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The 15th Environmental Researchers' Colloquium was hosted by Sligo Institute of Technology in January 2005. This annual meeting is organised by the host institution and by the Environmental Sciences Association of Ireland (ESAI) and it aims to provide postgraduate researchers with a supportive forum in which to present papers and learn about research being undertaken in other universities, both in Ireland and elsewhere.

Standards have always been high, but increasing funding for environmental research has meant that both research and resultant papers have tended to increase over time in quality and interest.

At the 2005 ESAI Annual General Meeting, the decision was taken to publish selected papers and an invitation was extended to all paper and poster postgraduate authors to submit short papers for review. All papers were peer-reviewed and went through an editing process comparable to that required by a scientific journal. In this way, students gained practical experience in publishing their work and ESAI was able to create a permanent record of research in progress and completed by some Irish postgraduates.

Much hard work went into the production of these proceedings, and many already busy people volunteered to take on tasks to ensure that a high standard of presentation was achieved. Special thanks are due to the paper authors, ESAI Council members and Chairperson, Sinead Macken, the referees, and to Cóilín MacLochlainn for his careful and thorough work in preparing the text for the printers. ESAI provided funds to cover formatting and publication costs.

It is the intention of ESAI to publish proceedings of the 2006 and subsequent Environmental Researchers' Colloquia.

Professor Richard Moles,
Editor

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THE ENVIRONMENTAL IMPACTS OF PRIVATE CAR TRANSPORT ON THE SUSTAINABILITY OF SATELLITE AND FAST-GROWING IRISH SETTLEMENTS

Walter Foley, B. O'Regan, Richard Moles and John Morrissey

ABSTRACT

Spiralling population growth amongst small-sized satellite settlements is a challenging issue for planners and scientists in contemporary Irish society. Data for a range of environmental indicators were collected for selected settlements from a 79-settlements sample nationwide. Identification of data required and data available, and the means of gaining information both necessary and valuable but currently not available, were prime aims of this research. An extensive questionnaire survey was carried out in each of the 79 settlements. Transport was identified as a critical parameter in settlement sustainability in Ireland. Utilising the data collated permitted evaluation of private car use at settlement level and provided important empirical evidence to support sustainability policies through the application of settlement-sensitive CO_2 (a greenhouse gas adopted in this study as an indicator for sustainability) emission values. Recent patterns of change in the distribution of people, likely to continue into the future, are increasing the extent to which personal transport is unsustainable by enhancing dependency on private cars and increasing distance between residences and journey destinations. This study will allow effective, practical recommendations to be made regarding the integration of sustainability goals into Irish settlement planning.

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INTRODUCTION

This paper reports on a study on the impacts of private transportation on the sustainability levels in a cross-section of Irish settlements located in three study clusters, namely, the polycentric Midland cluster and the monocentric Sligo and Limerick clusters (Fig. 1) (a monocentric model has one centre as its focal point, whereas polycentricity implies many centres).

An analysis is provided, for some of the results of this study, on the effects on sustainability of private transport choices in Irish settlements. This work is part of a larger study titled *Sustainability and Future Settlement Patterns in Ireland* (SFSPI).

The growth centre debate is not restrained to a simple concentration versus dispersion issue. The appropriate policy for one region or settlement may not necessarily be suitable for another region or settlement. The question is subsequently asked: "Does the proliferation of housing estates in smaller settlements and the creation of new residential settlements result in increased transport flows in contemporary Ireland?"

In order to address this question, the study undertook characterisation of the types of cars used and the total distance travelled in settlements. Aggregation of survey data for each of the 79 settlements into seven distinct settlement classes based on many variables including population size, population change from 1996-2002, distance to Gateway settlements and level of service was a critical exercise. The aggregation of the survey data ensured increased robustness of data and additionally made the wide-ranging data more user-compatible.

Service levels were measured using a Service Index. This index is a useful stand-alone tool for settlement classification. Settlements were ranked according to levels of specific tertiary services exclusively. The Service Index, which accounts for a range of settlement services, provided an ideal medium for transport behaviour comparisons. (The service level had a range of scores from 4 to 79 for all settlements).

METHODOLOGY

Individual questionnaire responses were divided on the basis of the settlement class in which respondents lived. For each settlement class, responses were selected where the private car was the travel mode, either driver alone or with passengers. Engine capacities were then aggregated into capacity classes using fuel consumption as the primary control. Consumption values were taken from the Sustainable Energy Ireland report *Analysis of New Car Registrations in Year 2000* (SEI 2003).

For each settlement class, data on the number of cars in each engine class were linked with data on total distance travelled per week. To calculate emissions per settlement, Small Area Population Statistics (SAPS) data (CSO 2002) on proportions of individuals using private car as their mode of transport were combined with SFSPI questionnaire data.

Taking an average value (in relation to fuel type) for all cars for CO_2 emission values, and accounting for average consumption with respect to year of registration, and making the assumption that the private car fleet accounts for 50% of road transport, an estimate for road transport CO_2 emission was derived for each individual, and subsequently each settlement, based on the following equation:

Total kilometres (km) ∗ fuel consumption (l/km) ∗ CO_2 produced by combustion of a litre of petrol (t-CO_2/litre) = tonnes CO_2 emissions (t-CO_2).

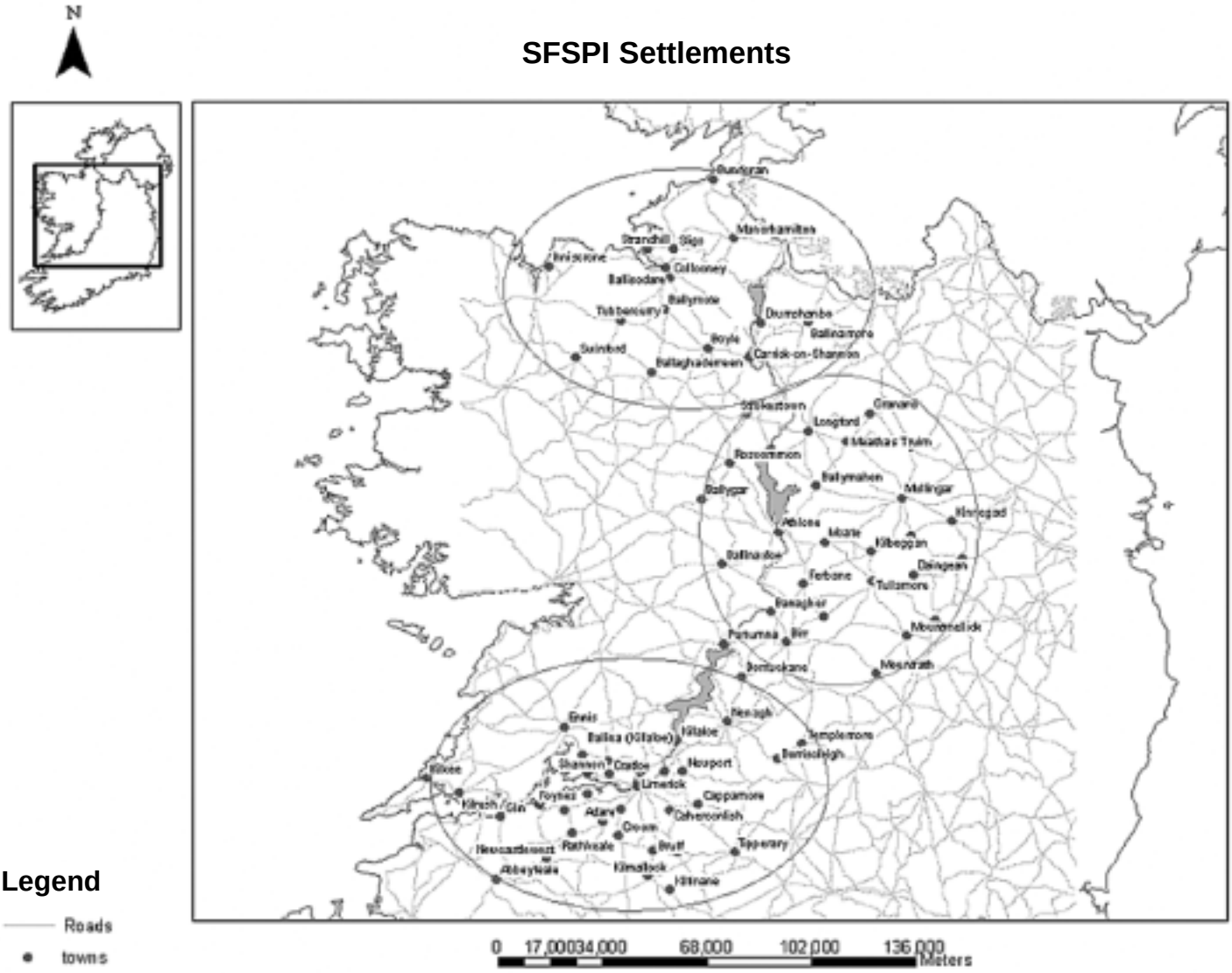

Fig. 1 – Distribution of the 79 SFSPI settlements.

RESULTS AND DISCUSSION

Transport related t-CO_2 emissions were calculated for all 79 settlements. A pattern emerged which suggested that residential satellite settlements and/or fast-growing settlements have an impact on road transport t-CO_2 emissions (Table 1 and Fig. 2).

Satellite settlements, and in particular satellite settlements which exhibited population growth during the period 1996-2002, are encouraging increased car dependence. Compare the CO_2 emissions in Table 1 to Tullamore, Mullingar or Sligo, which all are established large settlements, have a population above 10,000 and road transport emissions of 1.20, 1.27 and 1.15 tonnes-CO_2 per capita respectively. The population growths for Tullamore, Mullingar and Sligo, for the period 1996-2002, were 10.5%, 25.0% and 6.6% respectively.

Moreover, there is no evidence to suggest that the trend of increasing satellite centres will cease. In fact, of all settlements studied, those with highest population growth between 1996 and 2002 (that is, over 30%) were satellites, providing dormitory functions for larger settlements nearby. Some had recent rapid population growth, but others not. All showed relatively high dependence on private car transport (over 40% of journeys to work, school and college were by car), little use of public transport, and few journeys on foot. As a result, per capita CO_2 emissions related to transport were high. Of the ten settlements with the highest per capita CO_2 emissions, all were satellites within 5-15km of a larger settlement, with Kinnegad and Rochfortbridge being close to a main road serving Dublin. In monocentric clusters, of respondents residing in a satellite but working in a regional centre: more than 70% travelled 5-10km to Sligo and 5-15km to Limerick. Very few individuals both resided and worked within the satellites, presumably because they are not sited around industrial or other sources of employment. Satellite settlements also had low Service Index scores (Table 1) so that it can be presumed that longer journeys were not only required to destinations of work, but to all other destinations, for example, shops and recreation. Interestingly, satellite settlements with lower growth had slightly higher service levels (Table 1). These settlements may be older and more established satellites and accordingly more suitable for future development. However, there is no indication from the CO_2 transport emissions that car dependency is reduced in the higher service level satellites.

Table 1 – The settlements with the highest transport related t-CO_2 emissions from the 79 settlements sample are all satellite settlements.

Settlement	Satellite of:	CO_2 Per Capita, t-CO_2	% Population Growth or Decline 1996-2002	Service Level
Annacotty	Limerick	1.55	129	4
Newmarket-on-Fergus	Shannon/Limerick	1.56	-3	17
Moate	Tullamore/Athlone	1.58	5	14
Kinnegad	Dublin/Mullingar	1.62	151	11
Rochfortbridge	Dublin	1.71	91	12
Sixmilebridge	Shannon/Limerick	1.79	16	20
Adare	Limerick	1.99	6	27
Castleconnell	Limerick	2.09	-5	13
Ballina	Limerick	2.14	98	6
Strandhill	Sligo	2.39	31	11

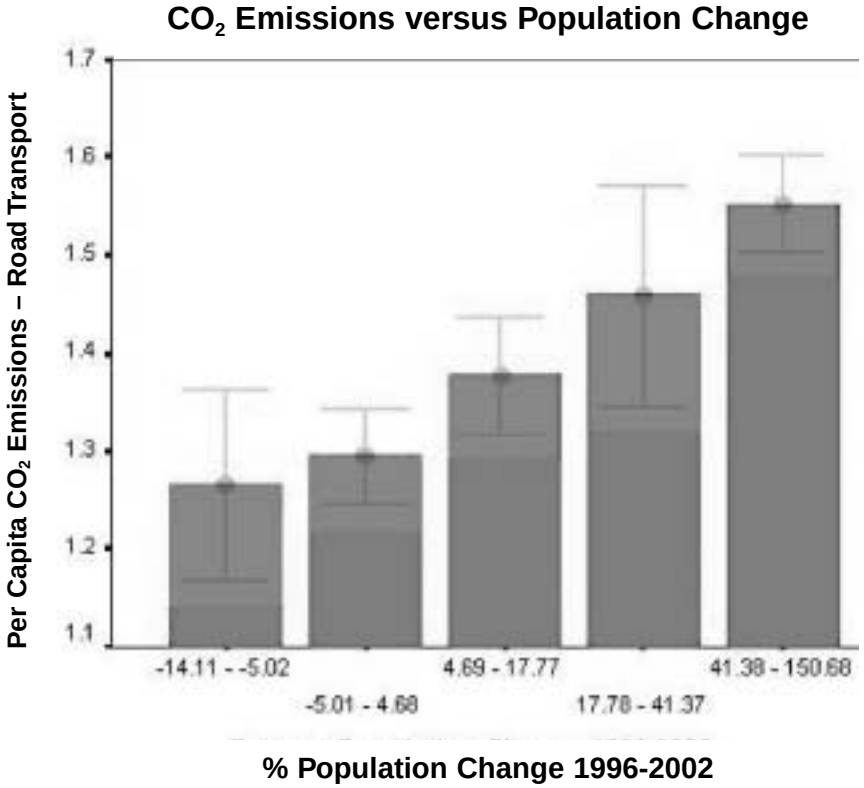

Fig. 2 – Population Change in relation to per capita road transport emissions. (Error bars are ± 1 standard error.)

CONCLUSION

In contemporary Ireland, transport within and between settlements is an important source of carbon dioxide emission and without the implementation of strict transport and land use policy regulations the situation will worsen not only in satellite and fast-growing settlements but in all settlement types.

ACKNOWLEDGMENTS

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PHYTOPLANKTON SPRING BLOOM DECAY IN THE LIFFEY ESTUARY

Tim O'Higgins, Jim Wilson and S.A. Ward

ABSTRACT

A YSI 6600 multiprobe sonde was deployed in the Liffey estuary in order to determine the timing and magnitude of the phytoplankton spring bloom. A diatom bloom with total phaeopigment concentration up to 77.9 mg m^{-3} developed from the 23rd of April until the 3rd of May 2004 and declined over the following six days. *Leptocylindrus danicus* was the principal species. Of the total phaeopigments, only 20% was made up of chlorophyll *a*. The bloom was triggered by a reduction in tidal stirring and an increase in surface water temperature, indicating the onset of stratification and typical spring bloom pattern. The bloom lasted 10 days and its decay coincided with a short period of anoxia. The observed pattern is consistent with the advection into the well-mixed water of the Liffey estuary of an offshore phytoplankton bloom and its subsequent decay.

Key words: spring bloom; eutrophication; Liffey; phytoplankton

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INTRODUCTION

Under the EU Water Framework Directive (WFD EC/2000/60), phytoplankton are the primary biological quality element in determination of the ecological status of transitional waters (i.e. waters in the vicinity of a river mouth which are partly saline in character). "High" status is afforded the water body if phytoplankton are consistent with undisturbed conditions; slight changes in biomass from undisturbed conditions lead to "good" quality status; and, where phytoplankton cause an "undesirable disturbance," "moderate" quality is assigned. The directive also dictates that the frequency of operational sampling be sufficient to produce an "acceptable level of confidence and precision" in monitoring of transitional waters. Changes in the estuarine environment occur on timescales from those of ebbing and flowing tides (hours), spring neap cycles (weeks), to seasonal changes (months) and inter-annual changes (Cloern *et al.* 1985) and are often influenced by stochastic processes such as rainfall and riverine input. Hence traditional infrequent spot sampling has the potential to alias data which may occur on timescales shorter than the sampling interval.

The Liffey estuary stretches 11km from its freshwater end to its mouth in Dublin Bay. The estuary is a highly modified environment which receives effluent from a municipal wastewater treatment plant and a thermal power station along its length. A range of biological problems exists in the estuary, from intense summer phytoplankton blooms in the upper estuary (O'Higgins & Wilson 2005), to hypoxic and anoxic sediments (Wilson *et al.* 1986). Results are presented here from a YSI 6600 multiprobe sonde which was deployed in order to determine the timing and magnitude of the spring bloom in the Liffey.

MATERIALS AND METHODS

The sonde was deployed at the North Bank Lighthouse (Fig. 1) which is adjacent to an outflow of combined thermal and sewage effluent. This site is 7.8m deep and vertically well mixed (Crisp 1979). The sonde was deployed on the 1st of April 2004, hanging freely from the lighthouse until the 12th of May, and was set to record temperature, salinity, dissolved oxygen, fluorescence and depth at intervals of 15 min. Samples for chlorophyll *a* and phytoplankton were collected in Dublin Bay on board the R.V. *Celtic Voyager* from the ship's underway water supply. One-litre samples for the determination of chlorophyll and phaeopigment concentrations were filtered onto Whatman GF/F filter papers and measured according to the method of Aminot and Rey (2000). The fluorescence signal was converted to an estimate of total phaeopigments by reference to data collected in Dublin Bay on the 1st of May 2004, with the mean measured total phaeopigment concentrations being equated to the mean measured fluorescence signal. Phytoplankton were settled according to the method of Utermöhl (1931), enumerated under inverted phase contrast microscopy and identified according to Tomas (1997). Estimates of phytoplankton carbon were made for different phytoplankton species using biovolume calculations (Kovala and Larrance 1966). Tidal velocities (u) were estimated by plotting mean values of hourly published tidal velocity data (ERU 1992) for spring and neap tides against tidal range for these dates. A linear fit was produced and applied to measured tidal range data from the sonde. These u values were used to calculate a stratification parameter $1/u^3$ (Simpson & Hunter 1974).

RESULTS

Salinity varied between 34 and 32 over a typical tidal cycle with a mean salinity of 32.7 leading to a classification of euhaline under the WFD. Dissolved oxygen concentrations also varied over a tidal cycle, with the fresher waters having values approximately 10% lower than the more saline waters. A daily cycle in temperature also occurred, with fresher waters being warmer than the more saline waters due to differential heating of land and sea.

During the early part of the study period daily average water temperature was negatively correlated with daily tidal range ($r^2 = 0.7331$, $p<0.001$). From the 19th of April to the 26th of April there was an increase in daily average temperature of 0.2°C. Minimum tidal ranges occurred on the 28th of April and water temperatures remained consistently

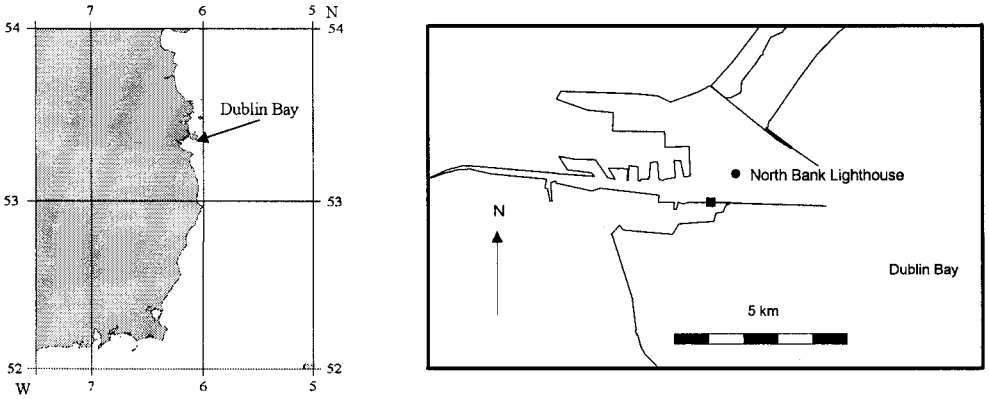

Fig. 1 – a) Location of Dublin Bay. b) The Liffey estuary showing position of sonde (large black dot) at the North Bank Lighthouse. The black square marks the combined power station and sewage outflow.

at or above 10.8°C following this date. At this time the relationship with tidal velocity became weaker and less significant ($r^2 = 0.4936$, $p<0.05$) (Fig. 2a). Maximum daily averaged temperature of 11.7°C (s.d. 0.8) occurred on the 2nd of May. Highest temperature values on this date were associated with thermal inputs from the local power station. Following initial low total phaeopigment concentrations, values began to rise on 25th of April. Total phaeopigments rose continuously until the 3rd of May, reaching a maximum 77.9 mg m^{-3} (Fig. 3). During the time of maximum fluorescence, maximum total phaeopigment concentrations were associated with maximum salinities indicating a marine origin for the phaeopigments. The total phaeopigment concentrations measured at near peak levels (1st of May) had a mean composition of 20% chlorophyll *a,* suggesting phytoplankton population in decline. The phytoplankton were dominated by small diatoms, with the principle species being *Leptocylindrus danicus*, making up 95.6% of total estimated phytoplankton carbon, and *Thalassionema nitzschoides*, making up a further 2%. The estimated maximum phytoplankton carbon concentration as calculated by biovolume was 906 mg C m^{-3}. Anoxia occurred for a brief period in the early hours of the morning on the 2nd of May (Fig. 3). The period of anoxia was accompanied by a drop in salinity coupled with a rise in temperature of 4.5°C. At this time temperature and salinity were negatively correlated ($p<0.001$, $r^2 = 0.737$); the freshwater temperature predicted from this relationship was 31.6°C, indicating a non-natural source of thermal input. There was a strongly significant correlation between the range in daily average total phaeopigment concentration and daily range in dissolved oxygen ($r^2 = 0.648$, $p<0.001$).

