, however, would like to use the best available condition but in many cases the best available may actually be quite poor. Historical data, expert opinion, models, palaeolimnology and outside-state reference sites may be used to recreate reference conditions rather than just accept the best available.

Establishing baseline reference conditions against which to measure the effects of human activities is only the first step in the WFD's requirement of being able to determine the ecological status of inland waters. The WFD also stipulates that the level of human impact on the structure and function of aquatic ecosystems needs to be defined in terms of ecological quality elements (Johnson, 2001). There are five biological quality elements in Annex V of the WFD that require examination: macroinvertebrates, phytobenthos, phytoplankton, macrophytes and fish fauna. In addition to this, member states must be able to identify five levels of impact (or ecological status) using these ecological quality elements.

In Irish terms, the ecological status of a river is classified into the following categories based on the Q-value Rating System:

- Q5, Q4-5 classified as ‘High’ status
- Q4 classified as ‘good’ status
- Q3-4 classified as ‘moderate’ status
- Q3, Q2-3 classified as ‘poor’ status
- Q1, Q1-2, Q2 classified as ‘bad’ status

The final classification will, however, depend on the results of the official EU intercalibration exercises. In addition to measuring quality elements or organism groups for classifying the ecological status, the WFD also specifies that a number of hydromorphological and physico-chemical elements need to be examined. This in turn will support and interpret the organism-based monitoring/assessment decision of whether or not a site deviates from good ecological quality. Good ecological status does not differ significantly from undisturbed, high ecological status or reference conditions, whereas moderate, poor and bad ecological quality differ from the expected and the biological communities show varying degrees of human induced stress ranging from moderate to severe changes in community structure and composition (Johnson, 2001).

1.4.5 Benthic macroinvertebrates

Benthic macroinvertebrates, together with algae, serve as the most common groups used for assessing the ecological river quality (Hellawell, 1986; De Pauw and Hawkes, 1993; Rosenberg and Resh, 1993). Generally, benthic macroinvertebrates are capable of reflecting different anthropogenic perturbations through changes in structure or function in the assemblages and thus enable an overall assessment of ecological status. Besides organic pollution, which can be assessed using a large number of biological indices, benthic macroinvertebrates can also be used to detect acid stress, habitat loss and overall stream degradation (Hering *et al*., 2004). For these reasons, benthic macroinvertebrates are likely to play a major role in future stream assessment in coherence with the Water Framework Directive.

The individual European countries have very different traditions in the use of benthic macroinvertebrates for biomonitoring purposes. Overviews were

given by Knoben *et al*., (1995), Nixon *et al*., (1996) and Birk and Hering (2002). The differences in the methods are inherent in the intensity of sampling and sorting, the taxonomic resolution, the storing and the statistical treatment of the data, the metrics used for assessment and the quality assurance procedures used in this process. Therefore, comparisons of results obtained with different national methods are complex and in many cases hindered by these differences. While existing methods in some of the countries are partly fulfilling the requirements of the Water Framework Directive (UK: Wright *et al*., 2000; France: Agences de l'Eau, 2000), existing methods need adaptation in other countries (Germany: DEV, 1992; Austria: Austrian Standards M 6232, 1997; Chovanec *et al*., 2000; Netherlands: Peeters *et al*., 1994; Italy: Ghetti, 1997) or no official method is available (Greece, Portugal). In Ireland's case the Quality Rating System must be made WFD compliant by converting it to an ecological quality ratio (EQR) system with a score that ranges from 0 to 1.

1.4.6 Conclusions

The WFD is a wide ranging and ambitious piece of European environmental legislation setting clear objectives to ensure that 'good status' is achieved for all European Waters by 2015. The aims of the WFD present major challenges to everyone involved in protection, use and management of the aquatic environment. Significant resources have been mobilised to meet the challenges and meet the tight time schedule for the activities, which must be undertaken. It is hoped that the current study can help in the overall development of a WFD-compliant macroinvertebrate monitoring system by helping to better understand the response of some of the key indicator species to eutrophication and other pressures.

1.5 Eutrophication

1.5.1 Background

Eutrophication, whether in a river or lake, is the enrichment of water by plant nutrients that are normally in short supply, which consequently cause an imbalance in the food web, resulting in high levels of plant and algal growth. The predominant nutrients that control plant and algal growth, are phosphorus and nitrogen. Phosphorus is usually available to aquatic organisms in water as phosphates and since it is naturally present in lower concentrations than nitrogen, phosphate usually acts as the limiting growth factor. In Ireland, one of the most serious environmental pollution problems is the over-enrichment of surface waters by phosphorus and to a lesser extent, nitrates (Environmental Protection Agency, 2002).

1.5.2 Nutrient sources

This type of pollution poses a threat to Ireland's game fish populations and has resulted from excessive inputs of nutrients from a number of sources. Estimates indicate that agriculture is responsible for the largest inputs of phosphorus and nitrates to waters. Sewage is another major source of these nutrient inputs. These sources are usually classified according to the type of discharge, namely 'point' and 'non-point' sources. Point sources include discharges from sewage and the run-off from open farmyards. Non-point sources originate by diffuse losses from land to which excessive amounts of animal waste or artificial fertilisers rich in organic phosphates have been applied. Phosphates are also derived from natural sources due to erosion. This source is generally not as biologically available for plant growth and is usually a very small source in comparison with anthropogenic sources. Rainfall and dry deposition from the atmosphere are further sources but generally to a lesser extent (Lucey *et al.*, 1999).

Large point sources such as sewage discharge are often the major, and more easily controlled, source of phosphorus loads but diffuse agricultural sources can be even larger than municipal sources especially in less densely

populated countries such as Ireland. There is now a significant body of evidence to suggest that phosphorus from agricultural sources can represent a significant input to freshwater and the increase in phosphorus concentrations in agricultural drainage water over time reflects the accumulation of phosphorus in soils (Sharpley *et al.*, 2000). Even losses from Irish grasslands, without any farmyards, can reach 3kg P/ha/annum where the soil burden is high and the soil type is prone to losses (Jordan *et al.*, in press). This is many times the typical background or reference condition loss rates of less than 0.1 kg P/ha/annum.

1.5.3 Eutrophication Trends in Ireland

National river quality surveys were first carried out in Ireland in 1971 on a river channel length of 2,900km. It became apparent in the late 1970s that river eutrophication was an actual or potential problem in many river catchments (McGarrigle, 2001). One of the first studies to highlight this problem was that undertaken for the original water quality management plans (WQMP), particularly those prepared for the south-eastern rivers like the River Suir (e.g. Horkan, 1980). By the early 1980s, there was a widespread national trend of increased eutrophication in rivers in Ireland. This prompted a series of studies aimed at gaining a better understanding of river eutrophication which would help to control and reverse the increasing trend of eutrophication in Irish rivers (e.g. McGarrigle 1983, 1984a, 1984b; McGarrigle and Lucey 1989, 1990; McGarrigle *et al.*, 1987).

The Environmental Protection Agency expanded its river quality investigations to 12,700km between 1991-1994 and to 13,500km between the period 1995 to 1997 (Lucey *et al.*, 1999). The third biological investigation was carried out during the period 1998-2000 in which the national baseline of some 13,100km of river channel was assessed. The most recent assessment of river quality in Ireland shows an improvement in water quality for the first time since the surveys began (Environmental Protection Agency, 2002). Unpolluted channel length increased from 67 % in the period 1995-1997 to almost 70% in the period 1998-2000. However, the overall status is still unacceptably poor, in comparison to what it was 20 years ago.

Over 30% of the national river channel is now considered to be polluted to some extent. This pollution is attributed in the main to eutrophication. The degree of pollution is minor in many cases but it is of concern in view of its potential impact on the pollution-sensitive trout and salmon in rivers and lakes. In Ireland this is of particular concern because most rivers are salmonid waters, capable of supporting game fish such as trout, salmon or sea trout. Luxuriant plant growth in salmonid rivers may cause diurnal fluctuations in Dissolved Oxygen (DO) resulting in fish kills due to low DO levels, particularly the pre-dawn period. In 1990, Moriarty found that up to 50% of fish-kills reported in Ireland were due to this type of phenomena in eutrophic waters during warm weather.

It is worth noting that the observed improvements have mainly occurred in catchments that have had intensive management programmes implemented over the last five to seven years (Environmental Protection Agency, 2002). The recent EU Water Framework Directive provides for a more integrated approach to controlling water pollution. Its full implementation will be a major policy challenge for Ireland, but should ultimately lead to a significantly improved water quality and water management across the State.

1.5.4 Effect of eutrophication on macroinvertebrates

As with many other environmental stresses two interrelated trends are readily discerned when increasing enrichment occurs. The first is a reduction in the diverse macroinvertebrate community characteristics of clean water, in which many species are represented by relatively few individuals, towards a condition, in which under the influence of severe pollution, a few species are represented by very large numbers of individuals. These species are those which are able to take advantage of the changes which the pollutant induces and to exploit the increased food supply which is provided. The second change, which is more relevant to that which occurs during eutrophic conditions, is the progressive disappearance of particular indicator species until very few remain and their place is taken by species not previously present or, at least, not abundantly

present (Hellawell, 1986). In Ireland, *Ecdyonurus* is a key water quality indicator genus and little is known about the factors responsible for its disappearance from eutrophic or other polluted rivers.

Although intermediate levels of organic enrichment may favour certain suspension or deposit feeding macroinvertebrate groups (such as blackfly and chironomid larvae), changes in the substratum (through increased sedimentation of organic matter) and low dissolved oxygen concentrations that often occur at high levels of organic pollution and under eutrophic conditions, usually result in the disappearance of intolerant taxa (Hynes, 1960; Hellawell, 1986). It is worth noting that during the preliminary stages of eutrophication, species numbers may increase due to favourable conditions for the particular invertebrate species.

While the Q-value System was originally based on the sensitivity of invertebrates to organic pollution, it has been found that the invertebrates show a definite response to eutrophication, which is related to but separate from, organic pollution. Rivers with low BOD and other organic pollutants like ammonia may suffer from diurnal oxygen fluctuations due to nutrient-induced growth of excessive plant biomass resulting in loss of sensitive species. As eutrophication progresses, other symptoms may arise, for instance causing changes in algal species or resulting in habitat siltation (McGarrigle, 2001).

1.5.5 Diurnal dissolved oxygen (DO) variation

One of the main impacts of eutrophication is the increase in primary production, combined with respiration, giving rise to a diurnal pattern of dissolved oxygen concentrations in rivers (Odum, 1956; Edwards and Owens, 1965). Dissolved oxygen depletion usually occurs at night in these rivers caused by the respiratory demands of the plant biomass and sediment bacteria. A clean river will normally display a DO reading near the 100% saturation level with little deviation above or below this level over the day in response to photosynthesis and respiration of plant material (McGarrigle, 1998).

A river unaffected by eutrophication will have low to medium plant biomass and the phosphorus levels will be low. Since phosphorus is normally the limiting nutrient, the addition of bioavailable phosphorus will cause an increase in plant growth. It has been shown that the variations in DO levels increases as the plant biomass increases causing diurnal fluctuations thereby exerting a significant strain on salmonid populations. This may be somewhat overlooked in routine monitoring programmes as practically all national and local river monitoring programmes over the past 30 years, measure day time DO levels only (McGarrigle, 2001).

1.6 Research Objectives

The status of *Ecdyonurus* as a key indicator species is based largely on empirical evidence. In particular there are clear statistical links between the presence and absence of *Ecdyonurus* and water quality parameters such as BOD, ammonia, nitrate and phosphate that are important chemical indicators of water quality (McGarrigle, 1998). The Irish Phosphorus Regulations are based on such a link between biological Q-values and phosphate concentrations. While these strong empirical links exist, the precise mechanisms controlling the distribution of *Ecdyonurus* in eutrophic and polluted rivers are not that well understood.

Ecdyonurus is one of the most useful indicators of pollution because it is almost ubiquitous in unpolluted waters, but is sensitive to pollution. It can be expected to occur in river types ranging from large to small, from hard water to moderately acidic ones and from fast flowing to all but the slowest-flowing stretches of river, provided the river is not polluted.

In the light of the Water Framework Directive's need for ecological assessment of rivers, it will be necessary to adapt the Irish Q-value System in order to provide a broader ecological assessment of river status. A more comprehensive ecological assessment needs to take into account, for example, the expected faunal and floral composition for a given type of site under pristine conditions and factors such as river hydromorphology and invasive species.

An important objective of this project is to obtain an improved understanding of reference conditions as revealed by changes in the macroinvertebrate fauna and phytobenthos when a river's status begins to depart from its pristine state. To this end, this project attempts to refine our knowledge of the ecology of *Ecdyonurus* and other members of the Heptageniidae family in relation to a range of potential controlling factors in the riverine environment.

1.7 Study locations

A GIS map outlining the ten study locations and catchment geology selected for this study is shown in Fig. 1.1. The five high status and five impacted rivers and associated information are shown in Table 1.2 and Table 1.3, respectively.

Each of the ten rivers are described separately in detail below followed by the catchment characteristics showing CORINE 2000 landuse, GSI rock units (bedrock geology) and river network indicating Strahler stream order (Fig. 1.2 -Fig. 1.11).

Table 1.2 List of the five high status rivers studied in this investigation.

River	Code	Station number	Location	GPS	Q-value 2001-2003
Owengarve	34O03	0200	Bridge upstream of the Moy River confluence (Dawros Bridge)	54 00 784 N 8 50 154 W	5
Castlebar	34C01	0020	Bridge near Graffa More upstream of Lough Mallard	53 51 42 N 9 21 00 W	4-5
Brusna (North Mayo)	34B07	0400	Bridge west of Cloonta	54 07 58 N 9 04 10 W	4-5
Dunneill	35D06	0100	Bridge 2km upstream Dromore West	54 14 15 N 8 51 42 W	5
Callow Loughs Stream	34C08	0300	Bridge upstream Yellow River	54 01 20 N 9 02 00 W	4-5

Table 1.3 List of the five impacted rivers studied in this investigation.

River	Code	Station number	Location	GPS	Q-value 2001-2003
Cartron	33C02	0100	Bridge west of Lough Gall	53 56 13 N 9 49 38 W	3-4
Lough na Corralea Stream	30L03	0400	Bridge east of Cloonee	53 42 51N 9 19 02 W	3
Robe	30R01	0400	Hollymount Bridge	53 39 42 N 9 07 19 W	3
Mullaghanoe	34M03	0100	Bridge WNW of Bellahy	53 58 04 N 8 48 01 W	3
Mad	34M04	0100	Bridge in Cloonacool	54 06 07 N 8 46 23 W	3

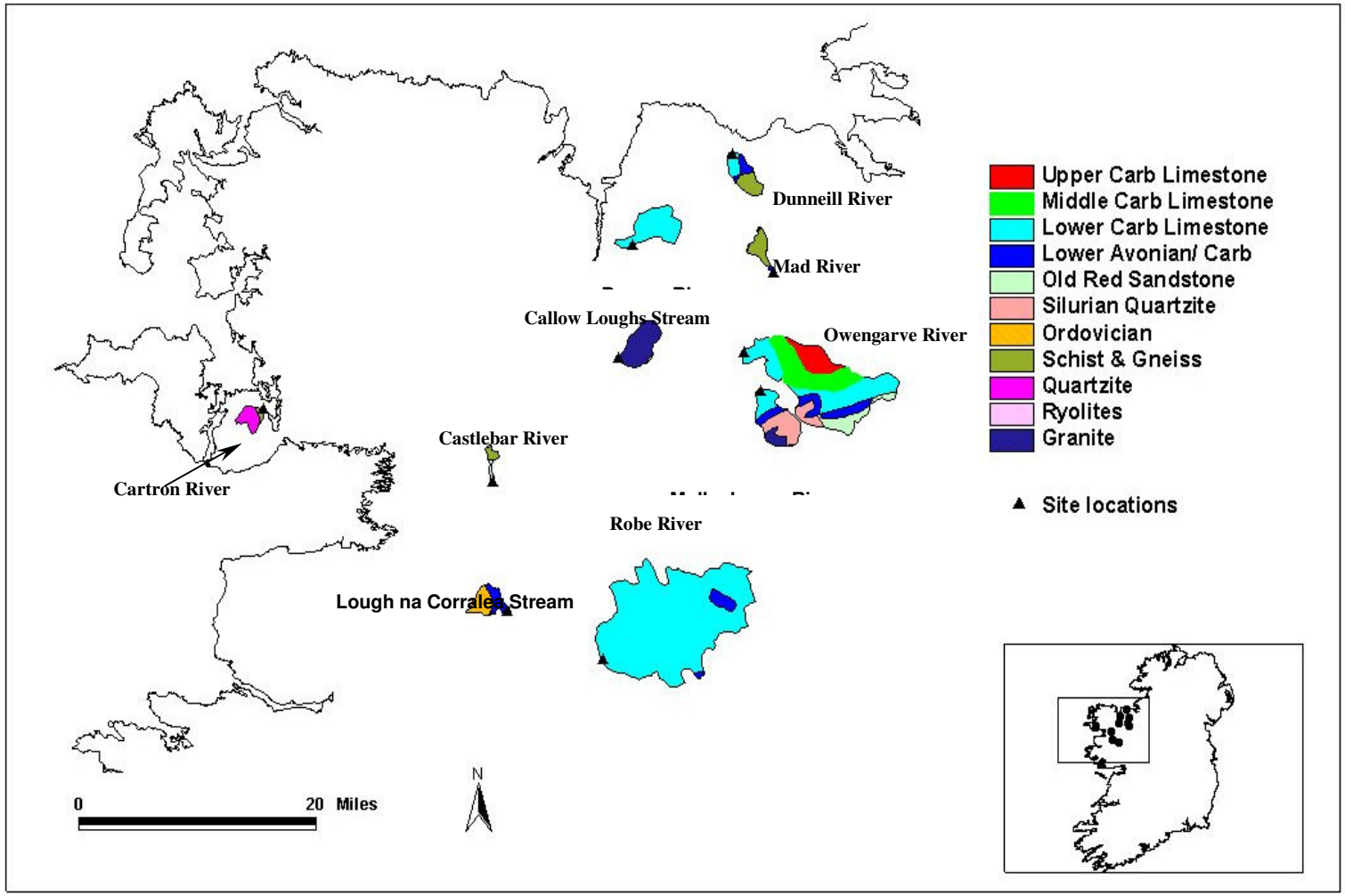


Fig. 1.1 GIS map showing the location of the ten river catchments and associated geology.

1.7.1 Site 1: The Owengarve River

The Owengarve River site is situated around 5km west of Curry village in Sligo. The study site (EPA national river code 34O03-0200), was located approximately 100m downstream of the bridge which is situated upstream of the Moy River confluence. Monthly samples were taken in an area measuring approximately 15m x 6m (depending on the water level on the day of sampling). Its co-ordinates are E: 145303, N: 3074170; Latitude: 54° 00' 784" N, Longitude: 8° 50' 154" W. The Owengarve is a fourth order river with a catchment area of 121.27 km^2. The catchment is dominated by pasture and peat bog.

The average wet width was 6-12m for the duration of the sampling period. Pebbles and cobble were the main substratum type in this river. The dominant tree species present were alder (*Alnus* spp.), willow (*Salix* spp.), Birth (*Betula* spp.) and hawthorn (*Crataegus monogyna*). The vegetation along the banks consisted mainly of mint (*Mentha* spp.), butterbur (*Petasites hybridus*), puccinellia (*Glyceria* spp.) and forget-me-not (*Myosotis* spp.). The main species of emergent macrophytes present were bur-reed (*Spraganium erectum*), duckweed (*Lemna* spp.), watercress (*Nasturtium* spp.), wild celery (*Apium* spp.) and horsetail (*Equisetum* spp.). The floating plants found were pondweed (*Potamogeton natans*) and iris (*Iris pseudacorus*). The main submerging plants were water-milfoil (*Myriophyllum* spp.), *Fontinalis* spp. and other moss varieties.

Monitoring by the EPA began in the Owengarve River in 1980 and it continues to retain a Q-value of 5.

Fig. 1.2 outlines the catchment characteristics of the Owengarve River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order.

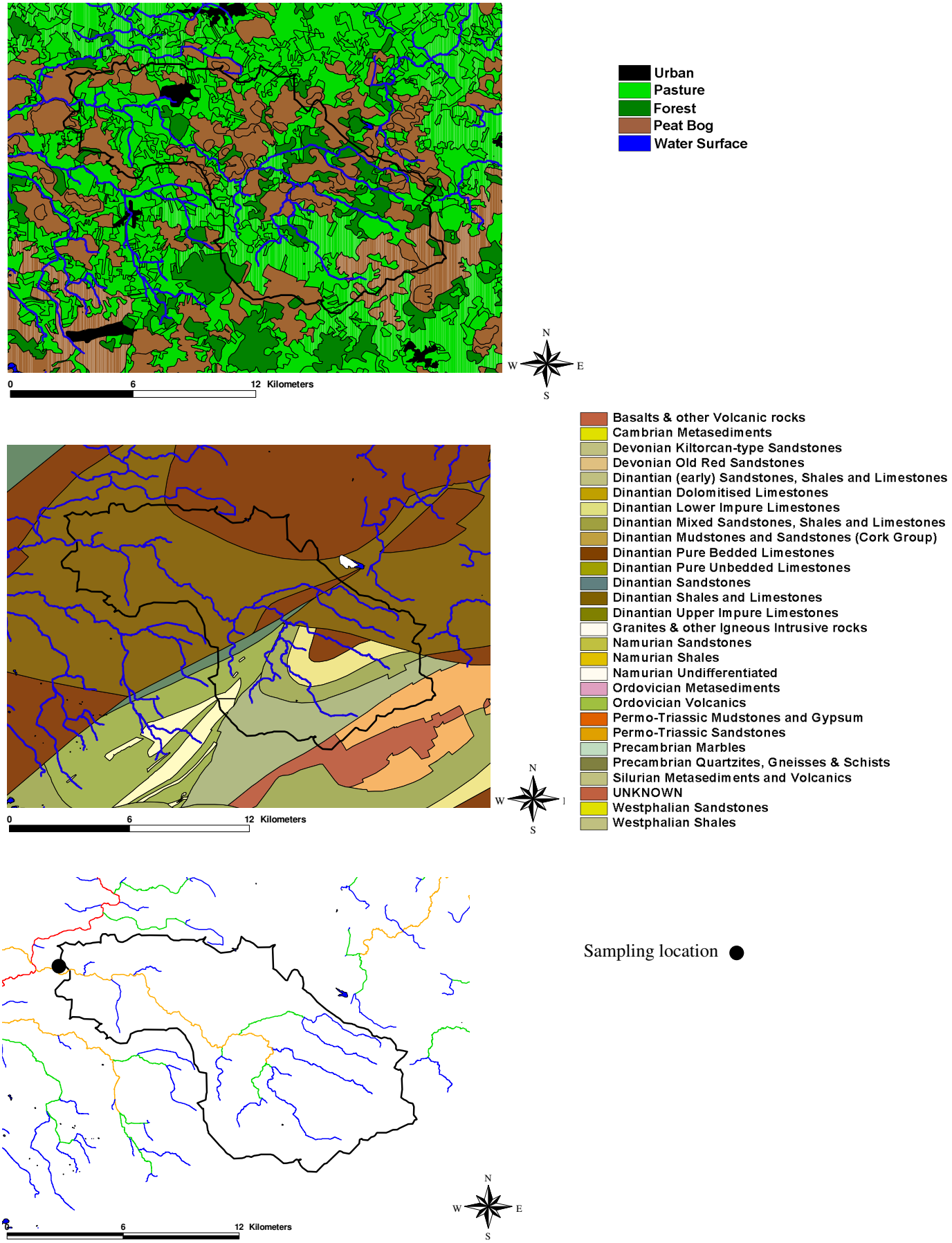


Fig. 1.2 Catchment characteristics for Catchment of the Owengarve River Sample Site 34O030200 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.2 Site 2: The Castlebar River

The Castlebar River site is located approximately 6km west of Castlebar town. The study site (EPA national river code 34C01-0020), was located approximately 200m downstream of the bridge near Graffa More, upstream of Lough Mallard. Monthly sampling was carried out in an area measuring approximately 9m x 3m (depending on the water levels on the day of sampling). Its co-ordinates are E: 110930, N: 291732; Latitude: 53° 51' 20" N, Longitude: 9° 20' 55" W. The Castlebar River is a second order river with a catchment area of 5.25 km^2. Its catchment is dominated by agricultural land and peat bog with a small forest located a few miles upstream of the sampling site.

The average wet width of the river during the sampling period was 3-4m. Pebbles and cobbles dominated the riverbed substrate. The main riparian trees present were hawthorn, willow and ash (*Fraxinus excelsior*). The vegetation on the riverbanks consisted of montbretia (*Crocosmia* x *crocosmiflora*) while bur-reed was the main emergent macrophyte. The submerged macrophytes were *Fontinalis* spp and other mosses.

The quality of this river is good and has maintained a Q-value of 4-5 since monitoring began in the 1970s.

Fig. 1.3 outlines the catchment characteristics of the Castlebar River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating the Strahler stream order.

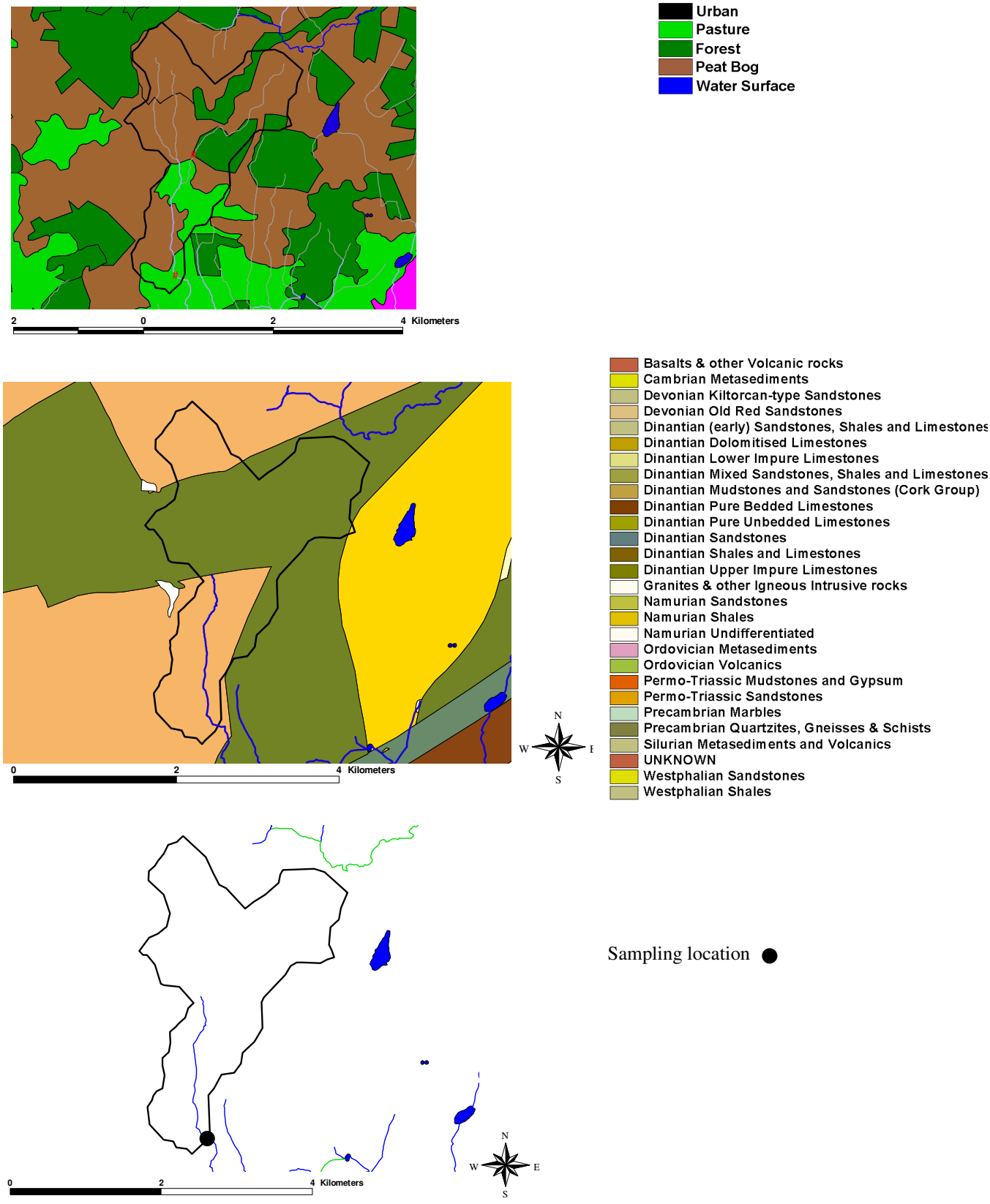


Fig. 1.3 Catchment characteristics for Catchment of the Castlebar River Sample Site 34C010020 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.3 Site 3: The Brusna River

The Brusna River is situated approximately 7km north east of Ballina town, in north Mayo. The study site (EPA national river code 34B07-0400), was located 10-20m downstream of the bridge west of Cloonta. Monthly sampling was carried out in an area measuring approximately 17m x 6m (depending on the water level on the sampling day). Its co-ordinates are E: 130196; N: 320988; Latitude: 54° 07' 58" N, Longitude: 9° 04' 10'' W. The Brusna is a fourth order stream with a catchment area of 28.15 km^2. Pasture and peat bogs are the main land use.

The average wet width of the river was 6-8m during the study period. The substratum consisted mainly of large and small cobble, pebbles and sand and gravel. The dominant tree species were sycamore (*Acer pseudoplatanus*), willow, ash and alder. The vegetation along the banks of the river consisted of meadow sweet (*Filipendula ulmaria*), willow-herb (*Epilobium* spp), purple loosestrife (*Lythrum salicaria*), snowberry (*Symphoricarpos albus*), *Petasites* spp and montbretia. The emergent plants in this river were bur-reed, wild celery, mint and watercress. The main submerged macrophytes were *Fontinalis* spp, other species of moss and water-milfoil. The floating Pondweed was also present.

The Brusna River has maintained good water quality standards since monitoring commenced in the late 1980s and presently holds a Q-value of 4-5.

Fig. 1.4 outlines the catchment characteristics of the Brusna River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

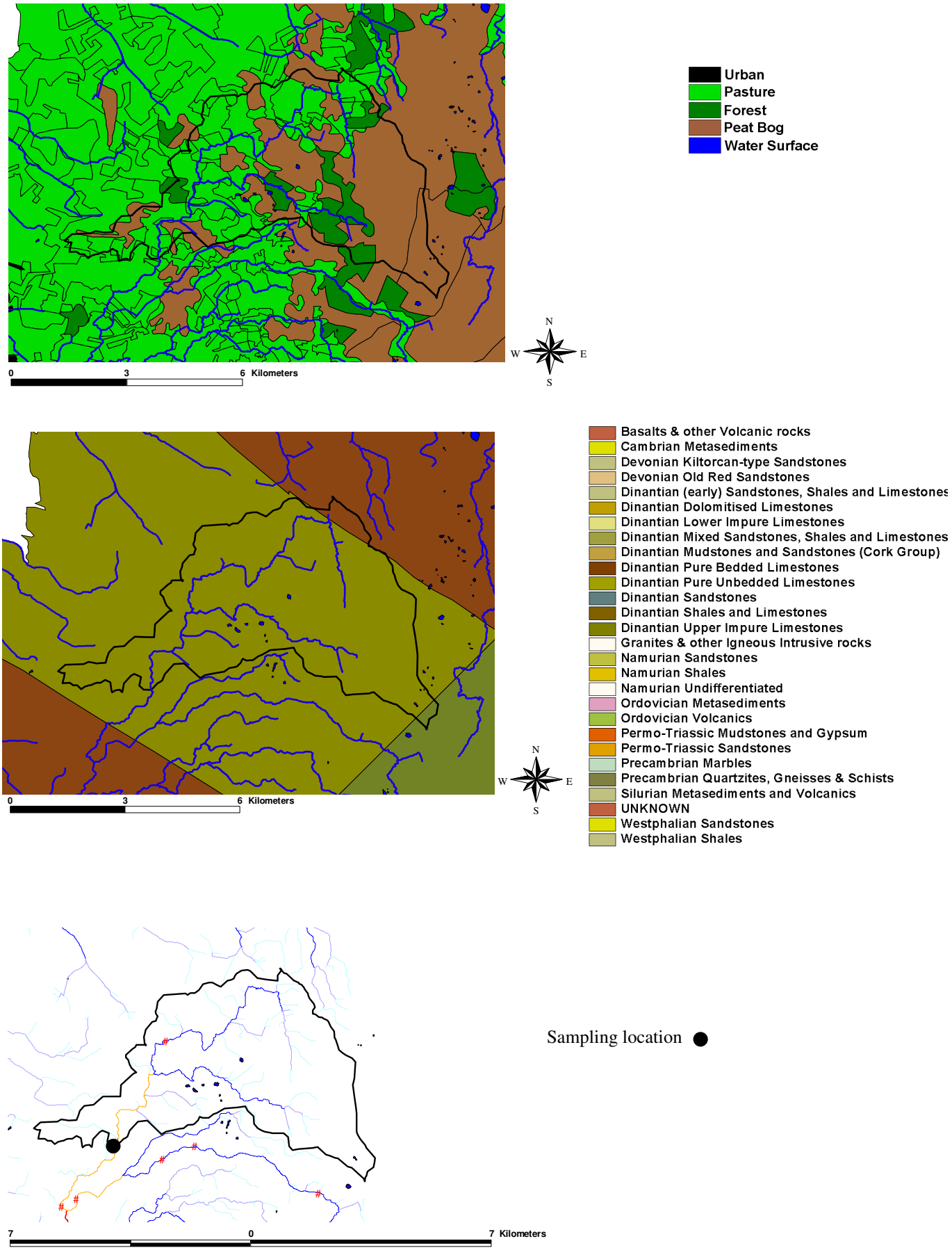


Fig. 1.4 Catchment characteristics for Catchment of the Brusna River Sample Site 34B070400 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.4 Site 4: The Dunneill River

The Dunneill River is located approximately 2km south, south east of the village of Dromore West in Sligo. The study site (EPA national river code 35D06-0100), was located roughly 40m downstream of the bridge at Dromore West. Monthly samples were taken in an area measuring approximately 14m x 8m (depending on the water level on the day of sampling). Its co-ordinates are E: 144492; N: 329228; Latitude: 54° 14' 15'' N, Longitude 8° 51' 42'' W. The Dunneill is a third order stream with a catchment area of 12.02 km^2. Pasture and peat bogs are the dominant land uses in the catchment.

During the sampling period, the average wet width of the river was approximately 8-10m. The dominant substrate in the river consisted of pebbles and cobbles and to lesser extent boulders. The mains tree species were ash, alder, willow and fuchsia (*Fuchsia magellanica*). The bankside vegetation consisted of rush (*Juncus* spp.) and *Petasites* spp. The main emergent plants were mint, *Carex* spp. and *Caltha* spp., while moss was the dominant submerged macrophyte. The land use in this catchment is mainly pasture.

The Dunneill River has maintained a high water quality standard since the monitoring programme employed by the EPA commenced. It continues to have a Q-value of 5.

Fig. 1.5 outlines the catchment characteristics of the Dunneil River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

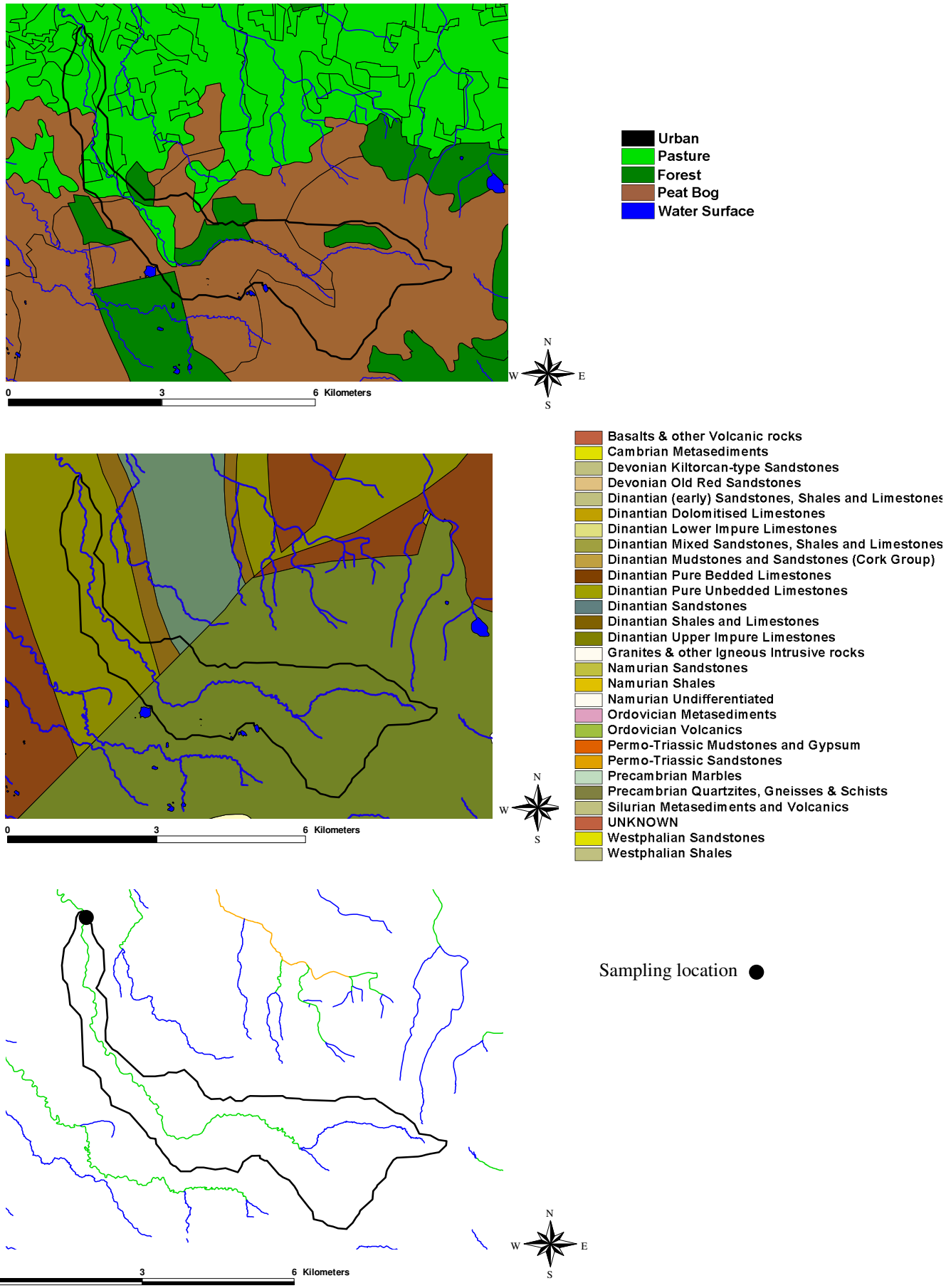


Fig. 1.5 Catchment characteristics for Catchment of the Dunneill River Sample Site 35D060100 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.5 Site 5: Callow Loughs Stream

Callow Loughs Stream is located approximately 3 km north east of Foxford town in Mayo. The study site (EPA national river code 34C08-0300), was located just downstream of the bridge situated upstream of the Yellow River confluence. Monthly sampling was carried out in an area approximately measuring 7m x 15m (depending on the water level on the sampling day). Its co-ordinates are E: 129356, N: 305629; Latitude: 54° 01' 120" N, Longitude: 9° 02' 00" W. Callow Loughs Stream is a second order stream with a catchment area of 8.76 km^2. The predominant land use in the area is pasture and there is also a working quarry upstream and a disused one downstream.

The average wet width of the river was between 6-8m for the sampling duration. Large and small cobbles were the main substratum types. The species of trees consisted of a mixture of alder, ash and willow. Bankside vegetation consisted mainly of hart's tongue (*Asplenium* spp.), blackthorn (*Prunus spinosa*) and fern species. The emergent macrophytes were mint, wild celery and speedwell (*Veronica* spp.). Moss and water-milfoil were the most common submerged macrophyte species.

Callow Loughs Stream has good water quality and since monitoring began in 1989, it fluctuated between Q4 and Q4-5. Since 1998, it continued to maintain a Q4-5 rating.

Fig. 1.6 outlines the catchment characteristics of Callow Loughs Stream showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

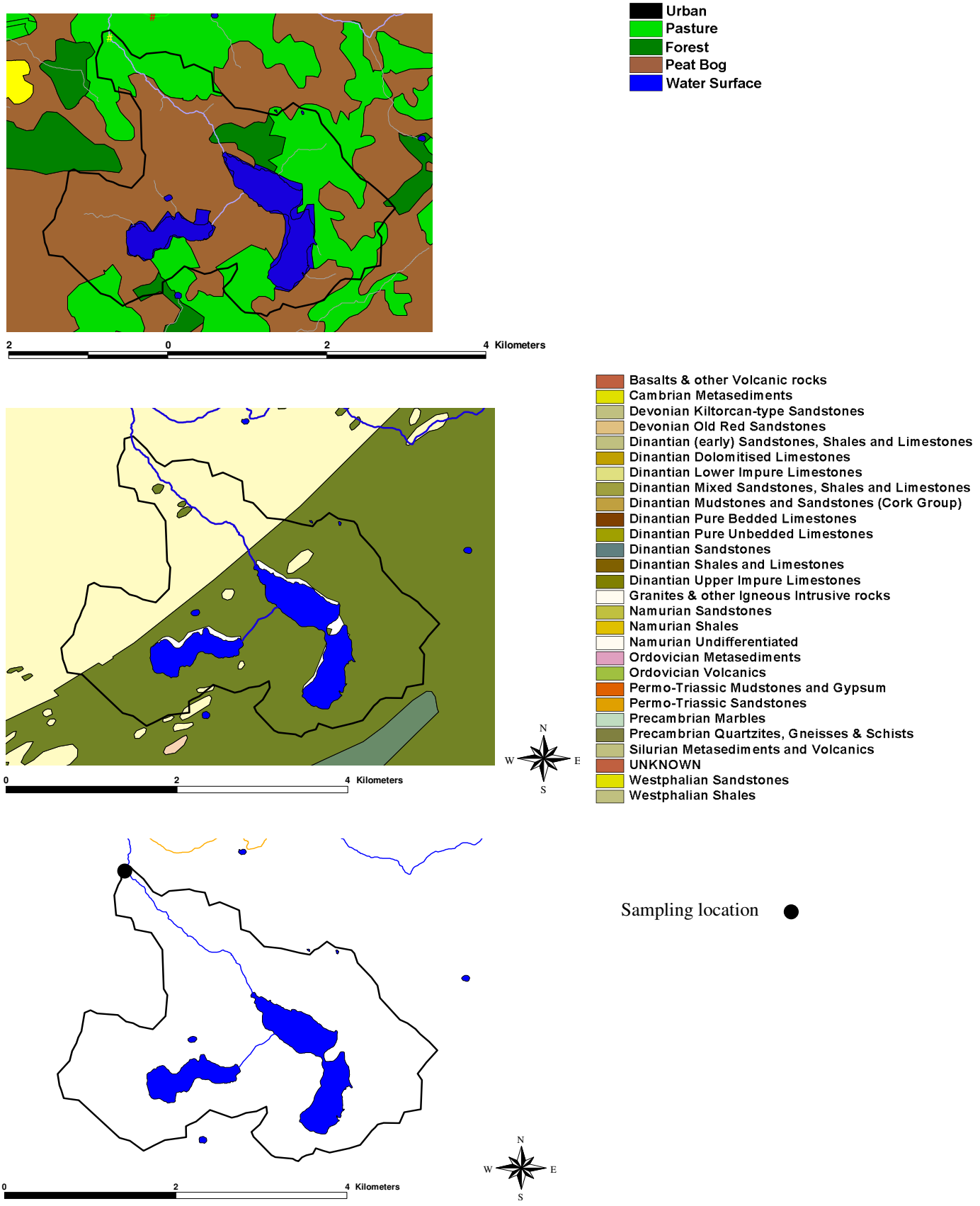


Fig. 1.6 Catchment characteristics for Catchment of the Callow Loughs Stream Sample Site 34C080300 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.6 Site 6: The Cartron River

The Cartron River is located approximately 4km north west of Mulranny village in Co.Mayo. The study site (EPA national river code 33C02-0100) was located roughly 100m upstream of the bridge west of Lough Gall. Monthly sampling was carried out in a designated area measuring approximately 15m x 8m (depending on the water level on the day of sampling). Its co-ordinates are E: 80000, N: 300184; Latitude: 53° 56' 13''N, Longitude: 9° 49' 38'' W. The Cartron River is a fourth order river with a catchment area of 10.47 km^2. A large forestry plantation upstream and peat bog surrounding the river dominate the land use.

The average wet width of the river during the sampling programme was 6-12m. The substratum of the Cartron River was dominated by large and small cobble interspersed with gravel. A large conifer plantation was located upstream of the river site. The bankside vegetation consisted of rush. There were no emergent macrophytes and moss and liverworths were the only submerged vegetation in this river.

In 1982, the Cartron was assigned a Q-value of 4-5 and retained this standard until it dropped to a Q4 when assessed in 1994. The quality declined thereafter to a Q3-4 in 1997 and from 1999 to the present study, it was assigned a Q3. There has been a notable decline in the numbers of ephemeropteran species over the years. This river is prone to quite a substantial amount of algal growth particularly during the spring and autumn months.

Fig. 1.7 outlines the catchment characteristics of the Cartron River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

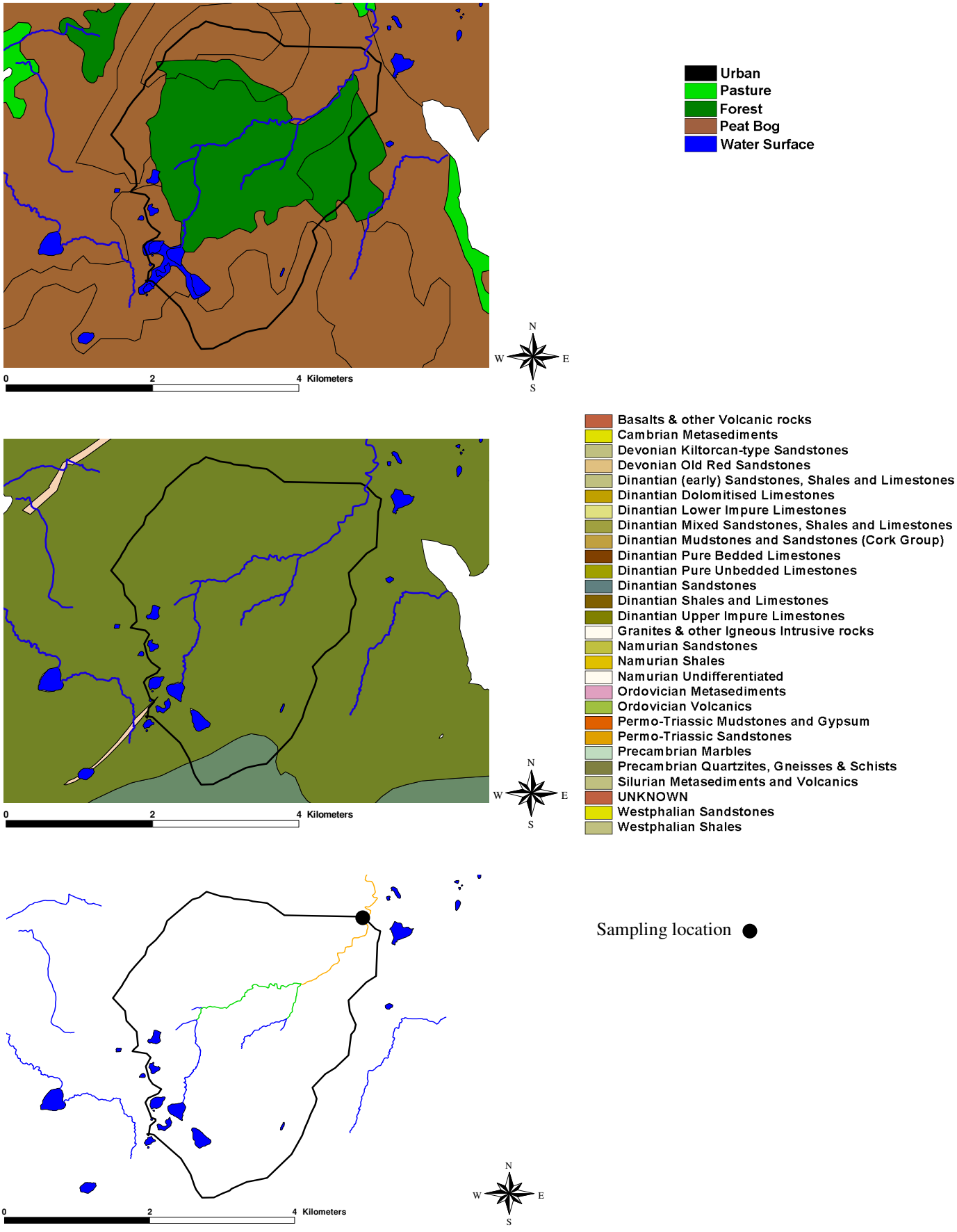


Fig. 1.7 Catchment characteristics for Catchment of the Cartron River Sample Site 33C020100 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.7 Site 7: Lough na Corralea Stream

Lough na Corralea Stream is situated approximately 2km north west of Party in Co. Mayo. The study site (EPA national river code 30L03-0400) was located just downstream of the bridge east at Cloonee. Monthly sampling was carried out in an area measuring approximately 16m x 4m (depending on the water level on the day of sampling). Its co-ordinates are E: 113055; N: 274654, Latitude: 53° 42' 51''N, Longitude: 9° 19' 02'' W. Lough na Corralea Stream is a fourth order river with a catchment area of 12.58 km^2. Pasture is the dominant land use.

The average wet width in the river during the study programme was between 5 and 7m. Lough na Corralea Stream is a fast flowing stony river system with quite a lot of bedrock and large boulders but is largely dominated by cobble and gravel. The main riparian trees are sycamore, willow and ash. The main vegetation growing on the riverbanks were montbretia and mint. The submerged macrophytes consisted of rush, moss and *Callitriche* spp. The emergent plants in this river were wild celery, pondweed and water-plantain (*Alisma* spp.).

The quality of Lough na Corralea Stream has varied from a Q4 in 1989 to a Q4-5 in 1996. In 2000, the river was assessed and assigned a Q3-4 with a distinct absence of species from the Heptageniidae family. (An *Oscillitoria* type of algal mat grows readily on the substrate in this river).

Fig. 1.8 outlines the catchment characteristics of Lough na Corralea stream showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

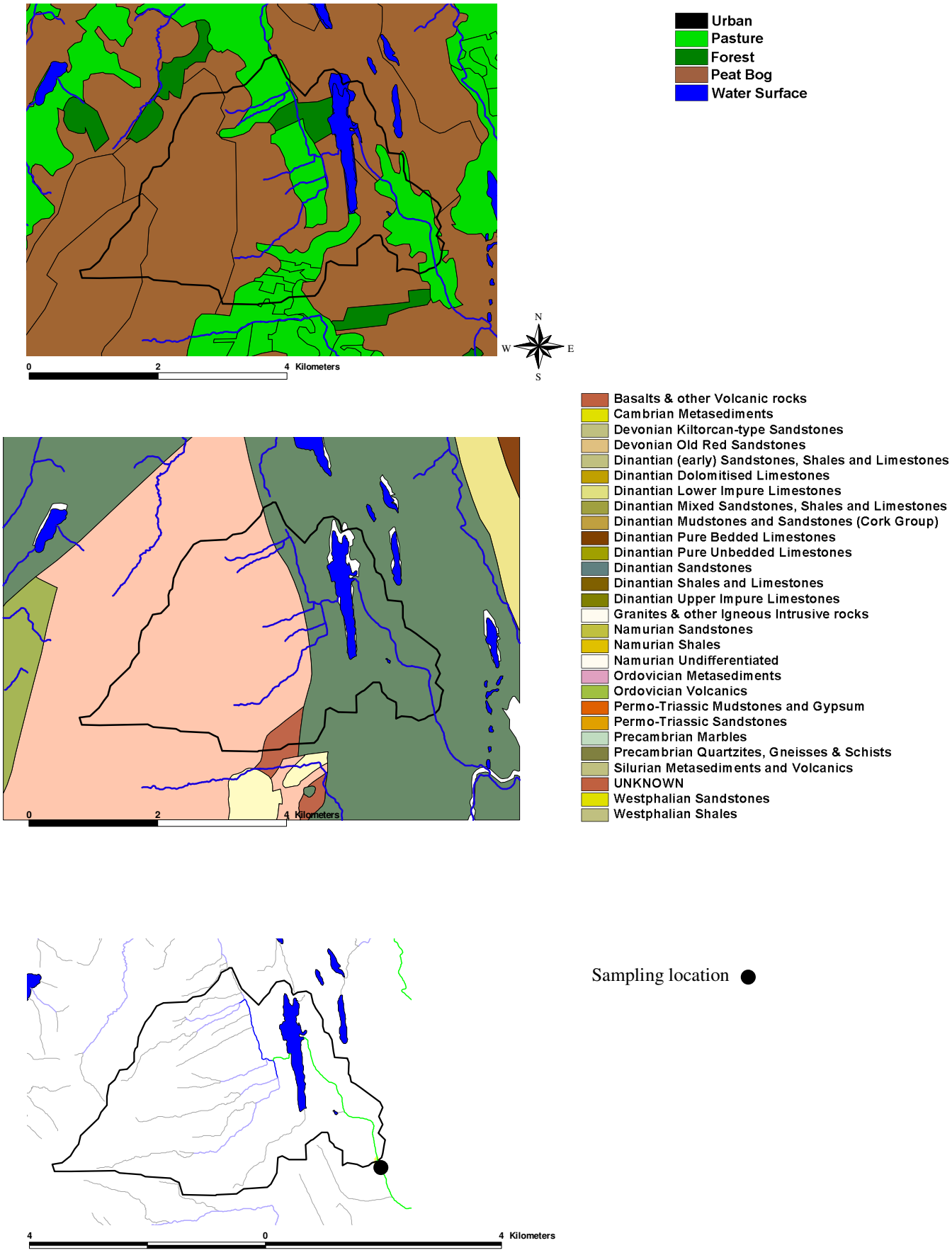


Fig. 1.8 Catchment characteristics for Catchment of Lough na Corralea Stream Sample Site 30L030400 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.8 Site 8: The Robe River

The Robe River flows through the village of Hollymount, which is located approximately 6km north east of the town of Ballinrobe in Co. Mayo. The study site (EPA national river code 30R01-0400) was located approximately 12-15m downstream of the bridge at Hollymount. Its co-ordinates are E: 125839, N: 268640; Latitude: 53° 39' 42''N, Longitude: 9° 07' 19''W. Monthly sampling was carried out in an area measuring approximately 12m x 25m (depending on the water level on the day of sampling). The Robe River is a fourth order river with a catchment area of 193.20 km^2. The predominant land use is pasture where sheep and cattle graze close by.

The average wet width in the river during the study period ranged between 12-15m. Cobble and gravel dominated the substrate with boulders interspersed. There was a high content of silt and gravel in this river, particularly around the marginal areas. The riparian vegetation consisted of gorse (*Ulex europaeus*), alder and hawthorn. The submerged macrophytes present were forget-me-not and water-milfoil. The main emergent plants were watercress, bur-reed and *Scirpus*.

In 1984, weirs were constructed just downstream of the bridge where the study site is located which appear to have changed the energy of the river and in spite of the presence of good riffled areas, the water quality of this river has deteriorated over the last few decades from a Q4 to a Q3 with a substantial loss of sensitive indicator species. This river is prone to eutrophication quite regularly.

Fig. 1.9 outlines the catchment characteristics of the Robe River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

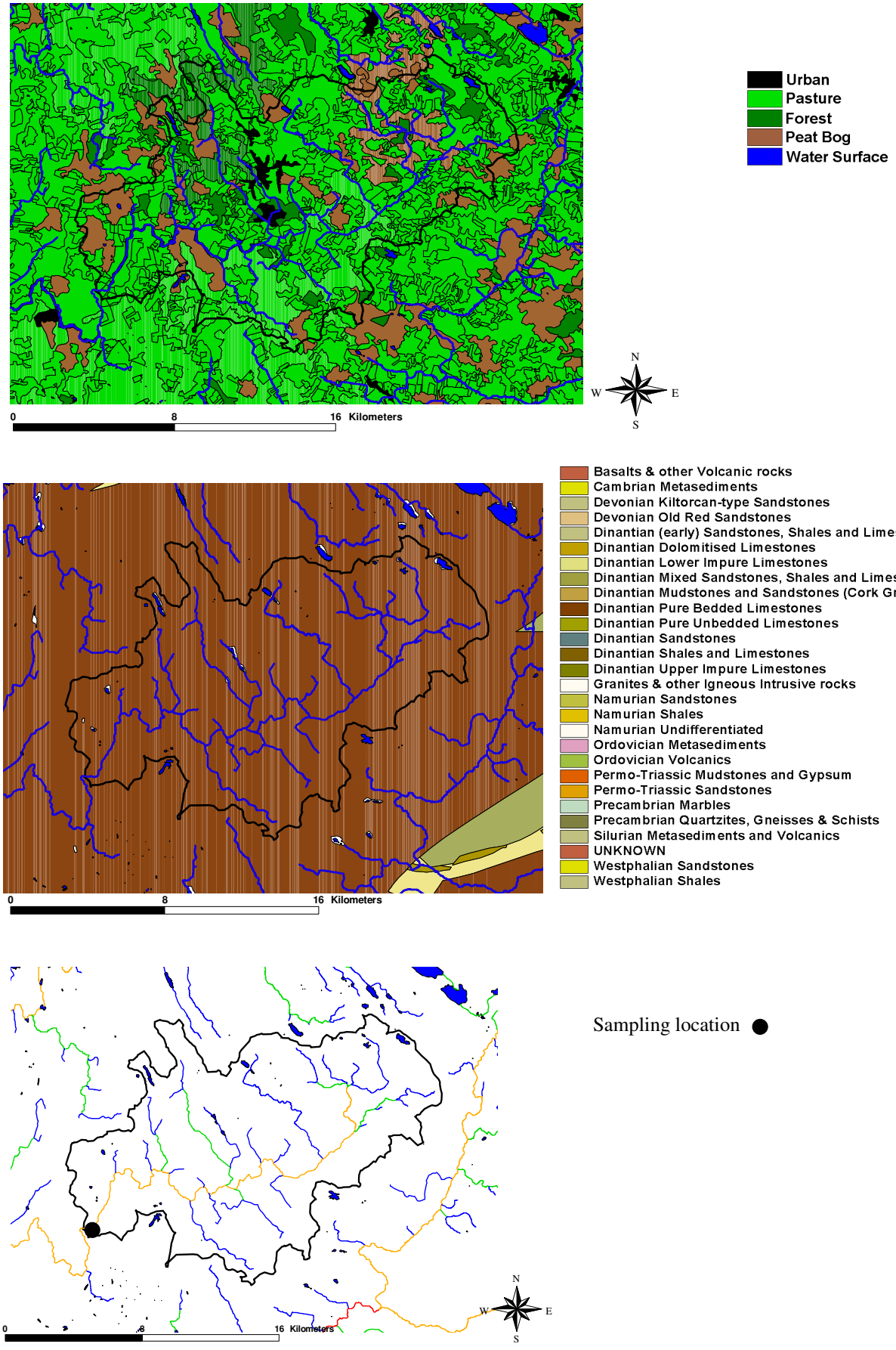


Fig. 1.9 Catchment characteristics for Catchment of the Robe River Sample Site 30R010400 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.9 Site 9: The Mullaghanoe River

The Mullaghanoe River is situated 0.5km west north west of Bellahy village in Co. Mayo. The study site (EPA national river code 34M03-0100) was located approximately 2m downstream of the bridge. Monthly sampling was carried out in a designated area measuring approximately 10m x 4m (depending on the water level on the day of sampling). Its co-ordinates are E: 147505, N: 302542, Latitude: 53° 58' 04''N, Longitude: 8° 48' 01'' W. The Mullaghanoe River is a fourth order river with a catchment area of 25.19 km^2. Pasture and peat bogs are the main land use within the catchment.

The average wet width during the sampling programme was 4-8m. Large and small cobble and gravel dominated the substratum type. The main tree species were ash, willow, sycamore and hawthorn. The bankside vegetation consisted of *Glyceria*, willow herb (*Epilobium*), meadowsweet (*Airgead luachra*) and purple loosestrife (*Lythrum salicaria*). The emergent macrophytes were *Lemna*, wild celery and watercress. The submerged macrophytes consisted of pondweed, moss and *Callitriche*.

The quality of the Mullaghanoe River has varied considerably over the last two decades. It fell from a Q4 in 1983 to a Q3 in 1989 and rose again to a Q4 in 1993. By 1995 the quality had declined again and it was assigned a Q2-3 which it maintained until it rose again to a Q3 in 2000. This river becomes very enriched and contained sewage fungus and solids quite a few times during the study programme.

Fig. 1.10 outlines the catchment characteristics of the Mullaghanoe River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

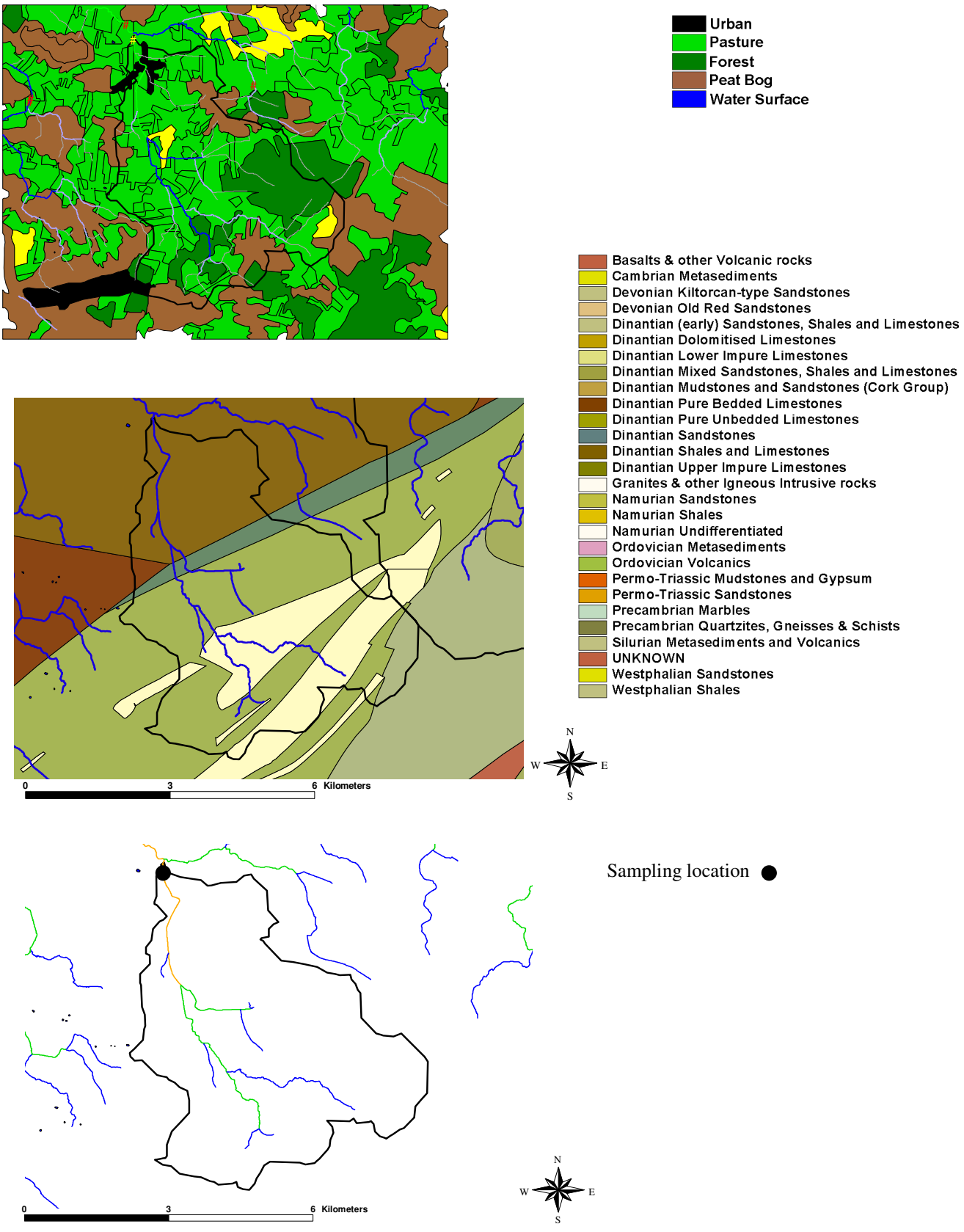


Fig. 1.10 Catchment characteristics for Catchment of the Mullaghanoe River Sample Site 34M030100 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

1.7.10 Site 10: The Mad River

The Mad River flows through the village of Cloonacool, which is approximately 6km west of Tobercurry in Sligo. The study site (EPA national river code 34M04-0100), was located approximately 300m upstream of the bridge in Cloonacool. Monthly sampling was carried out in an area measuring 4m x 15m. Its co-ordinates are E: 149230, N: 317304; Latitude: 54° 06' 07'' N, Longitude: 8° 46' 23'' W. The Mad River is a third order river with a catchment area of 7.77 km^2. Pasture is the predominant land use and there is a large forestry plantation located upstream of the study site.

The average wet width in the study area was 4-6m during the sampling period. Boulders and cobble dominated the substratum type with a moderate degree of siltation. The dominant tree species present were hawthorn, willow, ash, sycamore, alder and Gorse. During the course of this study there were very few macrophytes found in the river. Moss and liverworths were the most common submerged plants. The bankside vegetation consisted of rush and *Petasites* spp.

Owing to the steep gradient of this mountain river it is prone to flash floods. Sheep graze in upstream pasture where overgrazing is evident and cattle have access to the river near the bridge for drinking purposes. The macroinvertebrate diversity has diminished in recent years with a notable absence of *Ecdyonurus* spp., *Perla* spp. and *Gammarus* spp. In 1989 it was assigned a Q5 and from 1993 to the present study, the river has fluctuated between a Q3 and a Q3-4.

Fig. 1.11 outlines the catchment characteristics of the Mad River showing the CORINE 2000 landuse, GSI rock units (bedrock geology) and the river network indicating Strahler stream order

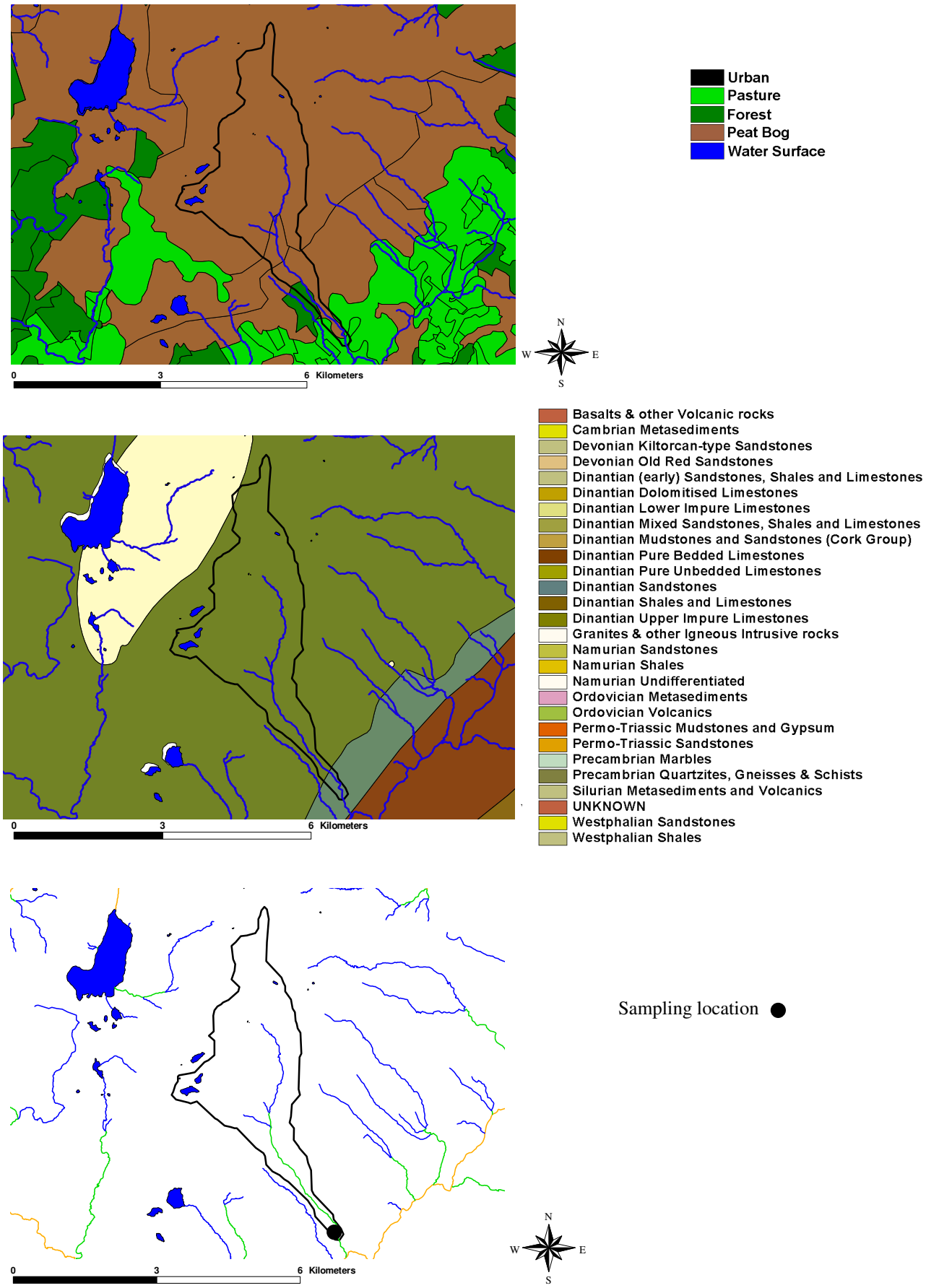


Fig. 1.11 Catchment characteristics for Catchment of the Mad River Sample Site 34M040100 showing CORINE 2000 landuse GSI rock units (bedrock geology) and river network indicating Strahler stream order.

Chapter 2

Experimental enrichment of a high status river in the West of Ireland: Effects of nutrient manipulation on the genus *Ecdyonurus* and benthic chlorophyll levels.

2.1 Introduction

This study was undertaken in response to a perceived eutrophication problem in Irish rivers and to investigate the effect of artificial eutrophication on an oligotrophic system. Taxa such as the key indicator genus *Ecdyonurus* are important in the Irish Water Quality Rating system and this study investigates its sensitivity to eutrophication. The approach adopted was to artificially increase the concentration of nutrients in a river and observe the response of *Ecdyonurus* using a novel split-stream system devised specially for this project. The experiment was predicated on the basis of a phosphorus limited river system.

The most significant threat to the quality of freshwater in Ireland is eutrophication. This is defined as the enrichment of waters by the nutrients phosphorus and nitrogen, beyond natural levels (Bowman and Clabby, 1998). The response of lake phytoplankton and periphyton to nutrient addition during the eutrophication process is well known (Lund, 1969; Kalff, *et al*., 1975; Schindler, 1975, 1985; Stockner and Evans, 1974). However, the changes in algal production and species composition following nutrient addition in streams and rivers are not fully understood or adequately documented (Stockner and Shortreed, 1978). There has been a substantial amount of work published on the limiting role of nutrients in primary production and detrital decomposition of lake and marine systems. However, efforts to understand their role in running waters has been a much slower process (Elwood *et al*., 1981). Despite the fact that detrital decomposition and algal production are known to be influenced by both nitrogen and phosphorus (e.g., Hynes and Kaushik, 1969; Rhee, 1978; Smith, 1979), there is little known about the mechanisms which drive nutrient limitation in natural streams.

Wetzel (1983) made comparisons between the relative amounts of different elements required for algal growth in freshwaters, emphasising the importance of phosphorus and nitrogen. The ratio derived for phosphorus was 80,000 while that of nitrogen was 30,000 (oxygen and hydrogen both have a ratio of 1). This compares with, for example, a ratio for carbon and silicon of 2,000 and 3,000, respectively. He pointed out that eventhough variations in conditions of solubility or availability may at times make abundant elements (e.g., silicon, iron and certain micronutrients), almost unattainable, phosphorus and secondarily nitrogen, are generally the first to impose limitation on the system.

Experiments on whole ecosystem enrichment have been well documented and have been used to improve our knowledge and understanding of the response of an ecosystem to man-made perturbation (Lock *et al*., 1990). In lake systems, this approach was pioneered by Schindler and his fellow workers (Schindler, 1985) at the experimental lakes in Canada. The levels of phosphorus, nitrogen, sulphate, organic carbon and pH were manipulated and findings showed that alterations of any one of these parameters had detectable consequences on the other. The most notable change occurred with the introduction of phosphorus. Primary production of phytoplankton standing crop was stimulated in all cases, indicating that phosphorus was a limiting nutrient in these oligotrophic lakes (Lock *et al*., 1990). Predicting quantitative algal responses to increased loadings of nutrients in river and stream ecosystems however is more difficult as they are in a constant state of flux, both spatially and temporally (Bothwell, 1985). Extensive laboratory research on P-limited growth kinetics of unicellular planktonic algae has clearly shown that steady state growth rates saturate at extremely low levels of ambient dissolved phosphorus (<10µgP/l) (Fuhs, 1969; Rhee, 1973; Tilman and Kilham, 1976; Brown and Button, 1979).

Natural phytoplankton populations contain carbon, nitrogen and phosphorus in an C:N:P molar ratio of 105:15:1, and on average 1µg phosphorus supports 1g chlorophyll production (Redfield, 1958; Ryther and Dunstan, 1971). Other studies on enrichment or eutrophication of running waters

have shown increases in periphytic biomass under certain conditions (e.g. Elwood *et al*., 1981; Stockner and Shortreed, 1976; Horner and Welch, 1981; Horner *et al*., 1983; Perrin *et al*., 1987). Peterson *et al*. (1985) reported the findings of a phosphorus enrichment experiment in a Tundra river. He showed that an increase of only 10µg/l PO_4-P above the background (1-4µg/l), either alone or in combination with NO_3-N, resulted in substantial increases in epilithic chlorophyll *a*, metabolic activity (heat output), bacterial activity and growth rates of two dominant riffle insects. This study also revealed a decrease in species richness of diatom population in response to enrichment.

Work carried out by Wuhrmann and Eichenberger (1975) seems to support this conclusion. However, many workers have found that much higher levels of phosphorus (>20µg/l PO_4-P) are required to produce algal bloom problems in streams and rivers (MacKenthun, 1968; Wong and Clark, 1976; Horner *et al*., 1983). Elwood *et al*. (1981) found that although biofilm biomass (grown on glass slides) in the phosphorus enriched section of a second-order woodland stream was consistently higher than in the unenriched section, only a small initial increase occurred in biofilm chlorophyll *a* in situ at the enriched site. Observations showed that the nitrogen-fixing cyanobacterium *Nostoc* sp. was significantly higher in the enriched sections than the control section of the stream. This suggests the operation of a dual N and P limitation effect in this stream (Lock *et al*., 1990). A seasonal pattern in benthic algal biomass development is often restricted to lowland streams with longer periods of stable discharge and low-to moderate water velocity (Kjeldsen, 1996). In high gradient rivers and streams with frequent changes in discharge, biomass development is more dependent on the interval since the last flood event than on the season (Tett *et al*., 1978; Fisher *et al*., 1982; Biggs and Close, 1989; Scarsbrook and Townsend, 1993).

The impact of eutrophication on sensitive indicator taxa like *Ecdyonurus* has not been investigated in detail to date in Ireland. This study examines the effects of nutrient manipulation on *Ecdyonurus venosus* in two high status rivers in the West of Ireland.

2.2 Study outline

Eutrophication is the main cause for the decline in water quality in Irish rivers. There is a considerable lack of understanding in what controls the disappearance of this sensitive indicator species as eutrophication impacts on a river system. This investigation attempts to refine our knowledge of the detailed mechanisms a river experiences as it departs from its pristine state. It was hypothesised that the addition of orthophosphate to one section of an oligotrophic river would cause an increase in the algal biomass thereby affecting the distribution of the genus *Ecdyonurus* within the experimental area.

2.3 Material and Methods

The first study was carried out in the Clydagh River during the summer of 2002 and repeated again during the summer of 2003. On completion of the experiment in the Clydagh River at the beginning of July 2003, the split-stream structure was moved to the Castlebar River. In total, the split-stream experiment was carried out on three separate occasions.

2.3.1 Site 1: The Clydagh River

The Clydagh is a small river located 10km north of Castlebar town, Co.Mayo. The study site (EPA national river code 34C05-0030) was located at Latitude 53° 53' 20" N: Longitude 9° 15' 40" W. Discharge was variable in this river: Flow patterns were "flashy" and corresponded rapidly to precipitation. The mean flows in the river during the summer of 2002 and 2003 were 0.05 m^3/s and 0.03 m^3/s, respectively. The site had an average wet width of 7m during both sampling periods. The experimental section was uniform with respect to gradient, substrate and canopy cover. The bottom substrate consisted of a mixture of pebbles, cobble and large boulders. The principal land use was predominately pasture with forestry located upstream. The dominant riparian tree species were willow (*Salix* spp.), hawthorn (*Craetagus sp*.) and birch (*Betula pendula)* while *Fontinalis* spp., and other mosses were the submergent macrophytes. *Sparganium erectum* was the predominant emergent plant.

2.3.2 Site 2: The Castlebar River

Details of the Castlebar River are outlined in Chapter 1, section 1.7.2. Discharge in the river was regular and the mean flow during the study period was 0.02 m^3/s.

2.3.3 Preliminary investigations

Prior to setting up the split-stream experiment, an initial investigation into the N:P ratio of eight potential river sites was undertaken. The Clydagh river was sampled on 10/07/02 revealing a molar N:P ratio of 19; (N:P>15, P is assumed to be limiting; N:P<15, N is assumed to be limiting). The background orthophosphate concentrations were analysed in the form of unfiltered Molybdate Reactive Phosphate (MRP µ/L P) and concentrations were low (0.012-0.05 µ/l P). Other water quality parameters were also examined throughout the study (see Table 2.1).

A three-minute kick sample for macroinvertebrates was taken on the same day to establish the biological quality of the river. The Q-value of the river was 4-5 with abundant numbers of the genus *Ecdyonurus* present. The Clydagh River was chosen as the site for the split-stream experiment due to suitable background water chemistry (P limited) and the presence of the genus *Ecdyonurus*.

The experiment was replicated in the Castlebar River during the summer of 2003. The river was biologically and chemically assessed displaying a high Q-value of 4-5, with abundant numbers of the genus *Ecdyonurus* present, meeting the requirements for good water quality (low MRP concentration). Both rivers were chosen as appropriate reference condition sites for the nutrient manipulation experiments.

2.3.4 Experimental design and nutrient addition

2.3.4.1 Split-stream construction

The objective of the design was to split the river longitudinally into two equal sections using a clear perspex barrier. This would prevent the river water mixing on each side of the barrier. One side of the stream was to receive an input of a known concentration of nutrient to continuously enrich this section (treated section) while the other side was the untreated control section. Installation of the split-stream barrier commenced on the 16th July 2002 in the Clydagh River.

The study site was selected in a straight stretch of the river just downstream of the bridge where a 10m long clear perspex barrier was constructed down the centre of the river. Clear perspex sheets (2m x 0.5m x 5.5mm) were chosen to avoid creating shadows on the riverbed or water surface thereby introducing potential shading factors that could effect periphyton growth. Angle irons (0.5m) were driven into the substratum until vertically stable at 1m intervals. It was essential that the perspex was flush with the riverbed or, if possible, driven 4-5cm into the substratum. This was achieved firstly by creating a longitudinal channel between the angle irons and then pushing the perspex down into the river substrate.

Five perspex sheets were fixed in series to the iron bars using stainless steel bolts. Once in place the 10m structure was supported vertically allowing the natural free movement of water on either side of the barrier. The barrier was fitted to the riverbed and any gaps were sealed with sand and small pebbles to prevent mixing of water between the left and right hand sides of the river. The river was left to settle for 3 weeks prior to the commencement of the sampling programme. This enabled the river to readjust to the disturbances it experienced during the instillation of the barrier. To test the barrier for any leaks and to verify rapid homogenous mixing fluorescent dyes (Rhodamine-B and Fluorescein disodium salt) were then added to the experimental section. Fig. 2.1 and Fig. 2.2 show the detailed layout of the split-stream sites.

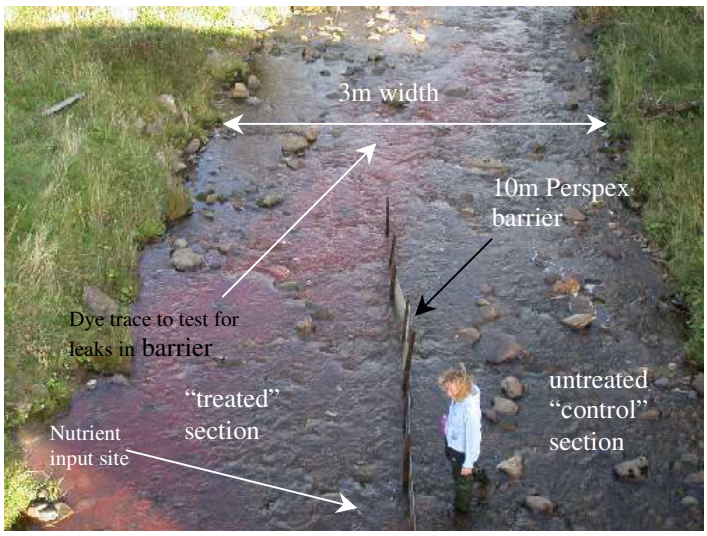


Fig 2.1 Detailed view of the split-stream structure in the Clydagh River during 2002 and 2003.

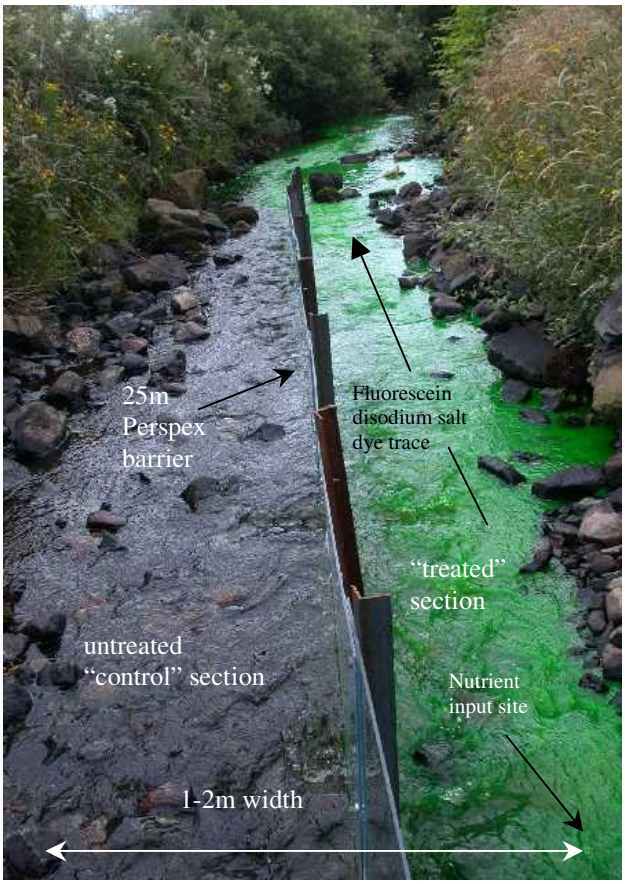


Fig 2.2 Detailed view of the split-stream structure in the Castlebar River during 2003.

2.3.4.2 Nutrient addition

Based on previous studies carried out by Peterson and his co-workers in 1985 who studied the effects of nutrient enrichment on a Tundra River in northern Alaska over a 6 week period, it was proposed to increase the background orthophosphate concentration of the river water by 10µg/l in an attempt to artificially enrich one side of the split-stream. Such a low level also ensured that the river was not 'polluted' or over enriched by the experiment. On each site visit the flow in the manipulated section was measured in m^3/s. The nutrient solution was made up to a concentration of 20g P/l using potassium dihydrogen orthophosphate salt (KH_2PO_4) which was stored in a 20litre rectangular polycarbonate carboy. This was placed in a steel box at an elevated position on the bank of the river. The water chemistry of the river in its natural state was analysed 1-2 days before each visit thereby supplying information on the background concentration of orthophosphate in the river. Depending on the flow in the river at each site visit, the nutrient stock concentration was adjusted accordingly, thereby delivering a continuous supply of P, in order to raise the river water concentration by 10µg/l. The target concentration was achieved using the following mass balance equation:

Concentration downstream = $(f \times c + F \times C)/(f + F)$

f = flow in peristaltic pump (l/s)
c = concentration of the nutrient stock solution in mg/l
F = Flow in the experimental channel being dosed (l/s)
C = assumed upstream concentration of P in mg/l

The solution was added continuously to one side of the river using a gravity fed Mariotte siphon feeding system at a rate of approximately 1ml per minute. The site was visited 2-3 times per week to check flows, carry out routine sampling and change the battery supplying the pump. This close monitoring of the system ensured a continuous supply of nutrient to the manipulated section at the correct concentration. The physico-chemical parameters listed in Table 2.1 were monitored at various intervals within each side of the split-stream on a weekly basis. Water samples were taken at the upstream end of each side of the split-stream (0m at nutrient input and

0m control section). Depending on the experiment, the water was analysed at 10m and 20m distances from the top end in both sides and again upstream and downstream (20m, 30m) of the experimental site.

The Mariotte siphon feeding system was used to deliver nutrient to the Clydagh River in 2002 over a period of 6 weeks. In June 2003, the experiment was repeated by manipulating the other side of the river. The nutrient was released into the opposite side for the duration of 5 weeks using a battery powered peristaltic pump, which provided a more reliable nutrient delivery system. The same system was used again when the experiment was moved to the Castlebar River in July 2003 and nutrient was added over a 9 week period. The split-stream structure was extended in this river by a further 10m to 20m to allow for increased sampling within the study area and an improved opportunity to assess the response over distance from the nutrient delivery point.

2.3.4.3 Chlorophyll a estimations

Algal biomass, was estimated using chlorophyll per unit area measurements obtained from scrapings taken from the stone surfaces in the river. The standard hot methanol chlorophyll *a* extraction method adopted by the EPA was used to estimate photosynthetically active biomass on the surfaces sampled. Within each of the "control" and "treated" sections, riffled areas were selected for sampling. All stones sampled were covered by at least 20 cm of water for 4 weeks before sampling commenced. Using a stiff toothbrush, a $10cm^2$ section of stone surface was scraped from 5 stones in series within the first 2m of each side of the experimental site. These were then pooled into a 500ml container of river water. At 2m intervals along the 10m longitudinal gradient, this process was carried out until a total of 5 pooled samples were gathered from both the treated (experimental) and untreated (control) sides of the split-stream (see Fig. 2.3). Samples were taken back to the laboratory and analysed on the same day. Chlorophyll *a* concentrations were measured once prior to the addition of nutrient and again over a period of weeks (depending on experiment) during the nutrient manipulation experiment.

As previously mentioned, the split-stream structure was extended in the Castlebar River in 2003 from 10m to 20m. During the last three weeks of the programme in this experiment the sampling regime was intensified. Using random numbers in a grid-like representation of the experimental site, 25 stone scrapings were taken separately along each side of the 20m split. The flow of the river water was concentrated along the spilt-stream structure, particularly in low flows so it was decided to take algal scrapings randomly, 15cm and 30cm from the perspex barrier (see Fig. 2.4). The chlorophyll *a* concentrations were measured individually instead of measuring 5 pooled samples, in order to improve the power of the statistical analyses. In the Clydagh River during the summer of 2002, algal biomass estimations were taken on four separate occasions over a 6-week period. In the summer of 2003, algal biomass scrapings were measured on nine visits over a 5-week period. Finally, in the Castlebar River in 2003, ten algal measurements were established over a period of 8-weeks.

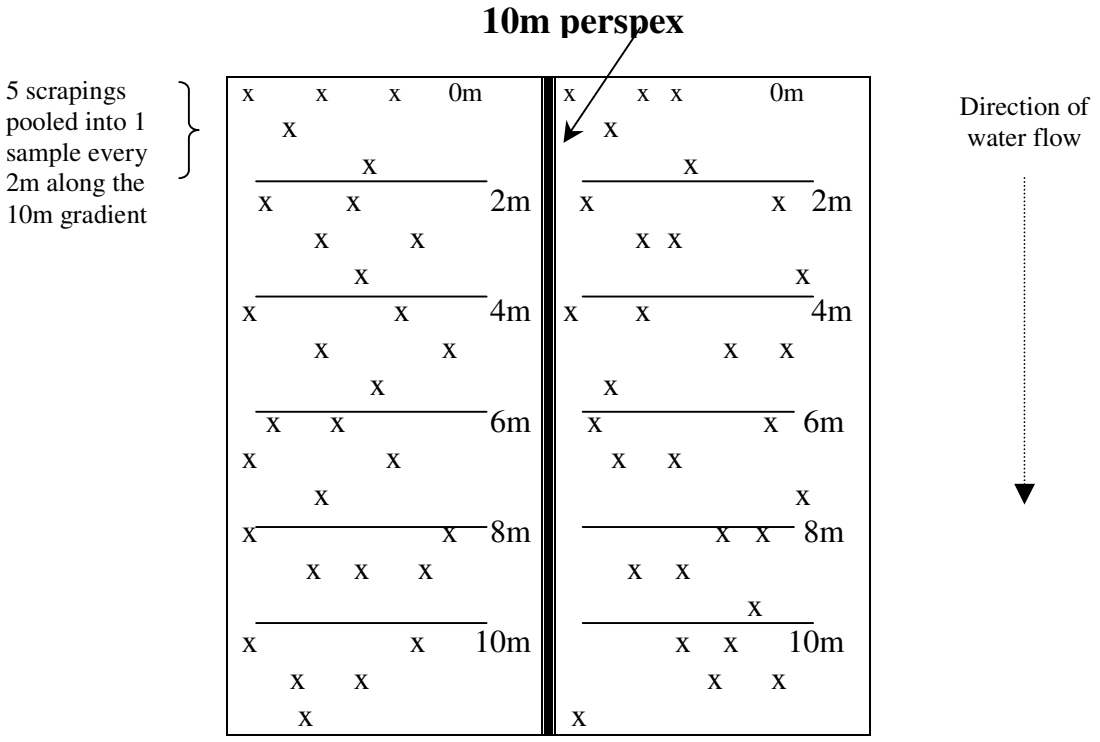


Fig 2.3 Sampling regime in the Clydagh River in 2002 and 2003.

