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by

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The Water Quality Section of the Environmental RTDI Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in this area. The reports in this series are intended as contributions to the necessary debate on water quality and the environment.

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1 Summary

An important objective of this project was to obtain an improved understanding of why ecological communities depart from reference conditions as pollution and eutrophication impact 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 (P) 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.

The experiments did reveal surprising results showing the importance of nitrogen (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 molybdate-reactive P (MRP) concentrations (<0.05 mg/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 (WFD), there may be a case for introducing tighter regulations on the limits of nitrogen emitted to waterbodies 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, *E. 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 *Ecdyonurus* 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, *E. 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 cycles of both *E. insignis* and *E. 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 percentage Ephemeroptera/Plecoptera/Trichoptera (%EPT). Results from feeding and microhabitat investigations suggest that as eutrophication and the impacts of organic pollution progress, a change occurs in the feeding guilds and microhabitat preferences among the macroinvertebrate communities.

2 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 impact 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 (Schindler, 1985) and his fellow workers 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. Stockner and Shortreed, 1978; Elwood *et al.*, 1981; 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 10 µg/l PO_4-P above the background (1–4 µg/l P) in river water channels, either alone or in combination with NO_3-N, resulted in substantial increases in epilithic chlorophyll (Chl) *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 were taken from the river for water chemistry purposes. 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 in 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 such as 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: Dubé *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 (<0.05 mg/l P). This implies that a small proportion of high-status rivers are N-limited rather than P-limited. Thus, in terms of the WFD, there may be a case for introducing tighter regulations on the limits of nitrogen emitted to waterbodies 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.

2.1 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.

3 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 *E. 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 *E. 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-phenylindole (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 on 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 *E. 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, *E. venosus* can be classified as both a herbivorous grazer and a 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 to September 2003.

The *Ecdyonurus* 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 to 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 for 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 the 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. 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 were 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.

3.1 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 of 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.

4 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, *E. 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 *E. venosus* as having a bivoltine life cycle. It may be that the life cycle 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 *E. 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 *E.* or *E. insignis* in Ireland. *Ecdyonurus insignis* had a univoltine life cycle in the rivers studied in this investigation. Macan (1970) also described it as having a similar life cycle.

The absence of both *E. insignis* and *E. dispar* from the benthos during the summer and winter months suggests that these species develop quite differently to the more common species *E. 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 1973, 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, *R. 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. *Heptagenia* spp. emerged a bit later than *R. 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.

4.1 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 provides 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 (DO) 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 *E. insignis* and in particular *E. 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, *E. 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*.

5 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 total organic N (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. 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 *versus* 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 benthic 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 occurs.

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* spp. (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 land-use 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* spp. 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 may occur 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 be 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; Dadd, 1982; Lamberti and Moore, 1984; Cargill *et al*., 1985). 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.

5.1 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 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 DO 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.

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