DISCUSSION

The co-occurrence of the change in the linear relationship between tidal range and surface water temperature (Fig. 2a) and the minimum tidal current velocities (Fig. 2b) indicates that the onset of neap tides was instrumental in promoting thermal stratification. The fluorescence signal fits the pattern of a typical marine spring diatom bloom. Such blooms generally occur in temperate shelf seas when thermal stratification occurs and phytoplankton present in the surface layer of the water column are no longer mixed below the "critical depth" (Sverdrup 1953). While Dublin Bay itself is not thermally stratified (due to the shallow water column and strong tidal currents), stratified waters are widespread in the western Irish Sea (Horsburgh *et al.* 2000) and advection of Irish Sea waters is of great importance in Dublin Bay (Wilson 2005).

It is reasonable to assume that the date of minimal tidal stirring (28th of April) was the date of the peak bloom in plankton since reduced vertical mixing would lead to maximum exposure to light for the phytoplankton on this date. However, there was a 5-d lag between minimum tidal range and maximum fluorescence, which suggests that the bloom took place offshore and was advected into Dublin Bay. Modelled residual currents from waters in the vicinity of Dublin Bay reach values of up to 8 cm s^{-1} (Horsburgh *et al.* 2000), meaning that in 5-d a bloom could travel up to 34.5km; however, the actual origin of this bloom is not known.

The short-lived period of anoxia, the relationship between dissolved oxygen saturation and fluorescence, and the predominance of phaeopigments, rather then chlorophyll *a,* all point to a phytoplankton population that was in decline rather than actively growing during the peak in the fluorescence signal. This suggests a dying bloom producing an oxygen demand rather than supply.

The hypoxia observed on the 2nd of May during the fluorescence peak could have deleterious consequences for macrofauna in the Liffey estuary, particularly for sedentary species. Reduction in secondary producers such as filter-feeding polychaetes or molluscs can decrease an estuary's capacity to absorb any increase in primary productivity due to eutrophication (Cloern 2001). However, this hypoxia is not solely attributable to the decline of the phytoplankton bloom, which, given the stoichiometry of organic matter breakdown, would have an oxygen demand of approximately 0.9 mg l^{-1} constituting approximately 15% of dissolved oxygen in saturated waters with salinity 28 at 12°C (approximately the conditions observed at the time). This suggests an additional local source of biological oxygen demand which may be exacerbated by increased temperatures due to the input of thermal effluent from the local power plant. It should be noted, however, that spikes in the temperature signal occurred throughout the study period but only this one (coincidental with the spring bloom) produced a dramatic decrease in oxygen concentrations.

The observed bloom appears to be of non-anthropogenic nature, having been advected into Dublin Bay from the Irish Sea, but its consequences may certainly be considered an "undesirable effect". A combination of oxygen demand from the sewage effluent, high natural phytoplankton abundance and increased temperatures due to the addition of cooling waters from the local power station combined to produce anoxic conditions for a short period during the spring bloom.

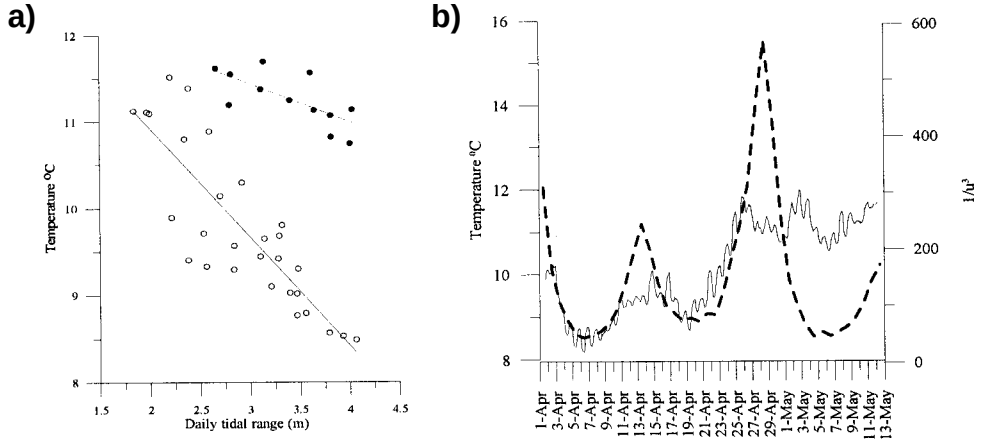

Fig. 2 – a) The relationship between daily tidal range and mean daily water temperature in April (open circles) and May (solid circles). b) Mean daily measured temperature (solid) plotted with the stratification parameter 1/u3 (dashed).

CONCLUSION

The use of electronic instrumentation has allowed observation of estuarine processes on temporal scales smaller than those of traditional spot sampling. This has demonstrated the sensitivity of the Liffey system to organic loading from natural and anthropogenic sources which can combine to produce undesirable effects.

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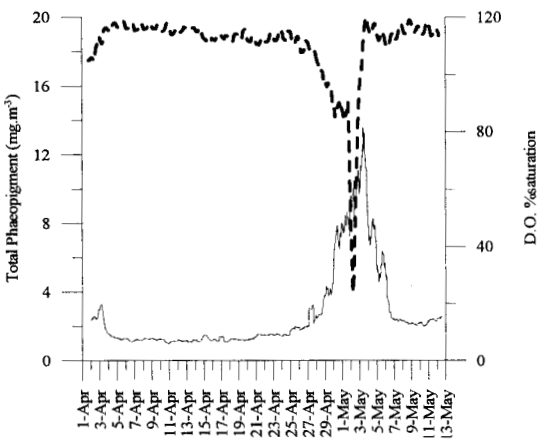

Fig. 3 – Mean daily dissolved oxygen % saturation (dashed) and running average total phaeopigment concentration (solid).

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TEMPORAL MONITORING OF TOTAL DISSOLVED COPPER IN SURFACE SEAWATER OF GALWAY BAY, IRELAND, USING A MOBILE VOLTAMMETRIC ANALYSIS SYSTEM

Sarah Knight, R. Cave, N. Morley and D. Leech

ABSTRACT

A mobile voltammetric laboratory, using Adsorptive Cathodic Stripping Voltammetry (AdCSV), has been assembled for the real-time analysis of dissolved copper from fresh to marine waters. This system can be used for both shipboard and shore-based monitoring. Results are presented here from 13-hour tidal cycle surveys of total dissolved copper in surface seawater, completed at two selected sites in Galway Bay, west coast of Ireland. The first site, on the north shore of Galway Bay, was located at the outflow of the Corrib River, which passes through Galway City. In contrast to the first site, at the second site near the mouth of a small bay on the south side of Galway Bay there are no direct riverine freshwater sources. However, fresh groundwater flow out of the karst limestone bedrock into this small bay is significant. The distinctly different geographies and geologies of these two sites are reflected in the contrasting correlations of total dissolved copper with salinity. We show that using dissolved copper in conjunction with salinity as a chemical tracer of water-masses in coastal waters allows us to discern between "clean" versus anthropogenically-impacted freshwater inputs.

Key words: copper; Adsorptive Cathodic Stripping Voltammetry (AdCSV); Galway Bay; *in situ;* tidal cycles

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INTRODUCTION

Copper is present in seawater in nanomolar concentrations, primarily in the dissolved phase as organic complexes of the 2+ ion (Donat & Bruland 1995). Marine biota require copper in small concentrations; however, dissolved copper may also be toxic, as it has been found to antagonistically compete with other essential trace elements at cellular uptake sites (Sunda & Huntsman 1998), and can also cause oxidative damage by interfering with the normal function of anti-oxidant defence enzymes (Company *et al*. 2004) and through the production of toxic hydroxyl radicals (Geret *et al*. 2002). Biological availability and hence nutritive or toxic potential is largely dependent on the dissolved phase speciation (Eriksen *et al*. 2001; Stauber *et al*. in press).

In addition to the biological significance, the speciation of copper in seawater may also have physical applications. Generally the organic ligands that dominate copper speciation bind to copper much more strongly than do inorganic ligands, and this strong complexation is reflected in the magnitude of the conditional stability constants of the strongest class of organic copper complexes, which are typically of the order of 10^{12}–10^{14} (Muller, 1996; Vasconcelos *et al*. 2002; Zamzow *et al*. 1998).

The strength of the organic copper complexes render them chemically resilient, allowing for the possibility of dissolved copper to serve as a physical tracer of water masses and their movement. In southern Spain, dissolved copper and other trace metals have been used to trace the metal enriched Tinto/Odiel river plume through the strait of Gibraltar and into the Western Mediterranean (Elbaz-Poulichet *et al*. 2001).

For trace metal analysis in seawater, Adsorptive Cathodic Stripping Voltammetry (AdCSV) is a frequently used technique as it is very sensitive and generally no sample preconcentration or matrix dilution is required. The increased sensitivity of this technique over other voltammetric methods lies in the adsorption step of the determination. During this step the potential at the working electrode is set at a voltage that promotes adsorption of the analyte ion/complex onto the surface of the working electrode, thus concentrating the analyte at the electrode out of the bulk solution. For copper, only two reagents need be added to the sample: an electrolyte/pH buffer and an excess of a model-complexing ligand to bind with all of the available copper ions. The signal output is recorded as the magnitude and voltage position of the current response due to the forced reduction at the working electrode of the copper ions in the model complex. The magnitude of the current is proportional to the concentration of the model copper complex in solution, and precise determinations are completed using standard additions. For total (as opposed to labile) determinations of dissolved copper in natural seawater, the sample is filtered to remove particulate material and UV-digested to break down organic material and release the copper ions, prior to reagent addition.

For accurate determinations of copper and all trace metals in seawater, greater care must be taken during all aspects of sample collection, storage, transport and treatment to ensure that the risk of contamination is kept to a minimum. For this reason, *in situ* analyses of dissolved copper in seawater are preferable to land-based analyses of previously collected discrete samples. Various voltammetric techniques lend themselves well to adaptation for *in situ* use as the instrumentation is small and relatively portable, and little sample pre-treatment is required.

A description of the mobile voltammetric laboratory assembled for the purpose of ship-board or shore-based monitoring of total dissolved copper in fresh and sea waters is given below. This laboratory has been successfully used for both spatial and temporal surveys. Results are presented from two shore-based tidal surveys, where total dissolved

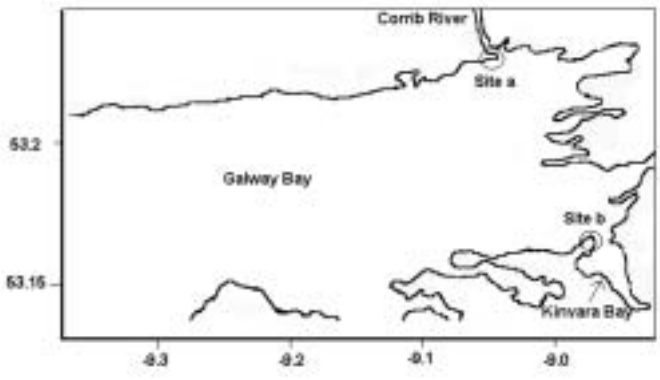

Fig. 1 – Study area (Galway Bay site (a) and Kinvara Bay site (b)).

copper and salinity were monitored over a 13-hour period, and advantages of using total dissolved copper in addition to salinity for tracing water masses are discussed.

MATERIALS AND METHODS

STUDY AREA

Shore-based surveys have been completed at a number of sites around Galway Bay, located on the west coast of Ireland. Results are presented here from two tidal surveys completed at (a) the Corrib River outflow at the Galway Harbour Docks, Galway City, and (b) Parkmore Quay, at the mouth of Kinvara Bay, on the southwestern shores of Galway Bay (Fig. 1). The only major city on Galway Bay is Galway City, which is Ireland's fourth largest with a population of ~70,000 (Central Statistics Office 2003). Mutton Island Sewage Treatment Plant officially opened in May 2004, and provides primary and secondary treatment for Galway City's wastewater. The Corrib River drains a largely granitic catchment area of ~3000 km^2 with an average discharge of 74 m^3/s (Marine Institute 1999). In contrast to the northern side of Galway Bay, the bedrock of the southern side is karst limestone. There are no riverine sources of freshwater in Kinvara Bay, but because of the porous limestone geology the fresh groundwater input to the bay is significant (Cave, personal communication). Situated at the head of this small bay sits the village of Kinvara, with a population of ~450 (Central Statistics Office 2003). There are no sewage treatment facilities for the town proper, and the waste from some residences, businesses and a local hotel are pumped directly into the bay (Roden 1999).

REAGENTS

All reagents were made up in water purified by reverse osmosis (Milli-RO, Millipore) followed by ion-exchange (Milli-Q, Millipore). 8-hydroxyquinoline (Fisher Scientific) was used as the complexing ligand and 2-amino-2-hydroxymethyl-1,3-propanediol (Fisher Scientific) as the pH buffer/electrolyte (pH 8.2). An aqueous stock solution of 10 mM 8-hydroxyquinoline (oxine) was prepared in 0.15 M Ultrar (Merck) grade HCl, and a 1.0 M aqueous stock solution of 2-amino-2-hydroxymethyl-1,3-propanediol (tris) in 0.48 M HCl. A Cu(II) stock solution of 10 µM was prepared by dilution of a 1000 ppm atomic-adsorption standard solution (Merck) in Milli-Q water and acidified using 1 µl concentrated HCl per ml solution. A second solution of 0.5 µM Cu(II) was prepared by dilution of the 10 µM solution in Milli-Q water and similarly acidified.

All containers and equipment that came in contact with the sample and/or reagents had been previously acid-cleaned, and precautions to ensure minimal risk of sample contamination were taken at all stages of analyses.

MOBILE AdCSV LABORATORY

Copper determinations were completed using AdCSV with a Metrohm 757VA Computrace combined electrode/potentiostat unit connected to a supply of nitrogen gas and controlled by a Dell Latitude portable PC. The working electrode was a hanging mercury drop, the counter electrode a platinum wire, and the reference electrode a silver wire. Square wave pulse modulation was used for all determinations as this scanning waveform typically yields the greatest sensitivity.

For shore-based monitoring, all instrumentation was transported and set up in the back of a Ford Transit van and powered using a generator. The inner two-thirds of the walls, ceiling and floor of the van were covered with sheets of clear polyethylene, and this area is herein referred to as the "outer clean area". This space housed the "inner clean area", which consisted of a previously constructed wooden frame

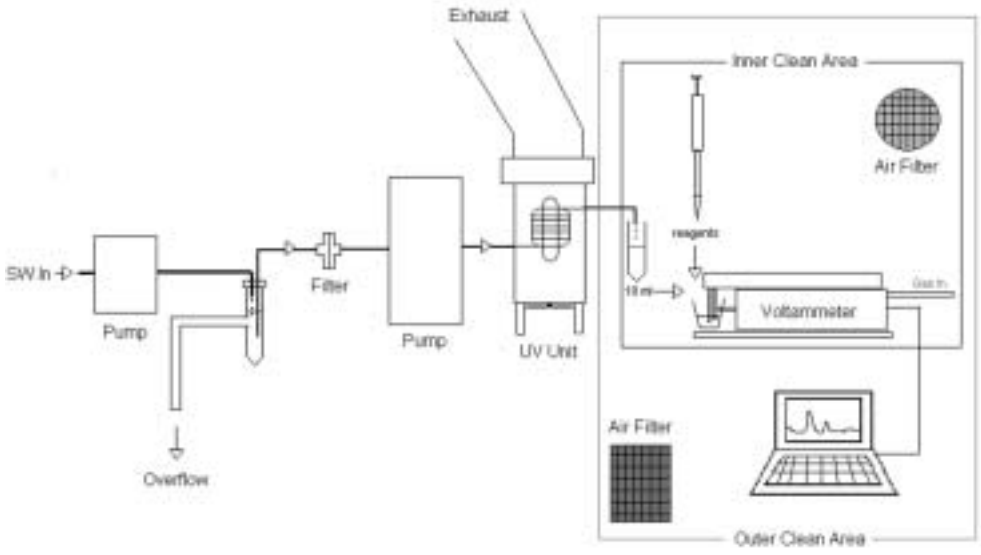

Fig. 2 – Mobile AdCSV laboratory.

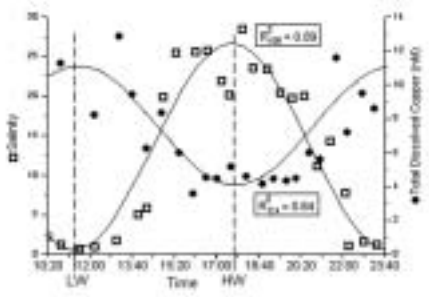

Fig. 3 – Galway Docks site time-series of Total Dissolved Copper and Salinity.

covered in sheets of polyethylene. Both the inner and outer clean areas were fed with clean air from portable HEPA air filter units. All manual sample manipulations and AdCSV analyses were completed in the small inner clean area, with the researcher and PC situated in the outer clean area.

Surface seawater was continuously sampled through a 10m length of polypropylene tubing (i.d. 1.2cm) using a peristaltic pump. The end of the tubing was submerged ~30cm below the surface of water and attached to a buoy which was anchored at a distance of ~1m from the side of the pier.

Fig. 2 is a diagram of the set-up of the mobile laboratory in the rear of the van. The water was pumped through the first length of tubing and into a collection vessel. A second peristaltic pump subsampled from this vessel and pulled the sample through an in-line filter fitted with a 0.45 µm membrane filter to remove particulates. From there the sample entered the purpose-built UV digestion unit. This unit consists of a quartz coil (total length ~3.5m, internal diameter 1.0mm) through which the sample travels around a 260 W mercury discharge tube (Ultralight AG). The lamp and coil are housed in an aluminium casing, and the unit is cooled with a fan fitted at the bottom of the housing, and exhausted via a length of exhaust tubing fitted to the top. The sample exited the UV unit and was collected in a second vessel, housed in the inner clean area. In addition to the second sample collection vessel, the inner clean area held the Metrohm 757VA Computrace and the reagents. From the second collection vessel the sample and reagents were manually pipetted into the voltammetric cell, to give final concentrations of 10 µM oxine and 10mM tris. Two replicate determinations by AdCSV were performed on the sample and two 1.5nM Cu(II) standard additions, with a voltage protocol as follows:

a) 300 s purge using nitrogen gas to remove oxygen (presence of oxygen creates a broad interfering peak in the scan)
b) 90 s deposition/preconcentration time at a potential of –1.2 V
c) 5 s quiescence period at a potential of –0.2 V
d) square wave scan in a negative direction from –0.2 V to –0.8 V (step height 0.00366 V, pulse amplitude 0.025 V, frequency 50 Hz)
e) 100 s nitrogen gas purge after each copper standard addition, then steps b-3 repeated.

The concentration of the sample was automatically determined by the program using a linear regression standard addition curve of the formula $y = mx + b$, where x = additive concentration of the standard addition and y = mean peak height of the replicates of each addition. The negative x-intercept of the fitted line (with slope m) then yields the positive sample concentration. Separate samples for salinity analysis were taken every 20-30 minutes.

RESULTS

In April 2004, total dissolved copper and salinity were monitored over a 13-hour tidal cycle at the outflow of the River Corrib using the mobile AdCSV laboratory. The results are displayed as a time series in Fig. 3, with each of the salinity and total dissolved copper data sets correlated to the tidal sine function, and as an x-y scatter plot in Fig. 4.

At low water, the sample was predominantly fresh, and copper values were highest, close to 10.5 nM. With the incoming tide the water became more saline, to a maximum of ~28, and total dissolved copper concentrations decreased to ~ 4.5 nM. Extrapolating the best fit line of the copper-salinity data (Figure 4) to the y-axis gives a zero salinity endmember total dissolved copper concentration of 10.3 nM. Extrapolating to the typical salinity of 35.5 for the northeast Atlantic gives a figure of 1.7 nM total dissolved copper for the seawater endmember.

In May 2004, the tidal cycle at Parkmore Quay, Kinvara Bay, was completed. Figure 5 displays the results, again as a time series. Much smaller variation in both parameters were observed than at the Galway Docks site. Salinity values ranged from just below 27 at low water to just above 32 around high water. Total dissolved copper concentrations fluctuated about a mean of 3.9 +/- 0.7 nM.