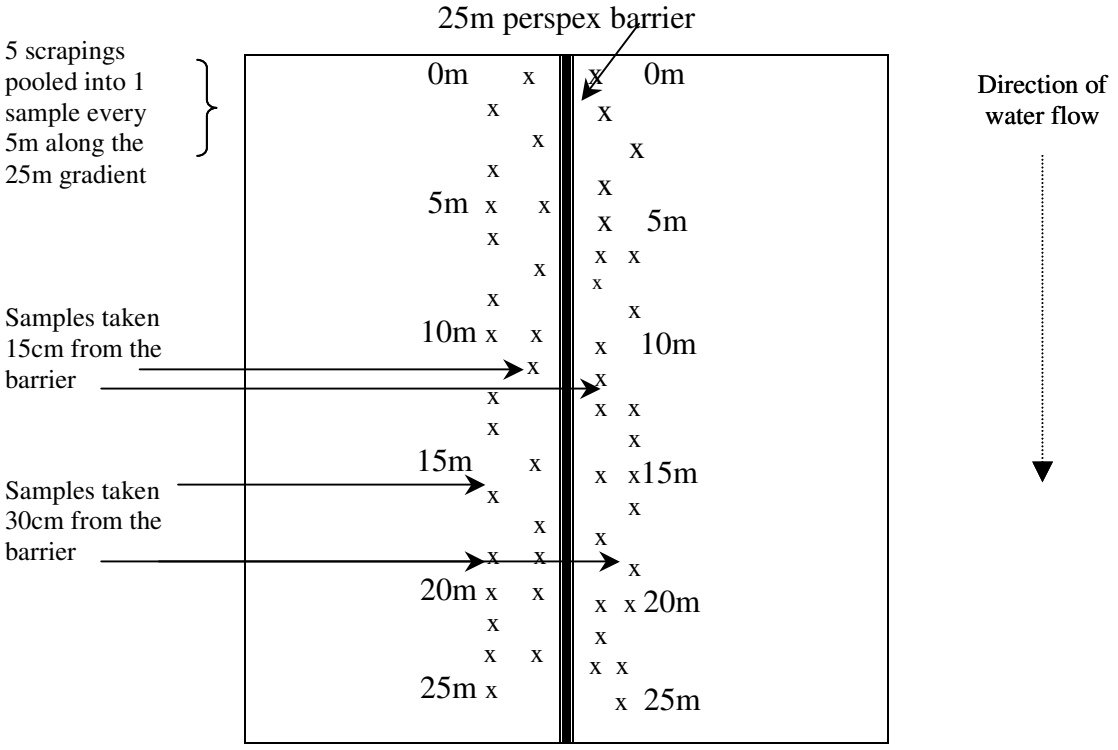


Fig 2.4 Sampling regime in the Castlebar River in 2003.

2.3.4.4 Macroinvertebrate sampling

Quantitative sampling, using a 36cm x 36cm metal-framed Surber sampler, was carried out prior to the addition of the nutrient and on subsequent visits during the nutrient manipulation experiment. In the Clydagh River during the summer of 2002, macroinvertebrates were sampled on four separate occasions over a 6-week period. No macroinvertebrates were sampled in the Clydagh River in 2003 due to low numbers of the *Ecdyonurus* in the river. In the Castlebar River in 2003, macroinvertebrate samples were taken on seven occasions over a period of 9 weeks.

Sampling was initiated within riffled sections of the split-stream sites and 5 Surbers were taken on either side of the split-stream during each sampling trip. The Surber was placed on the riverbed facing upstream and the substrate within the metal frame disturbed. All macroinvertebrates were dislodged into the open net and special care was taken when collecting the *Ecdyonurus* specimens. The samples were preserved in 70% IMS on site for later identification. As with the collection of stone scrapings, Surber sampling was carried over a period of weeks during the nutrient manipulation experiment (time scale depending on particular experiment). The macroinvertebrate Surber samples were taken at 2m and 5m intervals (depending on the experiment) along the longitudinal gradient of the split-stream barrier (Fig 2.3 and Fig 2.4).

Comparisons between periphyton biomass and macroinvertebrates could then be made between the treated and control sections. All macroinvertebrates were counted and identified to family level while the *Ecdyonurus* specimens were identified to species level.

2.4 Results

Results are shown comparing chlorophyll levels in the treated and control sides of the split-stream. Macroinvertebrates are also compared on either side. The minimum, mean, median, maximum and standard deviation values for the background physico-chemical parameters measured during the three experiments are outlined in Table 2.1. As indicated above, phosphate was added in order to raise the ambient concentration in the treated side of the stream by 10µgP/l when mass balanced. Water samples also collected for physico-chemical analysis immediately downstream of the split-stream during the enrichment process and at 20m, 30m, 50m, 100m downstream showed no detectable downstream gradients in the MRP concentrations.

The effects of nutrient addition on chlorophyll *a* ($\mu g/cm^2$) concentrations and macroinvertebrates between the treated and control sections during the three experiments were examined using a repeated measures analysis of variance (ANOVA). Statistical analysis was performed using SPSS version 11.0 software. This analysis examines the temporal variation of the chlorophyll *a* and macroinvertebrate Surber samples along the gradient of the split-stream barrier (positional information) during the course of the experiment.

The general linear model was used to analyse the data. In SPSS, the Within-Subjects Variables correspond to the number of weeks the experiment was carried out for. The Between-Subjects Factors relates to the "treated section" (manipulated) and the "control section" (non-manipulated) of the experimental divide. The positional information (distance from the top to the bottom of the perspex barrier) representing the spatial variation along the gradient during the course of the experiment are specified using the covariance structure for the residuals.

Results are outlined in Table 2.2. Chlorophyll *a* ($\mu g/cm^2$) and macroinvertebrate data were transformed as needed to stabilise variance and to improve normality.

Table 2.1 Maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters examined in the Clydagh River in 2002, 2003 and in the Castlebar River in 2003 during the split-stream experiments.

	Temperature °C	DO % Saturation	pH pH units	Conductivity µS/cm	Orthophosphate mg/l P	TON mg/l N	Ammonia mg/l N	Chloride mg/l Cl	Alkalinity mg/l $CaCO_3$	BOD_5 mg/l O_2	Colour Hazen units
Clydagh River 2002											
n	*6*	*6*	*12*	*12*	*12*	*12*	*12*	*11*	*12*	*10*	*11*
Minimum	10.3	97.0	7.4	68.0	0.012	0.05	0.005	11	14.0	0.40	33.0
Mean	13.3	102.0	7.9	172.0	0.027	0.05	0.007	13.7	60.5	0.79	141.8
Median	13.9	100.5	8.1	164.0	0.025	0.05	0.005	14	54.0	0.80	110.0
Maximum	15.7	109.0	8.2	276.0	0.050	0.05	0.02	16	116.0	1.00	309.0
Standard deviation	1.9	4.9	0.2	68.9	0.012	1.12E-09	0.005	1.7	34.9	0.22	81.0
Clydagh River 2003											
n	*14*	*14*	*14*	*14*	*15*	*15*	*15*	*15*	*11*	*7*	*13*
Minimum	5.8	94.0	7.1	87.0	0.012	0.05	0.005	14.0	10.0	1.00	26.0
Mean	12.9	102.7	8.1	228.8	0.021	0.05	0.005	17.7	87.1	1.01	71.6
Median	13.4	102.0	8.2	255.0	0.019	0.05	0.005	17.0	92.0	1.00	54.0
Maximum	18.4	111.0	8.3	287.0	0.030	0.05	0.005	21.0	120.0	1.10	153.0
Standard deviation	3.7	4.7	0.3	59.7	0.006	7.04E-10	8.8E-11	2.21	32.9	0.04	40.9
Castlebar River 2003											
n	*15*	*15*	*16*	*18*	*18*	*18*	*18*	*18*	*9*	*5*	*10*
Minimum	9.6	96.0	7.1	83.0	0.028	0.05	0.005	13.0	14.0	1	40.0
Mean	13.9	102.9	7.9	197.6	0.036	0.06	0.005	20.6	52.0	1	110.2
Median	14.0	103.0	7.9	220.5	0.037	0.05	0.005	21.0	52.0	1	94.5
Maximum	18.9	112.0	8.3	252.0	0.044	0.155	0.005	25.0	82.0	1	220.0
Standard deviation	2.9	4.17	0.3	49.9	0.004	0.02	5.64E-11	2.5	26.1	0	59.5

Table 2.2 Repeated measures analysis of variance of chlorophyll *a*, *Ecdyonurus* genus numbers and total macroinvertebrate numbers.

River/Parameter	Source of variation (between groups)		Source of variation (within-subjects effects)		
	Treated vs control section	Gradient			
Clydagh River 2002			**Week**	**Week * Gradient**	**Week * treated vs control section**
Chlorophyll *a*	$F_{1,7} = 0.004$ $P = 0.950$	$F_{1,7} = 2.48$ $P = 0.159$	$F_{3,21} = 2.153$ $P = 0.124$	$F_{3,21} = 0.901$ $P = 0.457$	$F_{3,21} = 0.345$ $P = 0.793$
Ecdyonurus genus numbers/m^2	$F_{1,7} = 0.029$ $P = 0.869$	$F_{1,7} = 1.507$ $P = 0.259$	$F_{3,21} = 2.190$ $P = 0.119$	$F_{3,21} = 1.370$ $P = 0.279$	$F_{3,21} = 0.654$ $P = 0.589$
Total number macroinvertebrates/m^2	$F_{1,7} = 2.113$ $P = 0.189$	$F_{1,7} = 2.877$ $P = 0.134$	$F_{3,21} = 3.569$ $P = 0.031$*	$F_{3,21} = 1.316$ $P = 0.295$	$F_{3,21} = 0.158$ $P = 0.923$
Clydagh River 2003					
Chlorophyll *a*	$F_{1,7} = 26.867$ $P = 0.001$***	$F_{1,7} = 6.725$ $P = 0.036$*	$F_{8,56} = 1.837$ $P = 0.089$	$F_{8,56} = 2.595$ $P = 0.017$*	$F_{8,56} = 4.290$ $P = 0.001$***
Castlebar River 2003					
Chlorophyll *a*	$F_{1,7} = 18.630$ $P = 0.003$**	$F_{1,7} = 0.170$ $P = 0.692$	$F_{9,63} = 6.068$ $P = 0.001$***	$F_{9,63} = 0.538$ $P = 0.841$	$F_{9,63} = 2.674$ $P = 0.011$*
Ecdyonurus genus numbers/m^2	$F_{1,7} = 5.414$ $P = 0.053$	$F_{1,7} = 0.384$ $P = 0.555$	$F_{6,42} = 0.499$ $P = 0.806$	$F_{6,42} = 1.042$ $P = 0.412$	$F_{6,42} = 2.005$ $P = 0.086$
Total number macroinvertebrates/m^2	$F_{1,7} = 0.054$ $P = 0.823$	$F_{1,7} = 17.339$ $P = 0.004$**	$F_{6,42} = 4.092$ $P = 0.003$**	$F_{6,42} = 3.923$ $P = 0.003$**	$F_{6,42} = 2.990$ $P = 0.016$*

p values:
* $p<0.05$; ** $p<0.01$; *** $p<0.001$; ns – not significant

The physico-chemical measurements outlined in Table 2.1 are average values of the "natural" background concentrations of the parameters in the river (upstream of split-stream site) taken over the experimental periods. The N:P ratios of the rivers in their "natural" state are outlined in Table 2.3. N:P ratios were determined as TON (Total Oxidised Nitrogen) + ammonia divided by the orthophosphate concentration in molar quantities.

Table 2.3 Variation in the background N:P molar ratios of the Clydagh River and the Castlebar River during the three experimental sampling periods.

Date	Clydagh River 2002	Date	Clydagh River 2003	Date	Castlebar River 2003
10/07/02	19	13/03/03	9	08/04/03	4
24/07/02	6	20/03/03	8	23/04/03	4
20/08/02	3	26/03/03	6	08/05/03	4
22/08/02	3	03/04/03	9	19/05/03	4
29/08/02	5	08/04/03	8	10/06/03	3
04/09/02	6	23/04/03	9	30/06/03	3
12/09/02	8	19/05/03	10	07/07/03	3
19/09/02	5	05/06/03	6	14/07/03	3
02/10/02	5	16/06/03	4	29/07/03	4
03/10/02	6	19/06/03	4	24/07/03	4
09/10/02	6	23/06/03	4	28/07/03	4
15/10/02	5	26/06/03	5	31/07/03	4
		30/06/03	5	05/08/03	3
		03/07/03	5	07/08/03	3
		07/07/03	6	11/08/03	3
				14/08/03	3
				20/08/03	3
				09/09/03	8
				17/09/03	9

2.4.1 Effects of nutrient enrichment on periphyton growth

2.4.1.1 Experiment 1 - Clydagh River: 26th July - 15th October 2002

On initial examination of the river in July 2002, an N:P ratio of 19 indicated that the river was P-limited (Table 2.3). The N:P ratio however dropped substantially over the remainder of the experiment to low levels that suggested N limitation rather P limitation. There was no significant difference in the chlorophyll *a* biomass between the "treated" section and the "control" section of the spilt-stream experiment throughout the experiment (Table 2.2). Algal standing crop did not respond to phosphorus enrichment and biomass levels remained low during the entire experiment (Fig. 2.5).

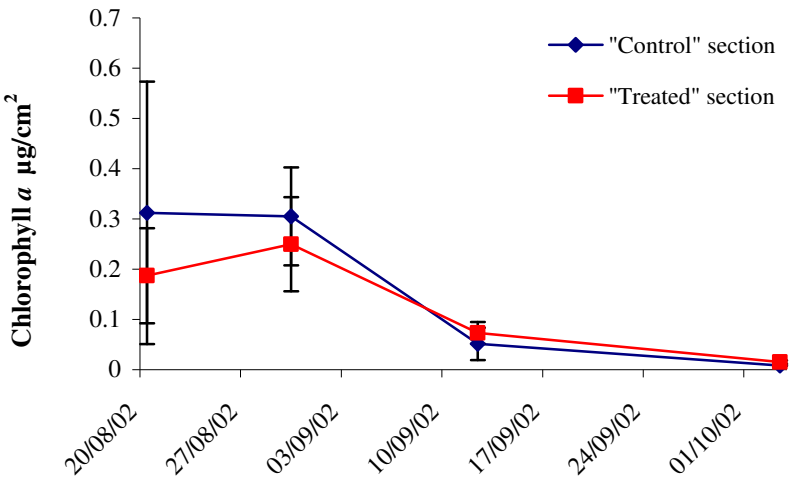


Fig. 2.5 Mean chlorophyll *a* concentration (µg/cm^2) in the "treated" section (+10µgP/l) vs the "control" section. Bars are means ± standard deviation: *n* = 5.

2.4.1.2 Experiment 2 – Clydagh River: 5th June - 11th July 2003

The N:P ratios from March to July 2003 in the Clydagh River also remained low throughout the entire study (Table 2.3). There was an overall significant difference (Table 2.2) between the "treated" and the "control" sides of the Clydagh River along the temporal gradient during the sampling period. Visible sloughing of the periphyton mats began to occur after day 28. The chlorophyll *a* concentrations began to increase after day 32, particularly in the P-treated section (Fig. 2.6).

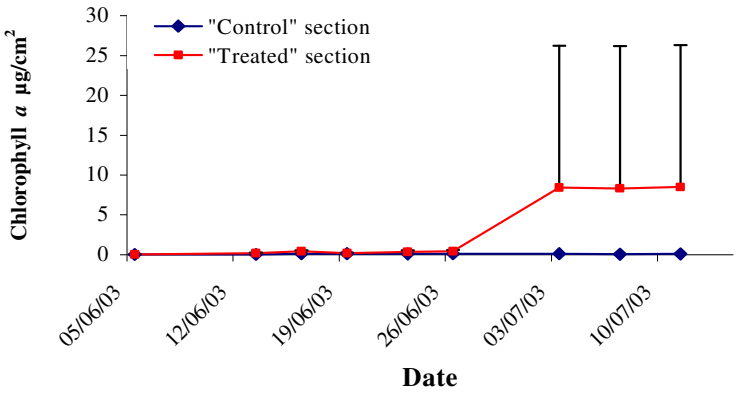


Fig. 2.6 Mean chlorophyll *a* concentration (µg/cm^2) in the "treated" section vs the "control" section. Bars are ± standard deviation: n = 5. Negative values have been omitted for clarity.

2.4.1.3 Experiment 3 – Castlebar River: 18th July – 12th September 2003

The N:P ratios in the Castlebar River stayed low throughout the entire study (Table 2.3). The mean chlorophyll *a* concentrations increased substantially in the last three weeks of the study (Fig. 2.7) with a significant difference in the periphyton biomass (Table 2.2; p=0.003) between the "treated" and the "control" sections during the course of the study. There was also a significant trend through time in the chlorophyll *a* concentration (Table 2.2: p=0.001) with a quadratic fit to the trend line of increasing chlorophyll. There were no significant differences in the chlorophyll *a* concentrations along the gradient however indicating that even though there were significant increases in the concentrations over time, chlorophyll *a* concentrations did not increase along the length of the perspex barrier. In other words, the concentrations were not higher at the bottom of the experimental divide compared to the top i.e. from 0-20m).

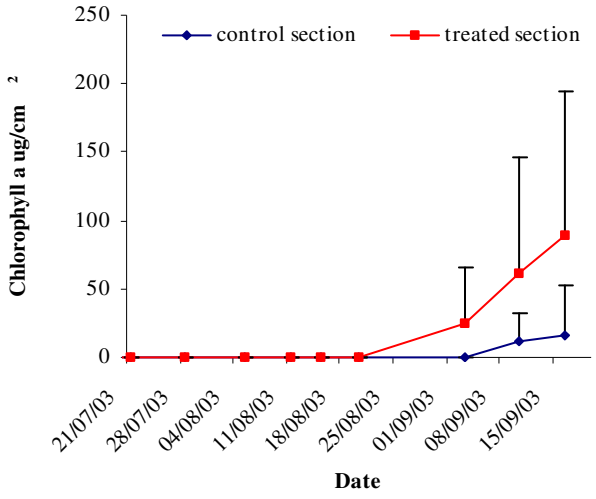


Fig. 2.7 Mean chlorophyll *a* concentration (µg/cm^2) in the "treated" section (manipulated) vs the "control" section. Bars are means ± standard deviation: n = 5. Negative values having been omitted for clarity.

2.4.2 Effects of nutrient enrichment on macroinvertebrates

2.4.2.1 Experiment 1 - Clydagh River: 26th July - 15th October 2002

The response of nymphs of the *Ecdyonurus* genus to nutrient addition showed no significant difference (Table 2.2) between the "treated" and the "control" sections of the Clydagh River during the 6-week sampling period. The graph in Fig. 2.8 shows the average numbers of *Ecdyonurus* genus per m^2 in both sections of the river over the experimental period. There was a significant difference in the total number of macroinvertebrate fauna on the temporal scale only (Table 2.2; p=0.031) and no other significant differences were observed at any level. The total numbers of macroinvertebrates per m^2 are shown in Fig. 2.9.

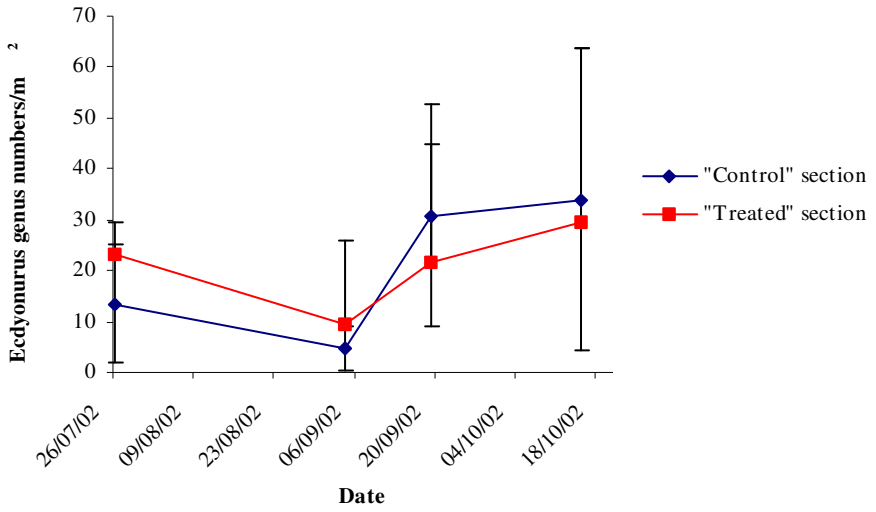


Fig. 2.8 *Ecdyonurus* genus numbers per m^2 in the Clydagh River during the split-stream experiment in 2002. Bars are means ± standard deviation: n = 5.

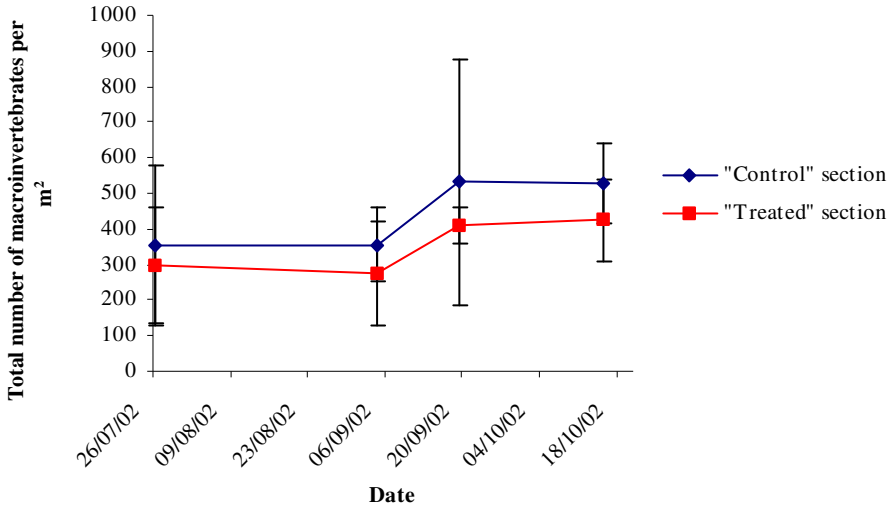


Fig. 2.9 Total macroinvertebrate numbers per m^2 in the Clydagh River during the experiment in 2002. Bars are means ± standard deviation: $n = 5$.

2.4.2.2 Experiment 2 - Clydagh River: 5th June - 11th July 2003

Weekly biological assessments were performed from mid-March 2003 to July 2003 prior to commencement of the experiment. Results revealed a marked absence of the genus *Ecdyonurus* during this time. Immature juveniles of this genus were present in low numbers on occasions with sporadic findings of mature larvae. This was in complete contrast to the previous summer where *Ecdyonurus* genus was abundant. Due to the "flashy" nature of the river, it is thought that eggs or the immature larvae may have been displaced from this site during a flood in early spring/summer (Appendix 5.3). For this reason, the effects of nutrient manipulation on the macroinvertebrate fauna were not examined during this experiment.

2.4.2.3 Experiment 3 – Castlebar River: 18th July – 12th September 2003

Here the macroinvertebrates were collected over a period of 7-weeks during the nutrient addition experiment. The graph in Fig. 2.10 represents the numbers of *Ecdyonurus* genus per m^2 in both sections of the river over the experimental period. The total numbers of macroinvertebrates per m^2 in both sides of the split-stream experiment are shown in Fig. 2.11. There was no significant difference in numbers of *Ecdyonurus* genus (Table 2.2).

A significant difference in the abundance of macroinvertebrate fauna (Table 2.2; p=0.004) was observed along the gradient only and there was no significant differences between the "treated" and the "control" sections per se. Significant differences were found at all levels for the "within-subjects effects" (Table 2.2).

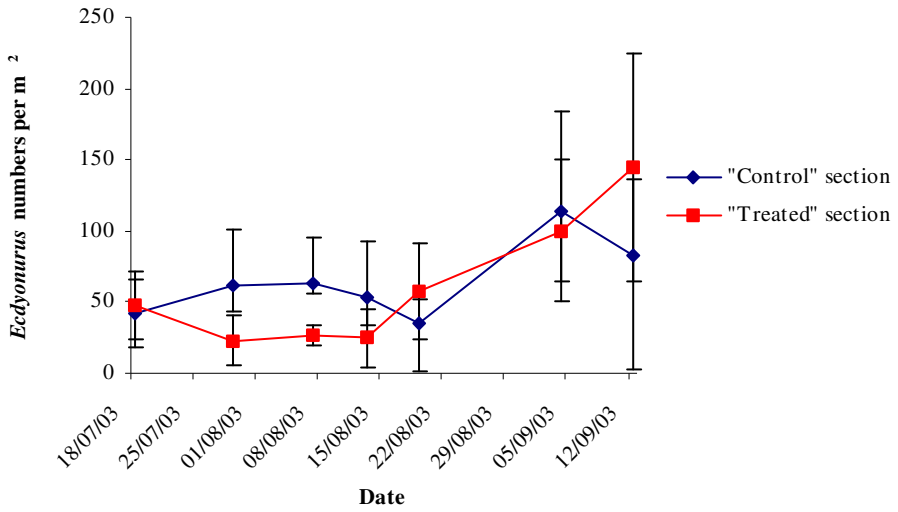


Fig. 2.10 *Ecdyonurus* genus numbers per m^2 in the Castlebar River during the split-stream experiment in 2003. Bars are means ± standard deviation: $n = 5$.

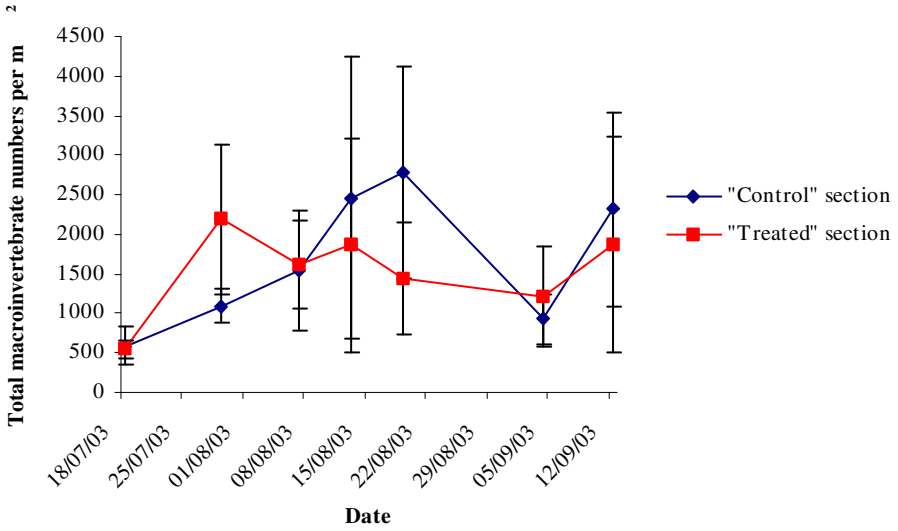


Fig. 2.11 Total numbers of macroinvertebrates per m^2 in the Castlebar River during the split-stream experiment in 2003. Bars are means ± standard deviation: $n = 5$.

2.5 Discussion

2.5.1 Introduction

While some of the nutrient manipulation experiments showed significant differences between the control and treated sections, not all did so. In addition, it is interesting to note that sites appeared to be N-limited but seemed to respond to P, particularly after 6-7 weeks of nutrient enrichment. The phosphorus enrichment experiments carried out in the Clydagh River in 2002 and 2003 and the Castlebar River in 2003 produced some interesting and valuable insights into the ecology of reference condition river stretches. Each of the three enrichment experiments are discussed individually and then comparisons are made later with other studies from the literature.

2.5.2 General findings in the Clydagh River 2002

There was no significant difference in the growth of periphyton biomass in the enriched section, compared with the untreated section of the Clydagh River in 2002. In addition, there were no significant differences in the numbers of *Ecdyonurus* genus between both sections during the study period. Significant differences in total macroinvertebrate numbers were observed over time only. Larval numbers increased in both sections of the experimental divide from mid-September on. This period (September to October) signals the hatching of eggs from the benthos into immature larvae which is quite typical in the life cycle of many macroinvertebrates. No significant differences were observed between both sections of the experimental divide and the increase in the abundance of macroinvertebrates more than likely corresponds to the natural changes that occurred in the life cycles of the larvae which are unrelated to the experiment.

The N:P ratio of the Clydagh River prior to setting up the split-stream experiment was 19. This was within the range of reported molar N:P ratios for algae 15:1 (Redfield, 1958) to 30:1 (Rhee, 1978) and would include the range of N:P ratios where dual N and P limitation would be expected. Unfiltered MRP concentrations averaged at 27-µgP/l over the experiment while the mean DIN concentration ($NO_3 + NO_2 + NH_3$-N) was 57µgN/l. At

this average P concentration, small changes in the concentration of either nutrient, particularly P, should have had a significant effect on the N:P ratio. The background N:P ratios dropped to low levels two weeks later and fluctuated between ratios of 3 and 8 as the experiment progressed until the middle of October.

The mean chlorophyll *a* concentrations highlighted the lack of response to phosphate addition. Concentrations remained similarly low on both sides of the split-stream during this period. The algae in the river appear to be under a dual N and P limitation as the N:P ratios fluctuate below or above the growth optimum. Manuel and Minshall (1978) found significantly higher levels of chlorophyll *a* and aufwuchs biomass in experimental streamside channels enriched with N and P, using stream water with an ambient N:P ratio of 12, a value at which N limitation would be expected. However, there were no significant increases in chlorophyll *a* or aufwuchs biomass when the stream itself was enriched in situ. These authors imply that increased grazing effects and sloughing of algal biomass in the natural river may have masked the effects of enrichment on the periphyton growth.

The low N:P ratios coupled with increased grazing by macroinvertebrates may have contributed to the lack of apparent algal response to enrichment in the present study. Unfortunately it was not possible to measure grazing pressures, an area which should be examined in future investigations. It is also possible that algal seed populations did not establish themselves during early spring 2002 or may have been depleted due to floods during this period (Appendix 5.3) caused by the "flashy" nature of this river. There may also be fluxing of nutrients from the forestry plantation, septic tanks and farms upstream of the site causing the fluctuations in the N:P ratio. The N:P ratios were only measured on approximately a weekly basis over a three month period so it would be important to monitor these ratios on a weekly basis over the course of an entire year.

2.5.3 General findings in Clydagh River 2003

The experiment was repeated in the Clydagh River in 2003, but the nutrient was applied to the opposite side of the river for this trial. There was a significant difference in the growth of periphyton biomass (chlorophyll *a* $\mu g/cm^2$) in the enriched section, compared with the untreated section. The average N:P ratio from March to July 2003 was 6, reaching a high of 10 in May 2003, appearing to indicate an N limited system. The mean chlorophyll *a* concentration in both sides of the river showed an increase in the periphyton biomass particularly during the last two weeks of the experiment. It is difficult to say what may be driving the growth of the periphyton during this experiment, when there was no significant difference in biomass levels during the experiment in the same river in 2002 and the fact that the river displayed such a low N:P ratio. There may be a year effect driving the change or differences found between both the experiments carried out in the Clydagh River (2002 and 2003). During the study visual observations suggested that algal growth appeared to be at its greatest towards the lower end of the split-stream (10m long). This suggested that uptake of the nutrient was not immediate and occurred further downstream of the point source. There is the possibility that there was fluxing of N through the river system that was not detected during the sampling programme. There may be nutrients pulsing from the forestry plantation upstream during rainfall events, causing intermittent releases of N into the system, thereby fluctuating the N:P ratios between sampling periods. This may have contributed to an increase in periphyton growth in the enriched section of the split-stream towards the end of the experiment but must remain as speculation. High resolution time-proportional sampling designed to pick up diurnal or day to day variations in N:P would be required to verify this hypothesis.

A biological assessment was carried out on a weekly basis (March – June 2003) prior to manipulation of the nutrient. The river did display a high Q-value of 4-5 but there was a marked absence of the genus *Ecdyonurus* during this period with the exception of the small numbers of immature juveniles that were periodically found. The abundance of *Ecdyonurus* in the same river was far greater during the summer of 2002 in comparison to the

summer of 2003. As previously mentioned, this river is prone to floods and due to the scouring nature of the substrate, it is thought that the eggs or the immature larvae of the genus *Ecdyonurus* may have been dislodged during a flood in the spring 2003. Because of this, the effects of nutrient enrichment on the macroinvertebrate fauna were not examined during this experiment.

2.5.4 General findings in the Castlebar River 2003

The experiment was replicated by moving it to the Castlebar River and the length of the split-stream was extended to 25m. A significant difference in chlorophyll *a* concentrations was detected over time between the control section and the treated sections of the experiment. The N:P ratios monitored from April to September 2003, gave a mean N:P ratio over this period of 4. As with the Clydagh River, this indicated an N-limited system. Interestingly, the highest significant difference in the chlorophyll *a* biomass levels between the enriched and control sections throughout all three experiments were found in this river. The lowest N:P ratios were also measured in this system. There was no significant difference in *Ecdyonurus* genus numbers per m^2 between either side of the split-stream. A significant difference in the total number of macroinvertebrates was found over time along the gradient but was not observed between the control and the treated sections of the experiment per se. As with the Clydagh River in 2002, the significant difference in macroinvertebrate abundance during the course of the experiment probably reflects the natural changes that occur in rivers during emergence and hatching processes.

After 6-weeks, the sampling regime for estimating periphyton biomass was modified to measuring individual stone scrapings as opposed to measuring a pooled sample of 5 scrapings over the 20m. This improved the power of the sampling regime and the statistical analyses. The chlorophyll *a* levels began to increase after 6-weeks of continuous nutrient enrichment. Algal standing crop appeared to respond to the phosphorus enrichment and biomass levels increased steadily during the last three weeks of the experiment. This was also reflected in the chlorophyll *a* concentrations which also showed a

significant trend through time with a quadratic fit to the trend line of increasing chlorophyll.

As in the Clydagh River, there is the possibility of the fluxing or pulsing of N through the river system, which are not detected between sampling periods. Domestic houses in close proximity to the river could possibly be introducing N sporadically into the river. Potential upstream sources include a concrete manufacturer, flushing of toilets by night, overflowing septic tanks or from waste grey water coming from washing machines and drains nearby. It appears that enough N is being released into the river e.g. from septic tank hydraulic pressure in the evenings, to allow all the background P to be taken up, plus the additional 10µgP/l released into the river via the nutrient manipulation experiment. The addition of N to the system in this manner may be contributing to the increased algal growth in the enriched section, but again, must remain as speculation.

2.5.5 Comparisons with other studies

Determining the likely biological impact of nutrient enrichment is especially complicated for flowing systems (van Nieuwenhuyse and Jones, 1996). The recognised need to include the influence of processes that occur upstream led to the development of the river continuum theory (Vannote *et al*., 1980). An improved understanding of the temporal aspects of nutrient loss with those of biological demand is necessary, particularly in view of the possibility of a seasonal dependence of N limitation (Axler *et al*., 1994; Peterson *et al*., 1997). Studies by Heathwaite *et al*., (1996) demonstrated in terms of nutrient loss, a good correlation between total area of agricultural land and river nitrate concentrations. Meyer *et al*., (1988) summarised into three areas, the reasons why a greater understanding of elemental cycling within lotic systems is necessary to improve understanding of ecosystem behaviour. These were: (1) nutrients regulate ecological processes in streams, especially when a nutrient is limiting; (2) nutrients link terrestrial and aquatic ecosystems: and (3) stream processes alter the timing, magnitude and form of elemental fluxes and therefore nutrient availability to downstream communities (Edwards *et al*, 2000).

Studies have also been carried out in which stimulatory effects of enrichment have been observed (Elwood *et al*., 1981). Work carried out by Wuhrmann and Eichenberger (1975) revealed no significant effect of P, N or N+P enrichment on periphyton growth receiving groundwater with a concentration of 10µgP/l. The N:P ratio in the stream channels was 96:1, a value where P-limitation would be expected. In a second experiment, a significant increase in biomass was observed when a mixture of trace elements was added to the stream water (Elwood *et al*., 1981). It may be possible that the primary producers were limited during this period by one or more essential elements but these were not examined in the experiments. Statistical analysis indicated there were no significant differences in macroinvertebrate numbers per m^2 and *Ecdyonurus* numbers per m^2 between both sides of the split-stream in the Clydagh River, suggesting there was equal grazing pressure on either side of the experimental divide.

2.5.6 N:P Ratios

The vast majority of rivers in Ireland are P limited and an analysis of the EPA database of the N:P ratios for 99 rivers in the West of Ireland found that approximately 4% of samples analysed are N-limited and with low MRP concentrations (<0.05 mg/l P; Appendix 5.14) so it was assumed that the river systems chosen for these experiments would also be P limited. The Clydagh River was predicated a P-limited system on the basis of displaying an N:P ratio of 19, measured in July 2002. The ratio dropped considerably thereafter and remained low when the experiment was repeated during the following summer. The study was replicated in the Castlebar River during the summer of 2003 but analysis of the water revealed yet again another apparently N limited site. In view of the fact that only a small percentage of rivers in Ireland are N limited, the dynamics of these high status rivers are interesting from an ecological point of view. It was envisaged that by artificially enriching a P limited river, algal growth would increase and the effects on the sensitive indicator genus *Ecdyonurus* could be described.

This genus was very abundant in the Castlebar River, even though the phosphorus concentrations exceeded the annual median 30µgP/l concentration set in the Phosphorus Regulations (DELG, 1998) on many occasions during the study although it was always satisfactory from a biological quality point of view. The mean background MRP concentration in the Castlebar River during the study in 2003 was 37µgP/l. At an N:P ratio of 4, it seems likely that the system was N-limited at the point when the samples were taken. Nonetheless, the fact that P additions apparently stimulated growth suggests that if nitrogen becomes available in the system, the available P is rapidly depleted and that the added 10µgP/l was also consumed. However, the river has a Q-value of 4-5 with low levels of algal growth.

In a study carried out by Edwards *et al.* (2000), the variations in nitrogen dynamics of an upland river in northeast Scotland were examined. He concluded that the source of nitrogen appeared to vary seasonally where it originated primarily from in-stream biological production during the

summer months while in the winter-spring period leaching from the plant-soil system appeared to be the major contributor. He found that on any individual sampling day, a wide range of N:P ratios occurred in the catchment studied. He observed the lowest N:P ratios during the summer and early autumn, particularly for upland catchments dominated by semi-natural vegetation. In many upland situations, physical scouring of stream systems during spates, or chemical-induced inhibitions by high molecular weight organic matter (Freeman *et al*., 1990), would be expected to limit algal productivity at certain times of the year (Twist *et al*., 1998). Findings from the Irish river nutrient enrichment experiment, clearly showed that both rivers during the three experiments were N limited throughout the summer/autumn months, as also found in studies carried out by Edwards and his co-workers (Edwards *et al*., 2000).

Chapter 3

Investigations into the feeding regime of the mayfly *Ecdyonurus venosus.*

3.1 Introduction

This study describes the feeding regime of the mayfly larvae *Ecdyonurus venosus* by examining the gut contents of specimens taken from the Castlebar River in the West of Ireland. This same river was used for the split-stream experiment (2003) outlined in Chapter 2. The gut contents of *Ecdyonurus venosus* larvae obtained from this river during the split-stream experiment were investigated to establish the diet of these mayflies. The genus *Ecdyonurus* is particularly important as an indicator species in the ecological assessment of river water quality in Ireland due to its sensitivity to pollution and widespread occurrence in Irish rivers (McGarrigle *et al*., 1998). To date, there have been no studies carried out on the diet of this species. It was hypothesised that the gut contents of the larvae may reflect the changes in the algal biomass exhibited in the experimental site and possibly reflect a variation in algal taxa between both sections of the experiment – i.e. its feeding regime would change during the nutrient manipulation experiment. The objective was to establish what these key indicator species fed on.

The feeding habits of organisms allow for their classification based on what they consume and therefore, the role this consumption plays in the ecological integrity of a community (Moog, 1995). Organisms are generally categorised into functional feeding-guilds based on the morphology of mouthparts, feeding behaviour and food consumed (Cummins, 1973, 1974; Cummins and Klug, 1979; Merritt and Cummins, 1984). Autecological studies on the feeding behaviour of specific species are rarely available and therefore there has been many false classification lists presented in the

literature. The following circumstances often prevent the unambiguous classification of organisms into functional-feeding groups:

- Few species are obligate feeders on a specific food resource and many use and select a wide range of items
- Certain organisms shift their diet during their life cycle and seasonally in response to food availability
- Feeding classifications can be difficult to define as some organisms are opportunistic feeders with unspecified nutritional requirements (Moog, 1995)

Cummins (1973) stated that the functional feeding group denotes a hypothetical particle size range ingested or mode of feeding, not food type or resource assimilation. Based on particle size ingested and mouthpart morphology, the functional-feeding groups are important in that they allow grouping of benthic communities into components. Functional feeding groups could therefore be helpful when describing the mode of feeding or food attainment and the role of the invertebrates in the processing of food (Cummins and Klug, 1979; Wallace and Webster, 1996).

It is currently recognised however, that the functional feeding group does not denote a specific, obligate trophic status for a given taxon and that the function and/or trophic status can change between life stages of that taxon (Cummins, 1988). It is difficult to classify an organism into a functional feeding guild simply through evaluating its anatomy or morphology. The role of the organism within the community also needs to be examined closely in conjunction with detailed descriptions of its feeding preferences. Ecologists are faced with the dilemma as to how to clarify the trophic position (e.g. detritivore, herbivore, omnivore) and food web connections of aquatic organisms. Food web investigations have traditionally been based upon gut analyses or literature sources of food habits. The exact sources of energy for animals are often not clear, especially for detritivores or omnivores making interactions in food webs poorly understood (Paine, 1988; Mihuc and Toetz, 1994). It is therefore important to obtain knowledge of the major components of the food web in an aquatic

ecosystem and how efficiently energy is transferred through the trophic levels. The use of stable isotopes is also a powerful research tool to identify interactions in food webs: e.g. detritus as an energy source in general (Deegan *et al*., 1990), energy sources for animals in subalpine lakes (Rau, 1980; Estep and Vigg, 1985), the role of predaceous zooplankton in pelagic food webs (Kling *et al*., 1992) and the use of allochthonous and authochthonous food in streams (Rounick *et al*., 1982). The type of food actually assimilated by the animal can be calculated using stable isotope analyses but this can be very costly and time consuming.

A basic aspect of the structure and function of a freshwater ecosystem is its material cycling and energy flow. A significant portion of such cycling and flow involves the processing of various forms of organic matter by freshwater invertebrates. This represents a basis for interest in aquatic trophic relations including food intake, tissue assimilation and waste release (Cummins, 1973). Studies carried out by Hynes (1970) found that the diversity of the ingested food greatly exceeds the diversity of the aquatic insects and that the majority of species appear to be generalists rather than specialists. Reports into feeding habits have been subject to considerable variation and require qualification with regard to habitat and species preferences.

The functional feeding group concept widely used in aquatic ecology (Cummins, 1973) is useful to specify how animals capture their food. Analysis of functional feeding guilds provides an insight into the dynamic ecological relations between construction, reconstruction and the mineralisation processes. A functional feeding guild distribution programme offers a method of indirect and empirical assessment of these processes (Moog, 1995). A shift in the equilibrium state of a river-type, from one governed by production to one dominated by decomposition would indicate a disturbance. In turn, this disturbance would be reflected in compositional changes in the feeding guild structure (Schweder, 1992; Kohmann, *et al*., 1993; Moog 1993, 1994).

There is little information in relation to assimilation of the various resources among lotic primary consumers. Some hypotheses have suggested that if taxa eliminate most competitive interactions by partitioning space and/or time, then generalist food utilisation would be expected (Schoener, 1974; Townsend, 1989; Pahl-Wostl, 1993). Recent work by Mihuc and Minshall (1995) and Mihuc (1997) has confirmed the argument put forward by Cummins (1973), that many species of stream invertebrates are trophic generalists capable of feeding and growing on a broad range of food types and that the invertebrates also switch among resources in space and time. Work carried out by Lamberti and Moore (1984) also suggest that most aquatic insects are opportunistic feeders that consume a wide variety of food items with seasonal and age-specific variation in feeding habits and diet. Assimilation of food resources however, cannot be inferred from diets unless the assumption is made that all of the material ingested by an individual is assimilated, this assumption being unacceptable (Cummins, 1973; Warren, 1989; Martinez, 1993; Mihuc and Minshall, 1995). Often assimilation efficiency values are inferred from limited published data, where the same values for related taxa (Benke and Wallace, 1980; Smock and Roeding, 1986), and sometimes for entire functional feeding groups were found (Lugthart and Wallace, 1992). Based on current literature, assimilation of both allochthonous (detrital fine and coarse particulate organic matter; FPOM, CPOM) and autochthonous (periphyton, algae) resources by lotic macroinvertebrates have been determined in relatively few studies (Mihuc, 1997).

Environmental stress can cause disruptions in ecological integrity where the primary effect is on autecological relations. Organisms respond to various changes in the environment, which in principal are definable but it is impractical to measure all but a few. There are species which react with specific environmental factors, and produce characteristic responses that alter the ecological function of a particular community (Moog, 1995). Disturbance of an aquatic ecosystem (e.g. de- or afforestation, nutrient input causing increased algal growth), can lead to changes in food availability (Stout *et al*., 1993). The ability of an aquatic system to cope with such disturbances is without doubt related to the feeding plasticity of the

organisms that inhabit it (Friberg and Jacobsen, 1994). For trophic relationships and feeding plasticity to be understood, studies of feeding preferences are essential, especially as invertebrates appear to prefer diets most favourable to survival (Otto, 1974; Inversen, 1974; Kostalos and Seymour, 1976).

Algal grazing is now acknowledged to be a very important link in the food webs of streams in general (Lamberti and Moore, 1984; Hildrew, 1992). Benthic algae form an important part of the biofilm that coats the upper surfaces of substrate in running water (Madsen, 1972; Rounick and Winterbourn, 1983; Lock *et al*., 1984). Other materials make up the mixed assemblages on substrate surfaces, like coccoid bacteria, organic matter and fungi. The living cells exude polysaccharide, which attaches them to the substratum and forms a slimy matrix into which exoenzymes and exudates are released (Lock, 1992). These activities enable interactions among the cells, which may affect the growth and viability of populations making up this complex community (Ledger and Hildrew, 1998).

It has been well documented that the rates of development and accrual of algae on stones in streams are affected by many environmental variables; among them are irradiance (light) (Ledger and Hildrew, 1998; Hill *et al*., 1995), nutrient concentrations (Elwood *et al*., 1981; Stockner and Shortreed, 1976; Peterson *et al*., 1985), temperature (Darley, 1982), current velocity (Peterson and Stevenson, 1992), substratum composition (Blinn *et al*., 1980; Bott, 1983), disturbance (Grimm and Fisher, 1989; Peterson and Stevenson, 1992; Biggs and Thomsen, 1995) and the pH of the water (Maurice *et al*., 1987). Deviations among these variables result in temporal and spatial variations in the biomass and composition of biofilm, which in turn affect its potential nutritional quality for invertebrate consumers (Ledger and Hildrew, 1998). Grazing by macroinvertebrates has been shown to have an overriding influence on periphyton in many situations. These studies highlight the effects of grazing by decreasing biomass levels and influencing or changing the taxonomic composition and community structure of the periphyton (Hunter, 1980; Kelser, 1981; Lamberti and Resh, 1983; McAuliffe, 1984; Cattaneo and Kalff, 1986; Steinman *et al*., 1991;

Hill *et al*., 1992). Welch *et al*. (1992) found that lack of suitable habitat for grazers was an important factor allowing the development of dense mats of filamentous green algae, like *Cladaphora*, in some environments. Stream herbivores can be as important as other factors such as light, in shaping periphytic assemblages, although their mode of action in this respect is quite different (Wellnitz and Ward, 1998). By eating or otherwise disturbing periphytic algae, herbivores may change algal composition and physical growth forms over others (Steinman, 1996). Mouthpart structure, ingestion rates and foraging behaviour vary between taxa, and each of these traits may influence the responses of periphytic standing crop, taxonomic composition and algal physiognomy to grazing (Feminella and Resh, 1991; Lamberti *et al*., 1987).

A close correspondence between food ingested and food assimilated would be expected from evolutionary processes but the presence of a material in the digestive tract does not prove nutritional importance. In other words food habits may yield no information about assimilation. Significant amounts of mineral sediment may be found in the guts of many periphyton grazers and fine particle detritivores (Coffman, 1976; Coffman, *et al*., 1971; Maciolek and Tunzi, 1968). The mineral substance is not nutritionally significant but coatings of adsorbed organic material and associated bacteria may be extremely important (Cummins, 1973). Furthermore, Mihuc, (1997) notes however that these organic materials and associated bacteria are important in establishing species food preferences and can be useful when combined with assimilation efficiency estimates from the literature (but this method does not actually measure assimilation directly).

The effect of food on regulating the life history characteristics of aquatic insects has been investigated mostly in shredder and collector species (Otto, 1974; Ward and Cummins, 1979; Fuller and Mackay, 1981; Vannote and Sweeney, 1985; Sweeney *et al*., 1986a, 1986b). Similar observations for grazer-scrapers are scarce. Studies carried out by Hill and Knight (1987), found that caddisfly larva with scraping mandibles were better at feeding on inconspicuous algae than mayfly larva with collector-gatherer (grazer) mouthparts. These collector-gatherers reduced the loose overlying layer

however, and they had a greater influence on community structure than the scrapers.

Moog (1995) classifies aquatic organisms into functional feeding guilds based on what they consume. He categorises the genus *Ecdyonurus* as a grazer and detritus feeder (gathering collector) which has a preference for epilithic algal tissues, biofilm and particulate organic matter (POM). Detritus feeders (gathering collectors) tend to eat sedimented fine particulate organic matter (FPOM). He implies that this genus may function as both a grazer and a detritus feeder and this area clearly requires further investigation.

In natural populations, gut contents of scraper-grazers usually contain variable proportions of two main kinds of food: periphyton and fine amorphous detritus (Chapman and Demory, 1963: Anderson and Cummins, 1979; Hawkins, 1985, Yule, 1986; Wallace and Gurtz, 1986). In general, mouthpart morphology may largely determine the kinds of food to which grazers have access (Gregory, 1983; Steinman *et al*., 1987); Karouna and Fuller, 1992). There are a variety of different mouthpart morphologies within the grazer community. The genus *Ecdyonurus* has brush-like labial palps that engage in broad sweeping motions to harvest food from substrata, which it then moves towards its mouth. It sweeps wide swaths of periphyton from the substrata with its labial palps. It displays a clinging mode of existence and possesses a dorsal-ventrally compressed head with prognathous mouthparts (Arens, 1989). As with other larva from the Heptageniidae family, *Ecdyonurus venosus* is a common grazer found in most Irish rivers but there have been no studies undertaken in this country to analyse its feeding regime, or to verify its assignment into this functional feeding group. Other autecological data including species-specific information about link relationships and energy transfer within the food webs would also be required to accurately characterise the community trophic relationships of this genus (Schoener, 1974; Schoenly *et al*., 1991; Hildrew, 1992: Pahl-Wostl, 1993; Closs and Lake, 1994; Polis, 1994). These investigations however were beyond the scope of this study, which focuses entirely on feeding regimes alone.

3.2 Study outline

This study focuses on the feeding ecology of the mayfly larva, *Ecdyonurus venosus*. One of the underlying hypotheses of this study is that as eutrophication progresses, the periphyton species change thereby affecting the food sources of *Ecdyonurus*. Specimens were obtained before and after the addition of phosphorus to the river in order to assess any resultant changes in feeding regime. In this study, a method permitting quantitative examination of particulate gut contents for gut analysis of the mayfly larvae *Ecdyonurus venosus* was adopted. This method allowed separation of particles that make up the gut, into several distinct classes that are described in detail below.

3.3 Materials and Methods

3.3.1 Site description: The Castlebar River

Details of the Castlebar River study site are given in Chapter 1.

3.3.2 Sampling design

The study was conducted at the split-stream experimental site in the Castlebar River during the period of the nutrient manipulation. The river was split using a 20m Perspex barrier separating each side uniformly. Prior to the addition of the nutrient, each section of the split-stream was sampled for macroinvertebrates. On 18th July 2003 (pre-nutrient addition), five Surber samples were taken at 4m intervals along each side of the divide. From each Surber, specimens of the genus *Ecdyonurus* were carefully collected and immediately preserved in 70% IMS (Industrial Methylated Spirits). There was only one species of *Ecdyonurus* found in this river during the study, namely *Ecdyonurus venosus*. Specimens were collected again at intervals over a 7-week period post-nutrient addition. The periphyton biomass was measured during the course of the experiment by estimating the chlorophyll *a* concentrations ($\mu g/cm^2$) from stone scrapings. The mean benthic chlorophyll *a* concentrations in the treated section showed statistically significant increases towards the end of the study in comparison with the control section (see Chapter 2). The concentrations seemed to increase particularly in the last three weeks of the study (Fig. 3.1). It was hypothesised that there may have been a change in the feeding regime of *Ecdyonurus* caused by the increase in the periphyton biomass on the stones in the treated section, found in the latter stages of the study. Specimens were chosen from the treated section for gut content analysis from samples obtained on 12th September 2003 when the chlorophyll *a* concentrations were elevated compared to the control section (Fig. 3.1).

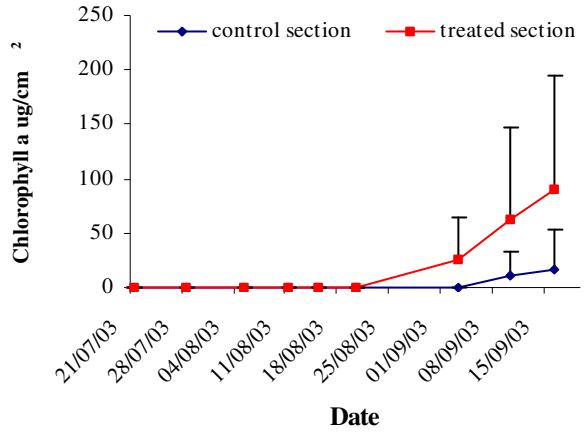


Fig. 3.1 Mean chlorophyll *a* concentrations (µg/cm^2) in the treated section (manipulated) vs the control section. Bars are means ± standard deviation: $n = 5$.

3.3.3 Gut content analysis

3.3.3.1 Cold acid hydrogen peroxide method

Larvae of *Ecdyonurus venosus* were sampled from a number of high status and impacted rivers in March 2003 (Table 3.7 and 3.8). The cold acid hydrogen peroxide method was the first and most simplistic technique used in an attempt to isolate and identify the material consumed by this species. This study was carried out to provide species-level information on the diatoms consumed by this larva and did not provide information on the overall particulate material eaten. The DAPI method described below was used to categorise the material consumed in more detail.

The cold acid hydrogen peroxide method was used to isolate the benthic diatoms in the gut contents. The gut was dissected from each animal and exposed to cold 30% hydrogen peroxide with potassium permanganate to aid oxidation and digestion. These samples were then washed several times with distilled or deionised water to removed any chemicals, centrifuged and allowed to sediment overnight. The supernatant was decanted the following morning. A known volume of material was mounted onto a microscope slide, covered with a cover slip and heated gently on a hotplate to fix. A total of 15-20 fields were observed using oil immersion (10x100 magnification). An attached camera was used to take photographs. The gut contents of eight larvae were oxidised and seven were left untreated and intact for direct observation under the microscope. These were used for comparative purposes, in the event that oxidation led to loss of sample.

3.3.3.2 DAPI method

Gut content particles ingested by the mayfly larvae were examined using a fluorochromatic stain 4'6 diamidino-2-phenylindole (DAPI) with nuclear DNA-specific binding properties; DNA-bound DAPI fluoresces blue when exposed to light at 365nm and viewed at high magnification (Wittekind, 1972). This stain was used in conjunction with epifluorescence microscopy (Walker *et al*., 1988) and with light microscopy to identify the particles consumed by the mayfly larvae. The DAPI stain allows for the enumeration of very small bacteria, below the resolution of a light microscope (<1.0µm) due to the fluorescent glow of the bacterial DNA. This stain has been successfully used to quantify bacterial counts in waters rich in organic material (Kondratieff and Simmons, 1985; Rassoulzadegan and Sheldon, 1986).

Five *Ecdyonurus* specimens of various sizes were selected from samples obtained from each section of the divide on 18th July 2003 and 12th September 2003. In total 20 individual *Ecdyonurus venosus* specimens were examined for gut analysis in this study. Ledger and Hildrew, (2000), in their study of the gut contents of stoneflies and chironomids, recommended removing food particles from the foregut, where food tends to be more intact than in the mid or hindgut. This area was difficult to pinpoint when dissecting the guts of the mayfly larva. As no descriptions could be found in the literature describing exactly where the fore-, mid- or hindguts were located in the digestive tract, the gut was visually split into three equal sections and assigned fore- mid- and hindgut accordingly. Occasionally there was no food in the foregut so the mid- and hindgut had to be taken as a whole, while there were other times when food was only present in the hindgut. Each area removed for examination was carefully noted. The animals were measured to the nearest millimetre from the top of the labium to the tip of the last abdominal segment. The gut of each larva was removed under a dissecting stereomicroscope using a fine forceps and the contents from the gut were separated from the peritrophic membrane with a scalpel and fine forceps into a drop of distilled water in a petri dish.

The material was pipetted from the drop into an Eppendorf tube with 2mls of distilled water and vortexed for 20-30seconds. DAPI solution was added (0.2mls) to each vial and held in the dark for 20-25mins. To provide a dark background for improved visibility of the fluorescing particles, 25mm black Isopore (0.2µm) filters were used. For even distribution, these filters were soaked in distilled water and placed on top of a damp backing filter (Millipore, 25mm diameter, 0.45µm pores size) in a small filter holder (Swinnex W/O filter). After staining, the sample was shaken and filtered through the Swinnex filtration system (Hobbie *et al*., 1977; Porter and Feig, 1980). A BX60 Olympus microscope with an Olympus U-RFL-T epifluorescence burner was used to view the fluorescent stained microscope slides. Photomicrography was done using an attached Olympus camera. The damp filters were removed and placed onto microscope slides previously smeared with immersion oil. A drop of immersion oil was added to the filter and a coverslip was then placed on top. The slides were stored horizontally in the dark at 4°C. All particles in 15 fields of view were counted and assigned to one of several groups.

Particles found in the gut of *Ecdyonurus venosus* were assigned to the following categories: (1) filamentous green algae (2) coccoid green algae (3) diatoms (4) biofilm matrix (5) algal agglomerates (6) inorganic debris (7) plant detritus (8) coccoid bacteria (9) peritrophic membrane and (10) unidentified particles.

When the DAPI stain is exposed to UV-light, bacteria and algal nuclei fluoresce bright blue while protozoans appear light blue with a distinct nucleus. Chlorophyll in algae autofluoresces a dull red/orange colour and organic plant detritus fluoresces dull yellow (Coleman, 1980; Porter and Feig, 1980; Rassoulzadegan and Sheldon, 1986). Illuminating the sample further with white light enabled clumps of algal agglomerates to be distinguished from the biofilm matrix. Algal agglomerates autofluroesce red under white light highlighting the algal chloroplasts. The bacterium attached to the matrix in biofilm does not autofluoresce under white light thereby disappearing when switched between channels (from UV-light to red). This was useful, as sometimes it was difficult to distinguish between

these categories as bacteria and algal nuclei fluoresce bright blue causing confusion on occasion. The structure of biofilm matrix consists of a thin film of brown matrix material with a porous or granular appearance, which generally contains algal matter, embedded with bacteria (Ledger and Hildrew, 2000). Algae were assigned to an 'algal agglomerate' category when the algae appeared as a mass of algal chloroplasts as opposed to a filamentous green algae or coccoid green algal particle. As Ledger and Hildrew (2000) found, some particles in this study could not be identified so they were assigned to an 'unidentified particles' category along with detrital material of unknown origin. On occasions, peritrophic membrane was found in the sample and was estimated as part of the material examined but not reported in the results. Therefore, peritrophic membrane was discounted when expressing percentage composition results.

The technique is a relatively time-consuming one. Some preliminary samples were analysed in order to develop the dissection technique and perfect the staining process. Once the techniques were developed the dissection, counting and photographing of the 20 *Ecdyonurus* gut contents took 5 weeks.

3.3.4 Analysis of Benthic stone scrapings

Stone scrapings were obtained from submerged cobbles prior to the addition of nutrient and again at intervals during the manipulation experiment. These were taken to quantify the composition of algal communities growing on the substrate and for taxonomic comparisons with those found in the *Ecdyonurus* gut analysis. Qualitative stone scrapings were taken from 5 stones at various intervals along each side of the divide and they were pooled into a 100ml-perspex glass bottle. The samples were preserved immediately by adding 0.7ml of Lugols iodine and stored in the dark for later analysis. Two samples from scrapings obtained on 21st July 2003 (pre-nutrient addition) and two samples from scrapings taken on 9th September 2003 (post-nutrient addition) were examined in this part of the investigation. These dates were chosen as they coincided with larval sampling periods.

Ecdyonurus venosus has been classified as a grazer and thus it would be expected to move in broad sweeping motions to harvest food from the substrate by brushing with its labial palps (Wellnitz and Ward, 1998). They collect various particulate types as they feed and it was hypothesised that there may be a similarity between the material taken from the stone scrapings and the food ingested by the larvae. In order to quantify the composition of the stone scrapings, they were analysed using the same epifluorescent microscopy technique as was used to examine the gut contents. Lugols iodine has historically been used to preserve phytoplankton but its use in combination with the DAPI stain to identify particulate material as described above was not previously documented. Stone scrapings obtained from each side of the experimental divide on 21/07/03 and 09/09/03 were chosen as they coincided with larval sampling periods. The Lugols acts as a fixative-preservative stain, that makes cells dark brown but can mask chlorophyll fluorescence (Gifford and Caron, 2000). Thus it was not known how effectively the DAPI stain would work in conjunction with the Lugols.

For quantitative algal taxa observations, a 1ml aliquot was taken from each preserved suspension, placed in a 10ml settling chamber, filled to the top

with distilled water and left to settle for 24 hours. The algal taxa were identified and enumerated along transects across the width and length of the chamber until a total of 15 fields were viewed per sample.

3.4 Results

3.4.1 Gut content analysis

The gut contents of *Ecdyonurus venosus* specimens were analysed for samples taken on five separate dates from the Castlebar River. Details of the sampling dates and the number of specimens dissected are outlined in Table 3.1.

A chi-squared analysis was carried out on different treatment combinations of the gut contents of *Ecdyonurus venosus* and the stone scrapings taken from the Castlebar River during the summer of 2003 to establish if there were any similarities between the gut contents and the food material scraped from the stone surfaces (Table 3.11). The results are outlined in section 3.4.3.

Table 3.1 Details of the sampling dates and the number of specimens examined for the gut content investigations in the Castlebar River in 2003.

Sampling date	**Number of specimens**	**Number of specimens**
Split stream nutrient addition comparison	Treated Side	Control Side
18th July 2003 (pre-treatment)	5	5
12th Sep 2003 (post treatment)	5	5

Additional samples (see Table 3.4 for details)	**Number of specimens**
29th July 2003	2
7th August 2003	1
19th August 2003	3

Specimens were examined and food items were assigned to one of ten food categories previously outlined. Coccoid bacteria were relatively common in the guts but these were not included in the percentage composition results of any of the specimens below as they generally represented less than 1% of the total particle area in the majority of the invertebrate gut contents investigated.

Details of the length of the individual specimens examined and the area of the digestive tract dissected are outlined in Tables 3.2, 3.3 and 3.4.

Table 3.2 Length of the *Ecdyonurus venosus* specimens (mm) examined and details of the area of the digestive tract dissected during the gut analysis investigations pre-nutrient addition (18th July 2003).

	Length specimen (mm)	Area of digestive tract dissected
Control section	10.0	Fore-, mid- and hindgut
	9.5	Fore-, mid- and hindgut
	8.0	Fore-, mid- and hindgut
	8.2	Mid- and hindgut
	8.0	Mid- and hindgut
Treated section	9.4	Fore-, mid- and hindgut
	11.5	Mid- and hindgut
	10.0	Fore-, mid and hindgut
	10.0	Fore- and midgut
	9.0	Mid- and hindgut

Table 3.3 Length of the *Ecdyonurus venosus* specimens (mm) examined and details of the area of the digestive tract dissected during the gut analysis investigations post-nutrient addition (12th September 2003).

	Length specimen (mm)	Area of digestive tract dissected
Control section	8.5	Mid- and hindgut
	9.0	Midgut
	8.5	Mid- and hindgut
	7.4	Mid- and hindgut
	8.0	Midgut
Treated section	8.0	Foregut
	8.0	Mid- and hindgut
	7.0	Fore- and midgut
	7.3	Midgut
	7.5	Mid- and hindgut

Table 3.4 Additional Samples: length of the *Ecdyonurus venosus* specimens (mm) examined and details of the area of the digestive tract dissected during the gut analysis studies.

	Date	Length specimen (mm)	Area of digestive tract dissected
Control section	29/07/03	10.2	Fore- and midgut
Treated section	29/07/03	11.4	Fore- and midgut
	07/08/03	11.7	Fore- and hindgut
	19/08/03	10.9	Fore-, mid- and hindgut
	19/08/03	11.8	Fore- and midgut
	19/08/03	11.1	Fore- and midgut

The mean percentage composition of the gut contents in the 'pre-nutrient addition' specimens (18th July 2003) and in the 'post-nutrient addition' specimens (12th September 2003) are outlined in Table 3.5.

Table 3.5 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the Castlebar River on 18th July 2003 and 12th September 2003.

	Pre-nutrient addition 18th July 2003	**Pre-nutrient addition 18th July 2003**	**Post-nutrient addition 12th September 2003**	**Post-nutrient addition 12th September 2003**
Food category	**Treated section**	**Control section**	**Treated section**	**Control section**
Diatoms	-	-	2%	7%
Desmids	1%	4%	-	-
Filamentous green algae	8%	4%	5%	4%
Coccoid green algae	11%	15%	8%	7%
Biofilm matrix	4%	5%	4%	2%
Inorganic debris	24%	16%	16%	30%
Unidentified particles	3%	4%	15%	7%
Plant detritus	45%	41%	37%	26%
Algal agglomerates	4%	11%	13%	17%

3.4.1.1 Gut content results 'pre-nutrient addition' (18th July 2003)

The mean percentage composition of gut contents in the treated section of the Castlebar River on 18th July 2003 (pre-nutrient addition) is shown in Fig. 3.2. The results for the control section of the Castlebar River on the 18th July 2003 are shown in Fig. 3.3. Photomicrographs of the DAPI stained particles found in the gut contents of *Ecdyonurus venosus* 'pre-nutrient addition' (18/07/03) are shown in Fig. 3.4A-F. Coccoid bacteria (not shown in graphs) represented 0.4% and 2.7% of the total particulate area of the gut contents of the specimens examined in the treated and control sections pre-nutrient addition, respectively.

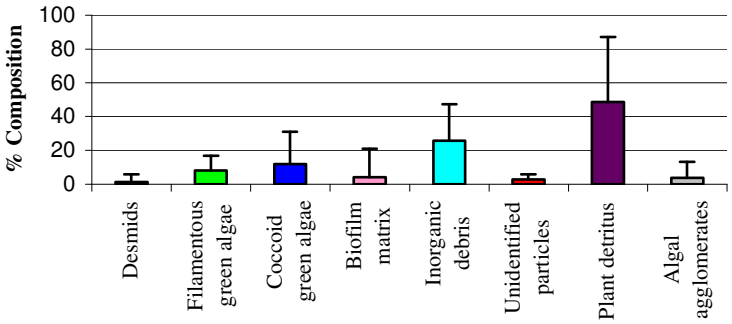


Fig. 3.2 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the treated section of the Castlebar River pre-nutrient addition. Bars are + standard deviation: $n = 5$.

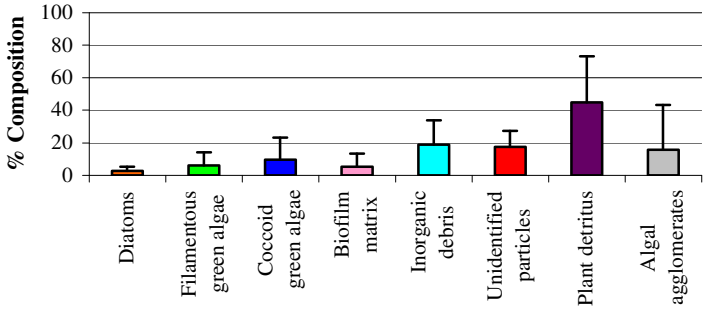


Fig. 3.3 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the control section of the Castlebar River pre-nutrient addition. Bars are + standard deviation: $n = 5$.

The gut contents of the specimens examined prior to the addition of nutrient, consisted of algae (desmids, coccoid green algae, filamentous green algae and algal agglomerates), quite a large proportion of plant detritus, biofilm matrix, inorganic debris and unidentified particles. The mean composition of ingested material in the larvae examined from both sides of the split-stream prior to nutrient addition, was proportionately relatively similar (Table 3.5) with no significant differences observed between the food items (Table 3.11; p=0.3). Plant detritus was the dominant material in the gut of these mayflies, comprising nearly half of the total particulate area. It

accounted for 41% in the control section compared to 45% in the treated side of the experiment. Filamentous green algae were found to represent 4% (control) and 8% (treated) of the larvae guts, coccoid green algae represented 11% (treated) and 15% (control) and algal agglomerates accounted for 4% (treated) and 11% (control) on either side of the experimental divide. Desmids were represented only by *Closterium* spp., which was found in low numbers accounting for 1% (treated) and 4% (control) of the gut contents of *Ecdyonurus venosus* during this sampling period. Biofilm matrix was present in low amounts comprising 4% (treated) and 5% (control) of the guts examined, as were unidentified particles ranging from 3% (treated) and 4% (control). Inorganic particles were present, representing 24% (treated) and 16% (control) of the total particle area in the guts examined between each section of the split-stream.

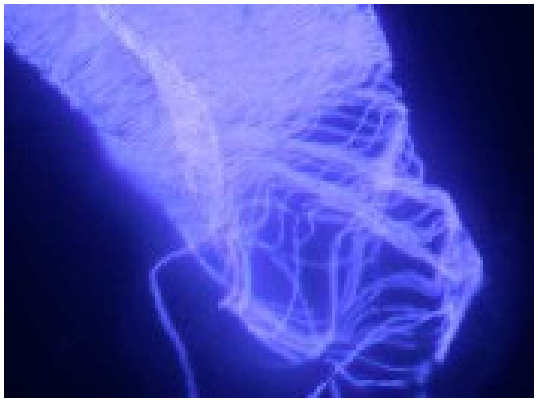


Fig. 3.4A 400x A possible nematode spp. Specimen unravelling in the gut of an 11.5mm specimen of *Ecdyonurus venosus* during the digestion process. This specimen was taken from the treated section of the split-stream experiment on 18th July 2003.

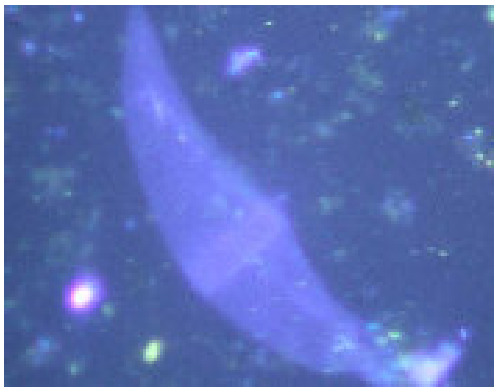


Fig. 3.4B 200x *Closterium* spp. (desmid) found in the gut of an 11.5mm *Ecdyonurus venosus* specimen in the treated section of the split-stream experiment on 18th July 2003.

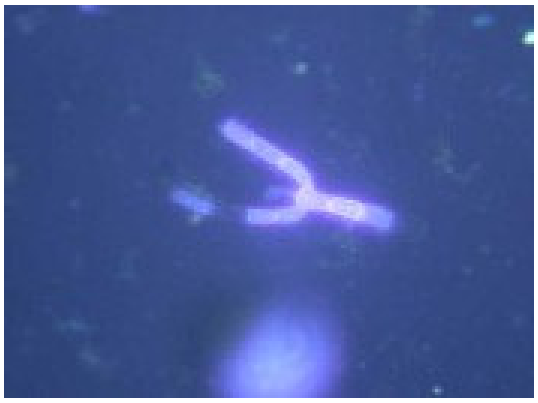


Fig. 3.4C 400x Branched filament of algae with nuclei brightly fluorescing in the centre of each cell. Found in the gut of a 12mm *E.venosus* specimen in the treated section of the split-stream experiment on 18th July 2003.

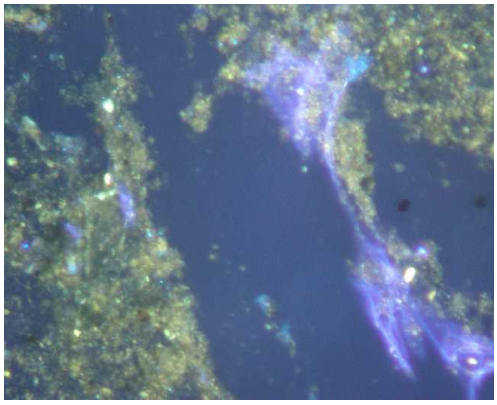


Fig. 3.4D 200x Detritus and inorganic particles found in the gut of a 9.5mm specimen of *E.venosus*. A section of peritrophic membrane of the animal is fluorescing bright blue. The animal was sampled from the control section of the experiment on 18th July 2003.

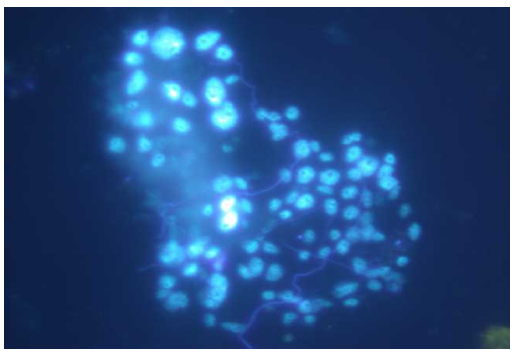


Fig. 3.4E 400x Algal agglomerate in the gut of a 7.4mm specimen of *E.venosus* taken from the control section of the split-stream experiment in the Castlebar River on 18th July 2003.

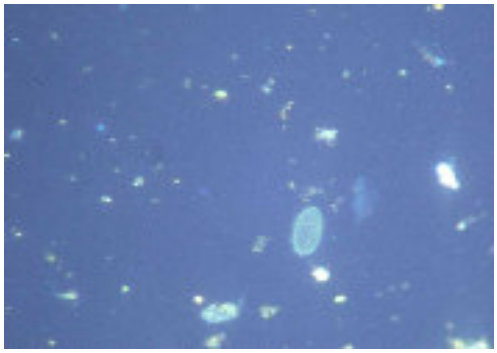


Fig. 3.4F 200x *Cocconeis* spp. (egg shaped) with another partially digested of same to the foreground. Plant detritus and inorganic particles are scattered throughout the sample. Found in the same gut as in Fig 3.6E

3.4.1.2 Gut content results 'post-nutrient addition' (12th September 2003)

The mean percentage composition of the gut contents from samples obtained from the treated section of the split-stream experiment, on 12th September 2003 (post-nutrient addition) is shown in Fig. 3.5. Results for the control section are given in Fig. 3.6. Photomicrographs of the DAPI stained particles found in the gut contents of specimens post-nutrient addition (12/09/03) are shown Fig 3.7A-F. Coccoid bacteria (not shown in graphs) represented 0.1% and 0.6% of the total particulate area of the gut contents of the specimens examined in the treated and control sections post-nutrient addition, respectively.

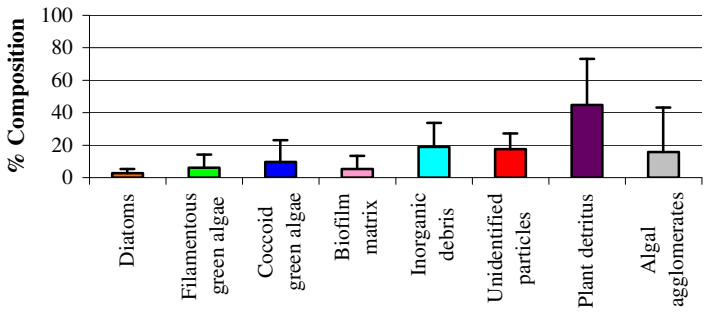


Fig. 3.5 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the treated section of the Castlebar River post-nutrient addition. Bars are + standard deviation: $n = 5$.

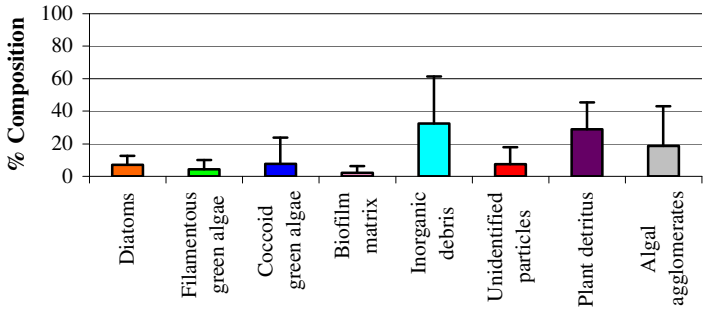


Fig. 3.6 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the control section of the Castlebar River post-nutrient addition. Bars are + standard deviation: $n = 5$.

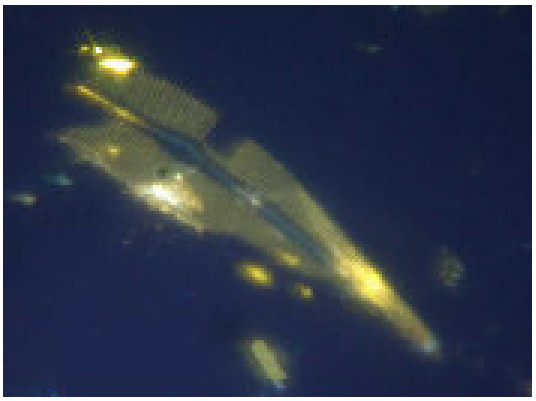


Fig. 3.7A 400x *Navicula* spp. found in the gut of a 7mm *E.venosus* in the treated section of the split-stream experiment on 12th September 2003.

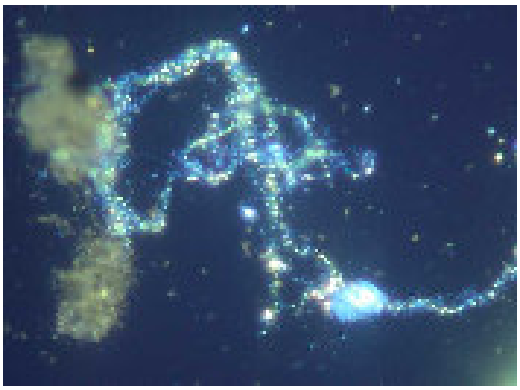


Fig. 3.7B 400x Algal agglomerate (green mass to left of photo) with a string of coccoid bacteria and plant detritus. Found in a 7.3mm *E.venosus* specimen in the treated section of the Castlebar River on 12th September 2003.

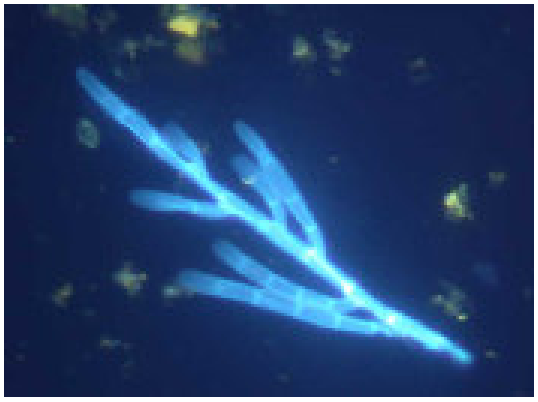


Fig. 3.7C 200x Branched filament of algae with plant detritus autofluorescing yellow. Found in the gut of an 8mm *E.venosus* specimen in the treated section of the Castlebar on 12th September 2003.

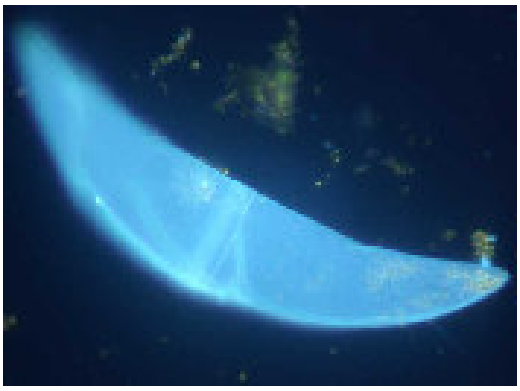


Fig. 3.7D 200x *Closterium* spp. (Desmid – 150µ) found in the gut of an 8mm specimen of *E.venosus* in the treated section of the Castlebar River on 12th September 2003.

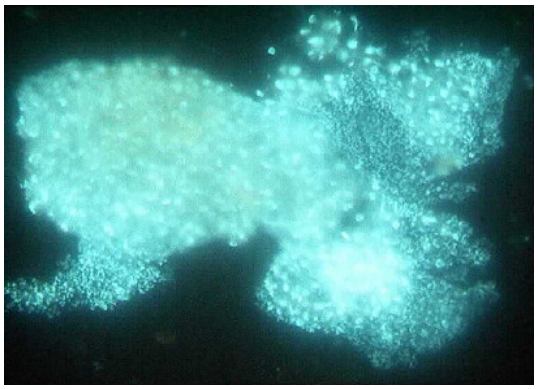


Fig. 3.7E 200x Biofilm matrix in the gut of an 8.5mm specimen of *E.venosus* in the control section of the Castlebar River on 12th September 2003.

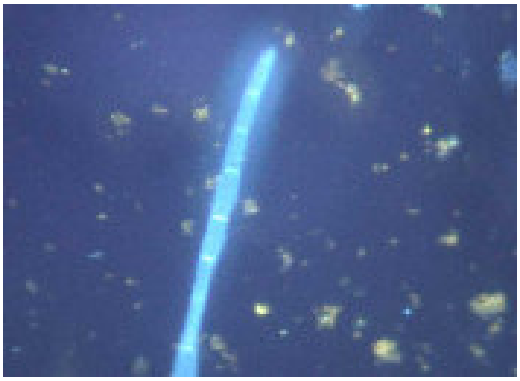


Fig. 3.7F 200x Filament of algae found in the gut of an 11mm specimen of *E.venosus* in the control section of the split-stream experiment. Plant detritus (dull yellow coloration) is also evident.

In this case the larval gut contents consisted primarily of algae (diatoms, coccoid green algae, filamentous green algae and algal agglomerates). They also contained quite a large proportion of plant detritus and inorganic debris. Unidentified particles and biofilm matrix made up a proportion of the larva guts. As with the specimens investigated on 12th July (pre-nutrient addition), the larvae studied on this occasion contained a good proportion of plant detritus (26% and 37%) in each side of the experimental divide. *Navicula spp*., was found in specimens sampled from the treated section representing 2% of total particulate content. This species accounted for 7% of the gut content of animals taken from the control section of the split-stream on the same day (12th September). Filamentous green algae represented 5% (treated) and 4% (control) of the gut contents in both study sections. Coccoid green algae and algal agglomerates represented 8% (treated) and 13% (treated) and 7% (control) and 17% (control) respectively. Biofilm matrix makes up for 2% (control) and 4% (treated) of the particulate material and inorganic debris accounts for 30% (control) and 16% (treated) of the contents of guts analysed on the treated and control sections of the experimental divide respectively (Table 3.5).

Significant differences in the gut contents were found between the treated and the control sections post-nutrient addition (Table 3.11 Treatment combination 6 – $p<0.001$). The main difference in food composition between summer and autumn sampling, was the absence of diatoms in the guts examined in July, which were present in the larval stomachs in September. Greater numbers of diatoms are present in rivers during the spring and autumn seasons, which may account for their presence in September. There were also desmids found on occasion in the specimens examined in July only. Other specimens from the Castlebar River studied during the investigation did not contain diatoms or desmids. These latter did however contain filamentous green algae, coccoid green algae, biofilm matrix and inorganic debris. Plant detritus also dominated the diet of these larvae. The most common diatom species found in the ingested material was *Navicula* spp. No other diatom was found in its gut contents, which suggests that *Navicula* spp. was the main diatom species consumed during this period. This diatom may also be more tolerant to the digestive

processes in the gut of these larvae compared with other taxa. The significant difference found in the gut contents between the treated and the control sections post-nutrient addition appear to be attributed to the natural variation in the food items available to *Ecdyonurus* during a particular feeding episode.

3.4.1.3 Gut content results from additional samples investigated

The gut contents of the larvae were also analysed for three other sampling dates. These were carried out before the main left/right (control v treated sections) comparison and formed a preliminary examination to verify that the technique worked but it was felt worthwhile to report on these results as well. Two guts were examined from samples obtained on 29th July, one specimen was investigated from samples taken on 7th August and three from samples taken on 19th August 2003. With the exception of the larva analysed on 29th July, which was taken from the control section of the experimental divide, all other specimens were taken from the treated section.

The percentage breakdown of the gut contents for these specimens is outlined in Table 3.6. The food contents of the guts of *Ecdyonurus venosus* examined are also shown in Figs. 3.8 to 3.11. Photomicrographs illustrating examples of the DAPI stained particles, found in the gut contents of the *Ecdyonurus venosus* specimens investigated on 29/07/03, are shown in Fig. 3.12A-F. More particulate material found in the gut contents of mayflies examined on 07/08/02 and 19/08/03, are presented in Fig. 3.13A-F.

In addition to the items in the Table 3.6 and Figs. 3.7 to 3.10, coccoid bacteria represented 0.3% of the total particulate area of the gut contents of the specimens examined on 29/07/03. Coccoid bacteria represented 0.1% and 0.2% of the total particulate area of the gut contents of the specimens examined on 07/08/03 and 19/08/03 respectively.

Table 3.6 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the additional samples investigated in the Castlebar River during the study.

	29/07/03	29/07/03	07/08/03	19/08/03
	N=1	N=1	N=1	N=3
Food category	**Treated section**	**Control section**	**Treated section**	**Control section**
Diatoms	-	-	-	-
Desmids	-	-	-	-
Filamentous green algae	13%	13%	1%	16%
Coccoid green algae	32%	9%	5%	16%
Biofilm matrix	0%	23%	5%	10%
Inorganic debris	16%	23%	20%	20%
Unidentified particles	23%	11%	5%	8%
Plant detritus	16%	21%	64%	30%
Algal Agglomerates	-	-	-	-

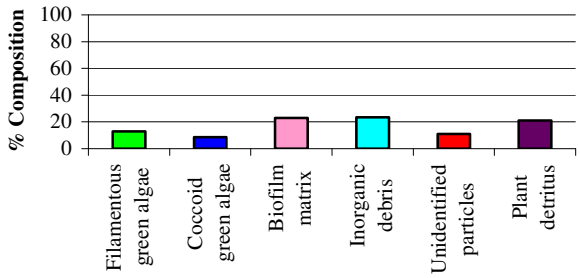


Fig. 3.8 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the control section of the Castlebar River on 29/07/03.

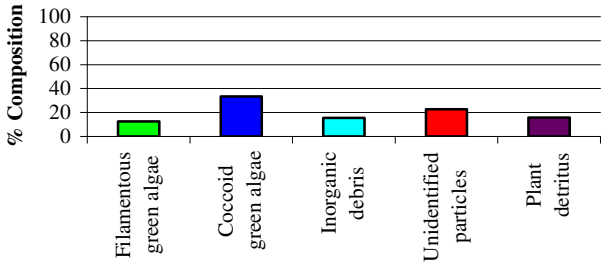


Fig. 3.9 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the treated section of the Castlebar River on 29/07/03.

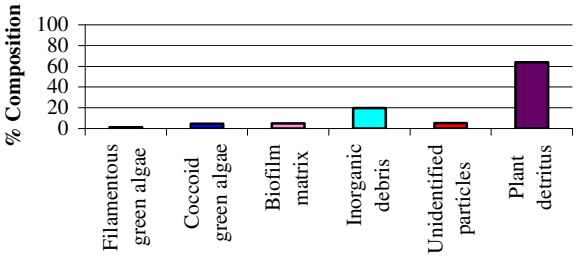


Fig. 3.10 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the treated section of the Castlebar River on 07/08/03.

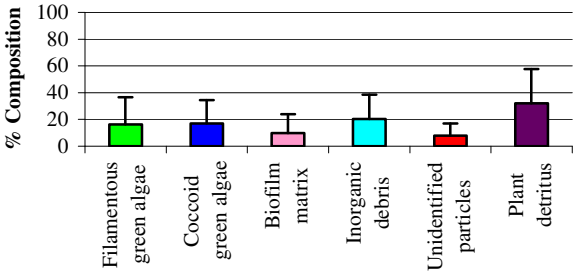


Fig. 3.11 Mean percentage composition of the gut contents of *Ecdyonurus venosus* in the treated section of the Castlebar River on 19/08/03. Bars are + standard deviation: $n = 3$.