DISCUSSION

GALWAY DOCKS SITE

What is immediately clear from Fig. 3 is the inverse relationship between total dissolved copper and salinity. As copper is primarily anthropogenically and terrestrially sourced, it was expected that copper concentrations would increase with increasing freshwater influence at this site because (a) the bedrock of the drainage basin is largely granitic, which is host to a variety of trace metals; (b) the River Corrib passes through Galway City; (c) the proximity of Galway Harbour Docks, an active commercial port, to the site.

Fig. 3 shows that salinity and total dissolved copper correlate to the tidal sine function. The R-sq value of this fit for total dissolved copper (0.64), although good, is somewhat less than that for salinity (0.89). Salinity is (almost) entirely conservative in its behaviour, and hence spatial or temporal changes in salinity values are only due to the

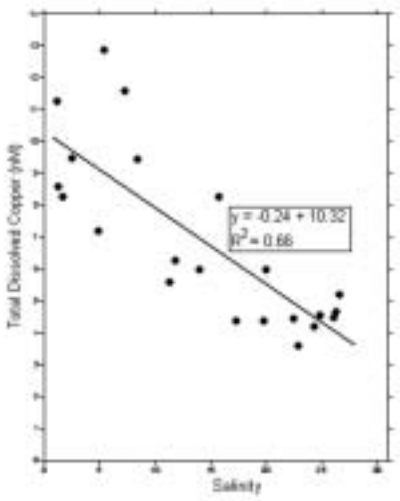

Fig. 4 – Galway Docks site Total Dissolved Copper – Salinity plot.

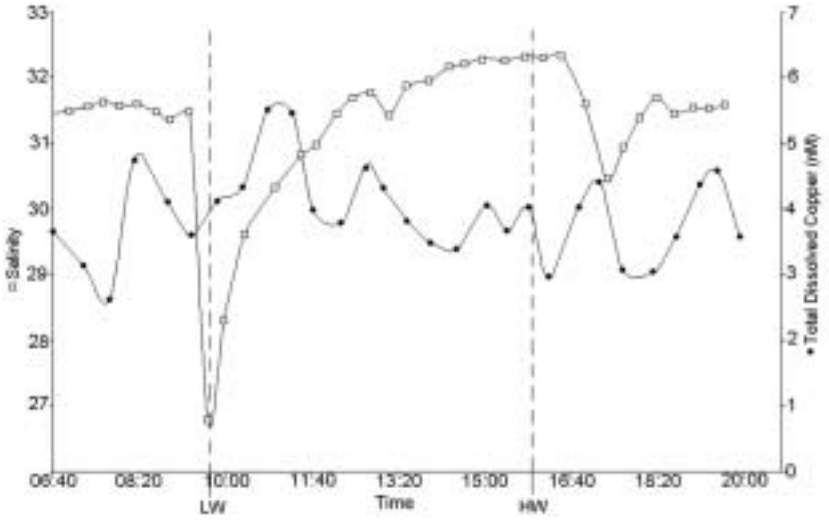

Fig. 5 – Kinvara Bay site time-series of Total Dissolved Copper and Salinity.

physical mixing of different water masses of different salinities. As previously mentioned, the strong organic-copper complexes that dominate the dissolved phase of copper in freshwater and seawater are resistant to chemical reactions but are not entirely unreactive. This would be especially true in the highly dynamic chemical environment of an estuarine mixing zone where copper would be subject to a higher degree of speciation and phase changes, through adsorption/ desorption reactions with particulates for example, than in the consistent ionic matrix of the open ocean.

The total dissolved copper-salinity plot (Fig. 4) again shows quite clearly the inverse linear relationship between the two variables. The freshwater endmember concentration of 10.3 nM is higher than non-contaminated estuarine concentrations (e.g. Moffett *et al*. 1997), but significantly lower than heavily industrialised estuaries such as the Humber, where total dissolved copper concentrations of up to 160 nM for the freshwater end have been reported (Comber & Gunn 1995).

Reports of the concentrations of biologically available or "labile" copper in seawater (free or very weakly complexed with organic ligands) that produce a toxic response to marine organisms vary greatly. In a 2003 review (Eklund & Kautsky), results from 27 different papers on the toxic effects of copper to marine macroalgae were summarised, with reported threshold values ranging from ~40 nM to upwards of 1 µM. From a biological point of view, the concentrations of total dissolved copper that were observed in Galway Bay likely poses little threat to marine organisms, as the copper complexing capacity of the seawater would be much greater than this value in an organic loaded coastal zone, rendering a much smaller fraction of the total as "labile" (and hence bioavailable). Future work is planned to use the mobile AdCSV laboratory to monitor "labile" as well as total dissolved copper at the Galway Docks site, to better assess the biological significance of copper in the bay.

A seawater endmember total dissolved copper concentration of 1.7 nM corresponds well to published values for the northeast Atlantic, which has been determined at 0.7–1.3 nM (Kremling & Pohl 1989; Saager *et al*. 1997). The fact that our estimated concentration is on the high side indicates that the Corrib River is the major copper source in Galway Bay, but not the only source.

KINVARA BAY SITE

The main freshwater influence at the site occurred sharply at low water, as seen in Fig. 5, with a smaller freshwater signal evident again at slack water at high tide. However, there was no correlated copper response, and values of total dissolved copper fluctuated randomly about a mean of 3.9 +/- 0.7 nM.

The absence of a correlated increase in total dissolved copper with increasing freshwater pressure (at low and slack waters) suggests that despite the moderate southerly wind blowing, the freshwater signal was not due to the tidal flushing of the contaminated inner bay but rather to a fresh groundwater resurgence. The bedrock geology of the surrounding landscape is limestone, which generally speaking does not harbour trace metals. As the salinity drops are not accompanied by an increase in dissolved copper, our results indicate that, as would be expected, the groundwater does not contain a significant concentration of copper.

CONCLUSION

The correlated response between total dissolved copper and salinity at the Galway Docks site indicates that total dissolved copper does have the potential to serve as a tracer of river plume movement. To contrast the correlated response at this site with the uncorrelated response at the Kinvara Bay site makes clear the advantage of monitoring total dissolved copper in conjunction with salinity, whereby the discrimination of contaminated *vs* non-contaminated freshwater sources is made possible. This work is the first of its kind in Galway Bay, where very little research on dissolved concentrations of metals has been conducted to date. The mobile AdCSV laboratory could be adapted to monitor a variety of other metals, with only changes to the reagents and possibly to the voltammetric parameters required. The main advantage of conducting trace metal determinations *in situ* using a mobile laboratory over collection of discrete samples for later analyses is a reduction in the number of potential sources of sample contamination: the sample is not exposed to the environment between leaving the sea and entering the clean working area, does not need to be stored in a sample bottle, and does not need to be acidified. Minimising the risk of contamination is particularly important when

sampling for trace metals, as they are often present in much lower concentrations in seawater than in airborne and terrestrial materials. A second benefit is in time saved to the researcher, who returns from the field with a data set, rather than with a multitude of samples still to be analysed. Future work is planned to assess the potential biological impact of copper-rich point source inputs to Galway Bay by measuring both the total and labile dissolved copper concentrations over a tidal cycle.

ACKNOWLEDGMENTS

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THE SOIL SEED BANK ASSOCIATED WITH INVASIVE POPULATIONS OF *HERACLEUM MANTEGAZZIANUM* SOMMIER & LEVIER

Margherita Gioria and Bruce Osborne

ABSTRACT

Alterations in soil seed bank richness, abundance and composition, together with the accumulation of viable seeds in the soil, could be major factors in determining the success of certain invasive species. Despite considerable efforts aimed at making invasion ecology a more predictive science, little information is available on the impact of invasive species on the soil seed bank, or the role of seed banks as reservoir of seeds for the restoration of previously-invaded ecosystems. Giant hogweed, *Heracleum mantegazzianum* SOMMIER & LEVIER, was selected among major invaders in Ireland, with the aim of assessing its impact and contribution to the seed bank at invaded sites. To do so, a comparison was made of soil samples collected at different depths and time of the year from areas with and without invasive colonies, using the seedling emergence method. At the time of assessment, *H. mantegazzianum* represented approximately 55% of the invaded seed bank, with a mean value of 9,613 (±1,203) seedlings m^{-2}. As a result of the invasion process, species richness in the invaded seed bank decreased, although the magnitude of the impact was species-specific and site-dependent. Monocotyledons and dicotyledons decreased both in richness and abundance, with grasses exhibiting the greatest reduction.

Key words: soil seed bank; biological invasion; biodiversity; *Heracleum mantegazzianum*

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INTRODUCTION

Biological invasions by alien species represent one of the most pervasive threats to global biodiversity (Cronk & Fuller 1995; Williamson 1996; Lonsdale 1999). Considerable efforts have been directed towards the identification of common traits that may contribute to the success of certain species (Rejmanek & Richardson 1996) or that may make some communities more invasible (Robinson *et al.* 1995; Burke & Grime 1996; Tilman 1997; Lonsdale 1999; Brown & Peet 2003). However, the impact of invasive species on natural ecosystems is still poorly understood. In particular, the impact of invasive species on the soil seed bank, or the role of previously-invaded seed banks as a reservoir of seeds for restoration programmes, has been normally neglected. Despite receiving less attention, alterations in soil seed bank diversity, composition and abundance are likely to be major factors in determining the success of certain invasive species. In addition, the accumulation of viable seeds of invasive species in the soil is likely to have implications for management, control and restoration programmes.

As part of an ongoing study on the impact of invasive herbaceous species on the soil seed bank, the giant alien *Heracleum mantegazzianum* was selected among different invaders in Ireland as a particular priority due to its potential for rapid spread, toxicity to humans (Drever & Hunter 1970) and the economic costs associated with its removal (Nielsen *et al*. 2005). This species, a member of the Umbelliferae and native to the North-West Caucasus, was reported (Wyse Jackson 1989) growing in the National Botanic Gardens, Glasnevin, prior to 1889. Today, *H. mantegazzianum* has a widespread distribution, primarily along watercourses, roadsides, hedgerows, waste ground and other damp habitats, and has been recorded in 29 of the 40 Irish vice-counties (Wyse Jackson 1988). *H. mantegazzianum* is the largest central European herbaceous species (Pyšek, 1994) and its remarkable stature, coupled with its early germination and ability to shade the surrounding vegetation (Dodd *et al*. 1994), have been regarded as major factors in out-competing native species. In Britain and Ireland *H. mantegazzianum* is a monocarpic perennial and reproduces exclusively by seeds (Tiley *et al.* 1996), producing 20,500 seeds on an average plant (Perglová *et al*. 2005), although numbers in excess of 100,000 have been recorded on a single plant (J. Caffrey, personal communications). Most seeds germinate in January-February (Caffrey 2001; Gioria, personal obs.) and release of seeds from the umbels occurs from September until late October. Seeds require a period of chilling and moisture to germinate (Tiley *et al*. 1996; Moravková *et al*. 2005). At present, water appears to be the main dispersal agent, followed by wind and human disturbance (Pyšek & Prach 1994; Tiley & Philip 1994; Gioria, personal obs.).

The purpose of this study was a) to provide an improved understanding of the type of seed bank formed by *H. mantegazzianum* in Ireland, and b) to assess its impact on the soil seed bank of invaded areas. Assessment of the seed bank of *H. mantegazzianum* and its impact on the seed bank of resident species was investigated through intensive soil sampling over two years, using the seedling emergence method to assess the presence of viable seeds. In order to understand which species were more likely to be affected by the process of invasion, comparable areas with and without invasive colonies were selected. Samples were collected in May, after the majority of seeds of *H. mantegazzianum* had germinated in the field, and again in October, after release of seeds from the umbels. Samples were collected at three different depth categories to provide information on the vertical distribution of seeds in the soil and their persistence. The results presented in this paper refer to data recorded in the year 2004 at two sites characterised by riparian grassland communities, and representative of the species' main habitat in Ireland.

MATERIALS AND METHODS

STUDY SITES AND PLANT SPECIES

Soil samples for seed bank analyses were collected at two riparian sites (53°23'N, 6°18'W and 53°26'N, 6°11'W, respectively) located in North Dublin City. Mean rainfall at Dublin Airport (approximately 10km north of the study sites) is 69.4mm in January and 49.9mm in July, with a mean annual value of 733mm. Mean maximum and minimum temperatures in the area are 7.6°C and 2.5°C in January, and 18.9°C and 11.4°C in July, respectively (www.met.ie/recentweather/monthlydata.asp).

The study sites were selected for their ability to provide representative areas with and without invasive colonies. According to local information, the plant had been present at the sites approximately 30 to 40 years (Lambert & Caffrey, personal communications). *H. mantegazzianum* dominated the colonised areas, together with *Petasites hybridus* (L.) P. Gaertn. at Site 1, and *Urtica dioica* L. at Site 2. For the uncolonised areas the dominant species were *Agrostis stolonifera* L., *Alopecurus pratensis* L., *Epilobium hirsutum* L., *Ranunculus repens* L. and *Rumex obtusifolius* L. at Site 1. The vegetation at Site 2 was dominated by *A. stolonifera*, *Chamerion angustifolium* (L.) Scop., *Poa trivialis* L. and *R. repens*.

SAMPLING DESIGN

Sampling of the seed bank was conducted by adopting a stratified random design. At each site, a sampling area of 100 m^2 was selected and colonised and uncolonised plots were identified. Each plot (10m x 5m) was then subdivided in two subplots (5m x 5m) according to the distance from the invaded area. Ten soil cores (5cm diameter and 15cm depth) were randomly collected within each subplot. Samples were divided into three depth categories (A = 0-5 cm, B = 5-10 cm, C = 10-15 cm) to provide information on both horizontal and vertical distribution of seeds in the soil. In total, 480 soil samples were collected in May and in October from colonised and uncolonised areas. In order to integrate the seed bank data, concentric circular plots of 0.50m^2 were set out around the soil sampling points to provide information on the standing vegetation and surveys conducted at monthly intervals from April to October 2004.

ASSESSMENT OF THE SEED BANK

Assessment of the seed bank of colonised and uncolonised areas was based on the seedling emergence method. This method is designed to provide an assessment of the seed flora present (Thompson and Grime 1979) and the lack of germination of certain species does not imply their absence from the seed bank. Samples were placed in unheated greenhouses located at the Horticultural Unit in Thornfield (University College Dublin), to provide them with conditions that are comparable to the ones experienced in the field. Soil samples were sieved using a coarse sieve (mesh size 2mm) to remove rhizomes, pebbles and other material. This procedure also enabled us to identify seeds of *H. mantegazzianum,* due to their large size. The samples were then mixed with sterile potting soil and spread in an approximately 2cm-deep layer over a layer of sterile sand in plastic pots (10cm diameter). Control pots, containing only sterile potting mix, over a layer of sterile sand were randomly positioned among the sample pots at a ratio of 1 to 5, to identify and quantify the presence of airborne seeds in the greenhouses. Pots were watered daily and seedling emergence was recorded at least once a week from the day of potting until June 2005 (approximately 13 and 8 months for the samples collected in May and October, respectively). Seedlings were removed soon after germination and, if not immediately identifiable, were transferred to separate pots of compost until identification was possible. To stimulate the germination of buried seeds in the pots, the soil was disturbed and inverted every two to four weeks.

DATA ANALYSIS

All seedling germination data are presented as the number of seedlings per square metre. The rate of seedling emergence was assessed as the percentage of total seedlings that emerged within the first four weeks. Seedling numbers were analysed using records made over 8-13 months from the samples collected in October and May, respectively.

To provide information on the impact of *H. mantegazzianum* on major plant groups present in the seed bank, seedlings were divided into monocotyledons and dicotyledons, and, by life-history, into annuals, biennials and perennials. For the final analysis, annuals and biennials were combined.

RESULTS

SEED BANK OF *HERACLEUM MANTEGAZZIANUM*

H. mantegazzianum accounted for 52-58% of the seedlings present in the colonised seed bank, with an average germination of 9,613 ±1,203 (mean ±SD) seedlings m^{-2} (Table 1). Seedling germination occurred exclusively from the upper 5cm soil layer and only from the samples collected in October. Germination started after samples had been exposed to winter temperatures for approximately nine weeks in unheated greenhouses (early January). Seeds of *H. mantegazzianum* showed high synchronous germination, with 98% of the germination occurring within seven days from the emergence of the first seedling, and no further germination was recorded after four weeks. No seeds of *H. mantegazzianum* were found in the samples collected in the uncolonised areas.

SEED BANK SPECIES RICHNESS

A total of 49 species germinated from the seed bank of colonised and uncolonised areas (Table 2). Invasion by *H. mantegazzianum* led to a significant decrease in species richness in the invaded seed bank. Forty-five species germinated from the uncolonised seed bank. In contrast, only 33 species were found in the colonised seed bank. Four species were recorded only in the colonised seed bank (*Fumaria officinalis* L., *Senecio jacobea* L., *Stachys sylvatica* L. and *Stellaria media* (L.) Vill.). Six grass species were recorded in the seed bank of uncolonised areas (*Agrostis capillaris* L., *A. stolonifera*, *Elymus repens* L., *Holcus lanatus* L., *Lolium perenne* L. and *Poa trivialis*). *E. repens* and *P. trivialis* did not emerge from the colonised seed bank. *H. lanatus* and *L. perenne* were common to the seed banks of both colonised sites. *Carlina vulgaris*, *Chamerion angustifolium*, *Cirsium arvense* (L.) Scop., *Epilobium hirsutum* L., *E. obscurum* L., *Ranunculus repens* and *Urtica dioica* were common in the colonised seed bank at both sites. Three *Juncus* spp. (*J. articulatus* L., *J. bufonius* L. and *J. effusus* L.) were recorded in both the colonised and uncolonised seed bank, although they were not recorded in the standing vegetation of colonised areas. Dicotyledons dominated (77-80%) the seed bank of both colonised and uncolonised areas. Perennial species represented approximately 77-78% of the colonised and uncolonised seed banks, respectively. Twenty-two species that were recorded in the standing vegetation did not germinate (Table 2).

Table 1 – Mean (± SD) number of seedlings m^{-2} (n=10) germinated from samples collected in May and October 2004 from colonised and uncolonised areas at two sites, North Dublin City.

Species/plant group	Colonised areas	Uncolonised areas
Heracleum mantegazzianum	9 613 (± 1 203)	0 (± 0)
Grasses	1 872 (± 1 026)	8 212 (± 6 536)
Herbs	5 717 (± 666)	12 045 (± 792)
Juncus spp.	407 (± 504)	140 (± 54)

SIZE AND COMPOSITION OF THE SEED BANK

Invasion by *H. mantegazzianum* profoundly altered the size and composition of the seed bank. In the colonised areas, the number of seedlings of resident species decreased by approximately 60%. Grass species exhibited the largest decrease, with a decline of approximately 80 and 68% at Site 1 and Site 2, respectively (Table 1). Germination of seedlings of herb species decreased by 58 and 46% at Site 1 and Site 2, respectively (Table 1), although the impact was species-specific and site-dependent. *Urtica dioica* was the most abundant species in the colonised seed bank after *H. mantegazzianum*, with 3,794 (± 2,386) and 2,445 (± 1,649) seedling m^{-2} recorded at Site 1 and Site 2, respectively (21% and 14% of the total seed bank). At Site 2, *Rumex sanguineus* L. and *U. dioica* were more abundant in the colonised seed bank by 76% and 18%, respectively. Other species found in the colonised seed bank included *Lolium perenne* (9%) and *Epilobium hirsutum* (5%) at Site 1, and *Cirsium arvense* (6%) and *Rumex sanguineus* (4%) at Site 2. *Juncus* spp. were better represented in the colonised seed bank, with *J. bufonius* and *J. effusus* more abundant at Site 1 (77%) and Site 2 (50%).

DEPTH DISTRIBUTION OF THE SEED BANK

The majority of seedlings (65-83%) germinated from the upper soil layer (5cm deep), and only 5-8% of seedling germinated from the lower layer (10-15cm deep) in both colonised and uncolonised areas. Invasion by *H. mantegazzianum* resulted in a decrease in both richness and abundance at each depth sampled. Richness decreased by 63% in the lower layers (5-15cm deep), with only 11 species recorded in the colonised seed bank, compared to 30 species emerging from the uncolonised areas. The total number of seedlings of resident species in the lower layers (B and C) decreased by approximately 48%, with 3,259 (± 1,979) and 1,324 (± 605) seedlings m^{-2} recorded in the colonised seed bank at Site 1 and Site 2, respectively, compared to 9,906 (± 5,479) and 3,234 (± 1,797) seedlings m^{-2} recorded in the uncolonised seed bank. *U. dioica* was the most abundant species recorded in the C soil layer, representing 36% and 78% of the colonised and uncolonised seed bank, respectively. Other common species germinating from the C soil layer were *A. stolonifera* and *L. perenne,* among the grasses, *Cirsium arvense* and *Epilobium hirsutum,* among the herbs, and *J. bufonius* and *J. effusus* among the Juncaceae.

VEGETATION

A total of 70 species were recorded in the standing vegetation: 8 grasses, 57 herbs and 5 *Juncus* spp. (Table 2). The standing vegetation was severely affected by the invasion, with a significant decrease in both the richness and abundance of resident species. Seventy species were recorded in the uncolonised areas, compared to only 11 species recorded in the colonised areas (16%). Four species (*A. stolonifera*, *Equisetum arvense* L., *Galium aparine* L. and *U. dioica*) were common to both colonised areas. Of 8 grass species growing in the uncolonised areas, only 2 species (*A. stolonifera* and *H. lanatus*) were recorded in the vegetation of the colonised areas. No *Juncus* spp. were recorded in the colonised vegetation. Dicotyledons were dominant (80%) in the vegetation of both colonised and uncolonised areas, with perennials representing approximately 80% of the standing vegetation.

DISCUSSION

GERMINATION OF *HERACLEUM MANTEGAZZANIUM*

At the time of assessment, *H. mantegazzianum* dominated the seed bank of the invaded areas, comprising approximately 55% of the seedlings recorded. The mean of seeds germinating averaged 9,613 (± 1,203) seedlings m^{-2} (Table 1). Germination occurred exclusively from the upper 5cm soil layer, and was restricted to the samples collected in October. Seeds of *H. mantegazzianum* showed high synchronous germination, with 98% of the germination occurring within the first week after the appearance of the first seedling, after the exposure of seeds to approximately two months of winter temperatures. The lack of germination from the samples collected in May, coupled with the high synchronous germination, suggests that the majority of seeds emerge early in the year (mid to late January in mild conditions), whereas the remaining seeds soon lose their viability. The lack of germination late in the spring seems to be confirmed by preliminary results obtained from samples collected in May 2005 at a third site (unpublished results). These germination patterns indicate that, in Ireland, *H. mantegazzianum* tends to form a transient type of seed bank (*sensu* Thompson *et al*. (1997)). These results differ from data reported in the literature from Central Europe, where germination was also observed with samples collected in July (Moravková *et al*. 2005), indicating greater seed viability in the soil and the formation of a short-term type of seed bank. These differences in seed survival could be attributed to differences in temperature and rainfall between Central European sites and those found in Ireland. No seeds were recorded in the samples collected in the uncolonised areas, confirming the limited importance of wind in seeds dispersal.

For *H. mantegazzianum*, early-season germination (usually January in mild conditions) and the development of an extensive canopy, could confer a significant competitive advantage through shade-associated reductions in the growth or emergence of native species.

IMPACT ON THE SEED BANK

Invasion by *H. mantegazzianum* led to significant alterations in soil seed bank richness, abundance and composition for each depth category. Three trends in the impact on the soil seed bank associated with the invader were identified, although the magnitude of the impact was species-specific and site-dependent. Firstly, species richness of grasses and herbs declined as a consequence of the invasion process. Secondly, the number of seedlings of both grass and herb species significantly decreased, the invasion resulting in a profoundly altered

Table 2 – List of species recorded from the seed bank and the standing vegetation in colonised (C) and uncolonised (U) plots at Site 1 and Site 2. The letter *V* indicates the presence of the species.

Species	Seed Bank					Vegetation			
	Site 1		Site 2			Site 1		Site 2	
	C	U	C	U		C	U	C	U
Agrostis capillaris	V	V					V		V
Agrostis stolonifera	V	V	V	V		V	V	V	V
Alliaria petiolata							V		V
Alopecurus pratensis								V	
Anthriscus sylvestris						V	V		
Armoracia rusticana								V	
Borago officinalis			V						V
Buddleja davidii							V		V
Calystegia sepium		V		V			V		V
Capsella bursa-pastoris				V					V
Cardamine hirsuta		V					V		
Cardamine pratensis	V	V					V		
Carlina vulgaris			V	V			V		V
Chamerion angustifolium	V	V	V	V			V		
Chenopodium album	V	V	V			V		V	
Cirsium arvense	V	V	V	V			V	V	V
Elymus repens		V		V			V		V
Epilobium hirsutum	V	V	V	V			V		V
Epilobium obscurum	V	V	V	V			V		V
Epilobium parviflorum		V					V		
Equisetum arvense						V	V	V	V
Festuca pratensis							V		V
Filipendula ulmaria						V		V	
Fumaria officinalis			V						V
Galium aparine		V				V	V	V	V
Geranium molle	V			V					V
Geranium robertianum								V	V
Heracleum mantegazzianum	V		V				V		V
Heracleum sphondylium						V	V		V
Holcus lanatus	V	V	V	V			V	V	V
Hypochaeris radicata	V	V						V	
Juncus articulatus	V	V					V		
Juncus bufonius	V	V		V			V		
Juncus conglomeratus							V		
Juncus effusus	V	V	V	V			V		
Juncus inflexus							V		
Lamium album							V		
Lathyrus pratensis							V		V
Lolium perenne	V	V	V	V			V		V
Lotus corniculatus				V			V		V
Medicago lupulina			V	V					V
Mercurialis annua				V					V
Petasites hybridus						V	V		V
Phalaris arudinacea	V	V		V			V		V
Plantago lanceolata			V			V		V	
Plantago major									V
Poa trivialis				V			V		V
Polygonum aviculare		V	V						
Potentilla anserina							V		V
Potentilla reptans				V			V		V
Ranunculus acris		V		V			V		V
Ranunculus repens	V	V	V	V			V		V
Rubus fructicosus		V		V			V		
Rumex acetosella	V		V	V			V		V
Rumex conglomeratus							V		V
Rumex crispus				V			V		V
Rumex obtusifolius	V	V	V	V			V		V
Rumex sanguineus	V	V	V	V			V		V
Senecio jacobaea	V						V		
Sinapis arvensis			V	V					V
Stachys sylvatica	V						V		
Stellaria media	V						V		
Taraxacum officinale	V	V	V				V		V
Trifolium pratense		V		V			V		V
Trifolium repens		V		V			V		
Urtica dioica	V	V	V	V		V	V	V	V
Veronica beccabunga						V			
Veronica chamaedrys		V						V	
Veronica hederifolia		V				V			
Vicia cracca				V				V	V
Vicia sativa									V
Vicia sepium		V					V		

composition of the seed bank. Only four perennial grass species, out of a total of 6 species recorded in the uncolonised areas, germinated and the number of germinating seeds decreased by approximately 75%. The same trend was identified for herb species. Richness declined (33 herbs were recorded in the colonised areas out of a total of 49 species emerged), and the number of germinating seeds of herbs decreased by approximately 50%. Exceptions to this generalisation were *Rumex sanguineus* and *Urtica dioica*. In relation to *Juncus* spp., *J. bufonius* and *J. effusus*, known for forming a highly persistent seed bank (Grime *et al*. 1988), were better represented in the colonised seed bank, although they were not recorded in the standing vegetation of colonised areas.

In conclusion, *H. mantegazzianum* forms a transient seed bank. This, coupled with poor wind dispersal, could limit the spread of this species if effective methods were implemented to control or eradicate standing plants. *H. mantegazzianum* had a significant impact on both species richness and the size of the resident seed bank, altering its composition and limiting its potential role as reservoir of diversity. The seed bank of species with long-lived seeds, e.g. *Juncus* spp. and *U. dioica* (Grime *et al*. 1988), was largely unaffected by the invasion process and species with effective wind dispersal, e.g. *Epilobium hirsutum* (Grime *et al*. 1988), persist in the seed bank of invaded areas.

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HIDDEN BIODIVERSITY: CHITONS IN IRELAND

Julia D. Sigwart

ABSTRACT

This paper presents the first checklist of Irish polyplacophoran molluscs, including confirmed records for twelve species in the class. The polyplacophorans, or chitons, are distinctive marine invertebrates found on rocky shores around Ireland. Very little is known about the species diversity or evolutionary relationships among Irish chitons, or even the class in general. Some baseline data is available for the distribution of these animals around Ireland, but their reactions to potential disturbances such as pollution or climate change are completely unknown, although as elements that are relatively rare in marine intertidal ecosystems, they are thought to be sensitive to disturbances. However, these molluscs are usually overlooked as 'difficult' in ecosystem surveys, as they are almost impossible to distinguish without dissection. Consequently, species lists and surveys of marine invertebrates often refer only to the erroneous '*Chiton* sp.' (a genus so far unrecorded from Ireland) or similar, and indeed there has never previously been an attempt to compile a complete list of the species found in Ireland.

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INTRODUCTION

The chitons (class Polyplacophora), or 'coat-of-mail shells' (*ciotón máille* in Irish language), are some of the most enigmatic of the familiar marine invertebrates from rocky intertidal ecosystems. Chitons are rare elements in Atlantic intertidal ecosystems (e.g. Bishop 2003). But identification challenges make them less accessible as potentially useful organisms in monitoring efforts. Chitons form a distinctive molluscan clade whose members normally have eight shell plates (valves) as adults. These animals are found in oceans all over the world, primarily in the intertidal zone of exposed rocky shores, but ranging to depths of more than 1000m (Kaas & Van Belle 1985). The shell plates of chitons, in a row on their dorsal surface, are the most distinctive characteristic of the group and often their only visible feature. Most polyplacophoran species are notoriously difficult to identify and old records are often unreliable due to common misidentification errors (Matthews 1967). Diagnosis is often dependent on the shape of insertion plates in the shell valves embedded in mantle tissue, not visible in the living animal. In ecological surveys (in Ireland and elsewhere), all polyplacophorans are often grouped together under the erroneous "*Chiton* sp." Here, for the first time, I present a checklist of Irish Polyplacophora, with confirmed records of a total of twelve species on Irish shores. The central aim of this project has been to address the question of how many species of polyplacophoran molluscs are present in Ireland, and what can be learned about sampling biases.

METHODS

A checklist was compiled, arranged in the accepted systematic order by family (Kaas & Van Belle 1985), and alphabetically by genus and species. Only validated records, from literature or museum specimens, are included in the list. The complete synonymy is compiled from recent monographical treatments (Kaas & Van Belle 1985a; 1985b; 1990; Jones & Baxter 1987), published checklists (Hoisaeter 1986; Seaward 1982; Smith & Heppell 1991) and from the online Check List of Marine Mollusca (CLEMAM 2004).

Museum records were examined from collections in Dublin (National Museum of Ireland, Natural History; NMINH) and London (The Natural History Museum, BMNH). Specimens were examined and species-level identity confirmed by the author, by dissection where necessary. Additional locality records were gathered from primary literature, and historical and contemporary field guides to seashells that make specific reference to chitons. Many older field guides (ca. 1960 and earlier) are aimed at shell-collecting enthusiasts, and offer species-level diagnoses for common shore animals (e.g. Duncan 1943). More contemporary field guides, which are designed more for use observing creatures in their own habitats, are often less specific for animals that are difficult to identify, such as chitons (e.g. Wye 2003). Records are only included in the summary of distribution data if multiple Irish records were found for a species, and if a relatively specific locality information could be determined.

RESULTS

Based on biological records from museum collections and literature, I have compiled a Checklist of the Irish chitons including twelve species found on the island of Ireland (Table 1). A field identification key and a complete synonymy of these species is available online (http://www.ucd.ie/zoology/sigwart.htm).

Taxonomically, of the 12 species found in Ireland, four are in the 'primitive' suborder Leptochitonina, considered to be the most basal polyplacophorans, typically inhabiting deep waters. The remainder are divided between the five species in the family Ischnochitonidae and three species in the Acanthochitonidae. In traditional classification, these three groups are arranged in 'ascending' evolutionary order, with acanthochitonids representing the crown group of Polyplacophora, and with the base of living chitons in a clade comprising Leptochitonidae and Hanleyidae within the Leptochitonina. This classical interpretation differs substantially from the results obtained through cladistic analysis, but has yet to be resolved (e.g. Okuso *et al.* 2003). All field records and literature references have been compiled to create rough distribution maps for the Irish chiton fauna (Fig. 1). Most historical records are at a fairly general level (e.g. Dublin Bay, Galway Bay), and therefore this map is intended to give a broad picture of where species are found around the coast of Ireland and Britain. A distinctive pattern emerges, with the majority of records coming from Galway, Cork, Dublin and popular localities in Northern Ireland (e.g. Mulroy Bay, Strangford Lough).

Fig. 1 – Distribution map of polyplacophorans records from Ireland and Great Britain, showing the groups represented in the fauna (Leptochitonina, black; Ischnochitonidae, dark grey; Acanthochitonidae, light grey). Distances from shore are not to scale; locations represent generalised records. The distribution patterns, especially those in Ireland, may represent popular collecting localities more accurately than biological species distributions.

DISCUSSION

Interestingly, the type genus (*Chiton*) is entirely absent from Ireland. Previous records from the southern coast of Great Britain are considered dubious. The family Chitonidae is present with several species in the Mediterranean, but seems to be confined to warmer sea temperatures (Poppe & Goto 1991). This is an important omission as the common name 'chiton' is very often confused with the generic '*Chiton*'.

The distributions of the various species around Ireland are most notable for their absences: despite widespread records from Britain in all three, the locality records around the island of Ireland are focused on a very few localities. This is an artefact of sampling, and not representative of the complete distribution of chitons in Ireland. For example, *Hanleya hanleyi* has been recorded only from Co. Cork and Co. Antrim. Future efforts will no doubt extend its recorded range to connect these isolated populations.

We know that marine faunas are sensitive environmental indicators for impacts of pollution, habitat destruction, invasive species, and over-exploitation. In particular, ecosystems in shallow seas (such as most of this material represents) may be especially sensitive to the effects of global warming and consequent increases in ocean temperature. However, studies of biodiversity often suffer from a lack of reliable baseline data with which to compare our current findings. Museum collections are physical databases of our planet's biota. Recent studies (e.g. Mikkelsen & Bieler 2000) have found that recent museum collections independently recover 80 per cent of species found in intensive nearshore surveys. Additional work by many ecologists indicates that nearshore fauna, including molluscs, are highly sensitive to changes in their marine environments (Southward 1980). Specimen databases in museums are one tool that allows us to analyse in detail patterns of biodiversity in space and time, in response to changing conditions.

Table 1 – List of the species of chitons recorded from Ireland.

Family	Species
LEPTOCHITONIDAE	*Leptochiton asellus* (Gmelin 1791)
	Leptochiton cancellatus (Sowerby G.B.II 1840)
	Leptochiton scabridus (Jeffreys 1880)
HANLEYIDAE	*Hanleya hanleyi* (Bean in Thorpe 1844)
ISCHNOCHITONIDAE	*Ischnochiton albus* (Linné 1767)
	Callochiton septemvalvis (Montagu 1803)
	Lepidochitona cinerea (Linné 1767)
	Tonicella marmorea (Fabricius O. 1780)
	Tonicella rubra (Linné 1767)
ACANTHOCHITONIDAE	*Acanthochitona crinita* (Pennant 1777)
	Acanthochitona discrepans (Brown 1827)
	Acanthochitona fascicularis (Linné 1767)

Chitons are found in a wide range of depths in the marine environment, from the low intertidal (e.g. *Tonicella*) to abyssal depths (e.g. *Leptochiton*). Also, in all ecosystems, chitons tend to be found in low densities (Jones & Baxter 1987). Many species included on this checklist are clearly under-sampled. Even with comprehensive data available from surveys like the BioMar (Picton & Costello 1998) project in the 1990s, there are still significant gaps in our knowledge. Distribution of Irish species is not well understood, and our current knowledge appears to primarily reflect patterns of intensive collecting. Additional survey work, with reliable species-level identification, will be needed to fill the gaps around the coast of Ireland.

Polyplacophorans are a diverse and important group of molluscs that are often overlooked because their identification is comparatively more difficult than other groups. This checklist of Irish chitons is a useful tool for the future investigation of a very difficult and under-studied group of marine invertebrates. The twelve species in Ireland represent a great deal more biodiversity than was previously recognised.

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ASSESSING THE CURRENT SUSTAINABILITY AND IMPACTS OF FUTURE SUSTAINABLE DEVELOPMENT POLICIES IN LIMERICK CITY

David Browne, Bernadette O'Regan and Richard Moles

ABSTRACT

The objectives of this paper are to:
– review a myriad of current methodologies, with the view of selecting the most appropriate methods for measuring and modelling sustainability in a city-region;
– empirically show the changes in Material Flow Accounting (MFA) indicators in the Limerick city-region from 1992-2002, including Direct Material Input (DMI), Domestic Material Consumption (DMC) and Domestic Processed Output (DPO);
– introduce the sectoral metabolic inefficiency indicator and highlight its potential usefulness as a sustainability indicator from the perspective of resource management and life cycle assessment.

It was found that Direct Material Input (DMI) per capita increased from 19.7 tonnes in 1992 to 28.37 tonnes in 2002, i.e. an increase of 44% or 4.4% per annum. Domestic Material Consumption (DMC) per capita increased from 14.05 tonnes per capita in 1992 to 22.55 tonnes in 2002, i.e. an increase of 60.5% or 6% per annum. A metabolic approach was taken in order to provide a holistic framework within which to study material and energy flows in the city-region and was useful to relate the final disposal of material and product wastes by various means to consumption and production.

Key words: sustainable urban development; material flow accounting; urban metabolism; Limerick City-Region

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INTRODUCTION

Sustainable development is a conceptual goal that is increasingly becoming dominant in official policy rhetoric and, ostensibly, means that environmental and social concerns are considered along with more traditional economic criteria in planning and development decisions, within the context of a more participatory institutional governance framework. Strategic planning and development are now the guiding operational principles at various organisational levels, including national and municipal government plans, policies and programmes (PPP). Although no one definition may be seen as being panacea in terms of encompassing all the complex dimensions inherent in sustainable development, a number of principles are intrinsic to the concept, including the precautionary principle, inter- and intra-generational equity, non-declining economic welfare, subsidiarity (as recognized in the Local Agenda 21 principle) and maintenance of the natural resource base and the waste absorptive capacity of the local and global environment.

Sustainability has been defined as the "capacity to create, test, and maintain adaptive capability" (Holling 1973) or as the resilience of socio-ecological systems (Carpenter *et al.* 2001). Others argue that sustainability implies "living within the regenerative capacity of the biosphere" (Wackernagel *et al.* 2002), or maintaining natural capital (Rees & Wackernagel 1996). Although the term 'sustainability' has been variously appended to describe states of biophysical/natural, financial/economic, manufactured and cultural or social capital, sustainable development is essentially more of a directional trajectory or an 'ethical guiding principle' along an aspirational path of human, social and economic development (Reid 1995, p16).

Methodologies for measuring sustainability range from sets of simple socio-economic and environmental quality or state indicators to complex, holistic integrated models with feedback loops and inter-linkages. The sustainability modelling methodologies considered include:

(i) macro-economic and socio-political indicators, including environmentally-adjusted net national product (EANNP), satellite environmental-economic accounting (SEEA), genuine savings approach, measure of economic welfare (MEW), index of sustainable economic welfare (ISEW) and genuine progress indicator (GPI);

(ii) environmental-economic indicators, including material flow or biophysical accounting and ecological footprint analysis;

(iii) systems-based approaches and integrated assessment (IA) frameworks, e.g. Integrated Sustainable Cities Assessment Method (ISCAM), multi-criteria decision analysis (MCDA), decision support systems, and analytical hierarchy process (AHP);

(iv) other modelling approaches, including input-output and econometric modelling of policies, computable general equilibrium (CGE) modelling, and systems dynamics models, e.g. Limits to Growth, POLESTAR, QUEST, Threshold 21.

The strengths, weaknesses, potential applications and stakeholders, data inputs, outputs and applicability at various spatial scales of these methodologies were compared and it was decided that material flow accounting (MFA), ecological footprinting and an integrated assessment (IA) method were the most suitable methods for measuring and monitoring sustainability at a sub-national level, as data are available for these methods to be implemented and they can be used for sectoral analysis, which allows for corroboration and analysis of individual results and indicates optimal pathways for defensive expenditure, policy formulation and objective-focusing. Other indicators, including welfare indicators and socio-political measures, are more operational at a macro-level,

whereas modelling approaches such as econometric modelling and CGE modelling are more useful for estimating the macro-economic effects of certain fiscal policies.

This paper focuses on material flow accounting within the Limerick city-region, using the metabolism framework, and proposes an innovative resource management indicator called sectoral metabolic inefficiency. The metabolism concept, as relating to urban planning, has been developed by Wolman (1965), Boyden *et al.* (1981) and Girardet (1992) and is a biological systems way of looking at the resource inputs and waste outputs of settlements (Newman 1999, pp220-221). Newman defined the goal of sustainability in a city as the reduction of the city's use of natural resources and production of wastes while simultaneously improving its liveability, so that it can better fit within the capacities of the local, regional and global ecosystems (Newman 1999, p220).

In terms of systems theory, city-regions or urban settlements are open, self-organizing, complex systems, which continuously degrade and dissipate available energy and matter (Rees & Wackernagel 1996, p237) by transforming low-entropy, high-quality materials into high-entropy residual wastes in a linear processing system. City-regions appropriate the ecological output and life support functions of the global hinterland through commercial trade and natural biogeochemical cycles (Rees & Wackernagel 1996, p236), appropriating large volumes of materials and discharging wastes, which place onerous demands on the assimilative capacity of the local environment. With levels of urbanisation in OECD countries approaching 80% (OECD 2001, p52), the focused concentration of population and consumption in large urban settlements give cities enormous leverage in the quest for global sustainability. Mitlin & Satterthwaite (1994) argue that urban settlements have lower costs per capita for public infrastructure amenities, greater opportunities for material recycling and re-use, high population density, which reduces the per capita demand for occupied land and per capita use of fossil fuel for space-heating, and potential for reducing personal transport energy consumption through walking, cycling and public transit (Rees & Wackernagel 1996, p242).