Fig. 3.12A 100x *Ecdyonurus* spp. gill with distinct filament found in the gut of a 10.2mm *E.venosus* specimen in the control section of the split-stream experiment on 29th July 2003.

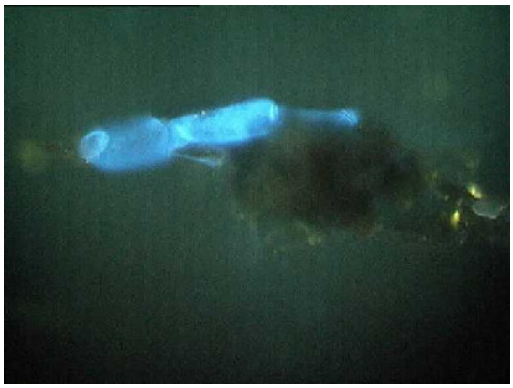


Fig. 3.12B 400x Femur of early invertebrate instar with agglomerate of detritus in the gut of a 10.2mm *E.venosus* specimen in the control section of the split-stream experiment on 29th July 2003.

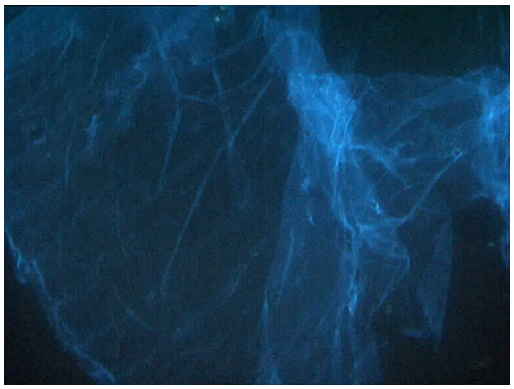


Fig. 3.12C 400x Peritrophic membrane of the gut of a 10.9mm *E.venosus* specimen fluorescing taken from the treated section of the split-stream experiment on 29th July 2003.

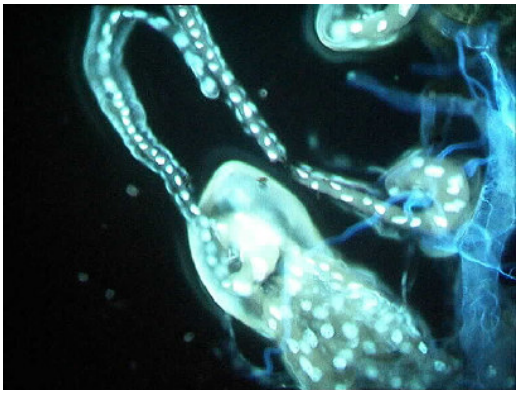


Fig. 3.12D 400x Filament of algae in spiral formation with nematode spp. in the gut of *E.venosus* in the treated section of the Castlebar River on 29th July 2003.

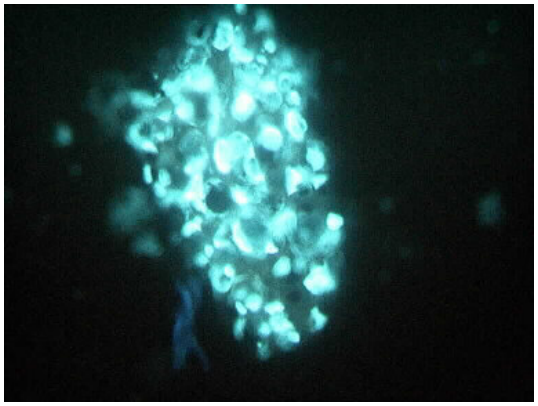


Fig. 3.12E 400x Coccoid green algal mass showing the chloroplasts and nuclei fluorescing under UV-light found in the gut of a 10mm *E.venosus* specimen in the treated section of the split-stream experiment.

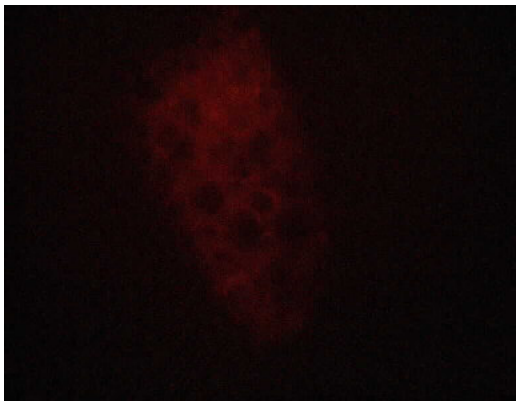


Fig. 3.12F 400x Coccoid green algae mass as in Fig. 2.20E but viewed under red light . Notice how the bright blue fluorescence of the chloroplasts and nuclei fade under this light. The algae cell walls are highlighted.

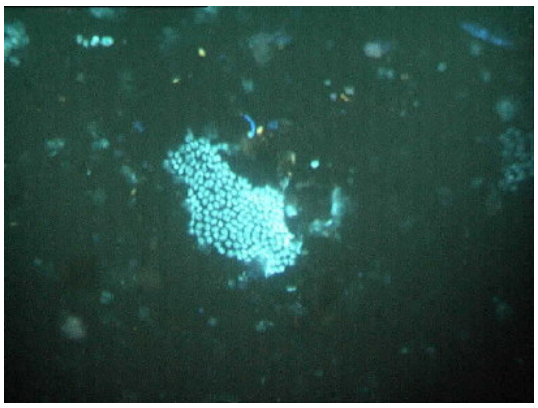


Fig. 3.13A 400x Coccoid green algae fluorescing bright blue in the gut of an 11.4mm *E.venosus* specimen in the control section of the split-stream experiment on 7th August 2003.

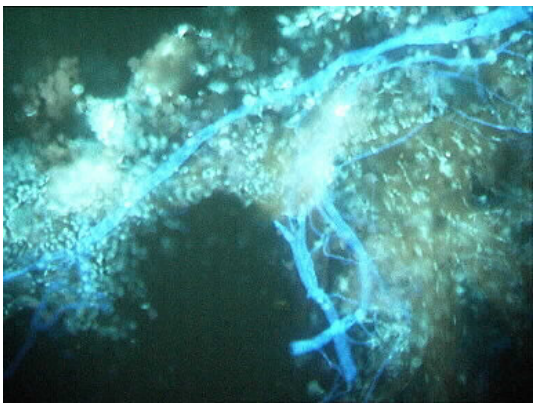


Fig. 3.13B 400x Coccoid green algae embedded in algal mass with a nematode sp. entwined in the agglomerate. Found in the gut of an 11.4mm *E.venosus* specimen in the control section of the Castlebar River on 7th August 2003.

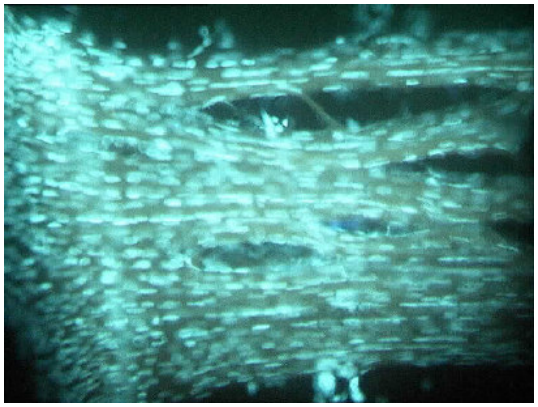


Fig. 3.13C 400x Algal agglomerate found in the gut of a 10.5mm *E.venosus* specimen in the treated section of the Castlebar River on 7th August 2003.

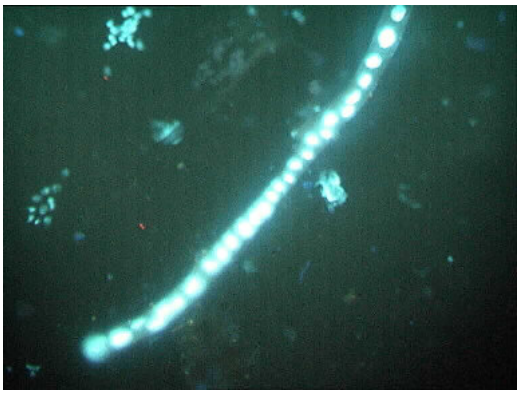


Fig. 3.13D 400x Filament of algae showing nuclei and chloroplasts fluorescing bright blue. Found in the gut of a 10mm *E.venosus* specimen in the treated section of the Castlebar River on 19th August 2003.

Fig. 3.13E 400x Coccoid bacteria fluorescing bright blue under UV-light in the gut of an 11mm *E.venosus* specimen taken from the control section of the split-stream experiment on 19th July 2003.

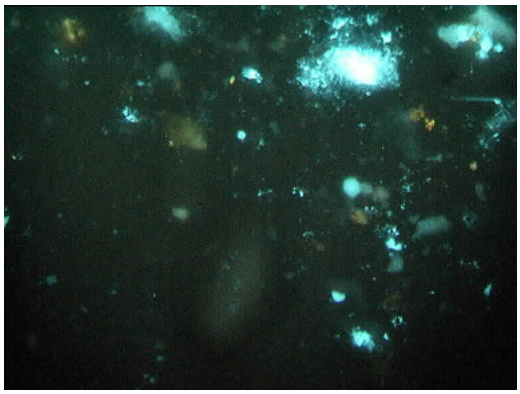


Fig. 3.13F 400x Photomicrograph taken from the gut of a 9mm *E.venosus* specimen in the control section of the Castlebar River on 19th July 2003. Plant detritus is clearly autofluorescing.

The gut contents of the larvae investigated on these occasions (Table 3.6) were similar to those studied on the 18/07/03 and 12/09/03 (Table 3.5). In comparison to those investigated pre- and post-nutrient addition, all specimens contained more filamentous algae with the exception of the sample examined on 07/08/03. There were no diatoms and desmids found in gut contents of the larvae examined on these other visits. Biofilm matrix, plant detritus and coccoid green algae made up a large proportion of the gut contents of these mayflies. The larvae ingested reasonable quantities of inorganic debris (mineral particles) and unidentified particles (Table 3.6). There were no algal agglomerates found in the ingested material of any of the larvae studied. The gut contents of one specimen showed some quite unexpected inclusions (Fig. 3.12A and B) containing the gill of an early instar of *Ecdyonurus* with the filament intact, along with the femur of an invertebrate instar with an agglomerate of detritus. This suggests that *Ecdyonurus* is relatively unselective in its feeding pattern as the animal appeared to ingest these animal remains together with its regular food items while it moved across the surface of the river substrate during a feeding episode.

The examination of these specimens provided preliminary results that contributed to knowledge of the diet of *Ecdyonurus venosus*. These results successfully validated the technique in isolating and identifying the material in the gut contents. This method was then employed on the larvae examined pre- and post-nutrient addition, which supplied the bulk of the information on gut content analyses in this investigation.

3.4.1.4 Results of gut analysis of Ecdyonurus venosus during spring 2003 investigation

As part of the investigation into the feeding regime of *Ecdyonurus venosus*, various other rivers were also sampled to obtain specimens for the gut analysis studies. Specimens of this mayfly species were collected from five high status rivers and five impacted rivers during March 2003. This investigation was carried out to provide information on the types of benthic diatoms consumed by these larvae. As diatoms are abundant in spring, it was decided to examine the gut contents of specimens sampled during this period. Intact samples were only present in three of the high status sites and two of the impacted sites investigated (N=5). The specimens were preserved in 70% IMS in the field and retained for later analysis. As mentioned in the Materials and Methods section, some of the gut contents were oxidised after dissection with 30% hydrogen peroxide, while the remainders were examined without treating. This was carried out for comparative purposes. The gut contents of each specimen was dissected and viewed under a compound light microscope without using any staining materials. Photomicrographs of the material found in these samples are illustrated in Fig. 3.14A-I. A list of algal taxa that were found in the gut contents of four *Ecdyonurus venosus* specimens taken from three high status sites in March 2003 are presented in Table 3.7. A list of algal taxa that were found in the gut contents of three specimens taken from two impacted sites are presented in Table 3.8.

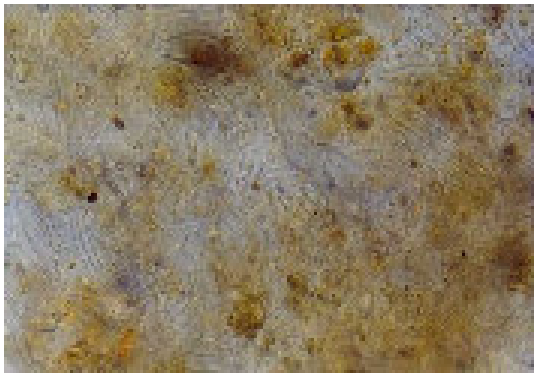


Fig. 3.14A 400x The gut contents of a 10mm specimen of *E.venosus* sampled from the Dunneill River in March 2003 showing an abundance of diatoms.

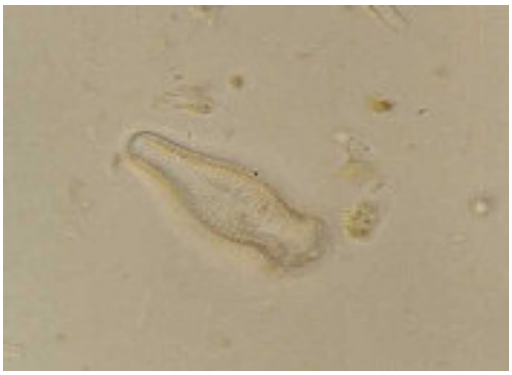


Fig. 3.14B 400x *Gomphonema trancatum* (Bacillariophyceae) found in the gut of a 10mm *E.venosus* specimen in the Dunneill River in March 2003.

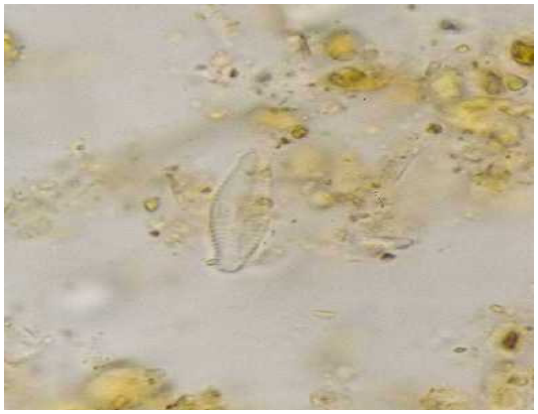


Fig. 3.14C 400x *Achnanthes lancelota* (Bacillariophyceae) found in the gut of a 14mm specimen of *E.venosus* in the Mullaghanoe River in March 2003.

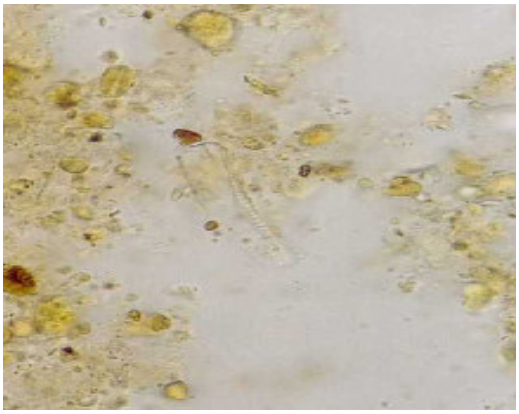


Fig. 3.14D 400x *Rhoicosphenia abbreviata* (Bacillariophyceae) in broken girdle view in the gut of a 14mm *E.venosus* specimen in the Mullaghanoe River during March 2003.

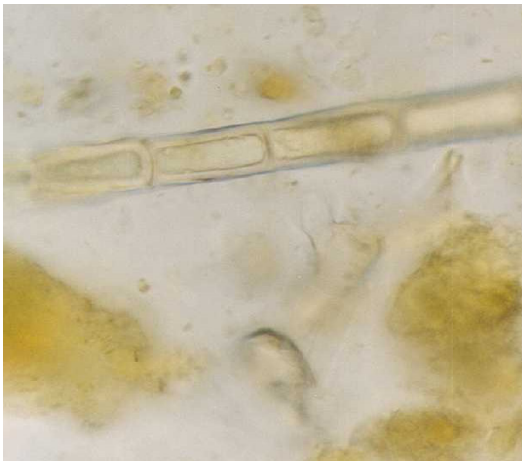


Fig. 3.14E 400x Filament of green algae found in the gut of a 14mm *E.venosus* specimen in the Castlebar River in March 2003.

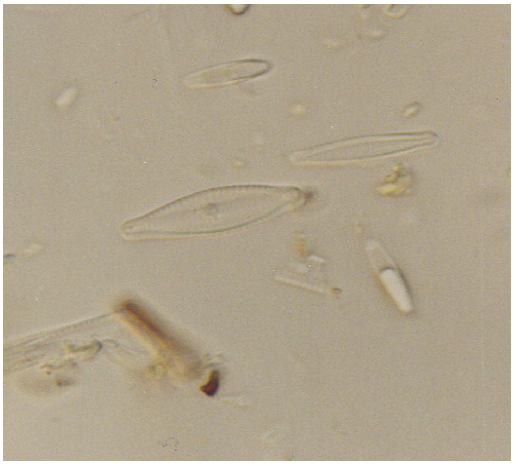


Fig. 3.14F 400x *Gomphonema parvulum* (Bacillariophyceae) in the gut of a 10mm *E.venosus* in the Dunneill River during March 2003.

Fig. 3.14G. 400x *Navicula lancelota* (Bacillariophyceae) found in the gut of a 14mm specimen of *E.venosus* the Castlebar River in March 2003.

Fig. 3.14H. 400x *Eutonia* spp. (Bacillariophyceae) found in the gut of a 15.6mm specimen *E.venosus* in Callow Loughs Stream River during March 2003.

Fig. 3.14I. 400x *Cocconeis placentula* (Bacillariophyceae) found in the gut of a 14mm specimen of *E.venosus* the Castlebar River in March 2003.

Table 3.7 Algal taxa found in the gut contents of four specimens of *Ecdyonurus venosus*, in three high status rivers during March 2003.

River	Body length (mm)	Algal taxa
Castlebar **Non-oxidised sample**	14	*Achnanthes lanceolata* *Cocconeis placentula* *Cymbella minuta* *Fragilaria capucina* *Fragilaria capucina var. vaucheriae* *Gomphonema clavatum* *Gomphonema parvulum* *Meridion circulare* *Navicula gregaria* *Navicula lanceolata* *Nitzschia dissipata* *Reimeria sinuata* *Rhoicosphenia abbreviata* Green filament–not long enough to identify
Dunneill **Non-oxidised sample**	13	*Achnanthidium minutissimum* *Diatoma moniliformis* **Gomphonema olivaceum** *Gomphonema parvulum* *Navicula lanceolata* *Reimeria sinuata*
Dunneill **Non-oxidised sample**	10	*Achnanthidium minutissimum** *Achnanthes lanceolata* *Cocconeis placentula* *Cymbella minuta* *Cymbella silesiaca* *Diatoma moniliformis* *Gomphonema parvulum* *Gomphonema truncatum* *Navicula lanceolata* *Nitzschia dissipata* *Reimeria sinuata* *Achnanthes* spp.
Callow Loughs Stream **Oxidised sample**	15.6	*Achnanthes biasolettiana* *Achnanthidium minutissimum* *Cocconeis placentula* *Eunotia* spp. *Fragilaria capucina var. vaucheriae* *Gomphonema olivaceum* *Meridion circulare* *Navicula lanceolata* *Nitzschia dissipata* *Synedra ulna*

**Achnanthidium minutissimum* was the dominant algal taxa found in the gut contents of these specimens of *Ecdyonurus venosus*.

Table 3.8 Algal taxa found in the gut contents of three specimens of *Ecdyonurus venosus* in two-impacted rivers during March 2003.

River	Body length (mm)	Algal taxa
Robe **Non-oxidised sample**	8.5	*Reimeria sinuata** *Achnanthes lanceolata* *Achnanthidium minutissimum* *Amphora pediculus* *Cocconeis placentula* *Gomphonema olivaceum* *Gomphonema parvulum* *Navicula tripunctata* *Achnanthes* spp.
Mullaghanoe **Oxidised sample**	15.6	*Achnanthes lanceolata* *Achnanthidium minutissimum* **Cocconeis placentula** *Fragilaria capucina* var. *vaucheriae* *Gomphonema angustum* *Gomphonema micropus* *Gomphonema parvulum* *Meridion circulare* *Navicula gregaria* *Navicula lanceolata* *Surirella brebissonii*
Mullaghanoe **Non-oxidised sample**	14	*Achnanthes lanceolata* *Achnanthidium minutissimum* *Cocconeis placentula* *Fragilaria capucina* var. *vaucheriae* *Gomphonema angustum* *Gomphonema micropus* *Gomphonema parvulum* *Meridion circulare* *Navicula lanceolata* *Nitzschia dissipata* *Reimeria sinuata* *Rhoicosphenia abbreviata*

* *Reimeria sinuata* was the dominant algal taxa found in the gut contents of this specimen of *Ecdyonurus venosus*.

The specimens examined from the high status and impacted rivers yielded some interesting findings, showing an abundance of diatoms in the gut contents (Tables 3.7 and 3.8) which corresponds with increased diatom growth during the spring season. The ingested food of the invertebrates that were not subject to oxidation tended to contain more diatoms than the treated ones, suggesting loss of algal taxa when using the oxidation technique. Only three of the eight oxidised samples examined contained diatoms, which were present in small numbers. Four out of the seven untreated samples contained diatoms and were particularly abundant in the Castlebar and Dunneill Rivers.

The dominant algal taxon found in the stomachs of the animals in the Dunneill River, the Castlebar River and Callow Loughs Stream (high status rivers) was *Achnanthidium minutissimum*. *Cocconeis* spp. was also common in the guts of these animals. *Achnanthidium minutissimum* is a broad spectrum species often recorded as abundant in epilithic diatom river studies in Ireland, Britain, Europe, Canada and the US (Ní Chatháin, 2002; Sabater *et al*., 1988; Tomas and Sabater, 1985; Cox, 1990; Dixit and Smol, 1994; Pan *et al*., 1996; Rott *et al*., 1998). It is a ubiquitous species, one that generally dominates diatom assemblages on cobbles in rivers of varying water quality, therefore it is not a great indicator of any particular environmental condition.

It does however seem to be more abundant in high status rivers, which was also found in studies carried out during the RIVTYPE project in Ireland in 2002 and 2003 (Ní Chatháin *et al*., 2004). Empty filaments of green algae were also found in the gut contents of *Ecdyonurus venosus* in the Castlebar River but could not be identified with confidence due to the effects of the digestion process on its structure. The impacted sites also contained an abundance of diatoms. *Achnanthidium minutissimum* and *Achnanthidium lanceolata* were also found in the guts of larvae sampled from the impacted sites but were not as abundant. The most abundant diatom present in the gut of one animal sampled from the Robe River (impacted site) was *Reimeria Sinuata*. Overall the diversity of diatoms found in the high status and impacted sites were quite similar. Based on the small number of animals

dissected in this spring study, it is very difficult to say whether there was a difference between the high status and the impacted samples.

3.4.2 Analysis of stone scrapings

The following groups were quantified from the stone scrapings using a combination of both the Lugols and DAPI stains: diatoms, filamentous green algae, plant detritus and inorganic debris. This technique did not stain biofilm matrix, coccoid green algae, algal agglomerates and coccoid bacteria as effectively as using DAPI stain alone and unfortunately these items could not therefore be included in the percentage compositional breakdown of the stone scrapings for comparison purposes. A summary of the percentage composition of the stone scrapings taken from a pooled sample on either side of the experimental divide on 21st July and 9th September 2003 is outlined in Table 3.9.

Table 3.9 The mean percentage composition of particulate material in the stone scrapings observed on the 21st July and 9th September 2003.

	21st July 2003	21st July 2003	9th September 2003	9th September 2003
Food category	**Treated section**	**Control section**	**Treated section**	**Control section**
Diatoms	17%	24%	32%	32%
Filamentous green algae	2%	10%	2%	6%
Inorganic debris	23%	10%	-	3%
Plant detritus	58%	48%	66%	59%
Unidentified particles	-	8%	-	-

3.4.2.1 Stone scraping results 'pre-nutrient addition' (21st July 2003)

The mean percentage composition of the particulate material in the stone scrapings taken in the treated and control sections of the split-stream experiment on 21st July 2003 (pre-nutrient addition) are graphically represented in Fig. 3.15, 3.16.

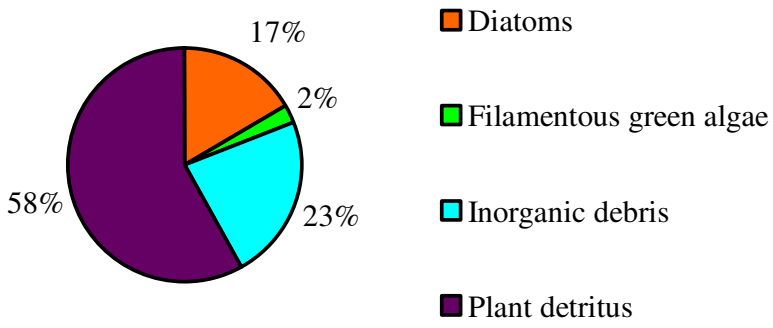


Fig. 3.15 Mean percentage composition of particulate matter in the stone scrapings in the treated section of the Castlebar River on 21st July 2003.

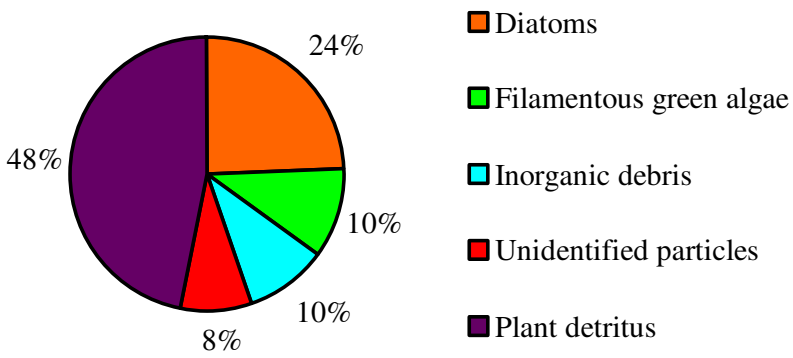


Fig. 3.16 Mean percentage composition of particulate matter in the stone scrapings in the control section of the Castlebar River on 21st July 2003.

3.4.2.2 Stone scraping results 'post-nutrient addition' (9th September 2003)

The mean percentage composition of the particulate material in the stone scrapings taken from both sides of the experiment on 9th September 2003 (post-nutrient addition) are presented in Fig. 3.17 and 3.18.

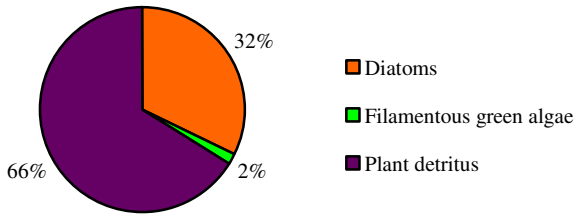


Fig. 3.17 Mean percentage composition of particulate matter in the stone scrapings in the treated section of the Castlebar River on 9th September 2003.

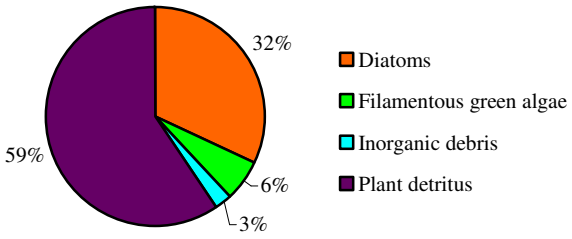


Fig. 3.18 Mean percentage composition of particulate matter in the stone scrapings in the treated section of the Castlebar River on 9th September 2003.

The mean percentage composition of particulate material in the treated and control sections of the experiment on the 21st July 2003 (post-nutrient addition) appeared to be similar to those observed on the 9th September 2003 (pre-nutrient addition). The particulate material in the stone scrapings taken from the treated and control sections of the split-stream experiment on the 21st July 2003 contained 17% and 24% of diatoms respectively (Table 3.9). The most common diatom found in these samples was *Navicula* spp.

Filamentous green algae represented 2% and 10% of the material studied respectively. Plant detritus represented a large proportion of the total particulate area examined in these samples, accounting for 58% and 48% of the samples in the treated and control sections respectively. The particulate matter found in the stone scrapings from the treated and control sections contained 23% and 10% inorganic debris respectively. Unidentified particles make up 8% of the sample examined in the control section, while there was none found in the treated side.

The mean percentage composition of diatoms was higher in both sections of the study in September 2003, where they represented 32% of the total particulate material in each sample analysed (Table 3.9). Plant detritus was present in large amounts, accounting for 66% and 59% of the samples studied in the treated and control, respectively. Inorganic debris was found in the control section of the study corresponding to 3% of the material in the sample.

The material found in the stone scrapings did not fluoresce well, as explained earlier, due to the preservative Lugols iodine interfering with the DAPI stain which is also evident in the photomicrographs illustrated in Fig. 3.19A-F. The algal cells, in particular filamentous green algae, coccoid green algae and algal agglomerates, do not fluoresce as effectively when stained initially with Lugols iodine (Fig. 3.19D, E and F). A claw from an early invertebrate instar was found in the stone scrapings taken from the treated section of the split-stream experiment on 9th September 2003 (Fig. 3.19A).

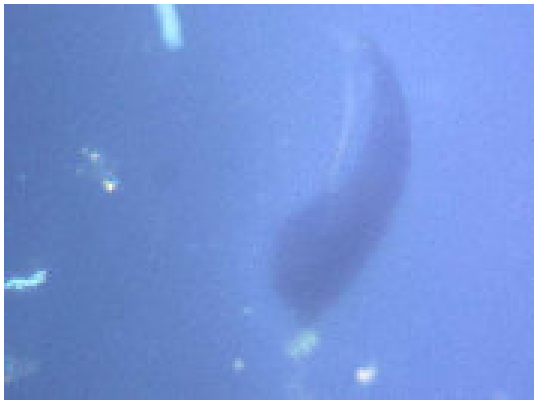


Fig. 3.19A 100x Invertebrate claw found in the stone scrapings taken from the treated section of the split-stream experiment on 9th September 2003.

Fig. 3.19B 400x Plant detritus and unidentified particles in a sample of stone scrapings taken from the control section of the experiment on 9th September 2003

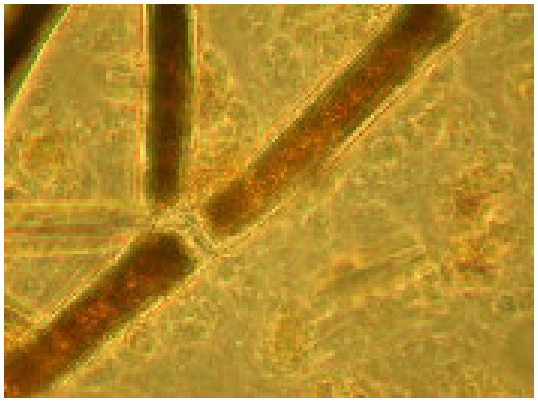


Fig. 3.19C 200x Filament of algae stained using Lugols iodine highlighting the brown chlorophyll taken from the stone scrapings in the control section of the split-stream experiment on 21st July 2003.

Fig. 3.19D 200x Filament of algae taken from a stone scraping from the treated section of the split-stream experiment on 21st July 2003. The sample was preserved in Lugols and subsequently stained with DAPI.

Fig. 3.19E 200x Filament of algae and unidentified mass to the left. Material did not fluoresce as effectively as when using DAPI stain alone and appeared dull due to the Lugols in the sample.

Fig. 3.19F 400x Plant detritus and algal agglomerate. Nuclei and chloroplasts are not fluorescing due to the Lugols staining the cells.

3.4.2.3 Algal composition in pre- and post-nutrient addition samples

The algal material in the stone scrapings was examined quantitatively to compile a list of taxa present in each of the four samples investigated. The mean percentage composition of the algal taxa found in the stone scrapings (15 fields viewed per sample) are summarised in Table 3.10. The mean percentage composition of each algal species found in each sample studied, are graphically represented in Figs. 3.20 to 3.23. Examples of the algal taxa found in the stone scrapings are illustrated in Figs. 3.24A-F.

Table 3.10 Mean percentage composition of algal taxa in four samples obtained from stone scrapings in the Castlebar River on two separate occasions during 2003.

	21st July 2003	21st July 2003	9th September 2003	9th September 2003
Algal list	**Control section**	**Treated section**	**Control section**	**Treated section**
Fragilaria spp.	24%	55%	35%	22%
Navicula spp.	12%	18%	3%	9%
Cocconeis spp.	30%	12%	14%	6%
Meridion spp.	11%	-	13%	7%
Cryptomonas spp.	14%	-	17%	39%
Cyclotella spp.	5%	-	4%	2%
Gomphonema spp.	3%	-	3%	4%
Trachelomonas spp.	1%	-	4%	2%
Melosira spp.	-	3%	1%	1%
Anabaena spp.	-	8%	-	-
Oscillatoria spp.	-	4%	-	-
Cymbella spp.	-	-	6%	4%
Oedogonium spp.	-	-	-	3%
Mougeotia	-	-	-	1%

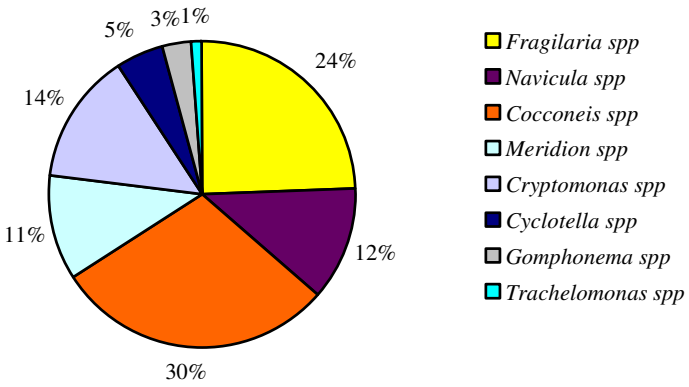


Fig. 3.20 Mean percentage composition of epilithic algal taxa in the control section of the Castlebar River on 21st July 2003.

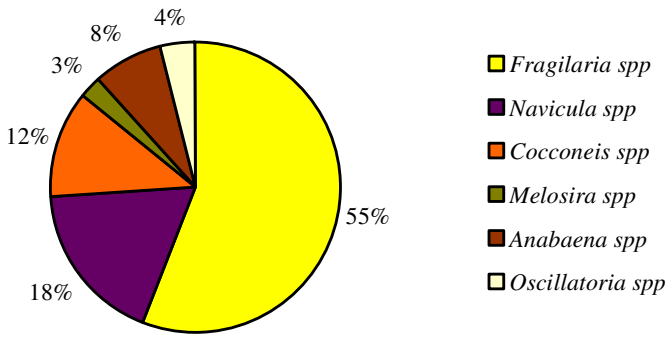


Fig. 3.21 Mean percentage composition of epilithic algal taxa in the treated section of the Castlebar River on 21st July 2003.

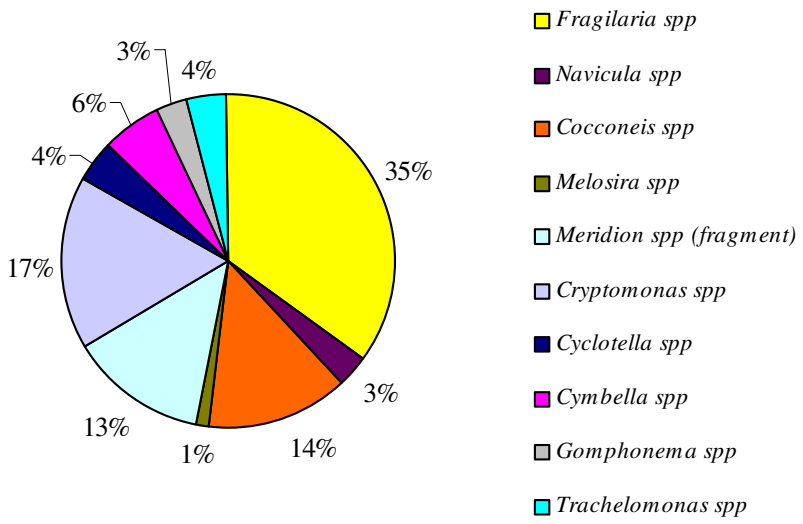


Fig. 3.22 Mean percentage composition of epilithic algal taxa in the control section of the Castlebar River on 9th September 2003.

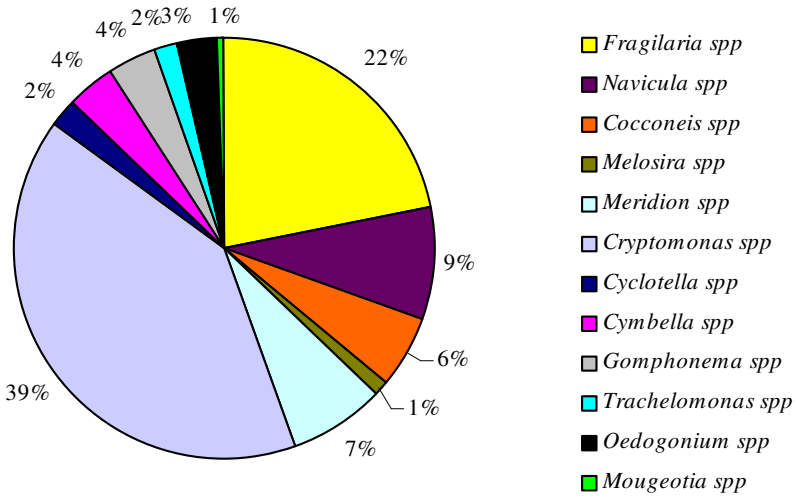


Fig. 3.23 Mean percentage composition of epilithic algal taxa in the treated section of the Castlebar River on 9th September 2003.

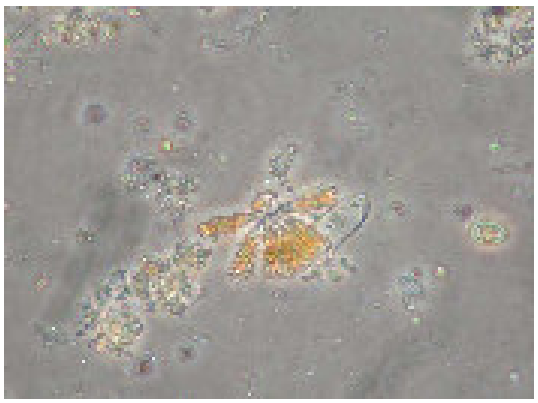


Fig. 3.24A 200x *Meridion* spp. (Bacillariophyceae) from the stone scrapings taken form the treated section of the Castlebar River on 9th September 2003 (post nutrient addition).

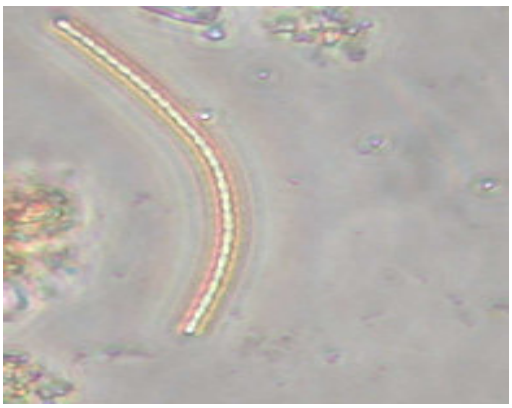


Fig. 3.24B 200x *Oscillatoria* spp. (Cyanobacteria) taken from stone scrapings in the control section of the Castlebar River on 21st July 2003 (pre-nutrient addition)

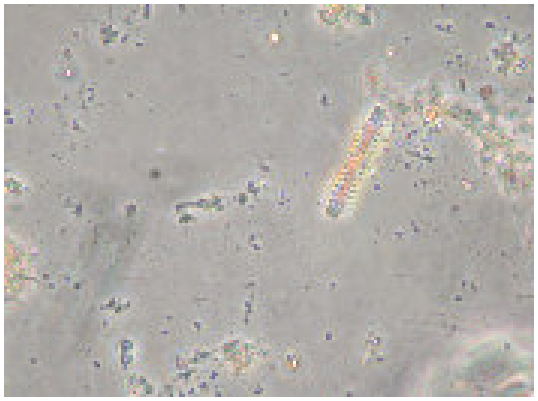


Fig. 3.24C 200x *Fragilaria* spp. (Bacillariophyceae) in the stone scrapings from the control sections of the Castlebar River on 21st July 2003.

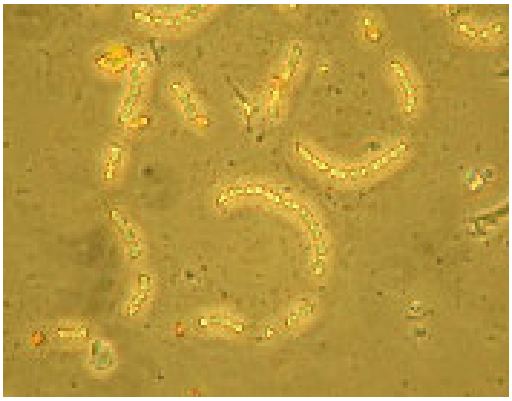


Fig. 3.24D 200x *Anabena* spp. (Cyanobacteria) found in the stone scrapings taken from the treated section of the Castlebar River on 21st July 2003.

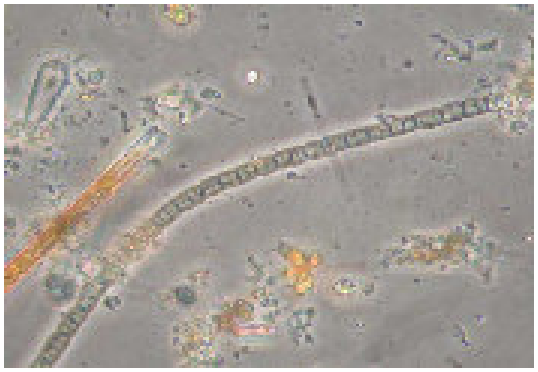


Fig. 3.24E 200x *Melosira* spp. (Bacillariophyceae) found in the stone scrapings in the treated section of the Castlebar River on 21st July 2003.

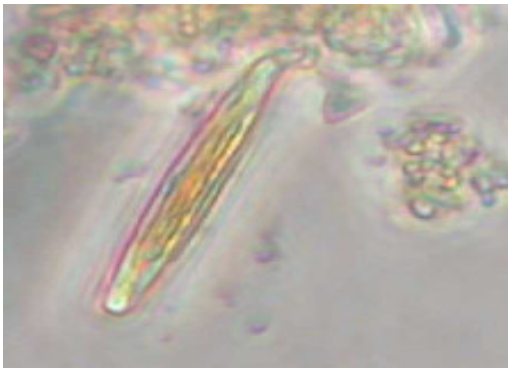


Fig. 3.24F 200x *Navicula* spp. (Bacillariophyceae) common to all samples taken on both sampling occasions during the study.

The algal taxa found in the stone scrapings are all common species present in Irish rivers and are listed in Table 3.10. The most common genera found in this river were *Fragilaria* spp., *Navicula* spp., and *Cocconeis* spp., which were present in all four samples on both sampling occasions. *Fragilaria* spp. was the most abundant algal species in the river during the study. The mean percentage composition of *Fragilaria* spp. in the treated section dropped from 55% when sampled on 21st July (pre-nutrient addition) to 22% when sampled on 9th September (post-nutrient addition). The mean percentage composition of this species in the control section on 21st July was 24% compared to 35% in the same section on the 9th September. The abundance of *Fragilaria* spp. appeared to fluctuate between sampling occasions. The mean percentage composition of *Navicula* spp. and *Cocconeis spp*. were lower in the autumn samples (9th September) in both the control and the treated sections of the experiment, compared to the summer samples (Table 3.10). On both sampling occasions, the mean percentage composition of algal taxa in the control sections of the experiment was quite similar (Fig. 3.20 and Fig. 3.22).

Some algal species were found only in the treated section of the experimental divide post-nutrient addition and were not present in the same section when sampled prior to the addition of nutrient (Table 3.10, Fig. 3.21 and Fig. 3.23). It is not evident why this should happen, but may be due to the natural variation in the algal community located within the river system or to differences between sampling locations. The blue green algae, *Anabaena* spp. and *Oscillatoria* spp. were found in the samples taken from the treated section on 21st July 2003. They were represented in small amounts, accounting for 8% and 4% of the total sample taken on this sampling date. Both of these genera are best known as 'nuisance organisms' but as they were not found in any of the other samples investigated during this study, in particular in the samples taken post-nutrient addition, they do not indicate eutrophic conditions in the river. They may, however, be favoured by the fact that the system appears to be nitrogen limited as some species at least are nitrogen fixers. *Cymbella* spp. was only present in the samples analysed post nutrient addition accounting for 6% and 4% of the total algal taxa found in the control and the treated

sections respectively. This species was not present in the samples analysed pre-nutrient addition. It is a large genus that contains many common genera and some species occur in all water types so its presence/absence is not indicative of the trophic status of this river. The algal taxon, *Oedogonium* spp. was found on one occasion only. It was present in the sample taken in the autumn (post-nutrient addition) in the treated section, representing 3% of the total algal taxa in this sample. These algae are abundant in stagnant or slow-flowing shallow waters and are most frequently found in midsummer (Pentecost, 1984). Samples were only taken on two occasions, on both sides of the experimental divide. Ideally, sampling frequency would need to be increased to a weekly sampling regime.

3.4.2.4 Summary of results from epilithic scrapings

The stone scrapings were examined quantitatively using inverted microscopy to compile a list of algal taxa. The scrapings were also examined using the same epifluorescent technique that was applied to the gut contents. Filamentous green algae, algal agglomerates and coccoid green algae were identified in the stone scrapings using this technique but it was impossible to categorise these into more precise taxonomic groups. This epifluorescent technique did not detect the array of algal taxa that were found in the stone scrapings, compared with that found when using inverted microscopy.

The stone scrapings were firstly assessed by use of the mean percentage composition of particulate matter, which was estimated using the same technique as was applied to the gut analysis specimens. As mentioned previously, the preservative Lugol's stains algal cells a dark brown but it can mask chlorophyll fluorescence (Gifford and Caron, 2000). As a result, not all of the particulate food categories found in the gut contents of the *Ecdyonurus venosus* specimens were identifiable in the stone scraping samples. Plant detritus was again the dominant material found in all of these samples. Proportions were slightly higher in September compared to July, where the largest amount (66%) was found in the 'treated' section. Diatoms were present in all samples and were particularly abundant in September 2003, accounting for 32% of the total particulate matter in each of the samples taken from the 'treated' and 'control' sections of the experiment. Filamentous green algae were again present in reasonable amounts in the stone scraping samples throughout the study. Overall, there were no marked differences proportionally in the percentage composition of particulate matter between the stone scraping samples.

Secondly, the mean percentage composition of epilithic algal taxa was examined during the study. The dominant species of algae on the stones in the river throughout the study were *Fragilaria* spp., *Navicula* spp. and *Cocconeis* spp. with the most common being *Fragilaria* spp. The percentage mean composition of all three species in the 'treated' section

sampled on 21st July was nearly double the amount found in the same section on 9th September. The stone scrapings taken in the autumn had half the amount of *Navicula* spp. and *Cocconeis* spp. present when compared with the summer samples, in terms of their percentage mean composition. The dominant diatom found in the guts of *Ecdyonurus venosus* in this river was *Navicula* spp. albeit diatoms as a group were a relatively small proportion of the total food items found in the gut of *Ecdyonurus* (2% and 7% in July and September respectively). These findings suggest that grazing pressures by this mayfly may have reduced *Navicula* spp. as its abundance increased on the substrata in the autumn months. It is also very important to note that the relative amounts of diatoms in the gut contents studied vs the stone surfaces seems to be lower indicating an avoidance of diatoms by *Ecdyonurus*. They also suggest that grazing on *Fragilaria* spp. and *Cocconeis* spp. increased, owing to the reduction of their proportions in the autumn as the diatom growth reached a peak. Due to the absence of these diatom species in the gut contents of *Ecdyonurus venosus*, it suggests that they were grazed on by other macroinvertebrates in the river system. Alternatively, it may be the result of changes in taxonomic composition occurring in the river caused by direct manipulation of the river using phosphorus but due to the small sample number investigated in this study, this must remain as speculation.

Interestingly, *Cocconeis* spp. is often considered to be a grazer resistant diatom species (Pan and Lowe, 1994). This adnate diatom is commonly present and often dominant in algal communities grazed by caddisflies (Lamberti *et al*, 1987; Steinman *et al*, 1987), mayflies (Colletti *et al*, 1987; Hill and Knight, 1987), or snails (Steinman *et al*., 1987; Lowe and Hunter, 1988). *Cocconeis* spp. and *Navicula* spp. may have experienced nutrient limitation and light limitation causing a reduction in their abundance, due to the growth of other overstory species like *Fragilaria* spp. *Cocconeis* spp. can secrete a tough mucilage which can secure the firm attachment of the entire valve to substrate (Patrick, 1948) making grazing difficult. This may account for the lack of *Cocconeis* spp. in the gut contents of *Ecdyonurus venosus* and its abundance in the stone scraping samples. *Fragilaria* spp.

may also be difficult for *Ecdyonurus venosus* to graze on due to the physiognomy of its structure.

3.4.3 Comparison of Ecdyonurus Gut Contents with Stone Scrapings

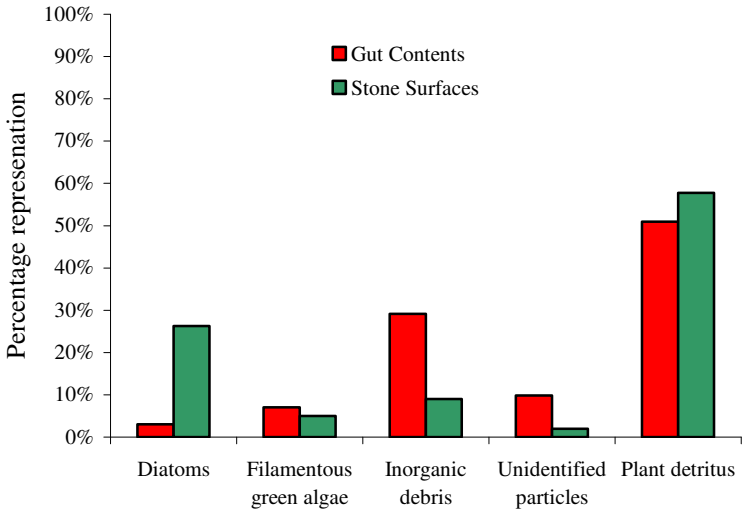


Fig. 3.25 Comparison of *Ecdyonurus* gut contents and stone surfaces for those items that could be identified in both sets of samples: diatoms, filamentous green algae, inorganic detritus, plant detritus and unidentified particles.

On a sample basis, the mean percentage composition of diatoms in the stone scrapings was higher than that found in the gut contents of *Ecdyonurus venosus* (Fig. 3.25). It is thought that direct sampling of the river substrate, may have contributed to the higher proportion of diatoms being detected in these samples on both sampling occasions. As the samples were taken fresh from the cobble substrates, more diatoms and algae were sampled without damaging the algal community structures. In addition to this, the content of the material available on the stone surfaces, during a particular feeding episode, would have been a factor determining the overall composition of food items in the invertebrate gut. Diatom growth is at its peak during the spring an autumn months, owing to their abundance in the stone scrapings in September compared to July 2003. Some of the stomachs may have been altered due to the length of time between sampling and dissection.

Fig. 3.25 attempts to make a comparison between the *Ecdyonurus* gut contents and the epilithic material taken from the stone surfaces on which they live and feed. The percentage breakdown is based on the average of all the samples taken. The graph suggests that *Ecdyonurus* do not select diatoms in that they are generally under represented in comparison with the stone surfaces whereas inorganic debris seems to be present in greater quantities than would be expected for a non-selective grazer. Plant detritus dominates the samples of both gut contents and the stone surfaces with little difference apparent.

A Chi-square analysis was carried out on a number of different treatment combinations of the gut contents of *Ecdyonurus venosus* and the stone scrapings taken from the Castlebar River during the summer of 2003 (Table 3.11) to establish if there were any similarities between the gut contents and the food material scraped from the stone surfaces.

Due to the variability in the techniques used in analysing the gut contents and the stone scrapings, only the following food groups were assessed in the Chi-square analysis: Diatoms, filamentous algae, Inorganic detritus, unidentified particles and plant detritus. As in Fig. 3.25, the analysis is based on the average of all the samples taken from the gut contents and the stone scrapings during the entire study and results are outlined in Table 3.11.

Table 3.11 Results of the Chi-square analysis carried out on different treatment combinations of the gut contents of *Ecdyonurus venosus* and the stone scrapings taken from the Castlebar River during the summer of 2003.

Treated v Control sections		Treatment combinations	Chi-square X^2	Degrees of freedom (df)	*p*-value
Treated section	**1**	Post-nutrient addition gut contents v Pre-nutrient addition gut contents	68.5	4	<0.001***
Control section		Post-nutrient addition gut contents v Pre-nutrient addition gut contents	31.8	4	<0.001***
Treated section	**2**	Post-nutrient addition stone surfaces v Pre-nutrient addition stone surfaces	37.3	4	<0.001***
Control section		Post-nutrient addition stone surfaces v Pre-nutrient addition stone surfaces	19.7	4	<0.001***
Treated section	**3**	Pre-nutrient addition stone surfaces v Pre-nutrient addition gut contents	12.1	4	<0.05*
		Post-nutrient addition stone surfaces v Post-nutrient addition gut contents	330.8	4	<0.001***
Control section	**4**	Pre-nutrient addition stone surface v Pre-nutrient addition gut contents	15.9	4	<0.01**
		Post-nutrient addition stone surfaces v Post-nutrient addition gut contents	84.7	4	<0.001***
	5	Pre-nutrient addition gut contents **treated section** v Pre-nutrient addition gut contents **control section**	5.1	4	0.3 ns
	6	Post-nutrient addition gut contents **treated section** v Post nutrient addition gut contents **control section**	96.1	4	<0.001***
	7	Pre-nutrient addition stone surfaces **treated section** v Pre-nutrient addition stone surfaces **control section**	35.4	4	<0.001***
	8	Post-nutrient addition stone surfaces **treated section** v Post-nutrient addition stone surfaces **control section**	6.5	4	0.164 ns

p-values: *P<0.05; ** P<0.01; *** P<0.001; ns – not significant

Significant differences in the gut contents were shown in the treated section (p<0.001) and the control sections (p<0.001) both pre- and post-nutrient addition (Treatment combination 1). There were also significant differences found in the stone scrapings between the treated (p<0.001) and the control sections (p<0.001) both pre- and post nutrient addition (Treatment combination 2). While the p-values show significant differences in the gut contents and the stone scrapings, these are merely reflecting the natural variability in the diatoms and plant detritus in the river during the experiment, as the differences were present both before and after the nutrient was added to the experimental section. The significant differences may also reflect the natural changes that occur in plant biomass in the river.

Significant differences between the gut contents of *Ecdyonurus venosus* and the material found on the stone scrapings were found in the treated section (Treatment combination 3) both pre-nutrient addition (<0.05) and post nutrient addition (<0.001). Significant differences were also found in the control section (Treatment combination 4) both pre-nutrient addition (<0.01) and post nutrient addition (<0.001). There appears to be more diatoms and plant detritus on the stone surfaces in both the control and treated sections post nutrient addition. Diatoms are generally more abundant in the spring and autumn months compared to the summer season which may explain the increase in diatoms in the stone surfaces post-nutrient addition (September) compared to the scrapings taken pre-nutrient addition which is contributing to the overall increased significant value. Plant detritus was slightly higher in the stone scrapings post-nutrient addition and may have been due to the natural variation in plant material along the sampling gradient in the experiment.

No significant differences in the gut contents were found between the treated and the control sections pre-nutrient addition (Treatment combination 5 – p=0.3). Significant differences in the gut contents were found between the treated and the control sections post-nutrient addition (Treatment combination 6 – p<0.001). There was more plant detritus found in the guts of the larvae taken from the treated section (49%) compared to the control section (35%) however the control section did contain slightly

more diatoms (9%) compared to the treated section (3%). Inorganic debris was higher in the gut contents taken from the control section (41%) compared to the treated section (21%). The significant difference found in the gut contents between the treated and the control sections post-nutrient addition appears to be attributed to the natural variation in the food items available to *Ecdyonurus* during a particular feeding episode.

A significant difference in the content of the stone surfaces ($p<0.001$) between the treated and the control section (treatment combination 7) was evident pre-nutrient addition highlighting the natural variability in the epilithic material on both sections of the experimental divide prior to the addition of the nutrient. No significant difference ($p=0.164$) was found in the content of the stone surfaces between the treated and the control sections post-nutrient addition (Treatment combination 8).

3.5 Discussion

3.5.1 Introduction

Ecdyonurus is widely regarded as a sensitive taxon and indicator of good water quality. A number of different hypotheses have been proposed for the sensitivity of macroinvertebrates such as *Ecdyonurus* ranging from toxicity effects, low oxygen concentrations, habitat siltation to food chain changes due to enrichment.

In Ireland *Ecdyonurus* appears to be a particularly useful indicator of eutrophication. In this context, therefore, the feeding regime of *Ecdyonurus* is of interest. Thus, in this study, it was aimed to establish a method to access the feeding habits of *Ecdyonurus venosus* by examining their gut contents, in a small river in the West of Ireland. In a separate experiment, a nutrient manipulation trial was set up in the Castlebar River, in an attempt to artificially enrich one section of the river, during the summer of 2003 (Chapter 2). This provided an opportunity to assess whether such direct manipulation had any obvious impact on the diet of *Ecdyonurus*. The addition of phosphorus nutrient to one side of the split-stream experiment, designed to raise the ambient nutrient concentration by 10 µgP/l, appeared to cause an increase in the periphyton biomass, especially in the last three weeks of the study. This experiment was used to investigate the diet of *Ecdyonurus venosus* in this river before and after the nutrient manipulation. It was hypothesised that the gut contents of the larvae would reflect the changes in the algal biomass variation exhibited in the experimental site and possibly reflect a variation in algal taxa between treated and control sections of the experimental section.

Thus, the overall objectives were to establish what this key indicator species was feeding on and to assess, the changes, if any, in its feeding regime during the nutrient manipulation experiment.

3.5.2 Summary of findings

This study investigated the gut contents of *Ecdyonurus* over a number of sampling occasions and initial findings give a good indication as to what these larvae are eating. The study did not try to document the entire range of feeding behaviour of the species, or to determine the nutritional significance of the material ingested. It is also important to remember that gut content studies only allow for determination of what is being consumed and not what is being assimilated.

A preliminary examination of the gut contents of *Ecdyonurus* larvae sampled during spring 2003, using the cold acid hydrogen peroxide oxidation technique, was not very successful in isolating diatoms from the specimens. Untreated guts were also examined and appeared to be more effective in isolating the diatoms and the occasional filament of algae. Other particles were found in the guts but were unidentifiable. This technique however, was unable to detect any other items ingested that would assist in increasing our knowledge of the diet of this mayfly.

The epifluorescent DAPI technique proved a very effective method in isolating the dietary gut contents of these larvae. The results from the preliminary investigation carried out on a few specimens, successfully validated this method in isolating and identifying a variety of food categories. The technique helped greatly in the categorisation of *Ecdyonurus* into its functional feeding group in this study.

The stone scrapings were assessed and quantified using a combination of both DAPI and Lugols stain. This method failed to stain biofilm matrix, coccoid green algae, algal agglomerates and coccoid bacteria due to the masking effect of Lugols on chlorophyll fluorescence. A new technique is being developed to stain Lugols fixed plankton samples with DAPI. This method is being prepared for publication (Struder-Kype *et al*., in prep) and is outlined in the methods section in the Guide to UK Coastal Planktonic Ciliates (2001). This allows the nuclear shape of microplankton to be used as a diagnostic feature in routine plankton analysis.

On the basis of the findings from the gut analysis studies carried out in the Castlebar River in 2003, the diet of the mayfly *Ecdyonurus venosus* mainly consisted of epilithic algal tissue, plant particulate matter (detritus), biofilm matrix and inorganic debris (mineral material). The *Ecdyonurus* guts sampled in March 2003 included diatoms and sporadic findings of filamentous green algae. It can therefore be classified as both a herbivorous grazer and detritus feeder. Due to the presence of dead animal remains in the guts on occasion, this genus may have an opportunistic feeding tendency or it may be simply be that this type of material is ingested as the animal grazes across the substrata. Interestingly, Moog (1995) also suggests that this genus may function as both a grazer and a detritus feeder. The food preference of these larvae appears to be strongly dependent on the food particles present in the environment at a given time. Thus the diet of the larvae of the studied species depends on the food resources of the environment. From our findings, this mayfly feeds on those particles which occur in the habitat in the greatest quantities or else those that are easily accessible. This may explain the differences in the proportions of diatoms in the guts of the animals investigated in March 2003 compared to those studied from July-September 2003.

The majority of authors place aquatic insects in the trophic chain in the position of primary consumers – herbivores, or secondary consumers – predators (Cummins *et al*., 1966; Minshall, 1967; Hynes, 1970; Coffman *et al*., 1971; Cummins, 1973; Shapas and Hilsenhoff, 1976; Gray and Ward, 1979). Ephemeropteran larvae are described by many authors as herbivores (Minshall, 1967; Coffman *et al*., 1971; Edmunds *et al.*, 1976) or as herbivore-detritivores (Shapas and Hilsenhoff 1976; Gray, Ward, 1979). According to Klonowska (1986), *Ecdyonurus venosus* larvae can be considered mainly as grazers/scrapers feeding chiefly on diatoms and sessile algae covering the mineral or organic substratum. The structure of its mouthparts is well adapted for scraping and grazing the substrata. There seems to be a relationship between the habitat of the mayfly and the type of food, while the morphology and behaviour of the animal is also an influencing factor.

Grazing studies carried out in a river in Switzerland by Wellnitz and Ward (1998), suggest that *Ecdyonurus* to removes periphyton more evenly than *Baetis*, producing a relatively thinner, cleaner looking mat. They imply that this difference in architecture may have resulted from *Ecdyonurus* "brushing" vs *Baetis* "scraping". Their findings reveal that *Ecdyonurus* harvest overstorey layers and that grazing by this invertebrate caused a taxonomic shift to species that were richer in chlorophyll content. The study hypothesised that the brushing mode of feeding employed by *Ecdyonurus* would have greater influence on "tall" forms of algae whose physiognomy would place then relatively high off the substratum which is the 'scraping and gathering' zone; and indeed, stalked physiognomies were influenced exclusively by *Ecdyonurus*.

In addition to this, diatom taxa may be predictably prostrate or erect with respect to the substratum, however, the substratum to which they are attached can be detritus or other algae within the periphytic matrix (Stevenson, 1996). This action may also account for the apparently greater proportion of inorganic debris in the gut of *Ecdyonurus* vis a vis the stone scrapings as proposed by the work carried out in this study. The Swiss study also reported that filamentous chlorophytes, for example, became clearly more abundant in response to *Ecdyonurus* grazing. This increase was due to *Ulothrix* and it may have resulted from *Ecdyonurus* cleaning diatoms from this alga's tough filament. Most overstorey periphyton removed by *Ecdyonurus* appeared to be an amalgam of diatoms, silt and detritus. These findings give an indication of the algal taxa preferred by *Ecdyonurus,* which also agree well with the Castlebar findings. Unlike Baetids, which make most of their large-scale movements by swimming (Richards and Minshall, 1988), Heptageniids typically crawl with their wide, flattened bodies pressed closely to the substratum. Non consumptive losses of periphyton by mayfly grazing can be important (Scrimgeour *et al*., 1991) and foraging *Ecdyonurus* undoubtedly detach substantially larger amounts of periphyton than foraging *Baetis*. Lamberti *et al*. (1989) suggested that removal of the periphytic overstorey can enhance the productivity of underlying layers by easing nutrient and light limitation.

The dietary composition and feeding regime of four mayfly species were examined in detail in a small stream in Poland (Klonowska, 1986). The author found that it was those species that occurred most frequently in the environment in a given season, which were ingested in the largest amounts. An average of 32% of the food contents of *Ecdyonurus venosus* composed of diatoms, the preferred types being *Achnanthidium* spp. (formerly known as *Achnantes* sp.), *Cymbella ventricosa*, *Meridion circulare*, *Navicula* spp. and *Surirella ovata*. These species were found in fairly large amounts in the guts of almost all the specified larval age classes in all seasons of the year. Other species were found only at particular times e.g. *Achnanthidium linearis* var. *pusilla* (formerly know as *Achnanthes linearis* var. *pusilla*) in winter and *Navicula minima* and *Cocconeis placentula* in winter and spring.

It was found by Klonowska (1986) that young *Ecdyonurus venosus* larvae ate few diatom species, the number of diatom species ingested increasing concurrently with their growth. He indicated that *Ecdyonurus venosus* larvae preferred two diatom species in all seasons of the year i.e. *Achnanthidium minutissimum* and *Cocconeis placentula* var. *klinoraphis*. Other species of diatom were preferred only in specific seasons or by younger or older larvae only, e.g. *Achnantes lanceolata* and *Meridion circulare*. Findings from our studies on specimens collected in March 2003 showed that the dominant algal species found in *Ecdyonurus venosus* was also *Achnanthidium minutissimum*. *Cocconeis spp* was again a common taxon in the gut contents of specimens. Results from the investigations on *Ecdyonurus* larvae sampled in the Castlebar River from July-September 2003, showed that the most common algal species found in their guts was *Navicula* spp. It therefore appears that the food selected by *Ecdyonurus venosus* for the diatom species ingested, are connected to a considerable degree with the presence in the habitat in a given season. It may be that in certain cases this selection is also brought about for purely mechanical reasons – a greater ease in scraping some diatom species off the substratum than others.

Due to the increase in algal biomass in the treated section of the nutrient manipulation experiment towards the end of the study, it was hypothesised that there may be a change in the algal taxa between the control and the treated sections. This type of transformation was not evident, however, in the stone scrapings (Table 3.11). As mentioned in the results section, the only notable difference in the gut contents between summer and autumn sampling, was the absence of diatoms in the guts examined in July, which were present in the larval stomachs in September. This may be attributed to the greater numbers of diatoms present naturally in rivers during the spring and autumn seasons and was unlikely due to the addition of nutrient during the manipulation experiment. Studies carried out by Paul and Duthie (1989) on algal species responses to nutrients have shown that the growth of overstorey algae became the most important component of community change while understorey algal species density remained largely unchanged as algal communities developed. They suggest that regulatory forces may vary at the population level through the experiments. For example, understorey species (adnate diatoms like *Cocconeis* and *Achnantes*) may experience nutrient limitation and then light limitation due to growth of overstory species (erect diatoms such as *Synedra*) (Meulemans, 1987; Paul and Duthie, 1989).

A comparison was made between the *Ecdyonurus* gut contents and the epilithic material taken from the stone surfaces on which they feed in the Castlebar River in 2003. The diversity of algal taxa observed in the stone scraping samples was not reflected in the gut contents of the *Ecdyonurus* larvae. The results may be explained by the suggestion that *Ecdyonurus* appears to feed on the upper layers of epilithic material rather than the forms closer to the stone surfaces (Wellnitz and Ward, 1998).

In order to establish the feeding regime of *Ecdyonurus* in Ireland and to investigate whether there was a temporal variation in the composition of the diet, a more in-depth study like the structure of the following investigation, could be undertaken. Ledger and Hildrew (2000) examined 15-20 individuals per month, over a 12-month period to establish the diet and feeding routine of three nemourid stonefly species. Their findings suggest

that they ingested a large quantity of algae and biofilm in spring and winter when substrata were cleared by flushing flows, but consumed fine particulate organic matter and associated microflora in later summer and autumn when discharge fell and these materials accumulated. They summarised that, nemourids were strong opportunistic feeders that switched their diet according to the availability of attached (biofilm) and loose (FPOM) resources in Lone Oak over the study period. Temporal variation in the food consumed by invertebrates was also observed by Chapman and Demory (1963), working in two small Oregon streams. The possibility of seasonal resource partitioning by macroinvertebrates is often forgotten by aquatic ecologists who assume obligate trophic function for all taxa in a given functional feeding group (Mihuc and Toetz, 1994).

The present study provides information on the feeding preferences of *Ecdyonurus* in Ireland for the first time. They appear to be relatively indiscriminate grazers of the upper storey layers of the periphyton and associated detritus. There appeared to be a selection for non-diatom species although diatoms were present in the diet especially in the Spring and Autumn.

The hypothesis that *Ecdyonurus* would show diet changes in response to mild phosphorus enrichment of a river was not supported by the results; perhaps because no obvious changes in periphyton species communities were not demonstrated either.

Chapter 4

Life history studies of the Heptageniidae family in five high status rivers in the West of Ireland.

4.1 Introduction

To date the life history of *Ecdyonurus* has not been studied in detail in Ireland and information on its larval growth of this genus is limited. A more complete knowledge of the life cycles of this genus is required in order to improve the sensitivity of the EPA Q-value System in the light of the requirement for broader ecological assessment under the EU Water Framework Directive. In particular, a more detailed knowledge of the life cycles of the four most common Irish *Ecdyonurus* species would be beneficial in helping to interpret the impact of pressures on the ecological status of rivers. The peak pressures that occur during low-flow warm summer periods are of particular interest in this respect.

Many other indicator species aestivate during the summer months – some stoneflies and *Rhithrogena semicolorata* (Curtis), for example, and thus, typically they are not found in benthic samples over much of the summer. Generally speaking, however, at least one *Ecdyonurus* species is found throughout the year. In cases where *Ecdyonurus* is not found during the summer this is taken to be an indication of water pollution if habitat requirements are met. *Ecdyonurus* may reappear later in the winter and spring periods at such sites. It is important therefore to determine the emergence periods and life cycle of the individual species of *Ecdyonurus* in order to improve the interpretation of Q-values especially in cases where *Ecdyonurus* larvae are not found during the summer months. By studying the life cycles of *Ecdyonurus* species in a number of rivers with low levels of environmental stress it is hoped to gain an insight into the likely impact of pollution and other stresses on these indicator species at locations where they have been eliminated from the faunal community.

Therefore, the aim of this study was to obtain quantitative information on the life cycles of the Heptageniidae, in particular the genus *Ecdyonurus*, in five high status rivers in the West of Ireland. Historically, the EPA biologists and other freshwater biologists nationwide carry out routine biological assessments for water quality purposes during the summer months, a time when pollution pressures are at their greatest. It was hypothesised at the commencement of the present study that the various species of *Ecdyonurus* emerge in overlapping phases such that during the summer months (when biological monitoring programmes are being undertaken) larvae of at least one *Ecdyonurus* species will always be present in the benthic riffle fauna of Irish rivers.

To understand and predict the response of organisms to variation and change within and between freshwater ecosystems, one needs information about their life histories (Power *et al*., 1988). Studies carried out by freshwater biologists studying temperate stream sites over the course of a year or two will have observed the seasonal succession of benthic species, many appearing, others disappearing as the seasons progress. The sequence of changes in size of individuals of various species over time will also have been noted. These patterns reflect the growth and development of individuals through their life cycle. A life cycle is the general sequence of these morphological stages and physiological processes through which an individual of a species passes during its life, effectively linking one generation to the next (Giller and Malmqvist, 1998). The qualitative and quantitative details of events associated with the life cycle make up the life history, such as growth, development, dormancy, dispersal, number of generations per year, etc. (Butler, 1984).

While the life cycle is essentially fixed for the species, the life history can vary. For example, all aquatic Diptera pass through a life cycle involving egg, larval, pupal and adult stages, but as far as life history is concerned, the duration of stages, numbers of larval instars, activity of pupa and emergence and flight duration of the adults can vary within and between populations and species of flies. Two aspects of life history patterns are especially important: voltinism which refers to the frequency with which the life cycle

is completed within a year and phenology which refers to the seasonal timing of the various life cycle processes and the population synchrony of these (Giller and Malmqvist, 1998). To determine life histories, biologists try to follow the development and progression of individuals derived from one reproductive period through the various life cycle stages or size classes over time. These are based on linear measurements of body parts, usually body length from tip of the labrum to the last abdominal segment or the head capsule width etc. (Wallace and Anderson, 1996). The life history of *Ecdyonurus* can vary between species and is often complicated by an overlap of cohorts. A cohort is a group of individuals that were born at the same time or in practice born over a short period. These cohorts must be recognised and separated before estimates can be made for growth rates, mortality rates and production (Elliott and Humpesch, 1980).

Within a species, prevailing conditions can determine how many generations can be squeezed into the year. For example, the short summers at high altitudes allow fewer generations than at lower latitudes. Univoltine species, with a single generation each year, tend to be found only in certain seasons. The life cycle of Ephemeroptera includes four stages: egg, larvae or nymph, and two adult stages, subimago and imago. This cycle exhibits incomplete metamorphosis, since it lacks the pupal stage (Engblom, 1996). Extensive literature was summarised by Clifford (1982), included data on 718 life cycles for 297 species, the majority of which occur in Europe and North America. He found that a large proportion of the Ephemeroptera were univoltine. This type of life cycle exhibits a single generation each year accounting for 60% of all ephemeropteran life cycles. A multivoltine species, one that displays more than one generation per year, represented 30%, while 4% of all ephemeropteran life cycles were semivoltine (one generation every two, or even three, years) and the remainder were variable. The life cycles of most species vary slightly according to environmental conditions (Elliott *et al*., 1988).

There is a great variation and flexibility in life history within and between populations, including egg diapause, duration of larval stages and growth rates, degree of synchrony in emergence and the number of generations per

year. This plasticity in life history can occur from intrinsic differences between populations across the geographical range of a species (Giller and Malmqvist, 1998). Temperature, for example, is inversely related to duration of egg development in non-diapausing invertebrates and faster development of arthropod instars occurs at higher temperatures. Nutritional effects can also influence voltinism (Butler, 1984). In response to different climates, therefore, many insect species may display more generations a year at lower altitudes and latitudes. This is most apparent in widely distributed species like *Baetis rhodani*, where due to changes in environmental conditions, life cycles vary from being univoltine in northern Europe to being bivoltine with both a winter generation and a summer generation throughout most of Europe. It also presents itself as being bivoltine with one winter and two summer generations in warmer streams of Southern Europe (Clifford, 1982).

Genetic constraints may also limit voltinism, as in the mayfly *Leptophlebia* which is always univoltine over a wide geographical and climatic range, but variation does occur in most other ephemeropteran species (Brittain, 1982). Life history patterns vary for the stonefly *Nemoura trispinosa*, from a univoltine slow seasonal type to a univoltine fast seasonal type with extended egg development dependent on maximum annual water temperature (Williams *et al*., 1995). This species exhibits eurythermal egg development – the ability to show major differences in the number of degree-days needed for egg development among local populations of the same species (Lillehammer *et al*., 1989). This allows for species to switch between one- and two-year generation times depending on the local temperature range and food supply.

The basic components for life history plasticity are prolonged hatching and emergence periods and a wide range of larval stages at any one time. This distributes the life cycle stages over time and thus decreases the risk of eradication by short-term catastrophes (Dietrich and Anderson, 1995). Drought during the summer is predictable and life cycle adaptations such as drought-resistant eggs ensure rapid recolonisation following a very dry spell. However, unpredictable winter or spring droughts do not allow for

such adaptations and hence the great value attached to life history plasticity (Giller and Malmqvist, 1998). Coexisting caddis in the Glenfinnish River in Ireland, demonstrate a wide variability in life history flexibility (Sangpradub, Giller and O'Connor, 1999). These variations in rate of development, including the ability to overwinter in different larval stages and the asynchronous, extended flight periods, give increased flexibility to cope with year-to-year differences in weather and unpredictable disturbances, so called 'spreading of risk' (c.f. den Boer, 1968).

Life history parameters are also part of the framework underlying 'ecological plasticity' in mayflies (Brittain, 1991) and basically control the ability to withstand both natural and man-made ecological disturbances. The different life cycles of the Ephemeroptera (Landa, 1968; Sowa, 1975) clearly influence both the completeness and comparability of samples. Furthermore, among the Heptageniidae (which comprise about 45% of the mayfly fauna of Central Europe) only the fully grown nymphs can be reliably identified to species level (Bauernfeind and Moog, 2000). Investigations that continue to neglect these considerations impair the ecological and faunistic interpretations (Bauernfeind, 1998). As Reusch (1985, 1995) and others have pointed out, monthly samples over at least one year are the only means to obtain objective data on insect community structure in streams and rivers.

From a scientific point of view, indicator organisms must be accurately identified to species level (De Pauw and Vanhooren, 1983; Furse *et al*., 1984; Hilsenhoff, 1987; Marten and Reusch, 1992; Moog *et al*., 1997; Resh and Unzicker, 1975; Rosenberg *et al*., 1986). Categorisations of macroinvertebrate life cycles are dependent on correct identifications. Better taxonomy produces more accurate results and clearly increases the precision of site classifications, improving the ability to detect subtle changes in environmental quality (Lenat and Barbour, 1994). Modern regional keys and revisions for identification of the Ephemeroptera are available for most of Europe (Studemann *et al*., 1992; Bauernfeind, 1994; Engblom, 1996). In Ireland, the keys used to identify the Ephemeroptera are those published by the Freshwater Biological Association (Elliott *et al*.,

1988). Keys and descriptions published before 1980 contain valuable information for the specialist but should be used with caution by the less experienced: the evaluation of diagnostic characters has changed considerably and many species have since been newly described (Bauernfeind and Moog, 2000). Larval identifications require much care because most key characters only apply to fully grown mature nymphs. No reliable keys are available at present for early instars and misidentification even at the genus or family level is possible.

Positive identification of species requires examination of all life stages for most aquatic insects. However, in most cases, identification of species has been based on nymphs or adults often with no association being made between the two (Hynes, 1970; Smock, 1996, Merritt *et al*., 1996). Field collecting of nymphs and adults in one location is an accepted method of identifying all insect life stages but has the inherent problem with discriminating between different species, especially if one has to rely on immature nymphs for initial identification. An insect reared from an immature stage to an adult, with the subsequent larval skin moult kept for comparison, provides the definitive association. The rearing of mayflies can be especially difficult because of the presence of a fragile subimago stage which has characteristics different to those of the adult (Finlay, 2001).

The Heptageniidae possibly represent the most difficult family to identify and instruction by trained taxonomists is recommended. In *Ecdyonurus*, mature nymphs and male imagines can usually be correctly identified (Bauernfeind, 1997; Hefti *et al*., 1989), but some training is essential. The use of reference specimens is advisable to acquire the necessary experience. Detailed information on the life cycle of *Ecdyonurus* in Ireland is quite limited due to insufficient studies carried out in this area and to the difficulty in the identification of this genus. This study addressed this knowledge gap.

4.2 Study outline

This study was undertaken in order to characterise the life history of the Heptageniidae family, with particular emphasis on the genus *Ecdyonurus*, in a number of clean-water (high status) rivers in the West of Ireland.

4.3 Material and Methods

The site descriptions for each of the five high status rivers (Owengarve River, Castlebar River, Brusna River, Dunneill River and Callow Loughs Stream) are already described in detail in Chapter 1.

4.3.1 Experimental design and sampling regime

Quantitative macroinvertebrate sampling was carried out on a monthly basis in all five rivers between July 2001 and October 2002 (see Table 5.4. in Chapter 5 details of sampling dates). During each visit five individual macroinvertebrate samples were taken per river using a 36cm x 36cm metal-framed Surber sampler (500 microns mesh size). It was important that the sampling area within each river included riffles, glides and margins so as to fully represent all potential habitats within the system. A homogenous sampling stretch was then chosen which included all relevant habitats and cross-sectioned into $1m^2$ quadrats. Details of the length of the sampling area for each of the ten river sites are outlined in the site descriptions in Chapter 1. Five quadrats were chosen randomly at each visit and a Surber sample taken within each taking care to represent riffles, glides and marginal areas. The substrate was carefully disturbed within the frame and all macroinvertebrates were collected with particular care observed when handling the *Ecdyonurus* specimens. All samples were preserved in 70% IMS immediately on site for later identification.

Due to increased water levels in the rivers over the winter months, routine Surber sampling had to be postponed until the levels receded. To continue with the life history studies, *Ecdyonurus* specimens were collected by taking a single kick sample in each river from November 2001 to January 2002 inclusive. Due to extreme weather conditions in February 2002, sampling was halted completely and recommenced in March 2002.

The following chemical 'quality' parameters were also examined in each river on a monthly basis: temperature and dissolved oxygen, % oxygen saturation (both taken in the field), pH, conductivity, orthophosphate,

T.O.N., ammonia, chloride, BOD, alkalinity and colour. These are further analysed in Chapter 5.

4.3.2 Laboratory studies: Identification and categorisation of monthly samples

The Heptageniidae larvae were separated from all other macroinvertebrates collected for the detailed life history studies. Larvae for the life history studies were counted, identified and categorised into the three main genus groups: *Ecdyonurus* spp., *Rhithrogena* spp. and *Heptagenia* spp. The body lengths of individual larvae were carefully measured to the nearest 0.1mm from the tip of the labrum to the end of the last abdominal segment, under a Nikon Stereo Microscope. The larva of the genus *Ecdyonurus* and *Rhithrogena* were then identified to species level using the Freshwater Biological Association Key to Ephemeroptera (Elliott *et al*., 1988). The genus *Ecdyonurus* was particularly difficult to identify so other keys were also consulted, namely the ephemeropteran key compiled by Engblom (1996). The *Heptagenia* specimens were identified to genus level only due to time constraints.

One specific feature examined in the identification of the various species of *Ecdyonurus* was the shape of the pronotum. The curvature differs between species (Fig. 4.1 to Fig. 4.3). It tends to be more rounded and curved in *Ecdyonurus dispar* (Curtis) and longer and less curved in *Ecdyonurus venosus* (Fabricius). All genera of *Ecdyonurus* contain seven gills but *Ecdyonurus insignis* (Eaton) is the only species that has a filament on all seven gills, thus making it relatively easy to identify intact specimens (Fig. 4.4). The other three species are more difficult to separate and rely on an examination of the curvature of the pronotum and setae on the prostheca. These setae number less than 10 in the larva as *Ecdyonurus torrentis* Kimmins whereas more than 10 setae are usually present in specimens identified as *Ecdyonurus venosus*. This particular feature was very difficult to examine and highly variable so it was therefore abandoned quite early on in the studies.

Length frequency distribution charts were compiled for each month to examine the life cycles of the individual species over the entire sampling period (Appendices 4.1 to 4.20). Due to the difficulty in identifying the small immature specimens of *Ecdyonurus* to species level, a separate category designated "*Ecdyonurus* species" was also used. All specimens that were not large enough to be accurately identified were placed in this category, which are outlined in the life cycle histograms.

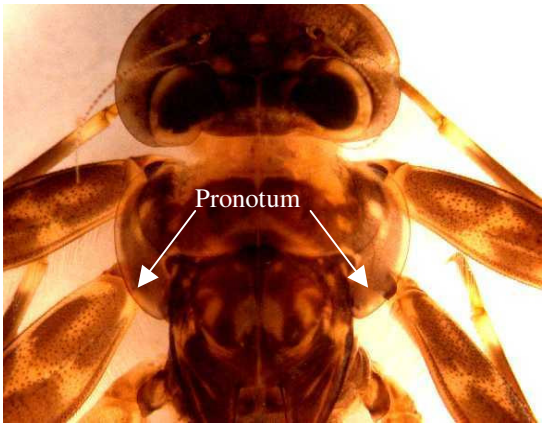


Fig. 4.1 Photomicrograph of the head and thorax of *Ecdyonurus venosus* highlighting the long thin curvature of the pronotum**.**

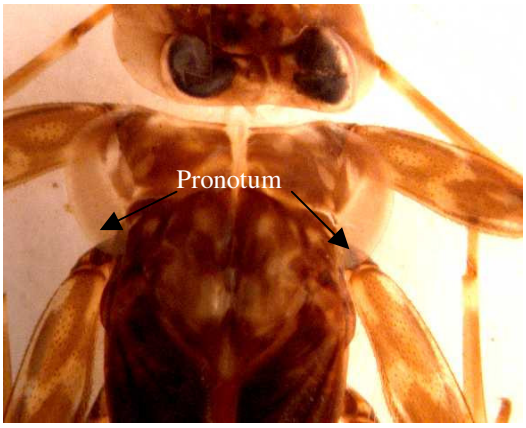


Fig. 4.2 Photomicrograph of the head and thorax of *Ecdyonurus dispar* displaying a shorter and more curved pronotum in comparison to that in Fig. 4.1.

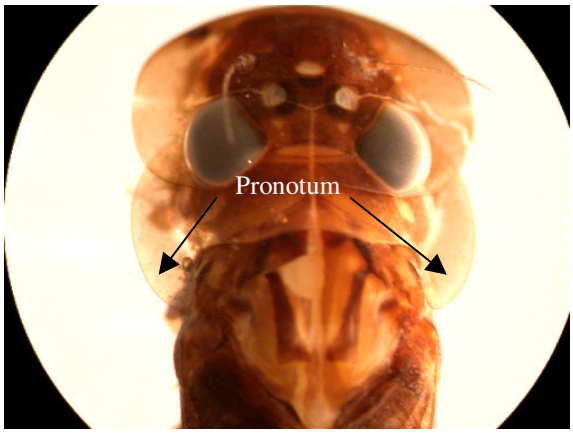


Fig. 4.3 Photomicrograph of the head and thorax of *Ecdyonurus insignis* highlighting a longer and wider pronotum than those found in *Ecdyonurus venosus* and *Ecdyonurus dispar*.

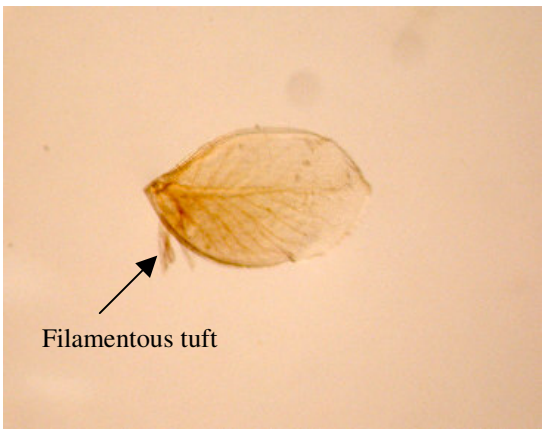


Fig. 4.4 Photomicrograph of the 7th gill found on the abdomen of *Ecdyonurus insignis* containing a filamentous tuft.

4.3.3 Rearing experiments

In order to verify the identifications of the various species of *Ecdyonurus*, in particular, those species that were more difficult to identify, such as *Ecdyonurus dispar*, larvae were collected for rearing from a number of the high status rivers during the summer of 2002. These rearing experiments were established for taxonomic review rather than for other experimental purposes. It was envisaged that this would be particularly helpful in verifying species from rivers where several species could be present during the same sampling period.

4.3.3.1 Rearing chamber

An uncovered glass tank measuring 55cm x 8cm x 40cm was used to house the rearing chambers. The system ran on a continuous flow basis using an electrically powered circulating pump and reservoir to circulate the water supply back into the aquarium. The water level in the aquarium generally remained at a level of approximately 5cm. As water was pumped around the system, overflow was directed into the reservoir via a 5mm diameter copper pipe that was fitted to one end of this aquarium. This ensured a continuous supply of water to the aquarium on a 24-hour basis.

4.3.3.2 Data collection

Larvae for rearing were collected from the five high status rivers from June to September 2002. From studying the literature, this phase represented the main period for emergence of the genus *Ecdyonurus*. Late instar nymphs with developing black wing pads were carefully removed from the substrate with a paintbrush and placed in a bottle of stream water with a small stone for attachment. The bottle was sealed and placed in a box, in this way live larvae were successfully transported to the laboratory.

4.3.3.3 Laboratory rearing

The aquarium was filled with laboratory tap water until it reached the 5cm-overflow pipe. Likewise, the reservoir was filled to the appropriate height and the circulating pump attached. The water was allowed to circulate overnight to oxygenate fully and to reach ambient room temperature and also in order to reduce the dissolved chlorine burden of the original tap water. The water temperature ranged from between 18°C to 21°C during the hatching period.

Each chamber housed an individual specimen to ensure identification of the individual and to enable verification by examining the larval skin and imago left behind. The chambers (25cm x 10cm) were made of plastic mesh to allow water to flow freely through the system. The live larvae were placed in a petri dish of water and identified to species level if possible under a stereomicroscope. Twigs and stones were collected from each site and added to each chamber for the insect to use as a food source and as a platform for emergence. They were then transferred quickly to a chamber, the lid placed on top and secured tightly using bull clips. A nylon mesh was tightly wrapped around the entire chamber using rubber bands to ensure no animals escaped and to capture the adults as they emerged. Three large aqua fizz aerators used to regulate airflow were also placed near the rearing chambers in an effort to recreate stream flow conditions.

The aquarium was placed near a window exposing it to the normal photoperiod of a 24-hour day but not direct sunlight. The larvae were checked every day and the life stage of the individual noted. Once emergence (or death) occurred the animals were removed and species and sex determined by observation using a stereomicroscope. Empty chambers were thoroughly washed and nymphs replaced as required. These new nymphs were acclimatised to the controlled temperatures for a period of 20 to 30 minutes. After about 5-7 days, an algal slime had grown on the base of the aquarium covering the stones and the rearing chambers. Larvae appeared to be sensitive to this growth and quite a few died within 2-3 days of placing them in the chambers. As a result, the aquarium was cleaned

every week with a phosphate free detergent (neutracon) to remove any algal build up that may have affected the survival of these sensitive invertebrates.

4.4 Results

4.4.1 Physico-chemical data

The maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters are outlined and discussed in more details in Chapter 5 (Appendix 5.1). The results indicate that the sites chosen were of good water quality with low values for the indicators of organic pollution and eutrophication, BOD, ammonia, total oxidised nitrogen (TON) and phosphate (i.e. unfiltered MRP).

The temperatures observed in the rivers during the sampling period were within the normal seasonal ranges. The mean pH values ranged from 7.7 to 8.0. Conductivity and alkalinity levels varied between the rivers reflecting the different typologies, geology and fluctuation in the discharges among sites. Chloride levels showed a narrow range in values in the high status rivers. The mean BOD was low in all sites measuring approximately 0.8 mg/l O_2 throughout the study.

There was, however, some suggestion of eutrophication impacts in the Owengarve, the Dunneill and the Brusna Rivers. On occasion, the DO was elevated (>120%) in these rivers (Appendix 5.1). Elevated levels of ammonia were detected in the Owengarve River (0.04 and 0.07mg/l N) and in the Dunneill River (0.11mg/l N) on occasion. High levels of unfiltered MRP were also measured in some of the high status sites, particularly in the Castlebar and the Brusna Rivers. TON concentrations fluctuated from 0.3 to 1.4 mg/l N in the Owengarve River and from 0.2 to 0.95 mg/l N in the Brusna River.

The results outlined in Appendix 5.1 emphasise the changes and constant fluxing experienced in the high status rivers during the life cycle studies.

4.4.2 Results from life history studies

Interpretations of the life history of each individual species studied are outlined in Sections 4.4.2.1 to 4.4.2.5 below. The life cycles of the

Heptageniidae in the five high status rivers are graphically represented in Figs. 4.5 to 4.28 and then some overall conclusions about the life cycles are drawn for West of Ireland rivers as a whole.

4.4.2.1 Interpretation of the life history of *Ecdyonurus venosus*

Ecdyonurus venosus was present throughout the year in all five rivers studied and appeared to show the emergence of two distinct broods. It is therefore considered to be bivoltine.

Summer/Autumn 2001

The first brood emerged from August to September 2001 in the Owengarve River (Fig. 4.5), from July to September 2001 in the Dunneill River (Fig. 4.6) and in August 2001 in the Brusna River (Fig. 4.7). Adults emerged from July to August 2001 in both the Castlebar River (Fig. 4.8) and in Callow Loughs Stream (Fig. 4.9). Hatching was evident in late autumn and continued on into early winter in all rivers.

Spring 2002

The larvae of *Ecdyonurus venosus* grew slowly and steadily throughout the winter months in all five rivers (overwintering larvae) and generally emerged as the first brood from March to April/May 2002 (Fig. 4.6, 4.7, 4.8, 4.9) with the exception of the Owengarve River where they emerged until slightly later from March to June 2002 (Fig. 4.5).

Summer 2002

The adults emerged throughout the summer months in the five rivers, laid their eggs and matured very quickly to hatch in early June 2002. This fast growing summer generation (second brood) grew rapidly to emerge as the summer stock in July and August 2002 in four of the five rivers. Larvae in the Castlebar River emerged on into September 2002 (Fig. 4.8). *Ecdyonurus venosus* emerged in July 2002 in Callow Loughs Stream (Fig. 4.9).

The Brusna River represented the lowest abundance of *Ecdyonurus venosus* of all the rivers studied and the life cycle here also appeared to be bivoltine. Numbers were very sporadic throughout the sampling programme, which

made it quite difficult to interpret its life history with accuracy. A few specimens were found in December 2001 having reached body lengths of up to 15mm (Fig. 4.7). Larvae had not reached this size during December in any of the other rivers studied, so it is difficult to say whether this corresponds to an emergence period when such few numbers were found during the sampling period. There is a possibility that these could be univoltine specimens that emerged in the spring and didn't manage to emerge in August/September forcing them to overwinter.

Ecdyonurus venosus specimens ranged in size among river sites (Table 4.2 to 4.6). Throughout the emergence period of autumn 2001, the length of the largest larvae in the Owengarve River ranged from 15-16mm. Those measured in the other four rivers were 1-2mm shorter. A similar pattern was observed the following autumn (2002) and all larvae were 1-2mm smaller than those found the previous autumn (2001). The largest larvae emerging in Callow Loughs Stream in spring 2002 measured from 14-15mm, 1-2mm less than those found in the other four rivers. The Owengarve River contained the largest specimens of *Ecdyonurus venosus* among all sites studied while Callow Loughs Stream had the smallest larvae throughout the study. There was no statistical difference (p=0.397) between the mean size range of the largest larvae during the emergence period in all rivers in 2001 compared to 2002.

In summary, *Ecdyonurus venosus* was present in all five rivers throughout the year with the exception of brief episodes after emergence periods and its life cycle appears to be bivoltine. Similar overwintering patterns with slow growing larvae followed by rapid growth during the summer months were also observed.

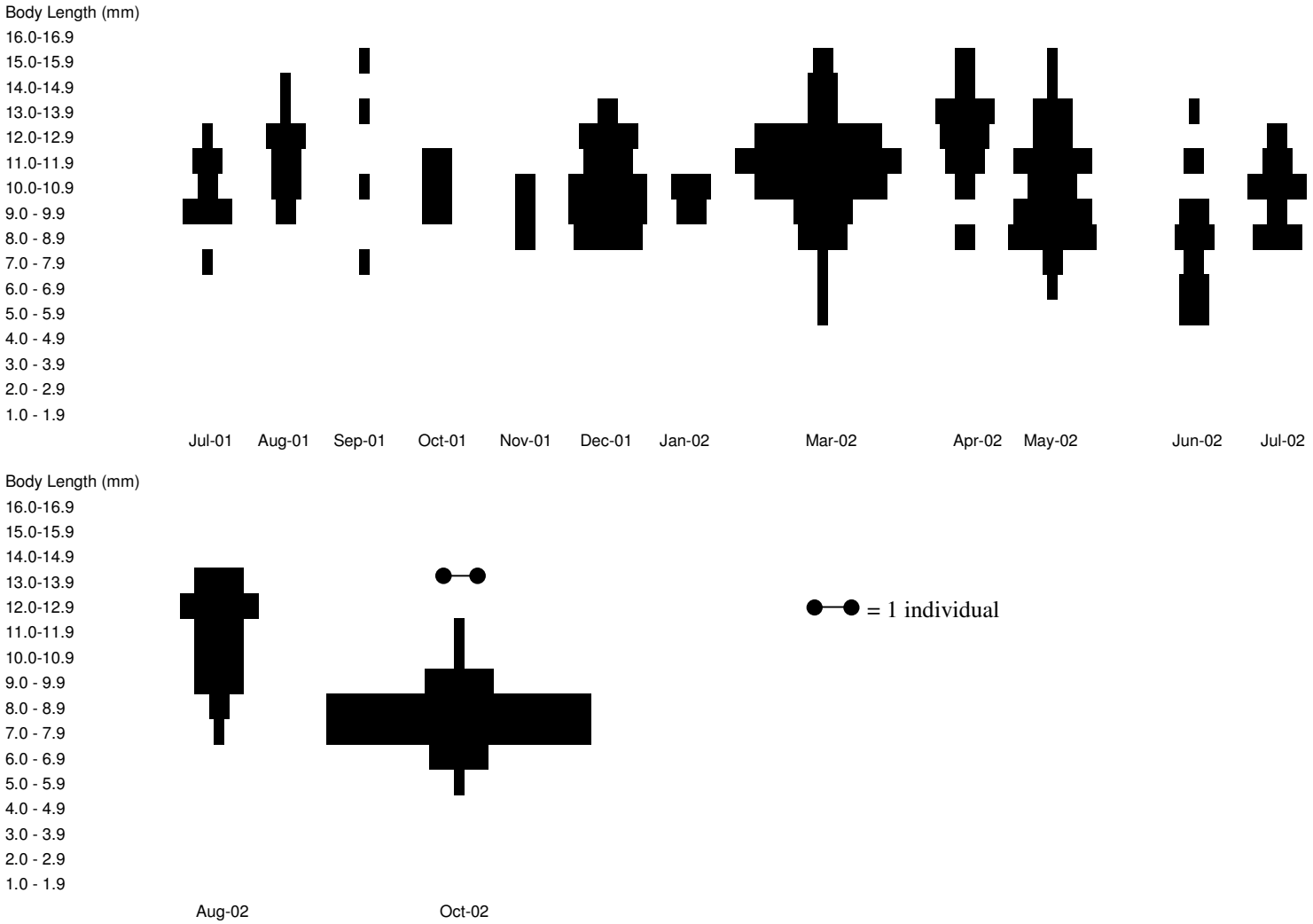


Fig 4.5 Life cycle of *Ecdyonurus venosus* in the Owengarve River from July 2001 to October 2002.

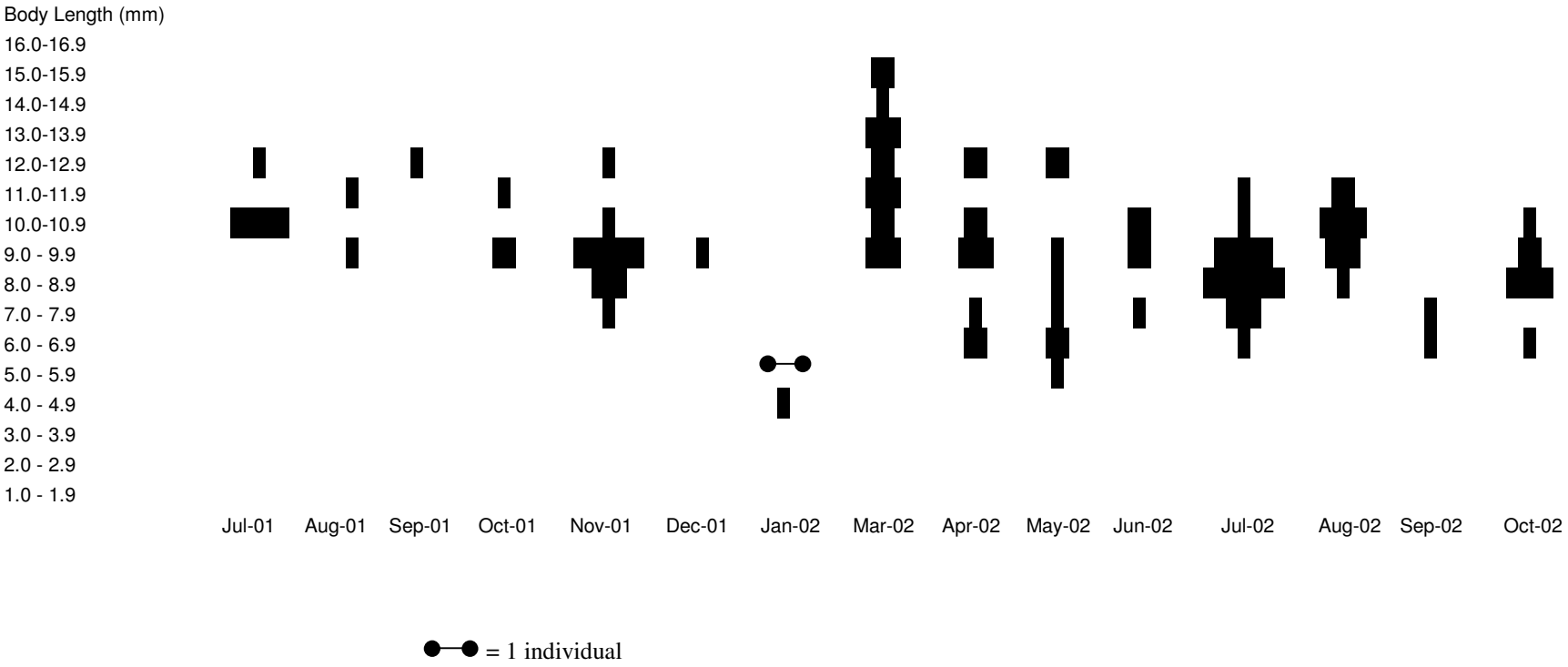


Fig 4.6 Life cycle of *Ecdyonurus venosus* in the Dunneill River from July 2001 to October 2002.

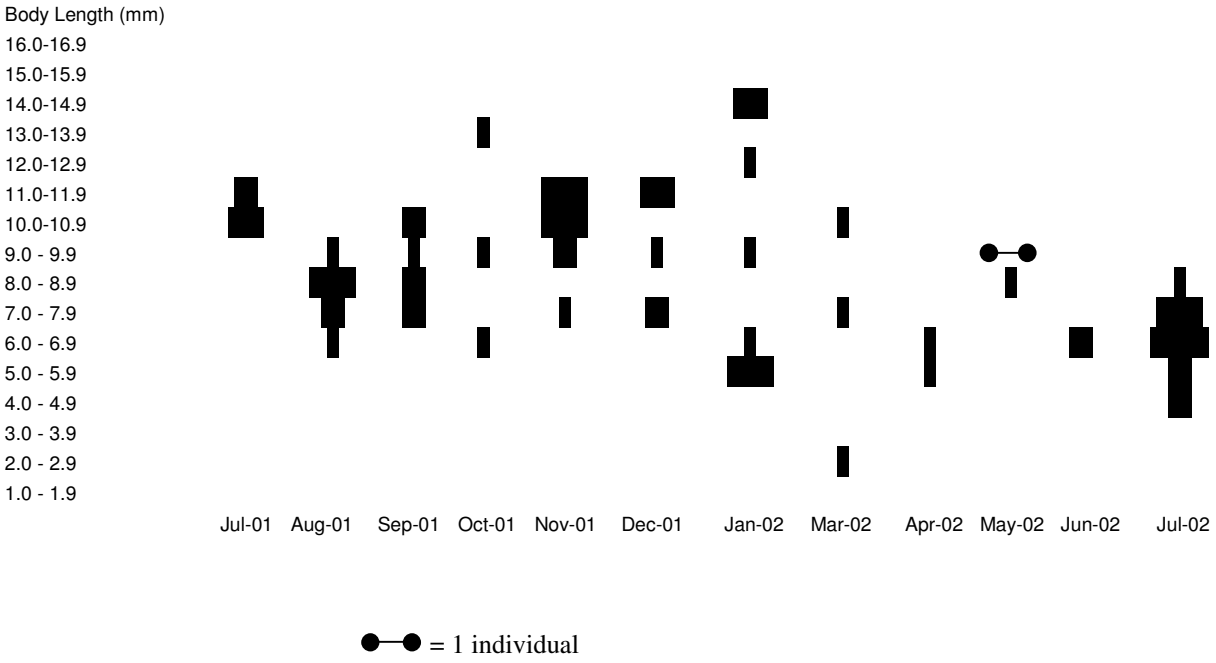


Fig 4.7 Life cycle of *Ecdyonurus venosus* in the Brusna River from July 2001 to October 2002.

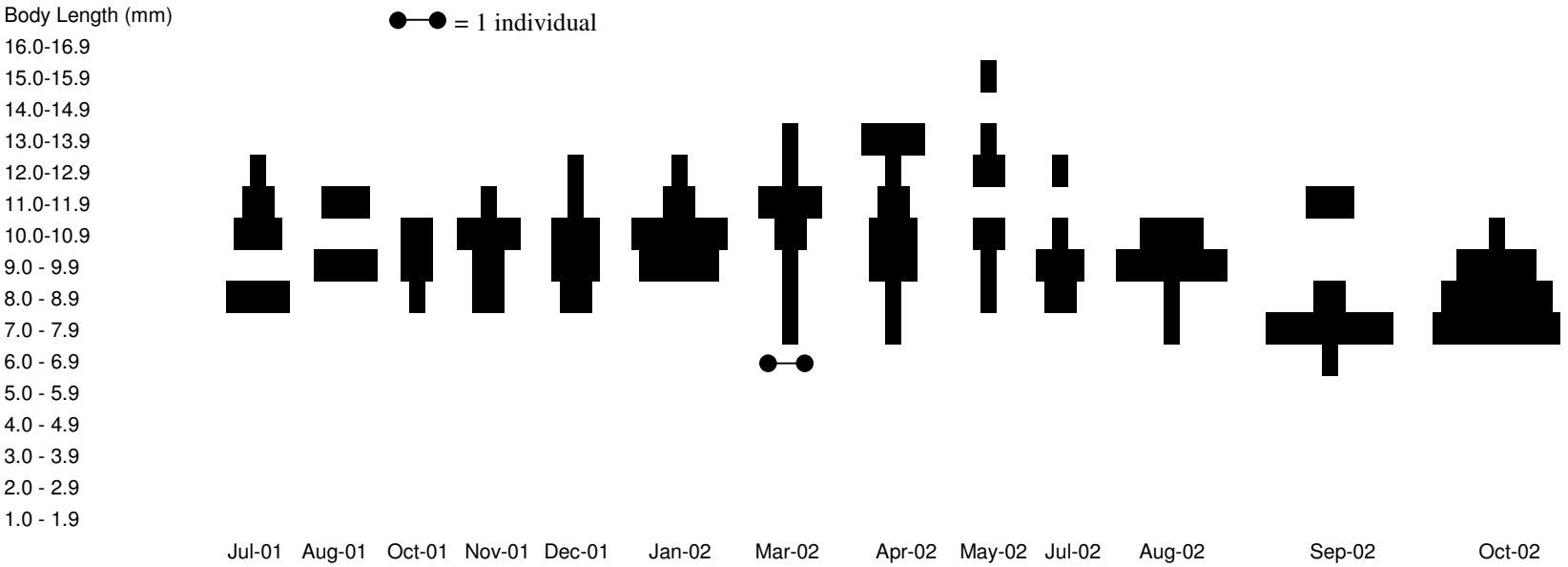


Fig 4.8 Life cycle of *Ecdyonurus venosus* in the Castlebar River from July 2001 to October 2002.

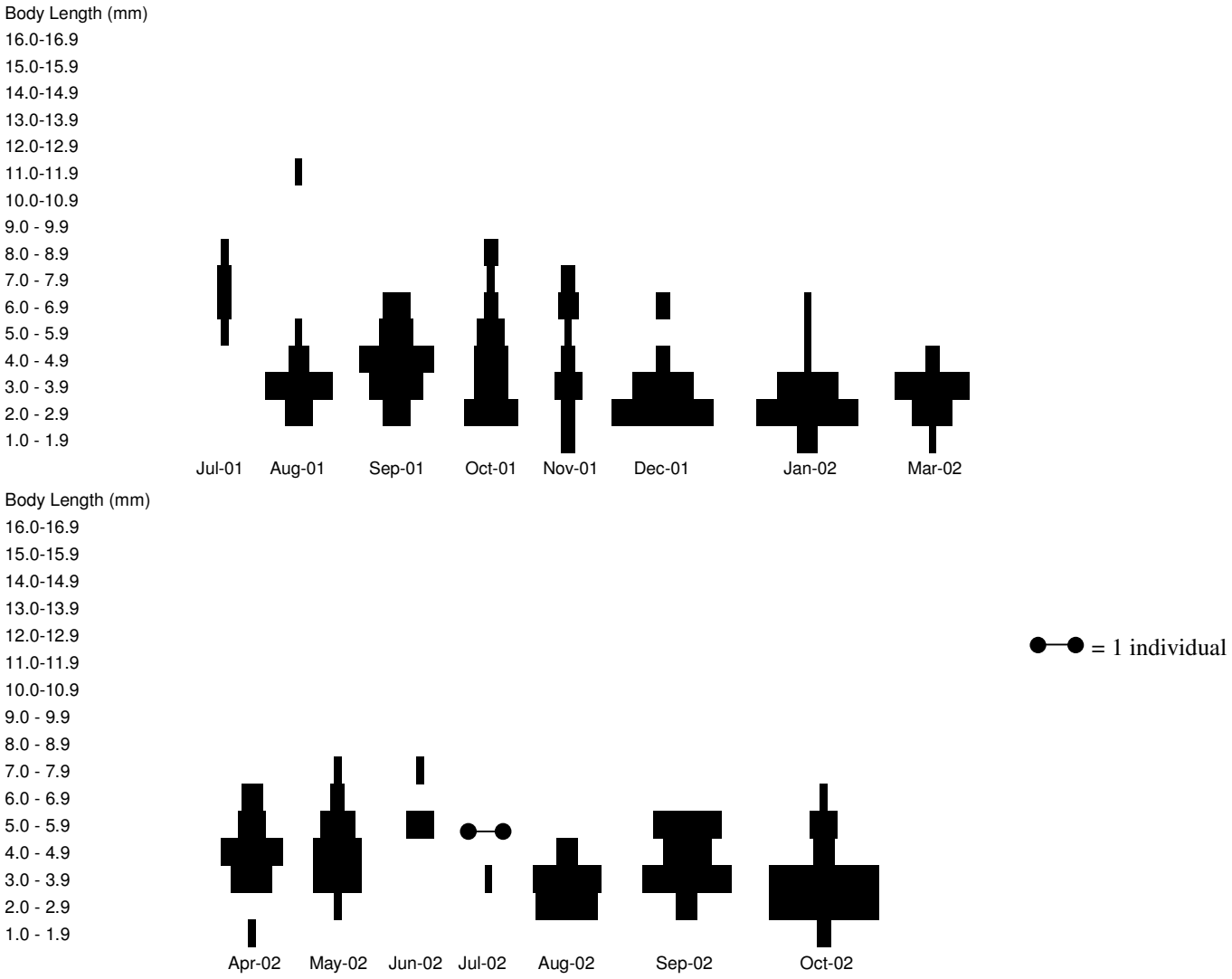


Fig 4.9 Life cycle of *Ecdyonurus* species in Callow Loughs Stream from July 2001 to October 2002.

4.4.2.2 Interpretation of the life history of *Ecdyonurus insignis*

Ecdyonurus insignis was found in four out of the five rivers, being absent from the Castlebar River entirely. The life cycle of this species showed one distinct brood and for that reason was described as being univoltine with overwintering larvae. It was present in the river benthos during the summer months only.

Summer 2001

Larvae were not found in the Owengarve River during 2001. Larvae emerged from the Dunneill River in July and August 2001 (Fig. 4.10) and in August 2001 in Callow Loughs Stream (Fig. 4.11). One specimen was found in Callow Loughs Stream in August 2001 which probably marked the end of the emergence period for this species. Emergence in the Brusna River occurred in August 2001 and September 2001 (Fig. 4.12) which appeared to be slightly later than in the other rivers. As with Callow Loughs Stream, only one specimen was found in the Brusna River in August 2001 signifying the end of the emergence period.

Summer 2002

Ecdyonurus insignis was found in the Owengarve River between June and August 2002 (Fig. 4.13). Larvae emerged in the Dunneill River from May to July (Fig. 4.10) and in the Brusna River from May to August 2002 (Fig. 4.12) while they emerged in Callow Loughs Stream from July to August 2002 (Fig. 4.11).

The length of the largest specimens of *Ecdyonurus insignis* in the four rivers ranged from 11 to 15mm. The largest larvae were found in the Owengarve River (14-15mm) while the smallest larvae were found in Callow Loughs Stream (10-11mm). There was no significance difference (p=0.411) in mean larval sizes between summer 2001 and summer 2002. In summary, *Ecdyonurus insignis* was present in four out of the five rivers and displayed a univoltine life cycle.

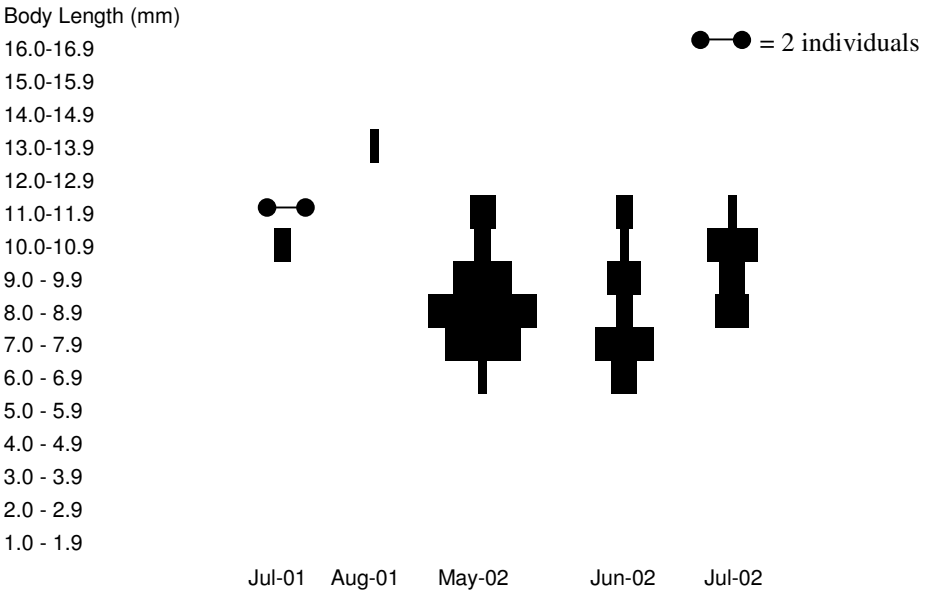


Fig 4.10 Life cycle of *Ecdyonurus insignis* in the Dunneill River from July 2001 to October 2002.

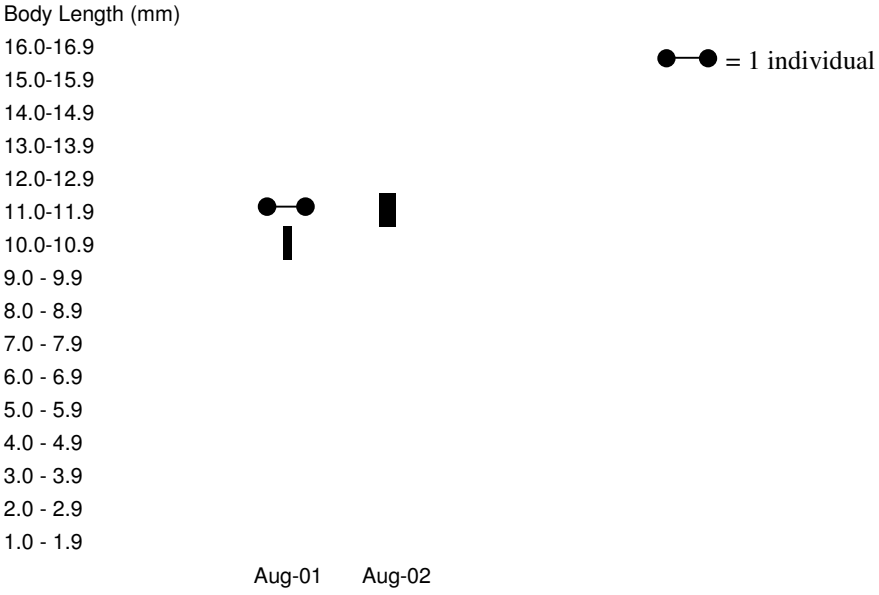


Fig 4.11 Life cycle of *Ecdyonurus insignis* in Callow Loughs Stream from July 2001 to October 2002.

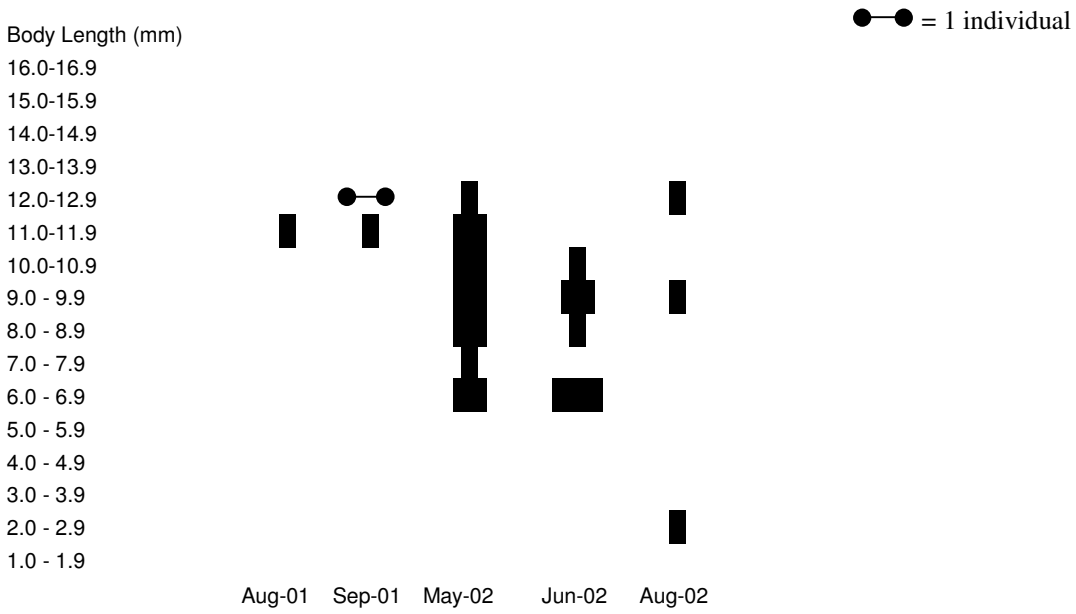


Fig 4.12 Life cycle of *Ecdyonurus insignis* in the Brusna River from July 2001 to October 2002.

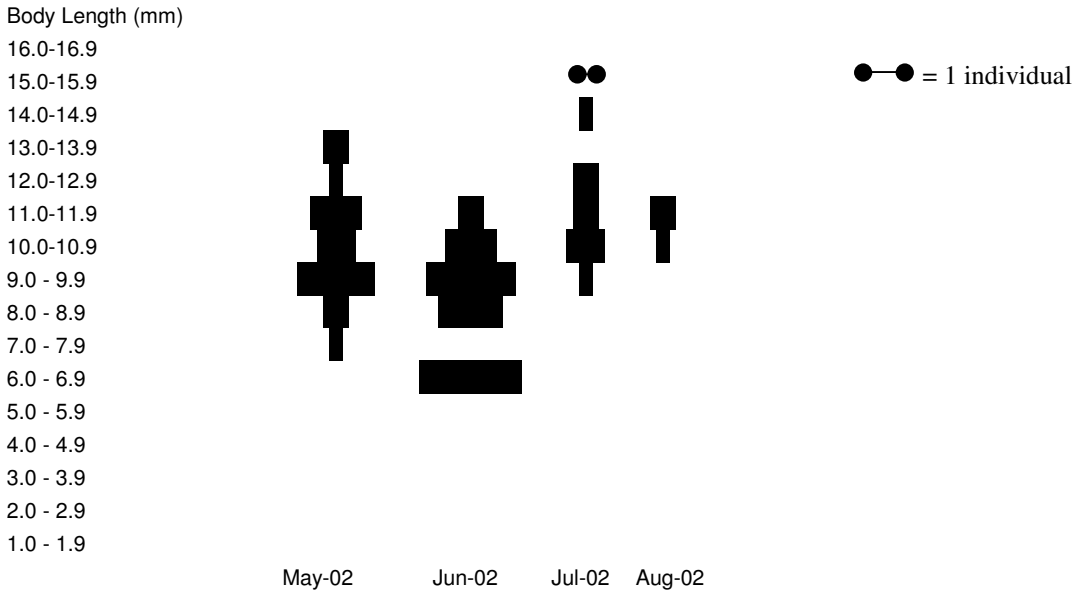


Fig 4.13 Life cycle of *Ecdyonurus insignis* in the Owengarve River from July 2001 to October 2002.

4.4.2.3 Interpretation of the life history of *Ecdyonurus dispar*

Numbers of *Ecdyonurus dispar* were low and were found as mature larvae only during the summer months. This species was found in all five rivers during the sampling programme and had a univoltine life cycle with overwintering larvae.

Summer 2001

Ecdyonurus dispar emerged in all five rivers in August 2001 (Figs. 4.14, 4.15, 4.16, 4.17 and 4.18). It is thought that recruitment of *Ecdyonurus dispar* began in June/July 2001 when juveniles hatched and grew very quickly to emerge a month or two later in August 2001.

Summer 2002

Larvae emerged during this season in the Owengarve River only in June 2002 (Fig. 4.14). The largest specimens found in the Owengarve River were 11-12mm in August 2001 and since *Ecdyonurus dispar* was relatively uncommon, however, it is possible that fully grown specimens larger than 12mm could have been missed due to their rarity in samples or due to rapid growth in the period between two sampling dates at emergence time. Recruitment more than likely commenced in late April/early May 2002 in this river in order for the larvae to reach 14mm on emergence in June.

In summary, *Ecdyonurus dispar* was present in all five rivers studied and had a univoltine life cycle. Numbers were low and it was the rarest of all three species of *Ecdyonurus* found in these rivers during the study.

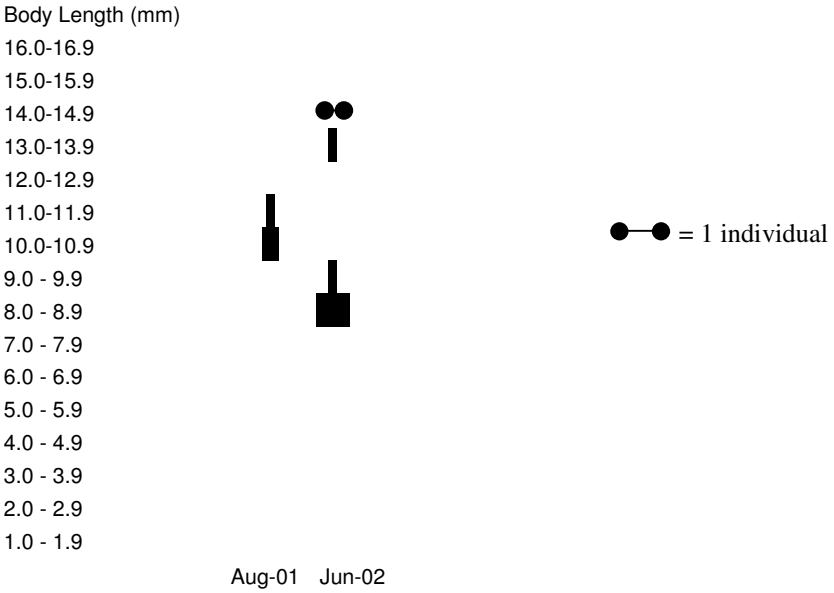


Fig 4.14 Life cycle of *Ecdyonurus dispar* in the Owengarve River from July 2001 to October 2002.

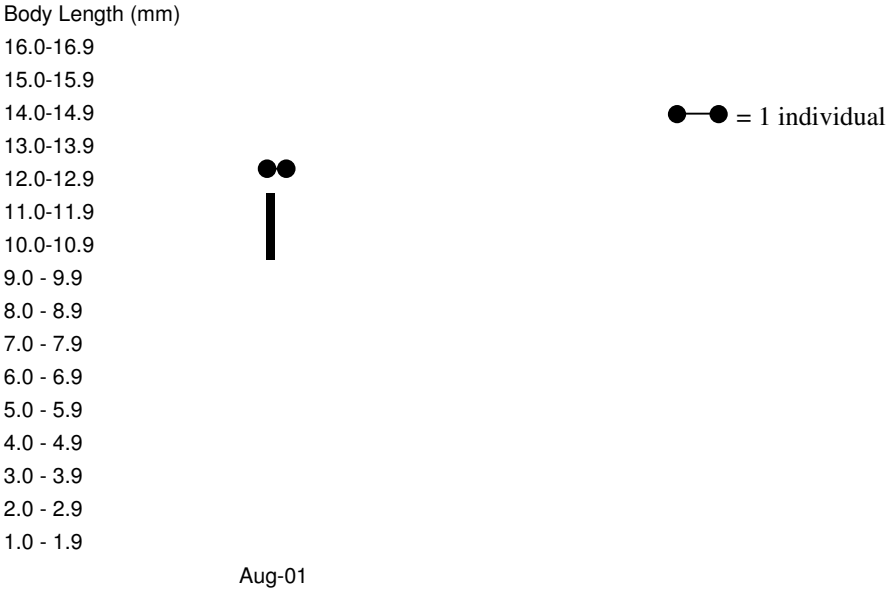


Fig 4.15 Life cycle of *Ecdyonurus dispar* in the Dunneill River from July 2001 to October 2002.

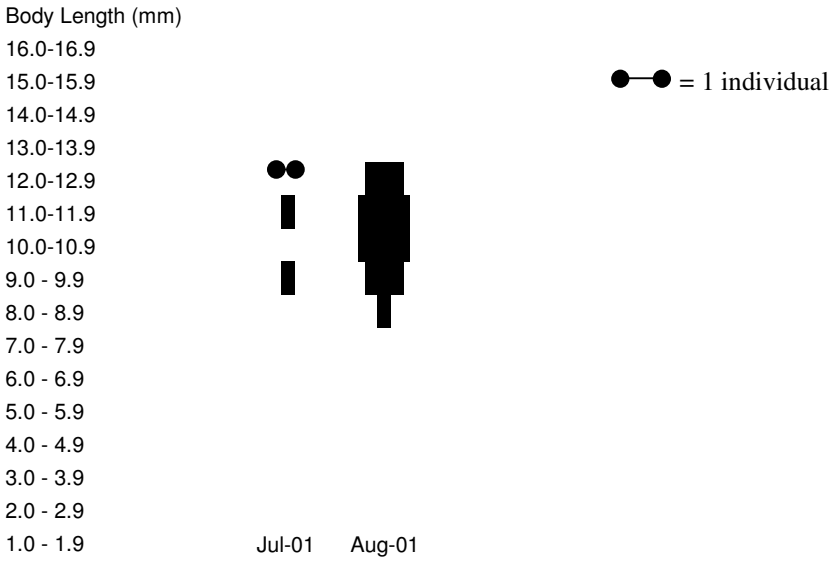


Fig 4.16 Life cycle of *Ecdyonurus dispar* in the Castlebar River from July 2001 to October 2002.

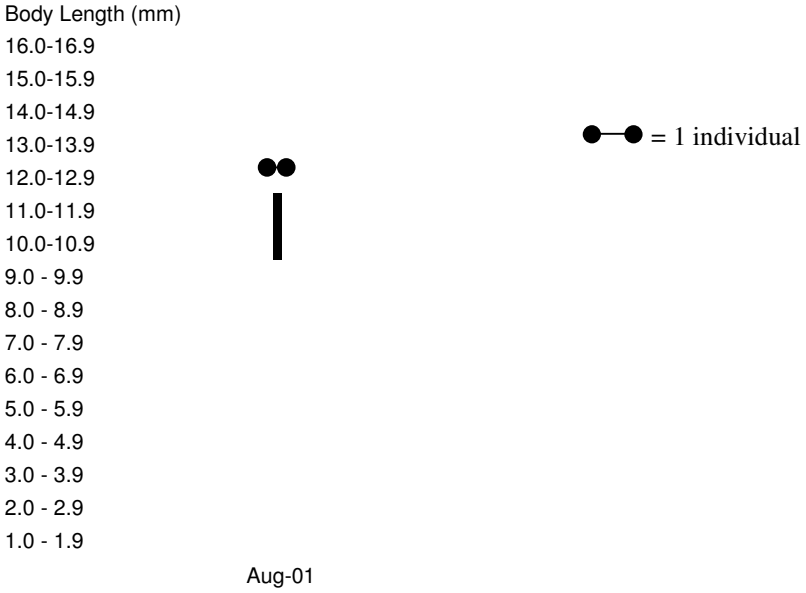


Fig 4.17 Life cycle of *Ecdyonurus dispar* in Callow Loughs Stream from July 2001 to October 2002.

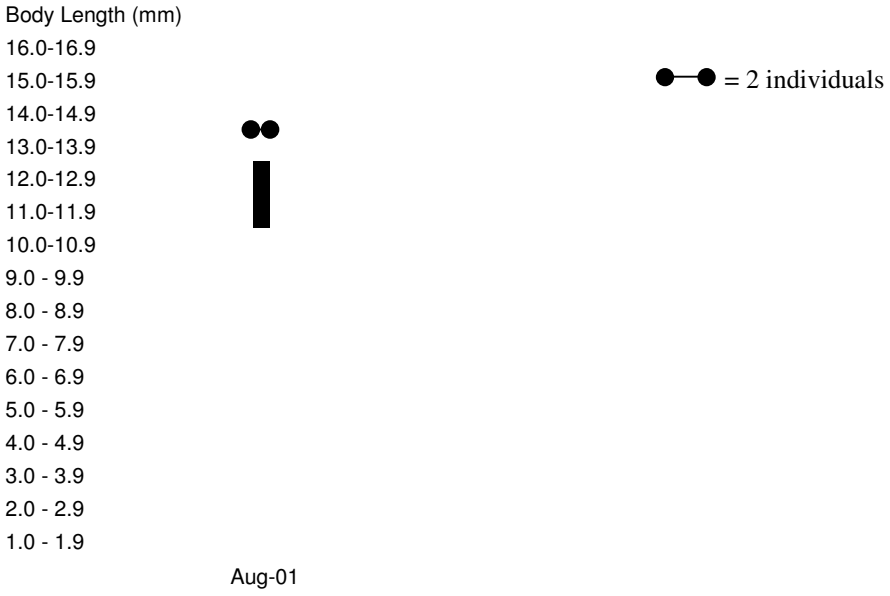


Fig 4.18 Life cycle of *Ecdyonurus dispar* in the Brusna River from July 2001 to October 2002.

4.4.2.4 Interpretation of the life history of *Rhithrogena semicolorata*

Rhithrogena semicolorata was present in all five rivers studied and showed a univoltine life cycle with substantial numbers represented. The overwintering larvae grew progressively during the winter months and final instars emerged in March and April 2002 in the Owengarve River (Fig. 4.19). The emergence period was slightly longer in the Dunneill River (Fig. 4.20), the Castlebar River (Fig. 4.21), Callow Loughs Stream (Fig. 4.22) and the Brusna River (Fig. 4.23) lasting from March to May 2002.

The larvae varied in size just before they emerged. The length of the largest specimens in the rivers ranged from 8-13mm. The smallest of these were found in the Owengarve River (8-9mm) while the largest were present in the Dunneill River (12-13mm).

Eggs laid by the adults that emerged in March and April 2002 began to hatch in September/October 2002 (depending on the river) creating a new generation and numbers increased as expected during these months. This is a pattern commonly observed by EPA biologists in the West of Ireland. *Rhithrogena* is typically missing from river benthos particularly in July and August with the earliest nymphs appearing in late August to early September (McGarrigle pers. Comm.).

In summary, *Rhithrogena semicolorata* was found abundantly in all five rivers and displayed a univoltine life cycle typical of this species.

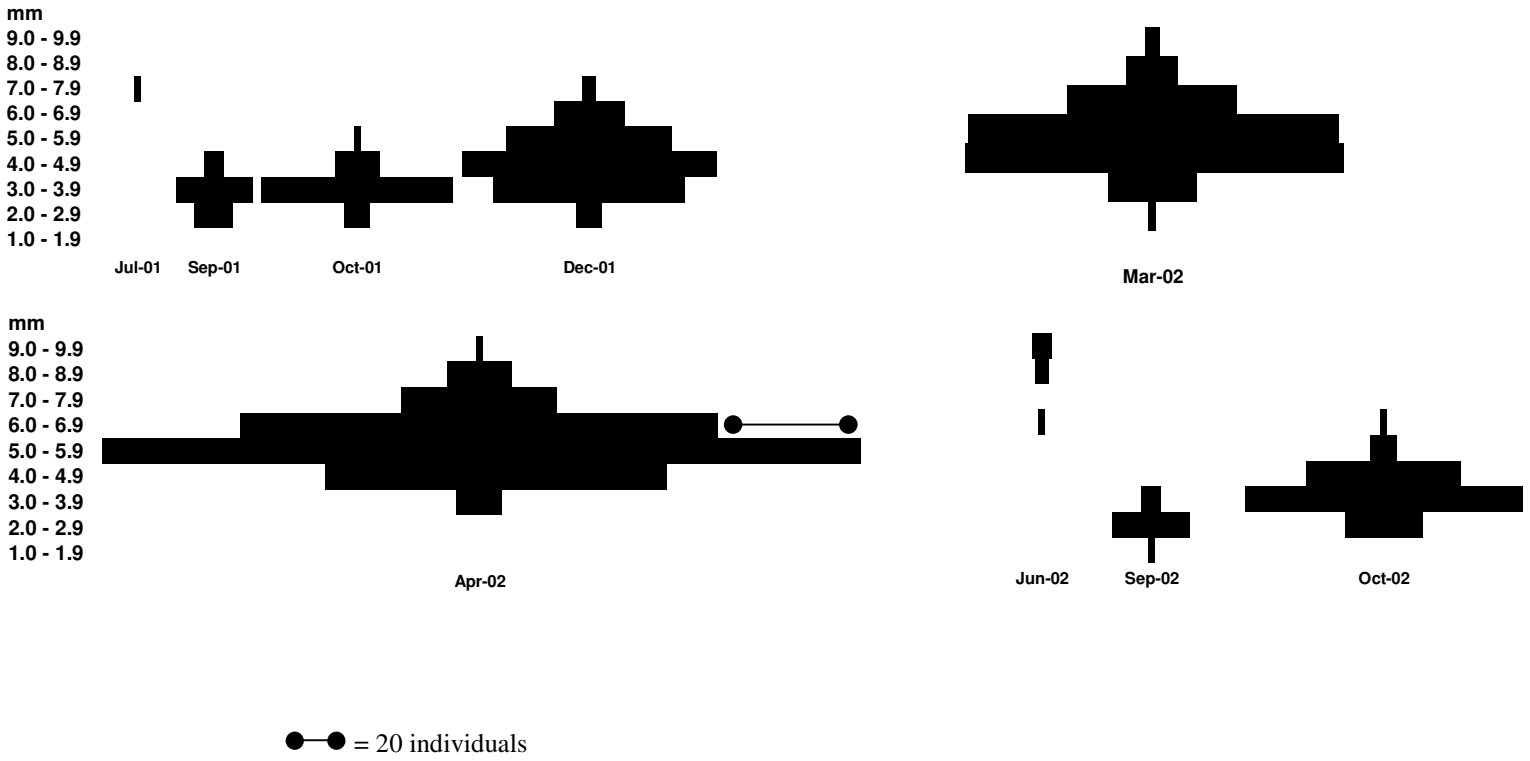


Fig 4.19 Life cycle of *Rhithrogena semicolorata* in the Owengarve River from July 2001 to October 2002.

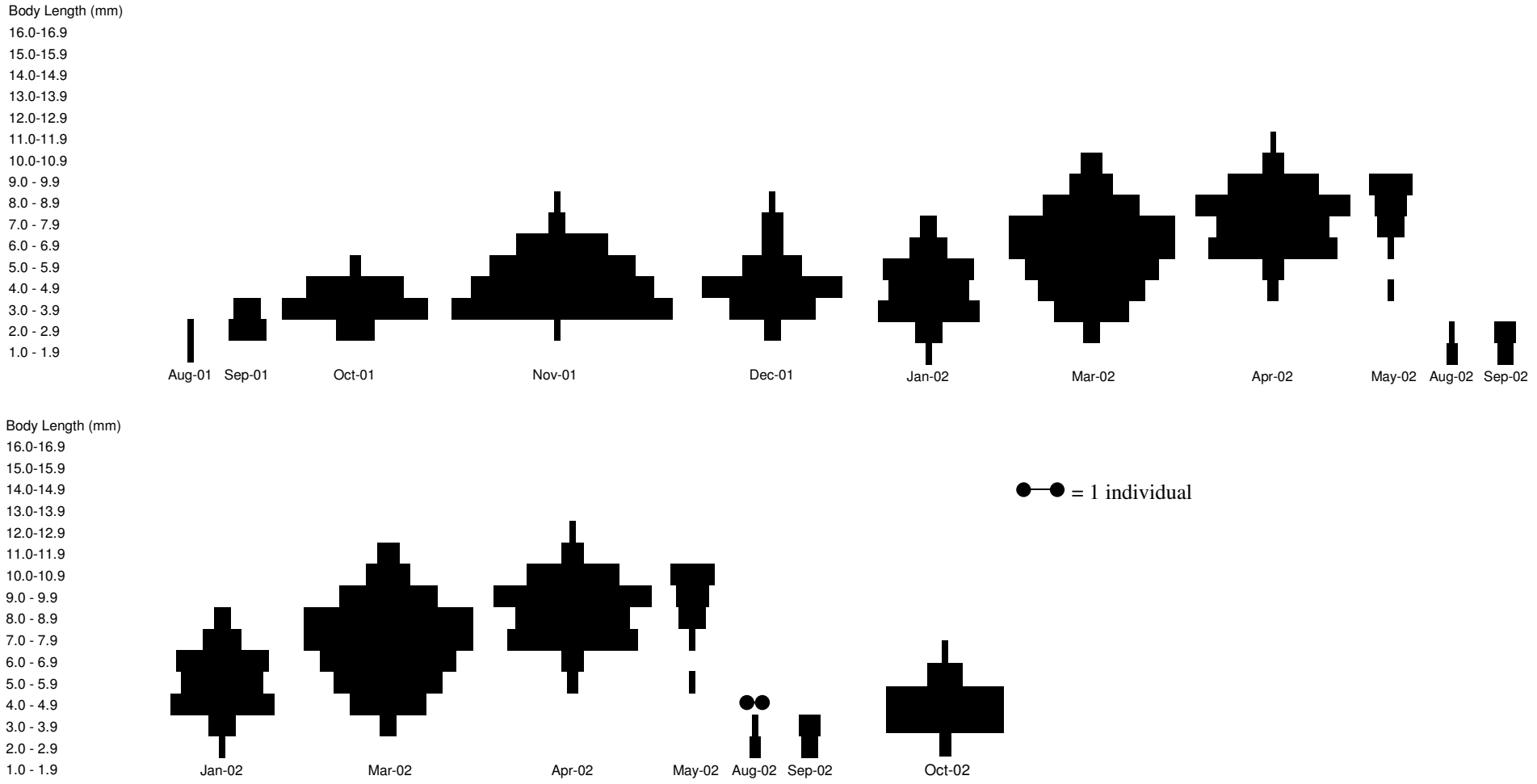


Fig 4.20 Life cycle of *Rhithrogena semicolorata* in the Dunneill River from July 2001 to October 2002.

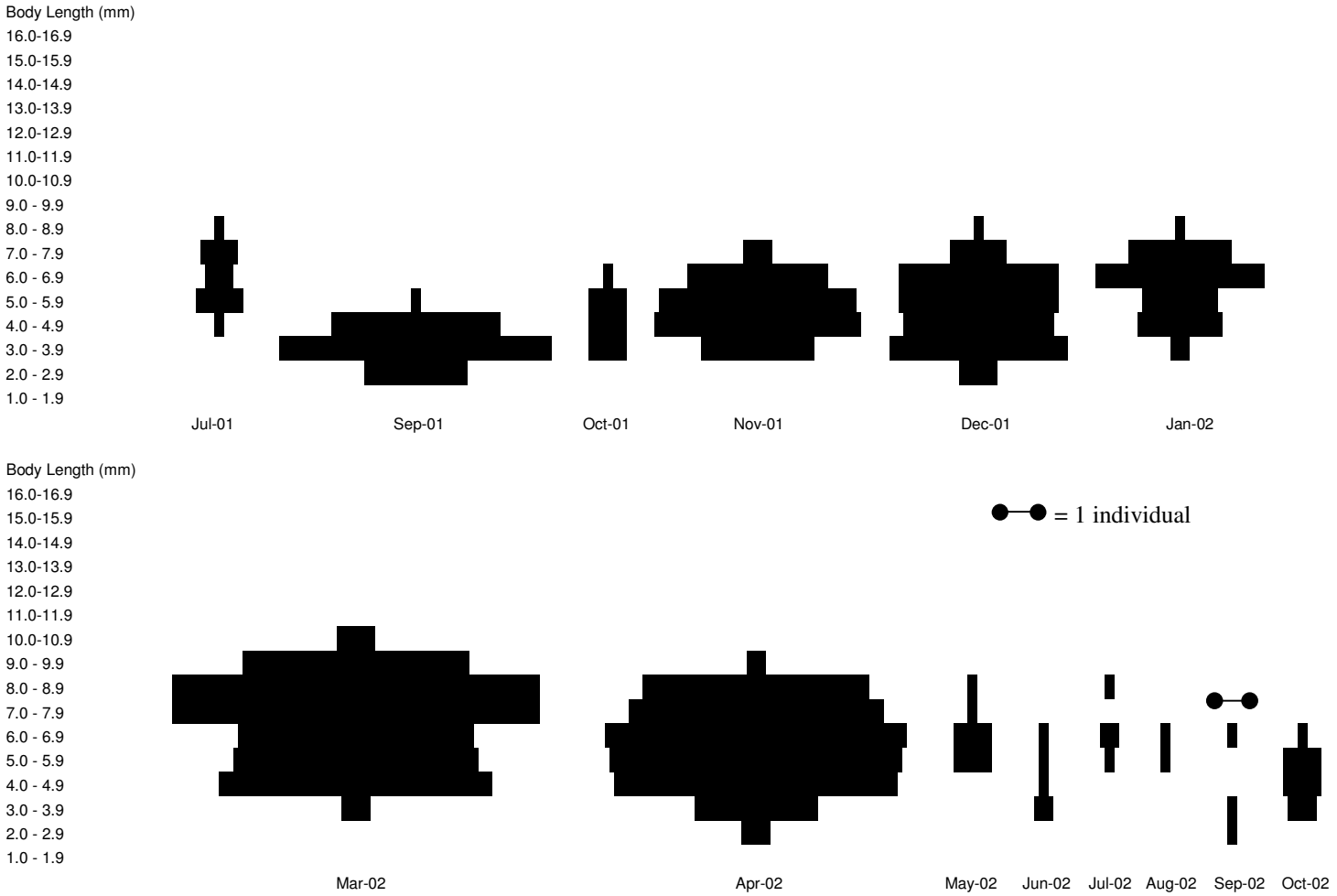


Fig 4.21 Life cycle of *Rhithrogena semicolorata* in the Castlebar River from July 2001 to October 2002.

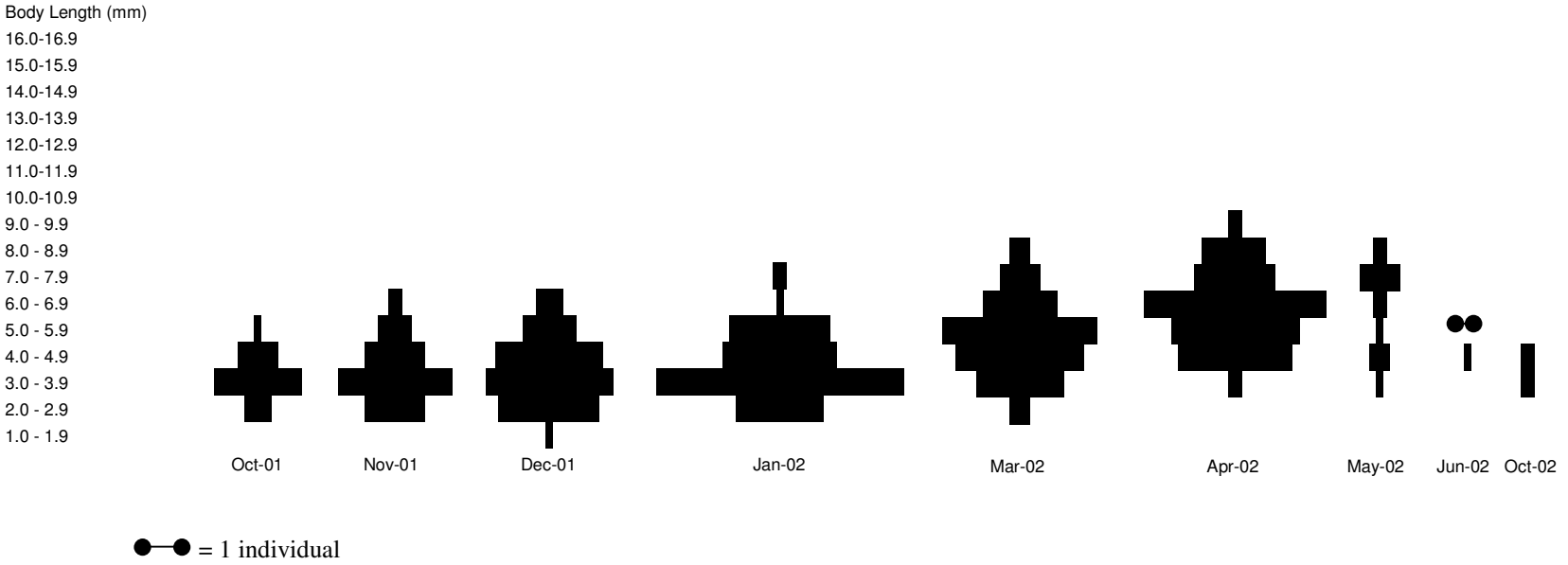


Fig 4.22 Life cycle of *Rhithrogena semicolorata* in Callow Loughs Stream from July 2001 to October 2002.

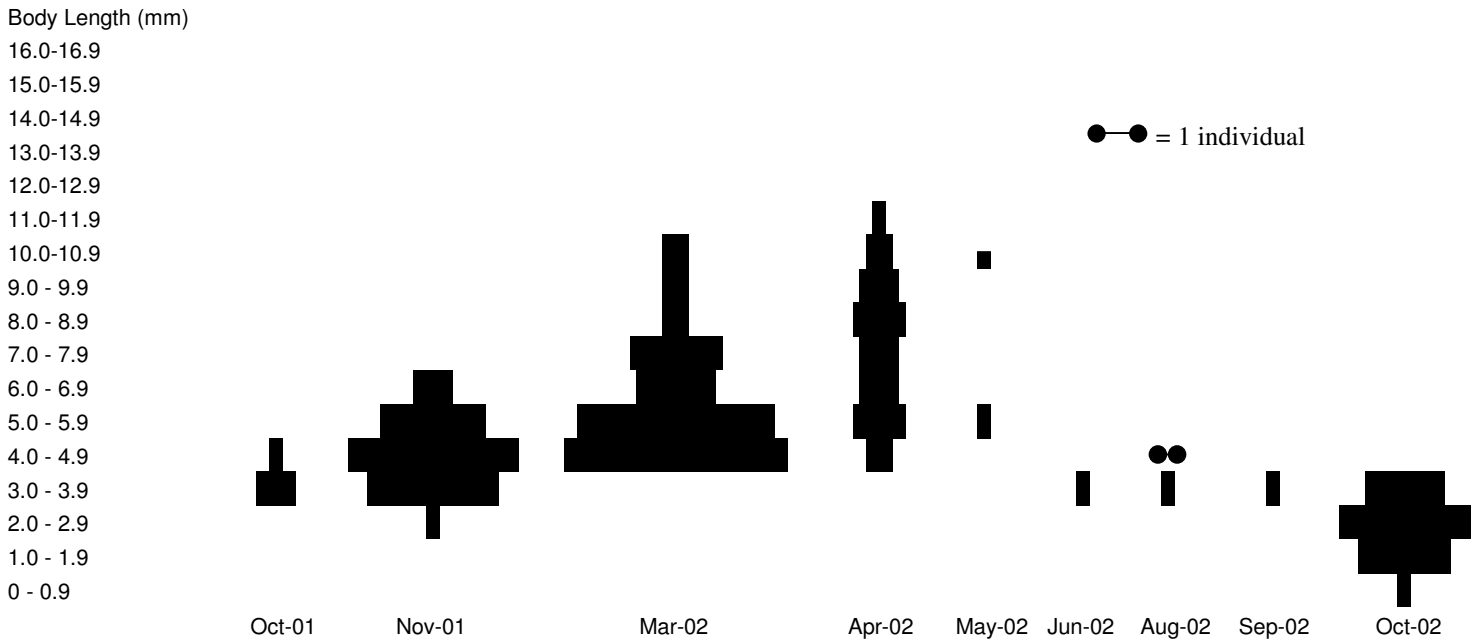


Fig 4.23 Life cycle of *Rhithrogena semicolorata* in the Brusna River from July 2001 to October 2002.

4.4.2.5 Interpretation of the life history of *Heptagenia* species

The *Heptagenia* specimens were not identified to species level, therefore, the life cycle of *Heptagenia* was examined at the genus level only. Adult specimens of *Heptegenia sulphurea* (Müller) were however identified from the Owengarve rearing trials (see Table 4.7). The life cycle of the genus was univoltine and was present in all five high status rivers.

The life cycle was very difficult to interpret in both the Dunneill and the Brusna Rivers as larvae were poorly represented. They were found on only one occasion in the Dunneill River (Fig. 4.24) and varied in size. *Heptagenia* spp. was found in November 2001, January 2002 and March 2002 (Fig. 4.25) in the Brusna River only.

Adults emerged in the other three rivers from May to July 2002. The Castlebar River contained the largest larvae and emerged as adults from May to June 2002 (Fig. 4.26). Some sporadic specimens were still in the benthos in July and August 2002. The larvae in Callow Loughs Stream were poorly represented measuring 1-2mm less than those in the Castlebar River and emerged from June/July 2002 (Fig. 4.27). A sporadic specimen was found in August marking the end of the life cycle. *Heptagenia* emerged in the Owengarve River from June to July 2002 (Fig. 4.28) with larvae of similar size (11-12mm) to those found in the Castlebar River.

In summary, the life cycle of *Hetagenia* genus was univoltine. It was quite difficult to interpret due to low numbers of larvae present in the rivers during the sampling programme. The interpretation is therefore based on findings from three (Owengarve River, Castlebar River and Callow Loughs Stream) of the five high status rivers studied.

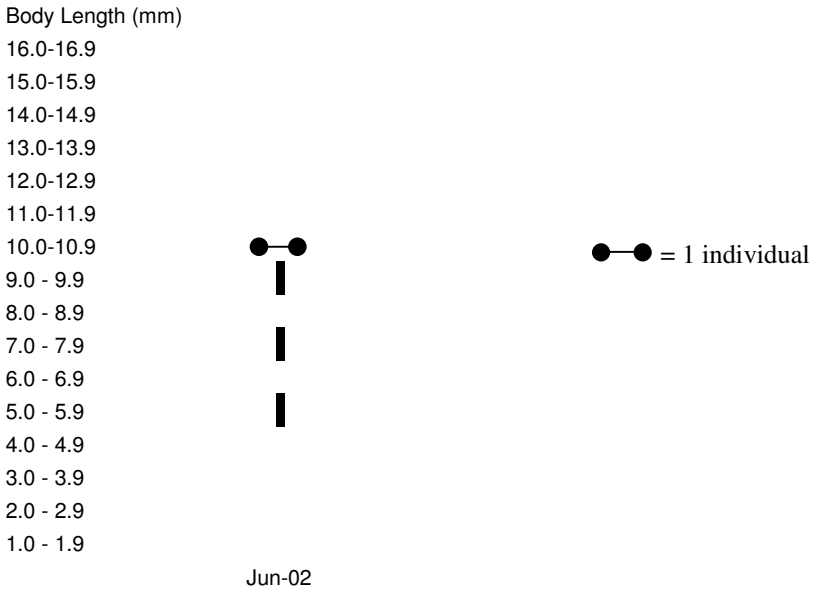


Fig 4.24 Life cycle of *Heptagenia* species in the Dunneill River from July 2001 to October 2002.

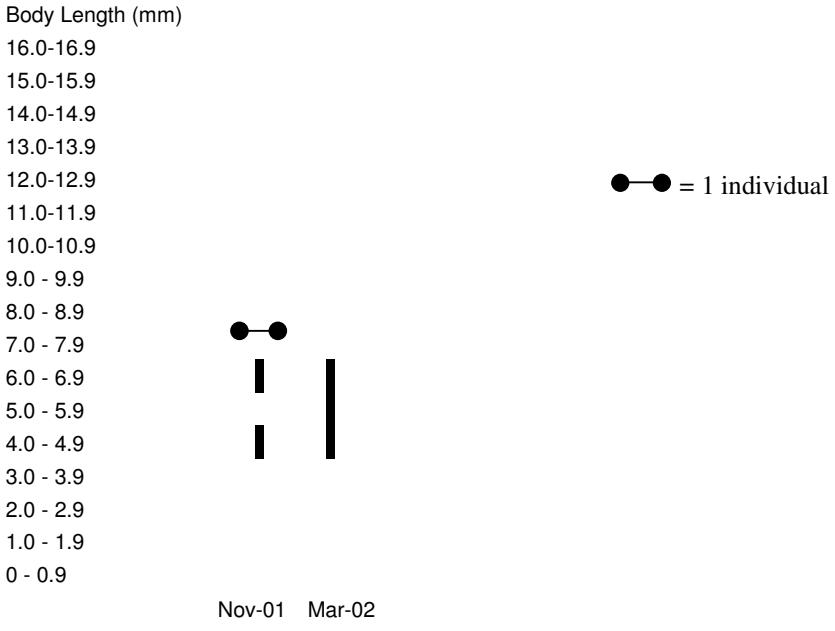


Fig 4.25 Life cycle of *Heptagenia* species in the Brusna River from July 2001 to October 2002.

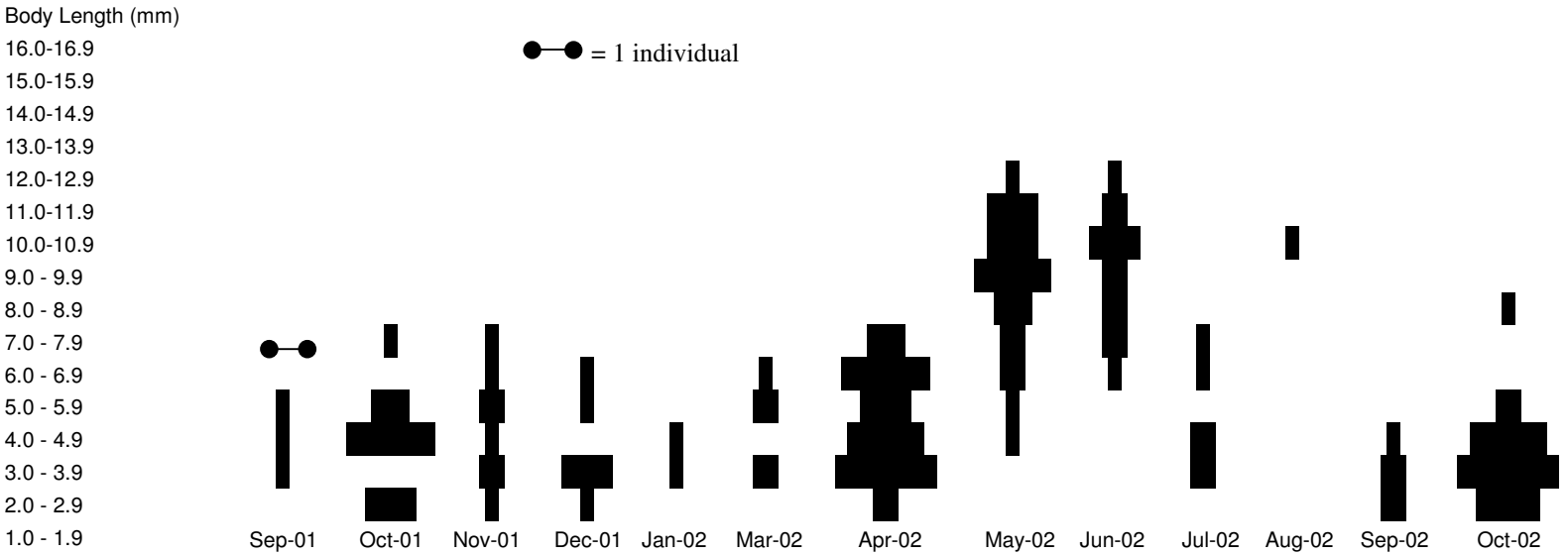


Fig 4.26 Life cycle of *Heptagenia* species in the Castlebar River from July 2001 to October 2002.

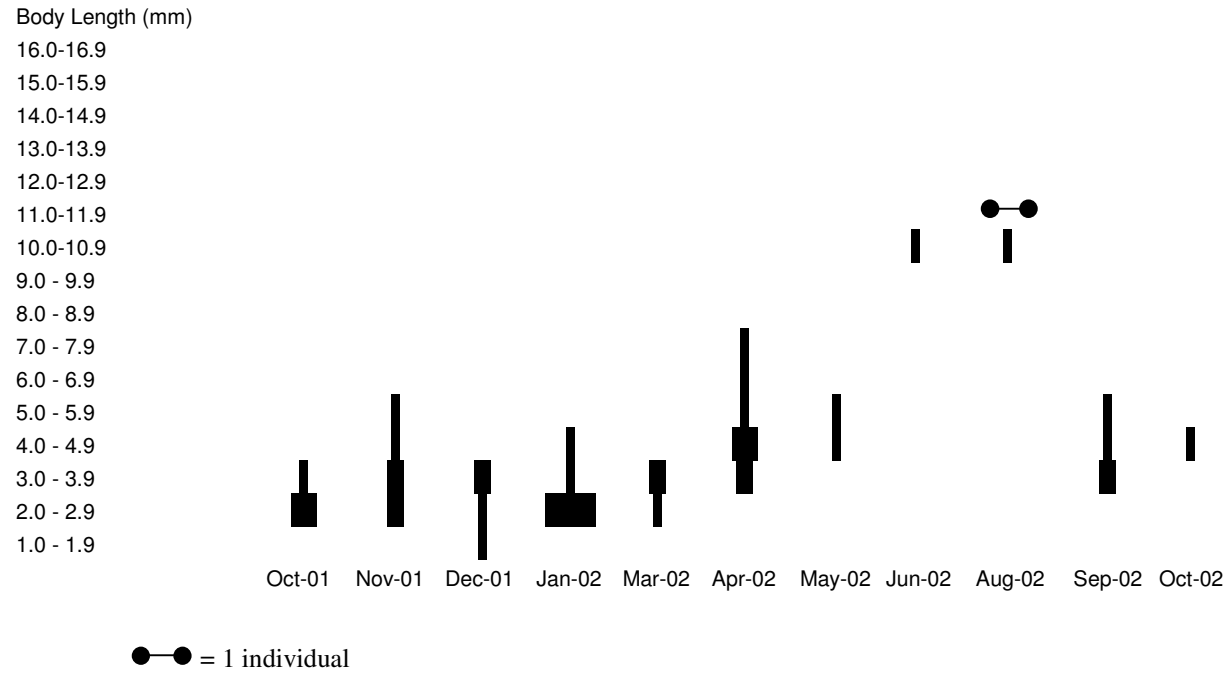


Fig 4.27 Life cycle of *Heptagenia* species in the Callow Loughs Stream from July 2001 to October 2002.

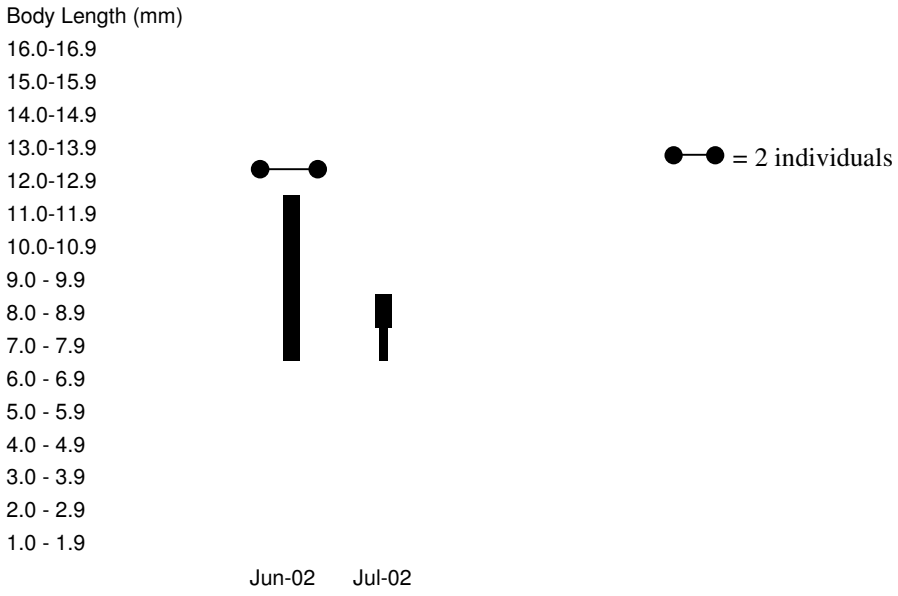


Fig 4.28 Life cycle of *Heptagenia* species in the Owengarve River from July 2001 to October 2002.

Table 4.2 Summary of life history of the Heptageniidae in the Owengarve River from July 2001 to October 2002.

Species	Emergence period Total	Emergence period Main	Length of largest larvae	First appearance of small larvae in large numbers	Over-wintering larvae
Ecdyonurus venosus	August-Sept 2001 March-June 2002 July-August 2002	August-Sept 2001 March-June 2002 August 2002	15-16mm 15-16mm 13-14mm	July 2001 Sept-Oct 2001 Aug-Sept 2002	yes
Ecdyonurus insignis	June-August 2002	July 2002	14-15mm	June 2002	yes*
Ecdyonurus dispar	August 2001 June 2002	August 2001 June 2002	11-12mm 13-14mm	July-August 2001 June 2002	yes*
Rhithrogena semicolorata	March-April 2002	March-April 2002	8-9mm	September 2001	yes
Heptagenia species	**June-July 2002**	June 2002	11-12mm	September 2002	yes

* Assumptions based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980).

Table 4.3 Summary of life history of the Heptageniidae in the Dunneill River from July 2001 to October 2002.

Species	Emergence Period Total	Emergence Period Main	Length of largest larvae	First appearance of small larvae in large numbers	Over - wintering larvae
Ecdyonurus venosus	July-September 2001 March-April 2002 July-August 2002	July-August 2001 March-April 2002 July-August 2002	12-13mm 15-16mm 11-12mm	July 2001 Sept-Oct 2001 Sept-Oct 2002	yes
Ecdyonurus insignis	July-August 2001 May-July 2002	July-August 2001 May-July 2002	13-14mm 11-12mm	 May 2002	yes*
Ecdyonurus dispar	August 2001	August 2001	10-12mm	July 2001	yes*
Rhithrogena semicolorata	March-May 2002	March-May 2002	12-13mm	August and September 2001	yes
Heptagenia species	June 2002	June 2002	10mm	June 2002	yes

* Assumptions based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980)

Table 4.4 Summary of life history of the Heptageniidae in the Castlebar River from July 2001 to October 2002.

Species	Emergence period	Emergence Period	Length of largest larvae	First appearance of small larvae in large numbers	Over-wintering larvae
	Total	Main			
Ecdyonurus venosus	July-August 2001 April-May 2002 July-September 2002	July-August 2001 April-May 2002 July-August 2002	12-13mm 15-16mm 12-13mm	August 2001 June 2002 Sept-Oct 2002	yes
Ecdyonurus dispar	August 2001	August 2001	12-13mm	July-August 2001	yes*
Rhithrogena semicolorata	March-May 2002	March-April 2002	10-11mm	September 2001	yes
Heptagenia species	May-June 2002	June 2002	12-13mm	September 2001	yes

* Assumptions based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980).

Table 4.5 Summary of life history of the Heptageniidae in Callow Loughs Stream from July 2001 to October 2002.

Species	Emergence period Total	Emergence period Main	Length of largest larvae	First appearance of small larvae in large numbers	Over-wintering larvae
Ecdyonurus venosus	July-August 2001 March-May 2002 July 2002	July-August 2001 March-May 2002 July 2002	11-12mm 14-15mm 10-11mm	Sept-Oct 2001 August-Sept 2002	yes
Ecdyonurus insignis	August 2001 July-August 2002	August 2001 July-August 2002	10-11mm 11-12mm	June-July 2001 June-July 2001	yes*
Ecdyonurus dispar	August 2001	August 2001	11-12mm	Only found in Aug 2001 in very small number	yes*
Rhithrogena semicolorata	March-May 2002	April 2002	9-10mm	October 2001	yes
Heptagenia species	June-July 2002	June/July 2002	10-11mm	October 2001	yes

* Assumptions based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980).

Table 4.6 Summary of life history of the Heptageniidae in the Brusna River from July 2001 to October 2002.

Species	Emergence Period Total	Emergence Period Main	Length of largest larvae	First appearance of small larvae in large numbers	Over-wintering larvae
Ecdyonurus venosus	August 2001 March-April 2002 June-August 2002	August 2001 April 2002 June-July 2002	11-12mm 15-16mm 11-12mm	August-September 2001 Sept-Oct 2002	yes
Ecdyonurus insignis	August-Sept 2001 May-August 2002	August-Sept 2001 May-August 2002	11-12mm 12-13mm	 May 2002	yes*
Ecdyonurus dispar	August 2001	August 2001	12-13mm	August 2001	yes*
Rhithrogena semicolorata	March-May 2002	April 2002	11-12mm	October 2001	yes
Heptagenia species	Insufficient data	Insufficient data	6-7mm	November 2001	yes

* Assumptions based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980).

4.4.2.6 Temperature variation among seasons

Seasonal variation in the monthly water temperatures from July 2001 to October 2002 is given in Fig. 4.29. Measurements of the cumulative degree-days for 2001, 2002 and 2003 are presented in Fig. 4.30.

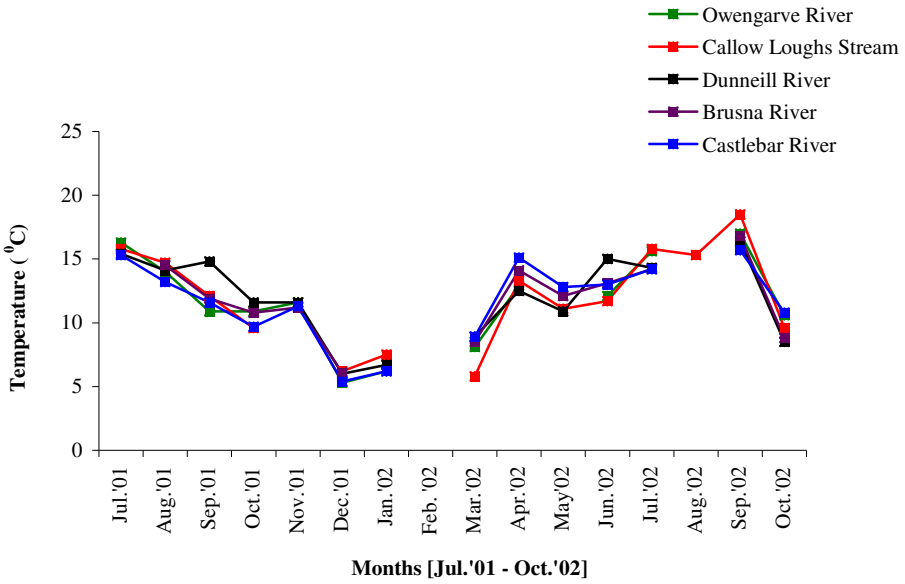


Fig 4.29 Monthly changes in temperature in the five river sites from July 2001 to October 2002.

Water temperatures increased substantially from March to April 2002 (Fig. 4.29) which appears to coincide with the emergence of *Ecdyonurus venosus* and *Rhithrogena semicolorata* larvae in all five rivers studied (Tables 4.2 to 4.6 and life cycle graphs below). Larvae of *Ecdyonurus venosus*, *Ecdyonurus insignis*, *Ecdyonurus dispar* and *Heptagenia* spp. emerged at different points during the summer seasons from July to September 2001and subsequently from May to September 2002.

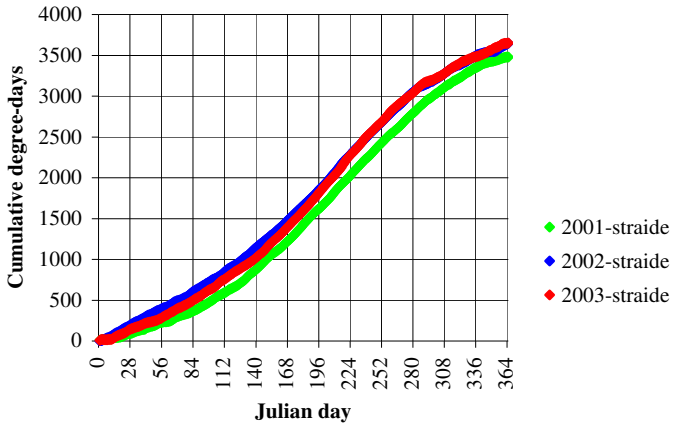


Fig. 4.30 Cumulative degree-days (air temperature) recorded at Straide weather station for 2001, 2002 and 2003. Straide represents the nearest station to the rivers studied in this investigation.

The cumulative degree-days data for 2001, 2002 and 2003 (Fig. 4.30) show that 2001 was a cooler year compared to 2002. This is also reflected in the changes observed in the water temperatures over the course of the study (Fig. 4.29). Unfortunately, the sampling programme did not commence until July and August 2001 so data from March to June (and July in the case of the Brusna River) were not available. It was therefore not possible to investigate whether larvae emerged later during this period in 2001 compared to 2002. It is important to note, however, that temperature, in conjunction with other factors such as food quality and quantity, is an important aspect controlling the life cycle of the Heptageniidae (Benke, 1984; Ward, 1992).

4.4.3 Results from the rearing experiments

In general, mortality of the nymphs was quite high during the rearing trials. A list of the species that successfully emerged into the imago stage is presented in Table 4.7.

Table 4.7 Species list of successfully reared adults from the rearing trials.

River	Date collected	Species identified	Number of specimens/Sex
Owengarve River	24/06/03	***Heptagenia sulphurea***	2 x sex unknown
	24/06/03	*Ecdyonurus insignis*	6 x Females: 2 x Males
	04/07/03	*Ecdyonurus insignis*	2 x Female: 5 x Males
	04/07/03	*Ecdyonurus dispar*	2 x Female
	11/09/03	*Ecdyonurus dispar*	3 x Female: 5 x Male
Dunneill River	24/06/03	*Ecdyonurus insignis*	3 x Female
	24/06/03	*Ecdyonurus venosus*	1 x Male
Castlebar River	18/06/03	*Ecdyonurus venosus*	2 x Male
	28/07/03	*Ecdyonurus dispar*	2 x Male
	10/07/03	*Heptagenia sulphurea*	1 x sex unknown
Brusna River	04/07/03	*Ecdyonurus insignis*	1 x Male

The duration of the emergence period varied throughout the trials ranging from 1-7 days and generally depended on the stage of development of the larvae when collected.

The emergence success of larvae of *Ecdyonurus venosus* was quite low in comparison with larvae of *Ecdyonurus insignis* and *Ecdyonurus dispar*. Larvae obtained from the Owengarve River appeared to have the greatest survival rate in the aquarium and emerged quite successfully to the imago stage. This was true in particular for larvae of *Ecdyonurus dispar* and *Ecdyonurus insignis*. Due to the low numbers of *Ecdyonurus* larvae in the Brusna River, there was difficulty in collecting adequate amounts for the rearing trials from this river. Mortality was high in specimens obtained from the Castlebar and the Dunneill Rivers, especially with specimens of *Ecdyonurus venosus*. These tended to die within a few days of being moved

into the aquarium. This may in itself be an added indication of the relatively high sensitivity of *Ecdyonurus venosus* to environmental stress in general.

4.5 Discussion

4.5.1 Introduction

Mayflies play an important role in almost all undisturbed freshwater communities and their larvae frequently form a considerable part of the material sampled during biomonitoring procedures. The genus *Ecdyonurus* in particular, is widely accepted as a bioindicator for water quality and as a key indicator species that forms an integral part of the EPA biological Q-Value system for many years. It is important therefore to have a good knowledge of the life cycle in order to be able to interpret the absence of *Ecdyonurus* in particular. It is necessary to be able to state definitively that at least one species of *Ecdyonurus* can be expected to occur in the riffle benthos of unpolluted rivers, at any time of the year.

The EPA carries out routine biological assessments on rivers in Ireland on a yearly basis, with an emphasis on summer months when pollution stresses are expected to be at their highest. It was hypothesised that all species of *Ecdyonurus* would be found during the summer season.

The overall objective of this study, therefore, was to establish the life cycle of various species within the Heptageniidae family with specific emphasis on the life cycle of the genus *Ecdyonurus* in five high status rivers in the West of Ireland.

The Heptageniidae species examined in the five rivers in this study are widely distributed in Europe. *Ecdyonurus venosus*, *Ecdyonurus insignis*, *Ecdyonurus dispar*, *Rhithrogena semicolorata* and *Heptagenia* spp. are all southern/central European species (Illies, 1978). The catchment areas of the five rivers studied here occupy a somewhat intermediate latitudinal position in Europe but located in a mild Atlantic-influence region of Ireland.

4.5.2 Interpretation of the life history of the genus *Ecdyonurus*

Ecdyonurus venosus was the dominant species in all five rivers studied in this investigation. It was found throughout the study period with the exception of a few months in some sites where it was absent prior to the onset of hatching and new recruitment processes. The abundance of this species in the benthos gradually increased rapidly initially, while small larvae were being added to the population and maximum numbers usually occurred towards the end of a hatching period. The life cycle of *Ecdyonurus venosus* was bivoltine in all five high status rivers, displaying slow-growing over-wintering generations that emerged in spring 2002 and fast-growing summer generations that emerged during late summer and autumn of 2001 and 2002. It would be important to distinguish bivoltine populations from overlapping univoltine populations that are just out of synch. The data allowed us to say that we are not dealing with univoltine populations that for example emerge in March/April and grow right through from then until the following March/April with another group that emerge in September growing through until the following September also.

Ecdyonurus venosus has been described in the literature as having two types of life cycles (Fahy, 1973). It is described as being univoltine with overwintering larvae, adults being found from April to July, sometimes to October (Elliott, 1967; Landa, 1968; Thaibault, 1971; Sowa, 1975; Wise, 1980). On the other hand, studies carried out by Connolly and McCarthy (1993), showed that the life cycle of *Ecdyonurus venosus* was univoltine in the Corrib catchment, while it is capable of completing two life cycles per year in continental Europe. However, the maximum sizes of nymphs of this species recorded in the former study were up to 7mm longer than those recorded in other European studies (Whitney, 1939; Hynes, 1961; Elliott, 1967 and Thaibault, 1971). Whelan (1980) described variations in the life cycle of *Ephemera danica* populations in Irish lakes and attributed them to differences in lake temperature regimes and other environmental factors.

Studies carried out by Fahy (1973) on the growth patterns of ten species of Ephemeroptera in a small stream system in the West of Ireland (the

Altahoney system) described the life cycle of *Ecdyonurus venosus* as being bivoltine. He also compiled emergence data or more accurately, flight period data, by setting Mundie trappings, sweep netting the bankside vegetation and beating tray collections from bankside trees. This contributed to a more accurate description of the life cycle of this species. Wise (1980) described the life cycle of *Ecdyonurus venosus* in a Northumbrian River in England as univoltine. Elliot (1967) also noted that the life cycle of this species in a tributary of the East Dart, in central Dartmoor, was univoltine. This was in contrast to the findings of Rawlinson (1939), in a study on the River Alyn, who found that *Ecdyonurus venosus* had a fast growing summer generation in addition to a slow-growing winter generation (bivoltine).

The emergence periods of *Ecdyonurus venosus* showed only slight variations between the river sites studied. It is widely known that the temperature together with the quantity and quality of the food available are the main factors that effect the life history of aquatic insects (Benke, 1984; Ward, 1992). The duration of the larval life of a species may vary with the season of the year and also with its geographical distribution. The life cycle of *Ecdyonurus venosus* herein described, showed a seasonal variation in the length of larval life and was more than likely directly dependent upon environmental conditions, particularly on temperature and food supply. It is reasonable to suggest that body sizes may therefore vary with local conditions, which will determine the length of the whole aquatic period of life and the season of emergence. This may account for the slight variation in emergence patterns between the rivers studied in this investigation. As temperatures increased in the rivers during this study in spring 2002 and food became more abundant the over-wintered larvae began to grow more rapidly. The availability of more food more than likely accounted for the larger body sizes during this period.

The fast growing summer stock of *Ecdyonurus venosus* emerged slightly earlier in 2001 in the Castlebar River, the Dunneill River, Brusna River and Callow Loughs Stream, compared to the Owengarve River. The slow-growing winter generation emerged during approximately the same period

in spring/summer 2002 but extended into June in the Owengarve and into May in the Castlebar River and Callow Loughs Stream. Another fast growing summer generation emerged in the five river sites in late summer/early autumn of 2002 when the average larvae had reached only 11-12mm. It is postulated that the principal driving factor determining whether emergence can occur or not is body weight rather than body length and that the slow-growing overwintering larvae lose weight during the cold winter period even if they perhaps undergo an additional moult allowing them to grow significantly longer than the summer generation. When they emerge in March April, however, they have just reached the body weight necessary to emerge and reproduce as adult insects.

The slow growing, over-wintering generation that emerged in spring 2002 seemed to grow to larger sizes compared to the fast growing summer stock of both 2001 and 2002, although there were no statistical differences observed. Even though egg development was not studied in this investigation, it was assumed from the interpretation of the findings from these studies that the majority of the summer eggs hatch within one or two months and similarly the eggs laid by the late-autumn adults hatch fairly rapidly and overwinter as larvae and taking from eight to ten months to complete their growth. The winter stock appeared to have developed from eggs oviposited in the autumn that either hatched in late autumn or remained as eggs that slowly hatched over the winter into larvae with a slower rate of growth caused by low temperatures. The rapid growth in spring and early summer was no doubt due to the abundance of plant food and the higher river temperatures, the slower growth, to cold and the scarcity of food. The larvae probably browsed on stones covered with algae and plant debris (Chapter 2) but during the cold months food is usually relatively scarce and can be further depleted by the scouring action of the winter floods (Rawlinson, 1939).

One of the major difficulties encountered in constructing the life cycles of the genus *Ecdyonurus* was identifying accurately the small immature larvae to species level. These unidentified juvenile larvae were assigned to the "*Ecdyonurus* species" category in the length frequency histograms. It was

particularly difficult to identify small larvae of *Ecdyonurus dispar* as the main diagnostic feature in differentiating between the species of *Ecdyonurus*, namely the pronotum, was not fully developed in larval specimens under 7-8mm in length. The larvae of *Ecdyonurus insignis* were much easier to identify as all seven gills have a diagnostic tuft or filament attached.