METHODOLOGY

Material flow accounting (MFA) aims to quantify the flow of resources, in terms of mass, within a defined geographical area or industry sector over a set period of time and is a measurement framework for direct and indirect biophysical flows between natural and socio-economic systems and involves input, output and consumption resource use indicators, including Direct Material Input (DMI), Total Material Requirement (TMR), Domestic Processed Output (DPO) and Domestic Material Consumption (DMC). The strengths, weaknesses and applications of MFA are given in Appendices: Table 1. In order to complete a material, product and waste flow analysis for the study area and assess urban metabolic inefficiency, it was necessary to quantify:

- Material production for Standard International Trade Classification (SITC) Divisions 00-43, including agriculture and natural textiles, forestry and wood products, fishing and aquaculture, coal, lignite and peat extraction, oil and gas extraction, construction materials and other crude minerals and mining and quarrying of metalliferous ores;
- Material imports, exports and consumption;
- Manufactured goods production and trade;
- Municipal, industrial and priority waste production;
- Methods of waste management and treatment, i.e. rates of disposal and recycling;
- Energy balances and emissions.

The study area selected for this research is the Limerick city-region, including the County Borough and its environs, and is part of a wider research programme currently being undertaken within the Centre for Environmental Research (CER) in the University of Limerick on community sustainability and future settlement patterns in Ireland. Within the context of the 2002 National Spatial Strategy (NSS), Limerick is regarded as a national/international gateway and, thus, has critical mass, strategic location, capacity for innovation and connections to the national transport network, serving as a locus for inward investment.

Data were collected for material flow analysis (MFA) and product flow analysis (PFA) from a number of sources, including the Central Statistics Office (CSO), PRODCOM, Census of Industrial Production (CIP), the Food and Agriculture Organisation (FAO), Organisation of Economic Co-operation and Development (OECD) and EUROSTAT and these data were then used to derive and calculate for 1992-2002:

- Material Input Indicators, including Direct Material Input (DMI), Total Material Input (TMI) and the Total Material Requirement (TMR);
- Material Output Indicators, including Domestic Processed Output (DPO) and the Total Material Output (TMO);
- Material Consumption Indicators, including Domestic Material Consumption (DMC), Total Material Consumption (TMC) and the Physical Trade Balance (PTB);
- Net Addition to Stocks (NAS) / Stock Changes;
- Product Consumption Flows.

In order to determine total urban metabolism and sectoral metabolic inefficiency, it was necessary to relate final waste disposal to household, commercial and industrial material and product consumption across a number of sectors, including food and organic waste, textiles and leather, paper and cardboard, chemicals, rubber and plastic products, metallic products and other household durable manufactured goods, industrial machinery and equipment, transport machinery and equipment, wood products and furniture, construction materials and non-metallic mineral products and other manufacturing. Where national data was used for consumption, relative weekly expenditure was used to adjust for Limerick residents. Domestic/residential, commercial and public services, industrial and personal and freight transport energy use were also included in the assessment of total metabolism and related to their sectoral emissions in order to determine sectoral energy metabolic inefficiency.

Limerick figures for household and commercial waste generated were used as well as the urban district recycling rate for household waste and these were used to estimate rate of disposal and, thus, the quantity of material household waste disposed of by various means. The national material recovery rates in the municipal waste stream were used to estimate rate of disposal and, thus, the quantity of material commercial waste disposed of by various means. Of the industrial waste generated in the different economic sectors, the quantities that were landfilled, incinerated, disposed of by various other means and reused/recycled were used to estimate the total rates of disposal and recovery and these were used to estimate the amount of industrial waste generated from that sector that was disposed of by various means.

RESULTS

Total Domestic Material Consumption (DMC) of agricultural and fish products, forestry and wood products, fossil fuels, construction materials and non-metallic mineral products and metalliferous ores by

Limerick residents increased from 1,070,259 tonnes in 1992 to 1,961,764 tonnes in 2002, i.e. an increase of 83.3% or 8.33% per annum (Tables 2 & 3). Domestic Material Consumption (DMC) per capita increased from 14.05 tonnes in 1992 to 22.55 tonnes in 2002, i.e. an increase of 60.5% or 6.05% per annum (Fig. 1). Total Direct Material Input (DMI) was calculated by summing annual national material production and material imports and this increased from 1,500,939 tonnes in 1992 to 2,468,311 tonnes in 2002, i.e. an increase of 64.45% or 6.45% per annum (Appendices: Tables 2 & 3). Direct Material Input (DMI) per capita increased from 19.7 tonnes in 1992 to 28.372 tonnes in 2002, i.e. an increase of 44% or 4.4% per annum (Fig. 2).

Total waste generated in Limerick and its environs, including municipal, i.e. household, commercial and litter and street sweepings waste as well as industrial and priority wastes, is given in Tables 4 & 5 and was calculated by aggregating the various waste stream components. Total waste generated per capita increased from 2.737 tonnes per capita in 1992 to 3.32 tonnes in 2003, i.e. an increase of 21.3% or 1.94% per annum. Fig. 3 shows the per capita increase in waste generation by Limerick residents from 1992-2003.

The Domestic Processed Output (DPO) per capita of Limerick residents increased from 11.93 tonnes in 1992 to 14.962 tonnes in 2000, i.e. an increase of 25.415% or 3.177% per annum. DPO per capita then fell to 14.3 tonnes in 2003, i.e. a decrease of 4.425% or 1.475% per annum. Direct Material Output (DMO) per capita increased from 14.848 tonnes in 1992 to 18 tonnes in 2000, i.e. an increase of 21.23% or 2.65% per annum. DMO per capita then fell to 17.247 tonnes in 2003, i.e. a decrease of 4.183% or 1.4% per annum. DPO and DMO for 1992-2002 are given in Tables 4 & 5. Fig. 4 shows the changes in per capita Domestic Processed Output (DPO) and Direct Material Output (DMO) from 1992-2003.

Metabolic inefficiency was estimated by dividing the sum of wastes from the household, commercial, industrial and priority wastes that were disposed of by various means by consumption. Total household, commercial, industrial, packaging and other priority waste, except for solid agricultural waste, disposed of by various means in Limerick in 1996 was 106,432.34 tonnes. Consumption of durable and non-durable products and construction materials was 827,010.544 tonnes and metabolic inefficiency was 12.87%. Total household, commercial, industrial, packaging and other priority waste disposed of by various means in 2002 was 125,733.423 tonnes. Consumption of durable and non-durable products and construction materials was 1,352,626.32 tonnes and metabolic inefficiency was 9.3%. Figs. 5 & 6 show the materials and waste sectoral and overall metabolic inefficiency for 1996 and 2002. Fig. 7 shows the comparative difference in sectoral and overall metabolic inefficiency between 1996 and 2002.

Total air emissions, including ammonia, carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4), nitrogen oxides (NO_X), nitrous oxide (N_2O), other greenhouse gases, sulphur dioxide (SO_2) and volatile organic compounds (VOC), by Limerick residents in 1996 were 770,785.17 tonnes (Central Statistics Office (CSO) 2004, pp22-27). Total residential, commercial and public services, industrial and transport coal, lignite, peat, oil and gas consumption in 1996 by Limerick residents was 140,258.455 tonnes and urban metabolic inefficiency was 549.55%. Total air emissions by Limerick residents in 2002 were 982,952.167 tonnes (Central Statistics Office (CSO) 2004, pp22-27). Total residential, commercial and public services, industrial and transport coal, lignite, peat, oil and gas consumption in 2002 by Limerick residents was 173,722.67 tonnes and metabolic inefficiency was 565.82%. Figs. 8 & 9 show the sectoral and overall energy and emissions metabolic inefficiency for 1996 and 2002. Fig. 10 shows the comparative difference in sectoral and overall metabolic inefficiency between 1996 and 2002.

CONCLUSION AND FUTURE WORK

In this paper, material flow accounting of a sub-national city-region was undertaken and it was found that both Domestic Material Consumption (DMC) per capita and Direct Material Input (DMI) per capita show an increase over the period 1992-2002, i.e. 6.05% and 6.45% per annum. Possible future work may be to relate these material flow indicator results to macro-economic indicators such as total Gross Domestic Product (GDP) and GDP per sector in order to determine trends in relative decoupling. The urban metabolism and metabolic inefficiency methods, which are proposed, aim to integrate the lifecycle of product consumption with environmental impact by comparing final waste disposal with household consumption in the city-region.

The metabolic inefficiency indicator developed within this paper is a novel empirical method for assessing urban sustainability and is useful in that it highlights the most inefficient sectors in terms of waste production relative to useful consumption. For example, it was found that in 2002 the most unsustainable sectors in terms of materials and waste were other household manufactured products, paper and cardboard and textiles while industry and commercial public services were the most unsustainable sectors in terms of energy use and emissions. It may be useful as a dematerialisation indicator or used concomitantly with municipal or industrial waste intensity indicators, i.e. per capita or Gross Domestic Product (GDP) to see if inefficiency indicators correlate with the largest sectoral material flows in terms of volume or waste flows in higher 'value-added' industrial sectors.

Although material consumption and waste production have increased from 1996-2002, metabolic inefficiency decreased from 12.87% in 1996 to 9.3% in 2002, suggesting a more efficient production and consumption system. However, the reduction in metabolic inefficiency was largely due to the increased consumption of construction materials in Limerick in 2002 and the fact that construction and demolition (C & D) waste produced showed little change. Energy and emissions metabolic inefficiency shows a slight increase from 549.55% in 1996 to 565.8% in 2002 with the commercial and public services and industrial sectors showing considerable increases in sectoral metabolic inefficiency.

In order to measure sustainability, sectoral metabolic efficiency, component ecological footprint analysis and sectoral integrated assessment are being used and it is hoped that these methods will corroborate and validate individual results and indicate optimal pathways for defensive expenditure and policy-making. At present, component ecological footprints for food, materials and waste, domestic and services energy, personal transport, freight transport, water metabolism and construction and the physical infrastructure are being calculated for 1996 and 2002 as well as the total ecological footprint for Limerick and its environs in order to estimate the environmental effects of material and product consumption. Consumption data and current and proposed national and European Union policy measures are also being used to calculate Trend-to-Target Indices and Sectoral Indices for various sectors, as part of the Integrated Sustainable Cities Assessment Method (ISCAM).

Although single and aggregate indicators can be useful in highlighting individual and overall trends, particularly at a macro level or when the indicators are selected on the basis of policy relevance, they are limited in terms of measuring sustainability of complex systems or highlighting synergies and feedback loops in society-nature interactions. Complex holistic models capture synergies and

homeostatic or self-regulatory feedback loops but are data-intensive and less transparent. Therefore, the ideal may be a complex model allowing for multiple-perspectives and scenario-analysis with a transparent interface, which allows the stakeholder to vary critical system parameters and simulate alternative scenarios.

It is anticipated, therefore, that a systems dynamics model will be developed, which can simulate component and aggregate ecological footprints within the timeframe of a strategic policy, plan or programme, allowing for alternative policy simulation and scenarios analysis. Systems dynamics will improve the dynamic and predictive capability of ecological footprint analysis, whilst providing a transparent interface with which stakeholders and end-users may explore alternative scenarios and simulate results of possible alternative policy strategies.

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Table 1 – Strengths, applications, weaknesses of Material Flow Accounting (Hinterberger *et al.* 2003, p11-12; Giljum & Hinterberger 2004).

Strengths	**Applications**	**Weaknesses**
• Complete accounting of the biophysical dimensions of economic activities • Ability to monitor rebound effects and shifts of pollution between different environmental media • Focuses on "persistent" environmental problems related to scale rather than toxicity-related environmental problems • Data organization is compatible with System of National Accounts • Indicators on all levels of aggregation (micro, sectoral, macro, input, output, consumption, trade) • Provides macro indicators, e.g. total environmental pressure equivalent to GDP, employment figures	• Integrated environmental-economic-social accounting • Calculation of resource productivities and role of technological and demand changes • Indicator of globalization, international trade and structural change and distribution of environmental burdens between developed and developing countries • Analyses of rebound effects by linking the macro and the micro perspective • Integrated sustainability modelling • Derivation of indicators for resource productivity and eco-efficiency • Provision of indicators for the material intensity of lifestyles • Allow policy-maker to react flexibly and quickly to new policy demands • Permit analytical uses, including estimation of material flows and land use induced by imports and exports as well as decomposition analyses separating technological, structural and final demand changes	• Aggregated indicators provide no information on material substitution potential • Weak links between MFA indicators and environmental impacts • No weighting of material flows and no consideration of qualitative aspects • Link to the actors responsible for the activation of material flows is not established and, therefore, it is not clear which groups of society should contribute to a strategy of dematerialisation • Economy as a black-box, i.e. no separation between material inputs for production versus consumption • MFA studies in most cases focus on methodological issues and the presentation of material balances and aggregated indicators and do not reflect the policy-related uses of results

Table 2 – Domestic Material Consumption (DMC) and Direct Material Input (DMI) by Limerick Residents and per capita, 1992-1997 (tonnes).

	1992	*1993*	*1994*	*1995*	*1996*	*1997*
Domestic Material Consumption (DMC)	1,070,259	1,112,936	1,264,530	1,329,624	1,402,029	1,669,859
Production	1,107,083	1,109,626.65	1,248,363	1,387,476	1,374,150	1,623,508
Imports	393,856	410,481	446,944.26	469,715	482,080.7	514,275
Direct Material Input (DMI)	1,500,939	1,520,107.65	1,695,307	1,857,191	1,856,231	2,137,783
Population	76,176.2	76,916.4	77,656.6	78,396.8	79,137	80,447
DMC per capita	14.05	14.47	16.284	16.96	17.716	20.757
DMI per capita	19.7	19.763	21.83	23.69	23.456	26.574

Table 3 – Domestic Material Consumption (DMC) and Direct Material Input (DMI) by Limerick Residents and per capita, 1998-2002 (Tonnes).

	1998	*1999*	*2000*	*2001*	*2002*
Domestic Material Consumption	1,814,241	2,098,628	1,746,762	1,988,551	1,961,764
Production	1,720,706	2,020,119	1,659,487.75	1,872,000	1,784,273
Imports	511,448	616,968	622,894	670,451	684,038
Direct Material Input (DMI)	2,232,154	2,637,087	2,282,381.75	2,542,451	2,468,311
Population	81,757	83,067	84,377	85,867	86,998
DMC Per Capita	22.19	25.264	20.7	23.16	22.55
DMI per Capita	27.3	31.75	27.05	29.61	28.372

Table 4 – Domestic Processed Output (DPO) and Direct Material Output (DMO) of Limerick Residents and per capita, 1992-1997 (tonnes).

	1992	*1993*	*1994*	*1995*	*1996*	*1997*
Total Waste Generated	208,495.71	210,124.6	226,545.03	235,223.28	245,693.1	268,142.05
Total Air Emissions	700,295.965	694,285.432	722,231.26	740,864	770,785.17	821,068.8
Greenhouse Gas Emissions	1,183.447	1,180.555	1,221.701	1,250.666	1,291.54	1,357.187
Domestic Processed Output	908,791.68	904,410.03	948,776.29	976,087.28	1,016,478.27	1,089,210.85
Population	76,176.2	76,916.4	77,656.6	78,396.8	79,137	80,447
DPO Per Capita	11.93	11.758	12.218	12.45	12.845	13.54
National Physical Exports	10,347,793	9,966,296	10,085,614	10,790,051	11,269,195	11,411,458
Population Proxy	0.02148	0.02157	0.021656	0.02174	0.0218	0.02189
Physical Exports	222,270.6	214,973	218,414	234,575.71	245,668.45	249,796.82
Direct Material Output	1,131,062.28	1,119,383.03	1,167,190.29	1,210,663	1,262,146.72	1,339,007.67
DMO Per Capita	14.848	14.553	15.03	15.443	15.95	16.64

Table 5 – Domestic Processed Output (DPO) and Direct Material Output (DMO) of Limerick Residents and per capita, 1998-2003 (tonnes).

	1998	*1999*	*2000*	*2001*	*2002*	*2003*
Total Waste Generated	285,500	296,890.55	312,826.42	257,693.92	282,111.68	293,199.47
Total Air Emissions	867,649	910,655	949,589.93	1,000,770.916	982,952.167	969,538.5
Greenhouse Gas Emissions	1,404.714	1,454.988	1,506.498	1,540.441	1,508	1,482.585
Domestic Processed Output	1,153,149	1,207,545.55	1,262,416.35	1,258,464.84	1,265,063.85	1,262,737.97
Population	81,757	83,067	84,377	85,867	86,998	88,308
DPO Per Capita	14.1	14.537	14.962	14.656	14.541	14.3
National Physical Exports	11,991,886	11,682,097	11,642,647	12,034,098	11,803,067	11,687,780
Population Proxy	0.02196	0.022	0.022088	0.0222	0.02221	0.02227
Physical Exports	263,341.82	257,006	257,162.8	267,157	262,146.12	260,286.86
Direct Material Output	1,416,491	1,464,551.55	1,519,579.15	1,525,621.84	1,527,209.97	1,523,024.83
DMO Per Capita	17.326	17.631	18	17.767	17.555	17.247

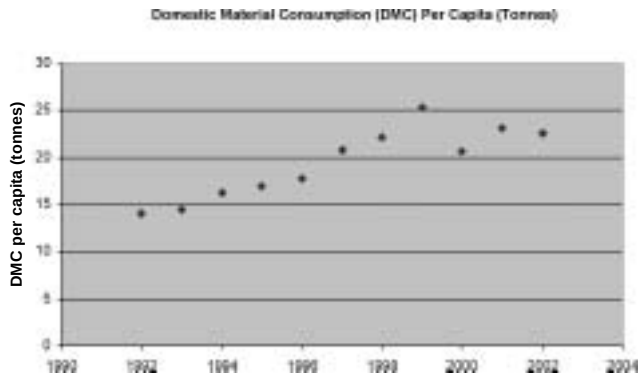

Fig. 1 – Domestic Material Consumption (DMC) per capita of Limerick residents, 1992-2002 (tonnes).

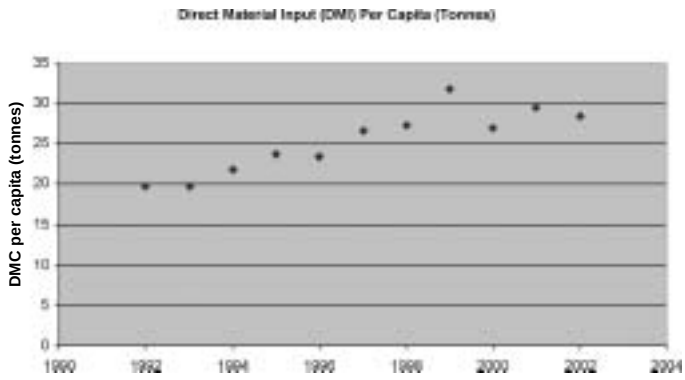

Fig. 2 – Direct Material Input (DMI) per capita of Limerick residents, 1992-2002 (tonnes).

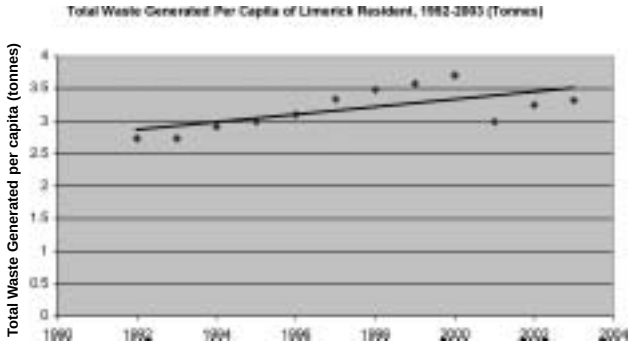

Fig. 3 – Total Waste Generated per capita by Limerick Residents, 1992-2003 (tonnes).

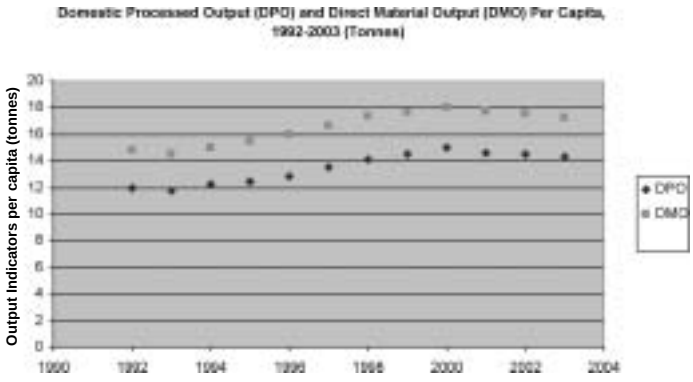

Fig. 4 – Domestic Processed Output (DPO) and Direct Material Output (DMO) per capita of Limerick Residents 1992-2003 (tonnes).