Even after the majority of the life cycles were constructed, it was still difficult to assign these immature larvae to a particular species. This proved even more complicated when the three different species of *Ecdyonurus* were found in a sample at the same time of year, like that found in the Owengarve River during the summer of 2002. Unfortunately, studies on the duration of egg development of the genus *Ecdyonurus* was not carried out during these investigations. There was an attempt to examine the flight periods of this genus, particularly in the more productive systems but it proved unsuccessful due the difficulty in finding adult specimens and time constraints. It was abandoned after a number of weeks when no adults were captured. Detailed studies on both the emerged adult populations and knowledge of the development of eggs are essential to complete the full life history of this sensitive indicator genus.

Ecdyonurus dispar emerged in all rivers during August 2001 only but emerged again the following June 2002 in the Owengarve River owing to the productive nature of this system. It emerged in August 2001 in all five rivers and appeared to happen at a time when the majority of the other *Ecdyonurus* species had already emerged or were about to reach the end of their emergence period. It was hard to say whether larvae of *Ecdyonurus dispar* grew over the winter months as the juveniles measuring under 8mm in length were extremely difficult to identify. Larvae greater than 8mm in length however were only identified in August 2001. The life cycle of *Ecdyonurus dispar* in these high status rivers was univoltine.

Studies carried out by Macan and Maudsley (1968) in Lake Windermere in Northern England and in the River Lune in 1979 and 1981 reveal a similar life cycle. The larvae grew all through the winter and some had reached full

size by May or June, though specimens of all sizes were still present. Observations on emergence patterns were recorded by Kimmins (1972) who recorded a flight period from June to October. In June the number of small nymphs increased and Macan and Maudsley (1968) interpret this to be the appearance of a second generation. However this interpretation was found to be incorrect because Humpesch (1980), on the rate of development of eggs and larvae at different temperatures showed that these small larvae could not have come from the eggs laid the same year; they could only have come from eggs laid late during the previous season when the temperature was falling to a level at which the development was slow. It is possible that eggs laid by the earliest adults gave rise to a quick summer generation emerging late in the season, but most of the populations achieve one generation in a year. This was also observed during his studies in 1981. The explanation favoured as to why the larvae were not present in the samples taken in July are that the larvae were prevented from colonising the superficial stones by the larger larvae of the other species like *Ecdyonurus venosus*.

Studies carried out by Wise (1980), showed that *Ecdyonurus dispar*, *Ecdyonurus venosus* and *Ecdyonurus torrentis* were all univoltine and that *Ecdyonurus torrentis* and *Ecdyonurus venosus* were both winter-growing species which emerged in the spring. *Ecdyonurus dispar* exploited the same niche during the mid-summer period when the other species of the genus were absent from the benthos. Macan (1957) found a similar cycle for *Ecdyonurus torrentis*. However, he also found that growth and emergence continued throughout the summer. Harker (1952) suggested that *Ecdyonurus torrentis* had a cycle of three generations in 2 years but the nymphs which hatched between August and April in the Coquet in Britain are thought to belong to the same cohort. However, Macan (1970) stated that *Ecdyonurus dispar* is absent from other lakes in the winter, though often abundant for a brief period in the summer.

Macan and Maudsley (1968) also observed the absence of small (2mm) and medium-sized larvae of *Ecdyonurus dispar* throughout the winter and in spring in some English lakes, e.g. Ennerdale Water (Macan, 1970) and in

English and Central European rivers (Landa, 1968; Sowa, 1975; Macan, 1979; Wise, 1980) which led to their conclusion, that there was only a quick summer growing generation. As far as the Ennerdale population was concerned, the latter interpretation was found to be incorrect because there was no evidence for an egg or larval diapause in winter and indeed, in 1977-78 when this study was carried out, small larvae (2mm) of *Ecdyonurus dispar* were collected during the winter. In May 1978, tiny larvae were also captured and these could not have been progeny of adults emerging in 1978. The absence of specimens in previous studies in this system may have been due to an irregular distribution and a scarcity of larvae in some lakes.

Ecdyonurus insignis was found in four out of five of the high status rivers in this study. It was completely absent from the Castlebar River. This species displayed a univoltine life cycle. It emerged from the Dunneill River, the Brusna River and Callow Loughs Stream towards the end of July and on into August 2001. The larvae of this species usually emerged when the numbers of larvae of other species of *Ecdyonurus* were low which usually indicated the end of an emergence period. Emergence periods for *Ecdyonurus insignis* varied slightly between the rivers. During 2001, this species emerged mainly during August and September 2001. The following year, in some of the rivers, the larvae began to emerge as early as in May 2002 while others emerged on into August 2002. With the exception of a study carried out in the River Lune by Macan in the late 1970's (1981), no previous investigations on *Ecdyonurus insignis* have been discovered. In his study, Macan found a generation of *Ecdyonurus insignis* during the summer months only and described it as having a univoltine life cycle with overwintering larvae. In central Europe this species overwinters in the egg stage (Landa, 1968; Sowa, 1975, 1979). Adults of this species have been found from May to October in the British Isles (Elliott and Humpesch, 1983).

As with *Ecdyonurus dispar*, *Ecdyonurus insignis* was found as mature larvae only during the summer months, predominantly during the months of May through to August. Again, there was no evidence of this species in the rivers during the winter or spring seasons. The absence of larvae of both

Ecdyonurus dispar and *Ecdyonurus insignis* during the winter and spring months suggests that these species develop quite differently to the more common species *Ecdyonurus venosus*. No examination of egg development of these species was carried out so it was difficult to ascertain whether they overwintered as eggs and larvae. Interpretations are based on the suggestions and material obtained from previous studies in the literature, which were mostly based in Britain in particular those from Elliott and Humpesch (1983) and Macan and Maudsley (1968). Findings from studies carried out in Ireland by Wise (1980) also support the suggestion that *Ecdyonurus venosus* and *Ecdyonurus dispar* overwinter as larvae. There have been no further studies to date of this nature in Ireland prior to this present investigation.

The absence of larvae of *Ecdyonurus dispar* during the winter and spring in localities where other *Ecdyonurus* spp. occur as well and where a definite pattern of the flight period of the latter has been observed, for example, in the River Lune. This led Macan (1981) to the following hypothesis: in the presence of larger larvae of another species, smaller ones are confined to the deeper parts of the substratum and do not colonise the stones at the surface until the larger have emerged. He suggests that while confined to the deeper regions larvae grow less rapidly than when at the surface. This so called 'size hierarchy effect' (Brown, 1957; Ricker 1979) is a common feature in trout hatcheries for fish having started at about the same size and it is understood that when the food supply is limited the establishment of a social hierarchy can result in an 'adequate' food supply and rapid growth for the dominant fish, and a 'less than adequate' food supply and slow growth for subordinate fish. When larger fish are removed, smaller ones recommence rapid growth once again (e.g., Coates, 1980). In some situations, the growth of smaller fish in a group of brown trout fry would not accelerate even under the conditions in which an adequate supply of food was available to support rapid growth by all (Peter, 1979).

Similar effects of intra-specific competition, shown for the freshwater crayfish *Oronectes virilis* (Hagen) by Momot and Jones (1977), indicate reverse relationship between growth rate and total standing stock biomass.

Variation in size of *Ecdyonurus dispar* of the same brood was observed in the experiments of groups of larvae at 20, 14, 9°C, and similar observations have been made by several other workers, e.g., Rawlinson (1939); Hunt (1953); Trost and Berner (1963); Bohle (1978), for other ephemeropteran species. It is not known what may have caused this variation and if the latter occurs in the field as well. However, different growth rates of larvae of the same brood maintained under the same environmental conditions may explain the existence of a long summer emergence period in some species or the split of the emergence period of an autumnal cohort into late autumn and early spring one (Humpesch, 1981). The fact that species with a long flight period tend to produce larger females and hence more fecund females early in the season has been reported by several workers, e.g., Beneche (1972), Elliott and Humpesch (1980). Sweeney and Vannote (1978), have proposed that such changes in North American species are due chiefly to temperature affecting adult size and fecundity by altering the larval growth rate and the timing and rate of adult tissue development.

Information on hatching time is therefore essential for the recognition and separation of cohorts of ephemeropteran species in the field, and cohorts must be separated for accurate estimations of growth rates, mortality rates and production (Humpesch, 1980). As a result of extensive field studies, Landa (1968) determined the number of generations per year for several ephemeropteran species by observing larval development. He assumed that eggs of *Ecdyonurus dispar* and *Ecdyonurus insignis* passed through a diapause stage, because newly-hatched larvae or small larvae could not be found in the samples during the autumn and winter, long after the flight period. Several other workers have made similar interpretations. Studies by Humpesch (1980) indicates that the classifications of Landa (1968) are only partially correct as there is no evidence of an obligatory diapause in eggs of *Ecdyonurus insignis* (River Eden) and *Ecdyonurus dispar* (Windermere, Lake Ennerdale). He surmised that the newly hatched larvae must be present in autumn and winter in these rivers. There is some information on the occurrence of young stages of Ephemeroptera and some workers have pointed out that most of them live deep in gravel beds (e.g. Macan, 1958;

Tilzer, 1968) whereas most of the older larvae live nearer the surface. Therefore, it may be difficult to find or catch the smaller larvae.

In all of the rivers investigated, the most common species present was *Ecdyonurus venosus* and it was also the largest of the larvae. The suggestion by Macan (1981) that in the presence of larger larvae of another species, in this case *Ecdyonurus venosus*, smaller ones are confined to the deeper parts of the substratum and do not colonise the stones at the surface until the larger have emerged, appears plausible in explaining why both *Ecdyonurus insignis* and *Ecdyonurus dispar* are only found in the benthos during the summer months. It reinforces the idea that the larvae grew very slowly during the winter months and began to grow steadily when the temperatures rose during the spring and summer seasons to emerge in late summer. Their absence from the benthos in winter months may also suggest that the eggs overwintered and hatched into a fast growing summer generation.

Partitioning of emergence periods appeared to be evident in this investigation, particularly in the Owengarve River as this river contained an abundance of *Ecdyonurus* specimens. The winter generation of *Ecdyonurus venosus* emerged between March and June 2002, *Ecdyonurus dispar* predominantly emerged in June 2002 while the larvae of *Ecdyonurus insignis* emerged in July 2002. Finally, the fast growing summer generation of *Ecdyonurus venosus* emerged in August 2002. Each species appears to divide itself into separate emergence periods during the summer months in order to maximise survival rates. Depending on the number of *Ecdyonurus* species present in the rivers, each individual species appeared to have adapted their life cycles to emerge at the most suitable time, in order to ensure the survival of the next generation. This phenomenon was also evident in studies carried out by Macan (1981). He investigated the life history of *Ecdyonurus torrentis* and *Ecdyonurus dispar* in the Lune River and found them to be similar, except that, at the same temperature, *Ecdyonurus torrentis* emerged earlier than *Ecdyonurus dispar* and its eggs hatched earlier. He implies that this may account for the way in which the two species divide the Lune between them in the manner observed.

Some other workers have found that the flight periods of the different species in the same biotype may follow a definite pattern, e.g. adults of *Ecdyonurus torrentis* appear first followed by *Ecdyonurus insignis* and *Ecdyonurus dispar* (Landa, 1962). From these field studies, some authors have suggested that these variations in the life cycle or this succession of species may be due to different patterns of egg development or larval growth e.g., it is suggested that the eggs of *Ecdyonurus torrentis* hatch about one month after oviposition, while those of *Ecdyonurus dispar* and *Ecdyonurus insignis* do not hatch until the following year (Landa, 1968; Sowa, 1975). Again, this information underpins the suggestion from our findings that different species in the same river system, like those observed in some of the rivers investigated in this study, follow chronological patterns of emergence periods in order to ensure the survival of each individual species. Therefore, different patterns in life cycle and succession of *Ecdyonurus* spp. can be partly explained by variation in their hatching times at different temperatures and in different localities but further studies need to be carried out in Ireland which would include egg development and flight period studies.

4.5.3 Interpretation of the life history of *Rhithrogena semicolorata*

Rhithrogena semicolorata was found in the five high status rivers investigated and was well represented at all sites. It clearly displayed a univoltine life cycle where emergence periods ranged from three months (March to May 2002), to two months (March to April 2002). The majority of the final instars emerging during March and April 2002. It possessed a slow-growing generation that over-wintered as larvae and grew steadily as the temperatures increased in the spring. Larvae were absent from the benthos after the emergence period for only one month in some rivers while this was extended to up to three months in others (typically from June to August) prior to the onset of another hatch. The eggs laid by the emerged females began to hatch in September/October 2002 into young larvae indicating that there was no embryonic diapause.

The larvae found in our studies varied in size prior to emergence. The smallest larvae to emerge were found in the Owengarve River ranging from 8-9mm while the largest to emerge were captured in the Dunneill River measuring between 12-13mm. Studies carried out by Fahy (1973) display some variation from the typical pattern found by other authors, chiefly in that there was a fall in the numbers of instars from March to April 1971. Harker (1952) indicated a final instar size of 14mm and Macan (1957) final instar size of less than 12mm. Hynes (1966) reported a final instar size of less than 10mm. In the work described by Fahy (1973) the largest nymphs were 13mm long at the beginning of the emergence period but later only smaller ones could be found and the last to emerge were between 9 and 10mm long. He points out that the size difference may be the result of the emergence of smaller individuals as the hatch progresses, a phenomenon reported as widespread among the Ephemeroptera (Coleman and Hynes, 1970).

Eventhough the temperature in the rivers was only taken once a month (with a gap of up to 4 weeks between measurements) there was a notable increase of between 5-7°C in the water temperatures from March to April 2002. This rise in water temperature appeared to signal the onset of the emergence of

Rhithrogena semicolorata in the rivers studied. Irrespective of what size the larvae had already reached during this period, they began to emerge from the rivers apparently triggered by the warmer waters (13-15°C) and ensuring the survival of the next generation by emerging as temperatures were on the increase.

Rhithrogena semicolorata occurs in South and Central Europe, Britain and Denmark, but is not found in the North European region of the Soviet Union or in Scandinavia (Sowa, 1975). With the exception of the study carried by Fahy (1973) there have been no investigations into the life cycle of *Rhithrogena semicolorata* in Ireland to date therefore comparative information on the life history of this species is taken from studies carried out in England and Switzerland. According to Elliott and Humpesch (1980), nymphs of *Rhithrogena semicolorata* appear to grow very slowly after hatching and the life cycle takes about one year from oviposition to emergence of the adults. Studies by Wise (1980) in a Northumbrian river, showed that the life cycle of *Rhithrogena semicolorata* was also univoltine, possessing a slow-growing winter generation which commenced hatching in early autumn and emerged during the spring. Nymphs were absent from the benthos during mid-summer. The life cycle of *Rhithrogena semicolorata* in the rivers studied in the present investigations reflected a similar pattern. In studies carried out in a prealpine stream system in Switzerland, this species also had a univoltine life cycle (Breitenmoser-Wursten and Sartori, 1995).

Wise (1980) also found that the temporal distribution and development of *Rhithrogena semicolorata* typically a 'winter' species was apparently limited by the warmer temperatures which prevailed for a longer period in the lower reaches during the summer. Thus, in these lower reaches, emergence was sooner and hatching took place later than in the hill region. Hence, the period during mid-summer when the nymphs were absent from the benthos was of correspondingly longer duration. This pattern was also observed during the present study. For example, in Callow Loughs Stream, *Rhithrogena semicolorata* was absent in July, August and September 2001 and hatched in October into young larvae. It emerged predominantly in March and April 2002 with a few remaining in May. The nymphs were

absent for a period of three months and began hatching into immature larvae in October 2002. The situation was slightly different in the Dunneill River. Young larvae were only absent in July 2001 and began to hatch into the system in August to commence a new generation. It began to emerge in March 2002 and continued until the end of June. Immature larvae were absent for a shorter period (two months) in this river and appeared again in the system in August 2002. Interestingly, the larvae in the Dunneill River grew to larger sizes (12-13mm) prior to emergence in comparison to the smaller sized larvae (9-10mm) found in Callow Loughs Stream at the onset of the emergence period. It is reasonable to suggest, therefore, that this was due to the larvae spending a shorter period of time (7-8 months) growing in the benthos, particularly during the winter period when temperatures were low, producing smaller sized animals. The larvae in the Dunneill River grew to larger sizes presumably as a consequence to spending a longer period of time (8-9 months) in the benthos reaching body lengths of between 12-13mm.

4.5.4 Interpretation of the life history of *Heptagenia* species

Heptagenia spp. was found in the five highs status sites but due to insufficient numbers captured during the sampling period in the Brusna and the Dunneill Rivers the life cycle was not interpreted in these sites. It was the least abundant of all the Heptageniidae species studied in this investigation. The specimens were identified to genus level only so it is possible that a number of species were among this genus. The *Heptagenia* specimens were therefore described as a genus group and appeared to adopt a univoltine life cycle with an overwintering larval generation.

Wise (1980) noted that *Heptagenia lateralis* (Curtis) was also univoltine with an overwintering generation that grew rapidly prior to emergence in early summer. He noted overall that *Heptagenia lateralis* grew little during the winter and emerged later than *Rhithrogena semicolorata* after a period of rapid spring growth. *Rhithrogena semicolorata* was absent from the benthos for a period during the summer between the completion of emergence and the beginning of the next generation.

Heptagenia spp. was poorly represented in the Dunneill River, Callow Loughs Stream and in the Brusna River. The life history of this genus displayed very similar patterns in the Owengarve and the Castlebar River. In both, larvae were absent from the benthos in July and August 2001 and hatched in September 2001. During the autumn and winter small instars were present but growth was minimal. In March and April, an outburst of hatching activity coincided with an increase in growth rate followed by its emergence in June 2002. A new generation hatched a month earlier in the Owengarve River (August 2002) compared to the Castlebar River where the next generation hatched in September 2002. Recruitment continued on into October 2002 as juvenile larvae increased in numbers. It is worth noting that in all sites, *Heptagenia* spp. emerged later than *Rhithrogena semicolorata*

4.5.5 Rearing trials

In general, the success of the rearing trials was quite low. This was especially true for the successful emergence of *Ecdyonurus venosus* from nymph to adult. Quite a large number of larvae of this species were identified in the rivers but failed to survive beyond a few days after transferring to the aquarium. Interestingly, larvae of *Ecdyonurus insignis* and *Ecdyonurus dispar* had better survival rates and emerged more successfully than *Ecdyonurus venosus*. This species may have been more sensitive to the type of algal slime that grew in the tank causing death. Alternatively, the copper pipe used to connect the reservoir to the aquarium tank may have caused a small degree of toxicity that this species is more sensitive to compared to the others, as all three species were reared at the same time and exposed to the same water. These hypotheses however remain purely speculative.

Finlay (2001) found greater rearing success of mayfly larvae in small individual chambers with much less water (1.25 litre plastic soft drink bottle cut in two and oxygenated) compared to large flow tanks powered by propellers. He considered this to be indicative of an inherent need for highly oxygenated water in some genera. For instance, a high rate of water movement may be necessary for the development of *Ecdyonurus venosus* that may not have been adequate in the aquarium used in our rearing experiments. It is possible that it requires a more concentrated supply of well oxygenated water to survive. It also indicates, that *Ecdyonurus insignis* and *Ecdyonurus dispar* may need lower current flow to complete its life cycle successfully. As mentioned previously, these rearing trials were designed solely for the verification of the various species of *Ecdyonurus*, particularly where a few species were present at the same time in the same river.

It was hoped that they would also help in confirming emergence periods among this genus and that the timing would be similar to those described from the studies carried out over 2001 and 2002. Due to high mortality rates however, it was difficult to confirm the exact timing of the emergence of the different species of *Ecdyonurus* in the rearing experiments. Rearing the nymphs to the adult stage did however assist in verifying specimens especially as in the case in the Owengarve River, where three species were present in the river during the summer months. It must be noted also, that due to the warmer temperatures of the aquarium water, the larvae tended to emerge earlier in comparison to how they might do in their natural state in rivers.

4.5.6 Concluding comments

In order to ensure that the Heptageniidae, in particular the genus *Ecdyonurus* are fully represented when carrying out routine biological assessments, the timing of sampling may be a critical factor, especially when attempting to capture species that are only present in the benthos for a few months during the year. Findings from the present studies appear to show that more intense sampling may be required during certain months of the summer. This would ensure a complete characterisation of the range of species present in a river system that may not be there if the sampling were carried out during the autumn/winter months.

From studying the life cycles of the Heptageniidae in five rivers in the West of Ireland it would appear that the life history of the genus *Ecdyonurus* can vary slightly from year to year and from river to river. Due to the short life cycle of *Ecdyonurus insignis* and in particular *Ecdyonurus dispar*, which are summer species only, it would be vital to sample from June to August in a given year to ensure capture of these species. The more common species of this genus, *Ecdyonurus venosus* was present in each of the rivers studied throughout the year, with the exception of a few months post emergence. Hence, one would expect to find this species when sampling during all seasons throughout the year. Apart from being absent from the benthos for a few months after the main flight period, particularly in July and August,

Rhithrogena semicolorata was also present throughout the year, so one would therefore expect to find this species from September to June also. *Hepatgenia* spp. emerged a bit later than *Rhithrogena semicolorata* so depending on the abundance in a river, would be expected to be found throughout the year when routinely sampling, apart from July and August and in some rivers in September.

As mentioned previously, positive identification of species requires examination of all life stages for most aquatic insects. The two main approaches are field and laboratory rearing. An attempt was made in this study to collect both nymphs (rearing trials in the laboratory) and adults (field studies) from each location to verify individual species. Unfortunately, these studies were unsuccessful due to high larval mortality in the laboratory aquarium and also to the difficulty in finding adults in the field caused by time restrictions that went beyond the constraints of the project. In addition, this study did not include an investigation into whether the eggs and larvae overwintered and as mentioned previously, assumptions that they did, were based on findings from Elliott and Humpesch (1983), Macan and Maudsley (1968) and Wise (1980). The interpretation of water quality assessment programmes may be limited due to the knowledge gaps that still remain therefore further studies in these particular areas are essential to complete a more accurate and detailed description of the life cycle of the Heptageniidae in the West of Ireland.

Chapter 5

Assessment of the physical, chemical and biological factors controlling the occurrence of *Ecdyonurus* in five high status and five impacted rivers in the West of Ireland.

5.1 Introduction

As previously mentioned in Chapter 4, *Ecdyonurus* is a key water quality indicator genus. Its sensitivity to water pollution is noted in a wide range of biological indicator systems, based on macroinvertebrates as are most members of the Heptageniidae (e.g. De Pauw and Vanhooren 1983; Moog 1995; Skriver *et al*., 2001). *Ecdyonurus*, a widely distributed genus (Kelly-Quinn and Bracken, 2000) is particularly important in Ireland for the Irish EPA's Quality Rating System (Q-value) (McGarrigle 1998; McGarrigle *et al*., 1998; McGarrigle pers. comm.). The Quality Rating System in turn shows clear statistical links between Q-values and water quality parameters such as BOD, ammonia, nitrate and phosphate, all being important chemical indicators of water quality (Clabby *et al*., 1992; McGarrigle *et al*., 1998). The Irish Phosphorus Regulations (DELG 1998) are based on a link between biological Q-values and unfiltered molybdate reactive phosphorus (MRP) concentrations. While these strong empirical links exist, the precise mechanisms controlling the distribution of *Ecdyonurus* in eutrophic and polluted rivers are not well understood or documented. *Ecdyonurus* is one of the most useful indicators of pollution because it is ubiquitous in unpolluted waters but sensitive to pollution.

The occurrence of *Ecdyonurus* is probably not controlled by one single factor. It is likely that three aspects of the river environment control the presence of this sensitive indicator i.e. chemical, physical and biotic factors. It is therefore necessary to integrate all three areas in order to provide an overall understanding of how *Ecdyonurus* and other sensitive species respond to pressures on their environment.

Monitoring the quality of water in a stream as an indicator of catchment health should include biological response attributes as well as an assessment

of the physico-chemical characteristics. Changes in the health of a system will be reflected in the aquatic biological community if they are exposed to environmental disturbance or stress as they act as integrators of the multiple present and past environmental effects (Cranston *et al*., 1996).

Bioindicators at the species level rather than higher taxonomic groups, appear to be more useful in detecting environmental stress due to the sensitivity that individual species have to environmental factors (Moog, 1995). The use of the indicator approach in aquatic ecosystems, in particular the use of macroinvertebrates, has received considerable attentions in biomonitoring programs (Rosenberg and Resh, 1993; Johnson, 1995). Biomonitoring of communities with an emphasis on taxonomic richness and composition is considered by many advocates to be a most sensitive means of detecting alterations in aquatic ecosystems. However, an even earlier warning of stress may be provided by physiological and morphological abnormalities in some aquatic biota (Clarke, 1994; Rosenberg and Resh, 1993; Madden *et al*., 1995; Clarke *et al*., 1995). Whole catchment responses are well reflected in the aquatic system because the water flowing from a catchment actually does the integration. Parameters measured at the bottom of the catchment integrate what goes on in the catchment per se. Thus, information obtained at a single monitoring site per catchment at its bottom-most point may summarise the total catchment response (Cranston *et al*., 1996). Obviously, however, questions of scale and extent of a particular impact also have to be considered especially when dealing with large catchments. There is also a suggestion that the true impacts on first order streams, which may be important for spawning of trout, for example, may not be adequately represented by sampling larger downstream streams (McGarrigle pers. comm.).

The primary physical characteristics or "key factors" which influence aquatic organisms are temperature, oxygen content, nutrient composition and availability and habitat structure or cover (Moog, 1995). Temperature has an important effect on the intensity of metabolic activity as well as most other biological processes of aquatic organisms. Temperature is also of decisive importance for the occurrence of specific organisms in an

environment and hence can determine the composition of a community. While mean temperatures and temperature sums (degree days) are of some importance (especially in determining growth rates and time of emergence for insects), it is primarily the temperature range or extreme values which determine the suitability of a site as habitat for a particular species. Both the optimal and the tolerable temperature ranges for some benthic organisms or their specific life stages e.g., egg, larvae, pupa and adult, are already known and can be used in assessing some temperature problems (Moog, 1995).

The response of macroinvertebrates to oxygen concentration is relatively unidirectional as their distribution is never limited by increasing oxygen saturation and only rarely is oxygen supersaturation considered damaging to organisms. Therefore, evaluating oxygen-dependent distributions is limited to determining an organism's sensitivity to low levels of oxygen particularly in regards to their ability to recover from extremely low oxygen concentrations. Organisms that are most sensitive to oxygen deficiencies are those with thick skins and no gills (many Plecoptera larvae) or immovable gills (Ephemeroptera larvae of the genera *Epeorus*, *Rhithrogena* etc.). Although oxygen content alone is insufficient in describing the saprobic conditions of a river it is nevertheless an important factor in discriminating water-quality classes (Moog, 1995). In eutrophic systems, supersaturated oxygen values measured in daylight may be regarded as a sign of potentially low oxygen saturation values at night time as the plant biomass which drives supersaturation by photosynthesis may be sufficient to cause oxygen depletion at night due to respiration (McGarrigle 2001). The Irish EPA regards supsersaturation values of over 120% as an indication of eutrophication in rivers and potential nocturnal deoxygenation (McGarrigle *et al*., 2002).

Nutrients and their availability are key factors in controlling trophic-relations within a river system. Seasonal changes in nutrients inputs causing algal blooms or even the sudden organic input of leaf-fall in autumn can cause changes in the abundance of benthic macroinvertebrates, in particular to sensitive indicator taxa such as the genus *Ecdyonurus* (McGarrigle *et al*., 1998).

Factors controlling the distribution and abundance of benthic invertebrates have been the subject of considerable debate (Stanford and Ward, 1983; Lake and Barmuta, 1986; Townsend, 1989; Townsend and Hildrew, 1994). The habitat or substrate structure requirements of benthic species is very important in determining their distribution within a river system. It has been suggested that there may be an interaction between areas of stream habitat that show variations in substrate content or that have been disturbed differently and invertebrate richness found in a stream (Lake and Barmuta, 1986; Townsend and Hildrew, 1994). Additionally, unexplained variability in the spatial distribution and abundance of stream invertebrates is the likely basis for the requirement to collect a large numbers of replicates to be representative of a site (Chutter, 1972; Schwenneker and Hellenthal, 1984; Norris *et al*., 1992), particularly when a Surber sampler is used.

Stream habitat forms an essential component of river 'health' (Maddock, 1999) that can be used to evaluate the overall ecological integrity of a river system (Muhar and Jungwirth, 1998). The condition of local stream habitat, otherwise known as the habitat template, influences the structure and organisation of biological communities (Hynes, 1970; Southwood, 1977; Swanson, 1980; Minshall, 1984; Sweeney, 1984; Downes *et al*., 1995). In the absence of water quality impairment, the local physical habitat will have a major influence over the biotic assemblages at a site. If water quality is of a high standard, diverse and abundant assemblages of stream biota are likely to exist if the local stream habitat is supportive (Plafkin *et al*., 1989; Barbour *et al*., 1999; Simpson and Norris, 2000).

Contemporary assessment of river health often involves some form of rapid biological assessment (Reynoldson *et al*., 1997; Norris and Thoms, 1999). Rapid assessment approaches have focused on aquatic organisms. Empirical models have been developed that predict the occurrence of macroinvertebrate taxa, based on their association with environmental variables at reference sites (Wright, 1995; Reynoldson *et al*., 1997; Simpson and Norris, 2000). This approach provides an independent way of matching new sites with reference sites, enabling predictions to be made. Thus, the

philosophy of this approach seems appropriate. However, these techniques have not been applied to features of streams other than their biota. Stream managers often require information about physical features of a river that need improving to enhance biological condition. Most of the rapid assessment approaches have limited ability to determine whether biological impairment results from poor water quality or from poor habitat. Therefore, the ability to predict local stream habitat features may be useful for distinguishing between the effects of water quality and the effects of habitat on biological condition, and may assist in river management (Davies *et al*., 2000).

Before a habitat can be identified as damaged, it is vital to know what the habitat should be like in the absence of effects from humans. Stream habitat may be influenced by a variety of factors operating at numerous spatial and temporal scales (Frissel *et al*., 1986; Richards *et al*., 1996). Habitat assessment is beginning to focus on nationally applicable and ecologically based approaches that incorporate comparisons to reference or target conditions. The River Habitat Survey (Raven *et al*., 1997, 1998; Jeffers, 1998) and the United States Environment Protection Agency Rapid Bioassessment Protocols (Plafkin *et al*., 1989; Barbour *et al*., 1999) use reference conditions to assess stream habitat at broad scales (state-wide or nationally).

5.2 Study outline

This study focuses on obtaining information on the value of the physico-chemical data in supporting the Q-value System and compares the physical, chemical and biological data as indicators of water quality between five high status and five impacted rivers studies in the West of Ireland.

5.3 Materials and Methods

Details of the ten river sites studied in this investigation are outlined in Chapter 1.

5.3.1 Preliminary site investigations

In order to establish the suitability of a river site to the project requirements, biological and chemical investigations were carried out in a number of rivers. A kick sample was taken in a total of 29 rivers and biologically assessed by assigning a Q-value to each river. The multihabitat kick sample was collected in each river using a 0.25 x 0.25m sweep net with a 500µm mesh. The net was trawled with an 'S' type movement behind feet kicking the substrate for three minutes. Where possible, all available habitats were sampled by kicking, stone washing and weed sweeping.

To assess the water quality of each river, water samples were analysed for the following physico-chemical parameters: Temperature (taken in the field), dissolved oxygen % saturation (taken in the field), pH, conductivity, ortho-phosphate, chloride, BOD, ammonia, Total Oxidised Nitrogen (T.O.N.), alkalinity and colour. Analytical methods for the water quality parameters are outlined in Table 5.1.

High status rivers which are potentially close to their reference condition were chosen on the basis of a high Q-value, the presence of the genus *Ecdyonurus* and good water chemistry. Impacted sites chosen had lower Q-values (3 or 3-4) where at one time *Ecdyonurus* survived but are now no longer present. Five high status and five impacted rivers were then chosen for this project. The list of high status rivers and impacted rivers selected for this study and associated information is shown in Table 1.1 and Table 1.2 outlined in chapter 1.

Water samples were collected in a 2-litre HDPE water-sampling bottle filled from below the surface of the water. All sampling bottles were washed thoroughly with water from each site before taking the final sample. Samples were transferred to the laboratory refrigerator (5°C) on the same day and analysed within 24 hours of sampling.

A summary of the methodologies used in analysing the water quality parameters listed above is outlined in Table 5.1.

Table 5.1 Summary of the methodologies used in analysing the water chemistry parameters.

Parameter	Methodology	Detection range	Unit
Temperature	WTW DO meter		°C
Dissolved Oxygen	WTW DO meter		mg/l O_2
pH	WTW inolab terminal level 3 meter	2-12	pH units
Conductivity	WTW inolab terminal level 3 meter	15-12,890	µS/cm 25^{o}C
Alkalinity	Titration using sulphuric acid	8-150	mg/l $CaCO_3$
Colour	DR4000 Hach Spectrophotometer	5-500	Hazen units
Ammonia	KONELAB 30 autoanalyser	0.03-1	mg/l N
TON	KONELAB 30 autoanalyser	0.4-10	mg/l N
Orthophosphorus	KONELAB 30 autoanalyser	0.012-0.5	mg/l P
Chloride	KONELAB 30 autoanalyser	2-100	mg/l Cl
BOD	5 day incubation @ 20°C	1-7	mg/l O_2

5.3.2 Physico-chemical parameters - procedures and analysis

Water samples were analysed in the ten rivers on a monthly basis for the chemical 'quality' parameters outlined in Table 5.1. Sampling began in July and August 2001 depending on the river and continued on a monthly basis until October 2002. The sampling programme took place over a period of 16-18 months depending on the river. Monthly samples were generally taken in all of the high status sites. However, due to adverse weather conditions some of the rivers were not sampled in January 2002. All sampling was completed halted in February because of flooding (chemistry samples were normally taken in conjunction with macroinvertebrate samples). Water samples were taken from the impacted sites from July to October 2001 and again from March to October 2002.

5.3.3 Macroinvertebrate collection

5.3.3.1 Sampling procedure

Quantitative macroinvertebrate sampling commenced in July 2001 in four (Owengarve River, Castlebar River, Dunneill River and Callow Loughs Stream) of the five high status rivers. Sampling began a month later in the Brusna River during August 2001. Monthly sampling continued for 16 months until October 2001. From November 2001 to January 2002 inclusive, kick samples were taken, as the water levels were too high to carry out routine Surber sampling. Due to floods in February 2002, sampling was completely suspended. Monthly Surber sampling recommenced in March and continued until October 2002. The sampling programme took place over a period of 16 months. These macroinvertebrate samples were also used to study the life cycle of the genus *Ecdyonurus* described in Chapter 4.

In three (the Mullaghanoe River, Lough na Corralea Stream and the Cartron River) of the five impacted rivers, monthly Surber sampling was carried out from July 2001 to October 2001 inclusive and again from March 2002 until the sampling programme ceased in October 2002. No sampling was carried out in these rivers from November 2001 to February 2002 inclusive (it was felt better to extend the seasonal range of the study into late 2002 than to cease three months earlier) – logistics dictated the total number of samples that could be sorted and identified within the project timescale. Surber sampling commenced earlier (May 2001) in two of the impacted sites, namely the Robe River and Lough na Corralea Stream. The Robe River and the Mullaghanoe River both contained specimens of *Ecdyonurus* on occasions. Depending on the water levels, it was decided therefore, to take a kick sample in these rivers during the winter months to obtain *Ecdyonurus* specimens. No further sampling was undertaken during the winter period.

The experimental design and sampling regime are described in section 4.3.1 of Chapter 4 and the sampling areas for each river have been detailed in Chapter 1. Within each quadrat the Surber was also placed randomly and the macroinvertebrate sample taken. Five Surbers were taken in each river

on each date. The Surber sampler covered an area of quantitative macroinvertebrate sampling in each river site and was carried out using a $^{1}/_{16}$ m^2 horizontal base (25cm x 25cm) and had an attached pond net with mesh of 650µm. The net was held open by a square-foot metal frame 36cm x 28cm. In operation, the frame that supports the net is in a vertical position, while the other frame is locked into a horizontal position against the bottom. The net opening was placed facing upstream, using the current to hold the net open and the horizontal frame was pushed into the river bottom (Standard Methods, 2001). The macroinvertebrates were collected from the riverbed by disturbing the substrate within the metal frame for 2 minutes and washing all stones thoroughly to dislodge animals. The large substrate material was removed and the remaining sample was transferred into a 1L plastic container and preserved immediately in 70% Industrial Methylated Spirits (IMS).

5.3.3.2 Determination of optimum numbers of Surber samples

A large variation is usually encountered in sampling natural macroinvertebrate populations with the result that small samples or small numbers of samples can be statistically inaccurate and imprecise. Therefore, when sampling quantitatively, it is vital that an adequate number of sampling units is taken to ensure an optimum level of accuracy in the sense of obtaining a mean population value that is as close as possible to the true mean for the population in question. In order to establish the optimum number of Surber samples for each river site, that maximises the return on effort, a series of samples were taken from a number of sites. Elliott (1979) suggests taking 5 sampling units at random and calculating the arithmetic mean. Next take 5 more units at random and calculate the mean for 10 units. He advises to continue to increase the sample size by 5-unit steps and plot the means for 5, 10, 15 etc. units against sample size. When the mean ceases to fluctuate, a suitable sample size has been reached and this sample size can be used for that particular station.

This process was carried out in one high status river (Dunneill River) and one impacted river (the Mad River). A total of 20 Surbers were taken in each site. On the basis of tolerating a target of 20% of the mean (as suggested by Elliott, 1979) the analysis showed that five Surbers was a suitable sample size to be taken from each site. For two of the rivers, increasing the number of Surber samples beyond this resulted in only a small improvement in accuracy and thus significant diminution of return on effort. Results of the percentage recovery of macroinvertebrates in 20 Surbers from the Dunneill River on 4th July 2001 are outlined in Fig. 5.1.

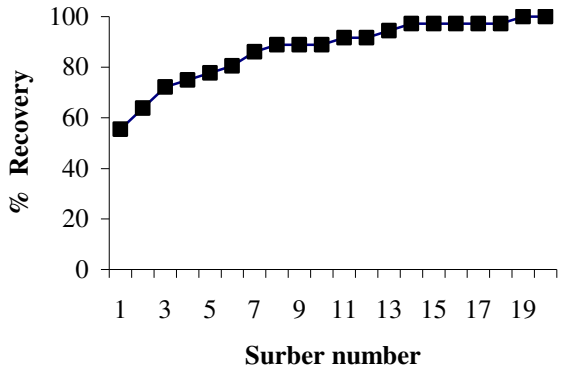


Fig. 5.1 Percentage recovery of macroinvertebrates collected from 20 Surbers in the Dunneill River on 4th July 2001.

Graphing the cumulative frequency of the presence/absence data showed, however, that a total of ten Surbers needed to be taken from the impacted site (Mad River) due to the high variance recorded there. Fig. 5.2 outlines the percentage recovery of the macroinvertebrates from the Mad River taken on 21st July 2001.

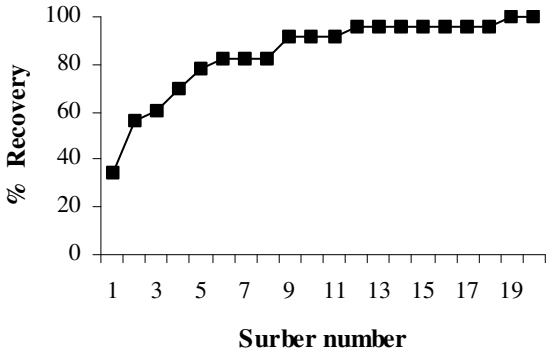


Fig. 5.2 Percentage recovery of macroinvertebrates collected from 20 Surbers in the Mad River on 31st July 2001.

5.3.3.2 Analysis of sample data

5.3.3.3

All macroinvertebrate Surber samples taken from the high status or potential reference condition rivers during the study programme were sorted entirely – i.e. five samples per month. Due to time constraints it was not feasible to fully examine all the Surber samples from the impacted sites. It was decided to concentrate firstly on the Robe and the Mullaghanoe Rivers as some of the Surber samples taken from these rivers contained *Ecdyonurus* on occasion. As many Surber samples as possible were sorted from the three remaining rivers within the time available thereafter. Table 5.2 outlines the number of Surbers that were sorted and identified for each of the impacted rivers investigated.

Table 5.2 The number of Surbers sorted per month in the impacted rivers during the study programme.

Month\River	Mullaghanoe	Robe	Mad	Cartron	Lough na Corralea Stream
May 2001	*	5	*	*	5
June 2001	*	*	*	*	*
July 2001	5	*	20	4	5
August 2001	4	2	2	4	5
September 2001	3	2	*	2	*
October 2001	1	*	*	3	*
March 2002	1	2	*	1	*
April 2002	5	*	*	5	*
May 2002	*	2	*	5	5
June 2002	4	2	*	*	5
July 2002	4	*	1	5	3
August 2002	4	*	1	5	2
September 2002	3	2	*	*	1
October 2002	3	2	*	*	*

* = No Surbers were sorted for this month

Total number of taxa (*S*), total number of individuals (*N*) (abundance) and percentage Ephemeroptera, Plecoptera and Trichoptera (% EPT) were complied for all sites and dates. Four indices were also calculated. These included included the Shannon-Wiener index (H'), Simpson index (λ), Margalef's index (*d*) and Pielous' evenness index. These metrics were used to describe the community structure and highlight any differences between the high status and impacted rivers examined in this study. Different diversity indices emphasise the species richness or equability components of

diversity to varying degrees. The most commonly used diversity measure is the Shannon (or Shannon-Wiener) diversity and the Simpson indices. These two non-parametric indices were used to test for heterogeneity.

The Shannon-Wiener index (H') defined as (Krebs, 1989)

$$H' = - \sum_i p_i \log (p_i)$$

where p_i is the proportion of the total count (or biomass etc.) arising from the *i*th species. H=0 if there is only one species in the sample and is maximum when all species are represented by the same number of individual (even distribution of abundance; Green, 1979; Ludwig & Reynolds, 1988).

The Simpson index (λ) defined as

$$\lambda = \sum pi^2$$

$$1-\lambda = 1 - (\sum pi^2)$$

$$\lambda' = \{\sum i \, N_i (N_i - 1)\}/\{N(N-1)\}$$

$$1-\lambda' = 1 - \{\sum_i N_i (N_i - 1)\}/\{N(N-1)\}$$

where N_i is the number of individuals of species *i*. The index λ has a natural interpretation as the probability that any two individuals from the sample, chosen at random, are from the same species (λ is always ≤ 1). It is a *dominance* index, in the sense that its largest values correspond to assemblages whose total abundance is dominated by one, or a very few, of the species present. Its complement, $1-\lambda$, is thus an equitability or *evenness* index, taking its largest value (of $1 - S'$) when all species have the same abundance. The slightly revised forms λ' and $1-\lambda'$ are appropriate when total sample size (*N*) is small.

Taxon richness is often given simply as the total number of taxa (*S*), which is obviously dependent on sample size (the bigger the sample, the more species there are likely to be). Alternatively, richness can be assessed using

Margalef's index (*d*), which incorporates the total number of individuals (*N*) and is a measure of the number of taxa present for a given number of individuals:

$$d = (S\text{-}1) / \log N$$

Equitability expresses how evenly the individuals are distributed among the different species, and is often termed evenness. It is expressed as Pielous' evenness index:

$$J' = H' / H'_{max} = H' / \log S$$

where H'_{max} is the maximum possible value of Shannon diversity i.e. that which would be achieved if all species were equally abundant (namely, log *S*).

The box and whisker plots included in Section 5.4.3.1 to 5.4.3.7 show the variations in the diversity indices and biotic metrics on a river by river basis across the entire sampling period. The various diversity indices emphasise the species richness or equitability components of diversity to varying degrees. The Shannon-Wiener index reflects an increasing diversity with a higher index number. For example, a value of 3 indicates a higher diversity than a value of 0. Simpson's index is similar to the Shannon-Wiener index and is a measure that accounts for both richness and proportion (percent) of each species. Margalef's index reflects the overall species richness within a community where increased species richness increases with a higher index number. Pielou's evenness is a component of diversity and compares and quantifies faunal equitability to taxa diversity for a given area. In general, all of the diversity indices in this study showed a similar trend across the study sites, with a higher diversity being found in the high status rivers compared to the impacted rivers (see results section).

The diversity indices described above were used to reduce the multivariate (multi-species) complexity of assemblage data into a single index (or small number of indices) which was then handled statistically by univariate analysis. ANOVA was run on the derived indices and metrics to test for differences in the community structure between the high status and

impacted rivers. It was decided to omit the data obtained during the winter months (November 2001, December 2001 and January 2002) from these analyses as they represented kick samples only and not Surber samples. It was thought that the data obtained from these months would be inconsistent with that compiled during the rest of the study.

In addition to the above indices, a set of metrics and indices to assess quality in lotic ecosystems (see Brabec *et al*., 2004; Buffagni *et al*., 2004; Ofenbock *et al*., 2004) were computed for both the high status and the impacted sites over the study period (Table 5.3). These were calculated using the recently available AQEM project software package (AQEM Version 2.3.4a, 2004). The indices were chosen based on their suitability for assessing the impact of organic pollution and eutrophication.

Table 5.3 Definition of selected indices/metrics.

Index/metric	Definition	Symbol	Reference
Belgian Biotic Index	Combination of richness a tolerance of selected	BBI	De Paw and Vanhooren, 1983
Danish Stream Fauna Index	Combination of richness a tolerance of selected	DSFI	Skriver *et al*., 2001
*German Saprobic Index	Indication or measure of the level of organic pollution	GERMAN	DEV, 1987, 1992; Pantle and Buck, 1955
**Czech Saprobic Index	Indication or measure of the level of organic pollution	CZECH	Sladecek, 1973; Rotschein, 1982
***Dutch Saprobic Index	Indication or measure of the level of organic pollution	DUTCH	Zelinka and Marvan, 1961
Indice Biotico Esteso	Sum of selected tolerance taxa	IBE	Ghetti, 1997
Average Score per Taxa	****BMWP divided by the existent selected taxa	ASTP	Alba Tercedor and Sanchez Ortega, 1988

Saprobic Index (Zelinka and Marvan, 1961) = $SI_{z\&m} = \frac{\sum^{s} z\&m^{s}_{i.}{}^{s} z\&m\, gi.\, n\, I}{\sum^{s} z\&m\, gi.ni}$

$SI_{Z\&M}$: Weighting factor (szg)

** The Czech Saprobic Index is calculated exactly the same way as the Saprobic Index (Zelinka and Marvan, 1961) including the weighting factor but with a slightly different taxa list.

*** The Dutch Saprobic Index is calculated in exactly the same way as the Saprobic Index (Zelinka and Marvan, 1961) but without the weighting factor.

****BMWP – Certain macroinvertebrate families are scored according to their sensitivity to organic pollution. The BMWP is the total of the scores of all families present in a taxa list. Each family in the sample is counted only one time, regardless of the number of species.

The objective of calculating the indices in Table 5.3 was to evaluate the robustness of the assessment methodologies to discriminate between the contrasting group of quality status rivers (high status and impacted sites). These metrics are commonly used to assess organic pollution. The Danish Stream Fauna Index (DSFI) (Skriver *et al*., 2001) have been also used to assess general degradation (AQEM consortium, 2002). For some of the metrics and indices, boundaries between quality classes are already established. These boundaries are referred to in Table 5.4. Interpretation of the boundaries for the remaining indices that are not outlined to in Table 5.8 were taken from the output data in the software.

Table 5.4 Class boundaries used as scoring criteria for some of the studied metrics.

Boundary Metric	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
BMWP	100	60	30	15
ASPT	0.50	0.43	0.34	0.25
BBI	8	6	4	2
IBE	8	6	4	2
GERMAN	1.5-2.2	2.2-3.0	3.0-3.5	>3.5
CZECH	1.5-2.2	2.2-3.0	3.0-3.5	>3.5

The AQEM software delivers results at different levels, which can be used to specify management implications and procedures. The AQEM assessment method is based on a "multimetric" procedure. A multimetric index combines several individual formulas (e.g. saprobic indices, feeding type composition, microhabitat etc.), the results of which are finally combined into a multimetric result. Thus, multimetric indices integrate multiple attributes of stream communities ("metrics") to describe and evaluate a sites condition (AQEM consortium, 2002).

One-way ANOVA was then carried out on the indices/metrics, microhabitat preferences and the feeding types derived from the AQEM software to assess the differences between the high status and the impacted sites.

5.3.4 Sediment analysis

As part of the overall habitat assessment, substrate sampling was carried out in all ten rivers between the 21st and the 27th March 2003. Sampling and analysis of the river bed material was carried out in accordance with the Central Fisheries Board standard methodology for sediment analysis (King and Kelly, 1999).

5.3.4.1 Sampling procedure

Sampling was carried out in an area of uniform appearance with regard to bed material. A metal-framed Surber sampler with a horizontal metal frame measuring $^1/_{16}$ m^2 (25cm x 25cm), with a 650µm pond net attached was placed on the stream bed as described in the previous section. A fine plankton (35µm) net with screw off bottle at end was placed over the coarser Surber net to retain fine silt material. Using a spade to dig vertically to loosen material inside the quadrat, particles were removed directly into a 10L metal bucket or sampling bag. Fine material worked backwards into the fine mesh net in a downstream manner after passing through the Surber net. Material was collected to a depth of approximately 0.3m. Three random Surber samples were taken in each river across a transect as follows: close to the right bank, central channel area and close to the left bank.

5.3.4.2 Sample Treatment

The substrates were allowed to air dry for 4 weeks prior to analysis. The entire sample was hand sieved using a 256mm-aperture sieve to remove the larger fraction. The sediments were then sieved manually through 4 grades of steel sieves (64mm, 32mm, 16mm, 8mm). All remaining material was dried completely and sieved for 20 minutes through a stack of sieves (aperture 128 to 0.03mm) using an intermittent amplitude mode capable of sorting the coarsest fractions of the sample. Each fraction retained in each sieve was weighed to the nearest gram and recorded.

5.3.4.3 Analysis of samples

The relationship between sieve aperture (mm) and the commonly used descriptive scale American Geophysical Union Descriptive Scale (A.G.U.D.S.) for substrate analysis is shown in Table 5.5. Particle size is frequently expressed on a logarithmic scale in which each successive size fraction covers twice the range of the proceeding one, e.g. 0.25, 0.5, 1, 2, 4mm etc. or for each phi unit increase in size, the corresponding particle size in millimetres is halved and for each phi-unit decrease in size the corresponding particle size is doubled (Table 5.5).

Table 5.5 Phi-unit to millimetre and log-scale conversion table and the size ranges of the particle size categories and sub-categories. Adapted from Gordon *et al*. (1992).

A.G.U.D.S	Sieve Aperture (mm)	Phi units	Log scale
Boulders	256-512	-9	2.7
Large cobble	128-256	-8	2.4
Small cobble	64-128	-7	2.1
Very coarse gravel	32-64	-6	1.8
Coarse gravel	16-32	-5	1.5
Medium gravel	8-16	-4	1.2
Fine gravel	4-8	-3	0.9
Very fine gravel	2-4	-2	0.6
Very coarse sand	1-2	-1	0.3
Coarse sand	0.5-1	0	0
Medium sand	0.25-0.5	1	-0.3
Fine sand	0.125-0.25	2	-0.6
Very fine sand	0.063-0.125	3	-0.9
Coarse silt	0.032-0.063	4	-1.2

5.3.4.4 Data presentation

The percentage composition by weight (g) of each fraction from each sample was calculated. The arithmetic mean percentage composition of each fraction was also calculated.

Cumulative percentage frequency curves were plotted for each of the three samples together with the calculated arithmetic mean. Log $_{10}$ sieve aperture (mm) was employed on the x-axis, as sediment particle sizes tend to follow a logarithmic distribution.

High status and impacted sites were all analysed individually. Comparison of fines content (sediment particles < 1mm) in samples was analysed using ANOVA to identify any differences between the individual sites and to highlight any differences between the high status and impacted sites. The relationship in the mean percentage frequency of all substrate fractions between the high status and impacted sites were also analysed using ANOVA.

5.3.5 Night-time Dissolved Oxygen (DO) measurements

Eutrophication has a major impact on dissolved oxygen levels in river water. Excessive plant growth can cause significant diel fluctuations in dissolved oxygen levels in rivers (Moriarity, 1990; McGarrigle 2001). In order to establish whether minimum dissolved oxygen saturation at night was an important factor controlling the occurrence of *Ecdyonurus*, it was essential to carryout night-time DO measurements in all ten rivers. This was undertaken on the 7th and 14th August 2003 at a time of low flows and high water temperatures. Single measurements for both DO and temperature was taken in each river using a WTW (Wissenchaftlich-Technische Werkstatten) oxygen probe during the hours of darkness.

5.4 Results

5.4.1 Physico-chemical results

The maximum, minimum, mean, median and standard deviation values for the water chemistry parameters examined in the high status and impacted sites during the sampling programme are outlined in Appendices 5.1 and 5.2 respectively. Rainfall data for the 2001-2002 period is shown in Appendix 5.3 as an aid to the interpretation of the water chemistry data and in order to give an idea of the potential flow variation in the rivers studied.

Graphical illustration of the mean monthly variations are presented for temperature (Fig 5.3) and pH (Fig 5.4), conductivity (Fig 5.5) and alkalinity (Fig 5.6), dissolved oxygen (DO; Fig 5.8) and biochemical oxygen demand (BOD; Fig 5.9), colour (Fig. 5.10) and chloride levels (Fig 5.11), ammonia (Fig 5.12), total oxidised nitrogen (TON; Fig 5.13) and unfiltered molybdate reactive phosphorus (MRP; Fig 5.14). Sampling was not carried out during some of the winter months due to bad weather thereby creating the gaps in the dataset shown in some of the graphs.

5.4.1.1 Temperature (°C) and pH

The temperatures observed in the high status and impacted sites during the sampling period were within the normal seasonal ranges (Fig. 5.3). The pH values were mostly circum-neutral or alkaline but were slightly lower on occasion in three of the impacted sites (Cartron River, Lough na Corralea Stream and the Mad River) compared to all other rivers investigated in this study reflecting the different geology and typology in the individual catchments (Fig. 5.4)

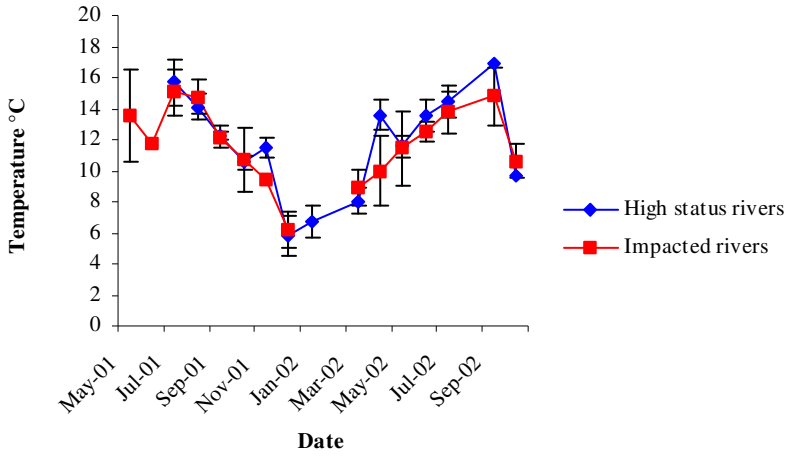


Fig. 5.3 Variation in mean temperature values in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

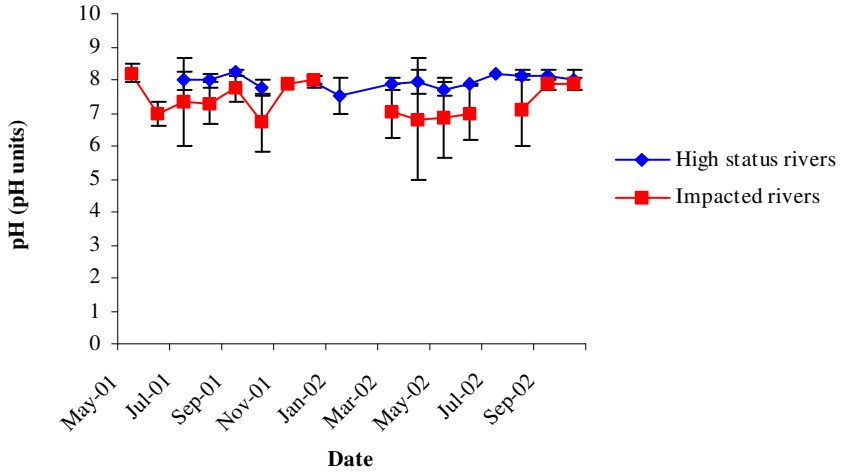


Fig. 5.4 Variation in mean pH values in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

5.4.1.2 Conductivity (µS/cm) and alkalinity (mg/l $CaCO_3$)

Conductivity and alkalinity levels varied considerably during the study programme in two of the high status sites, namely in the Brusna River and in particular in the Dunneill River (Fig. 5.5 and 5.6 respectively). These variations were not observed to the same extent in the other three high status sites.

Conductivity levels reached a high of 588 µS/cm on the 26th March 2002 in the Dunneill River, which is an unexpectedly high value but it followed a long spell of dry weather – only 2.3mm rainfall fell during the previous week at the Straide rainfall station – effectively this was the first extended dry period in 2002. The high conductivity value was not accompanied by, for example, high B.O.D., ammonia or nutrient concentrations that might indicate a pollution incident. Minimum levels of 73 µS/cm were recorded in both January and July 2002 in the Dunneill and mean values for conductivity were 260.5 µS/cm throughout the study (Appendix 5.1).

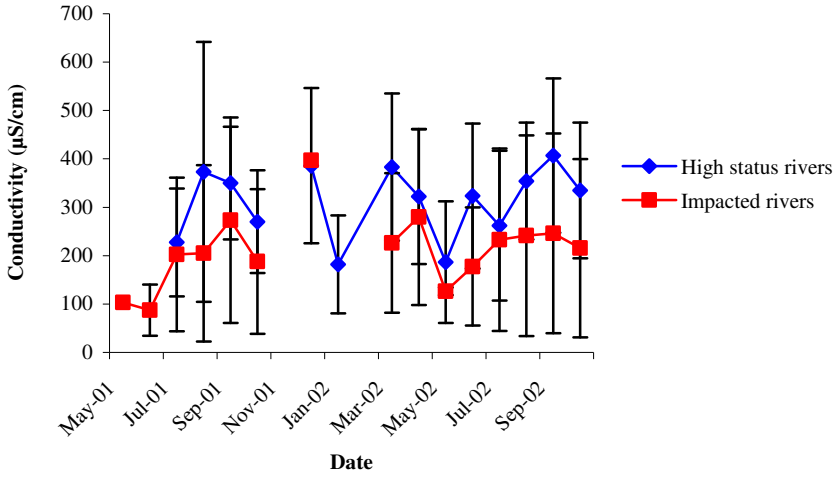


Fig. 5.5 Variation in mean conductivity levels in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

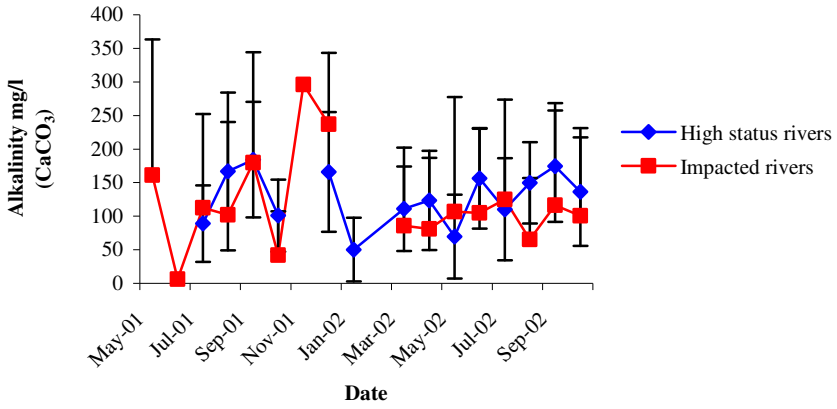


Fig. 5.6 Variation in mean alkalinity levels in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

Mean values for alkalinity in the Dunneill River (high status) were 104.4 mg/l $CaCO_3$ and maximum concentrations of 298 mg/l $CaCO_3$ were recorded in August 2001. Concentrations ranged between 30-91 mg/l $CaCO_3$ from March to July 2002 and rose dramatically to 162 mg/l $CaCO_3$ in August 2002 where they remained at a similar concentration until the end of the study in October 2002. Mean alkalinity concentrations in the Brusna River (high status) were 230.7 mg/l $CaCO_3$ (Appendix 5.1). One of the highest alkalinity values was recorded in this river in September 2001 (292 mg/l $CaCO_3$) and it dropped to 136 mg/l $CaCO_3$ in October 2001. A strong relationship was apparent between alkalinity in the Dunneill and preceding rainfall when graphed against 7-day cumulative rainfall for the day of sampling (Fig. 5.7). This was possibly due to groundwater influence during low flow periods.

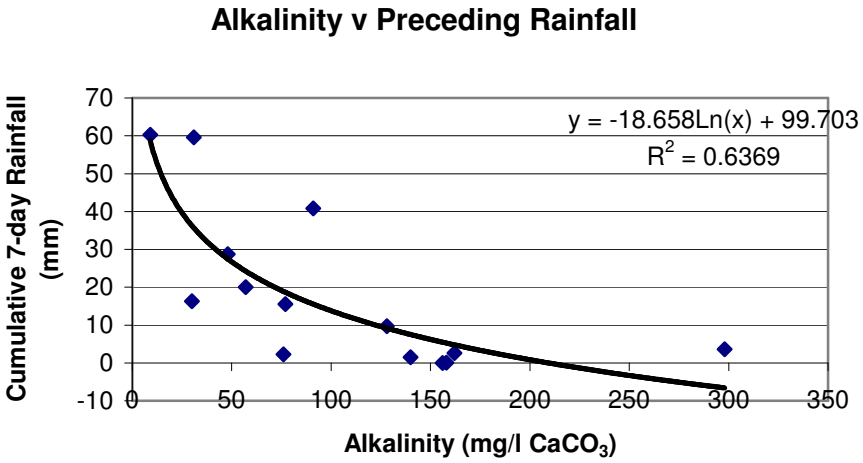


Fig. 5.7 Relationship between alkalinity and preceding rainfall in the Dunneill River against 7-day cumulative rainfall for the day of sampling.

Alkalinity concentrations and conductivity levels fluctuated in the Owengarve River (high status) also but not to the same extent as in the other two rivers. Alkalinity concentrations rose from 130 mg/l $CaCO_3$ in August 2001 to 252 mg/l $CaCO_3$ in September 2001.

Conductivity and alkalinity also varied in the Robe River (impacted site) but to a lesser extent (Fig. 5.5 and 5.6). These parameters did not vary considerably in the remaining four impacted sites or in the other two high status sites (Callow Loughs Stream and the Castlebar River).

The high alkalinity in the Robe and the Mullaghanoe Rivers (impacted river -mean values of 308.3 and 159.2 mg/l $CaCO_3$ respectively) and mean conductivity levels of 594.0 µS/cm (Robe River) and 395.8 µS/cm (Mullaghanoe River) reflected the calcareous nature of the catchment geology especially in the vicinity just upstream of the site sampled and resultant hard waters of these rivers. The Cartron, the Mad River and Lough na Corralea Stream (impacted rivers) were all soft-water rivers having catchments with predominantly siliceous bedrock geology with mean alkalinity levels ranging from 8.4 to 18.2 mg/l $CaCO_3$. In four out of the five high status rivers the mean alkalinity values ranged from 104.4 to 230.7 mg/l $CaCO_3$. The Castlebar River (high status) had the lowest alkalinity

level (mean value of 42.9 mg/l $CaCO_3$) among the high status rivers reflecting its soft-water nature.

In general, the variation in conductivity levels and alkalinity concentrations reflects the catchment geology and variation in the discharges among sites.

5.4.1.3 Dissolved Oxygen (% saturation) and BOD (mg/l O_2)

Mean values ranged from 101.1 to 107.2 % in the high status sites. On occasion however, the DO was elevated (>110%) in some of the high status rivers, particularly in the Owengarve and the Brusna Rivers (Appendix 5.1 and Fig 5.8). Mean values in the impacted sites ranged from 97.4 to 110.7% during the study period. The Robe (impacted river) displayed the highest DO level (158% saturation) among all ten rivers on 25th September 2001 (Appendix 5.2). This is taken to indicate a greater degree of eutrophication in the impacted river sites leading to higher levels of oxygen supersaturation during daylight hours as a result of more intense photosynthesis.

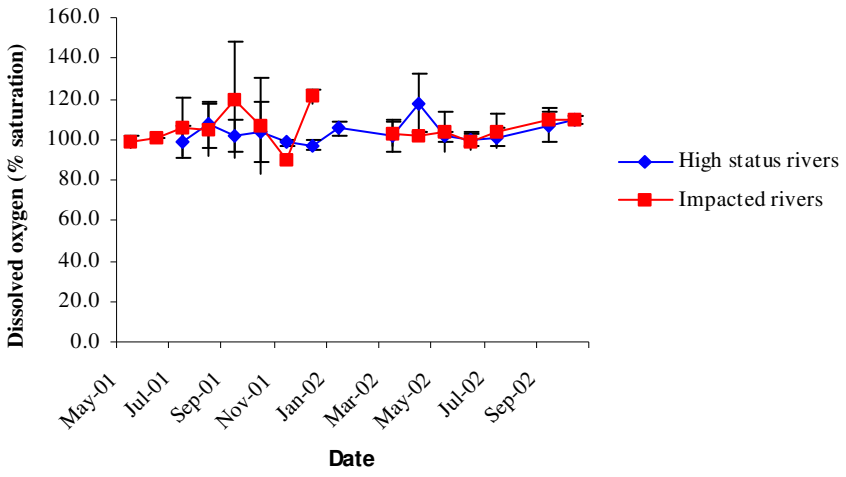


Fig. 5.8 Variation in mean dissolved oxygen levels in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

BOD levels were slightly higher in the impacted sites compared to the high status sites (Fig. 5.9). Concentrations fluctuated considerably in Lough na Corralea Stream (impacted) ranging from 0.4 to 3.5 mg/l O_2. Levels also

fluctuated in the Mullaghanoe River (impacted) ranging from 0.3 to 2.5 mg/l O_2 during the study. Mean values varied from 0.5 to 0.8 mg/l O_2 in the high status rivers and from 0.8 to 1.1 mg/l O_2 in the impacted rivers.

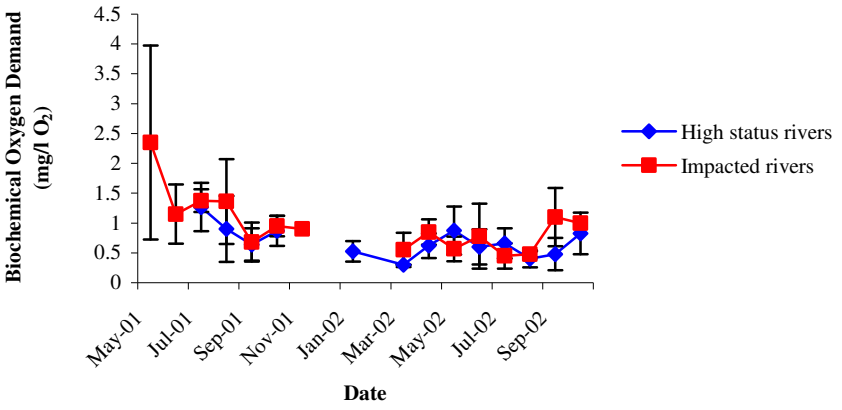


Fig. 5.9 Variation in mean BOD concentrations in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

5.4.1.4 Colour (Hazen units) and Chloride (mg/l Cl)

Colour appeared to vary more in the high status sites than in the impacted sites (Fig 5.10). Mean values in the high status rivers ranged from 53.3 in Callow Loughs Stream to 109.5 in the Castlebar River (Fig 5.10; Appendix 5.1). Mean values in the impacted sites ranged from 43.9 in the Mullaghanoe River to 145.8 in the Cartron River (Appendix 5.2).

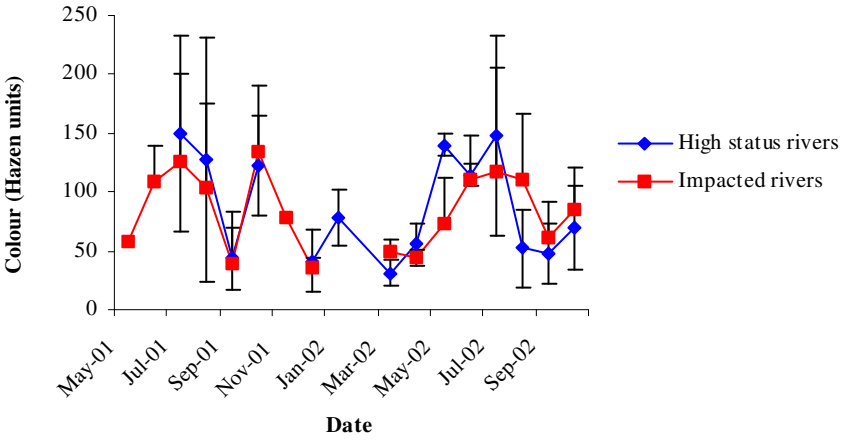


Fig. 5.10 Variation in mean colour measurements in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

Chloride levels showed a narrow range in values in most rivers (mean values from 10.1 to 24.5 mg/l Cl (Fig. 5.11; Appendix 5.1 and 5.2). The Cartron River (mean value of 24.5 mg/l Cl) is located near the sea and that may explain the broad range in values recorded here. Atlantic gales are known to cause elevated chloride levels in inland rivers.

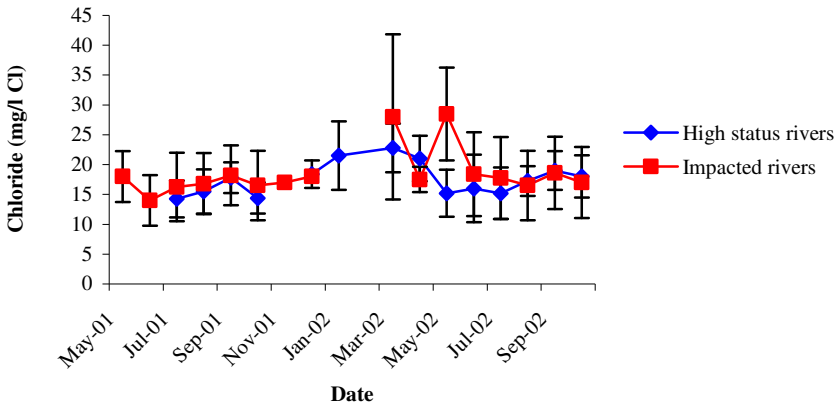


Fig. 5.11 Variation in mean chloride concentrations in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

5.4.1.5 Ammonia (mg/l N)

Mean concentrations of ammonia in the high status and impacted rivers are shown in Fig 5.12. Ammonia concentrations maintained a level of 0.01mg/l N in the Dunneill River (high status) throughout the study with the exception of an elevated measurement of 0.11mg/l N that was recorded in September 2001 (Appendix 5.1). Elevated levels (0.04 and 0.07mg/l N) were detected in the Owengarve River (high status) on occasion indicating perhaps that these rivers are not entirely pristine in nature. The concentration of ammonia in the other three high status sites remained low throughout the study (Appendix 5.1).

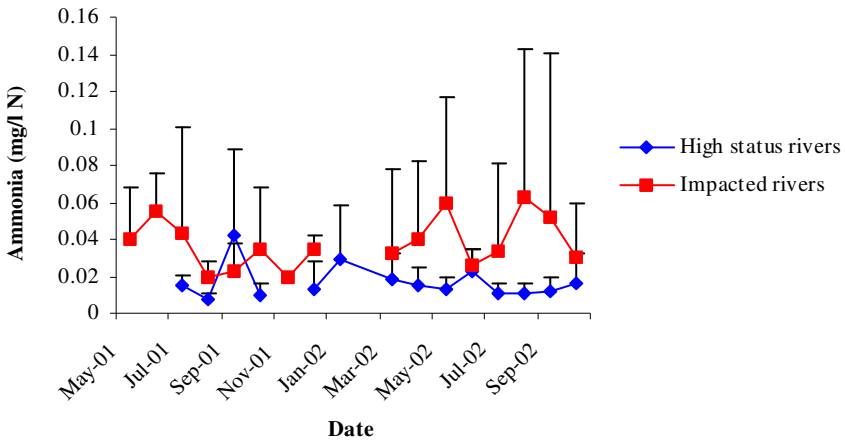


Fig. 5.12 Variation in mean ammonia concentrations in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

Elevated levels were detected in some of the impacted rivers also (Fig. 5.12). The highest concentration (0.21 mg/l N) was recorded in the Mullaghanoe River (impacted river) in August 2002 and displayed a mean of 0.08 mg/l N throughout the study period (Appendix 5.2). High concentrations were also detected in Lough na Corralea Stream (impacted river) with values ranging from 0.02 to 0.1 mg/l N (Appendix 5.2).

5.4.1.6 TON (mg/l N)

TON concentrations were elevated on occasion in some of the high status rivers and fluctuated from 0.3 to 1.4 mg/l N in the Owengarve River and from 0.2 to 0.95 mg/l N in the Brusna Rivers (Fig. 5.13). The median, upper quartile and 90 percentile values for TON for 99 rivers in the West of Ireland (1996-2001) were 0.687, 1.286 and 2.024 mg N/l respectively (Appendix 5.4). Concentrations in the other three high status rivers ranged from 0.1 to 0.5 mg/l N.

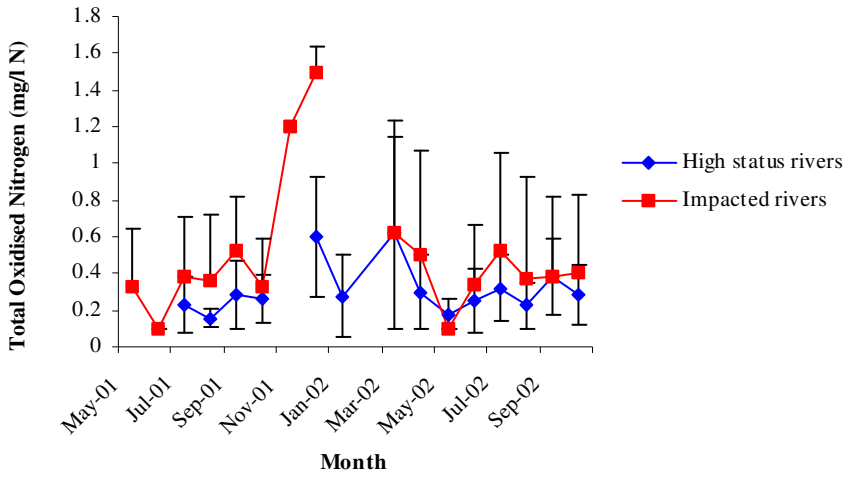


Fig. 5.13 Variations in mean TON concentrations in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

The highest TON levels in the impacted rivers were recorded in the Robe (1.6 mg/l N) and the Mullaghanoe Rivers (1.4 mg/l N) (Appendix 5.2).

5.4.1.7 Unfiltered MRP (mg/l P)

Fluctuations in the MRP concentrations in both the high status and impacted rivers during the course of the study period are shown in Fig 5.14. Mean values ranged from 0.032 mg/l P to 0.042 mg/l P in the high status rivers throughout the study (Appendix 5.1). In comparison with 99 rivers sampled over the period 1996 to 2001 in the West of Ireland by the EPA, Castlebar, the mean, median, 75 and 90th percentile MRP concentrations were 0.034, 0.018, 0.032 and 0.050 mg P/l (Appendix 5.4). Elevated levels of MRP were recorded in the Castlebar River (0.098 mg/l P), the Brusna River (0.105 mg/l P), the Dunneill River (0.112 mg/l P) and Callow Loughs Stream (0.082 mg/l P) during September 2001 (Fig. 5.1). Interestingly, these high levels appeared to coincide with increased conductivity and alkalinity values for the same period (Section 5.4.1.2). These appear to coincide with a low flow period in September 2001.

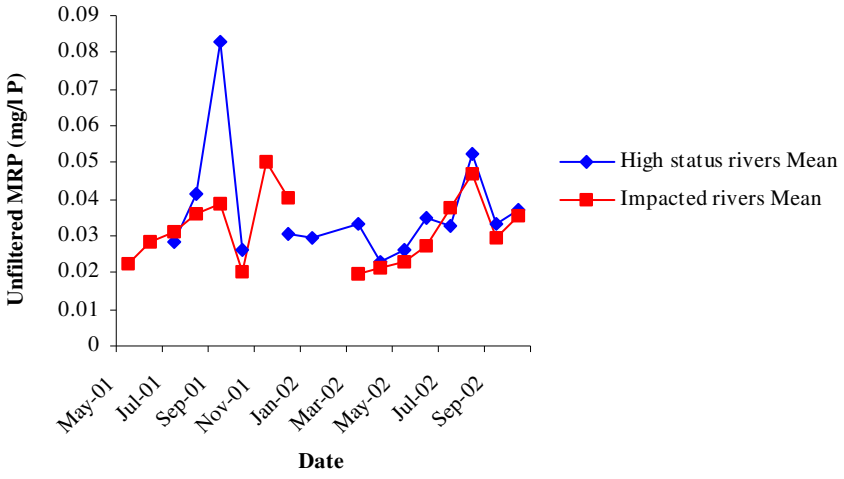


Fig. 5.14 Variations in mean unfiltered MRP concentrations in samples of stream water collected on a monthly basis from the high status and impacted rivers during the study.

Mean MRP concentrations in the impacted rivers ranged from 0.010 to 0.053 mg/l P (Appendix 5.2). The Mullaghanoe River displayed the highest recorded value of 0.098 mg/l P in August 2001 (Appendix 5.2).

5.4.1.8 Statistical comparisons of physico-chemical results between the high status v impacted sites

The physico-chemical data were transformed where appropriate and a repeated measures analysis (general linear model, SPSS) was carried out to assess if any significant differences in the parameters existed between the high status and impacted sites. Results are outlined in Table 5.6.

Table 5.6 Comparisons of the physico-chemical parameters between the high status and impacted sites using the general linear model in repeated measures (SPSS).

Parameter	Mean concentration compared	Source of variation (between groups)	Source of variation (within-subjects effects)	
			Month	Month*status
		High status vs Impacted rivers		
pH (pH units)	Reference < Impacted	$F_{1,3} = 3.239$ $P = 0.170$	$F_{8,24} = 1.343$ $P = 0.271$	$F_{8,24} = 0.403$ $P = 0.908$
Temperature (°C)		$F_{1,3} = 0.903$ $P = 0.412$	$F_{7,21} = 15.126$ $P = 0.001$***	$F_{7,21} = 0.409$ $P = 0.886$
Colour (Hazen units)		$F_{1,3} = 0.767$ $P = 0.446$	$F_{10,30} = 5.467$ $P = 0.001$***	$F_{10,30} = 1.383$ $P = 0.235$
Chloride (mg/l Cl)		$F_{1,2} = 4.740$ $P = 0.161$	$F_{9,18} = 2.337$ $P = 0.06$	$F_{9,18} = 0.397$ $P = 0.921$
Conductivity ($\mu S/cm^2$)	Reference < Impacted	$F_{1,3} = 4.104$ $P = 0.136$	$F_{9,27} = 0.764$ $P = 0.650$	$F_{9,27} = 0.721$ $P = 0.685$
Alkalinity (mg $CaCO_3$)	Reference < Impacted	$F_{1,2} = 0.710$ $P = 0.488$	$F_{9,18} = 1.984$ $P = 0.103$	$F_{9,18} = 0.619$ $P = 0.766$
Dissolved Oxygen (% O_2 saturation)		$F_{1,3} = 0.492$ $P = 0.724$	$F_{7,21} = 1.266$ $P = 0.314$	$F_{7,21} = 1.103$ $P = 0.397$
Biochemical Oxygen Demand (mg/l O_2)		$F_{1,2} = 2.435$ $P = 0.259$	$F_{7,14} = 2.312$ $P = 0.086$	$F_{7,14} = 0.496$ $P = 0.822$
Total Oxidised Nitrogen (mg/l N)		$F_{1,2} = 0.136$ $P = 0.747$	$F_{9,18} = 1.827$ $P = 0.132$	$F_{9,18} = 1.107$ $P = 0.406$
Unfiltered MRP (mg/l P)	Reference > Impacted	$F_{1,2} = 0.06$ $P = 0.83$	$F_{9,18} = 0.902$ $P = 0.544$	$F_{9,18} = 0.444$ $P = 0.893$
Ammonia (mg/l N)	Reference < Impacted	$F_{1,3} = 1.534$ $P = 0.001$***	$F_{7,21} = 28.337$ $P = 0.001$***	$F_{7,21} = 32.548$ $P = 0.001$***

p-values:

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Significant differences in ammonia concentrations (p=0.001), an indicator of organic pollution, were detected between the high status and impacted sites. There were no other significant differences in the physico-chemical parameters between the high status and impacted rivers. Somewhat unexpectedly, the mean MRP values in the 'high' status rivers were higher than those recorded in the 'impacted' sites which was mainly due to low N:P ratios (see Table 5.7 below). It appears that many of the clean rivers in the study were N limited for considerable periods.

The MRP concentrations were elevated on occasion in some of the high status rivers, particularly in the Castlebar and the Brusna Rivers, causing a high variance during the study period (Fig. 5.14). In September 2001, the MRP concentration in the Brusna River was 0.105 mg/l P and this contributed to the overall high variance in this river. On removing the MRP value for this month, the average concentration across the sampling period was 0.036 mg/l P. During the same month, the MRP level taken in the Castlebar River was also high, measuring 0.098 mg/l P, again, increasing the variance across the study. Again, when this value was eliminated, the average MRP concentration across the study was 0.032 mg/l P. Due to the unexpectedly high MRP values in the high status sites and to the suspicion that they were not actively P-limited, it was decided to take a closer look and investigate the variation in the N:P ratios in each river over the entire sampling period (Table 5.7).

5.4.1.9 Investigation in N:P ratios between high status v impacted sites

The N:P ratios fluctuated on a monthly basis between the study sites (Table 5.7). The variation between the high status and impacted sites is shown in Fig. 5.15.

Table 5.7 Monthly variation in the N:P Ratios recorded in the high status and impacted rivers during the study period. N:P ratios <15 points to an N limited system (values in bold).

	Reference rivers					Impacted rivers				
	Owengarve	**Castlebar**	**Brusna**	**Dunneill**	**Callow Loughs Stream**	**Cartron**	**Lough na Corralea Stream**	**Robe**	**Mullaghanoe**	**Mad**
May-01							75	32		
Jun-01						**7**			36	34
Jul-01	27	**13**		19	**14**	19		33	35	**14**
Aug-01	**6**				**13**	24		28	42	**11**
Sep-01	62	**4**	**7**	**2**	**5**	38	70	38	33	**5**
Oct-01	30	**14**	35	20	15	57	53			41
Nov-01								54	80	
Dec-01	66	27	54	32	32			88		
Jan-02	32	19		**11**	21					
Mar-02	71	17	57	20	29	35	55	89	71	
Apr-02	61	**9**	49	**14**	26				57	49
May-02	20	**9**	16	**13**	26	20	63			
Jun-02	24	**9**	22	**10**	17	16	38	40	33	34
Jul-02	25	**7**	17	87	**14**	17		49	40	**13**
Aug-02	22	**4**		**8**	**9**	15	**13**		31	**5**
Sep-02	47	**13**	35	15	23	**8**	44	31	66	**8**
Oct-02	26	**8**	23	**12**	15	10	22	30	41	**11**

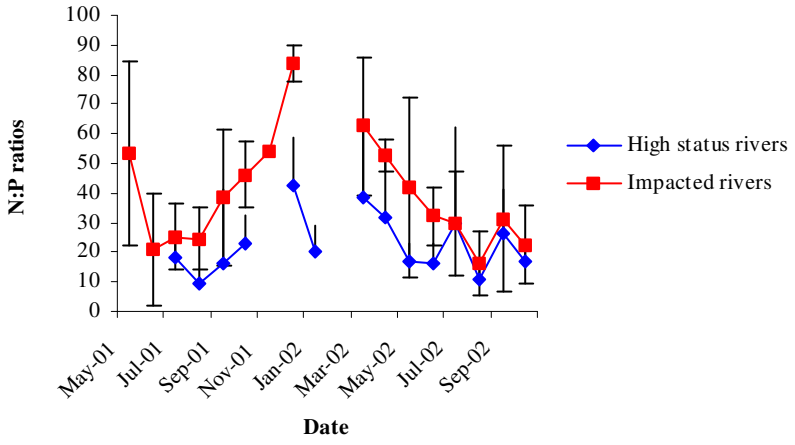


Fig. 5.15 Variation in the N:P ratios between the high status and impacted rivers during the sampling programme.

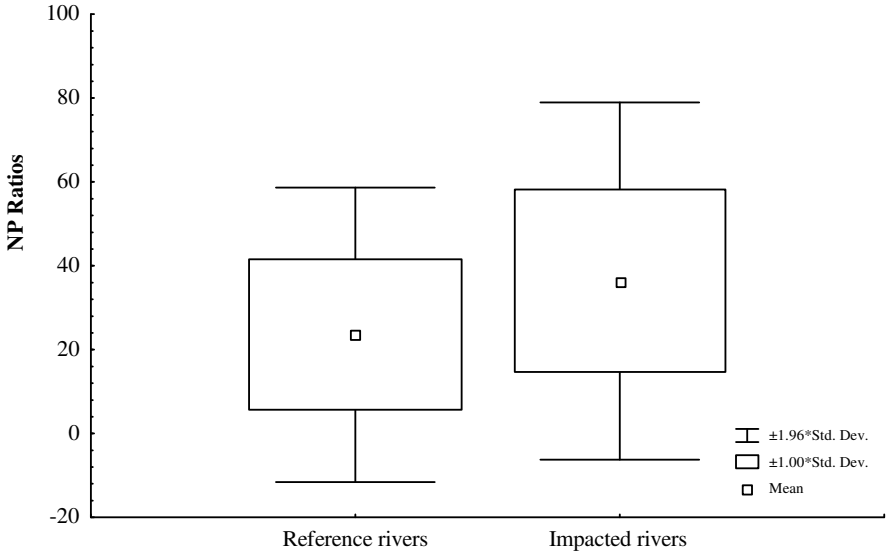


Fig. 5.16 Box and whisker plot showing the variation in the NP ratios in the ten rivers during the sampling period (p =0.001).

The N:P ratios did vary considerably between the high status and impacted rivers (Fig. 5.15). The lowest N:P ratios were found in the Castlebar River and Callow Loughs Stream, both high status rivers. With the exception of one sampling occasion (August 2001, Table 5.7), the Owengarve River was P-limited for the entire sampling period. The same was true for the Brusna River, which was P-limited throughout aside from being N-limited in September 2001. The Castlebar River was practically N-limited right throughout the study apart from December 1001 and March 2002 (Table 5.7, see also Chapter 2 split stream study). The Dunneill River fluctuated considerably on a month to month basis between N and P-limitation (Table 5.7). Callow Loughs Stream was N-limited from July to September 2001 inclusive and switched to a P-limited system from December 2001 to June 2002. As with the previous year, the river became N-limited in July and August 2001. In September and October 2002 the system changed back to being P-limited.

In terms of the impacted sites, the Mad River was N-limited on seven of the eleven sampling occasions and was the dominant impacted river to display N-limitation. With the exception of three sampling occasions in the Cartron River and one in Lough na Corralea Stream, four out of five of the impacted rivers remained P-limited for the duration of the study (Table 5.7).

In general, the impacted rivers were found to be more P-limited during the study in comparison to the high status rivers (Fig. 5.16; Table 5.7). The box and whisker plot in Fig. 5.16 compares the N:P ratios between the high status and impacted rivers throughout the study showing an overall significant difference ($p<0.001$) when a one-way ANOVA was applied to the data. In general, the N:P ratios in the high status rivers, were slightly lower during the summer of 2001 and 2002, compared to the other seasons (Table 5.7). Overall, in the impacted rivers, the N:P ratios were similar in the summer and autumn months. In some of the impacted rivers, however, the ratios seemed higher during winter 2001/2002 and spring 2002. A similar pattern was also observed in the high status sites during these seasons. The results here and also those in Chapter 2 demonstrate that some higher status rivers may be nitrogen limited particularly during the late

summer months rather than the more generally found P-limitation. An analysis of the EPA database of the N:P ratios for 99 rivers in the West of Ireland found that approximately 4% of samples analysed are N-limited and with low MRP concentrations (<0.05 mg/l P; Appendix 5.4). There is also a definite tendency for nitrogen limitation to occur during the summer months – a Chi-Square analysis of observed versus expected frequency of occurrence of N limitation at low MRP concentrations suggested a strong bias towards the summer months (Fig. 5.17). An initial analysis of the catchment characteristics of sites exhibiting low N:P ratios with low MRP concentrations has not revealed any obvious controlling factor such as geology or particular forms of land use.