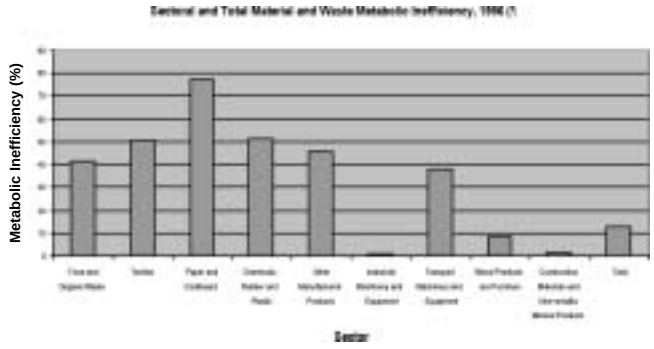

Fig. 5 – Sectoral and Total Material and Waste Metabolic Inefficiency, 1996 (%).

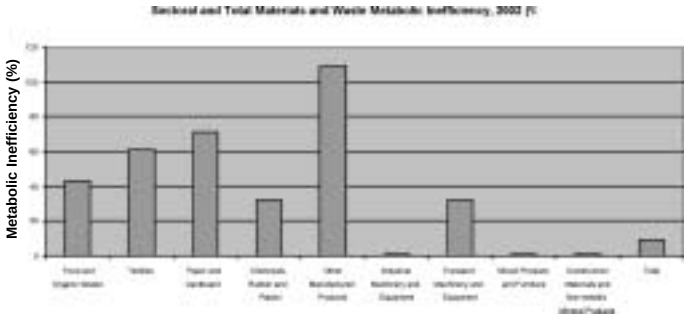

Fig. 6 – Sectoral and Total Material and Waste Metabolic Inefficiency, 2002 (%).

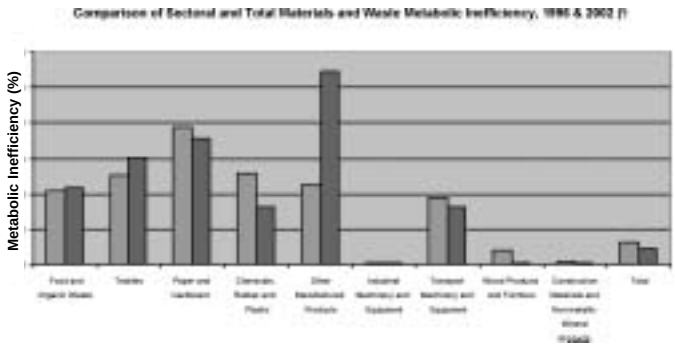

Fig. 7 – Comparison of Sectoral and Total Material and Waste Metabolic Inefficiency, 1996 & 2002 (%).

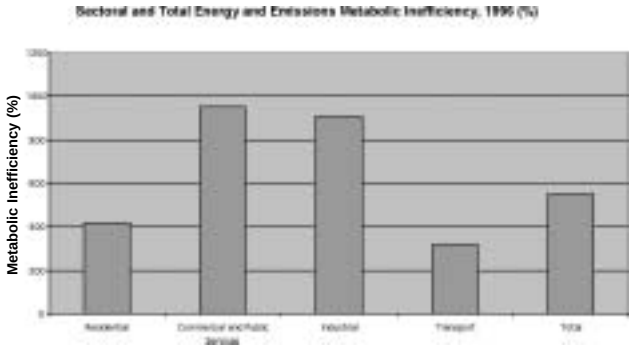

Fig. 8 – Sectoral and Total Energy and Emissions Metabolic Inefficiency, 1996 (%).

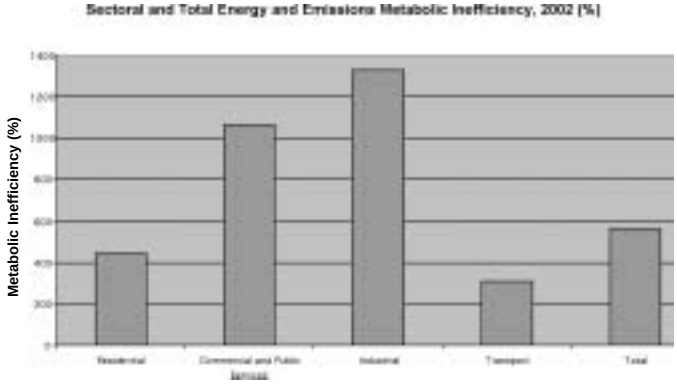

Fig. 9 – Sectoral and Total Energy and Emissions Metabolic Inefficiency, 2002 (%).

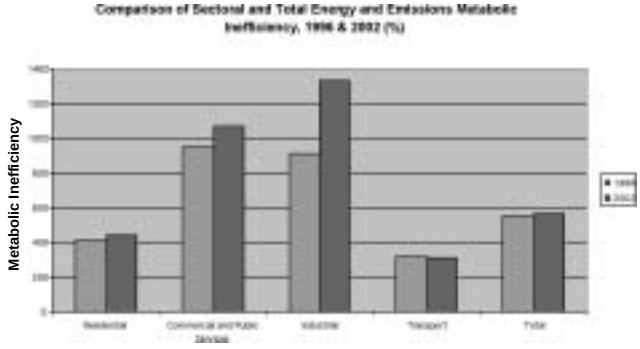

Fig. 10 – Comparison of Sectoral and Total Energy and Emissions Metabolic Inefficiency, 1996 & 2002 (%).

IDENTIFICATION OF THE SOURCE OF FAECAL CONTAMINATION IN WATER USING PCR

Siobhan Dorai-Raj, S. McHugh and Emer Colleran

ABSTRACT

In this study, six different PCR primer sets, designed to detect host-specific *Bifidobacteria* or *Bacteroides-Prevotella* species, were selected from recent literature. DNA was extracted from serial dilutions of artificially contaminated water and tested with these PCR primers in order to evaluate their sensitivity. Five of the primer pairs were specific to human faeces and could detect between 9.34×10^{-4} to 9.34×10^{-6} g (dry weight) of faeces/L. The sixth primer was ruminant-specific and had a sensitivity threshold of between 9.01×10^{-6} to 9.01×10^{-7} g (dry weight) faeces/L. The results obtained indicate that the more sensitive of these primers show promise as tools for tracking the source of faecal contamination in water.

Key words: faecal contamination; faecal source identification; water quality

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INTRODUCTION

It is estimated that there are in excess of 5,500 Group Water Schemes (GWS) in Ireland, providing water to approximately 200,000 households, mainly in rural areas. These GWS can be divided into two distinct groups, those that obtain water from the sanitary authority and distribute it themselves ('public' GWS) or those that source and distribute their own water ('private' GWS). It is the private GWS, which serve an estimated 50,000 domestic connections nationally, that are a source of ongoing concern to authorities, due to the unacceptably high level of faecal contamination present within the water supply. In 2003, 36% of private GWS monitored were contaminated by faecal coliforms (EPA 2004a). This level of faecal contamination is unacceptable and must be remedied. The EPA report on the "Quality of Drinking Water in Ireland for the year 2002" (EPA 2004b) highlighted the importance of source water protection and stated that to protect water resources, local authorities required detailed knowledge of the potential risks to water quality that exist in their functional areas so that these risks could be managed. To assess the potential risks and thus protect the source water, one must first identify the source of the pollution.

The most widely used faecal indicator microorganisms (coliforms, faecal coliforms, *Escherichia coli* and enterococci) are found in the faeces of both human and animals and, thus, can give no indication from which source the contamination originates. The need to identify the origin of faecal contamination in water has led, in recent years, to the development of faecal source identification (FSI) methodologies. Although the field of FSI is still in its infancy, researchers have used microbiological, genotypic, phenotypic and chemical methods to predict and identify sources of faecal pollution in water, with varying degrees of success (Gilpin *et al.* 2003; Scott *et al.* 2002; Simpson *et al.* 2002; Sinton 1998).

This study aims to assess the potential of an FSI method that is both library and culture independent and utilises molecular techniques, such as the polymerase chain reaction (PCR), to detect host-specific bacteria in contaminated water. The research is focused on the *Bacteroides-Prevotella* group and on the genus *Bifidobacterium. Bacteroides* and *Bifidobacterium* are the first and third most numerous bacterial populations in the human intestine, respectively (Nebra & Blanch 1999). The genus *Prevotella* contains 16 species that, before 1990, were all classified as *Bacteroides*, and, consequently, for many studies they are still grouped together (Shah & Collins 1990). Members of both of these groups are strict anaerobes, are restricted to warm-blooded animals and make up a significant portion of faecal bacteria. Most importantly, some species of the microorganisms are of human origin, whereas others are exclusively found in animals. The use of these organisms as indicators, however, has been limited because strict anaerobes are often difficult to grow. The use of molecular methods, such as PCR and probing, to detect the organisms can circumvent these difficulties.

The aim of this study was to evaluate the sensitivity of six different PCR primer sets, designed to detect host-specific *Bifidobacteria* or *Bacteroides-Prevotella* species. To achieve this, DNA was extracted from serial dilutions of water contaminated by either cow or human faeces and tested with these PCR primers.

MATERIALS AND METHODS

Sample collection

(i) Faecal samples: Human sewage samples were collected in sterile containers from the Mutton Island sewage treatment plant, Galway, Ireland, and from the Clarenbridge sewage treatment facility, Galway, Ireland. Fresh cow faecal samples were collected in sterile containers from three dairy farms in Co Galway, Ireland.

(ii) Water samples: Water samples were collected in 10-litre containers from the Corrib river, Galway, Ireland. The water was filtered through a GF/C Whatmann filter to remove debris and filter-sterilised using a 0.2mm pore-size Sartorius cellulose nitrate filter. The sterile water was stored in sterile 1l containers at room temperature.

Sensitivity analysis

Serial tenfold dilutions of fresh cow faecal or raw human sewage were made using filter-sterilised river water. The final concentrations ranged from $1 ¥ 10^{-2}$ to $1 ¥ 10^{-8}$ g (wet weight) faeces/L. The samples were filtered through a 0.2mm pore-size Sartorius cellulose nitrate filter and stored at -80°C. DNA was extracted from the filters and amplified with the various primers, as described below. The dry weights of the faecal samples were estimated by, firstly,

Table 1 – PCR primers used in this study.

Primers	Target	Faecal Host	Annealing Temperature	Reference
BV 1 & 2	*Bacteroides vulgatus*	Human	50°C	Wang *et al.* 1996
BT 1 & 2	*Bacteroides thetaiotamicron*	Human	50°C	Wang *et al.* 1996
BiADO 1 & 2	*Bifidobacteria adolescentis*	Human	55°C	Matsuki *et al.* 1998
BiCAT 1 & 2	*Bifidobacteria catenulatum*	Human	55°C	Matsuki *et al.* 1998
HF183F & Bac708R	*Bacteroides-Prevotella*	Human	59°C	Bernhard & Field 2000
CF128F & Bac708R	*Bacteroides-Prevotella*	Cattle	58°C	Bernhard & Field 2000

weighing replicate samples of wet faeces, then drying the samples for 24 hours in a 100°C oven, before re-weighing the samples and calculating the dry weights.

DNA extraction

(i) Faecal samples: DNA was extracted from 10-25 mg of cow or human faeces using the MOBIO UltraClean Soil DNA Isolation Kit, following the manufacturer's protocol, with the following modifications: following addition of Solution S1, a 10 min incubation step at 70°C was performed; following addition of Solution IRS, the bead tubes were vortexed using a Biospec Mini-Beater for 5 min; and the washing step with Solution S4 was performed 3 times.

(ii) Water samples: The water samples were filtered with a 0.2mm pore-size Sartorius cellulose nitrate filter and DNA was extracted from the filters as follows: 500 µl of DNA extraction buffer (Zhou *et al.* 1996) was added to the filter, along with 500 µl of lysis buffer (50mM Tris-HCl, 50mM EDTA, 750mM sucrose) and 40 µl of lysozyme (10 mg/ml). The samples were incubated at 37°C for 30 min, followed by the addition of 200 µl of 10% SDS and a further incubation step at 70°C for 60 min in a shaking waterbath. Six microlitres of proteinase K (10 mg/ml) was then added to each and the samples were incubated at 50°C for 30 min. The DNA was then purified by phenol-chloroform-isoamyl alcohol extraction, precipitated with one volume of 100% isopropanol and washed with 70% ethanol, before resuspension in 50 µl of TE buffer.

Extracted DNA was cleaned using Spin-X microcentrifuge 0.22 µm cellulose acetate filters and Sephadex G-50 (Sigma-Aldrich) and stored at -20°C. DNA was quantified by electrophoresis on a 1% agarose gel, using Hyperladder I (Bioline) for comparative purposes, on FluorChem gel imaging software.

Quantification of faecal bacteria

The numbers of *E. coli* and *Enterococci* in the artificially contaminated water samples were measured using the IDEXX Colilert® and Enterolert® tests, respectively, according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR)

The DNA was amplified with the primers listed in Table 1, following the conditions specified in the source paper with the exception of the HF 183 F & Bac 708 R and CF 128 F & Bac 708 R primer set. The cycling conditions followed were 35 cycles consisting of 94°C for 30 sec, the relevant annealing temperature for 1 min, and 72°C for 30 sec followed by a final 7 min extension at 72°C. DNA extracted directly from the human and cow faecal samples was used as a positive template control for the relevant primers.

RESULTS AND DISCUSSION

One of the major problems encountered during the course of this research was the difficulty in the extraction of pure contaminant-free DNA. This is a common problem with faecal samples since faeces, both human and animal, can contain a number of substances such as bile salts, humic acids or phenolic acids that will inhibit PCR (Kreader 1995; Matsuki *et al.* 1999; Nebra *et al.* 2003; Satake *et al.* 1997). The problem was overcome when extracting DNA from the faecal samples by including additional washing steps in the protocol supplied with the Ultraclean™ soil DNA kit (MOBIO). However, this modified protocol was unsuccessful in extracting DNA from the membranes used to filter the serial dilutions of artificially contaminated water. Furthermore, attempts to extract DNA from these membranes using the DNeasy tissue kit (QIAGEN), as was recommended, (Dick & Field 2004; Field

Table 2 – Detection limits of host-specific PCR Primers.

Primers	Source of DNA	Detection limit (g of wet faeces)	Detection limit (g of dry faeces)	Number of *E.coli* in equivalent weight of faeces	Number of *Enterococci* in equivalent weight of faeces
BV 1 & 2	Human	1×10^{-3}	9.34×10^{-5}	2.4×10^{2}	1.4×10^{2}
BT 1 & 2	Human	1×10^{-2}	9.34×10^{-4}	2.4×10^{3}	1.4×10^{3}
BiADO 1 & 2	Human	1×10^{-3}	9.34×10^{-5}	2.4×10^{2}	1.4×10^{2}
BiCAT 1 & 2	Human	1×10^{-3}	9.34×10^{-5}	2.4×10^{2}	1.4×10^{2}
HF183F & Bac708R	Human	1×10^{-4}	9.34×10^{-6}	2.4×10^{1}	1.4×10^{1}
CF128F & Bac708R	Cattle	1×10^{-4} to 1×10^{-5}	9.01×10^{-6} to 9.01×10^{-7}	3.8×10^{0} to 3.8×10^{-1}	4.1×10^{0} to 4.1×10^{-1}

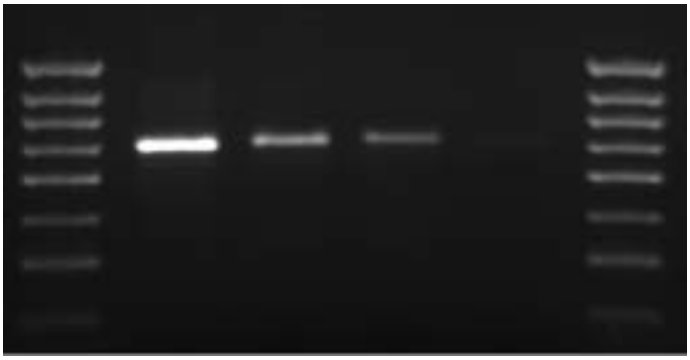

Fig. 1 – Gel electrophoresis of DNA amplified using the CF128F & Bac708R primer pair Lane 1: Hyperladder IV; Lane 2: 1×10^{-2} g (wet weight) cow faeces/L; Lane 3: 1×10^{-3} g (wet weight) cow faeces/L; Lane 4: 1×10^{-4} g (wet weight) cow faeces/L; Lane 5: 1×10^{-5} g (wet weight) cow faeces/L; Lane 6: Hyperladder IV.

et al. 2003) also failed (data not shown). These kits were only successful in extracting DNA from membranes used to filter river water that was not previously filter sterilised. It would appear, therefore, that filter sterilisation reduced the microbial load and, thus, the DNA to a level undetectable by the kits. Use of the SDS-based chemical extraction method, described in the methods section, allowed successful extraction of DNA from all membrane filters, although an extra purification step using Spin-X microcentrifuge 0.22 µm cellulose acetate filters and Sephadex G-50 (Sigma-Aldrich) was required. Despite this extra cleaning step, some contaminants remained and it was necessary to dilute the DNA (1/5 to 1/50 dilution) before all PCR inhibitors were removed.

The BV 1 & 2 and BT 1 & 2 primers (Wang *et al.* 1996) and BiADO 1 & 2 and BiCAT 1 & 2 primers (Matsuki *et al.* 1998) were not designed for the purpose of faecal source identification. Consequently, in order to assess their specificity, they were tested with 6 human faecal DNA samples, 5 sheep faecal DNA samples, 5 horse faecal DNA samples and 11 cow faecal DNA samples. All four sets of primers amplified DNA from the human faecal samples alone (data not shown). The two sets of primers HF183F & Bac708R and CF128F & Bac708R were previously used under different PCR conditions to those employed in the present study (Bernhard & Field 2000). Therefore, the efficacies of these primer sets, under the new conditions of this study, were tested with all the above DNA samples. As expected, the HF183F & Bac708R primers amplified DNA from the human faecal samples only and the CF128 F & Bac708 R primers amplified DNA from the ruminant faecal samples only (results not shown).

Fig. 2 – Gel electrophoresis of DNA amplified using the HF183F & Bac708R primer pair Lane 1: Hyperladder IV; Lane 2: 1×10^{-2} g (wet weight) human sewage/L; Lane 3: 1×10^{-3} g (wet weight) human sewage/L; Lane 4: 1×10^{-4} g (wet weight) human sewage/L; Lane 5: 1×10^{-5} g (wet weight) human sewage/L; Lane 6: Hyperladder IV.

Following evaluation of the specificity of the primers, their sensitivity was investigated using DNA extracted from serial dilutions of filter sterilised river water artificially contaminated by either cow or human faeces. The results are summarised in Table 2.

The primers specifically designed for the purpose of faecal source identification (HF 183F & Bac 708R and CF 128F & Bac708R) were the most sensitive. The DNA amplified using these primer sets can be seen in Figures 1 and 2. The HF 183F & Bac 708R primer set could detect 9.34×10^{-6} g (dry weight) of faeces/L and the CF 128F & Bac708R primer set could detect 9.01×10^{-6} to 9.01×10^{-7} g (dry weight) faeces/L. The more sensitive nature of these primers may be due to the fact that, unlike the other primers, they were not designed to detect a single species of bacteria, using sequences obtained from pure cultures, but were designed using *Bacteroides-Prevotella* sequences recovered from faecal and contaminated water clones (Bernhard & Field 2000). This may suggest that these primers are more relevant for use in faecal source identification.

The BV 1 & 2, BiAdo 1 & 2 and BiCat 1 & 2 primer sets amplified DNA at a faecal contaminant level of 9.34×10^{-5} g (dry weight) faeces/L, which corresponds to 24 *E. coli* or 14 *Enterococci* cells per 100 ml. This very sensitive detection limit merits the further use of these primers and, along with the HF 183F & Bac 708R and CF 128F & Bac708R primer sets, should be used in future faecal source identification experiments.

CONCLUSION

Although the sensitivity of the primers vary, all with the exception of the BT 1 & 2 primer pair, show promise as tools for tracking the source of faecal contamination. Extensive field-testing under various environmental conditions, however, will be required before they can be used in routine water monitoring. The survival time of *Bifidobacteria* and the *Bacteroides-Prevotella* group has not been assessed in water. These primers should, therefore, be tested in both artificially contaminated water and environmental water samples over a defined period of time to assess if the primers are indicative of more than just recent faecal contamination. The CF 128F & Bac 708R primers are the only pair specific for ruminant faeces. Although these primers exhibit adequate sensitivity, an additional set of primers specific for ruminant faeces would improve the accuracy of the PCR assay panel. In addition, the possibility of developing primers specific for porcine or poultry faeces also merits investigation.