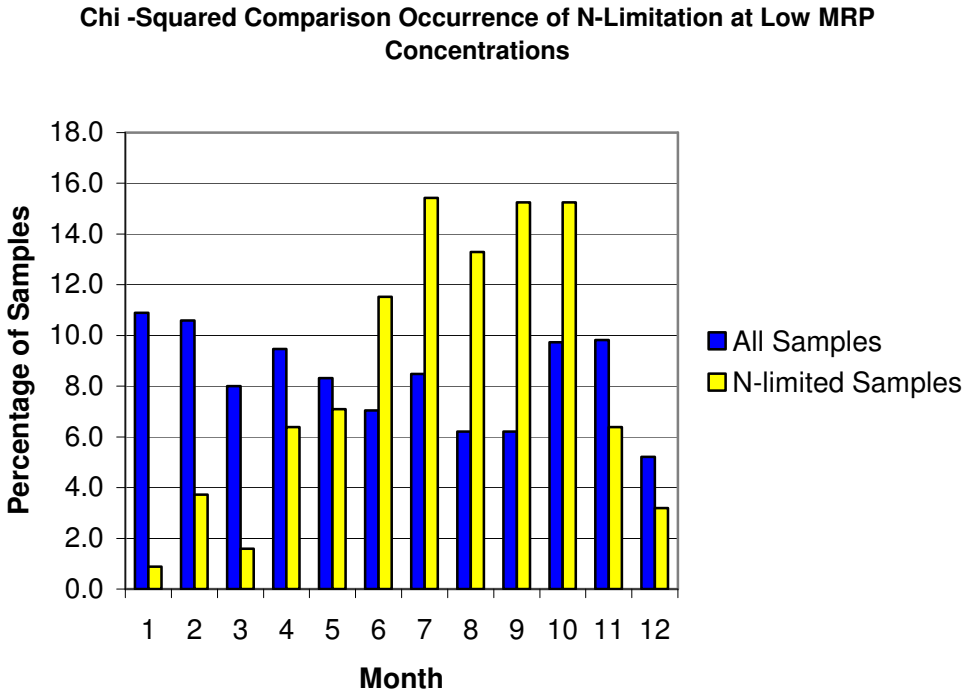


Fig. 5.17 Occurrence of N-limitation at low MRP concentrations in 99 rivers in the West of Ireland (1996-2001). N-limitation is more likely to occur between July and October ($X^2 = 54.9$, $p<0.001$).

5.4.2 Night time dissolved oxygen (DO) measurements

It was important to establish whether minimum dissolved oxygen saturation during the hours of darkness could be an important factor controlling the occurrence of *Ecdyonurus*. Table 5.8 outlines the results for the night-time dissolved oxygen saturation measurements taken in August 2003 during low flow, warm conditions.

Table 5.8 Percentage Saturation of dissolved oxygen and temperature readings obtained during the night-time hours.

	Sampling date	Time	% DO saturation	DO mg/l O_2	Temperature °C
High status Rivers					
Owengarve	7th August 2003	02.40hrs	80	7.3	19.6
Castlebar	7th August 2003	01.40hrs	92	8.7	17.5
Brusna	7th August 2003	04.00hrs	88	8.2	18.0
Dunneill	7th August 2003	03.45hrs	90	8.4	18.6
Callow Loughs Stream	7th August 2003	05.00hrs	91	8.5	16.7
Impacted Rivers					
Cartron	14th August 2003	04.15hrs	92	9.4	13.1
Lough na Corralea Stream	7th August 2003	00.05hrs	85	7.5	20.2
Robe	7th August 2003	00.40hrs	85	7.9	21.0
Mullaghanoe	7th August 2003	02.10hrs	65	6.1	16.8
Mad	7th August 2003	03.15hrs	97	8.9	19.0

The National Salmonid Waters Regulations incorporating the requirements (78/659/EEC) of the Freshwater Fish Directive require that 50% of samples should be greater the 9 mg/l O_2 and that all samples should be greater than 6 mg/l O_2. Thus, even though only one measurement was taken during one night-time visit, the concentration in the Mullaghanoe River (impacted river; 6.1 mg/l O_2) is close to breaching these requirements. The DO concentration in the Owengarve River (high status river; 7.3 mg/l O_2), in Lough na Corralea Stream (Impacted river; 7.5 mg/l O_2) and in the Robe River (Impacted river; 7.9 mg/l O_2) was also less than the 9 mg/l O_2. More frequent sampling would be required to demonstrate the pattern of night time oxygen depletion and particularly at the lowest flow conditions during the growing seasons. Unfortunately this was beyond the scope of the current study.

5.4.3 Results of the macroinvertebrate analysis

The diversity indices and metrics were examined on a river-by-river basis across the 16-month sampling period (Section 5.4.3.1 to 5.4.3.7). The graphs in Figs. 5.18 to Fig 5.24 show the variation in these indices/metrics between the high status and impacted rivers. Table 5.9 outlines the mean values for the various indices/metrics applied to average density/m^2 macroinvertebrate data in each of the ten rivers. The variation in the diversity indices and biotic metrics was examined on a river-by-river basis across five seasons and are presented in Appendices 5.5 to 5.11 (Section 5.4.3.8). The diversity indices and metrics were compared (Kruskal-Wallis ANOVA) between the high status and impacted sites (Appendix 5.12; Table 5.10).

Table 5.9 Mean values for the various metrics/indices calculated in the high status and impacted rivers during the sampling period.

High status rivers	**Shannon-Wiener index (H')**	**Margalef's index (d)**	**Pielou's evenness (J')**	**Simpsons index**	**Total number of taxa (S)**	**Total number of individuals (N)**	**% EPT**
Owengarve	2.9	5.1	0.8	0.9	38	1625	0.43
Castlebar	2.1	3.3	0.7	0.8	23	1004	0.49
Brusna	1.81	4.2	0.5	0.7	34	2639	0.44
Dunneill	2.4	4.1	0.7	0.9	30	1375	0.36
Callow Loughs Stream	2.4	4.7	0.7	0.8	36	1703	0.49
Impacted rivers							
Cartron	2.1	2.7	0.7	0.7	20	1251	0.44
Lough na Corralea stream	2.5	3.6	0.8	0.9	26	1269	0.36
Robe	1.7	2.0	0.6	0.7	17	2823	0.27
Mullaghanoe	2.1	3.3	0.6	0.8	30	7913	0.35
Mad	1.9	2.6	0.7	0.8	17	454	0.23

The AQEM software was also applied to average density/m^2 data to calculate a wider range of biotic indices to further compare the high status and the impacted sites (Section 5.4.3.9; Appendix 5.13).

Table 5.10 Results for the Kruskal-Wallis ANOVA applied to the diversity indices and metrics. The variation between the high status and impacted rivers were examined.

Index/Metric	Reference x impacted rivers	
	***p*-value**	**Chi-square**
Shannon-Wiener diversity (*H'*)	*P*=0.239 ns	1.386
Margalef's index (*d*)	***p*<0.001***	34.662
Pielou's evenness (*J'*)	*P*=1 ns	0
Simpsons diversity (λ)	*p*=0.239 ns	1.386
Total number of taxa (*S*)	***p*<0.001***	12.478
Taxon abundance (*N*)	*P*=0.239	1.386
Percentage EPT	***p*<0.001***	19.689

p-values: **p*<0.05; ***p*<0.01; ****p*<0.001; ns – not significant

5.4.3.1 Shannon-Wiener Index (H')

Mean values for Shannon-Wiener index were slightly higher in the high status rivers compared to the impacted rivers (Fig. 5.18; Table 5.9). No significant difference (Table 5.10; *p*=0.239) between the high status and impacted sites was observed when compared using this index.

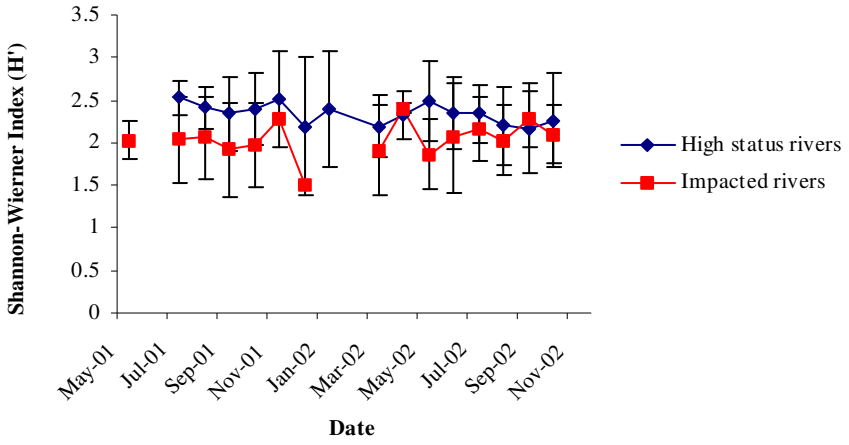


Fig. 5.18 Variation in the Shannon-Wiener diversity index (mean values) in the high status and impacted sites during the study.

5.4.3.2 Margalef's Index (d)

Mean values for Margalef's index (d) were generally higher in the high status than in the impacted rivers (Fig. 5.19) and ranged from between 2 to 5 (Table 5.9). A highly significant difference (Table 5.10; $p<0.001$) was found between the two groups of rivers using Margalef's index.

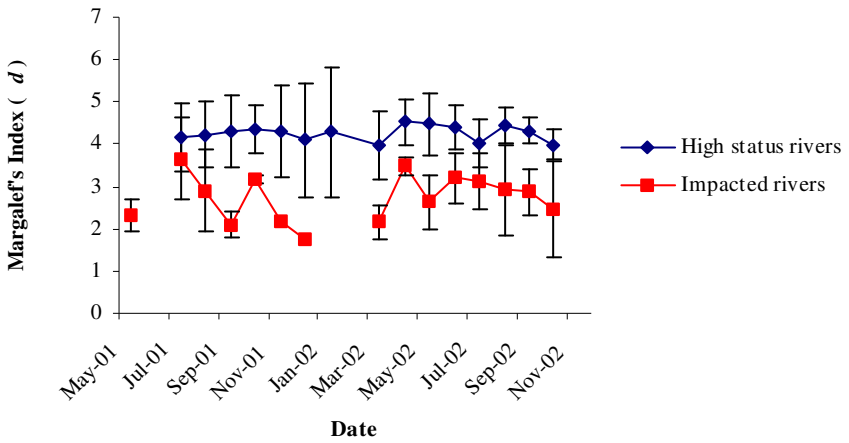


Fig. 5.19 Variation in Margalef's index (mean values) in the high status and impacted sites during the study.

5.4.3.3 Pielou's evenness (J')

The variation in Pielou's evenness (J') is shown in Fig. 5.20 with mean values ranging from 0.5 to 0.8 (Table 5.9). There was no significant difference (Table 5.10; p=1) between the high status and impacted sites using Pielou's evenness.

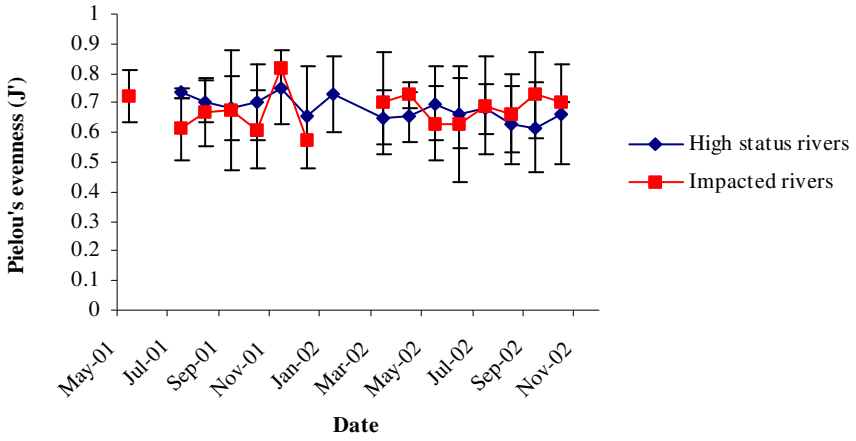


Fig. 5.20 Variation in Pielou's evenness index (mean values) in the high status and impacted sites during the study.

5.4.3.4 Simpsons diversity

Mean values for Simpsons index ranged from 0.7 to 0.9 (Fig. 5.21; Table 5.9). When comparing the two groups of rivers using this index, no significant difference was observed (Table 5.10; p=0.239)

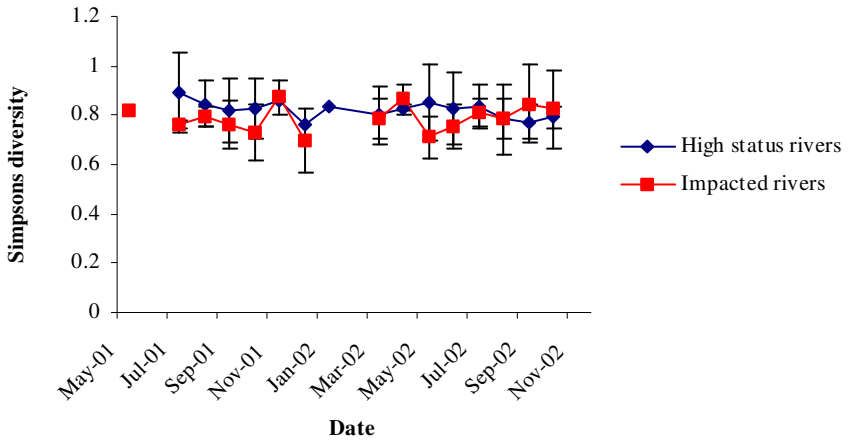


Fig. 5.21 Variation in Simpsons index (mean values) in the high status and impacted sites during the study.

5.4.3.5 Total number of taxa (S)

Mean values for the total number of taxa were generally higher in the high status than in the impacted sites (Fig. 5.22; Table 5.9). The high status and impacted sites were significantly different (Table 5.10; $p<0.001$) when compared using total number of taxa. The lowest mean value (17) was found in the Mad River and the Robe Rivers (impacted river) while the highest mean value (38) originated in the Owengarve River (high status river). It is worth noting that the Mullaghanoe (impacted by Charlestown sewage works) had a greater number of taxa than the Castlebar River site, which is recognised as perhaps one of the highest status sites in the study. Thus, reliance on number of taxa as a simple index of ecosystem health is not advisable in isolation. The number of invertebrate taxa present in rivers can increase in the early stages of eutrophication or organic pollution as, for example, the number of gastropod species increases in response to increased primary production. The addition of more tolerant grazers while at the same time some of the more sensitive taxa are still able to survive will boost the overall number of taxa as a response to eutrophication in this case.

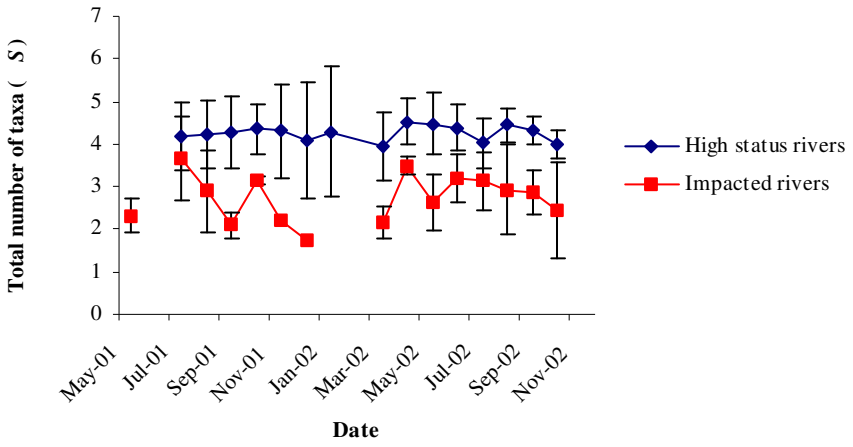


Fig. 5.22 Variation in the total number of taxa (mean values) in the high status and impacted sites during the study.

5.4.3.6 Taxon abundance (N)

The mean taxon abundance (N) (Fig. 5.23; Table 5.9) was quite similar in the Owengarve River (1624.9), the Castlebar River (1004.4) and the Dunneill River (1374.9). No significant differences (Table 5.10; p=0.239) were observed between the high status and impacted sites using this index. The Brusna River and Callow Loughs Stream contained slightly higher mean numbers (2639.3 and 1702.7 respectively). The highest abundance of invertebrates in all ten rivers was found in the Robe River (2823.2) and the Mullaghanoe River (7913.3) (both impacted sites).

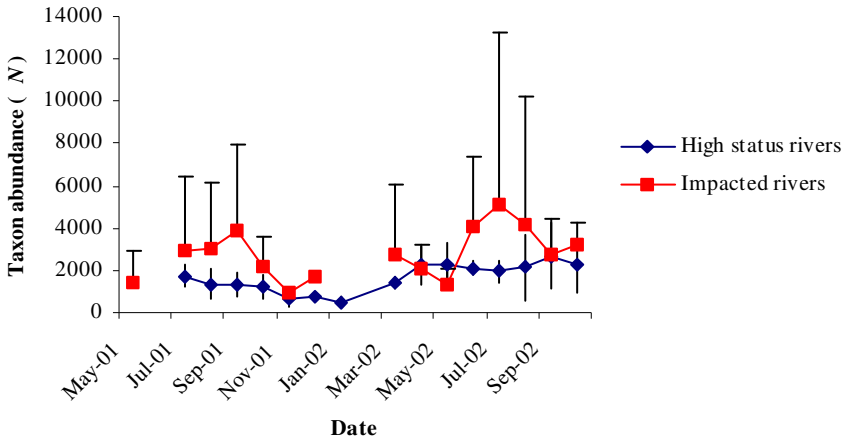


Fig. 5.23 Variation in the total number of individuals N (Abundance) in the high status and impacted sites during the study (mean values).

5.4.3.7 Percentage EPT

The highest mean percentage EPT value of 0.5 was found in the Castlebar River (high status river) while the Mad River displayed the lowest mean value of 0.2 (Fig. 5.24; Table 5.9). Significant differences were observed (p<0.001) between the high status and impacted sites when compares using percentage EPT. Generally speaking, with the exception of the Cartron River (impacted site), the mean percentage EPT was higher in the high status rivers compared to the impacted rivers. The Cartron River had a mean value of 0.4, which was quite high compared to the other impacted sites due to the high numbers of *Baetis* spp., *Leuctra* spp., and other plecopteran and trichopteran species in this river.

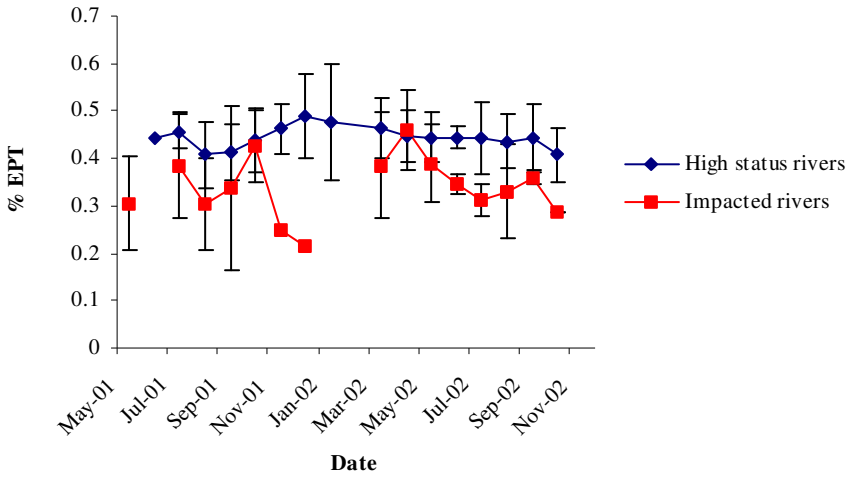


Fig. 5.24 Variation in percentage EPT (mean) in the high status and impacted sites during the study.

5.4.3.8 Seasonal variations

The variation in the diversity indices and biotic metrics examined on a river-by-river basis across five seasons results are presented in Appendix 5.5 to 5.11). The data for November 2001, December 2001 and January 2002 was omitted as only kick samples were taken during these months which would have been inconsistent with Surber data collected for all other seasons.

These plots give an indication of the variation in the range of indices and matrices among seasons. Not all samples were sorted in the impacted sites thereby creating some gaps in the seasonal data.

On a river-by-river basis, there were no substantial differences in the diversity indices between seasons in the high status sites (Appendix 5.5 to 5.11). Species richness (d) dropped slightly in the Dunneill River and Callow Loughs Stream in autumn 2001 (Appendix 5.5). The opposite was observed in the Owengarve, Castlebar and Brusna Rivers (high status) where species richness increased slightly during the same period. Different insect emergent patterns among the various species may have contributed to these variations. It would normally be expected that plecopteran species, in particular, would reappear in the benthic faunal macroinvertebrate community from early September onwards but typically many species will be absent during the summer months. The emergence pattern of *Rhithrogena* also typically will mean that few if any nymphs of this genus will be present from mid July to late August.

The total number of taxa/m^2 and the total number of individuals/m^2 (Appendix 5.6 and 5.7 respectively) increased slightly in spring 2002 in Callow Loughs Stream. An increase in the total number of taxa/m^2 in the Castlebar, Brusna and Dunneill Rivers was also observed during this season but not as pronounced as in Callow Loughs Stream (Appendix 5.5). The Owengarve on the other hand appeared to maintain a consistently high number of taxa during the studies with little change between seasons highlighting the homogeneity of this system.

The highest percentage EPT in the high status rivers was found in autumn 2002 in the Castlebar River (Appendix 5.8). A similar proportion was found in autumn 2001 in Callow Loughs Stream. The percentage EPT did not fluctuate dramatically between seasons and across sites. Percentage EPT also demonstrated strong differences between impacted and high status sites suggesting that, in combination with its consistency across seasons, it may be quite a useful indicator of ecosystem health in Irish rivers.

The Mullaghanoe River was the most diverse of all the impacted sites (Appendix 5.9 and 5.10). Species richness (Appendix 5.5) was highest in the Mullaghanoe River, particularly during summer 2001, 2002 and autumn 2002. Abundance (N) was also highest in summer 2001, summer 2002 and to a lesser extent in autumn 2002 (Appendix 5.7). Percentage EPT was generally low but consistent across all five seasons in this impacted river. Generally speaking, there was an increase in species richness during the summer 2002 in three of the five impacted rivers (Appendix 5.5). Tolerant species generally increase in impacted rivers therefore pollution impacts coupled with changes in species life cycles may be driving these changes.

Taking gaps in the dataset into consideration, with the exception of the Mad River, the diversity indices examined in the other three impacted rivers were generally consistent across seasons. As with the Mullaghanoe River, the total number of taxa in the Cartron River was highest in spring 2002 (Appendix 5.6). The total number of individuals/m^2 was quite consistent across the impacted sites (with the exception of the Mullaghanoe and the Robe rivers). The numbers increased in spring 2002 in the Robe River while they dropped in the Mullaghanoe River during the same period (Appendix 5.7).

There did not appear to be any variation in Shannon-Wiener index (Appendix 5.9), Simpsons diversity (Appendix 5.10) or in Pielou's evenness (Appendix 5.11) in the high status or impacted rivers across the seasons studied.

The diversity indices and biotic metrics were not as consistent across the five seasons in the impacted sites as they were in the high status sites. Gaps in the data set obviously contributed to a lack of information for particular seasons but it was still possible to gather knowledge of seasonal patterns within the community structure of these impacted rivers.

5.4.3.9 Calculation of AQEM metrics

The macroinvertebrate average numbers/m^2 data were analysed using the AQEM software (AQEM V2.3.4a, 2004) producing a series of metrics/indices.

A one-way ANOVA was applied to a selection of these metrics/indices, showing significant differences in all selected indices/metrics between the high status and the impacted sites (Appendix 5.13; Table 5.11). A summary of the *p*-values and the means recorded from comparisons (ANOVA) made between the high status and impacted sites for the indices/metrics (Appendix 5.13), microhabitat preferences (Appendix 5.14) and feeding types (Appendix 5.15) derived from the AQEM software is presented in Table 5.11.

These metrics focus mainly on organic pollution and eutrophication and based on the occurrence of the macroinvertebrate communities in the high status and impacted sites, the status of these rivers were confirmed with clear differentiation between the two groups.

The high status rivers were more species rich and contained more sensitive indicator taxa of organic pollution and eutrophication. The impacted sites, however, while only being moderately impacted (see BBI, IBE, ASPT in Appendix 5.13) did contain a higher percentage of tolerant taxa in comparison to the higher status sites.

As part of the AQEM assessment methodology a multimetric index is produced which integrates multiple attributes of stream communities ("metrics") to describe and assess the health of a river system. Key areas of interest included investigating the feeding measures and the microhabitat

preferences among the high status and impacted sites. Feeding measures comprise functional feeding groups and provide information of the balance of feeding strategies (food acquisition and morphology) in the benthic assemblage. Microhabitat preferences are based on individual macroinvertebrate tendencies to survive in a particular microhabitat type.

Table 5.11 Summary of the *p*-values and means recorded from comparisons made between the high status and impacted sites for the indices/metrics, microhabitat preferences and feeding types analysed from the AQEM software.

Variable	Status	Mean	*p*-values High status sites vs Impacted sites
Index/metric			
Czech Saprobic Indec	High status sites	1.3	$p<0.001$***
	Impacted sites	1.5	
Dutch Saprobic Index	High status sites	0.2	$p=0.009$**
	Impacted sites	0.1	
German Saprobic Index	High status sites	1.6	$p<0.001$***
	Impacted sites	1.7	
Danish Stream Fauna Index (DSFI)	High status sites	6.7	$p<0.001$***
	Impacted sites	5.8	
Indice Biotico Esteco (IBE)	High status sites	9.9	$p<0.001$***
	Impacted sites	8.6	
Belgian Biotic Index (BBI)	High status sites	8.8	$p<0.001$***
	Impacted sites	7.4	
Average Score per Taxon (ASTP)	High status sites	5.9	$p<0.001$***
	Impacted sites	5.6	
Microhabitat preference			
Lithal microhabitat type	High status sites	48.4	$p<0.001$***
	Impacted sites	33.9	
Phytal microhabitat type	High status sites	26.4	$p<0.001$***
	Impacted sites	21.2	
Pelal microhabitat type	High status sites	6.1	$p<0.001$***
	Impacted sites	13.2	
Akal microhabitat type	High status sites	7.3	$p<0.001$***
	Impacted sites	10.9	
Feeding type			
Grazers and scrapers	High status sites	58.2	$p<0.001$***
	Impacted sites	31.9	
Miners	High status sites	0.6	$p<0.001$***
	Impacted sites	1.7	
Gatherers/collectors	High status sites	22.6	$p<0.001$***
	Impacted sites	31.6	
Active filter feeders	High status sites	1.5	$p<0.001$***
	Impacted sites	3.5	
Passive filter feeders	High status sites	4.0	$p<0.001$***
	Impacted sites	11.7	
Predators	High status sites	4.5	$p=0.002$**
	Impacted sites	7.1	
Parasite	High status sites	0.7	$p<0.001$***
	Impacted sites	1.7	

p-values: * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

5.4.3.10 Microhabitat preferences

Descriptions of the microhabitat types derived in the AQEM project are outlined in Tables 5.12.

Table 5.12 Microhabitat types.

Microhabitat Type	Description of microhabitat
Pel	Pelal: mud; grain size < 0.063mm (%)
Arg	Argyllal: silt, loam, clay; grain size < 0.063mm (%)
Psa	Psammal: sand; grain size 0.063-2mm (%)
Aka	Akal: fine to medium-sized gravel; grain size 0.2-2cm (%)
Lit	Lithal: coarse gravel, stones, boulders; grain size > 2cm (%)
Phy	Phytal: algae, mosses and macrophytes including living parts of terrestrial plant (%)
POM	Particulate organic matter, such as woody debris, CPOM FPOM (%)
Oth	Other habitats (%)

Significant differences in the Pelal microhabitat type (p<0.001), Akal microhabitat type (p<0.001), Lithal microhabitat type (p<0.001) and Phytal microhabitat type (p<0.001) were observed between the high status and the impacted sites were observed (Table 5.12; Appendix 5.14).

A higher percentage of the invertebrate community in the impacted sites showed a preference for the Pelal (mud-grain size <0.063mm) and the Akal (fine to medium-sized gravel; grain size 0.2-2cm) microhabitat type compared to the high status sites.

In comparison, the high status rivers contained significantly higher percentages of invertebrate fauna preferring coarse gravel, stones and boulders (Lithal microhabitat; grain size > 2cm), algae, mosses and macrophytes including living parts of terrestrial plants (Phytal microhabitat) compared to the impacted sites.

The AQEM software produces scores (X out of 10 points) for each individual taxon or species in describing the autecological information: 10/10 being the most favoured and 0/10 being the least favoured for the

particular autecological feature expressed. As mentioned above, the microhabitat and feeding types were of particular interest in this study.

Ecdyonurus dispar and *Ecdyonurus insignis* scored 10/10 and *Ecdyonurus venosus* and the immature unidentified *Ecdyonurus* species scored 8/10 in preference for the Lithal microhabitat type. Results show that the sensitive indicator species like *Ecdyonurus* score highly in the rivers that contain coarse gravel, stones and boulders. In addition to this, the high status sites contain significantly higher percentages of taxa with a preference for this microhabitat type indicating that these high status sites have the capacity to sustain the more sensitive indicator species. Interestingly, sensitive indicator taxa like *Ecdyonurus*, *Perla* and some Trichopteran species preferred habitats (10/10) with gravel, stones and boulders only and are deemed to have a zero weighting or zero preference (0/10) for microhabitats containing mud, medium-sized gravel and habitats with algae, moss or macrophytes.

Taxa like Chironomidae species score 6/10, *Dicranota* spp. scored 4/10, *Oligochaetea* species 3/10 while *Tipula* species and *Tubificidae* species scored 5/10 with a preference for the Pelal microhabitat (mud loving taxa). In addition to this, these taxa all score 0/10 in preference to the Lithal microhabitat type. *Simulium* species scored 5/10 (highest score among all taxon) with a preference for the Akal microhabitat type (fine to medium-sized gravel). The results demonstrate that the impacted sites contained a higher percentage of mud loving taxa and these with a preference for fine to medium-sized gravel (0.2-2cm).

This is seen as an important result - especially when compared with the results based on particle size alone (Section 5.4.4.4). The particle size analysis of substratum did not show any significant differences between the high status sites and the impacted sites. When it is considered that all of the impacted sites were selected on the basis that they once were capable of supporting 'lithal' species such as *Ecdyonurus* – and indeed *Ecdyonurus* has been historically recorded at all sites; albeit absent or almost absent during this survey at the impacted sites. The distinct faunal community differences

relating to microhabitat preferences, however, suggest that quite subtle changes in the microhabitat of the impacted sites has taken place. The faunal community as a whole has apparently shifted from a Lithal-dominated one to a community that has a higher proportion of Pelal species and other taxa that prefer a finer-particle substratum.

5.4.3.11 Feeding types

Significant differences between the high status and impacted sites were observed when comparing the feeding types (Appendix 5.15; Table 5.11). The percentage of grazers and scrapers were significantly higher (Table 5.11; $p<0.001$) in the high status compared to the impacted sites. All other feeding types outlined in Table 5.11 were significantly higher in the impacted sites (Appendix 5.15) giving an indication into the feeding strategies among the invertebrate fauna within the two groups.

This further supports the idea that biotic influences may be quite significantly influence as eutrophication impacts progress. As with the microhabitat metrics statistically significant differences are apparent between the high status sites and the impacted sites. It is more difficult to understand the precise significance of having more grazers and scrapers in the 'cleaner' environment but it may be related to the condition of the substratum surface and interstices at a microhabitat level. A clean oligotrophic environment may favour grazers and scrapers whereas a more productive eutrophic system becomes 'clogged' knocking out the species which require clean stone surfaces with a modicum of algal growth to feed upon. If growth rates exceed a certain threshold and perhaps if algal species types change these invertebrate species are placed at a disadvantage and other types begin to replace them.

5.4.4 Results of sediment analysis

Substrate fraction dry weights (g) for each site is presented in Appendix 5.16. The mean percentage frequency distribution (by weight) from the individual sites is presented together with individual sample data in Appendix 5.17 (high status sites) and Appendix 5.18 (impacted sites). The results for the high status and impacted sites outlined below refer to the calculated mean values presented in Appendices 5.17 and 5.18.

The main aim of this section of the study was to determine whether significant differences in substrate composition were apparent when impacted sites were compared with high status sites and thus, to attempt to understand the importance of sediment and siltation in controlling the distribution of *Ecdyonurus* in particular.

5.4.4.1 Substrate fractions - High status rivers

The substrate fractions in the high status sites are presented in Figs. 5.25 to 5.29. The Dunneill River contained all particle sizes while samples from the other four high status sites had most size fractions with the exception of boulders (256-512mm) (Figs. 5.25 to 5.29). All rivers contained small cobble (64-128mm) and large cobble (128-256mm). Small cobble was the dominant particle size (by weight) in the Dunneill River, the Castlebar River, Callow Loughs Stream and the Brusna River, ranging from 45.01%, 48.65%, 40.62% and 45.06% respectively (Appendix 5.17). The Brusna River contained the highest percentage of large cobble among the five high status sites, representing 34.12% of the total sample weight. The other four river sites contained large cobble in smaller amounts ranging from 13.80% to 18.42%. The dominant fraction in the Owengarve River was coarse gravel accounting for 22.31% of the total fraction. Very coarse gravel (32-64mm)and large and small cobble were also recorded in this site accounting for 12.69%, 11.58% and 14.22% respectively of the total fraction weight. While carrying out routine monthly sampling, visual observations noted that this river regularly contained quite a lot of silt and sand. The silt or fine sediments (particles <1mm diameter) are examined separately in both the high status and impacted sites in section 5.4.4.4.

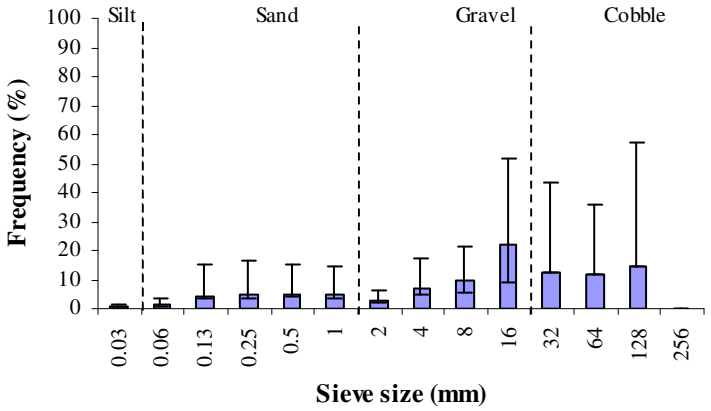


Fig. 5.25 Mean frequency particle size distribution (by % weight) from the Owengarve River in March 2003. Error bars indicate maximum and minimum values.

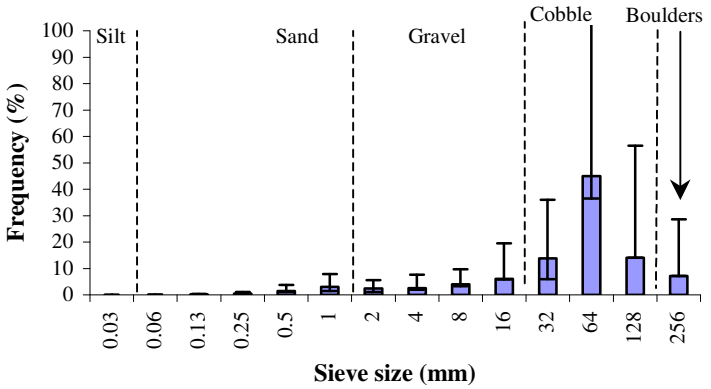


Fig 5.26 Mean frequency particle size distribution (by % weight) from the Dunneill River in March 2003. Error bars indicate maximum and minimum values.

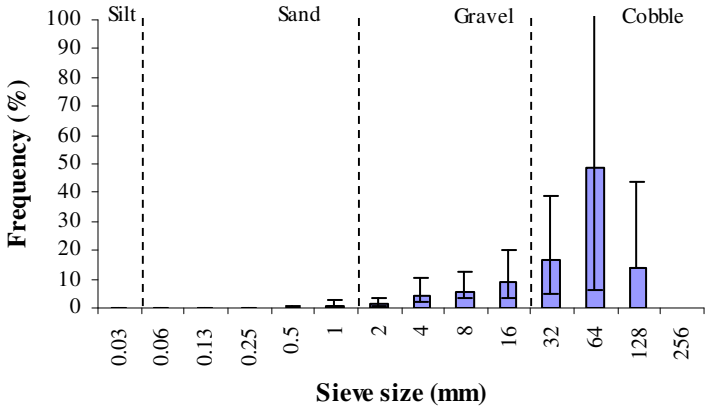


Fig. 5.27 Mean frequency particle size distribution (by % weight) from the Castlebar River in March 2003. Error bars indicate maximum and minimum values.

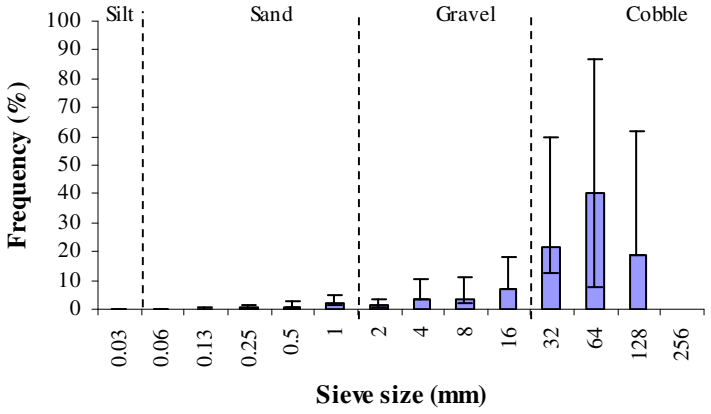


Fig. 5.28 Mean frequency particle size distribution (by % weight) from the Callow Loughs Stream in March 2003. Error bars indicate maximum and minimum values.

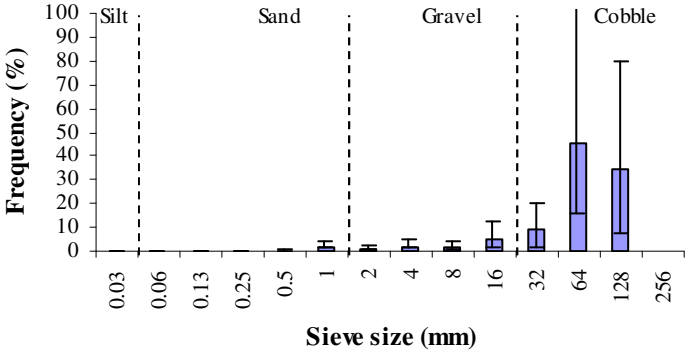


Fig. 5.29 Mean frequency particle size distribution (by % weight) from the Brusna River in March 2003. Error bars indicate maximum and minimum values.

5.4.4.2 Substrate fractions - Impacted sites

The substrate fractions in the impacted sites are outlined in Figs. 5.30 to 5.34. Boulders dominated the substratum in the Mad River and due to difficulty in sampling it was therefore decided to omit these from the results. Small cobble was recorded at all impacted sites while large cobble was found only in the Cartron River and Lough na Corralea Stream (Appendix 5.16). Small cobble generally dominated the fractions in the impacted sites followed closely by very coarse gravel. The Mullaghanoe River contained the highest percentage of small cobble accounting for 62.99% of the total fraction weight. Lough na Corralea stream contained the least amount representing 22.69% of the total fraction weight. Large cobble represented 5.91% and 20.36% of the substrate fraction in the Cartron River and Lough na Corralea Stream respectively. Very coarse gravel (32-64mm) was found in all impacted sites ranging from 14.01% to 27.89% (Appendix 5.18).

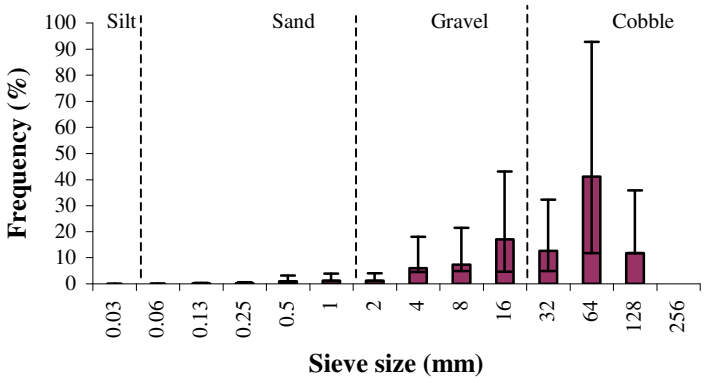


Fig. 5.30 Mean frequency particle size distribution (by % weight) from the Cartron River in March 2003. Error bars indicate maximum and minimum values.

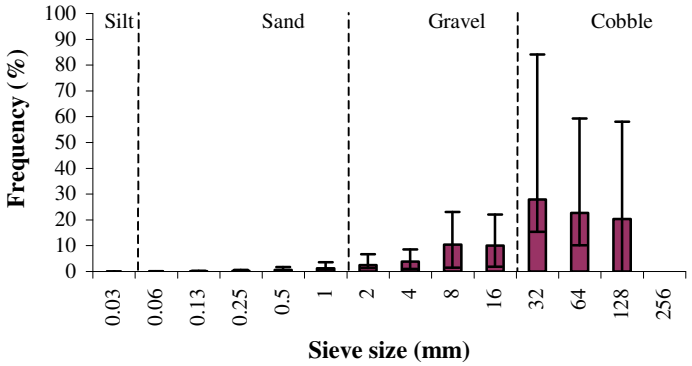


Fig. 5.31 Mean frequency particle size distribution (by % weight) from the Lough na Corralea Stream in March 2003. Error bars indicate maximum and minimum values.

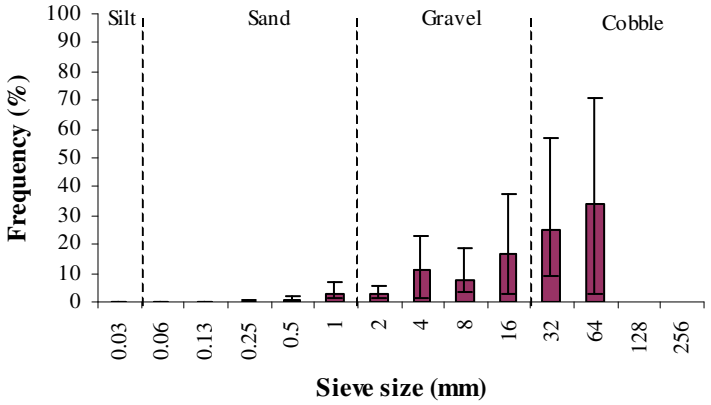


Fig. 5.32 Mean frequency particle size distribution (by % weight) from the Mad River in March 2003. Error bars indicate maximum and minimum values.

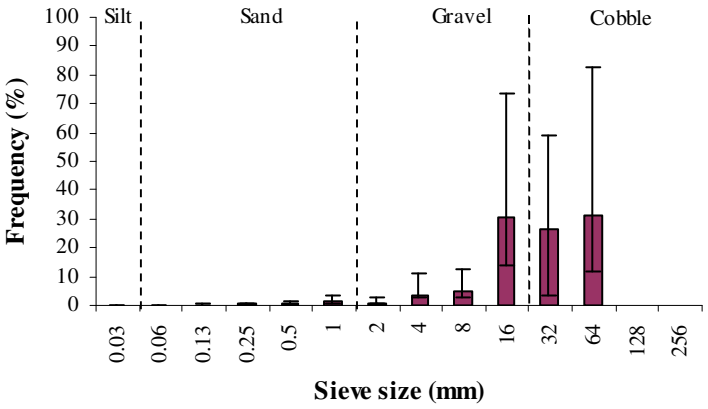


Fig. 5.33 Mean frequency particle size distribution (by % weight) from the Robe River in March 2003. Error bars indicate maximum and minimum values.

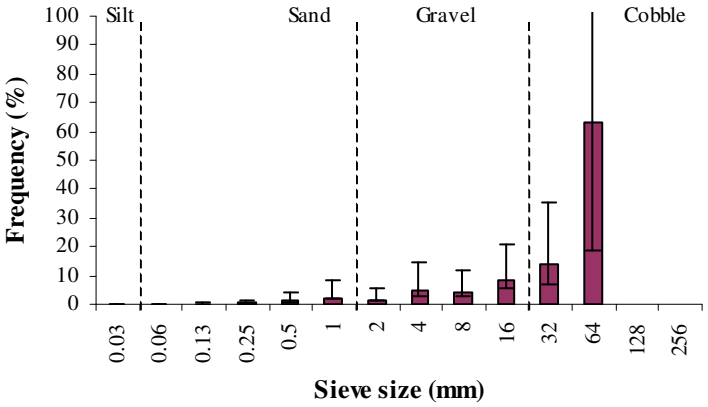


Fig. 5.34 Mean frequency particle size distribution (by % weight) from the Mullaghanoe River in March 2003. Error bars indicate maximum and minimum values.

5.4.4.3 Cumulative frequency curves

The data from individual high status and impacted sites are presented in cumulative frequency form in Appendices 5.19 and 5.20. Typically such data yield an S-shaped curve. The main function of the curve is to show the spread of particle sizes present. Mean values are used to report the results in most cases.

These graphs show the broad range of particle sizes present at all sites as can be observed by the flatness of the curve (i.e. tending to the horizontal). This pattern is indicative of considerable homogeneity of substrate between sites. Uniform samples would have a distribution that tends to the vertical. There was a substantial variation in fraction size from different samples, notably from one of the high status sites, the Owengarve River (Appendix 5.19A). This phenomenon was evident to a lesser extent in two of the impacted sites i.e. the Cartron River (Appendix 5.20A) and the Mullaghanoe River (Appendix 5.20E). At other sites, variation was considerably less. For example, three very similar samples were recorded from the Brusna River (Appendix 5.19E) the Mad River (Appendix 5.20C).

5.4.4.4 Fine sediments (particles < 1mm diameter)

Mean percentage fines were determined for each sample (Table 5.13). Mean percentage fines in the high status rivers ranged from 0.46 to 15.33. Mean percentage fines in the impacted rivers ranged from 1.03 to 1.95. The lowest maximum value of 0.33 for fines was recorded at the Brusna River. The highest mean, minimum and maximum values for fines were found in the Owengarve River (15.3, 0.36 and 4.87 respectively). Mean percentage fines for the high status sites are compared with the impacted sites (Fig. 5.35). Maximum and minimum values are included for each site. The Owengarve River (high status) displayed the highest recorded mean percentage fine value among all ten river sites.

Table 5.13 Mean percentage fines (particles <1mm diameter) from samples obtained from the high status and impacted river sites on the 21st and 27th March 2003.

River	Mean	Minimum	Maximum
High status sites			
Owengarve	15.33	0.36	4.87
Dunneill	2.11	0.01	1.49
Castlebar	0.46	0.01	0.34
Callow Loughs Stream	0.67	0.01	0.99
Brusna	0.50	0.01	0.33
Impacted sites			
Cartron	1.42	0.01	0.98
Lough na Corralea Stream	1.06	0.01	0.01
Mad	1.03	0.01	0.75
Robe	1.33	0.02	0.68
Mullaghanoe	1.95	0.12	1.19

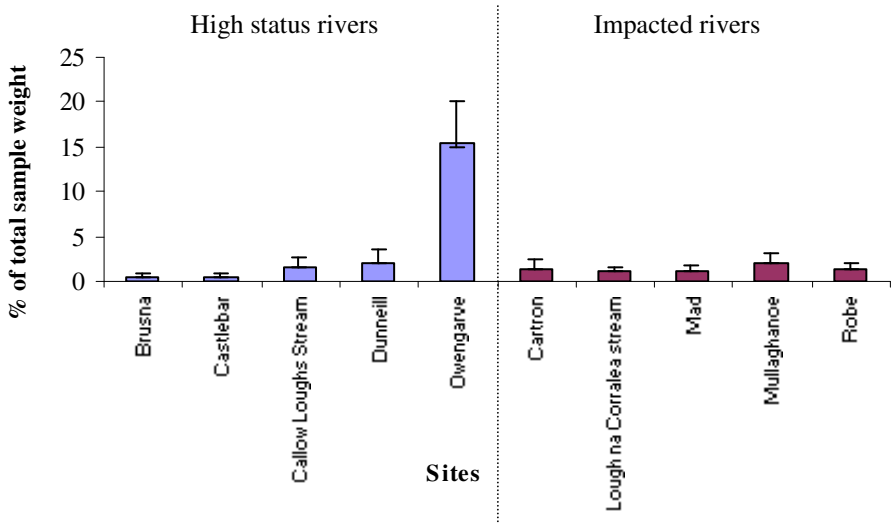


Fig. 5.35 Comparison of mean percentage fines (all particles < 1mm) from the high status and impacted rivers sampled in March 2003.

The box and whisker graph in Fig. 5.36 shows the relationship in the mean percentage fines (particles < 1mm diameter) between the high status and impacted sites. There was no significant difference in the mean percentage fines between the high status and impacted sites (Fig. 5.36: $p<0.79$). The difference in the mean percentage frequency of all substrate fractions between the high status and impacted sites is illustrated in Fig. 5.37 with no significant differences recorded ($p<0.71$). The mean percentage fines were compared between all ten river sites showing a significant difference (Fig. 5.38; $p<0.001$).

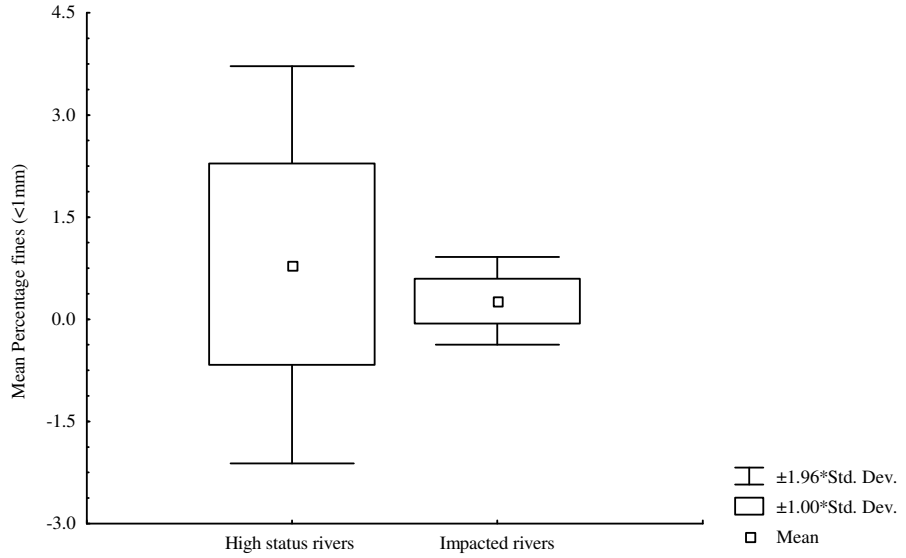


Fig. 5.36 Box and whisker graph showing the mean percentage fines (all particles < 1mm diameter) between the high status and impacted sites: $p<0.79$.

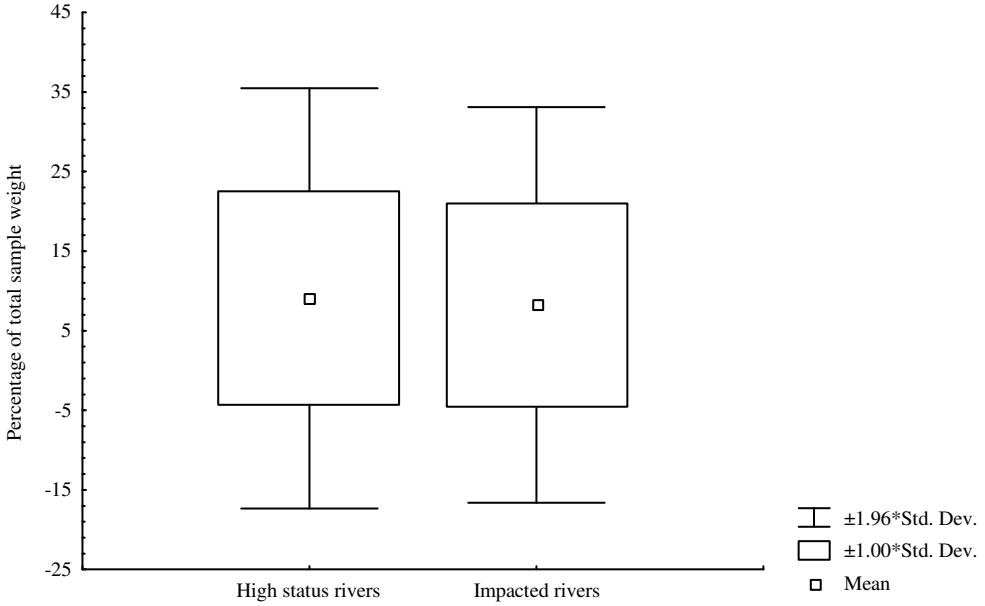


Fig. 5.37 Box and Whisker graph comparing the percentage frequency of all substrate fractions between the high status and impacted sites. $p<0.71$.

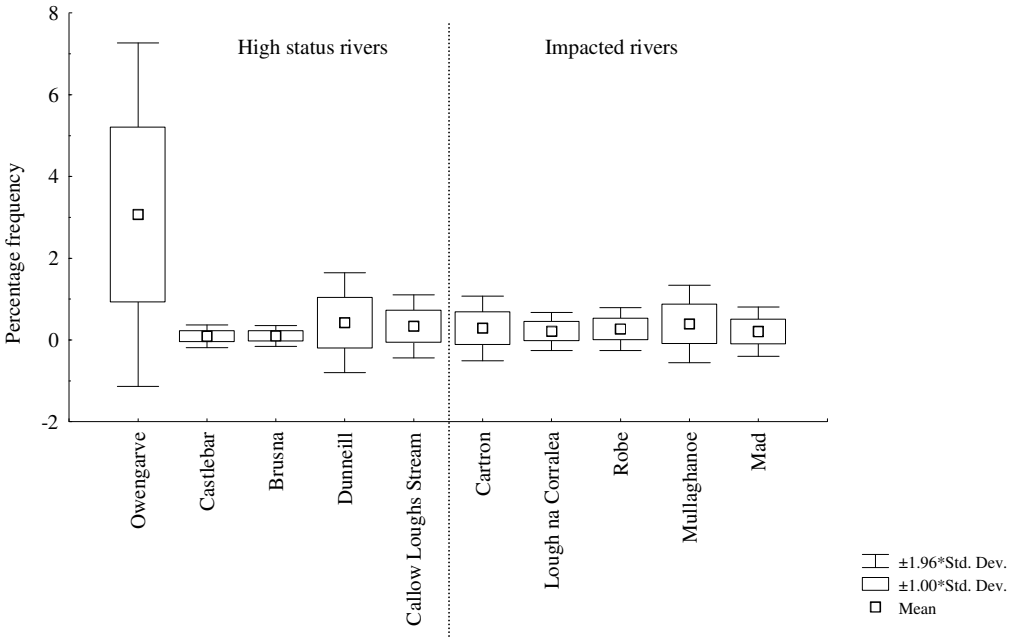


Fig. 5.38 Box and Whisker graph comparing the percentage fines between the individual river sites. $p<0.001$.

The aim of sampling the substrate in the ten rivers was to identify the particle sizes present at the various sites with particular reference to finer material. On a river by river basis the high status rivers contained a higher percentage of large cobble compared to the impacted sites. Small cobble was found in all ten rivers and showed slightly higher percentages in the high status rivers with the exception of one or two rivers. Boulders were only found in one of the nine rivers, namely the Dunneill River.

The sediment fractions in the ten rivers were all quite similar with the exception of the Owengarve River (high status river), which contained more fine silts and gravel than any other site. In general, cobble (large and small) and gravels (very coarse and coarse) dominated at all rivers. Overall, there were no significant differences in the sediment across the ten river sites.

5.5 Discussion

5.5.1 Introduction

The focus of this study was to establish information on the value of the physico-chemical data in supporting the Q-value System. It examines the chemical environment and biotic features like the macroinvertebrate communities and also the physical aspect with particular emphasis on the substratum type all associated with five high status and five impacted rivers in the West of Ireland.

An important objective of this project was to obtain an improved understanding of reference conditions as revealed by changes in the chemical parameters, macroinvertebrate fauna and physical changes when a river's status begins to depart from its pristine state. The project attempts to refine our knowledge of the ecology of *Ecdyonurus* in relation to a range of potential controlling factors in the riverine environment. In particular, it is necessary to understand what controls the disappearance of *Ecdyonurus* as eutrophication and organic pollution impacts on a river site.

5.5.2 General discussion

At the onset of the project, the high status rivers were chosen on the basis of the presence of *Ecdyonurus* and of good water quality as evidenced by water chemistry and high Q-values. The impacted sites were also chosen on the knowledge that at one point *Ecdyonurus* also survived in these rivers, and for reasons unknown, no longer exist. *Ecdyonurus* is a good indicator of pollution and the water chemistry results appear to support the hypothesis. The results show that ammonia was generally higher in the impacted rivers than in the high status rivers. As a consequence of eutrophication, on occasion however, some of the high status rivers displayed elevated DO levels, high unfiltered MRP and TO.N concentrations. This was particularly evident in the Owengarve River indicating that they were not all at reference condition as defined by the Water Framework Directive. Nonetheless, they were judged to be still of

high status according to the normative definitions of High Status in the Water Framework Directive.

The conservative indicators of water quality, chloride, colour and temperature displayed no significant differences between the high status and impacted rivers. There was a significant difference in pH, conductivity and alkalinity between the high status and impacted sites but these are most certainly due to different river typologies, particularly as determined by catchment geology.

Another hypothesis examined was whether low N:P ratios occur more commonly at high status sites than in more impacted sites. The phosphate levels were relatively high in some of the high status rivers and thus they may have been N-limited on occasion during the sampling period. Results from this study show that the impacted sites were more P-limited during the sampling period compared to the high status sites. Some of the high status rivers were N-limited, particularly the Castlebar and the Dunneill River which may have contributed to the increased levels of phosphate in these systems from time to time. Comparison with a wider range of physico-chemical samples taken in the West of Ireland shows that occasional N-limitation occurs when phosphate concentrations are less than 40µg P/l (approximately 4% of all samples analysed). The wider survey also showed that such N-limitation is significantly more likely to occur in the summer months (July to October).

The analysis of the sediments across the river sites revealed no significant differences. The sediment fractions in the ten rivers were all quite similar with the exception of the Owengarve River (high status) which contained more fine silt than any other site. This river was dredged a year prior to the commencement of the project so the consequence of this was still evident. The chalky nature of the surrounding banks and bank trampling by cattle upstream may also have contributed to the higher content of silt. It might have been expected that one would find less *Ecdyonurus* numbers in this situation but in fact it was the opposite as the Owengarve River contained the highest density and largest sizes of this species among all high status

sites. This, however, also is borne out by separate observations of Heptageniidae survival even in highly turbid waters caused by inorganic silt from sand quarry washings (McGarrigle pers. comm.). In general, the sediment size across the ten rivers did not appear to be a factor in controlling the presence or absence of *Ecdyonurus*. While the sediment particle size analysis did not reveal significant differences between the impacted and high status rivers, subjective observations of the substratum did suggest that the impacted rivers were generally more 'silted' than the high status group.

Understanding the changes that occur in macroinvertebrate communities in relation to pollution is a key issue for impact assessment. There have been numerous methods developed to measure community changes or to assess water quality including a variety of biotic and diversity indices and multivariate approaches (Hellawell, 1978, 1986; Metcalfe, 1989; Mason, 1991; Cairns *et al*, 1993; Rosenberg and Resh, 1993; Norris and Norris, 1995) and have been measured using a variety of terms, including biomass, species richness and species composition and density. However, as Boyle *et al*. (1990) pointed out, community changes are a complex function of species composition, species richness and the relative abundance and density of individuals.

The present study analysed the community differences within high status and impacted sites in conjunction with an examination of various water quality parameters. Several numerical indices were chosen in this study for analysis and interpretation of the macroinvertebrate fauna. The ability of the indices and metrics to highlight stressed sites varied. The diversity indices were examined on a river by river basis across the sampling period and the most significant differences between the high status and impacted rivers were found using Margalef's index, total number of taxa and % EPT. All three indices/metrics were higher in the high status rivers. These findings, therefore, also support the hypothesis that *Ecdyonurus* is a good indicator of water pollution at the community level. The variation in the diversity indices and biotic metrics was also examined across five seasons. Contrary to expectations, there does not appear to be any difference in

sampling across the seasons (Appendix 5.5 to 5.11). On a river by river basis one may see a change but there was not a marked, overall, seasonal change in the metrics used to describe macrinvertebrate communities. This may perhaps be due to the mild Irish climate which may produce more gradual changes in community structure across the seasons than might be expected in ecoregions where climate extremes are more pronounced.

Ecdyonurus was absent from three of the impacted rivers and was present only on occasion in the other two sites, the Robe and the Mullaghanoe rivers, which were both affected by eutrophication intermittently. These findings support the accepted view that sensitive species are reduced when water quality deteriorates (Chandler, 1970; Washington, 1984; Hellawell, 1986). However, it was also evident that polluted sites, particularly the intermediately polluted sites like the Robe and the Mullaghanoe Rivers had the highest abundance of invertebrates indicating an increase or influx of benthic-invertebrates more tolerant to pollution.

Use of the recently available AQEM software to calculate a much wider range of biotic indices including saprobic indices and modern European indices such as the Danish Fauna Index also demonstrated significant differences between impacted against high status sites. Trophic metrics like feeding measures are surrogates of complex processes (e.g. trophic interaction, production and food source availability) (AQEM consortium, 2002). Generalists, like gatherers and collectors have a broader range of acceptable food materials than the specialists (e.g. grazers and scrapers) and thus are more tolerant to pollution, which might alter availability of certain food types. This may explain why the percentage of grazers and scrapers was significantly higher ($p<0.001$) in the cleaner sites where foods ingested by these taxa are more widely available. The metric results also show the effect of eutrophication and organic pollution on the distribution of functional feeding groups. The percentage of the gatherers/collectors, filter feeders, predators, miners and parasites are significantly increased in the impaired sites. It appears that food resources for the more specialised grazers and scrapers may be more limited in these particular sites even

though they are only moderately polluted. This is confounded by the results from the microhabitat preferences produced from the AQEM programme.

A significantly higher percentage of the macroinvertebrate fauna in the higher status sites appeared to favour the Lithal microhabitat type (coarse gravel, stones, boulders) indicating that the food ingested by these taxa are more widely available on these substrate types. Results from AQEM show that taxa like the sensitive indicator *Ecdyonurus* favoured these substrate types suggesting that the surfaces contain good quality food sources for these grazers. In comparison, a higher percentage of taxa in the impaired sites favoured the Pelal microhabitat (mud loving) and the Akal microhabitat (fine to medium-sized gravel). Even though the sediment analysis did not show significant differences between the high status and the impacted sites, the results from the feeding and microhabitat investigations suggest a change in feeding guilds and microhabitat preferences among the macroinvertebrate community as organic pollution and eutrophication progresses.

Selection of the most appropriate indices and metrics were based primarily on the ability of the index/metric to provide a meaningful summary of the data as well as the applicability of the index to categorise the benthic community. Based on findings from this study, Margalef's index (d), total number of taxa (S) and % EPT were the most appropriate metrics and indices for distinguishing between high status and impacted sites in this study. Biotic indexes used in other European countries which focus mainly on organic pollution and eutrophication also gave clear differentiation between the two groups of sites based on their macroinvertebrate communities.

Studies carried out in the White River near Indianapolis investigated which diversity, similarity and biotic indices best reflected the observed changes in the water quality due to the effects of organic pollution (Lydy *et al*., 2000). Results showed that the most descriptive tool in analysing the data was not one of the indices but in fact the percentage EPT found at the site in a given year. The trends shown by this tool best reflected the changes in water

quality at the three sampling sites. The diversity indices tested were the least useful of all the indices; in fact, they indicated that the opposite result had occurred and water quality declined after the wastewater treatment plant located upstream of the site was upgraded (assuming high diversity corresponds to better water quality). Diversity indices failed to reflect the changes in the White River because the changes occurred in benthic-invertebrate community structure more so than in diversity. Pollution-intolerant EPT species replaced pollution-tolerant chironomids however, and this observation was not incorporated or detected by the diversity indices.

On the other hand, other studies have shown that taxon richness is a powerful tool in distinguishing between non or slightly impaired and stressed sites. Ofenbock *et al*. (2004) investigated the effects of various types of pollution (including organic pollution) on a number of rivers in four different bioregions in Austria. Richness measures reflect the diversity of an assemblage and are known to be most useful in indicating impairment (Resh *et al*., 1995). Elimination of taxa from a naturally diverse system can be easily detected (Barbour *et al*., 1996). In particular, species belonging to the insect orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) are generally regarded as sensitive to impairment and the loss of taxa richness within this group indicates perturbation (Wallace *et al*., 1996). Only metrics representing taxa richness and diversity (e.g. % EPT, total number of taxa, intolerant taxa, diversity indices) showed a unidirectional response to impacts like organic pollution. All were higher in the unimpacted rivers. Margalef's diversity index (Margalef, 1958) gave reliable results in separating the stream types and this measure contributed to the indices of all stream types with the exception of one. Unimpaired sites were more species rich than the impaired ones.

Although the number of sites investigated in this study was comparatively small, the findings are supported by other recent studies in highlighting the high discriminatory power of Margalef's index, total number of taxa and % EPT in differentiating between impacted and unimpacted systems. These may be very useful indicators of ecosystem health when assessing the

biological status of Irish rivers as demanded by the Water Framework Directive. The Water Framework Directive defines high ecological status and reference conditions very strictly. This project, however, was designed to investigate the subtle changes a river experiences as it departs from a its high status during the initial stages of eutrophication and the results may be useful in providing input into the definition of the Good/Moderate boundary for Irish rivers.

Chapter 6

6.1 General Discussion

6.1.1 Split-stream experiment

An important objective of this project was to obtain an improved understanding of why ecological communities depart from reference conditions as pollution and eutrophication impacts on individual species and in particular indicator taxa such as *Ecdyonurus*. The effects of eutrophication on *Ecdyonurus* were studied using a novel split-stream experiment which involved artificially increasing the phosphorus concentrations in two oligotrophic rivers in the West of Ireland. Nutrient enrichment experiments usually involve the use of artificial enclosures or channels in lakes and rivers but this experiment, the first of its kind undertaken in Ireland, involved manipulating a river in its natural state.

Whole ecosystem enrichment experiments in lakes were pioneered by Schindler and his fellow workers (Schindler, 1985) at the experimental lakes in Canada. Similar experiments in rivers using artificial channels studying enrichment or eutrophication have shown increases in periphytic biomass under certain conditions (e.g. Elwood *et al*., 1981; Stockner and Shortreed, 1976; Horner and Welch, 1981; Horner *et al*., 1983; Perrin *et al*., 1987; Bourassa and Cattaneo, 2000). Peterson *et al.*, (1985) showed that an increase of only 10µg/l PO_4-P above the background (1-4µg/l P) in river water channels, either alone or in combination with NO_3-N, resulted in substantial increases in epilithic chlorophyll *a* and other biological activities. Other workers (MacKenthun, 1968; Wong and Clark, 1976; Horner *et al.*, 1983) involved in nutrient manipulation experiments in rivers, found that much higher levels of phosphorus were needed to increase algal biomass.

The first hypothesis examined in this investigation was whether *Ecdyonurus* was directly or indirectly affected by the limiting nutrients phosphorus and nitrogen. It was envisaged that by artificially enriching a P limited river,

algal growth would increase and the effects on the sensitive indicator genus *Ecdyonurus* could be described. These experiments revealed surprising results showing the importance of N-limitation in the rivers studied. Some of the nutrient manipulation experiments showed significant differences in algal biomass nonetheless between the control and treated sections, but not all did so.

The results underline that experimental response is also dependent on the N:P ratios and the temporal scale of the experiment. Due to a low N:P ratio in the Clydagh River in 2002, no effect was observed in the manipulated section after sampling on four occasions over a 6 week period. There was a positive effect on periphyton growth in the same river in 2003 towards the end of the 5-week experiment, even though the river apparently had a low N:P ratio. The most significant differences in periphyton Chl *a* between treated and untreated sections were observed in the Castlebar River in 2003, particularly in the last 3 weeks of the 9-week experiment, despite an apparently N-limited system.

There is the possibility that there was fluxing or pulsing of N through the Clydagh and the Castlebar River systems, which was not detected between sampling periods. Domestic houses and a concrete manufacturing facility located upstream of the Castlebar River may have been potential sources possibly introducing N sporadically into the river and particularly from septic tanks of houses that are only occupied in the evenings and overnight – i.e. outside times when grab samples from the river for water chemistry purposes were taken. These undetected nitrogen sources may have caused the fluctuating N:P ratios between sampling periods.

Experiments carried out by Bourassa and Cattaneo (2000) in an experimental lake in Canada, showed that despite a four-fold increase in phosphorus concentration, the periphyton Chl *a* was only slightly higher in the enriched than the non-enriched treatments. Significant relationships between periphytic chlorophyll and nutrients have often been observed (Aizaki and Sakamoto, 1988; Biggs and Close, 1989; Mundie *et al*., 1991; Lohman *et al*., 1992; Dodds *et al*., 1997; Harvey *et al*., 1998). When

nutrients are undetectable, loss processes like grazing or sloughing have been proposed to explain the lack of response (Jones *et al*., 1984; Welch *et al*., 1988; Kjeldsen, 1996; Bourassa and Cattaneo, 1998).

An increase in nutrients or light can augment invertebrate growth, density and biomass (Lamberti *et al*., 1989; Mundie *et al*., 1991; Hill *et al*., 1995: Dube *et al*., 1997; Bourassa and Cattaneo, 1998). In the present experiment, no significant differences in numbers of *Ecdyonurus* were found between the treated and untreated sections. These findings appear to indicate that *Ecdyonurus* is not directly affected by the consequences of eutrophication i.e. increased algal biomass at the relatively low levels of impact that were observed during the experiment at any rate. It is hypothesised that nitrogen spikes may have occurred that allowed available phosphate to be used thus apparently causing the increased algal growth in some of the experiments, particularly in the Clydagh River in 2003 and the Castlebar River in 2003 but having no direct effect on the abundance of *Ecdyonurus*.

On analysing the N:P ratios in 99 rivers in the West of Ireland it was found that approximately 4% of the samples were N-limited with low MRP concentrations (<0.05mg/l P). This implies that a small proportion of high status rivers are nitrogen limited rather than phosphorus limited. Thus, in terms of the Water Framework Directive, there may be a case for introducing tighter regulations on the limits of nitrogen emitted to water bodies as well as phosphorus. Results from these studies highlight the complexity of the in-stream processes driving these N-limited and P-limited high status rivers. It is clear that they are dynamic systems in a constant state of flux.

6.1.1.1 Comments and recommendations

Nitrogen limitation appears to be a summer phenomenon primarily but more detailed studies are required preferably over the course of an entire year, in conjunction with investigations into the effect of nutrient enrichment in the form of nitrogen on periphyton biomass growth and the associated responses of sensitive indicator taxa.

6.1.2 Investigation into the feeding regime of *Ecdyonurus venosus*

It was hypothesised that the changes exhibited in the biomass in the split-stream experiment would be reflected in the gut contents of *Ecdyonurus venosus*, possibly showing a variation in algal taxa between both sections of the manipulation experiment. This study provided an opportunity to assess whether such direct manipulation had any obvious impact on the diet of this species. The most significant differences between the enriched and unenriched sections were observed in the Castlebar River in 2003 therefore *Ecdyonurus* specimens sampled from this river were chosen for the main gut analysis investigation. Studies dealing with algal-grazer interactions within the mayfly community are under represented in the literature (Feminella and Hawkins, 1995; Steinman, 1996), especially regarding the influence of abiotic parameters on stream herbivory. Since the feeding preferences of *Ecdyonurus venosus* had not been studied in Ireland to date, this study also provided new information on its diet.

The hydrogen peroxide oxidation technique was not very successful in isolating the benthic diatoms from the specimens and did not provide information on the overall material consumed by these invertebrates. The fluorochromatic stain 4'6 diamidino-2-phenylin-dole (DAPI) and epifluorescent microscopy (Walker *et al*., 1988), in combination with light microscopy, did however successfully categorise the material consumed in detail. This provided much needed information into the classification of *Ecdyonurus* into its functional feeding group by documenting its preferred diet. Unfortunately, time constraints did not allow for any isotope analysis on the gut contents, so this cheaper and less time consuming method of analysing the gut contents provided new information into the diet of *Ecdyonurus*. It paves the way for future work in gut analysis in Ireland as the techniques for isolating the gut contents of *Ecdyonurus* are perfected and further works are carried out in this area.

The gut contents of *Ecdyonurus venosus* were examined over a number of sampling occasions and preliminary findings reveal promising results in relation to what these invertebrates feed on. The study documented the

material consumed but did not provide information on what was being assimilated so the nutritional significance of the ingested food or the manner in which they fed were not examined.

On the basis of the findings from the study in the Castlebar River in 2003, *Ecdyonurus venosus* can be classified as both a herbivorous grazer and detritus feeder with a tendency towards opportunistic feeding. Moog (1995) also proposes that this genus feeds in a dual action mode and describes it as both a grazer and a detritus feeder. The food ingested by these larvae appears to be strongly dependent on the food available in the environment at a given time and they seem to feed on particles that are most abundant during a particular season or those that are easily accessible during a feeding episode. This may explain the differences in the proportions of diatoms in the invertebrate guts investigated in March 2003 compared to those studied from July-September 2003.

The *Ecydonurus* gut contents consisted mainly of epilithic algal tissue, plant particulate matter (detritus), biofilm matrix and inorganic debris (mineral material). Results indicate a greater proportion of inorganic debris in the gut of *Ecdyonurus* in comparison with that found on the stone scrapings more than likely due to the brushing action of *Ecdyonurus* and its tendency to harvest overstorey layers. Studies carried out by Wellintz and Ward (1998) indicate that *Ecdyonurus* also harvested overstorey layers. Their findings suggest that most overstorey periphyton removed by *Ecdyonurus* appeared to be an amalgam of diatoms, silt and detritus. The most common algal species found in the guts of the invertebrates sampled in the Castlebar River from July-September 2003 was *Navicula* spp. The diet seems to depend on the presence of a particular diatom species in the habitat in any given season and preference to a particular species may be due to a greater ease in scraping some diatom species off the substratum than others.

Interaction between periphyton and grazers can be confounded by contrasting impacts for grazers and differential algal responses to grazing (Pan and Lowe, 1994). Periphytic algal biomass was stimulated in the final weeks of the experiments in 2003 (particularly in Castlebar River) and it

was hypothesised that there may be a change in the algal taxa between the control and treated sections of the split-stream experiment. As mentioned in Chapter 3, Paul and Duthie (1989) have shown that algal species respond to increases in phosphorus by changing their community structure. Similar community shifts resulting from nutrient manipulation was also reported by Pringle and Bowers (1984). This was not evident in this investigation, however, which was reflected in the similarity in the stone scrapings and the food content in the guts of the larvae examined on both sides of the experimental divide. The hypotheses that *Ecdyonurus* would demonstrate diet changes due to enrichment was not supported but neither were there any observed changes in the periphyton species community.

6.1.2.1 Comments and recommendations

An in-depth study, preferably over a 12-month period across seasons is essential in this area in order to provide a more complete understanding into the feeding regime of *Ecdyonurus*. It should also be possible to determine which foods are assimilated using stable isotope analysis. In terms of understanding the role of diet in the known sensitivity of *Ecdyonurus* to pollution and eutrophication this study has shown that it has a relatively specialised mode of feeding. It is suggested that as eutrophication progresses and filamentous algae such as *Cladophora* begin to blanket stone surfaces it will become increasingly difficult for *Ecdyonurus* to feed in its normal grazer mode.

6.1.3 Life history studies of the Heptageniidae in five high status rivers in the West of Ireland

To understand and predict the response of organisms to variation and change within and between lotic ecosystems, we need information about their life histories (Power *et al*., 1988). The life cycle of the genus *Ecdyonurus* has not been described in any great detail in Ireland to date therefore this investigation provided much needed new information on this topic.

Of the three species studied, *Ecdyonurus venosus* was the dominant species in all five high status rivers displaying a bivoltine life cycle with only a slight variation in emergence periods between sites. Conflicting findings in Ireland were documented by Connolly and McCarthy (1993) and they described its life cycle as being univoltine in the Corrib catchment. As in this study, Fahy (1973) describes *Ecdyonurus venosus* as having a bivoltine life cycle. It may be that the lifecycle is flexible, having a bivoltine or a univoltine cycle depending on the temperature regime of individual rivers or in a given year. Studies carried out in England by Elliott (1967) and Wise (1980) show that this species had a univoltine life cycle which disagrees with the findings of Rawlinson (1939) who found that it had a fast growing summer generation in addition to a slow growing winter generation (bivoltine).

The life cycle of *Ecdyonurus dispar* investigated in this study was univoltine. Similar findings were documented in English studies carried out by Macan and Maudsley (1968) and Wise (1980). There was no evidence in the literature relating to studies on the life cycle of *Ecdyonurus dispar* or *Ecdyonurus insignis* in Ireland. *Ecdyonurus insignis* had a univoltine lifecycle in the rivers studied in this investigation. Macan (1970) also described it as having a similar life cycle.

The absence of both *Ecdyonurus insignis* and *Ecdyonurus dispar* from the benthos during the summer and winter months suggest that these species develop quite differently to the more common species *Ecdyonurus venosus*.

Partitioning of emergence periods appears to be evident in this study and is particularly noticeable in the Owengarve River where all three species were present. Macan (1981) describes this phenomenon as a tool that may ensure the survival of the next generation of each species. Other studies described by Landa (1968) and Sowa (1975) underpins the suggestion from our findings that the different species in the same river follow chronological patterns of emergence periods maximising the survival of each individual species. Lotic species are often regionally different in age and size at first reproduction, in numbers of generations per year and in the degree of synchrony of life history stages (Newell and Minshall, 1978).

The life cycle of *Rhithrogena semicolorata* was more straightforward and easier to interpret and it clearly had a univoltine life cycle. Studies carried out by Wise (1980) and Elliott and Humpesch (1980) in England describe it as being univoltine. The only study relating to its life cycle in Ireland was carried out by Fahy in 1971, where he also describes it as having a univoltine life cycle. The *Heptagenia* specimens were identified to genus level only and were therefore described as a genus group that appeared to adopt a univoltine life cycle with an overwintering larval generation. It was the least common of the Heptageniidae family and apart from investigations carried by Wise (1980) in England, there were no other similar studies found in the literature.

Apart from being absent from the benthos for a few months after the main flight period, particularly in July and August, *Rhithrogena semicolorata* was also present throughout the year, so one would therefore expect to find this species during most seasons in a given year also. Its absence during July and August, however, does not have the same significance as does the absence of *Ecdyonurus* during the summer months. *Hepatgenia* spp. emerged a bit later than *Rhithrogena semicolorata* so depending on the abundance in a river, would be expected to be found throughout the year when routinely sampling, apart from July and August and in some rivers in September.

6.1.3.1 Comments and recommendations

In order to ensure that the Heptageniidae, and in particular the genus *Ecdyonurus*, are fully represented when carrying out routine biological assessments, the timing of sampling may be a critical factor, especially when attempting to capture species that are only present in the benthos for a few months during the year. Findings from the present studies support the hypothesis put forward that the various species of *Ecdyonurus* emerge in overlapping phases such that during the summer months larvae of at least one *Ecdyonurus* species will be present in the benthic riffle fauna of Irish rivers. Results indicate that more intense sampling may be required during certain months of the summer.

Thus, assuming adequate sampling, the overlapping life cycles of *Ecdyonurus* should always result in summer samples yielding at least one representative of *Ecdyonurus* if a river is of high status or at reference condition. This is important if the monitoring programme is concerned with water quality as opposed to biodiversity or taxonomic issues. Summer low flow and high temperature conditions will tend to aggravate the impact of pollution discharges and thus, the faunal community in summer and early autumn provide a 'minimum thermometer' measure of the impact of pollution. This is the time when fish kills or loss of sensitive macroinvertebrates is most likely to occur. While many species have life cycles that help them to avoid critical conditions the present study suggests that at least one species of *Ecdyonurus* is likely to be present in high status rivers throughout the summer months.

Autumn or winter sampling may produce more species both of *Ecdyonurus* and other pollution sensitive taxa even in polluted systems as aestivating eggs of plecopteran species and *Rhithrogena* hatch out and small nymphs begin to appear in macroinvertebrate samples. If the aim of the sampling, however, is to assess water quality or ecological status as impacted by anthropogenic effects in particular, then summer assessments provide a more reliable assessment of worst case conditions in a river. As it may only take one pollution event to severely alter the taxonomic composition of a

lotic community this is a critical point. Autumn or winter sampling may not be sufficiently sensitive to detect for example summer eutrophication effects such as low night time dissolved oxygen during warm low flow conditions.

From studying the life cycles of the Heptageniidae in five rivers in the West of Ireland it appears that the life cycles of the genus *Ecdyonurus* can vary slightly from year to year and from river to river. Due to the short life cycle of *Ecdyonurus insignis* and in particular *Ecdyonurus dispar*, which appear to be summer species, it would be vital to sample from June to August in a given year to ensure capture of these species. The more common species of this genus, *Ecdyonurus venosus* was present in each of the rivers studied throughout the year, with the exception of a few weeks post emergence. Hence, one would expect to find this species when sampling during all seasons throughout the year. It reinforces the indicator value of *Ecdyonurus*.

6.1.4 Examination of the biotic and abiotic factors controlling the distribution of the genus *Ecdyonurus*

There are many possible indicators of river health, including measures of structure and function both of the biotic and of the physical components. Physical and chemical indicators (mostly of water quality) are the most commonly used and largest variety available (e.g. Hart *et al*., 1999; Maher *et al*., 1999). One of the main objectives of this study is to understand what controls the disappearance of *Ecdyonurus* as eutrophication and organic pollution impact on a river. The occurrence of *Ecdyonurus* is controlled by a number of features including chemical, physical and biotic factors. Measuring the health of a river system should therefore include an assessment of the biological community and its physico-chemical characteristics.

Ecdyonurus is a good indicator of pollution and the water chemistry results appear to support the hypothesis that the presence of *Ecdyonurus* is associated with good water quality. Ammonia concentrations were generally higher in the impacted rivers than in the high status rivers. As a consequence of eutrophication, on occasions, some of the high status rivers displayed elevated DO levels, high unfiltered MRP and TON concentrations.

There were no significant differences in the conservative indicators of water quality, namely chloride, colour and temperature between the high status and impacted rivers. As mentioned in Chapter 5, significant differences in pH, conductivity and alkalinity between the high status and impacted sites were most certainly due to different river typologies, which were largely dependent on the catchment geology.

The presence/absence of *Ecdyonurus* in the high status v impacted sites supports its use as a significant bioindicator of water quality. The presence of *Ecdyonurus* did not appear to be controlled by any of the physico-chemical parameters examined in this experiment. This genus did survive on occasion in some of the impacted sites that were moderately polluted indicating that favourable conditions were present from time to time to

support their survival. Despite the fact that the ammonia levels were significantly higher in the impacted sites, *Ecdyonurus* was evident in a number of these rivers.

No significant differences were found in the sediments across the ten river sites. It was evident that some of the sites were affected by siltation during the sampling programme, in particular the Owengarve and the Mullaghanoe Rivers. The sampling technique applied to the sediment analysis did emphasise the large amount of fine silt in the Owengarve River but it did not detect this in the Mullaghanoe or the Robe River. The impacted rivers appeared to be more 'silted' than the high status rivers from time to time highlighting the inadequacy of sampling on just one occasion. The results suggest that traditional particle size analysis may not be sensitive enough to detect the changes in habitat that have occurred but that the known microhabitat preferences of the invertebrate community taxa may allow quite subtle changes to be detected. It is hypothesised that the condition and precise nature of the surface films on stones is perhaps more important than the absolute particle size distribution of the gross substratum samples that were taken.

A number of numerical indices and metrics were selected to analyse and interpret the macroinvertebrate communities in the high status and impacted sites. The ability of the indices and metrics to emphasise stressed sites varied and the results revealed that the most significant differences between the two groups of sites were found using Margalef's index, total number of taxa and % EPT. All three indices were higher in the high status rivers. A wider range of biotic indices became available from the recent introduction of the AQEM system also showing significant differences between the high status and impacted sites.

The most appropriate indices were chosen on their capacity and suitability for assessing the impact of organic pollution and eutrophication on the bentic faunal community among the two groups of sites. Results from the feeding and microhabitat indices used in AQEM suggest that as eutrophication and the impacts of organic pollution progress, a change in the

feeding guilds and microhabitat preferences among the macroinvertebrate communities occur.

It has been observed that increased sediment loads may reduce the abundance and diversity of invertebrates by smothering interstitial habitat and reducing periphytic abundance or quality (Lloyd *et al*., 1987; Newcombe and McDonald, 1991; Ryan, 1991; Wood and Armitage, 1997). Fine silts have been found to be unsuitable habitats for most New Zealand aquatic insects (Quinn and Hickey, 1990; Jowett *et al*., 1991; Death, 2000). Some New Zealand invertebrate species, notably *Deleatidium* spp. (Ephemeroptera, Leptophlebiidae) and *Pycnocentrodes* sp. (Trichoptera, Conoesucidae) show preferences for 'clean' rather than silted periphyton (Ryan, 1991) and in colonisation trials, Ryder (1989) showed that the occurrence of fine sediment in the algal matrix reduced invertebrate densities by about 30%.

Consequently, changes in the pattern of sediment deposition as a result of landuse change may modify the impact that invertebrate grazers have upon periphytic prey. The Hydrobiid snail *Potamopyrgus antipodarum* for example, is often dominant in the macroinvertebrate communities in low-order pasture streams throughout New Zealand, yet it is rare in otherwise similar afforested catchments in which the 'sensitive' ephemeropteran, plecopteran and trichopteran taxa usually dominate (Quinn and Hickey, 1990). Of these latter taxa, the Leptophlebiid mayfly *Deleatidium* sp. is often the most abundant species and this has been shown to be unable to separate food from silt prior to ingestion and to be less abundant on sediment-rich epilithic tiles than on sediment poor ones (Ryder, 1989; Ryan, 1991). In contrast, *P.antipodarum* was more abundant on the impacted tiles (Ryder, 1989) and has been shown to take (small) sediment particles into the buccal cavity, scrape the encrusting organic matter off and finally 'spit' the sediment particle out (Lopez and Kofoed, 1980). These various lines of evidence suggest that *P.antipodarum* may be more tolerant of sediment contamination in its food than *Deleatidium* sp. are.

It is surmised that the changes in microhabitat may predominantly be affecting surface films. As eutrophication progresses the nature of surface films on stones and in the interstices of riverine gravels may change. Thus, increases in absolute abundance and production of surface algae – diatoms, cyanobacteria and green algae, for example. Changes in algal species may occur. Based purely on subjective observation of surface films on stones, it may also by hypothesised that increased pick-up of inorganic silt and organic detritus is occurring. While the initial hypothesis suggested that particle size analysis would be sufficient to detect ongoing siltation effects it now appears that at the levels of deterioration experienced at the impacted sites sampled, this analysis was not sufficiently sensitive. It should be borne in mind that Irish catchments (and particularly in the West of Ireland) have very little tillage agriculture and thus, significant silt inputs of soil origin are not expected. Peat silt due to exploitation of bogs, forestry drainage or due to overgrazing of blanket bogs may, however, be quite significant in certain areas although the Mad River is likely to be the only site so affected in the impacted sites. Future work should examine changes in surface films in more detail in an attempt to support or refute the above hypothesis.

Food quality can influence key life history traits of aquatic insects such as growth rate, size at maturity, and the ability to complete metamorphosis and to reproduce (Anderson and Cummins, 1979; Cargill *et al*., 1985; Dadd, 1982; Lamberti and Moore, 1984). Several studies have shown that because of the low quality of detritus as food, it supports only a small portion of the growth of hydropsychid caddisflies despite its abundance in the insects guts (Beneke and Wallace, 1980; Fuller and Mackay, 1981; Haefner and Wallace, 1981). The nature and consequently the digestibility of the detritus, algal tissue, biofilm matrix (organic film on rocks) and other material may be different from that consumed by other insects. In establishing stability within the invertebrate community in rivers it is important therefore that each taxon has a good supply of its own required food sources. High status rivers display a balanced ecosystem where food is plentiful and grazing pressures are able to sustain an equilibrium with periphyton growth.

6.1.4.1 Comments and recommendations

The analyses support the findings of the split-stream study, suggesting that nitrogen limitation may be an important aspect of eutrophication and of reference conditions in certain river types and particularly during the summer months. More work is required to elucidate the reasons why a small number of high status sites are nitrogen limited rather than the more typical state of phosphorus limitation.

The results of the whole-community analysis give some potential insights into the manner in which the traditional biotic indexes of organic pollution and eutrophication work at a community level. The traditional pollution-sensitive indicator species have definite microhabitat and feeding requirements that are typically found in oligotrophic unpolluted rivers. As nutrient levels increase the microhabitat changes sufficiently to favour less sensitive species. At the extreme an anoxic, mud-dominated environment will favour only tubificid worms and *Chironomus* perhaps, but there is a continuum of change and microhabitat effects may be more important than simple deoxygenation effects or toxicity effects due to ammonium, for example, at the early stages of eutrophication.

Development of alternative sampling methodologies is required to measure the effects of siltation that were undetected by the technique used in this investigation. Further studies into the microhabitat and feeding preferences of *Ecdyonurus* are essential in conjunction with a more detailed examination into the changes in the food quality that occur as eutrophication and the effects of organic pollution progress.

6.1.5 Concluding comments

The results demonstrate that the factors controlling biological communities may be complex both in time and space. The limited nocturnal oxygen survey carried out was perhaps insufficient to demonstrate the true impact of low dissolved oxygen saturation in these rivers. Macroinvertebrates, however, act as 'minimum thermometers' in the sense that they are impacted by the worst conditions – e.g. the lowest oxygen or the highest ammonia concentrations – that prevail while they are present in the river. *Ecdyonurus* is known to be sensitive to low oxygen saturation and is generally absent from moderately polluted river sites in Ireland.