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ISOLATION AND CHARACTERIZATION OF HYDROPHOBIC AND HYDROPHILIC ACID FRACTIONS IN THE DRAINAGE WATER FROM UNDISTURBED MONOLITH IN LYSIMETERS

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ABSTRACT

Humic substances (HS) typically constitute 40-60% of the dissolved organic matter (DOM) in surface waters. However, little information is available with regard to why more DOM is lost from some soils than from others. Drainage water samples (45L-50L) were collected from an experimental lysimeters facility between January and June, 2003. Hydrophobic (Ho) and hydrophilic (Hi) acids were isolated from the drainage water using XAD-8 [(poly)methylmethacrylate] and XAD-4 (styrene divinylbenzene) resin columns in tandem. The Ho acids can be considered to be composed of humic acids (HAs) and fulvic acids (FAs), and the Hi acids are known as the XAD-4 acids, and would fall outside the classical definitions of HS. Characterizations were carried out using Fourier transform infrared spectroscopy (FTIR), and amino acids and neutral sugar analyses. The amounts of DOM recovered decreased in the order: *Castlecomer* > *Oakpark* > *Rathangan*> *Elton* > *Clonroche.* With the exception of the Oakpark DOM, the order could be related to the drainage regime and to soil mineralogy rather than to the organic matter contents of the surface soils. Ho and Hi acids ranged from 62-90% and 10–28% of the total DOM. Significant differences were observed between the DOM fractions. The data emphasize that considerations of the mineral composition and the drainage regimes of soils are likely to be major determining factors in the leaching of DOM from soils to surface waters. Soil management will also have a role. Studies of the nature of the organic matter (SOM) in the different soils are ongoing and awareness of the compositions of SOM fractions will give more definite indications of the nature of the SOM that can be expected in drainage DOM. This is important for estimates of losses of DOM to reservoirs and estuarine environments.

Key words: humic substances; dissolved organic matter; hydrophobic/hydrophilic acids fraction; soil lysimeter; amino acids; neutral sugars; FTIR

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INTRODUCTION

Dissolved Organic Matter (DOM) is the soluble organic material that passes through ~0.1 and 1μm mesh size filters (Najjar *et al.* 1991). It originates mainly in the transformed soil organic matter (SOM) of vegetation and biological debris, from root exudates, and from the lysis of microorganisms. Addition of biological waste materials, such as livestock manures and sewage sludge, increases the concentrations of DOM in soil, either by acting as direct sources of DOM, especially when biotransformed, or by enhancing the solubilization of the SOM (Daudén *et al.* 2004).

Humic substances are major components of SOM and of DOM. Aquatic HS compose approximately 40%-60% of the DOM (Malcolm 1985; Leenheer & Croue 2003). The compositions of HS in terrestrial water have been shown to relate to the HS in the soils of the watersheds (Watt *et al.* 1996).

Thus, the concentrations of DOM, and their compositions and chemistries are highly variable, and depend on the sources of the organic matter, the temperature, ionic strength, pH, the major cation compositions of the water, the surface chemistry of sediment sorbents that influence solubility, and on the presence of photolytic and microbiological degradation processes. Although extensive studies have been carried out on some aspects of DOM, little is known about the compositions and aspects of the structures of the dissolved organic carbon (DOC) leached in the solutes from different soil types under field or simulated field conditions. By relating the properties of the DOC with those of the indigenous SOM it will be possible to gain an understanding of the reasons why more DOM is released from some soils than from others. Water quality is closely linked with soil quality. There are no reliable estimates of the amounts of carbon lost in drainage water from Irish soils.

Soil lysimeters are applied widely for research related to nitrate leaching and the transport of soil contaminants (Logsdon *et al.* 2002; Daudén *et al.* 2004; Mikata *et al.* 2003). In the present study drainage water was collected from a lysimeter facility to investigate NO_3-N leaching from five representative Irish soils at the Teagasc, Johnstown Castle Research Centre (Ryan & Fanning 1996). Aquatic humic substances (HS) were isolated from the drainage water using XAD-8 and XAD-4 resins in tandem.

Humic acids (HAs) and fulvic acids (FAs) are the so-called Ho fractions retained by XAD-8 resin. The Hi fractions which pass through XAD-8 are adsorbed by XAD-4 resin and these relatively highly charged components are known as the XAD-4 acids rich in aliphatic acids, carbohydrates, amino acids, etc. In the classical definitions the XAD-4 were components of FA fractions. The XAD-8 and XAD-4 resin in tandem technique has previously been successfully used for isolation of aquatic HS (Malcolm 1992; Watt *et al.* 1996; Hayes & Graham 2000).

MATERIALS AND METHODS

SOIL LYSIMETERS

The lysimeter studies involve five soils (Oakpark, Clonroche, Elton, Rathangan, and Castlecomer, Table 1)

Table 1 – Soil descriptions and leachate volumes† from the different lysimeters.

Soil	Volume (l)	Soil descriptions
Castlecomer	50	Poorly drained organic clay loam over clay loam texture;
Rathangan	50	Poorly drained loam over clay loam texture;
Oakpark	50	Very well drained gravelly brown earth of coarse sandy loam texture;
Elton	50	Well drained gravelly loam over gravelly clay loam texture;
Clonroche	45	Well drained loam to clay loam, over shaley loam texture brown earth.

† Collections were made between January and June 2003. Drainage water was a combination from all treatment collected based on soil types. These lysimeters last received fertilizer in autumn 2003.

from major Irish soil types. Soils were collected as undisturbed monoliths, transported to the Teagasc Johnstown Castle Research Centre, and arranged randomly in an experimental facility (Ryan & Fanning 1996). Each soil type was sown with perennial ryegrass and subject to replicated treatments of 0, 225 and 390 kg/ha N between 2001 and 2003. Surface soils (0-15cm, 15-30cm) from Oakpark, Clonroche, Elton and Castlecomer have organic carbon contents of 3.39%, 2.85%; 4.90%, 3.95%; 5.65%, 3.30%; 5.30%, 3.69%; respectively. Carbon contents decrease significantly with soil depth. The organic C content at different depths in the Rathangan profile (0-15cm, 15-30cm, 30-45cm, 45-60cm) were 3.74, 3.07, 3.49, 1.34%, respectively.

ISOLATION OF HYDROPHOBIC AND HYDROPHILIC FRACTIONS

Isolation

Water was filtered under pressure (compressed air, 10 psi) through 0.2mm pore size, 142mm diameter Sartorius cellulose acetate membranes, in order to avoid biological activity and to remove particulate matter. When a degree of membrane clogging had taken place and the effective pore size was decreased, the water was again passed through the membranes (Hayes & Graham 2000). The filtered samples were adjusted to pH 2.0, using 6 M HCl, and pumped (using a peristaltic pump) slowly through XAD-8 and XAD-4 resins in tandem (40ml/min). Distilled water was passed through the column until the conductivity was <100 uS cm^{-1}. Then the column was back eluted with 0.1 M NaOH, and the eluate was passed through the IR-120 cation exchange resin and freeze dried.

Characterization

Amino Acid and Sugar Analyses. Procedures outlined by Watt *et al.* (1996) were used.

Infrared Spectroscopy. KBr pellets were prepared using 100 mg of KBr (IR spectroscopy grade, oven dried at 110^{o}C) and 1 mg of dry samples. A Bomen FTIR model Amwen/32 instrument was used.

RESULTS AND DISCUSSION

There were significant differences in the amounts of the DOM fractions in the five drainage waters (Table 2). Invariably Ho acids were most abundant, and the relative abundances decreased in the order *Castlecomer* > *Oakpark* > *Rathangan* > *Elton* > *Clonroche.* Ho and Hi acids accounted for 62-90% and 11 to 28%, respectively, of the total DOM, except in the case of the very low-yielding Clonroche water. Based on the SOM contents, DOM was released more readily from the Castlecomer, Oakpark and Rathangan soils than from the Elton and Clonroche soils.

Amino Acids and Sugars

The data in Table 3 indicate that the amino acids (AA) contents of the hydrophobic and hydrophilic acids were between 0.6% and 2.4% of the total masses of the DOM fractions. These values were in the range (0.6-3.8%) found also by Watt e*t al.* (1996), and by Foan (2001) for the HS isolated from British and Irish water and drainage water from grassland and cultivated soils. For the Ho fractions, the AA contents followed the order: *Elton* > *Castlecomer* > *Oakpark* >*Rathangan.* The AA contents in the Rathangan Ho and Hi acids were 0.6 and 2.0%, respectively. However, Oakpark Ho and Hi acids were 1.1 and 0.94%, respectively. That might suggest that there was a higher proportion of FAs than HAs in Rathangan drainage water.

The order of abundances of the different classes of AAs in the Ho acids from the Elton and Rathangan soils was: Total Neutral Hydrophilic (TNHi) ≈ > Total Neutral Hydrophobic (TNHo) > Total acidic (TA) > Total basic (TB). The same trend was observed (TNHi > TA > TNHo > TB) for the Hi acids.

Table 4 shows that the amounts of neutral sugars (NS) isolated from the drainage water from the lysimeters were in the range of 15 mg mg^{-1} – 43 mg mg^{-1}. The NS contents for the Ho acids decreased in the order: *Rathangan* > *Castlecomer* > *Oakpark* > *Elton* > *Clonroche.* The Hi acids, as expected, were more enriched in NS than the Ho acids, and in contrast to some drainage water (Watt *et al.* 1996), the HAs (data not shown) were more enriched in NS than the FAs. Based on suggestions by Oades (1984), the mass ratio values (Mannose + Galactose)/(Xylose + Arabinose) would suggest that the DOM components from Oakpark soil had microbial origins. Values of the order of 2 would indicate microbial sources, and values approaching 0.5 would suggest plant origins. These ratio values would suggest that the DOM in all of the water was more likely to be derived from microorganisms than from

Table 2 – Yields and concentrations (concn.) of DOM, and the percentages of the Ho and Hi fractions in the different drainage water.

Sample	Concn. of total HS†	Hydrophobic acids (Ho)		Hydrophilic acids (Hi)	
	mg L^{-1}	*mg*	*%*	*mg*	*%*
Castlecomer	3.56	158	88.7	20	11.2
Oakpark	2.12	66	62.2	40	37.7
Rathangan	1.08	44	81.4	10	18.5
Elton	0.70	25	71.4	10	28.5
Clonroche	≈0.24	10	>90.0	<1	<10.0

† Concentration of DOM was calculated from amounts of hydrophobic plus hydrophilic fractions.

Table 3 – Contents of amino acids in the digests of the hydrolysates of the hydrophobic (Ho) and hydrophilic (Hi) acids isolated from the drainage water from the soil lysimeters.

Amino acid	Castlecomer Ho†	Elton Ho	Rathangan Ho	Rathangan Hi§	Oakpark Ho	Oakpark Hi
			< nmol mg^{-1} >			
Gly	19.3	28.4	11.0	46.6	20.2	13.8
Ala	17.0	26.3	6.5	23.7	10.4	8.4
Ser	8.8	5.9	1.5	5.4	3.0	8.2
Thr	10.1	16.8	5.6	22.8	9.1	8.2
Total Neutral Hi	**55.3**	**77.4**	**24.6**	**98.5**	**42.8**	**38.5**
Asp	17.4	24.2	6.1	22.2	12.9	15.3
Glu	15.2	27.7	6.6	21.1	9.9	15.1
Total Acidic	**32.6**	**51.9**	**12.7**	**43.3**	**22.9**	**30.4**
Phe	4.1	11.7	2.1	5.9	0	2.2
Ileu	3.9	9.5	1.7	4.6	0	2.0
Leu	9.8	36.6	6.0	11.7	0	4.1
Tyr	2.4	3.8	0.8	2.2	1.4	1.4
Val	10.5	16.0	4.1	13.0	7.7	5.0
Total Neutral Ho	**30.7**	**77.5**	**14.6**	**37.4**	**9.1**	**14.7**
Lys	2.1	9.2	5.3	1.8	2.6	2.3
Arg	5.2	7.3	2.2	7.7	14.5	3.3
His	1.5	3.0	0.7	3.1	7.7	
Total Basic	**8.8**	**19.5**	**8.3**	**12.6**	**24.9**	**5.6**
Total	*127.4*	*226.3*	*60.1*	*191.9*	*99.7*	*89.2*
% AAs*	1.34	2.39	0.63	2.02	1.05	0.94

† Hydrophobic (Ho) acids; § Hydrophilic (Hi) acids; * % AAs refers to the percentage in DOM samples; Total amino acids were calculated on an oven-dry and ash-free basis.

plants. The same trends were evident from the (Rha + Fuc)/(Xyl + Ara) ratio values, although the evidence for microbial contributions were less evident for the Oakpark water than for the (Mannose + Galactose)/(Xylose + Arabinose) ratio values.

FTIR

The infrared spectra showed some similarities and differences (see Fig.1-a, Fig.1-b). With the exception of the Hi fractions from Oakpark and Castlecomer drainage water, a major absorption band is evident in all fractions at 1720cm^{-1}, indicative of the carbonyl of carboxyl. In the cases of the Oakpark drainage water, the DOM of both the Ho and Hi fractions have greater resolution in the fingerprint region than for the samples from the other drainage water. The strong band at 3400-3000cm^{-1} indicates of H-bonded OH stretching, and is often indicative of phenolic and other OH groups.

The major absorption bands in the cases of the XAD-8 fractions were at 3440cm^{-1}, 2990cm^{-1}, 1720cm^{-1}, 1620cm^{-1}, 1400cm^{-1}, 1260cm^{-1} and 108 cm^{-1}. The bands at 1080cm^{-1} can be assigned to saccharides. The absorbance attributed to C-O stretching and OH deformation of COOH and the C-O stretching of aryl ethers occurs at around 1260cm^{-1} for the XAD-8 fractions. The peak at around 1720cm^{-1} was less dominant in the cases of the XAD-4 acids than for the XAD-8 fractions. Hydrophilic acids are known to be less aromatic in nature and to contain less carbon and more nitrogen than the corresponding FAs and HAs that would compose the Ho fractions (Croue *et al.* 1993; Foan 2001).

Recent studies of HS indicate relatively small primary molecular structures (100-2000 Da) with macromolecular characteristics resulting from aggregates formed by hydrogen boning, non-polar interactions and polyvalent cation interactions (Piccolo *et al.* 2001; Hayes & Clapp 2001; Simpson *et al.* 2002; Leenheer & Croue 2003). The molecules composing the DOM are likely to have less associations than the more molecularly interactive HS in soil aggregates.

CONCLUSIONS

Using XAD-8 and XAD-4 resin columns in tandem, hydrophobic (Ho) and hydrophilic (Hi) acids fractions of DOM were isolated from the drainage water of five representative Irish soils. For the most part the amounts of DOM recovered could be related to the drainage regimes of

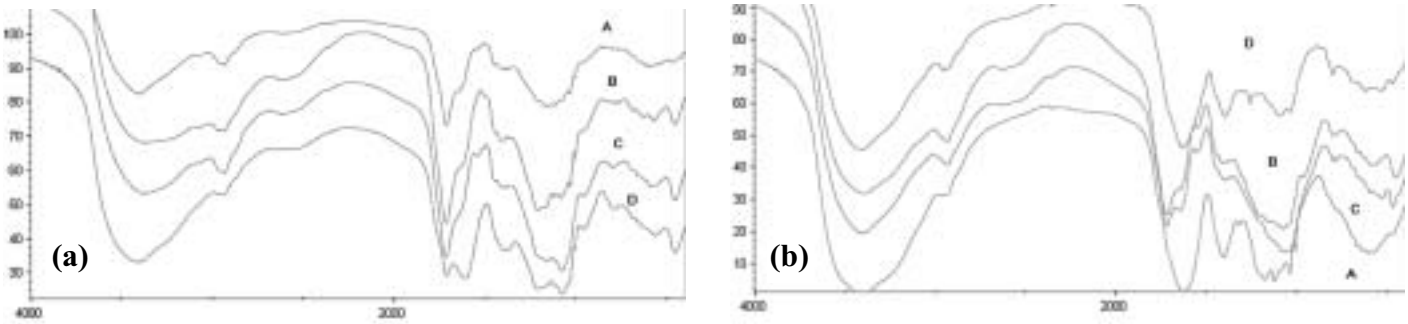

Fig. 1 – FTIR spectra for hydrophobic (Ho) and hydrophilic (Hi) acids from drainage water from the lysimeters. (a) Ho acids from A, Clonroche; B, Elton; C, Castlecomer; D, Oakpark. (b) Hi acids from A, Oakpark; B, Elton; C, Rathangan; D Castlecomer.

Table 4 – Contents of neutral sugars in digests of the hydrolysates of hydrophobic (Ho) and hydrophilic (Hi) acids isolated from the drainage water of the soil lysimeters.

	Elton	Rathangan		Oakpark		Castlecomer		Clonroche
mg mg^{-1}	Ho	Ho	Hi	Ho	Hi	Ho	Hi	Ho
Rhamnose	5.38	1.84	5.87	2.41	4.38	1.83	5.11	7.46
Fucose	4.11	1.43	3.35	1.89	3.29	1.48	3.97	6.35
Arabinose	4.55	1.63	3.39	2.38	4.17	2.00	4.42	6.22
Xylose	4.44	2.12	5.69	2.40	4.21	1.89	4.72	6.53
Mannose	6.27	2.96	7.75	5.58	10.33	2.32	6.54	10.38
Galactose	7.57	2.32	7.15	4.06	8.47	3.66	7.83	6.37
Glucose	6.56	2.73	9.37	3.05	8.12	3.63	7.77	8.69
Total	38.88	15.01	42.57	21.77	42.97	16.83	40.36	51.98
(Man+Gal)/ (Xyl+Ara)	1.54	1.41	1.64	2.02	2.24	1.54	1.57	1.31
(Rha+Fuc)/ (Xyl+Ara)	1.05	0.87	1.02	0.90	0.92	0.85	0.99	1.08

the soils which would reflect the mineralogy of the soils. There was no direct correlation between the amounts of DOM isolated and the organic matter contents of the surface soils. The Ho and Hi acids contents ranged from 62-90% and 10-28%, respectively of the total DOM.

The amino acids and neutral sugars contents of the DOM fractions from the drainage water contained much smaller concentration of HS than soils (data not shown here). A likely explanation is that the saccharide and peptide components have stronger associations with the HAs (especially) in soil organic matter than with the components that are readily dissolved in the drainage water. It is possible also that saccharides and peptides released from the surface organic matter were bound to the soil materials during passage through the soil column, or were metabolized by soil microorganisms as they passed down the soil column.

DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures associated with amide, carboxyl, hydroxyl, carbonyl and a variety of other functional groups (Simpson *et al.* 2002). Heterogeneous molecular aggregates in natural water increase DOM complexity (Leenheer & Croue 2003). An understanding of the compositional and structural chemistry of DOM components can have relevance to the assessments of carbon sequestering potential of agricultural soils, and especially such can allow predictions of the extents to which organic matter can be leached from soils and deposited in reservoirs and estuarine environments.

Ongoing studies relate the compositions of the SOM of the parent soils with those in the drainage water, and the relationships between the soil and drainage organic materials will provide a better understanding of management procedures for the conservation in soil of the potentially water soluble components of SOM.

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A GIS-BASED MODEL OF ARCTIC-ALPINE PLANT HABITAT DISTRIBUTIONS AND ITS APPLICATIONS FOR BIODIVERSITY CONSERVATION

A. Rico Santiago

ABSTRACT

Mapping species distributions is an integral component of the plant biodiversity strategy of Northern Ireland. Unfortunately, current species maps impart only a general understanding of distribution patterns and the factors affecting them. Particularly, Arctic-Alpine plants are known to have a restricted presence in Ireland, but their spatial distribution is poorly understood due to the remote and often inaccessible areas in which they occur. To address this, a GIS-based potential habitat model was developed as a mapping tool with which to investigate abiotic landscape factors affecting Arctic-Alpine plant distribution in Northern Ireland. The model's applications for biodiversity conservation are discussed.

Key words: GIS; habitat modelling; biodiversity conservation

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INTRODUCTION

Ireland's flora can be described as an impoverished subset of northwest European flora. Although the island possesses only a limited number of endemic populations, Curtis and McGough (1988) note that half of its species represent distinct distributional elements, such as Arctic-Alpine communities, which may be in decline in other locations. These unique floral assemblages are threatened by the progressive alteration and modification of large areas of habitats (Curtis & McGough 1988) in conjunction with environmental shifts affecting global biodiversity (Novacek & Cleland 2001). As a result of these threats, the government of Northern Ireland has implemented initiatives to protect its native flora within the frameworks of both domestic (e.g., the Wildlife (Northern Ireland) Order 1985) and international (e.g., the EU Habitats Directive 1992) legislation.

Northern Ireland's biodiversity conservation strategies rely on knowledge of species distributions, often disseminated through maps. These maps are necessary tools used by regulatory bodies to make informed judgements about the conservation status of species (Miller 1994). Traditionally, the spatial extents of species' ranges are derived from sightings datasets in a process where boundaries are drawn to encompass point data on maps. However, this method of delimiting species' ranges is plagued by coverage inconsistencies because it excludes areas that have not been searched or where species, though present, went undetected, and it includes both appropriate and inappropriate habitat (Butterfield, *et al.* 1994). The known distributions of Ireland's Arctic-Alpine plant species are based almost entirely on sightings data (Curtis & McGough 1988; Hackney 1992). In cases where detailed large-scale surveys have been done, they were usually of vegetation communities at specific sites covering small areas (Cross 1998). Consequently, knowledge of Arctic-Alpine plant distributions and the factors affecting them in Ireland is limited.