The life cycle analyses in this study suggest that at least one species of *Ecdyonurus* should be present at all times of the year. Thus, their utility as an indicator species is justified in the sense that if they are absent at a site it is most likely because of an adverse environmental pressure. It is postulated that the nature of the nutrient rich organic biofilms that once covered the stones in the impacted sites where *Ecdyonurus* previously survived have been adversely affected. Findings from the gut analysis studies show that epilithic algal tissue, plant particulate matter (detritus), biofilm matrix and inorganic debris (mineral material) are the main food items ingested by *Ecdyonurus*. The food quality of the biofilm, now deemed to be a very important source of nutrients for macroinvertebrates, may be reduced in these impacted sites due to adhesion of fine sediment particles undetected in this study. Our studies showed that algae are an important food source in the diet of *Ecdyonurus* and although not studied in detail in this investigation, changes in the epilithic algae in the impacted sites may have affected its feeding habits.

An understanding of the detailed ecology of key indicator species like *Ecdyonurus* is important in defining and comprehending the ecology of high status river sites. At high status sites the important indicator species are able to flourish and maintain sustainable populations. Increased knowledge of the detailed ecology of indicator species and reference conditions is necessary in order to enable cross-European comparisons between different

ecoregions and different ecotypes. Findings from this study have increased our knowledge in defining reference conditions, which is critical for the development of classification systems. In terms of the WFD, the results are beneficial and will be particularly useful when carrying out intercalibration exercises with other European countries and with other ecoregions.

References

Agences de l'Eau (2000) System for the evaluation of the biological quality of watercourses . SEQ Bio (Version 0). General principles. Water Agencies Study No. 77.

Aizaki, M. and Sakamoto, K. (1988) Relationship between water quality and periphyton biomass in several streams in Japan. *Verhandlungen der internationale Vereinigung fur theoretische und angewandte Limnologie*, 23, 1511-1517.

Alba Tercedor, J. and Sanchez Ortega, A. (1988) Un metodo rapido y simple para evaluar la calidad biologica de las aguas corrientes basado en el de Hellawell (1978). *Limnetica,* **4**, 51-56.

Allen, J.D. (1995) Stream ecology. Structure and function of running waters. *Chapman and Hall*, London, 388pp.

Anderson, N.H. and Cummins, K.W. (1979) Influences of diet on the life histories of aquatic insects. *Journal of the Fisheries Board of Canada***. 36**, 335-342.

AQEM consortium (2002) Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive. Version 1.0 February 2002, 198pp.

AQEM Version 2.3.4a August (2004) AQEM River Assessment Programme. Developed under EU 5th Framework Project Developing and testing of and Integrated Assessment system for the Ecological Quality of Streams and Rivers Throughout Europe using Benthic Macroinvertebrates. Contract EVK1-CT1999-00027. Website: www.aqem.de

Arens, W. (1989) Comparative functional morphology of the mouthparts of stream animals feeding on epilithic algae. (*Monographische Beiträge). – Archiv für Hydrobiologie*, **83**, 253–534.

Armitage, P.D., MacHale A.M. and Crisp, D.C. (1975) A survey of the invertebrates of four streams in the Moor House National Nature Reserve in North England. *Freshwater Biology*, **5**, 479-495.

Austrian Standards ONORM M 6232 (1997) Guidelines for the Ecological Survey and Evaluation of Flowing Surface Waters. Austrian Standards Institute, Vienna, 38 pp.

Axler, R.P., Rose, C. and Tikkanen, C.A. (1994) Phytoplankton nutrient deficiency as related to atmospheric nitrogen deposition in Northern Minnesota acid-sensitive lakes. *Canadian Journal of Fish and Aquatic Sciences*, **51**, 1281-1296.

Barbour, M.T., Gerritsen, J., Griffith, G.E., Frydenborg, R. McCarron, E., White, J.S. and Bastian, M.L. (1996) A framework for biological criteria for Florida streams using benthic macroinvertebrates. *J.N. Am. Benthol.Soc.* **15**, 185-211.

Barbour, M.T., Gerritsen, J., Snyder, B.D. and Stribling, J.B. (1999) *Revision to Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphy..ton, Benthic Macroinvertebrates and Fish*. US Environmental Protection Agency Assessment and Watershed Protection Division, Washington, DC. EPA 841-D-97-002.

Basu, B.K. and Pick, F.R. (1996) Factors regulating phytoplankton and zooplankton biomass in temperate rivers. *Limnol. Ocenaogr*, 41, 1572-1577.

Bauernfeind, E. (1994) Bestimmungsschlussel fur die osterreichischen Eintagsfliegen (Insecta: Ephemeroptera), 1. Teil. Wasser Abwasser Suppl. **4**, 1-92.

Bauernfeind, E. (1997) Discriminating characters in Central European species of *Ecdyonurus* EATON. *In* Landolt P. & M. Sartori (eds), Ephemeroptera & Plecoptera: Biology-Ecology-Systematics (= Proc. 8th Int. Conf. Ephemeroptera, Lausanne, 1995), Fribourg, 418-426.

Bauernfeind, E. (1998) Erganzugen und Berichtigungen zu den Eintagsfliegen des Lavanttales (Insecta: Ephemeroptera). Linzer. Biol. Beitr. **30**, 293-298.

Bauernfeind, E. and Moog, O. (2000) Mayflies (Insecta: Ephemeroptera) and the assessment of ecological integrity: a methodological approach. *Hydrobiologia*. **422/423**, 71-83.

Beneche, V. (1972) La fecondite be Baetis rhodani Pictet. *Freshwater Biology*, **2**, 337-354.

Benke, A.C. (1984) Secondary production of aquatic insects, pp. 289-322. *In*: The Ecology of Aquatic Insects, V.H. Resh and D.M. Rosenberg (eds), Praeger, New York.

Beneke, A.C. and Wallace, J.B. (1980) Trophic basis of production among net-spinning caddisflies in a southern Appalachian stream. *Ecology*, **61**, 108-118.

Biggs, B.J.F. and Close, M.E. (1989) Periphyton biomass dynamics in gravel bed rivers: the relative effects of flow and nutrients. *Freshwater Biology*, **22**, 209-231.

Biggs, B.J.S. and Lowe, R.L. (1994) Response of two trophic levels to patch enrichment along a New Zealand stream continuum. *New Zealand J.mar.freshwat.Res*., **28**, 119-134.

Biggs, B.J.F. and Thomsen, H.A. (1995) Disturbance of stream periphyton to perturbation in shear stress: time to structural failure and differences in community resistance. *Journal of Phycology*, **31**, 233-241.

Birk, S. and Hering, D. (2002) Waterview Web-Database: a comprehensive review of European assessment methods for rivers. FBA News 20 (winter 2002): 4.

Blinn, D.W., Fredericksen, A. and Korte, V. (1980) Colonization rates and community structure of diatoms on three different rock substrata in lotic system. *British Phycological Journal*, **15**, 303-310.

Bohle, H.W. (1978) Beziehugen zwischen dem Nahrungsangebot, der Drift und der raumlichen Verteilung bei Larven von *Baetis rhodani* (Pictet) (Ephemeropteren: Baetidae) Untersuchungen in Kunstlichen Fliesswasseranlagen. *Archiv fur Hydrobiologie*, **84**, 500-525.

Borchardt, M.A. (1996) Nutrients. In: *Algal Ecology*: *Freshwater Benthic Ecosystems* (eds R.J. Stevenson, M.L. Bothwell and R.L. Lowe), pp. 183-227. Academic Press, San Diego, CA.

Bothwell, M.L. (1985) Phosphorus limitation of lotic periphyton growth rates: an intersite comparison using continuous flow troughs. (Thompson River System, British Columbia). *Limnol. Oceanogr*, **30**, 527-542.

Bott, T.L. (1983) Primary production in streams. *Stream Ecology: Application and Testing of General Ecology Theory*. (eds. J. R Barnes and G.W. Minshall), pp. 29-54. Plenum Press, New York.

Bourassa, N. and Cattaneo, A. (1998) Control of periphyton biomass in Laurentian streams. *Journal of the North American Benthological Society*, **17**, 420-429.

Bourassa, N. and Cattaneo, A. (2000) Response of a lake outlet community to light and nutrient manipulation: effects on periphyton and invertebrate biomass and composition. *Freshwater Biology*, **44**, 629-639.

Bowman, J.J. and Clabby, K.J (1998) Water quality of rivers and lakes in the Republic of Ireland. Environmental Protection Agency. In: Wilson, J.G. (ed.) 1998 *Eutrophication in Irish waters*, 55-63. Dublin. Royal Irish Academy.

Boyle, T.P., Smillie, G.M., Anderson, J.C and Beeson, D.R. (1990) A sensitive analysis of nine diversity and seven similarity indices. *J. Wat. Pollut. Control. Fed*, **62**, 749-762.

Brabec, K. Zahradkova, S., Nemejcova, D., Paril, P, Kokes, J. and Jarkovsky, J. (2004) Assessment of organic pollution effect considering differences between lotic and lentic stream habitats. *Hydrobiologia*, **516**, 331-346.

Breitenmoser-Wursten, C. and Sartori, M. (1995) Distribution, diversity, life cycle and growth of a mayfly community in a prealpine stream (Insecta, Ephemeroptera). *Hydrobiologia*, **308**, 85-101.

Brittain, J.E. (1982) Biology of mayflies. *Annual Review of Entomology*, **27**, 119-47.

Brittain, J.E. (1991) Life history characteristics as a determinant of the response of mayflies and stoneflies to man-made environmental disturbance (Ephemeroptera and Plecoptera). *Proc. Vlth. Int. Conf.* Ephemeroptera and Plecoptera, Granada: 539-545.

Brown, M.E. (1957) Experimental studies on growth. *In*: The physiology of fishes (Ed. M. E. Brown), Vol. 1. pp.361-400. Academic Press London.

Brown, E.J. and Button, D.K. (1979) Phosphate-limited growth kinetics of *Selenastrum capricornutum* (Chlorophyceae). *J. Phycol*, **15**, 305-311.

Buffagni, A., Erba, S., Cazzola, M. and Kemp, J.L. (2004) The AQEM multimetric system for the southern Italian Apennines: assessing the impact of water quality and habitat degradation on pool macroinvertebrates in Mediterranean rivers. *Hydrobiologia*, **516**, 313-329.

Bunn, S.E., Edwards, D.H. and Lonergan, N.R. (1986) Spatial and temporal variation in the macroinvertebrate fauna of streams of the northern jarrah forest, Western Australia: community structure. *Freshwater Biology*, **16**, 67-91.

Butler, M.G. (1984) Life histories of aquatic insects. In *The ecology of aquatic insects* (ed. V.H.Resh and D.M. Rosenberg), pp.24-55. Praeger, New York.

Cairns, J. Jr, McCormick, P.V. and Niederlehner, B.R. (1993) A proposal framework for developing indicators of ecosystem health. *Hydrobiologia*, **263**, 1-44.

Canfield, D.E. Jr. (1983) Prediction of chlorophyll *a* concentrations in Florida lakes: the importance of phosphorus and nitrogen. *Wat. Res. Bull*,. **19**, 255-262.

Cargill, A.S., Cummins, K.W., Hanson, B.J. and Lowry, R.R. (1985) The role of lipids, fungi and temperature in the nutrition of a shredder caddisfly, *Clistoronia magnifica*. *Freshwater Invertebrate Biology*, **4**, 64-78.

Cattaneo, A. and Kalff, J. (1986) The effects of grazer size manipulation on periphyton communities. *Oecologia*, **69**, 612-617.

Chandler, J.R. (1970) A biological approach to water quality management. *Wat. Pollut. Control London*, **69**, 415-422.

Chapman, D.W. and Demory, R.L. (1963) Seasonal changes in the food ingested by aquatic insect larvae and nymphs in two Oregon streams. *Ecology*, **44**, 140-146.

Chovanec, A., Jager, P., Jungwirth, M., Koller-Kreimel, V., Moog, O., Muhar, S. and Schmutz, S. (2000) The Austrian way of assessing the ecological integrity of running waters: A contribution to the EU Water Framework Directive. *Hydrobiologia*, **422/423**, 445-452.

Chutter, F.M. (1972) A reappraisal of Needham and Usinger's data on the variability of stream fauna when sampled with a Surber sampler. *Limnology ann Oceanography*, **17**, 139-141.

Clabby, K.J., Lucey, J, McGarrigle, M.L., Bowman, J.J., Flanagan, P.J. and Toner, P.F. (1992) Water Quality in Ireland 1987-1990, Part One: General Assessment. Water Resources Division, An Foras Forbartha, Dublin. 189pp. ISBN 1 85053 180 3.

Clarke, G.M. (1994) Developmental stability analysis: an early-warning system for biological monitoring of water quality. *Australian Biologist*, **7**, 94-104.

Clarke, G.M., Arthington, A.H. and Pusey, B.J. (1995) Fluctuating asymmetry of chironomid larvae as an indicator of pesticide contamination in freshwater environments. Pages 101-109, *In* P.S. Cranston (ed.) Chironomids: from genes to ecosystems. CSIRO, Melbourne.

Clenaghan, C., Giller, P.S., O'Halloran, J. and Hernan, R. (1998) Stream macroinvertebrate communities in a conifer-afforested catchment in Ireland: relationships to physico-chemical and biotic factors. *Freshwater Biology*, **40**, 175-193.

Clifford, H.F. (1982) Life cycles of mayflies (Ephemeroptera), with special reference to Voltism. *Quaestiones Entomologicae*, **18**, 15-90.

Closs, G.P. and Lake, P.S. (1994). Spatial and temporal variation in the structure of an intermittent-stream food web. *Ecological Monographs*, **64**, 1-21.

Coates, D. (1980) Prey-size intake in humbug damselfish, *Dascyllus aruanus* (Pisces, Pomacentridae) living within social groups. *Journal of Animal Ecology*, **49**, 335-340.

Coffman, W.P., Cummins, K.W. and Wuycheck, J.C. (1971) Energy flow in a woodland stream ecosystem: I. Tissue support structure of the autumnal community. *Archiv fuer Hydrobiologie*, **68**, 232-276.

Coleman, A.W. (1980) Enhanced detection of bacteria in natural environments by fluorochrome staining of DNA. *Limnol. Oceanogr.* **25**, 948-951.

Coleman, M.J. and Hynes, H.B.N. (1970) The life histories of some Plecoptera and Ephemeroptera in a southern Ontario stream. *Can. J. Zool.* **48**, 1333-1339.

Colletti, P.J., Blinn, D.W., Pickart, A and Wagner, V.T. (1987) Influence of different densities of the mayfly grazer *Heptagenia criddlei* on lotic diatom communities. *J*. North. Am. *Benthol*. *Soc*. **6**, 270-280.

Connolly, B. and McCarthy, T.K. (1993) Maflies (Ephemeroptera) of the Corrib catchment, Ireland. *Biogeography of Ireland*: *past, present and future*. *M.J*. *Costello and K.S*. *Kelly* (*eds*.). *Occ*. *Publ*. *Ir*. *Biogeog*. *Soc*. *No* 2.

Cooke, G.D., Welch, E.B., Peterson S.A. and Newroth, P.R. (1993) *Restroation and Management of Lakes and Reservoirs.* 2nd edn, 548 p. Lewis Publishers, Boca Raton.

Cox, E.J., (1990) Studies on the algae of a small softwater stream I. Occurrence and distribution with particular reference to the diatoms. Archiv für Hydrobiologie**, 4**, 525-552.

Cranston, P.S., Fairweather, P. and Clarke, G. (1996) Biological indicators of water quality, pp 143-154. *In*: Walker, J and Reuter, D.J. (eds) Indicators of Catchment Health: a technical perspective. CSIRO, Melbourne.

Cummins, K.W. (1973). Trophic relations of aquatic insects. *Ann. Rev. Entomol*. **18**, 183-206.

Cummins, K.W. (1974). Structure and function of stream ecosystems, *BioScience*, **24**, 631-641.

Cummins, K.W. (1988). The study of stream ecosystems: a functional view, p. 320-363. *In*: J. R. Pomeroy and J. J. Alberts (eds.). Concepts of ecosystem ecology. Springer-Verlag, New York.

Cummins, K.W. and Klug, M.J. (1979). Feeding ecology of stream invertebrates, *Ann. Rev. Ecol. Syst.* **10**, 147-172.

Cummins, K.W., Coffman, W.P. and Roff, P.A. (1966) Trophic relationships in a small woodland stream. *Internationale vereinigung fur theroetische und angewandte lomnologie*. **16**, 627-638.

Dadd, R.H. (1982) Insect nutrition: relevance to marine invertebrates. Pages 33-54 *in* G.D. Pruder, C.J. Langon and D.E Conklin (eds.) Proceedings of the second International Conference on Aquaculture Nutrition. Division of Continuing Education, Louisana State University, Baton Rouge.

Darley, W.M. (1982). *Algal Biology: a Physiological Approach*. Blackwell Scientific Ppublications, Oxford.

Davis, W.S. and Simon, T.P. (1995) Introduction. Pages 3-6 *in* W.S. Davis and T.P. Simon (eds). Biological assessment and criteria.Tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida.

Davies, N.M., Norris, R.H. and Thoms, M.C. (2000) Prediction and assessment of local stream habitat features using large-scale catchment characteristics. *Freshwater Biology*, **45**, 343-369.

Death, R.G. (2000) Invertebrate-substratum relationships. In Colier, K.J. and Winterbourn, M.J. (eds.), New Zealand Stream Invertebrates: Ecology and Implications for Management. New Zealand Limnological Society, Christchurch: pp. 157-178.

Deegan, L., Peterson, B. and Portier, R. (1990) Stable isotopes and cellulose activity as evidence for detritus as a food for juvenile gulf Menhaden. *Estuaries*, **13**, 14-19.

DELG 1998. S.I. No. 258/1998: *Local Government (Water pollution) Act, 1977 (Water quality Standards For Phosphorus) Regulations, 1998*. Government Publications, Dublin.

Den Boer, P.J. (1968) Spreading of risk and stabilisation of animal numbers. *Acta Biotheoretica*, **18**, 165-194.

De Pauw, N. and Hawkes, H.A. (1993) Biological monitoring of river water quality. In Walley, W.J. and S. Judd (eds), Proceedings of the Freshwater Europe Symposium on River Water Quality Monitoring and Control. Birmingham: 87-111.

De Pauw, N. and Vanhooren, G. 1983. Method for biological quality assessment of watercourses in Belgium. *Hydrobiologia*,**100**,153-168.

DEV (Deutsches Institut fur Normung e.V.), 1987. Biologisch-okologische Gewasserguteuntersuchung (Gruppe M): Allegemeine Hinweise, Planung und Durchfuhrung von Fliebgewasserunter-suchungen (M1). In: Deutsche Einheitsverfahren zur Wasser- Wasser-Abwasser- und Schlammuntersuchng. VCH Verlagsgesellschaft mbH, Weinheim: 1-13.

DEV (Deutsches Institut fur Normung e.V.), 1992. Biologisch-okologische Gewasserguteuntersuchung: Bestimmung des Saprobienindex (M2). In Deutsche Einheitsverfahren zur Wasser-Abwasser- und Schlammuntersuchng. VCH Verlagsgesellschaft mbH, Weinheim: 1-13.

Dietrich, M. and Anderson, N.H. (1995) Life cycles and food habits of mayflies and stoneflies from temporary streams in western Oregon. *Freshwater Biology*, **34**, 47-60.

Dixit, S.S. and Smol, J.P., (1994) Diatoms as indicators in the environmental monitoring and assessment program-surface waters (EMAP-SW). Environmental Monitoring and Assessment, **31**, 275-306.

Dodds, W.K., Smith, V.H. and Zander, B. (1997) Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork river. *Water Research*, **31**, 1738-1750.

Downes, B.J., Lake, P.S. and Schreiber, E.S.G. (1995) Habitat structure and invertebrate assemblages on stream stones: a multivariate view from the riffle. *Australian Journal of Ecology*, **20**, 502-514.

Dube, M.G., Culp, J.M. and Scrimgeour, G.J. (1997) Nutrient limitation and herbivory: processes influenced by bleaching kraft pulp mill effluent. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2584-2595.

Edmunds, G.F., Jr., Jensen, S.L and Berner, L. (1976) The Mayflies of North and Central America. University of Minnesota Press, Minneapolis.

Edwards, R.W. and Owens, M. (1965) The oxygen balance of streams, ecology and the industrial society. Blackwell, Oxford. U.K.

Edwards, A.C. , Cook, Y., Smart, R. and Wade, A.J. (2000) Concentrations of nitrogen and phosphorus in streams draining the mixed land-use Dee Catchment, north-east Scothland. *Journal Applied Ecology*, **37**, 159-170.

Egglishaw, H.J. and Mackay, D.W. (1967) A survey of the bottom fauna of streams in the Scottish Highlands. Part 3. Seasonal changes in the fauna of three streams. *Hydrobiologia*, **30**, 305-334.

Elliott, J.M. (1967) The life histories and drifting of the Plecoptera and Ephemeroptera in a Dartmoor stream. *Journal of Animal Ecology*, **36**, 343-362.

Elliott, J.M. (1977) *Some methods for the statistical analysis of samples of benthic invertebrates*. Freshwater Biological Association, Windermere, Special Publication 25.

Elliot, J.M. (1979) Some methods for the statistical Analysis of samples of Benthic Invertebrates. Windemere: *Freshwater Biological Association*.

Elliott, J.M. and Humpesch, U.H. (1980) Eggs of Ephemeroptera. *Annual Report of the Freshwater Biological Association*, **48**, 41-52.

Elliott, J.M. and Humpesch, U.H. (1983) A key to the adults of the British Ephemeroptera with notes on their ecology. S*cientific Publications of the Freshwater Biological Association*, **47**, 101.

Elliott, J.M., Humpesch, U.H. and Macan, T.T. (1988) Larvae of the British Ephemeroptera: A key with ecological notes. *Freshwater Biological Association*, *Scientific Publication no* **49**.

Elwood, J.W., Newbold, J.D., Trimble, A.F. and Stark, R.W. (1981) The limiting role of phosphorus in a woodland stream ecosystem: effects of enrichment of leaf decomposition and primary producers. *Ecology*, **82**,146-158.

Engblom, E. (1996) Ephemeroptera, Mayflies. *In* Nilsson, A.N. (ed.), Aquatic Insects of North Europe – A Taxonomic Handbook, Vol. 1. Apollo Books, Stenstrup, 13-53.

Environmental Protection Agency (2002) *In* Environment in Focus. Key Environmental Indicators for Ireland. Eds, Lehane, M., Le Bolloch, O. and Crawley, P. Environmental Protection Agency, Wexford, Ireland.

Estep, M.L. and Vigg, S. (1985) Stable carbon and nitrogen isotope tracers of trophic dynamics of natural populations and fisheries of the Lahotan lake system. *Can. J. Fish. Aquat. Sci.*, **42**, 1712-1719.

European Comission (2000) Directive 2000/60/EC of the European Parliament and of the Council – Establishing a framework for the community action in the field of water policy. Brussels, Belgium, 23 October 2000.

Fahy, E. (1973) Observations on the growth of Ephemeroptera in fluctuating and constant temperature conditions. *Proc. R.I.A.*, **73**, 133-149.

Feminella, J. W. and Hawkins, C.P (1995) Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. *Journal of the North American Benthological Society*, **14,** 465-509.

Feminella, J.W. and Resh, V.H. (1991) Herbivorous caddisflies, macroalgae and epilithic microalgae: dynamic interactions in a stream grazing system. *Oecological*, **87**, 247-256.

Finlay, K.J. (2001) Construction and evaluation of a new laboratory system for rearing mayflies. *In*: *Trends in Research in Ephemeroptera and Plecoptera*, pp. 241-250.

Fisher, S.G., Gray, L. J., Grimm, N.B. and Busch, D.E. (1982) Temporal succession in a desert stream ecosystem following flash flooding. *Ecol. Monogr.*, **52**, 93-110.

Flanagan, P.J. and Toner, P.F. (1972) *The national survey of Irish rivers. A report on water qulaity*. Dublin. An Foras Forbartha.

Francoeur, S.N., Biggs, B.J.F, Smith, R.A. and Lowe, R.L. (1999) Nutrient limitation of algal biomass accrual in streams. An in situ bioassay. *Ecology*, **66**, 465-472.

Freeman, C.M., Lock, M.A., Marxsen, J. and Jones, S.E. (1990) Inhibitory effects of high molecular weight dissolved organic matter upon metabolic processes in biofilms from contrasting rivers and streams. *Freshwater Biology*, **24**, 159-166.

Frieberg, M. and Jacobsen, D. (1994) Feeding plasticity of two detritivore-shredders. *Freshwater Biology*, **32**, 133-142.

Frissel, C.A., Liss, W.J., Warren, C.E. and Hurley, M.D. (1986) Hierarchical framework for stream habitat classification: viewing streams in a watershed context. *Environment Management*, **10**, 199-214.

Fuhs, W. (1969) Phosphorus content and the rate of growth in the diatoms *Cyclotella nana* and *Thalassiosira fluviatilis*. *J. Phycol*. **5**, 312-321.

Fuller, R.L., and Mackay, R. J. (1981) Effects of food quality on the growth of three *Hyrdopsyche* species (Trichoptera: Hydropsychidae) *Can. J. Zool.* **59**, 1133-1140.

Furse, M.T., Moss, D., Wright, J. F. and Armitage, P.D. (1984) The influence of seasonal and taxonomic facts on the ordination and classification of running water-sites in Great Britain and on the prediction of their macro-invertebrate communities. *Freshwater Biology*, **14**, 257-280.

Ghetti, P.F. (1997) Manuale di applicazione Indice Biotico Esteso (I.B.E.). I macroinvertebrati nel controllo della qualita degli ambienti di acque correnti. Provincia Autonoma di Trento, Agenzia provinciale per la protezione dell'ambiente, 222pp.

Gifford, D.J. and Caron, D.A. (2000) Sampling, preservation, enumeration and biomass of marine protozooplankton. *In*. Harris, R.P. *et al*. (eds) ICES Zooplankton methodology manual. Academic Press, London, pp 193-221.

Giller, P.S. and Malmqvist, B. (1998) The biology of streams and rivers. Oxford University Press.

Gordon ND., McMahon TA. and Finlayson BL. (1992) Stream hydrology. An introduction for ecologists. John Wiley & Sons, Chichester. 526 pp.

Gore, J.A. (1985) *The restoration of Rivers and Streams*, 280 pp. Butterworth, Stoneham.

Gray, L.J. and J.V. Ward. (1979) Food habits of stream benthos at sites of differing food availability. *American Midland Naturalist*, **102**, 157-167.

Green, R.H. (1979) Sampling design and statistical methods for environmental biologists. John Wiley, New York, 257pp.

Gregory, S.V. (1983) Plant-herbivore interactions in stream ecosystems. Pages 157-189. In Barnes J.R. and G.W. Minshall (eds.) Stream Ecology Pleneum Press, New York: 157-189.

Grimm, N.B., and Fisher, S.G. (1986) Nitrogen limitation in a Sonoran Desert stream. *J. N. Am. Benthol*. Soc., **5**, 2-15.

Grimm, N.B. and Fisher, S.G. (1989) Stability of periphyton and macroinvertebrates to disturbance by flash floods in a desert stream. *Journal of the North American Benthological Society*, **8**, 293-307.

Haefner, J.D. and Wallace, J.B. (1981) Shifts in aquatic insect populations in a first-order southern Appalachian stream following a decade of old field succession. *Can. J. Fish. aquat. Sci*. **38**, 353-359.

Harker, J.E. (1952) A study of the life cycles and growth rates of four species of mayflies. *Proc. R. Ent. Soc. Lond.* (A), **27**, 77-85.

Hart, B.T., Maher, W. and Lawrence, I. (1999) New generation water quality guidelines for ecosystem protection. *Freshwater Biology*, **41**, 347-359.

Harvey, C.J., Peterson, B.J., Bowen, W.B., Hershey, A.E., Miller, M.C., Deegan, L.A. and Finlay, J.C. (1998) Biological responses to fertilization of Oksrukuyik Creek, a tundra stream. *Journal of the North American Benthological Society*, **17**, 190-209.

Hawkins, C.P. (1985) Food habits of species of *Ephemerella* mayflies (Ephemeroptera, Insecta) in streams of Oregon. *Am. Midl. Nat.* **113**, 343-352.

Hawkins, C., Murphy, M and Anderson, N. (1982) Effects of canopy, substrate composition and gradient on the structure of macroinvertebrate communities in Cascade range streams of Oregon. *Ecology*, **63**, 1840-1856.

Heathwaite, A.L., Johnes, P.J. and Peters, N.E. (1996) Trends in nutrients. *Hydrological Processes*, **10**, 263-293.

Hefti, D., Tomka, I. and Zurwerra, A. (1989) Revision of morphological and biochemical characters of the European species of the *Ecdyonurus helveticus* – group (Ephemeroptera, Heptageniidae). *Bull. Soc. Entomol. Sussie*. **62**, 329-344.

Hellawell, J.M. (1978) Biological Surveillance of Rivers: A Biological Monitoring Handbook. Water Research Centre, Medmenham and Stevenage.

Hellawell, J.M. (1986) Biological Indicators of Freshwater Pollution and Environmental Management. Applied Science Publishers, London

Hering, D., Moog, O., Sandin, L. and Verdonschot, P.F.M. (2004) Overview and application of the AQEM assessment system. *Hydrobiologia*, **516**, 1-20.

Hildrew, A.G. (1992) Food webs and species interactions. *The Rivers Handbook: Hydrological and Ecological Principles*, Vol. I (Eds P. Calow and G.E. Petts), pp.309-329. Blackwell Scientific Publications, London.

Hill, W.R (1996) Effects of light. *Algal Ecology*: *Freshwater Benthic Ecosystems*. *Aquatic Ecology Series* (eds. J.R. Stevenson, M.J. Bothwell and R.L. Lowe), pp. 121-144. Academic Press, Inc., San Diego.

Hill, W.R. and Knight, A.W. (1987) Experimental analysis of the grazing interaction between a mayfly and stream algae. *Ecology*, **68**, 1955-1965.

Hill, W.R., Boston, H.L. and Steinman. (1992). Grazers and nutrients simultaneously limit lotic primary productivity. *Can. J. Fish. Aquat. Sci.* **49**, 504-512.

Hill, W.R., Ryon, M.G. and Schilling, E.M. (1995) Light limitation in a stream ecosystem: responses by primary producers and consumers. *Ecology*, **76**, 1297-1309.

Hilsenhoff, W.L. (1987) An improved biotic index of organic stream pollution. *Great Lakes Entomol*. **20**, 31-39.

Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbial.* **33**, 1225-1228.

Horkan, J.P.K. (1980) *Interim report on eutrophication and related studies of the Thurles area of the River Suir May-July 1978*. Water Resources Division, Dublin. 213pp

Horner, R.R. and Welch, E.B. (1981) Stream periphyton development in relation to current velocity and nutrients. *Can. J. Fish. Aquat. Sci*. **38**, 449-457.

Horner, R.R., Welch, E.B. and Veenstra, R.B. (1983) Development of nuisance periphytic algae in laboratory streams in relation to enrichment and velocity. In *Periphyton of Freshwater Ecosystems* (Edited by Wetzel. R.G.) pp.121-134. Junk, The Hague.

Hughes, R.M. (1995) Defining acceptable biological status by comparing with reference conditions. Pages 31-47 *in* W.S. Davis and T.S. Simon (editors)> Biological assessment and criteria. Lewis Publishers, Boca Raton, Florida.

Humpesch, U.H. (1980) Effect of temperature on the hatching time of eggs of five *Ecdyonurus* spp. (Ephemeroptera) from Austrian streams and English streams, rivers and lakes. *J. Anim. Ecol*., **49**, 317-333.

Humpesch, U.H. (1981) Effect of temperature on larval growth of *Ecdyonurus dispar* (Ephemeroptera: Heptageniidae) from two English lakes. *Freshwater Biology*, **11**, 441-457.

Hunt, B.P. (1953) The life history and economic importance of a burrowing mayfly, *Hexagenia Limbata* in southern Michigan lakes. *Bulletin of the Institute for Fisheries Research, University of Michigan*, **4**, 1-151.

Hunter, R.D. (1980) Effects of grazing on the quantity and quality of freshwater aufwuchs. *Hydrobiologia*, **69**, 251-259.

Hynes, H.B.N. (1956) Effect of drought on the fauna of a small mountain stream in Wales. *Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie*, **13**, 826-833.

Hynes, H.B.N. (1960) The Biology of Polluted Waters. Liverpool Univ. Press, Liverpool, England.

Hynes, H.B.N. (1961) The invertebrate fauna of a Welsh mountain stream. *Arch. Hydrobiol.*, **57**, 344-388.

Hynes, H.B.N. (1966) The biology of polluted waters. Liverpool University Press.

Hynes, H.B.N. (1968) Further studies on the invertebrate fauna of a Welsh mountain stream. *Archives of Hydrolobiology*, **65**, 360-379.

Hynes, H.B.N. (1970) The ecology of stream insects. *Annual Review of Entomology*, **15**, 25-41.

Hynes, H.B.N. (1970) *The Ecology of Running Waters*, University of Toronto Press, Toronto.

Hynes, H.B.N. and Kaushik, N.K. (1969). The relationship between dissolved nutrient salts and protein production in submerged autumnal leaves. *Verhandlungen Internationale Vereingung fur Theoretische und Angewandte Limnologie*, **17**, 95-103.

Illies, J. (1978) *Limnofauna Europaea*. Gustav Fisher Verlag, Stuttgart, 2nd Ed.

Inversen, T.M. (1974) Ingestion and growth in *Sericostoma personatum* (Trichoptera) in relation to the nitrogen content of ingested leaves. *Oikos*, **25**, 278-282.

Jeffers, J.N.R. (1998) Characterisation of river habitats and prediction of habitat features using ordination techniques. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **8**, 529-540.

Johnson, R.K. (1995) The indicator concept in freshwater biomonitoring. Pages, 11-27, *In*: P.S. Cranston (ed.) Chironomids: from genes in ecosystems. CSIRO, Melbourne.

Johnson, R.K. (1999) Regional representativeness of Swedish reference lakes. *Environmental Management*, **23**, 115-124.

Johnson, R.K. (2001) Defining reference condition and setting class boundaries in ecological monitoring and assessment. Background document for the EU-funded REFCOND project.

Jones, J.R., Smart, M.M. and Burroughs, J.N. (1984) Factors relating to algal biomass in Missouri Ozark streams. *Verhandlungen der internationale Vereinigung fur theoretische und angewandte Limnologie*, **22**, 1867-1875.

Jowett, I.G., Richardson, J., Biggs, B.J.F., Hickey, C.W. and Quinn, J.M. (1991) Microhabitat preferences of benthic invertebrates and the development of generalised *Deleatidium* spp. habitat suitability curves, applied to four New Zealand rivers. *New Zealand J. mar. Freshwat. Res.* **25**, 187-199.

Kalff, J., Kling, H.J. Holmgren, S.H. and Welch, H.E. (1975) Phytoplankton, phytoplankton growth and biomass cycles in an unpolluted and in a polluted polar lake. *Verh. Int. Ver. Limnol*. **19**, 487-495.

Karouna, N. and Fuller, R.L. (1992) Influence of four different grazers on algal communities associated with clay tiles and leaf discs. *Hydrobiologia*, **245**, 53-64.

Karr, J.R. (1991) Biological integrity: a long-neglected aspect of water resource management. *Ecological Applications*, **1**, 66-84.

Kelly, M.G. and Whitton, B.A. (1998). Biological monitoring of eutrophication in rivers. *Hydrobiologia*, **384**, 55-67.

Kelly-Quinn, M. and Bracken, J.J. (2000) The distribution of the Ephemeroptera in Ireland. *Occasional Publication of the Irish* ***Biogeographical Society*** 5**.**

Kelser, D.H. (1981) Periphyton grazing by *Amnicola limnosa*: an enclosure-exclosure experiment. *J. Freshwat. Biol. Ecol.* **1**, 51-59.

Kimmins, D.E. (1972) A revised key to the adults of the British species of Ephemeroptera with notes on their ecology. *Scient. Publs. Freshwat. Biol. Assoc*. no. 15, 75pp.

King, J.J. and Kelly, F.L. (1999) Experimental Drainage Maintenance (EDM) and Urban Flood Relief Programmes (Impact of Works on fish stock and habitat): Survey methodologies. Central Fisheries Board (unpublished report).

Kjeldsen, K. (1996) Regulation of algal biomass in a small lowland stream: field experiments on the role of invertebrate grazing, phosphorus and irradiance. *Freshwater Biology*, **36**, 535-546.

Kjeldsen, K., Iversen, T.M., Thorup, J. and Winding, T. (1998) Benthic algal biomass in an unshaded first-order lowland stream: distribution and regulation. *Hydrobiologia*, **377**, 107-122.

Kling, G., Fry, B. and O'Brien, W.J. (1992) Stable isotopes and trophic structure in arctic lakes. *Ecology*, **73**, 561-566.

Klonowska, M. (1986) The food of some mayfly (Ephemeroptera) nymphs from the streams of the Krakow-Czestochowa Jura (Southern Poland). *Acta Hydrobiol*., **28**, 181-197.

Knoben, R.A.E., Roos, C. and van Oirschot, M.C.M. (1995) Biological assessment methods for watercourses. UN/ECE Task Force on Monitoring and Assessment, Volume 3. RIZA report no. 95.066, Lelystad.

Kohmann, F., Binder, W. and Braun, P. (1993) Leitbilder fur die Erstellung okologisch begrundeter Sanierungskonzepte kleiner Fliebgewasser, Langfassung eines Vortrages, Tagung "Wasser" Berlin (30.4.1993).

Kondratieff, P.F and Simmons, Jr. G.M. (1985) Microbial colonisation of seston and free bacteria in an impounded river. *Hydrobiologia*, **128**, 127-133.

Kostalos, M. and Seymour, R.L. (1976) Role of microbial enriched detritus in the nutrition of *Gammarus minus* (Amphipoda). *Oikos*, **27**, 512-516.

Krebs, C.J. (1989) Ecological methodology. Harper and Row, New York, 654pp.

Lake, P.S. and Barmuta, L.A. (1986) Stream benthic communities: persistent presumptions and current speculations. *Limnology in Australia*. (eds. P. DeDeckker and W.D. Williams), pp263-276. Commonwealth Scientific and Industrial Research Organization, Melbourne.

Lamberti, G.A. and Moore, J.W. (1984) Aquatic insects as primary consumers. *The Ecology of Aquatic Insects* (Eds V.H. Resh and D.M. Rosenberg), pp.164-195, Praeger, New York.

Lamberti, G.A. and Resh, V.H. (1983) Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. *Ecology*, **64**, 1124-1135.

Lamberti, G.A., Ashkenas, L.R., Gregory, S.V. and Steinman, A.D. (1987) Effects of three herbivores on periphyton communities in laboratory streams. *Journal of North American Benthological Society*, **6**, 92-104.

Lamberti, G.A., Gregory, S.V., Ashkenas, L.R., Steinman, A.D., McIntire, C.D. (1989) Productive capacity of periphyton as a determinant of plant animal interactions in streams. *Ecology*, **70**, 1840 1856.

Landa, V. (1962) Die Entwicklung der mitteleuropaischen Ephemeropteren. *International Congress of Entomology*, 11, *Vienna*, **3**, 250-254.

Landa, V. (1968) Developmental cycles of Central European Ephemeroptera and their interrelations. *Acta. entomologica. bohemoslovaca,* **65**, 276-284.

Ledger, M.E. and Hildrew, A.G. (1998) Temporal and spatial variation in the epilithic biofilm of an acid stream. *Freshwater Biology*, **40**, 665.

Ledger, M.E. and Hildrew, A.G. (2000) Herbivory in an acid stream. *Freshwater Biology*, **43**, 545-556.

Lembi, C.A., O'Neal, S.W. and Spencer, D.F. (1988) Algae as weeds: economic impact, ecology, and management alternatives. pp. 455-481. In *Algae and Human Affairs*. (Edited by Lembi, C.A. and Waaland, J.R.). Cambridge, England).

Lenat, D.R. and Barbour, M.T. (1994) Using benthic macroinvertebrate community structure for rapid, cost-effective, water quality monitoring:

rapid bioassessment. *In* Loeb, S.L. and Spacie, A. (eds), Biological Monitoring of Aquatic Systems. Lewis Publishers, Boca Raton FL. 197-215.

Lillehammer, A., Brittain, J.E., Saltveit, S.J. and Nielsen, P.S. (1989) Egg development, nymphal growth and life cycle, strategies in Plecoptera, *Holarctic Ecology*, **12**, 173-86.

Lloyd, D.S., Koenings, J.P. and LaPerriere, J.D. (1987) Effect of turbidity in fresh waters of Alaska. *North. Am. J. Fisheries. Mgmt.*, **7**, 19-33.

Lock, M.A. (1992) Attached microbial communities in rivers. *Aquatic Microbiology: An Ecological Approach* (ed. T. E. Ford), pp.113-139. Blackwell Scientific Publications, Oxford.

Lock, M.A., Wallace, R.R., Costerton, J.W., Ventullo, R.M. and Charlton, S.E. (1984) River epilithon: towards a structural-functional model. *Oikos*, **42**, 10-22.

Lock, M.A., Ford, T.E., Hullar, M.A.J., Kaufman, M., Vestal, J.R., Volk, G.S. and Ventullo, R.M. (1990) Phosphorus limitation in an arctic river biofilm – A whole ecosystem experiment. *Wat. Res.*, **24**, 1545-1549.

Logan, P. (2001) Ecological quality assessment of rivers and integrated catchment management in England and Wales. *J.Limnol*,. **60**, 25-32.

Lohman, K., Jones, J.R. and Baysinger-Daniel, C. (1991) Experimental evidence for nitrogen limitation in a northern Ozark stream. *J. N. Am. Benthol. Soc.* **19**, 14-23.

Lohman, K., Jones, J.R. and Perkins, B.D. (1992) Effects of nutrient enrichment and food frequency on periphyton biomass in northern Ozark streams. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 1198-1205.

Lopez, G.R. and Kofoed, L.H. (1980) Episammic browsing and deposit-feeding in mud snails (Hydrobiidae). *J.Mar.Sci*. **38**, 585-597.

Lowe, R.L. and Hunter, R.D. (1988) Effects of grazing by *Physa integra* on periphyton community structure. *J. North. Am. Benthol. Soc*. **7**, 29-36.

Lucey, J., Bowman, J.J., Clabby, K.J., Cunningham, P., Leane, M., MacCarthaigh, M., McGarrigle, M.L., Toner, P.F. (1999) Water quality in Ireland 1995-1997. Environmental Protection Agency, Wexford.

Ludwig, J.A. and Reynolds, J.F. (1988) Statistical Ecology. John Wiley, New York, 337pp.

Lugthart, G.J. and Wallace, J.B. (1992) Effects of disturbance on benthic functional structure and production in mountain streams. *Journal of the North American Benthological Society*, **11**, 138-164.

Lund, J.W.G. (1969) Phytoplankton, p. 306-330. *In* Eutrophication: Causes, consequences, correctives. Natl. Acad. Sci. Washington, D.C.

Lydy, M.J., Crawford, C.G. and Frey, J.W. (2000) A comparison of selected diversity, similarity and biotic indices for detecting changes in benthic-invertebrate community structure and stream quality. *Arch. Environ. Contam. Toxicol*., **39**, 469-479.

Macan, T.T. (1957) The life histories and migrations of the Ephemeroptera in a stony stream. *Transactions of the Society of British Entomologists*, **12**, 129-156.

Macan, T.T. (1958) Methods of sampling the bottom fauna in stony streams. Communications. *International Association of Theoretical and Applied Limnology*, **8**, 1-21.

Macan, T.T. (1970) *Biological Studies of the English Lakes*. Longman Group Limited, London.

Macan, T.T. (1979) *A key to the nymphs of the British species of Ephemeroptera with notes on their ecology*. Freshwater Biological Association Scientific Publication 20, Ambleside, UK.

Macan, T.T. (1981) Life histories of some species of *Ecdyonurus* (Ephemeroptera) in the River Lune, North-Western England.

Macan, T.T. and Maudsley, R. (1968) The insects of the stony substratum of Windermere. *Transactions of the Society for British Entomology*, **18**, 1-18.

Maciolek, J. A., and Tunzi, M.G. (1968). Microseston dynamics in a simple Sierra Nevada lake-stream system. *Ecology*, **49**, 60-75.

MacKenthun, K.M. (1968) The phosphorus problem. *J. Am. Water. Works. Assoc.* **60**, 1047-1053.

Madden, C.P., Austin, A.D. and Suter, P.J. (1995) Pollution monitoring using chironomid larvae: what is a deformity? Pages 89-100. *In* P.S. Cranston (ed.) Chironomids: from genes to ecosystems. CSIRO, Melbourne.

Maddock, I. (1999) The importance of physical habitat assessment for evaluating river health. *Freshwater Biology*, **41**, 373-391.

Madsen, B.L. (1972). Detritus on stones in small streams. *Memorie Dell'instituto Italiano Di Idrobiologia*, **29**, 385-403.

Maher W., Batley, G.E. and Lawrence I. (1999) Assessing the health of sediment ecosystems: Use of chemical measurements. *Freshwater Biology*, **41**, 361-372.

Manuel, C.Y. and Minshall, G.W. (1978) Limitations on the use of microcosms for predicting algal response to nutrient enrichment in lotic

systems. pp 645-667.Department of Biology, Idaho State University, Pocatello, Idaho.

Margalef, R. (1958) Information theory in ecology. *Entomol. Systematics*, **3**, 36-71.

Marten, M. and Reusch, H. (1992) Anmerkungen zum DIN 'Saprobienindex' (38 410 Teil 2) und Forderung alternativer Verfahren. Natur Landschaft **67**, 544-547.

Martinez, N.D. (1993) Effects of resolution on food web structure. *Oikos*, **66**, 403-412.

Mason, C.F. (1991) *Biology of Freshwater Polluiton*. Longman Scientific and Technical, Essex.

Maurice, C.G., Lowe, R.L., Burton, T.M. and Stanford, R.M. (1987) Biomass and compositional changes in the periphytic community of an artificial stream in response to lowered pH. *Water, Air and Soil Pollution*. **33**, 165-177.

McAuliffe, J.R. (1984) Resource depression by a stream herbivore: effects of distribution and abundance of other grazers. *Oikos,* **42**, 327-346.

McCormick, P.V. and Stevenson, R.J. (1991) Grazer control of nutrient availability in the periphyton. *Oecologia*, **86**, 287-291.

McGarrigle, M.L. (1983) *Development of Water Quality Management Methods Based on systems Analysis (River Suir)*. EEC Contract 6612/4, Department of the Environment and An Foras Forbartha, Dublin. 42pp.

McGarrigle, M.L. (1984a) *Development of a water quality model for Irish Rivers. Preliminary studies on the River Suir*. WR/R16, Water Resource Division, An Foras Forbartha, Dublin.

McGarrigle, M.L. (1984b) Experiments with the River Model. *Ir. J. Environ. Sci.* **3**, 40-47.

McGarrigle, M.L. (1998) Impact of eutrophication on Irish river water quality. In: *Eutrophication in Irish Waters* (Ed. J.G. Wilson) pp 1-9 Royal Irish Academy.

McGarrigle, M.L. (2001) *In* Went Memorial Lecture 2001: Eutrophication of Irish Waters – From Science to legislation and management.

McGarrigle, M.L. and Lucey, J. (1989) Waste assimilative capacity survey of the Athboy River. Report to Meath County Council. Environmental Research Unit.

McGarrigle, M.L. and Lucey, J. (1990) Assimilative capacity survey of the Athboy River. Report to Meath County Council. Environmental Research Unit.

McGarrigle, M.L., Horkan, J.P.K. and Lucey, J. (1987) Water quality survey of the Magherarney river. Report to Monaghan County Council, WR/C132, Water Resources Division, An Foras Forbartha, Dublin.

McGarrigle, M.L., O'Grady, M., Champ, W.S.T. and McCarthy, T.K.M. (1998) The Moy Catchment pp212-236. In Moriarty, C. (Ed.) *Studies of Irish rivers and Lakes*. Marine Institute, Dublin. 279pp. ISBN0529 191 29.

McGarrigle, M.L., Bowman, J.J., Clabby, K.J., Lucey, J., Cunningham, P., MacCarthaigh, M., Keegan, M. Cantrell, B., Lehane, M., Clenaghan, C. and Toner, P.F. (2002) Water Quality in Ireland 1998-2000. Envionmental Protection Agency.

Merritt, R.W. and Cummins, K.W. (1984) An introduction to the aquatic insects of North America, Second Edition. Kendall/Hunt Publishing Company.

Merritt, R.W., Cummins, K.W. and Resh, V.H. (1996) Design of Aquatic Insect Studies: collecting, sampling and rearing procedures, pp. 12 –28. In: R.W. Merritt and K.W. Cummins (eds.) An introduction to the Aquatic Insects of North America. 3rd edn. Kendall Hunt Publishing Co.Iowa.

Metcalfe, J.L. (1989) Biological water quality assessment of running water based on macoinvertebrate communities: history and present status in Europe. *Environ. Pollut.*, **60**, 101-139.

Meulemans, J.T. (1987) A method for measuring selective light attenuation within a periphytic community. *Arch. Hydrobiol.* **109**, 139-145.

Meyer, J.L., McDowell, W.H., Bott, T.L., Elwood, J.W., Ishizaki, C., Melack, J.M. Peckarsky, B.L., Peterson, B.J. and Rublee, P.A. (1988) Elemental dynamics in streams. *Journal of the North American Benthological Society*, **7**, 410-432.

Mihuc, T.B. (1997) The functional trophic role of lotic primary consumers: generalist versus specialists strategies. *Freshwater Biology*, **37**, 455-462.

Mihuc, T.B. and Minshall, G.W. (1995) Trophic generalists vs. trophic specialists: implications for food web dynamics in post-fire streams. *Ecology*, **76**, 2361-2372.

Mihuc, T.B. and Toetz, D. (1994) Determination of diets of alpine aquatic insects using stable isotopes and gut analysis. American Midland Naturalist, **131**, 146-155.

Minshall, G.W. (1967) Role of allochthonous detritus in the trophic structure of a woodland springbrook community. *Ecology*, **48**, 139-149.

Minshall, G.W. (1984) Aquatic insect-substratum relationships. *In*: The Ecology of Aquatic Insects (eds. V.H. Resh and D.M. Rosenberg), pp. 258-400. Praeger Publishers, pp, New York.

Momot, W.T. and Jones, P.D. (1977) The relationship between biomass, growth rate and annual production in the crayfish. *Oronectes virilis Freshwater Crayfish*, **3**, 3-31. (3rd International Symposium on Freshwater Crayfish, Finland)

Moog, O. (1993) Makrozoobenthos als Indikator bei okologischen Fragestellungen, Landschaftswasserbau 15, 103-143, Wien.

Moog, O. (1994) Okologische Funktionsfahigkeit des aquatiscehn Lebensraumes, Wiener Mitt. 120, 15-59.

Moog, O. (1995) Fauna Aquatica Austriaca. Version 1995. Wasserwirtschafts-kataster, Bundesministerium fur Land-und Forstwirtschaft, Wien. A comprehensive species inventory of Austrian aquatic organisms with ecological notes.

Moog, O., Bauernfeind, E. and Weichselbaumer, P. (1997) The use of Ephemeroptera as saprobic indicators in Austria. *In*: Landolt, P. and M, Sartori (eds), Ephemeroptera and Plecoptera: Biology-Ecology-Systematics (= Proc. 8th Int. Conf. Ephemeroptera, Lausanne, 1995). Fribourg: 254-260.

Moriarty, C. (1990) *Fish Kills in Ireland in 1990*. Fishery Leaflet 149. Dublin, Department of the Marine, Roinn na Mara.

Moss, B., Stephen, D., Alvarez, C., Becares, E., Van de Bund, W., Van Donk, E., De Eyto, E., Feldmann, T., Fernandez-Alaez, C., Fernandez-Alaez, M., Franken (2001) The determination of ecological quality in shallow lakes – a tested system (ECOFRAME) for implementation of the European Water Framework Directive.1-55.

Muhar, S. and Jungwirth, M. (1998) Habitat integrity of running waters – assessment criteria and their biological relevance. *Hydrobiologia*, **386**, 195-202.

Mullholland, P.J., Steinman, A.D., Palumbo, A.V., Elwood, J.W. and Kirschtel, D.B. (1991) Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. *Ecology*, **72**, 966-982.

Mundie, J.H., Simpson, K.S. and Perrin, C.J. (1991) Responses of stream periphyton and benthic insects to increases in dissolved inorganic phosphorus in a mesocosm. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 2061-2072.

Newcombe, C.P. and MacDonald, D.D (1991) Effects of suspended sediments on aquatic ecosystems. *North. Am. J. Fisheries. Mgmt*., **11**, 72-82.

Newell, R.L. and Minshall, G.W. (1978) Like history of a multivoltine mayfly. *Tricorythodes minutus*: an example of the effect of temperature on the life cycle. *Annals of the Entomological Society of America*, **71**, 876-881.

Ní Chatháin, B., (2002) Investigation of the ecology of benthic algae in the river Deel, South-West Ireland, Ph.D. thesis, University of Limerick, Ireland, 273 pp.

Ní Chatháin B., Harrington T. J., McCarthy J., Kelly-Quinn, M., Bradley, C., and Baars, J.R. (2004) Preliminary results from the investigation of benthic diatoms from potential reference river sites in Ireland. The International Journal of Oceanological and Hydrobiological Studies. In press.

Nixon, S.C., Mainstone, C.P., Milne, I., Inversen, T.M., Kristensen, P., Jeppesen, E. Friberg, N., Jensen, A. and Pedersen, F. (1996) The harmonised monitoring and classification of ecological quality of surface waters in the European Union. Draft Final Report. No. CO., 4096.

Norris, R.H. and Norris, K.R. (1995) The need for biological assessment of water quality: Australian perspective. *Aust. J. Ecol*., **20**, 1-6.

Norris, R.H. and Thoms, M.C. (1999) What is river health? *Freshwater Biology*, **41**, 197-209.

Norris, R.H., McElravy, E.P. and Resh, V.H. (1992) The sampling problem. *The Rivers Handbook, Hydrological and Ecological Principles* (eds P. Callow and G.E. Petts), pp.282-306. Blackwell Scientific Publications, Oxford.

OECD (Organisation for the Economic Cooperation and Development) (1982) Eutrophication of waters. Monitoring assessment and control. Final report. OECD Coopertive Programme on monitoring of Inland Waters (Eutrophication Control), Environmental Directorate, OECD, Paris, 154 p.

Odum, H.T. (1956) Primary production in flowing waters. *Limnol. Oceanogr*., **1**, 102-117.

Ofenbock, T., Moog, O., Gerritsen, J. and Barbour, M. (2004) A stressor specific multimetric approach for monitoring running waters in Austria using benthic macroinvertebrates. *Hydrobiologia*, **516**, 251-268.

Otto, C. (1974) Growth and energetics in a larval population of *Potamophylax cingulatus* (Steph.) (Trichoptera) in a South Swedish stream. *J. Animl. Ecol*. **43**, 339-361.

Pahl-Wostl, C. (1993) Food webs and ecological networks across temporal and spatial scales. *Oikos*, **66**, 415-432.

Paine, R.T. (1988) Food webs: road maps of interactions or grist for theoretical development. *Ecology*, **69**, 1648-1654.

Pan, Y. and Lowe, R.L. (1994) Independent and interactive effects of nutrients and grazers on benthic algal community structure. *Hydrobiologia*, **291**, 201-209.

Pan, Y., Stevenson, R.J., Hill, B.H., Herlihy, A.T. and Collins, G.B. (1996) Using diatoms as indicators of ecological conditions in lotic systems: a regional assessment. *J. N. Am. Benth. Soc*., **15**, 481-495.

Pantle, R. and Buck, H. (1955) Die biologische Uberwachung der Gewasser und die Darstellung der Ergebnisse. Bes. Mitt. Dt. Gewasserkundl. Jb. **12**, 135-143.

Parsons, M., and Norris, R.H., (1996) The effects of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology*. **36**, 419-434.

Patrick, R. (1948) Factors affecting the distribution of diatoms. *Botanical Review*, **14**, 473-524.

Paul, B.J. and Duthie, H.C. (1989) Nutrient cycling in the epilithon of running waters. *Can. J. Bot*. **67**, 2302-2309.

Peeters, E.T.H.M., Gardeniers, J.J.P. and Tolkamp, H.T. (1994) New methods to assess the ecological status of surface waters in the Netherlands. Part 1: Running waters. *Verh. Int. Ver. Limnol*., 1914-1916.

Pentecost, A. (1984) Introduction to Freshwater Algae. Richmond. Richmond Publishing Co.

Perrin, C.J., Bothwell, M.L. and Slaney, P.A. (1987) Experimental enrichment of a coastal stream in British Columbia: effects of organic and inorganic addition on atrophic periphyton production. *Can. J. Fish. Aquat. Sci*. **44**, 1247-1256.

Peter, R.E. (1979) The brain and feeding behaviour. *In*: Fish Physiology (Eds. W. S. Hoar, D.J. Randall and J.R. Brett), Vol. 8. pp.121-159. Academic Press, London.

Peterson, C. G. and Stevenson, R. J. (1992) Resistance and resilience of lotic algal communities: importance of disturbance timing and current. *Ecology*, **73**, 1445–1461.

Peterson, B.J., Bahr, M. and Kling, G.W. (1997) A tracer investigation of nitrogen cycling in a pristine tundra river. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2361-2367.

Peterson, B.J., Hobbie, J.E., Hershey, A.E., Loch, M.A., Ford, T.E., Vestal, J.R., McKinley, V.L., Hullar, M.A.J., Miller, M.C., Ventullo, R.M. and Volk, G.S. (1985) Transformation of a tundra river from heterotrophy to autotrophy by addition of phosphorus. *Science*, **229**, 1383-1386.

Plafkin, J.L., Barbour, M.T., Porter, K.D., Gross, S.K. and Hughes, R.M. (1989) *Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates & Fish*. US Environmental Protection Agency Assessment and Watershed Protection Division, Washington, D.C. EPA/440/4-89/001.

Polis, G.A. (1994). Food webs, trophic cascades and community structure. *Australian Journal of Ecology*, **19**, 121-136.

Porter, K.G. and Feig, Y.S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* **25**, 943-948.

Power, M.E., Stout, R.J., Cushing, C.E., Harper, P.P., Hauer, F.R., Matthews, W.J., Moyle, P.B., Statzner, B and Wais De Badgen, I.R. (1988). Biotic and abiotic controls in river and stream communities. *J.N. Am. Benthol. Soc.*, 7, **4**, 456-479.

Pringle, C.M. and Bowers, J. (1984) An in situ substratum fertilization technique: Diatom colonization on nutrient-enriched sand substrata. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 1247-1251.

Quinn, J.M. and Hickey, C.W. (1990) Magnitude of effects of substrate particle size, recent flooding and catchment development on benthic invertebrates in 88 New Zealand rivers. *New Zealand J. Mar. Freshwat. Res.* **24**, 411-427.

Rapport, D.J. (1991) Myths in the foundations of economics and ecology. *Biological journal of the Linnaean Society*, **44**, 185-202.

Rassoulzadegan, F. and Sheldon, R.W. (1986) Predator-prey interactions of nanozooplankton and bacteria in an oligotrophic marine environment. *Limnol. Oceanogr.* **31**, 1010-1021.

Rau, G.H. (1980) Carbon-13/Carbon-12 variation in subalpine lake aquatic insects: food source implications. *Can. J. Fish. Aquatic. Sci.*, **37**, 742-746.

Raven, P. J., Boon, P. J., Dawson, F. H., and Ferguson, A. J. D. (1998) Towards an integrated approach to classifying and evaluating rivers in the UK. Aquatic Conservation-Marine and Freshwater Ecosystems, **8**, 383-393.

Raven, P.J., Fox, P.J.A., Everard, M., Holmes, N.T.H. and Dawson, F.H. (1997) 'River Habitat Survey: a new system for classifying rivers according to their habitat quality', in Boon, PJ and Howell, D.L.(Eds), *Freshwater Quality: Defining the indefinable?*, The Stationery Office, Edinburgh, 215 – 234.

Rawlinson, D. (1939) Studies on the life-history and breeding of *Ecdyonurus venosus* (Ephemeroptera). *Proceedings of the Zoological Society of London, Series B.* **109**, 377-450.

Reckhow, K.H. and Chapra, S.C. (1983) *Engineering Approaches for Lake Management*, Vol.1, p.340. Ann Arbor Science, Butterworth Publishing Co., Woburn, MASS.

Redfield, A.C. (1958) The boloigical control of chemical factors in the environment. *Am. Sci.* **46**, 205-221.

REFCOND (2003) Final version 7.0. Guidance on establishing reference conditions and ecological class status boundaries for inland surface waters. CIS Working group 2.3. p 1- 93.

Resh, V.H. and Unzicker, J.D. (1975) Water quality monitoring and aquatic organisms: the importance of species identification. *J. Wat. Pollut. Cont. Fed.*, **47**, 9-19.

Resh, V.H., Norris, R.H. and Barbour, M.T. (1995) Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. *Austral. J. Ecol.*, **20**, 108-121.

Reusch, H. (1985) Limnofaunistische Untersuchungen uber die Eintags-, Stein-und Kocherfliegen (Ephemeroptera, Plecoptera, Trichoptera) der Ortze (Niedersachsisches Tiefland). Jb. Naturw. Verein Fstm. *Lutenburg*, **37**, 117-139.

Reusch, H. (1995) Planungsrelevante Aufbereitung und Bewertung faunistisch-okolgischer Daten vom Makrozoobenthon in Fliebgewassern. Sch.-R. *Landschaftspfl. Natursch*, **43**, 31-42.

Reynoldson, T.B., Bailey, R.C., Day, K.E. and Norris, R.H. (1995) Biological guidelines for the freshwater sediment based on **Be**nthic **A**ssessment of **S**edimen**T** (the **BEAST**) using a multivariate approach for predicting biological state. *Australian Journal of Ecology*, **20**, 198-219.

Reynoldson, T.B., Norris, R.H., Resh, V.H., Day, K.E. and Rosenberg, D.M. (1997) The reference condition: a comparison of multimetric and multivariate approaches to assess water-quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society*, **16**, 833-852.

Rhee, G.Y. (1973) A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. *J. Phycol*. **9**, 495-506.

Rhee, G Y. (1978) Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. *Limnology and Oceanography*, **23**, 10-25.

Richards, C. and Minshall, G.W. (1988) The influence of periphyton abundance on *Baetis bicaudatus* distribution and colonisation in a small stream. *Journal of the North American Benthological Society*, **7**, 77-86.

Richards, C., Host, G.E. and Arthur, J.W. (1993) Identification of predominant environmental factors structuring stream macroinvertebrate communities within a large agricultural catchment. *Freshwater Biology*, **29**, 285-294.

Richards, C., Johnson, L.B. and Host, G.E. (1996) Landscape-scale influences on stream habitats and biota. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 295-311.

Ricker, W.E. (1979) Growth rates and models. *In*: Fish Physiology (Eds. W. S. Hoar, D.J. Randall and J.R. Brett), Vol.8 pp. 677-743. Academic Press, London.

Roche, W. (1996) River Crana Habitat Monitoring Programme: Substrate Analysis Study No.1- 1994 Results. Central Fisheries Board, Dublin.

Rosenberg, D.M. and Resh, V.H. (1993) Introduction to Freshwater Biomonitoring and Benthic Macroinvertebrates. In Freshwater Biomonitoring and Benthic Macroinvertebrates (editors D.M. Rosenberg and V.H. Resh). Chapman and Hall, New York.

Rosenberg, D.M. Danks, H.V. and Lehmkuhl, D.M. (1986) Importance of insects in environmental impact assessment. *Envir. Manage*. **10**, 773-783.

Rotschein, J. (1982) Nove metody stanoveni indexu saprobity (New methods of saprobic index assessment) *Vodni hospodarstvi B*, **6**, 159-162.

Rott, E., Duthie, H.C., and Pipp, E., (1998) Monitoring organic pollution and eutrophication in the Grand River, Ontario, by means of diatoms. *Can. J. Fish. Aquat. Sci.,* **55**, 1443-1453.

Rounick, J.S. and Winterbourn, M.J. (1983) Formation structure and utilisation of stone surface organic layers in two New Zealand streams. *Freshwater Biology*, **13**, 57-72.

Rounick, J.S., Winterbourn, M.J. and Lyon, G.L. (1982) Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. *Oikos*, **39**, 191-198.

Ryan, P.A. (1991) Environmental effects of sediment on New Zealand streams: a review. *New Zealand J. mar. Freshwat. Res.* **25**, 207-221.

Ryder, G.I. (1989) Experimental studies of the effects of fine sediments on lotic invertebrates. Ph.D. thesis, University of Otago, Dunedin, New Zealand: 216 pp.

Ryding, S.O. and Rast, W. (1989) *The Control of Eutrophication of Lakes and Reservoirs.* Vol. 1, p. 314. UNESCO, Paris and The Parthenon Publishing Group, United Kingdom.

Ryther, J. and Dunstan, W.M. (1971) Nitrogen, phosphorus and eutrophication in the coastal and marine environment. *Science*, **171**, 1008-1013.

Sabater, S., Sabater, F. and Armengol, J. (1988) Relationships between diatom assemblages and physico-chemical variables in the River Ter (NE Spain), Int. Revue gesamt. *Hydrobiology*, **73**, 171-179.

Sangpradub, N., Giller, P. and O'Connor, J (1999) Life history patterns of stream-dwelling caddis. *Arch. Hydrobiol.* **146**, 471-493.

Sas, H. (1989) *Lake Restoration by reduction of Nutrient Loading: Expectations, Experiences, Extrapolations.* Academia Verlag, Richarz.

Scarsbrook, M.R. and Townsend, C.R. (1993) Stream community structure in relation to spatial and temporal variation: A habitat templet study of two contrasting New Zealand streams. *Freshwat. Biol.*, **29**, 395-410.

Schindler, D.W. (1975) Whole-lake eutrophication experiments with phosphorus, nitrogen and carbon. *Verh. Int. Ver. Limnol*. **19**, 3221-3231.

Schindler, D.W. (1985) The coupling of elemental cycles by organisms: evidence from whole-lake chemical perturbations. In *Chemical Processes in Lakes* (Edited by Stumm W.). Wiley-Interscience, New York.

Schoener, T.W. (1974) Resource partitioning in ecological communities. *Science*, **185**, 27-38.

Schoenly, K., Beaver, R.A. and Heumier, T.A. (1991). On the trophic relations of insects: a food-web based approach. *American Naturalist*, **137**, 597-638.

Schweder, H. (1992) Neue Indices fur die Bewertung des okologischen Zustandes von Fliebgewassern, abgeleitet aus der Makroinvertebraten-Ernahrungstypologie, Limnologie aktuell Bd. 3. 353-377, G. Fischer Vlg. Stuttgart-New York.

Schwenneker, B.W. and Hellenthal, R.A. (1984) Sampling considerations in using stream insects for monitoring water quality. *Environmental Entomology*, **13**, 741-750.

Scrimgeour, G.J., Culp, J.M., Bothwell, M.L., Wrona, F.L. and McKee, M.H. (1991) Mechanisms of algal patch depletion: importance of consumptive and non-consumptive losses in mayfly diatom systems. *Oecologia*, **85**, 343-348.

Shapas, T.J. and Hilsenhoff, W.L. (1976) Feeding habits of Wisconsin's predominant lotic Plecoptera, Ephemeroptera and Trichoptera. Great Lakes *Entomologist*, **9**, 175-178.

Sharpley, A., Foy, B., and Withers, P. (2000) Practical and innovative measures for the control of agricultural phosphorus losses to water: An overview. *J. Environ. Qual*, **29**, 1-9.

Simpson, J. and Norris, R.H. (2000) Biological assessment of water quality: development of AUSRIVAS models and outputs. *In*: Assessing the Biological Quality of Freshwaters: *RIVPAC and Similar Techniques* (eds J.F.Wright, Sutcliffe, D.W. and Furse, M.T., PP. 125-142. Freshwater Biological Association and Environment Agency, U.K.

Skriver, J., Friberg, N. and Kirkegaard, J. (2001) Biological assessment of running waters in Denmark: introduction of the Danish Stream Fauna Index

(DSFI). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **27**, 1822-1830.

Sladecek, V. (1973) System of water quality from the biological point of view. *Arch. Hydrobiol. Beih. Ergenbnisse. Limnol.*, **7**, 1-218.

Sladecek, V., Hawkes, H.A., Alabaster, J.S., Daubner, I., Notlich, I., Solbe, J.F.D. and Uhlamann, D. (1982). Biological examination. In Examination of Water for Pollution Control. A reference handbook. Vol. 3. Biological, Bacteriological and Virological Examination. (editors M.J Suess). Pergamon Press, Oxford.

Smith, V.H. (1979) Nutrient dependence of primary productivity in lakes. *Limnology and Oceanography*, **24**, 1051-1064.

Smith, V.H. (1982) The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. *Limnol. Oceanogr.* **27**, 1101-1112.

Smock, L.A. (1996) Macroinvertebrate movements: drift, colonisation and emergence, pp. 371-390. *In*: F.R.Hauer and G.A. Lamberti (eds.). Methods in Stream Ecology. Academic Press, San Diego.

Smock, L.A. and Roeding, C.E. (1986) The trophic basis of the macroinvertebrate community of a southeastern U.S.A. blackwater stream. *Holartic Ecology*, **9**, 165-174.

Southwood, T.R.E. (1977) Habitat, the templet for ecological strategies? *Journal of Animal Ecology*, **46**, 337-365.

Southwood, T.R.E. (1988) Tactics, strategies and templets. *Oikos*, **52**, 3-18.

Sowa, R. (1975) Ecology and biogeography of mayflies (Ephemeroptera) of running waters in the polish part of the carpathians. 2. Life cycles. *Acta. Hydrobiol.*, **17**, 319-359.

Sowa, R. (1979) Le developpement des Ephemeropteres de la riviere Dunajec aux environs de Piening. *Proceedings of the 2nd International Conference on Ephemeroptera*, 125-131.

Standard Methods (2001) For the examination of water and wastewater edition. 17th edition.

Standard Methods (2002) For the examination of Water and Wastewater, 20th edition.

Stanford, J.A. and Ward, J.V. (1983) Insect species diversity as a function of environmental variability and disturbance in stream systems. *Stream Ecology, Application and Testing of General Ecological Theory* (eds J.R. Barnes and G.W. Minshall), pp. 265-278. Plenum Press, New York.

Steinman, A.D. (1996) Effects of grazers on freshwater benthic algae. *Algal Ecology Series* (eds J. R. Stevenson, M. I. Bothwell and R.L. Lowe), pp.341-366. Academic Press, Inc., San Diego.

Steinman, A.D., Mulholland, P.J. and Kirschtel, D.B. (1991) Interactive effects of nutrient reduction and herbivory on biomass, taxonomic structure and P uptake in lotic periphyton communities. *Can. J. Fish. Aquat. Sci.*, **48**, 1951-1959.

Steinman, A.D., McIntire, C.D., Gregory, S.D. and Lamberti, G.A (1989) Effects of irradiance and grazing on lotic algal assemblages. *Journal of Phycology*, **25**, 478-485.

Steinman, A.D., McIntire, C.D., Gregory, S.V. and Lamberti, G.A. and Ashkenas, L.R. (1987) Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in lab streams. *J.* North. Am. *Benthol. Soc*. **6**, 175-188.

Stevenson, R.J. (1996) An introduction to algal ecology in freshwater benthic habitats. *Algal Ecology: Freshwater Benthic Ecosystems, Aquatic Ecology Series* (eds. J. R. Stevenson. M. I. Bothwell and R. L. Lowe), pp.3-30. Academic Press Inc., San Diego, CA.

Stockner, J.G. and Evans, D. (1974) Field and laboratory studies on the effects of nitrogen, phosphorus and NTA on attached algal communities. *Fish. Mar. Serv. Res. Dev. Tech. Rep*., **416**, 109p.

Stockner, J.G. and Shortreed, K.R.S. (1976) Autotrophic production in Carnation Creek, a coastal rainforest stream on Vancouver island, British Columbia. *J. Fish. Res. Board Can.* **33**, 1553-1563.

Stockner, J.G. and Shortreed, K.R.S. (1978) Enhancement of autotrophic production by nutrient addition in a coastal stream on Vancouver Island. *J. Fish. Res. Board Can*., **35**, 28-34.

Stout, B.M., Benfield, E.M. and Webster, J.R. (1993) Effects of forest disturbance on shredder production in southern Appalachian streams. *Freshwater Biology*, **29**, 59-69.

Struder-Kypke *et al*. in prep *In* Guide to UK Coastal Planktonic Ciliates © (2001) DJS Montagnes, University of Liverpool.

Studemann, D. Landolt, P., Sartori, M., Hefti, D. and Tomka, I. (1992) Soc. Entomol Suisse (ed.), Ephemeroptera. Insecta helvetica, Fauna 9. Fribourg, 1-174.

Swanson, F.J. (1980) Geomorphology and ecosystems. *In*: Forests: Fresh Perspectives from Ecosystem Analysis. Proceedings of the 40th Annual Biology Colluquium (ed. R.H. Waring). Oregon State University Press, Oregon.

Sweeney, B.W. (1984) Factors influencing life-history patterns of aquatic insects. *In*: The Ecology of Aquatic Insects (eds V.H. Resh and D.M. Rosenberg), pp.56-100. Praeger Publishers, New York.

Sweeney, B.W. and Vannote, R.L. (1978) Size variation and the distribution of hemimetabolous aquatic insects: two thermal equilibrium hypotheses. *Science*, **200**, 444-446.

Sweeney, B.W. and Vannote, R.L. (1981) *Ephemerella* mayflies of Clay Creek: bioenergetic and ecological relationships among six coexisting species. Ecology 62: 1353-1369.

Sweeney, B.W. and Vannote, R.L., and Dodds, P.J. (1986a) The relative importance of temperature and diet to larval development and adult size in the winter stonefly. *Soyedina carolinensis*. Plecoptera: Nemouridae) *Freshwater Biol*. **16**, 39-48.

Sweeney, B.W. and Vannote, R.L., and Dodds, P.J. (1986b) Effects of temperature and food quality on growth and development of a mayfly, *Leptophlebia intermedia Can. J. Fish. Aquat. Sci*. **43**, 12-18.

Tett, P., Gallegos, C., Kelly, M.G., Hornberger, G.M. and Cosby, B.J. (1978). Relationship among substrate, flow and benthic pigment density in Mecums Rivers, Virginia. *Limnol. Oceanogr*. **23**, 785-797.

Thibault, M. (1971) Le developpment des Ephemeropteres semivoltines et univoltins d'un ruisseau de Pays Basque Francais. Doctorat these. Universite de Paris.

Tilman, D. and Kilman, S.S. (1976) Phosphate and silicate growth and uptake kinetics of the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* in batch and semicontinuous culture. *J. Phycol*. **12**, 375-383.

Tilzer, M. (1968) Zur Okologie und Besiedlung des hochalpinen hyporheischen Interstitials im Arlberggebiet (Osterreich). *Archiv fur Hydrobiologie*, **65**, 253-308.

Tomas, X. and Sabater, S., (1985) The diatom associations in the Llobregat river and their relations to water quality, *Verh. Int. Verein. Limnol*., **22**, 2348-2352.

Townsend, C.R. (1989) The patch dynamics concept of stream community ecology. *Journal of the North Americam Benthological Society*, **8**, 36-50.

Townsend, C.R. and Hildrew, A.G. (1994) Species traits in relation to a habitat template for river systems. *Freshwater Biology*, **31**, 265-276.

Trost, L.M.W. and Berner, L. (1963) The biology of *Callibaetis floridanus* Banks (Ephemeroptera: Baetidae). *Florida Entomologist*, **46**, 285-299.

Twist, H. Edwards, A.C. and Codd, G.A. (1998) Algal growth responses to waters of contrasting tributaries of the River Dee, north-east Scotland. *Water Research,* **32**, 2471-2479.

Van Nieuwenhuyse, E.E. and Jones, J.R. (1996) Phosphorus chlorophyll relationship in temperate streams and its variation with stream catchment area. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 99-105.

Vannote, R.L. and Sweeney, B.W. (1985) Larval feeding and growth rate of the stream cranefly *Tipula abdominalis* in gradients of temperature and nutrition. Proc. *Acad. Nat. Sci. Philadelphia*, **137**, 119-128.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R. and Cushing, C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130-137.

Vollenweider, R.A. (1968) Scientific fundamentals of eutrophication of lakes and flowing waters, with particular reference to nitrogen and phosphorus as factors in eutrophication. Technical report DAS/CSI/68.27, Environmental Directorate, Organization for Economic Cooperation and Development (OECD), Paris, 154 p.

Walker, E.D., Olds, E.J. and Merritt, R.W. (1988) Gut content analysis of Mosquito larvae (Diptera: Culicidae). Using DAPI stain and epifluorescence microscopy. *Entomology Society Of America*. **25**, 551-554.

Wallace, J.B. and Anderson, N.H. (1996) Habitat, life history and behavioural adaptations of aquatic insects. In *An introduction to the aquatic insects of North America* (ed. R.W. Merritt and K.W. Cummins),pp. 41-73. Kendall/Hunt, Dubuque, IA.

Wallace, J.B., and Gurtz, M.E. (1986). Response of *Baetis* mayflies (Ephemeroptera) to catchment logging. *Am. Midl. Nat*., **115**, 25-41.

Wallace, J.B., and Webster, J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual Review of Entomology*, **41**, 115-139.

Wallace, J.B., Grubaugh, J.W. and Whiles, M.R. (1996) Biotic indices and stream ecosystems processes: results from an experimental study. *Ecol. Appl.* **6**, 140-151.

Walton, S.P., Welch, E.B. and Horner, R.R (1995) Stream periphyton response to grazing and changes in phosphorus concentration. *Hydrobiologia*, **302**, 31-46.

Ward, J.V. (1992) Aquatic Insect Ecology. 1. Biology and Habitat. J. Wiley and Sons, New York, 438p.

Ward, G.M., and Cummins, K.W. (1979) Effects of food quality on growth of a stream detritivore, *Paratendipes albimantus* (Meigen) (Diptera: Chironomidae). Ecology, **60**, 57-64.

Ward, J.V. and Stanford, J.A. (1979) The ecology of regulated streams. Plenum Press, New York.

Ward, J.V. and Stanford, J.A. (1984) Thermal responses in the evolutionary ecology of aquatic insects. *Annual Review of Entomology*, **27**, 97-117.

Warren, P.H. (1989) Spatial and temporal variation in the structure of a freshwater food web. *Oikos*, **55**, 299-311.

Washington, H.G. (1984) Diversity, biotic and similarity indices: a review with special relevance to aquatic ecosystems. *Wat. Res.* **18**, 653-694.

Watson, V., Berlind, P., and Bahls, L. (1990) Control of algal standing crop by P and N in the Clark Fork River. *Proc. Clark Fork River Symposium.*

Welch, E.B., Quinn, J.M. and Hickey, C.W. (1992) Periphyton biomass related to point-source enrichment in seven New Zealand streams. *Wat. Res.* **26**, 669-675.

Welch, E.B., Jacoby, J.M., Horner, R.R. and Seeley, M.R. (1988) Nuisance biomass levels of periphytic alage in streams. *Hydrobiologia*, **157**, 161-168.

Wellnitz, T.A. and Ward, J.V. (1998) Does light intensity modify the effect mayfly grazers have on periphyton? *Freshwater Biology*, **39**, 135-149.

Wetzel, R.G. (1983) *Limnology*. 2nd edn. Philadelphia. W.B. Saunders Co.

WFD CIS Guidance Document No.10 (March 2003) *Rivers and Lakes – Typology, Reference Conditions and Classification Systems*. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-5614-0, ISSN No. 1725-1087.

Whelan, K.F. (1980) *Studies on the life histories, distribution and production of the Ephemeroptera in selected Irish waters*. Ph.D. thesis, Zoology Dept., University College, Dublin.

Whitney, R.J. (1939) The thermal resistance of mayfly nymphs from ponds and streams. *J. Exp. Biol.* **16**, 374-385.

Whitton, B.A. (1979) Algae and higher plants as indicators of river pollution. In Biological Indicators of Water Quality (editors A. James and L. Evison). John Wiley, Chichester.

Wiederholm, T. (1980) Use of benthos in lake monitoring. *Journal of the Water Pollution Control Federation*, **52**, 537-547.

Williams, D.D., Williams, N. and Hogg, I. (1995) Life history plasticity of *Nemoura trisinosa* (Plecoptera: Nemouridae) along a permanent-temporary water habitat gradient. *Freshwater Biology*, **34**, 155-63.

Wise, E.J. (1980) Seasonal distribution and life histories of Ephemeroptera in a Northumbrian river. *Freshwater Biology*, **10**, 101-111.

Wittekind, D. (1972) Fluorescence properties of the monoaminoacridines and some 2-aminoacridine-derivatives, pp. 95-105. *In* A. A Thaer & M. Sernetz (eds.), Fluorescence techniques techniques in cell biology. Springer, New York.

Wong, S.L. and Clarke, B. (1976) Field determination of the critical nutrient concentratins for *Cladaphora* in streams. *J. Fish. Res. Board Can.* **33**, 95-96.

Wood, P.J. and Armitage, P.D. (1997) Biological effects of fine sediment in the lotic environment. *Envir. Mgmt*., **21**, 203-217.

Wright, J.F. (1995) Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology.* **20,** 181-197.

Wright, J.F., Sutcliffe, D.W., Furse, M.T. (eds), (2000) Assessing the Biological Quality of Fresh Waters – RIVPACS and Other Techniques. Freshwater Biological Association, Ambleside, Cumbria, U.K., 373 PP.

Wuhrmann, K. and Eichenberger, E. (1975) Experiment on the effect of inorganic enrichment of rivers on periphyton primary production. *Verhandlugen Internationale Vereinigung fur Theoretische und Angewandte Limnologie*, **19**, 2028-2034.

Yule, C. (1986) Comparison of the dietary habits of six species of *Dinotoperla* (Plecoptera: Gripopterygidae) in Victoria. *Aust. J. Mar. Freshwater Res*. **37**, 121-127.

Zelinka, M. and Marvan, P. (1961) Zur Prazisierung der biologischen Klassifikation der Reinheit fliebender Gewasser. *Arch. Hydrobiol*. **57**, 389-407.

Appendices

Appendix 5.1 Maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters examined in the high status rivers during the study programme, May 2001 to October 2002.

	Temperature °C	DO % Saturation	pH pH units	Conductivity µS/cm	Orthophosphate mg/l P	TON mg/l N	Ammonia mg/l N	Chloride mg/l Cl	Alkalinity mg/l $CaCO_3$	BOD_5 Mg/l O_2	Colour Hazen units
Owengarve River											
n	*14*	*14*	*12*	*14*	*14*	*14*	*13*	*13*	*13*	*11*	*14*
Minimum	5.3	90.0	7.6	316.0	0.018	0.12	0.01	13.0	116.0	0.3	27.0
Mean	11.7	104.2	7.9	397.7	0.037	0.54	0.03	16.6	167.2	0.8	107.7
Median	11.3	99.8	8.0	387.5	0.038	0.48	0.02	17.0	158.0	0.9	109.0
Maximum	17.0	129.9	8.2	500.0	0.049	1.40	0.07	22.0	252.0	1.4	246.0
Standard deviation	3.5	12.60	0.2	67.46	0.009	0.31	0.02	2.4	40.79	0.3	60.9
Dunneill River											
n	*14*	*14*	*13*	*14*	*13*	*12*	*13*	*14*	*14*	*12*	*14*
Minimum	6.0	99.0	6.7	73.0	0.012	0.10	0.01	8.0	9.0	0.3	19.0
Mean	11.9	105.2	7.9	260.5	0.032	0.19	0.02	13.7	104.4	0.7	83.7
Median	12.1	104.2	8.1	264.0	0.023	0.15	0.01	14.0	84.0	0.6	47.5
Maximum	16.4	123.0	8.3	588.0	0.112	0.50	0.11	19.0	298.0	1.5	243.0
Standard deviation	3.3	6.8	0.5	147.8	0.027	0.13	0.03	3.1	76.1	0.4	74.3
Castlebar River											
n	*14*	*14*	*13*	*15*	*14*	*14*	*14*	*13*	*14*	*12*	*15*
Minimum	5.4	95.4	7.3	111.0	0.025	0.10	0.005	16.0	23.0	0.3	44.0
Mean	11.5	101.1	7.7	168.2	0.036	0.16	0.015	21.5	42.9	0.8	109.5
Median	12.2	99.4	7.7	175.0	0.032	0.13	0.010	20.0	36.5	0.6	98.0
Maximum	15.7	110.0	8.2	215.0	0.098	0.3	0.040	30.0	81.0	1.8	228.0
Standard deviation	3.3	5.0	0.2	32.2	0.019	0.07	0.011	4.37	17.4	0.5	55.6

Appendix 5.1 continued. Maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters examined in the high status rivers during the study programme, May 2001 to October 2002.

	Temperature °C	DO % Saturation	pH pH units	Conductivity µS/cm	Orthophosphate mg/l P	TON mg/l N	Ammonia mg/l N	Chloride mg/l Cl	Alkalinity mg/l $CaCO_3$	BOD_5 Mg/l O_2	Colour Hazen units
Callow Loughs Stream											
n	*14*	*14*	*13*	*14*	*14*	*14*	*14*	*14*	*14*	*11*	*14*
Minimum	5.8	94.0	7.8	173.0	0.018	0.18	0.005	13.0	53.0	0.3	21.0
Mean	11.9	101.8	8.0	286.9	0.035	0.24	0.012	16.9	115.4	0.5	53.3
Median	11.9	99.1	8.0	311.0	0.031	0.20	0.007	17.0	120.0	0.3	33.0
Maximum	18.5	114.0	8.3	348.0	0.082	0.40	0.030	22.0	158.0	0.8	129.0
Standard deviation	3.9	6.9	0.2	61.13	0.016	0.08	0.008	2.8	34.5	0.3	35.6
Brusna River											
n	*12*	*12*	*11*	*11*	*11*	*10*	*11*	*12*	*12*	*10*	*12*
Minimum	6.1	98.4	7.7	352.0	0.023	0.20	0.005	12.0	136.0	0.3	26.0
Mean	11.8	107.2	8.0	515.0	0.042	0.57	0.011	21.3	230.7	0.6	69.8
Median	12.0	103.0	8.1	521.0	0.038	0.50	0.005	22.0	239.0	0.6	37.5
Maximum	16.8	137.0	8.2	826.0	0.105	1.00	0.030	27.0	296.0	0.9	167.0
Standard deviation	2.9	11.2	0.2	144.6	0.022	0.27	0.010	3.7	57.7	0.3	54.6

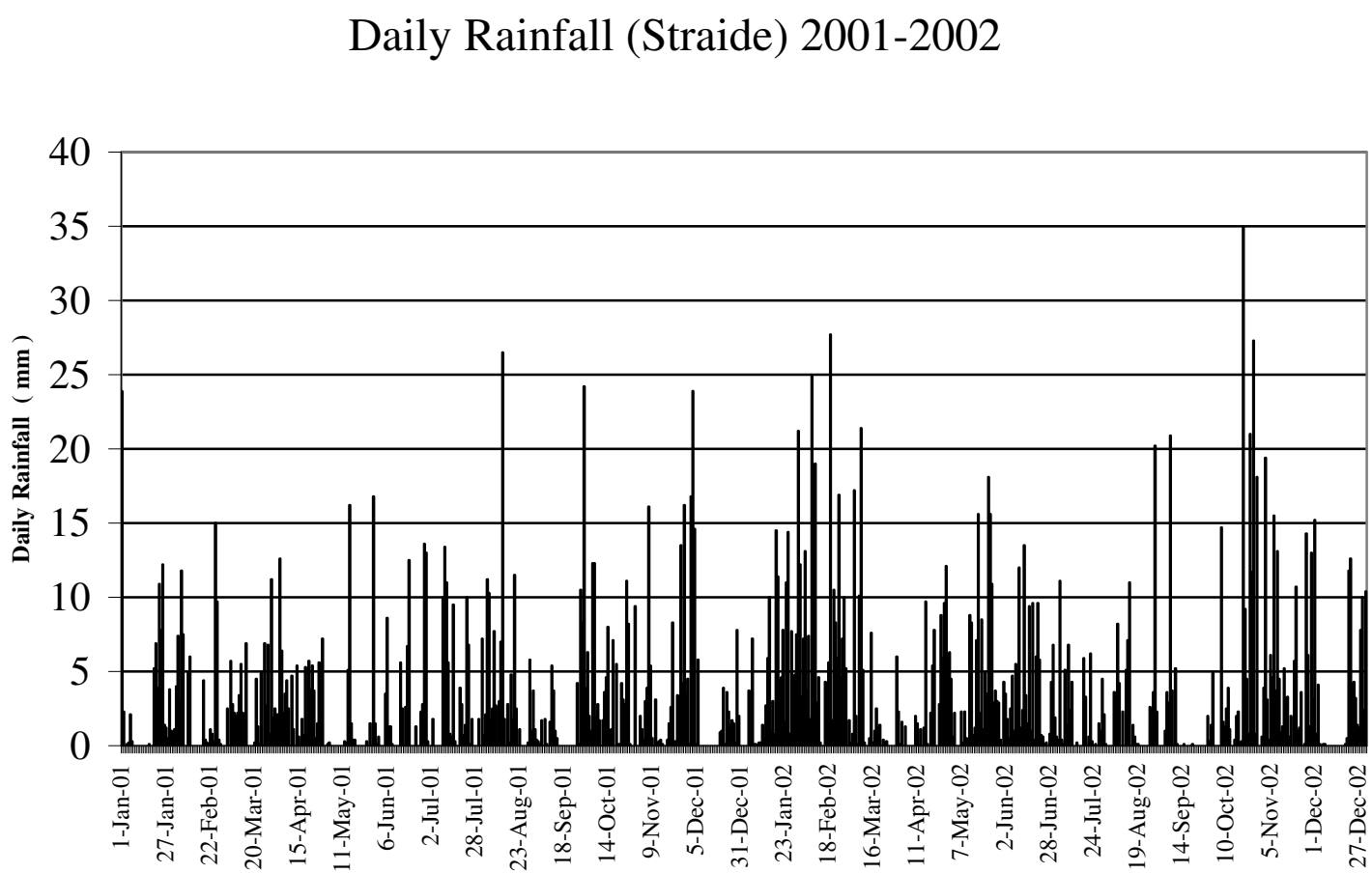
Appendix 5.2 Maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters examined in the impacted rivers during the study programme, May 2001 to October 2002.

	Temperature °C	DO % Saturation	pH pH units	Conductivity µS/cm	Orthophosphate mg/l P	TON mg/l N	Ammonia mg/l N	Chloride mg/l Cl	Alkalinity mg/l $CaCO_3$	BOD_5 Mg/l O_2	Colour Hazen units
Cartron River											
n	*10*	*10*	*10*	*10*	*11*	*11*	*11*	*10*	*9*	*9*	*10*
Minimum	9.4	94.6	5.3	80.0	0.007	0.10	0.01	19.0	6.0	0.3	12.0
Mean	12.5	106.6	6.6	106.3	0.018	0.16	0.01	24.5	8.6	0.8	145.8
Median	12.5	102.9	6.6	101.0	0.014	0.10	0.01	24.0	6.0	0.8	152.5
Maximum	16.1	138.2	8.1	132.0	0.036	0.60	0.02	34.0	24.0	1.3	206.0
Standard deviation	2.28	12.5	1.0	19.3	0.009	0.16	0.004	4.7	6.0	0.3	61.5
Lough na Corralea Stream											
n	*9*	*9*	*9*	*10*	*9*	*10*	*10*	*9*	*9*	*9*	*9*
Minimum	7.6	87.1	6.1	87.0	0.005	0.10	0.020	16.0	6.0	0.3	42.0
Mean	12.0	97.4	7.1	112.2	0.010	0.14	0.05	16.2	18.2	1.1	75.4
Median	11.5	96.0	7	104.5	0.008	0.1	0.04	15.0	17	0.9	78.0
Maximum	16.5	111.0	8	167.0	0.025	0.4	0.1	23	32.0	3.5	122.0
Standard deviation	2.9	7.6	0.6	23.6	0.006	0.1	0.03	2.8	8.0	1.0	25.0
Mad River											
n	*11*	*11*	*11*	*12*	*11*	*11*	*11*	*11*	*10*	*11*	*12*
Minimum	7.7	98.6	5.5	42.0	0.005	0.1	0.01	8.0	6.0	0.3	33.0
Mean	11.4	106.8	7.1	54.1	0.023	0.1	0.02	10.1	8.4	0.9	121.7
Median	11.8	103.4	7.3	51.0	0.019	0.1	0.01	10.0	8.0	1.0	130.0
Maximum	16.6	127.0	8.0	74.0	0.061	0.1	0.04	16.0	15.0	1.5	191.0
Standard deviation	2.7	9.6	0.9	10.1	0.018	0	0.01	2.3	2.8	0.5	48.2

Appendix 5.2 continued. Maximum, minimum, mean, median and standard deviation values for the physico-chemical parameters examined in the impacted rivers during the study programme, May 2001 to October 2002.

	Temperature °C	DO % Saturation	pH pH units	Conductivity µS/cm	Orthophosphate mg/l P	TON mg/l N	Ammonia mg/l N	Chloride mg/l Cl	Alkalinity mg/l $CaCO_3$	BOD_5 Mg/l O_2	Colour Hazen units
Mullaghanoe River											
n	*12*	*12*	*12*	*14*	*14*	*14*	*13*	*14*	*14*	*11*	*14*
Minimum	6.0	97.0	7.7	308.0	0.028	0.69	0.02	17.0	121.0	0.3	14.0
Mean	11.4	110.7	7.8	395.8	0.053	0.99	0.08	18.8	159.2	0.9	43.9
Median	11.8	109.6	7.9	400.5	0.048	0.95	0.07	19.0	162.0	0.9	42.5
Maximum	14.6	141.9	8.1	471.0	0.098	1.40	0.21	21.0	202.0	2.5	74.0
Standard deviation	2.65	12.8	0.11	50.8	0.018	0.26	0.06	1.37	22.3	0.6	19.8
Robe River											
n	*13*	*12*	*11*	*12*	*12*	*12*	*11*	*12*	*13*	*11*	*12*
Minimum	5.4	90.0	7.7	63.0	0.027	0.50	0.01	17.0	264.0	0.3	12.0
Mean	12.7	107.9	8.0	594.0	0.042	0.83	0.02	19.8	308.3	0.9	54.7
Median	14.1	102.3	8.0	635.0	0.040	0.70	0.01	20.0	304.0	0.9	47.5
Maximum	16.0	158.2	8.4	677.0	0.066	1.60	0.03	22.0	360.0	1.8	138.0
Standard deviation	3.2	18.2	0.2	169.6	0.010	0.33	0.01	1.48	25.7	0.5	30.3

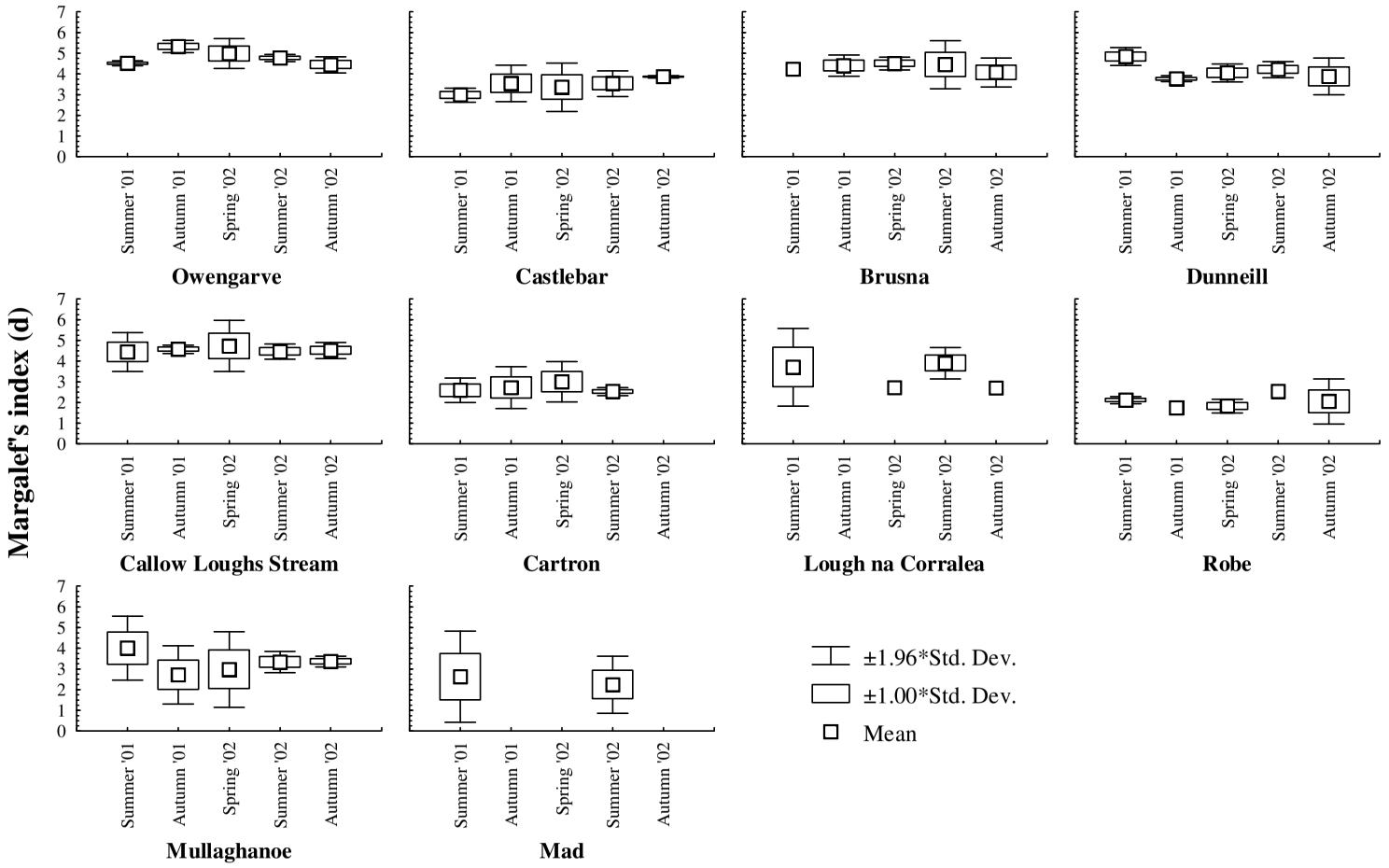
Appendix 5.3 Daily rainfall (mm) for calendar years 2001 and 2002 at Straide (National Grid E: 125780, N: 297380), Co. Mayo which is central to the study area and given as an indication of wet and dry spells rather than an absolute indication of rainfall for each of the 10 catchments studied. Total rainfall at Straide was 1024 mm in 2001 and 1374 mm in 2004. This compares with the long-term average annual precipitation of 1181 mm at Straide. Long-term evapotranspiration here is 456 mm - 378 mm in summer and 78 mm in winter giving long-term values of 725 mm annual runoff with 626 mm net winter runoff and 99 mm net summer runoff.



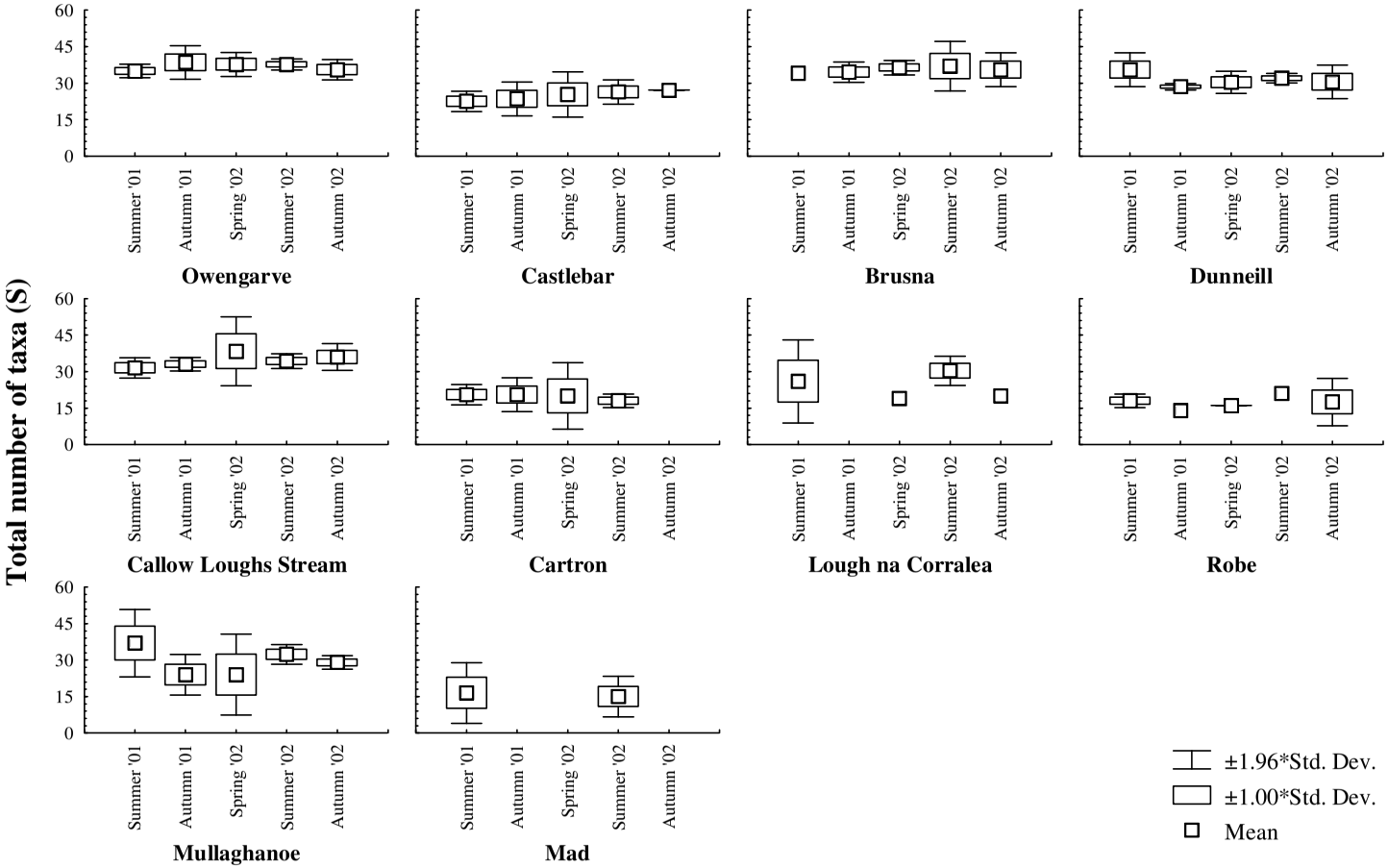
Appendix 5.4 Comparative descriptive statistics for 99 western rivers over the period 1996-2001 based on 12763 samples at 346 sample sites as analysed by the EPA Laboratory, Castlebar, Co. Mayo.

Determinand	Valid N	Mean	Median	Min	Max	Std.Dev.	Lower Quartile	Upper Quartile	10th percentl	90th percentl
Chloride (mg Cl/l)	12676	44.2	20	1	29320	523.3	17	23	15	27
Colour (Hazen)	12713	74.9	67	5	1250	50.5	39	100	22	142
Conductivity (µS/cm)	12743	466.7	439	7.8	36000	991	287	571	142	633
Hardness (Mg CaCo3/l)	913	179.0	168	28	529	72.6	118	230	99	282
pH	12744	7.9	7.9	5.1	9.3	0.4	7.7	8.1	7.4	8.3
Temperature (°C)	12667	10.63	10.1	0.0	102.3	4.31	7.4	14.0	5.4	16.3
Dissolved Oxygen %	12640	94.9	95	0	232	11.1	89	100	84	106
Ammonia (mgN/l)	12627	0.058	0.028	0.002	81.560	0.858	0.015	0.047	0.009	0.077
BOD (mg O2/l)	12520	1.39	1.2	0.0	240.0	2.43	0.9	1.6	0.6	2.2
Molybdate Reactive Phosphorus (mg P/l)	12739	0.034	0.018	0.001	33.250	0.322	0.009	0.032	0.008	0.050
Oxidised Nitrogen (mg N/l)	12692	0.918	0.687	0.009	41.512	0.923	0.271	1.286	0.100	2.024
Nitrite (mgN/l)	1298	0.007	0.004	0.000	0.200	0.012	0.002	0.007	0.002	0.011
Suspended Solids (mg/l)	1368	6.5	5	0	208	11.2	3	6	1	11
Copper (mg/l)	1332	0.005	0.001	0.001	1.200	0.043	0.001	0.002	0.001	0.007
Zinc (mg/l)	1331	0.044	0.020	0.001	13.600	0.444	0.004	0.025	0.002	0.025

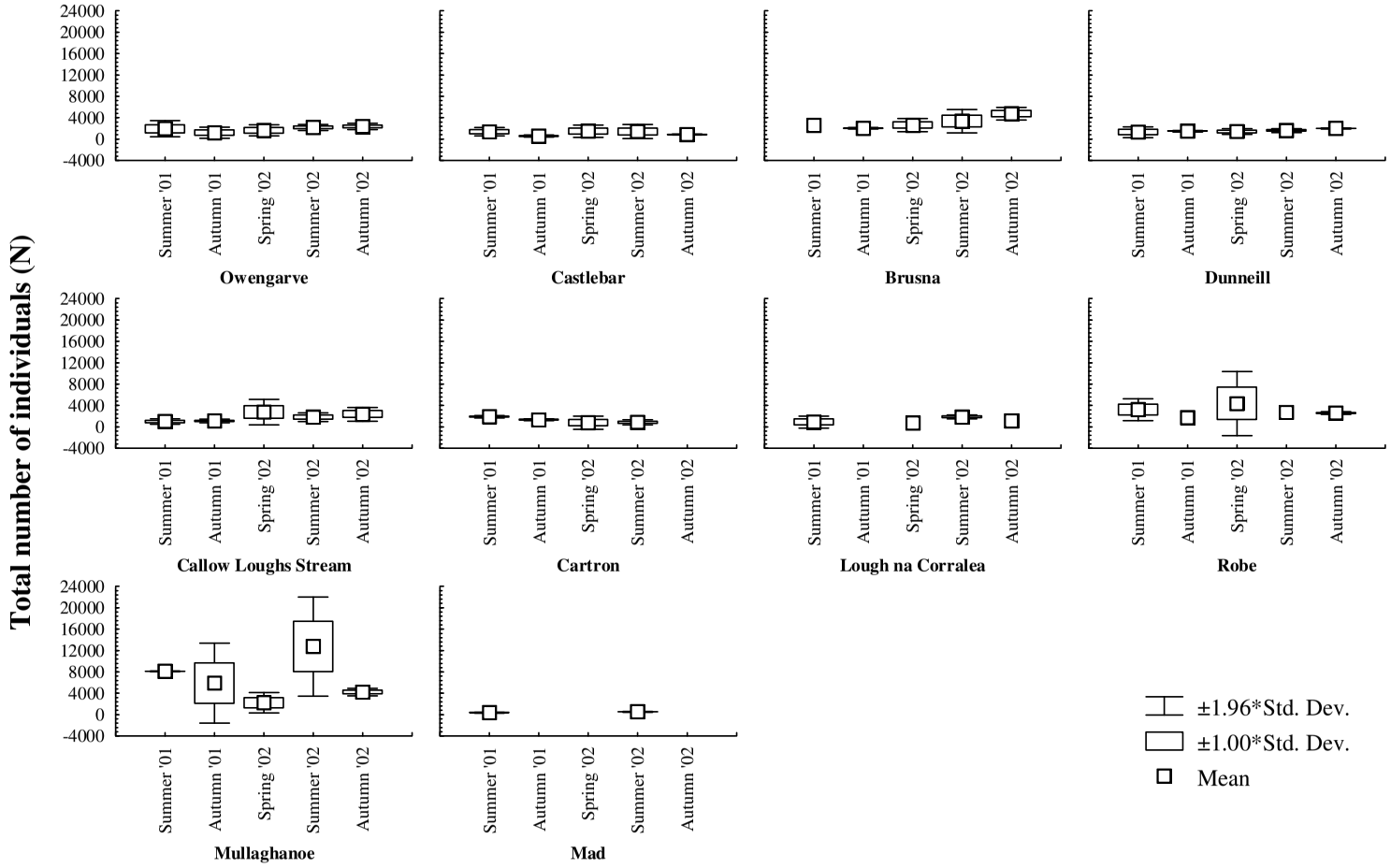
Appendix 5.5 Box and Whisker plots of the seasonal variation in the Margalef's index (d) (Species richness) over five seasons across the ten rivers.



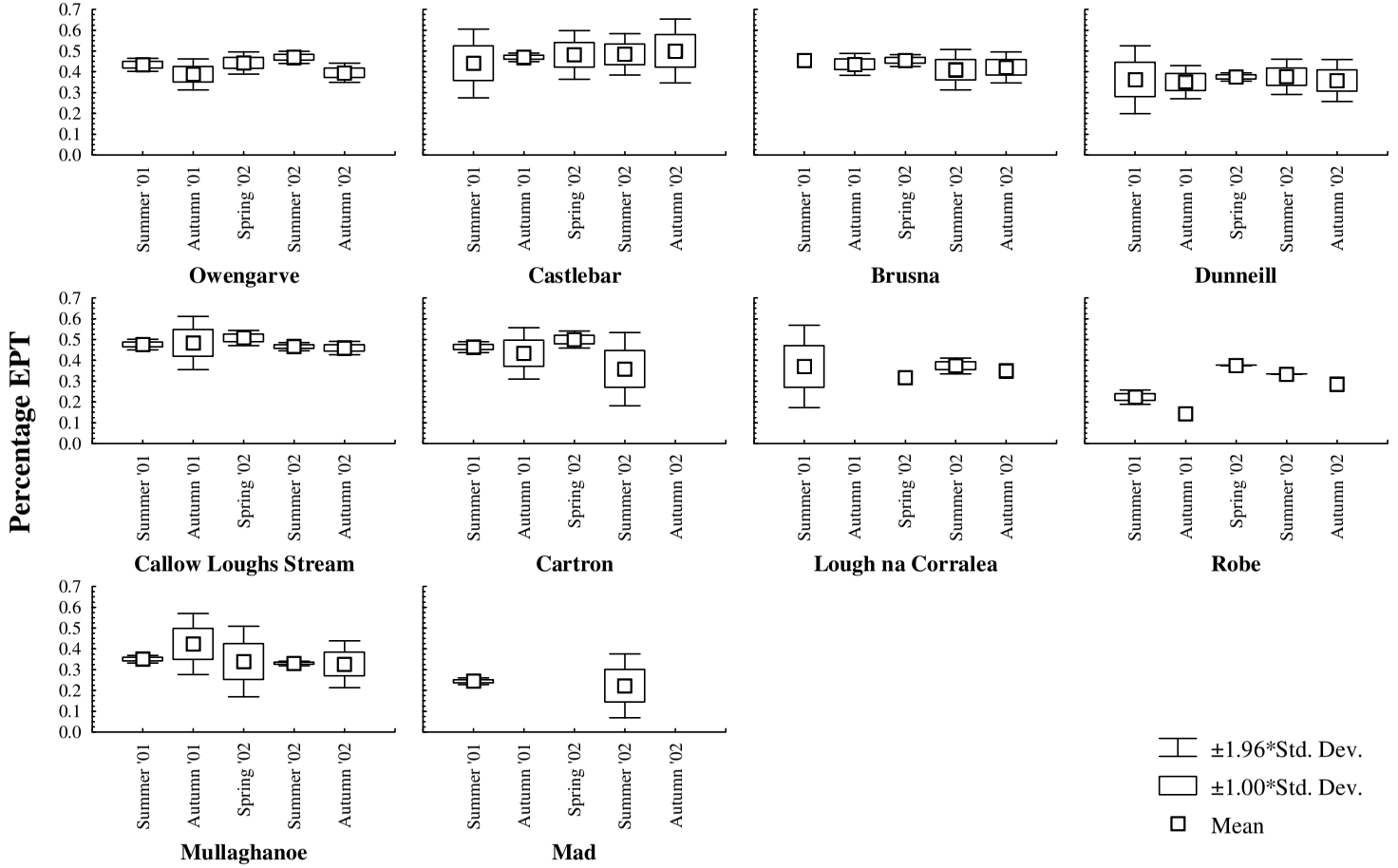
Appendix 5.6 Box and Whisker plots of the seasonal variation in the total number of taxa per metre square over five seasons across the ten rivers.



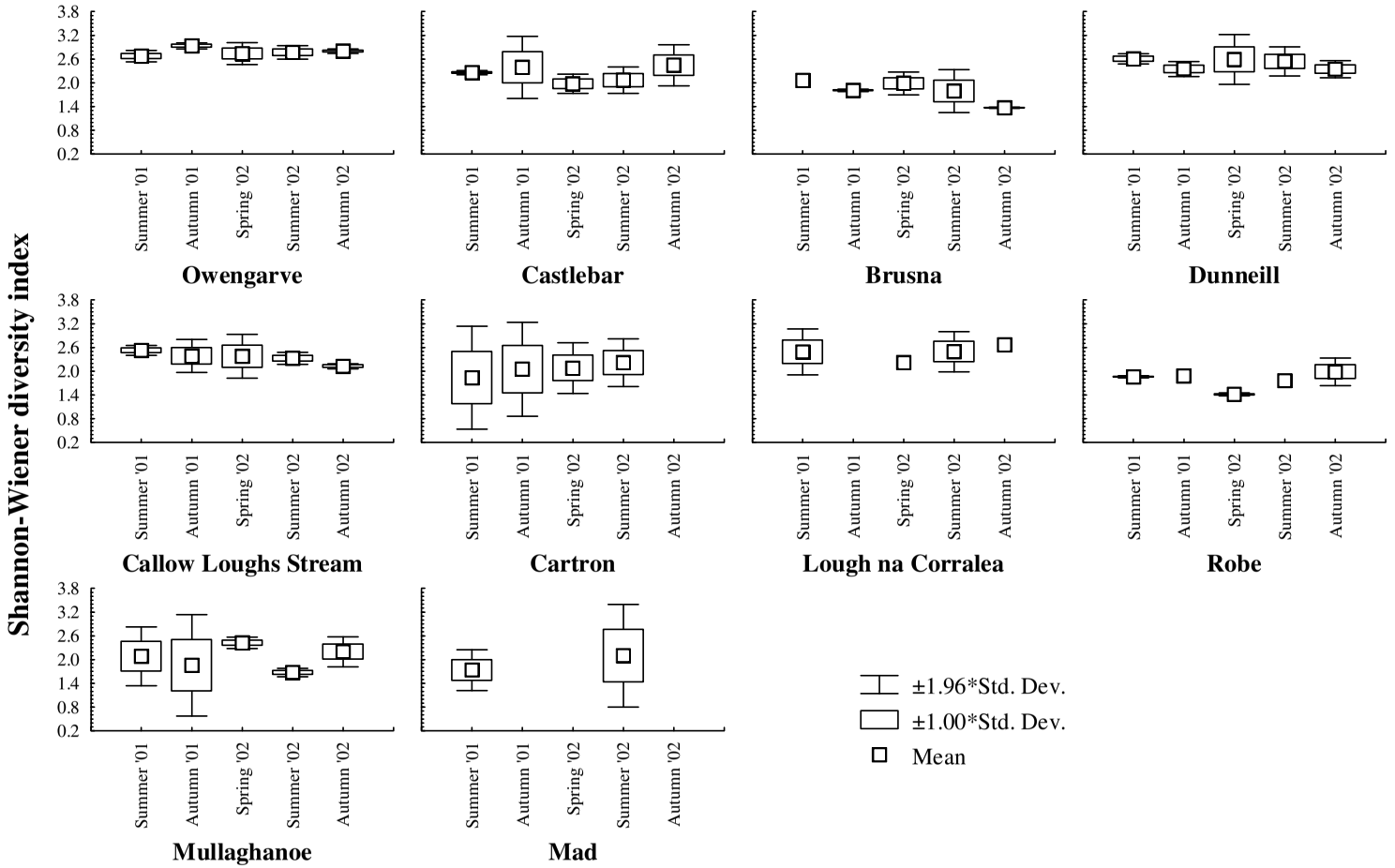
Appendix 5.7 Box and Whisker plots of the seasonal variation in the total number of individuals per metre square over five seasons across the ten rivers.



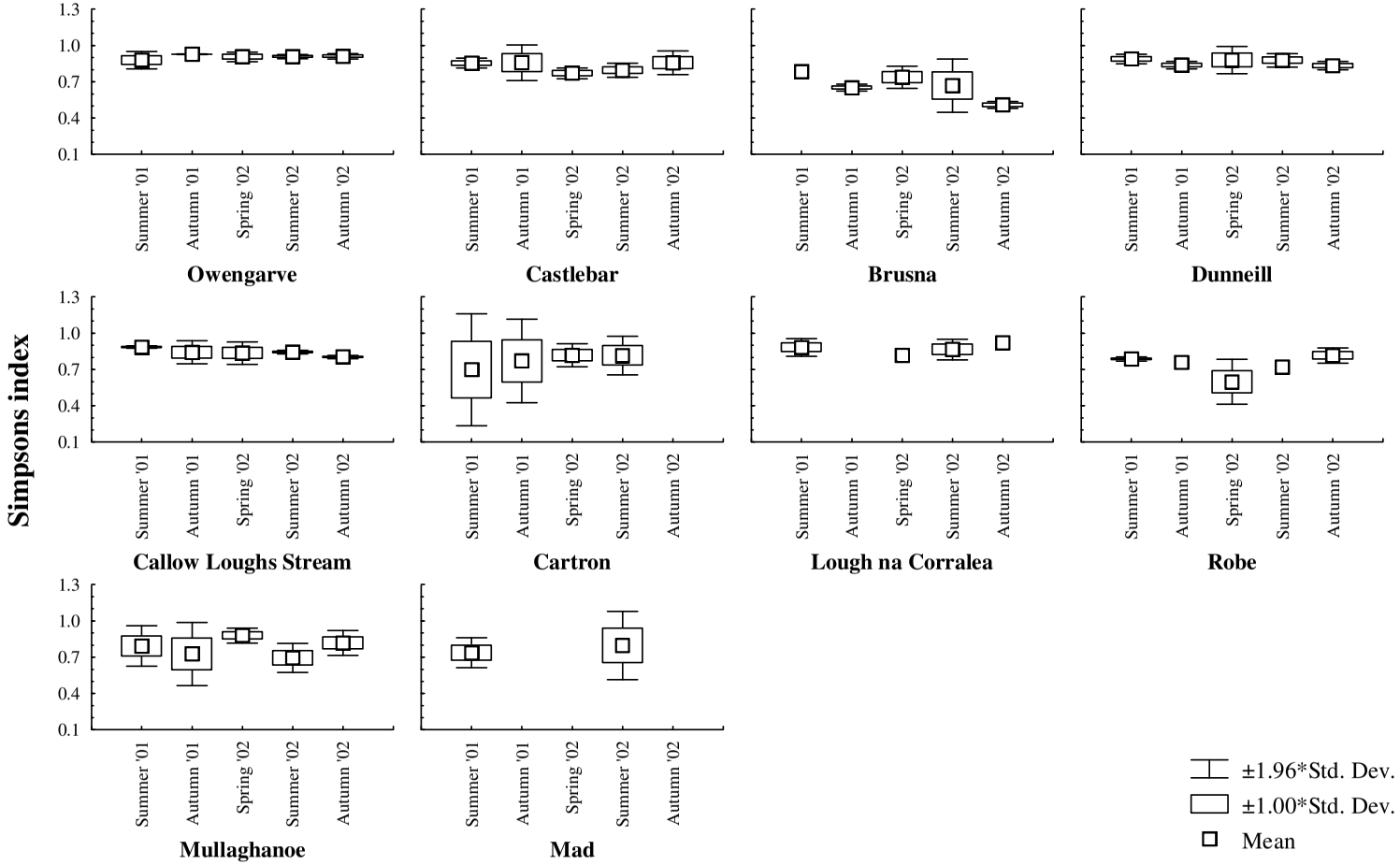
Appendix 5.8 Box and Whisker plots of the seasonal variation in the Percentage EPT per metre square over five seasons across the ten rivers.



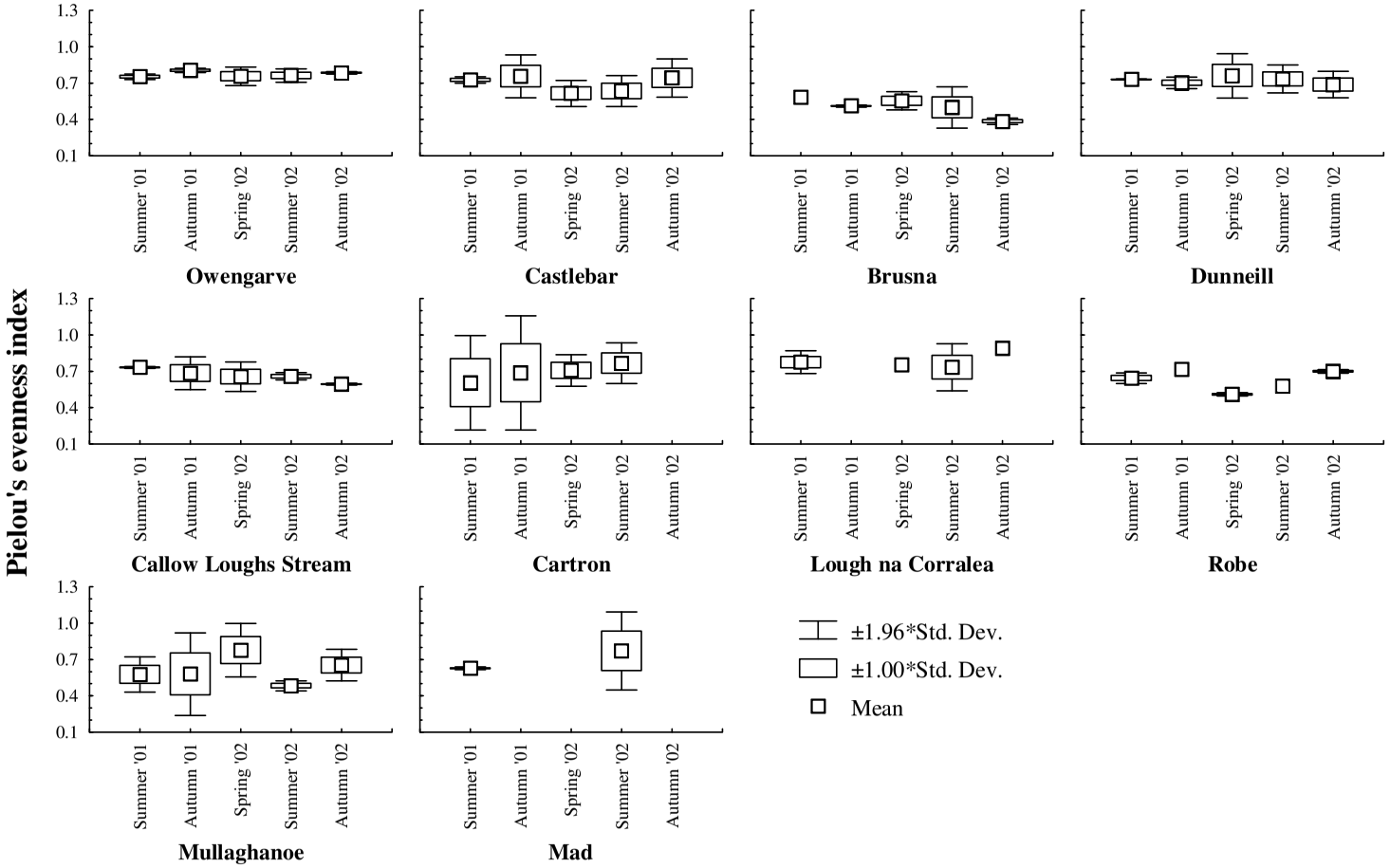
Appendix 5.9 Box and Whisker plots of the seasonal variation in the Shannon-Wiener index over five seasons across the ten rivers.



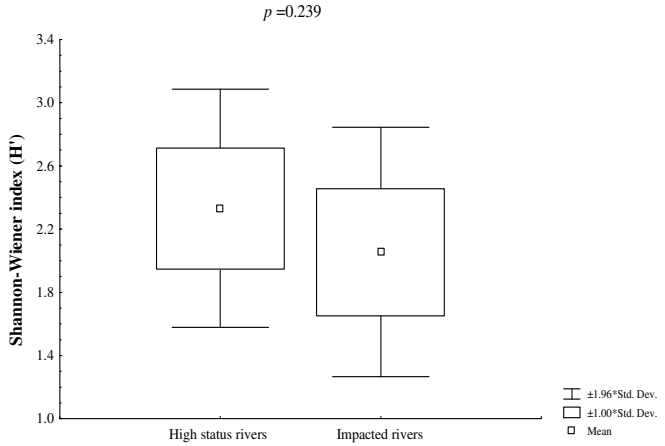
Appendix 5.10 Box and Whisker plots showing the seasonal variation in Simpson's index over five seasons across the ten river sites.

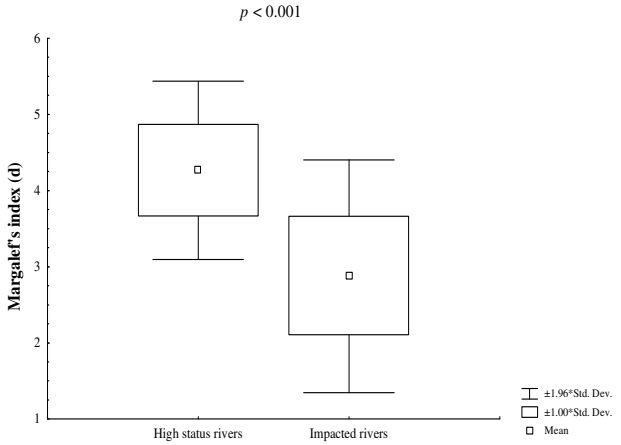


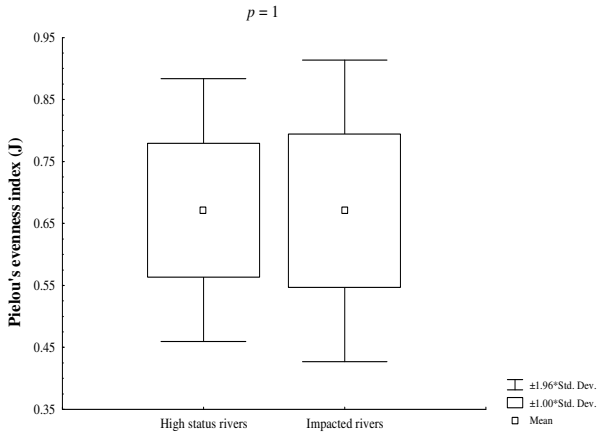
Appendix 5.11 Box and Whisker plots of the seasonal variation in Pielou's evenness index (J') over five seasons across the ten rivers.

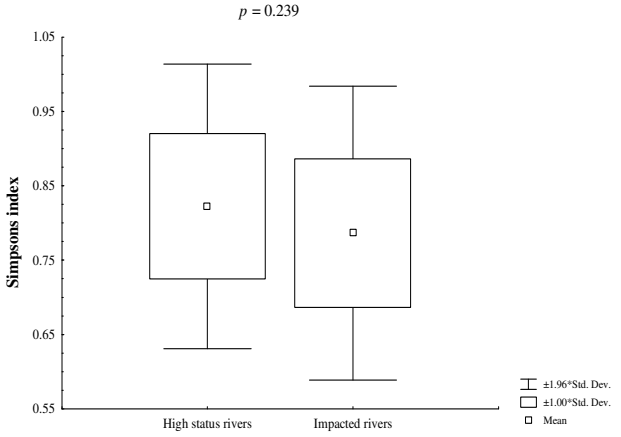


Appendix 5.12 Comparison of the diversity indices and matrices using ANOVA between the high status and impacted sites during the study period.

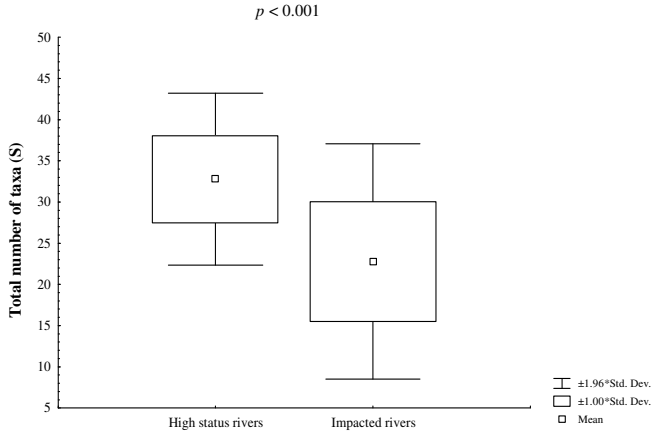


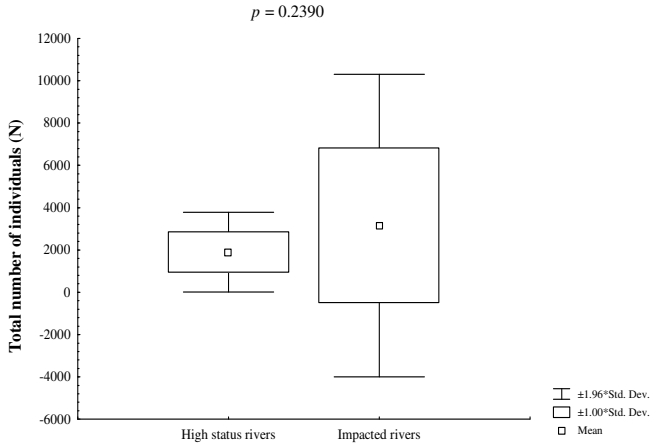


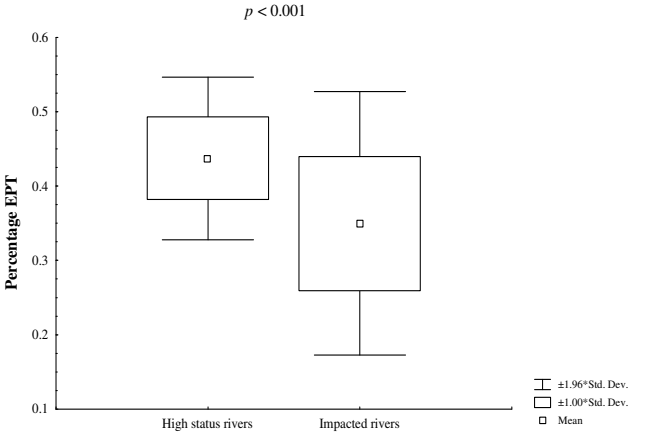




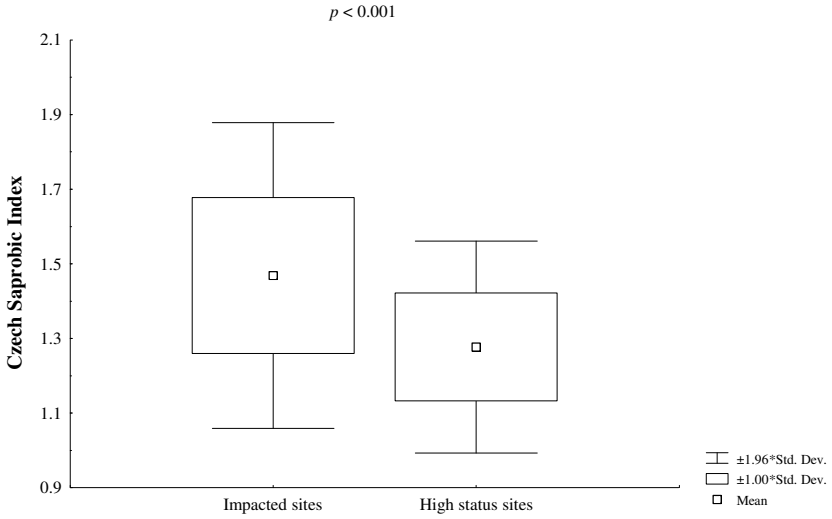
Appendix 5.12 continued. Comparison of the diversity indices and matrices using ANOVA between the high status and impacted sites during the study period.

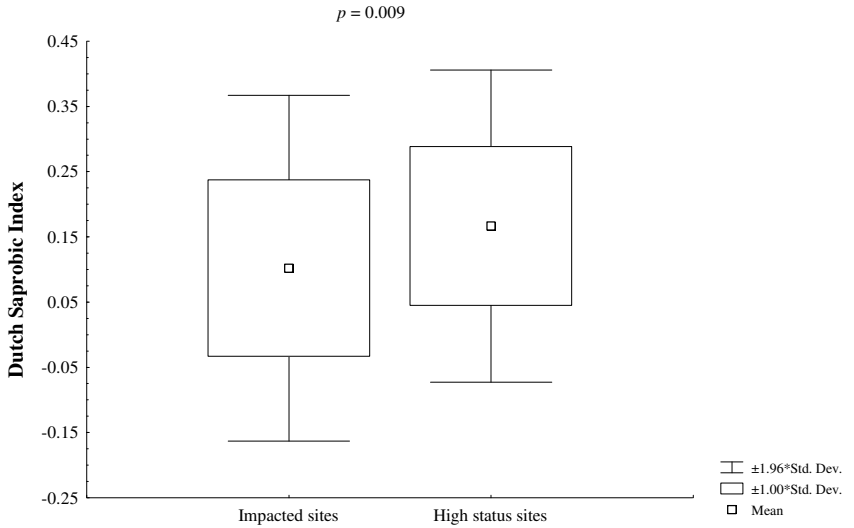


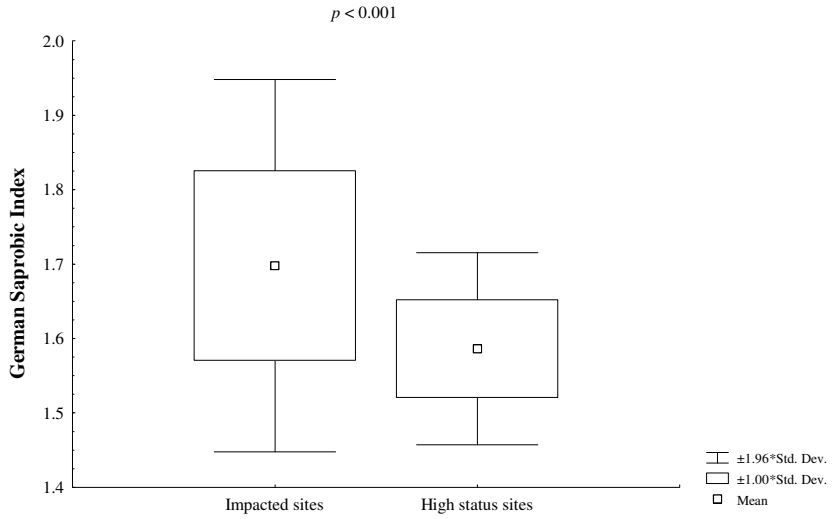


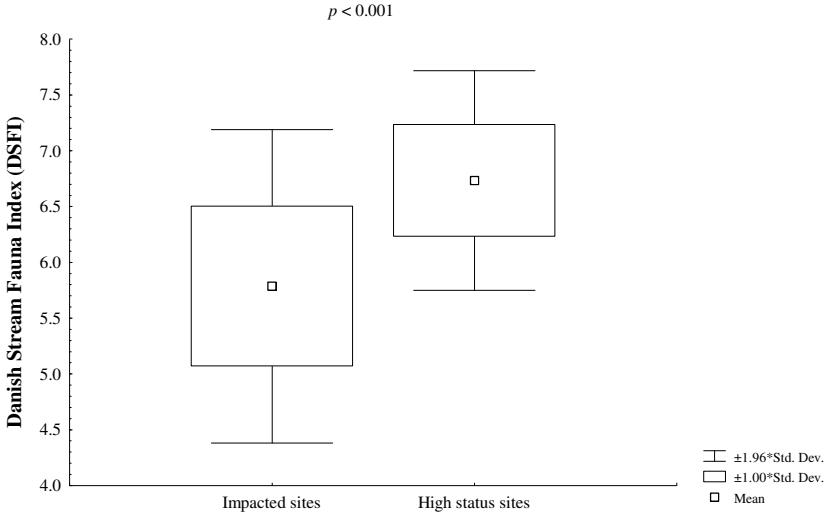


Appendix 5.13 Comparison of the biotic indices AQEM between the high status and impacted sites during the study period.

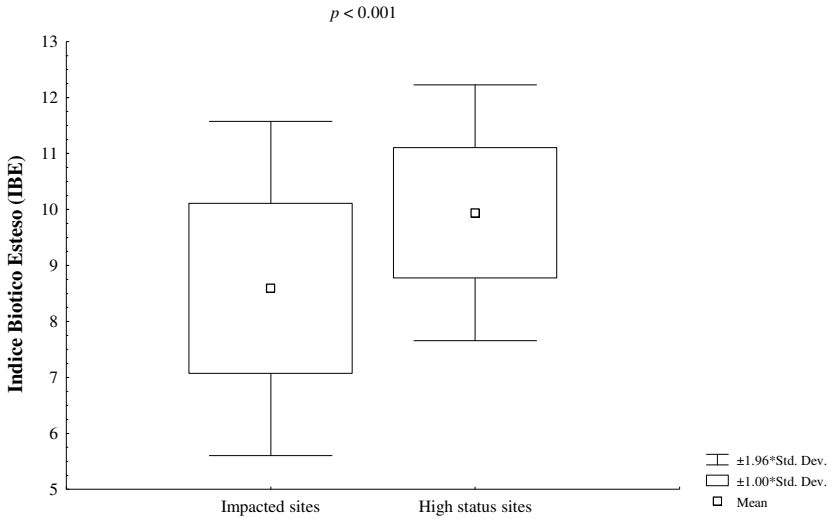


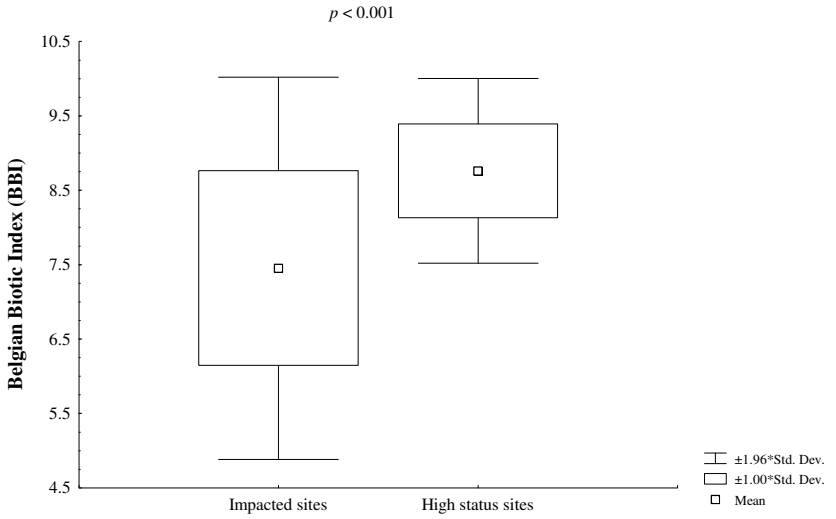


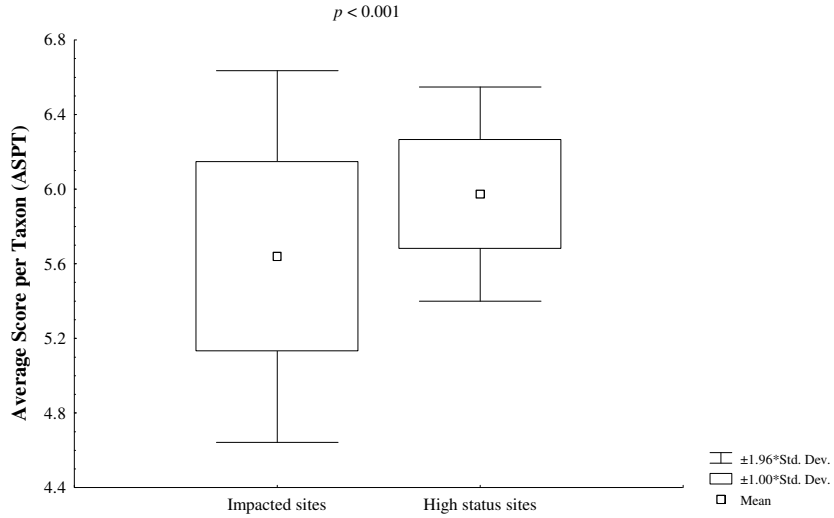




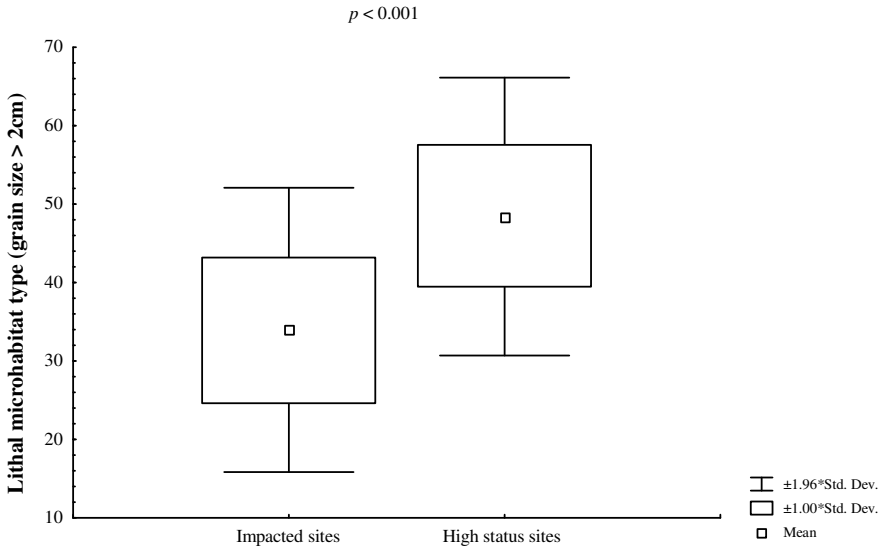
Appendix 5.13 Continued: Comparison of the biotic indices AQEM between the high status and impacted sites during the study period.

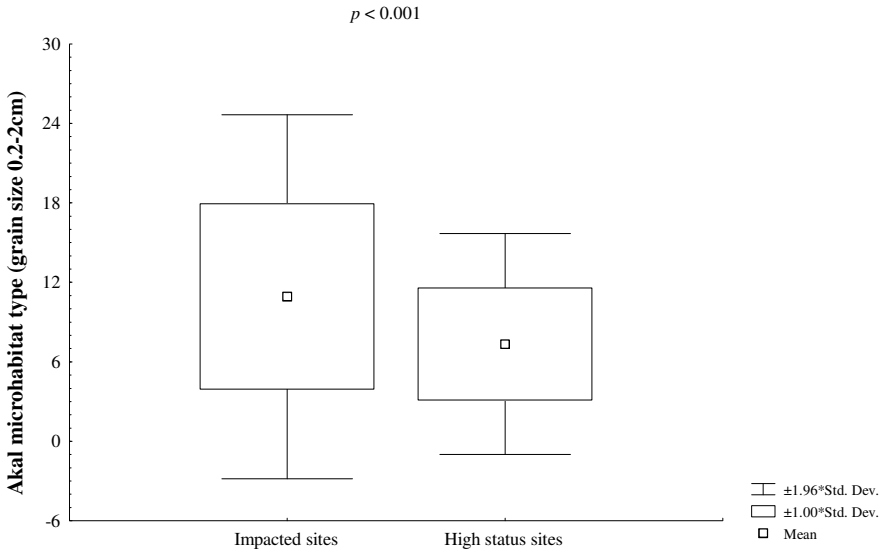


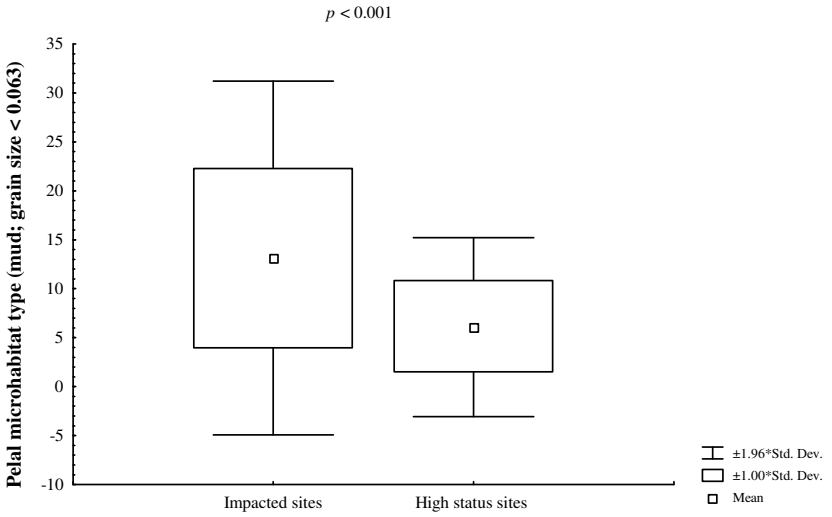


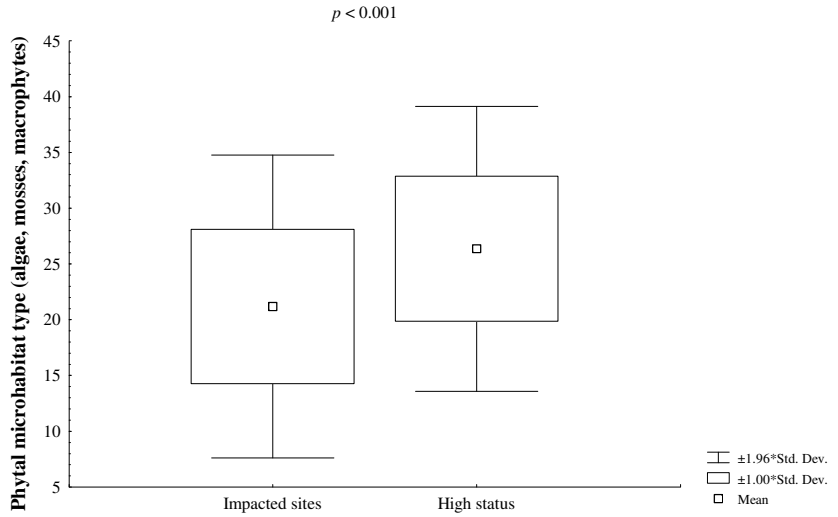


Appendix 5.14 Comparison of the microhabitat preferences calculated using AQEM between the high status and impacted sites during the study period.

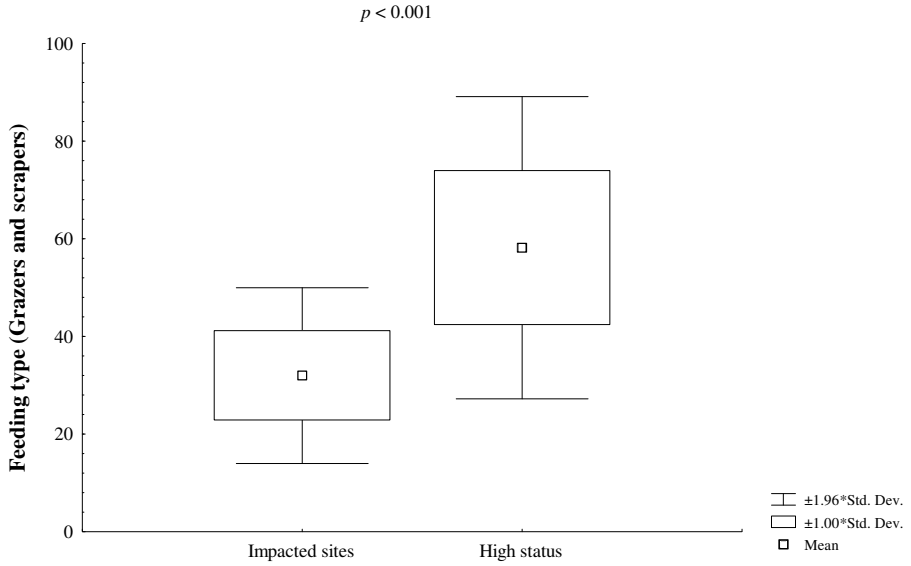


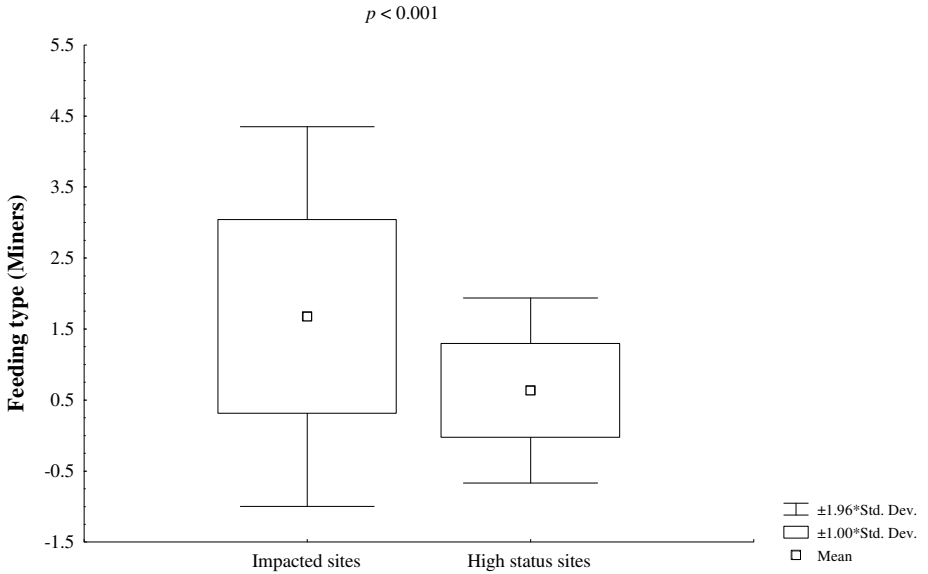


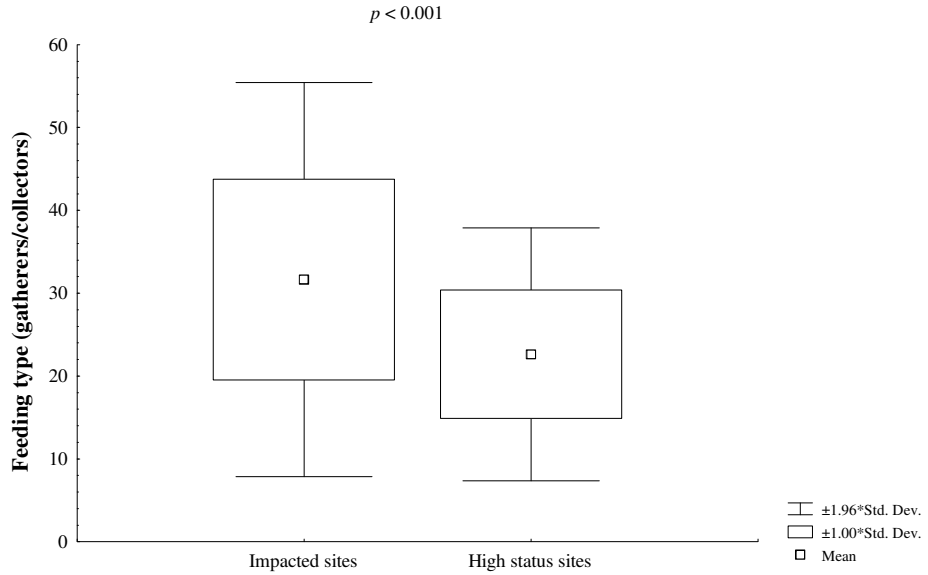


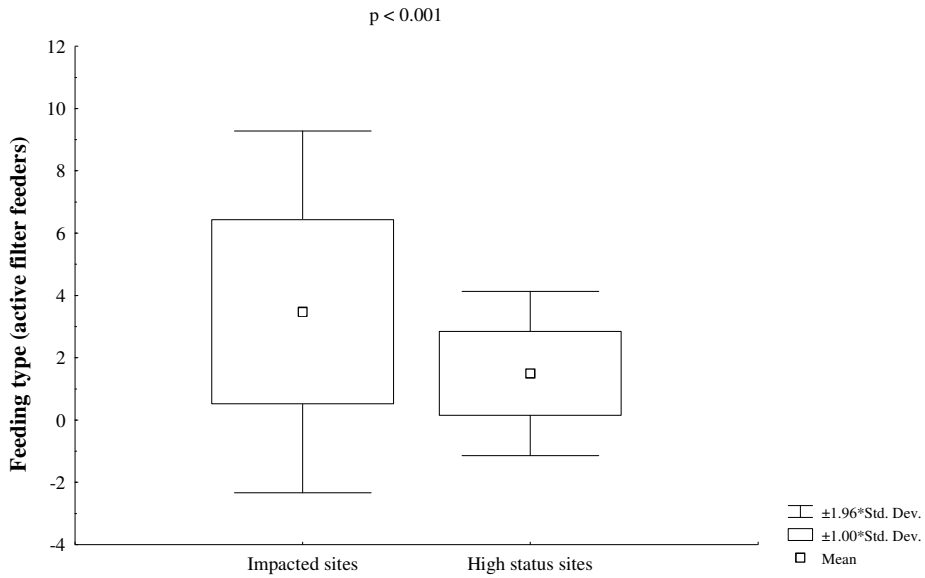


Appendix 5.15 Comparison of the feeding types calculated using AQEM between the high status and impacted sites during the study period.

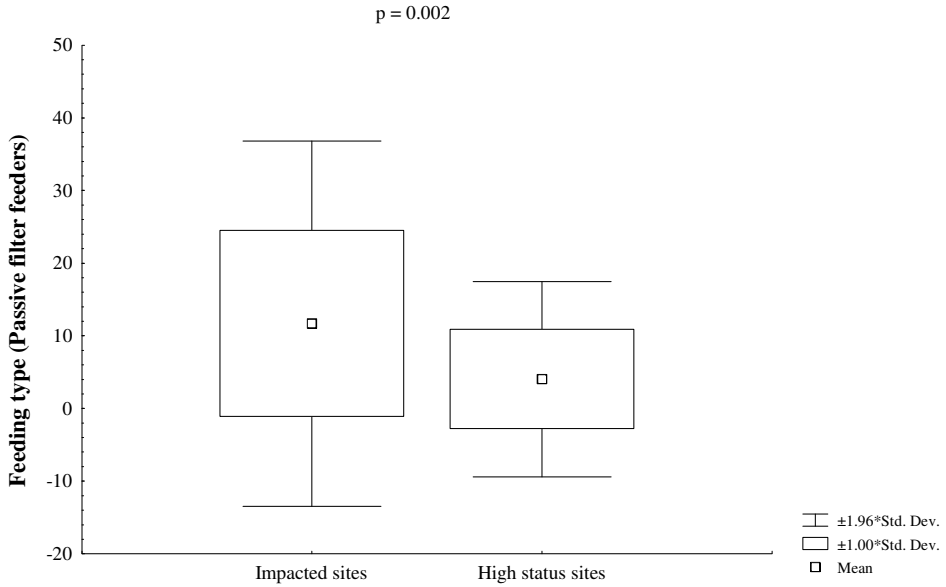


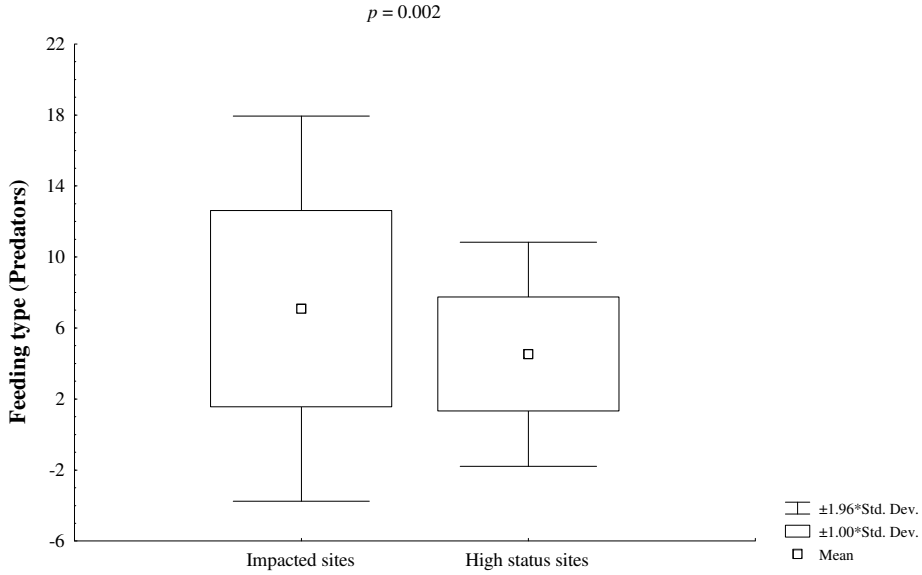


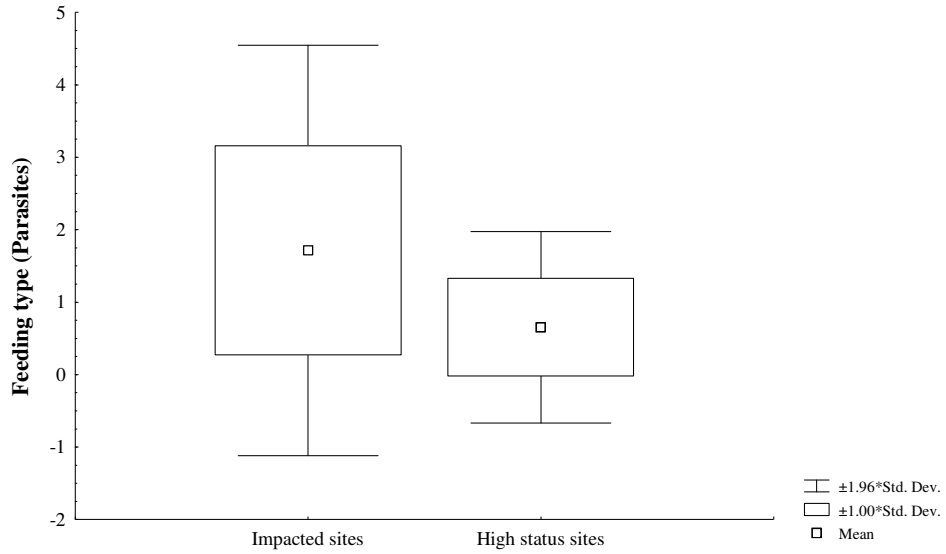




Appendix 5.15 Comparison of the feeding types calculated using AQEM between the high status and impacted sites during the study period.







Appendix 5.16 Substrate fraction weights (grams) for the high status and impacted rivers sampled in March 2003.

High status rivers	Owengarve River			Dunneill River			Castlebar River			Callow Loughs Stream			Brusna River		
Sieve (mm)	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**
0.03	2	13	13	1	1	1	1	0	0	1	0	1	1	0	1
0.063	7	46	24	5	2	3	2	2	1	2	3	5	2	2	2
0.125	25	188	84	11	6	10	2	3	1	2	10	20	3	2	5
0.25	42	202	68	35	15	41	6	6	1	6	12	43	5	2	11
0.50	61	180	35	127	51	138	38	29	5	38	13	119	30	6	37
1	109	160	68	278	188	190	113	56	26	113	23	195	197	12	126
2	69	68	32	180	332	93	131	38	48	131	20	110	115	3	57
4	240	154	132	34	224	388	408	152	187	408	14	250	229	30	144
8	286	215	208	38	672	430	440	199	380	440	44	135	180	24	68
16	589	511	717	11	537	1015	629	573	650	629	380	9	548	155	193
32	735		415	443	1383	1676	962	1114	1314	962	1327	550	570	471	430
64	246		1345	4475	1049	3555	2535	4648	3261	2535	1605	2003	2166	2528	2870
128			2337		5214		701	2873		701		2650	3412	1172	850
256					2636										
Total	2411	1737	5478	5638	12310	7540	5968	9693	5874	5968	3451	6090	7458	4407	4794

Impacted Rivers	**Cartron River**			**Lough na Corralea Stream**			**Mad River**			**Robe River**			**Mullaghanoe River**		
Sieve (mm)	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**	**Sample 1 Weight (g)**	**Sample 2 Weight (g)**	**Sample 3 Weight (g)**
0.03	1	1	0	0	2	1	0	1	1	2	1	1	1	1	0
0.063	3	9	1	1	6	3	1	3	1	6	5	5	2	4	2
0.125	8	24	2	1	10	8	1	6	3	11	13	11	6	14	6
0.25	9	44	21	12	21	16	4	9	9	24	30	20	16	33	10
0.50	14	66	136	9	41	47	18	37	43	43	64	28	72	32	10
1	8	114	165	19	82	99	64	172	149	52	155	41	155	31	9
2	13	88	171	119	84	178	53	183	115	36	134	36	95	25	19
4	129	526	739	220	388	117	357	729	410	45	492	146	259	149	45
8	213	620	868	728	727	413	261	712	138	93	541	269	195	225	49
16	1085	1400	1613	699	665	140	548	1287	518	2798	2071	1143	265	939	86
32	1690	866	641	3258	1213	520	1023	965	1178	2143	1532	1591	342	1600	227
64	4411	4835	1819	726	2963	791	1385	2061	1146	1276	1523	3487	1120	4468	2675
128	969	2735			1899	1572									
256															
Total	8553	11328	6176	5792	8101	4175	3715	6165	3711	6529	6561	6778	2528	7521	3138

Appendix 5.17 Percentage frequency of each substrate fraction (by weight) for the five high status sites studied in March 2003.

High status rivers	Owengarve River				Dunneill River				Castlebar River			
Sieve (mm)	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %
0.03	0.08	0.75	0.24	0.36	0.02	0.01	0.01	0.01	0.02	0.00	0.00	0.01
0.063	0.29	2.65	0.44	1.13	0.09	0.02	0.04	0.05	0.03	0.02	0.02	0.02
0.125	1.04	10.82	1.53	4.46	0.20	0.05	0.13	0.13	0.03	0.03	0.02	0.03
0.25	1.74	11.63	1.24	4.87	0.62	0.12	0.54	0.43	0.10	0.06	0.02	0.06
0.50	2.53	10.36	0.64	4.51	2.25	0.41	1.83	1.50	0.64	0.30	0.09	0.34
1	4.52	9.21	1.24	4.99	4.93	1.53	2.52	2.99	1.89	0.58	0.44	0.97
2	2.86	3.91	0.58	2.45	3.19	2.70	1.23	2.37	2.20	0.39	0.82	1.13
4	9.95	8.87	2.41	7.08	0.60	1.82	5.15	2.52	6.84	1.57	3.18	3.86
8	11.86	12.38	3.80	9.35	0.67	5.46	5.70	3.95	7.37	2.05	6.47	5.30
16	24.43	29.42	13.08	22.31	0.20	4.36	13.46	6.01	10.54	5.91	11.07	9.17
32	30.49	0	7.58	12.69	7.86	11.23	22.23	13.77	16.12	11.49	22.37	16.66
64	10.20	0	24.55	11.58	79.37	8.52	47.15	45.01	42.48	47.95	55.52	48.65
128	0	0	42.67	14.22	0	42.36	0	14.12	11.75	29.64	0	13.80
256	0	0	0	0	0	21.41	0	7.14	0	0	0	0
Total	100	100	100	100	100	100	100	100	100	100	100	100

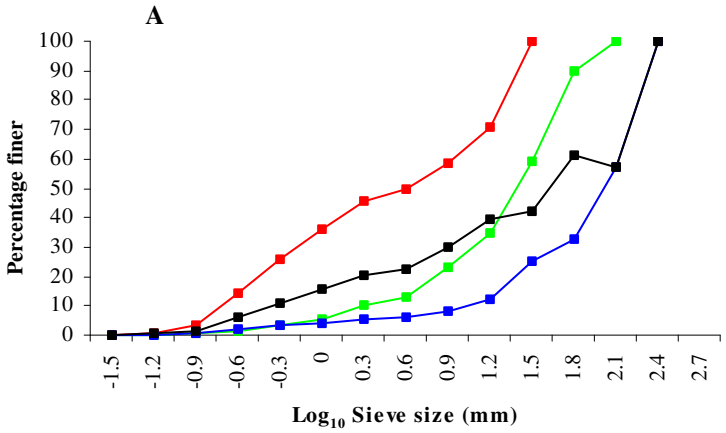
High status rivers	Callow Loughs Stream				Brusna River			
Sieve (mm)	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %
0.03	0.02	0.00	0.02	0.01	0.01	0.00	0.01	0.01
0.063	0.03	0.09	0.08	0.07	0.03	0.05	0.04	0.04
0.125	0.03	0.29	0.33	0.22	0.04	0.05	0.06	0.05
0.25	0.10	0.35	0.71	0.38	0.07	0.05	0.11	0.08
0.50	0.64	0.38	1.95	0.99	0.40	0.14	0.44	0.33
1	1.89	0.67	3.02	1.86	2.64	0.27	1.85	1.59
2	2.20	0.58	1.81	1.53	1.54	0.07	0.93	0.85
4	6.84	0.41	4.11	3.78	3.07	0.68	2.25	2.00
8	7.37	1.27	2.22	3.62	2.41	0.53	1.46	1.47
16	10.54	11.01	0.15	7.23	7.35	3.52	4.96	5.28
32	16.12	38.45	9.03	21.20	7.64	10.69	9.10	9.14
64	42.48	46.51	32.89	40.62	29.04	57.37	48.76	45.06
128	11.75	0	43.51	18.42	45.75	26.60	30.03	34.12
256	0	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100	100

Appendix 5.18 Percentage frequency of each substrate fraction (by weight) for the five impacted sites studied in March 2003.

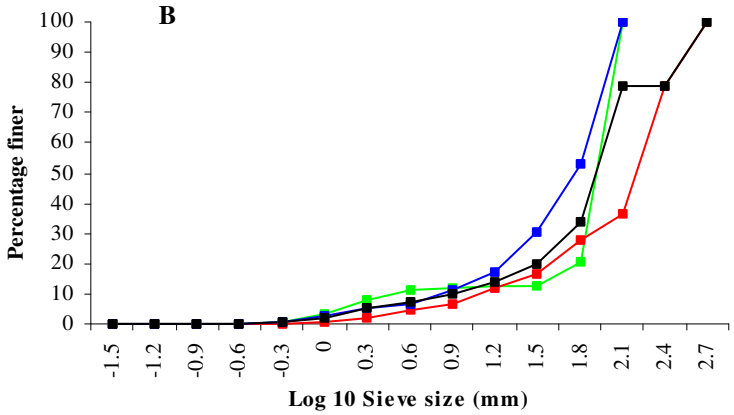
Impacted rivers	Cartron River				Lough na Corralea Stream				Mad River			
Sieve (mm)	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %
0.03	0.01	0.01	0.00	0.03	0.02	0.00	0.02	0.02	0.00	0.02	0.03	0.02
0.063	0.04	0.08	0.02	0.08	0.07	0.02	0.07	0.05	0.03	0.05	0.03	0.04
0.125	0.09	0.21	0.03	0.20	0.19	0.02	0.12	0.11	0.03	0.10	0.08	0.07
0.25	0.11	0.39	0.34	0.63	0.38	0.21	0.26	0.28	0.11	0.15	0.24	0.17
0.50	0.16	0.58	2.20	1.11	1.13	0.16	0.51	0.60	0.48	0.60	1.16	0.75
1	0.09	1.01	2.67	1.25	2.37	0.33	1.01	1.24	1.72	2.79	4.02	2.84
2	0.15	0.78	2.77	3.64	4.26	2.05	1.04	2.45	1.43	2.97	3.10	2.50
4	1.51	4.64	11.97	6.69	2.80	3.80	4.79	3.80	9.61	11.82	11.05	10.83
8	2.49	5.47	14.05	12.20	9.89	12.57	8.97	10.48	7.03	11.55	3.71	7.43
16	12.69	12.36	26.12	14.83	9.82	12.07	8.21	10.03	14.75	20.88	13.96	16.53
32	19.76	7.64	10.38	26.91	12.46	56.25	14.97	27.89	27.54	15.65	31.75	24.98
64	51.57	42.68	29.45	26.52	18.95	12.53	36.58	22.69	37.28	33.43	30.89	33.87
128	11.33	24.14	0	5.91	37.65	0.00	23.44	20.36	0	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100	100	100	100	100	100

Impacted rivers	Robe River				Mullaghanoe River			
Sieve (mm)	Sample 1 %	Sample 2 %	Sample 3 %	Mean %	Sample 1 %	Sample 2 %	Sample 3 %	Mean %
0.03	0.03	0.02	0.01	0.02	0.04	0.01	0.00	0.02
0.063	0.09	0.08	0.07	0.08	0.08	0.05	0.06	0.06
0.125	0.17	0.20	0.16	0.18	0.24	0.19	0.19	0.21
0.25	0.37	0.46	0.30	0.38	0.63	0.44	0.32	0.46
0.50	0.66	0.98	0.41	0.68	2.85	0.43	0.32	1.20
1	0.80	2.36	0.60	1.25	6.13	0.41	0.29	2.28
2	0.55	2.04	0.53	1.04	3.76	0.33	0.61	1.57
4	0.69	7.50	2.15	3.45	10.25	1.98	1.43	4.55
8	1.42	8.25	3.97	4.55	7.71	2.99	1.56	4.09
16	42.85	31.57	16.86	30.43	10.48	12.49	2.74	8.57
32	32.82	23.35	23.47	26.55	13.53	21.27	7.23	14.01
64	19.54	23.21	51.45	31.40	44.30	59.41	85.25	62.99
128	0	0	0	0	0	0	0	0
256	0	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100	100

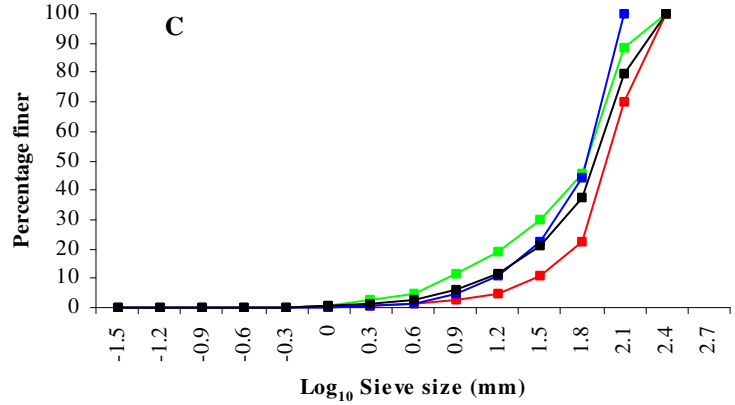
Appendix 5.19 Cumulative percentage frequency distribution of the substrate in the high status rivers measured during the sediment analysis programme.



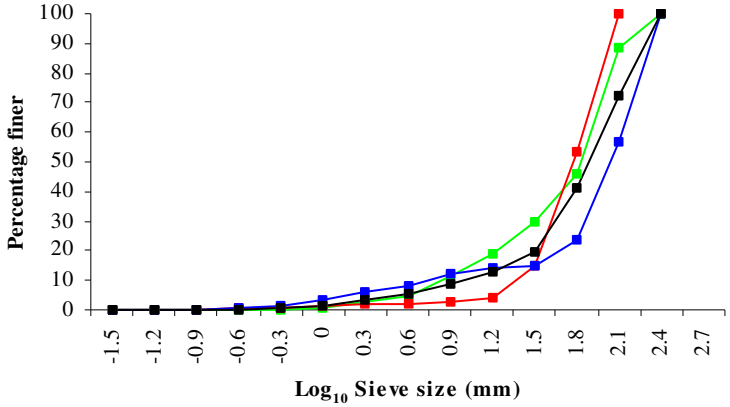
(A) Cumulative percentage frequency distribution of substrate in the Owengarve River, March 2003. Sample 1: Sample 2: Sample 3: Mean



(B) Cumulative percentage frequency distribution of substrate in the Dunneill River, March 2003. Sample 1: Sample 2: Sample 3: Mean

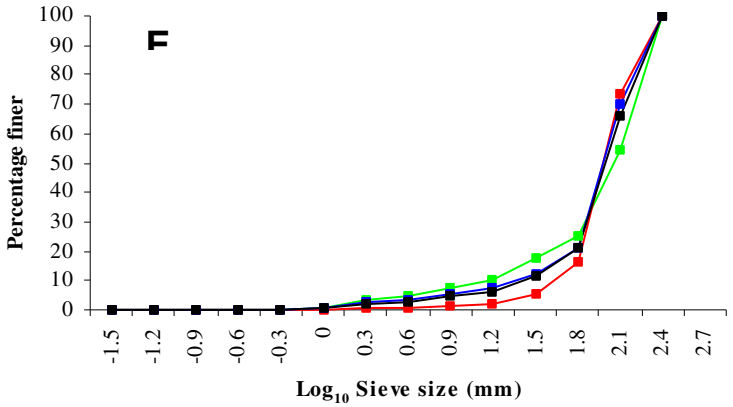


(C) Cumulative percentage frequency distribution of substrate in the Castlebar River, March 2003. Sample 1: Sample 2: Sample 3: Mean



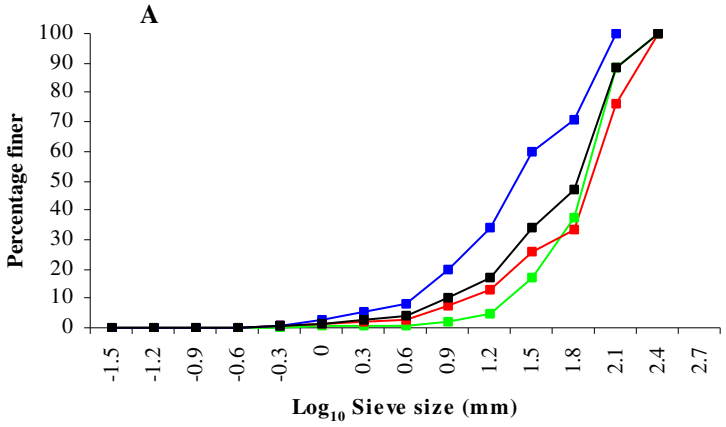
(D) Cumulative percentage frequency distribution of substrate in Callow Loughs Stream, March 2003. Sample 1: Sample 2: Sample 3: Mean

Appendix 5.19 Cumulative percentage frequency distribution of the substrate in the Brusna River (high status river) measured during the sediment analysis programme.

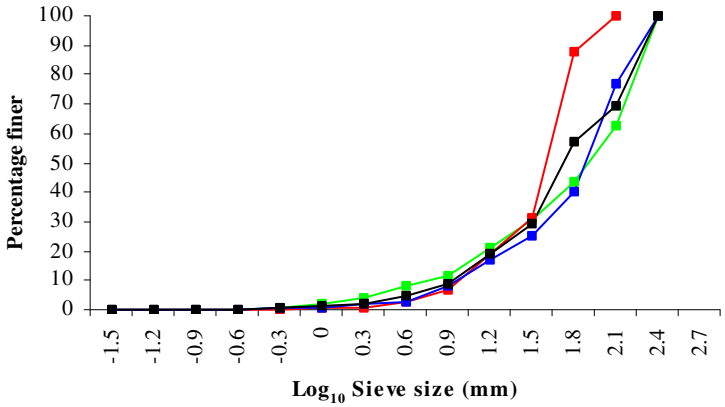


(E) Cumulative percentage frequency distribution of substrate in the Brusna River, March 2003. Sample 1: Sample 2: Sample 3: Mean

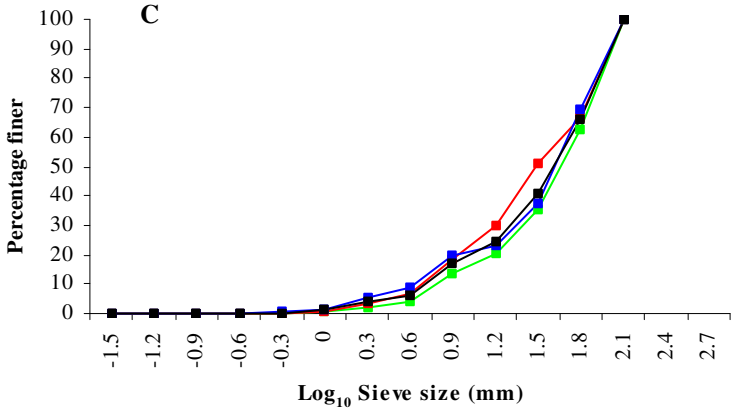
Appendix 5.20 Cumulative percentage frequency distribution of the substrate in the impacted rivers measured during the sediment analysis programme.



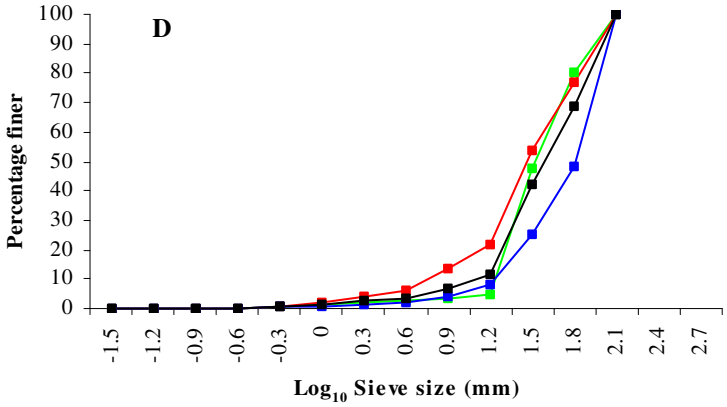
(A) Cumulative percentage frequency distribution of substrate in the Cartron River, March 2003. Sample 1: Sample 2: Sample 3: Mean



(B) Cumulative percentage frequency distribution of substrate in Lough na Corralea Stream, March 2003. Sample 1: Sample 2: Sample 3: Mean

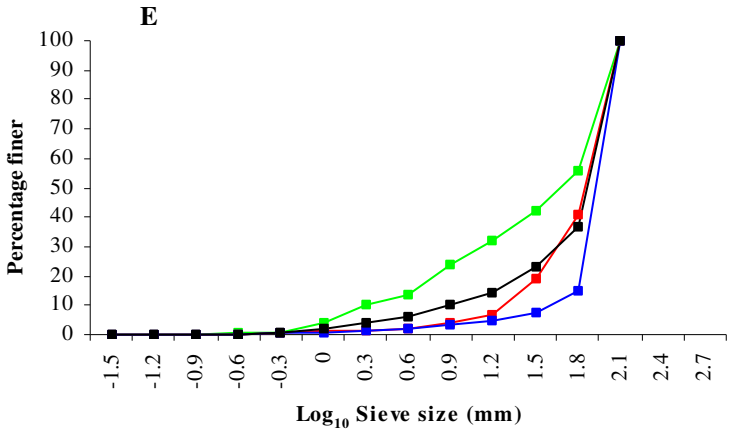


(C) Cumulative percentage frequency distribution of substrate in the Mad River, March 2003. Sample 1: Sample 2: Sample 3: Mean



(D) Cumulative percentage frequency distribution of substrate in the Robe River, March 2003. Sample 1: Sample 2: Sample 3: Mean

Appendix 5.20 Cumulative percentage frequency distribution of the substrate in the Mullaghanoe River (impacted rivers) measured during the sediment analysis programme.



(E) Cumulative percentage frequency distribution of substrate in the Mullaghanoe River, March 2003. Sample 1: Sample 2: Sample 3: Mean