The presence or absence of species is correlated to abiotic environmental features that cumulatively constitute their habitats (MacArthur 1958; Rosenzweig & Winakur 1969; Rosenzweig 1995; Pyatt 1996). Plant studies indicate topographical gradients are primary factors controlling the distributions of Arctic-Alpine species (Barrio, *et al.* 1997; Tappeiner, *et al.* 1998; Hoersch, *et al.* 2002) in conjunction with edaphic factors (Daubenmire 1968; Miller 1986; Pyatt, *et al.* 2001). Therefore, habitat distribution maps are useful complements to sightings-based range maps. Much work has been done on the use of Geographic Information Systems (GIS) for habitat mapping as well as the development of predictive habitat distribution models for conservation purposes (Miller 1994; Guisan & Zimmermann 2000). However, Irish datasets from which habitat data can be extracted are often characterised by coarse resolutions inappropriate for local mapping and which impart only general information on species distributions (Tubridy & O'Riain 2002). To date, there have been no published attempts to model plant habitat at relatively large scales and covering broad areas in either the Republic of Ireland or Northern Ireland.

To address these knowledge gaps, a GIS-based model that outputs areas of potential habitat for target Arctic-Alpine plant species was developed. Future work includes the use of the model outputs to analyse the extent of habitat fragmentation in Northern Ireland as well as the extent in which physiographic factors affect habitat distribution. Additionally, model outputs will be used to investigate the efficacy with which conservation management areas in Northern Ireland protect the target species. All analyses of model outputs will be done in both regional and sub-regional scales.

GIS-BASED MODEL

Because of their limiting influence on plant distributions, abiotic environmental factors were chosen as the input parameters for a GIS-based predictive model of Arctic-Alpine plant habitat. The model used the following parameters to cumulatively define potential habitat: elevation, slope steepness, slope aspect and soil type. The distributions of Arctic-Alpine species are also affected by temperature and precipitation, but these factors are highly correlated to topography (Boyko 1947; Peterson *et al.* 1997). This is especially true in Northern Ireland where terrain is the dominant feature affecting temperature

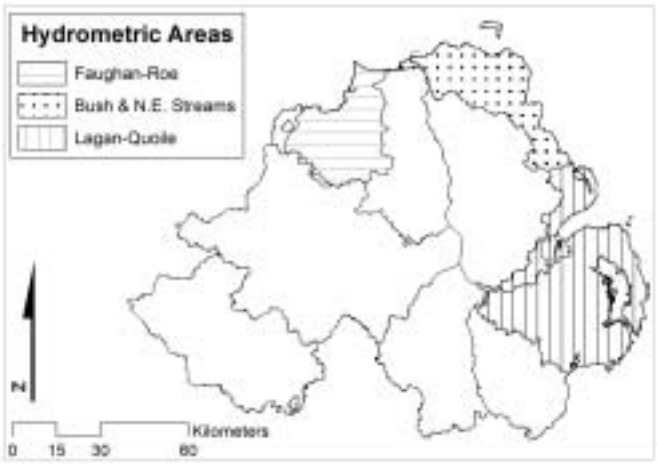

Fig. 1. – Northern Ireland hydrometric areas defining the spatial extents of model outputs.

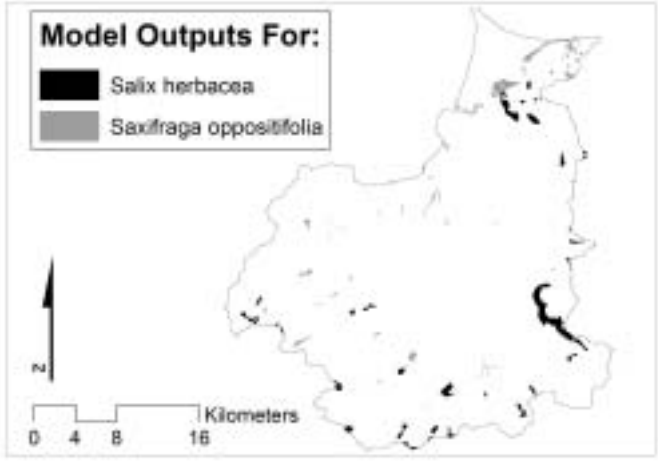

Fig. 2. – Model outputs for *S. oppositifolia* and *S. herbacea* in the Faughan-Roe hydrometric area.

variations and the distribution of rainfall throughout the region (Betts 1997). Therefore, the model implicitly employed topographic gradients as proxies for the effects of temperature and precipitation on species distribution.

Model parameters were extracted from the best available datasets for Northern Ireland. The ArcGIS 9 software package was used to derive elevation, slope steepness and slope aspect from a digital terrain model provided by the Centre for Ecology and Hydrology in Wallingford, United Kingdom. Soil types were derived from the Northern Ireland Soil Survey Project database created by the Department of Agriculture and Rural Development, Northern Ireland, and acquired from the Ordnance Survey, Northern Ireland. Both datasets are appropriate for mapping at scales of 1:50,000. After extraction, the parameters were converted to spatially referenced polygons and then combined using a simple, un-weighted overlay algorithm. Areas where all factors intersect were designated as potential habitat.

MAPPING POTENTIAL HABITAT

The habitat distributions of four plants were modelled. *Dryas octopetala* L., *Saxifraga oppositifolia* L., *Silene acaulis* (L.) Jacq., and *Salix herbacea* L. were chosen as a subset of Northern Ireland's vascular Arctic-Alpine flora. With the exception of *S. herbacea* L., these species are considered rare in the region and are protected under the Wildlife (Northern Ireland) Order (1985). An extensive literature review was undertaken to determine the species' topographic and edaphic requirements which were then used as inputs for the model.

The spatial extents of the model's output maps were defined by three hydrometric areas in Northern Ireland. The Faughan-Roe, Bush & N.E. Streams and Lagan-Quoile major watersheds are roughly associated with Co. Derry, Co. Antrim and Co. Down, respectively (Fig. 1). The areas were chosen because of similarities in size and climatic regimes as well as marked differences in landscape types, terrain and geology. These commonalities and distinctions provide the basis for planned analyses of the effects model parameters have on habitat distributions at regional and sub-regional scales.

Currently, only two of the four plant species have been modelled and only for the Faughan-Roe hydrometric area. Potential habitat areas for *S. oppositifolia* and *S. herbacea* are shown in Fig. 2. Unsurprisingly, there are similarities in their predicted habitat distributions. However, noticeable differences can be seen in their areas, extent of fragmentation and patch isolation. Quantitative analyses of these similarities and differences are planned.

APPLICATIONS FOR MODEL OUTPUTS

The potential habitat model is the initial component of a larger Ph.D. project. Future components will be based on the model outputs and will be oriented towards conservation applications, including watershed-level assessments of habitat fragmentation and analyses of the degree to which habitat areas are protected under current government initiatives. Model outputs will also be used to investigate the influences of environmental factors on habitat distribution and the scales in which they operate.

Fragmented landscapes cause changes in species distribution or local extinctions because of mechanisms stemming from physical changes in habitat as well as reductions in total habitat area (Saunders *et al.* 1991). The second component of this project will make use of landscape indices linked to ecological functions to assess the degree of Arctic-Alpine habitat fragmentation in the three study areas. These indices are established in ecological literature and appropriate for the quantification of *inter alia* habitat patch characteristics, patch isolation and aggregation in the landscape (O'Neill *et al.* 1988). Results of this component will be used to assess the extent in which areas managed for conservation purposes in Northern Ireland (e.g., national parks) protect the habitat areas of the four Arctic-Alpine target species.

Understanding habitat distribution patterns at various scales is also important for management purposes. For instance, planning a conservation strategy for rare habitat types in a localised area may not be necessary if the habitat types are abundant when viewed from a regional perspective. Conversely, habitat types that are regionally scarce may be abundant in certain locales. Therefore, the third component of this project will be a comparative analysis of habitat distributions at the sub-regional scale (e.g., watershed level) and the regional scale in terms of physiographic differences between the three hydrometric areas. Specifically, an investigation of how model parameters vary within and between the study areas is planned in order to contextualise the distribution patterns of Arctic-Alpine plant habitats.

NEXT STEPS

Terrain and soil are not the only abiotic factors affecting habitat. Land use and land cover, in the form of impervious surfaces (e.g., roads and buildings) and agriculturally intensive areas, play a crucial role in determining habitat availability and fragmentation (Stewart & Hutchings 1996). Therefore, a fifth parameter, land cover type, is expected for the model pending acquisition of the MOLAND land cover dataset from the European Commission's Joint Research Centre. It has a scale of 1:25,000 and is therefore appropriate for watershed-level mapping.

Once the model is completed, experts in the field will be asked to comment on its components and the methodology of its construction. Additionally, plant habitat requirements and model outputs will be sent to external advisors for review and comment. Finally, in an effort to ground-truth the model, outputs will be compared to sightings data acquired from the Ulster Museum's Centre for Environmental Data and Recording, Northern Ireland.

CONCLUSION

It is envisioned that this peer-reviewed GIS-based model of potential habitat distribution will provide resource managers with the methodology for a fully updateable and scaleable tool for assessing the current state of Arctic-Alpine habitat fragmentation anywhere in Northern Ireland. Additionally, this can be used to assess the efficacy of existing protected areas and prioritising conservation efforts on suitable habitat areas not currently protected. Finally, it is anticipated that model outputs will provide insights into the factors affecting habitat distribution at various spatial scales.

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THE POLLUTION STATUS OF NORTH DUBLIN BAY

Fionnuala McBreen and James G. Wilson

ABSTRACT

The pollution status of North Dublin Bay was determined through the use of the Biological Quality Index (BQI) and the Pollution Load Index (PLI). To determine the BQI, the quality of macrofauna and macroalgae was surveyed at the twenty-four sample sites in North Dublin Bay. The inner Tolka basin was found to be lacking in both macrofauna and macroalgae and was the only area to be designated abiotic. A BQI value of 4.72 was obtained for North Dublin Bay. The PLI was determined from the concentrations of organic content, total nitrogen, total phosphorus, lead, copper, iron, cadmium, chromium, zinc and manganese. The inner Tolka basin was found to have the highest concentration of pollutants in North Dublin Bay. Lead, total phosphorus and total nitrogen had the lowest contaminant PLI values. An overall PLI of 2.49 was obtained for North Dublin Bay. There was a close correspondence of contaminant levels with impacts on the macrofauna. The results suggest little improvement in some inner sections over the past 30 years, and suggest that these areas may have some difficulty in meeting the Water Framework Directive quality goal by 2015.

Key words: estuary; pollution; nutrients; metals; sediment; Dublin

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INTRODUCTION

As with most estuaries, anthropogenic impacts on Dublin Bay have been difficult to assess. Estuaries vary with depth in both salinity and stratification, tend to have high levels of organic input and nutrients and are naturally disturbed systems with a marked tendency toward heterotrophy (Heip *et al.* 1995). This can make their pollution status hard to evaluate, especially as some species will react more harshly to changes in salinity than to inputs of pollution (Wilson & Jeffrey 1987). Indeed, Roth & Wilson (1998) found that they could not distinguish between disturbances caused by pollution and those caused by the natural environment (e.g. tidal emersion) in intertidal areas of Dublin Bay.

North Dublin Bay starts at the mouth of the Tolka estuary and extends northward up to Sutton strand, and incorporates North Bull Island. Pollution in the Tolka Basin was first noted in Victorian times (Letts & Adeney 1908). The area around North Bull Island now consists of sand flats, *Salicornia* flats and mudflats, and the island itself is a mix of salt marsh and sand dunes (Jeffrey & Walsh 1983). North Bull Island has been recognised through international, EU and Irish legislation as an important area in North Dublin Bay and is host to five species of bird of international importance and twelve of national importance. Eutrophication has been observed in the lagoons behind North Bull Island with frequent blooms of the green algae *Ulva* and *Enteromorpha* (Jennings & Jeffrey 1998; Brennan *et al.* 1998). This has been ascribed to increased particulate nutrient input to the estuarine sediments from domestic sewage (Jeffrey *et al.* 1992). The nutrients act like fertiliser, leading to an increase in numbers of individuals, species and the total biomass. The excessive addition of nutrients also leads to oxygen depletion in the sediment. Heavy metals are also a concern in North Dublin Bay as they are conservative pollutants which are generally not subject to bacterial attack and hence become permanent additions to the estuarine environment (Clark 2001). Heavy metals are a serious source of concern as they are toxic to estuarine organisms over threshold levels (Kennish 1992). Wilson (1980) concluded that in general the heavy metals in the Tolka Estuary were coming from domestic and agricultural sources and not from specialised industries. Anthropogenic influence in North Dublin Bay diminishes rapidly with distance from the port and river, with no discernable impacts on either benthos (Walker & Rees 1980) or water column (O'Higgins & Wilson 2004) beyond the headlands.

In 1985, Jeffrey *et al.* outlined a new approach to qualitative estuary evaluation that addressed the problems with earlier procedures. The method involves using two complementary indices, the Biological Quality Index (BQI) and the Pollution Load Index (PLI) (Jeffrey & Wilson 1985). The purpose of these indices is to correlate the biological and chemical factors from the intertidal zone of estuaries and to identify any links with pollutants and macrobenthic community structures (Jeffrey *et al.* 1985). The BQI measures the biological health of an estuary and classifies estuaries as abiotic, opportunistic or stable depending on the species diversity (Jeffrey *et al.* 1985), with areas being respectively completely devoid of life, abundant in short-lived opportunistic species or dominated by longer-living organisms. The PLI is an index that rates the chemical pollution of the estuary. Both the BQI and the PLI are measured from baseline (unpolluted = 10) to threshold levels on a scale of 0 to 10 where 1 is the threshold level, the level at which significant damage is evident (Jeffrey *et al.* 1985). Sediment samples are used for the PLI as they are better indicators of past and present pollution (Wilson & Jeffrey 1987). In using sediment for the PLI, however, assumptions are made that a dynamic relationship exists between the water column, sediment and biota and that the intertidal zone reflects the general health of the estuary (Jeffrey *et al*. 1985). The importance of sediment as a basic component of Dublin Bay's energy flow network has been highlighted by Wilson and Parkes (1998). It is recommended that the six mandatory pollutants – nitrogen, phosphorus, cadmium, chromium, zinc and organic content – are tested for the PLI (Wilson & Jeffrey 1987).

The BQI/PLI method has been successfully used in estuaries in Ireland, France and the US (Wilson 2003). Wilson & Elkaim (1991) surveyed and compared results for French and Irish estuaries. They found the Avoca estuary to be the most polluted (BQI = 0.10, PLI = 0.00), followed by the Tolka estuary (BQI = 1.83, PLI = 0.12). In 1989, the BQI of the inner Tolka estuary had declined from 0.93 to 0.47 since 1979 (Jeffrey *et al.* 1991, Wilson & Elkaim 1991). The

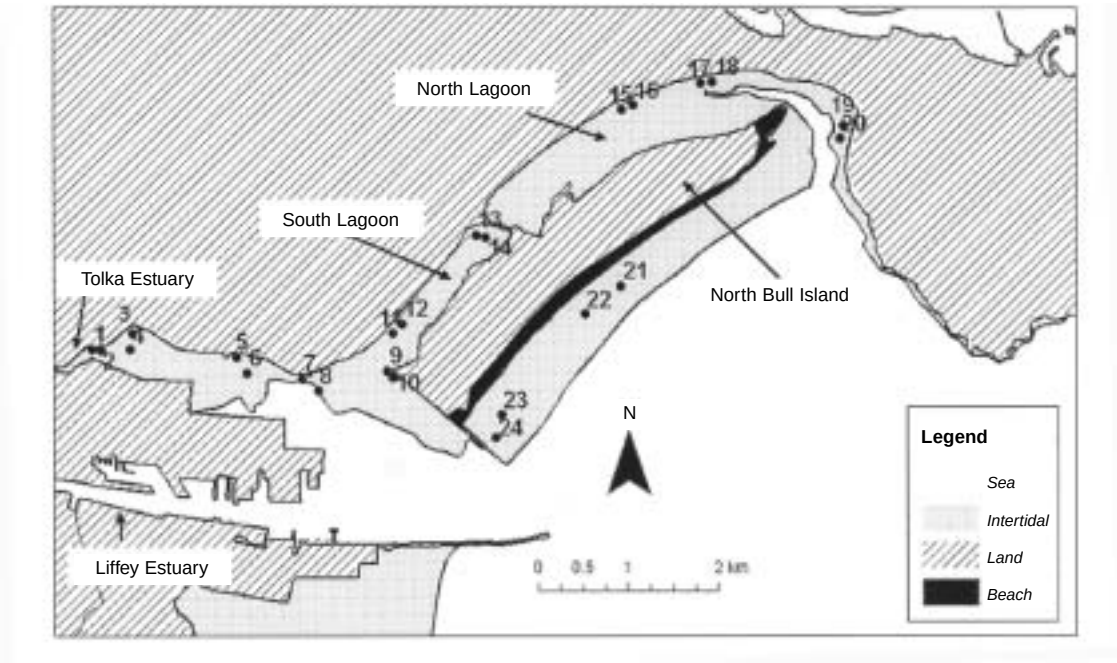

Fig. 1 – Map of sample sites 1 – 24 from North Dublin Bay for 2003 study.

PLI increased from 0.12 to 0.78 over the same time period. In both cases the main contaminant was phosphorus, indicating agricultural pollution. Ten per cent of the area surveyed in 1979 was stable; this was reduced to zero in 1989. Substantial organic input (probably sewage) to the estuary over the ten year period was thought to be the cause (Jeffrey *et al*. 1991). Subsequent surveys in 1996 and 1998 have shown a decrease in the PLI's below the original 1979 level (Wilson 2003). There has been a general decrease in the percentage of stable and abiotic areas in the Tolka estuary since 1977 (Wilson 2003). Wilson (2003) concluded that despite many efforts to clean up the Tolka estuary, these efforts must have only barely compensated for the increased pollution associated with the rise in population experienced in Dublin.

North Dublin Bay comes under the terms of the recent Water Framework Directive (WFD) (2000/60/EC). This has two major implications for management. Firstly, it sets quality standards for chemical and biological parameters, including an obligation to maintain, or to restore to 'good ecological quality.. Secondly, a timetable has been laid down for a series of actions, up to the final implementation of the WFD in 2015. Consequently, the WFD has been recognised as a major imperative by the interdepartmental discussion document, *A National Monitoring Programme for Transitional, Marine and Coastal Waters* (EPA, 2003). As the BQI/PLI approach addresses both biological and chemical parameters, it a useful method for assessing whether estuaries have reached the 'good ecological quality' required by the WFD. The method is easily transferable to estuaries both in Ireland and in Northern Europe.

MATERIALS AND METHODS

Field and laboratory methods for this study were based on *A Manual for the Estimation of Estuarine Quality (1985).* Twenty-four sites were sampled in October 2003 for PLI and BQI analysis (Fig. 1). For the PLI, sediment samples were taken from each site and placed in labelled polythene bags. Areas were zoned as abiotic, stable or opportunistic for the BQI depending on the species, species diversity and age classes of the macrofauna and algae present. The algae were identified on site and sediment was sieved through a 1mm sieve and the biota present identified. The fauna and flora were scored on an abundance scale from 0 (absent) to 5 (abundant). The boundaries between the zones were also identified, and, along with site location, were described and their position determined through the use of the Global Positioning System (GPS). Sediment types and the presence of rivers, overflows or discharge points were also recorded. The sediment samples were analysed for phosphorus, nitrogen, organic content and for the heavy metals cadmium, chromium, copper, lead, manganese, zinc and iron. Organic content was measured through loss-on-ignition (LOI), concentrations of heavy metals were determined using the Atomic Absorption Spectrometer (AAS) after digestion in hot concentrated HNO_3, total nitrogen concentrations were calculated using the LECO elemental analyser, and total phosphorus was measured using the method of Murphy & Riley (1962). The BQI was calculated through the use of GPS and the computer program ArcGIS from the co-ordinates recorded in the field with a handheld GPS. These Irish National Grid co-ordinates were put onto a digitised Irish Ordinance Survey map of North Dublin Bay. The zones were drawn by creating polygon features on the map, from which the area of each zone could be calculated. The PLI (Jeffrey *et al*. 1985) was calculated from the pollutant concentrations in $\mu g\ g^{-1}$ Dry Weight.

The BQI was calculated using the following formula:

$BQI = 10^{(A-C)}$, where A is the proportional area of the abiotic zone; C is the proportion of the stable zone.

The PLI was calculated from the following formulae:

$PLI = 10^{(1-(CP-B)/(T-B))}$

Site PLI = $(PLI_1 \times PLI_2 \times PLI_3 \ldots\ldots PLI_k)^{1/k}$

Zone PLI = $(\text{Site index}_1 \times \text{Site index}_2 \ldots\ldots \text{Site index}_j)^{1/j}$

Estuary PLI = $(\text{Zone index}_1 \times \text{Zone index}_2 \ldots\ldots \text{Zone index}_n)^{1/n}$

CP = Pollutant Concentration, B = Baseline level, T = Threshold level, k = number of sites, j = number of sites, n = number of zones.

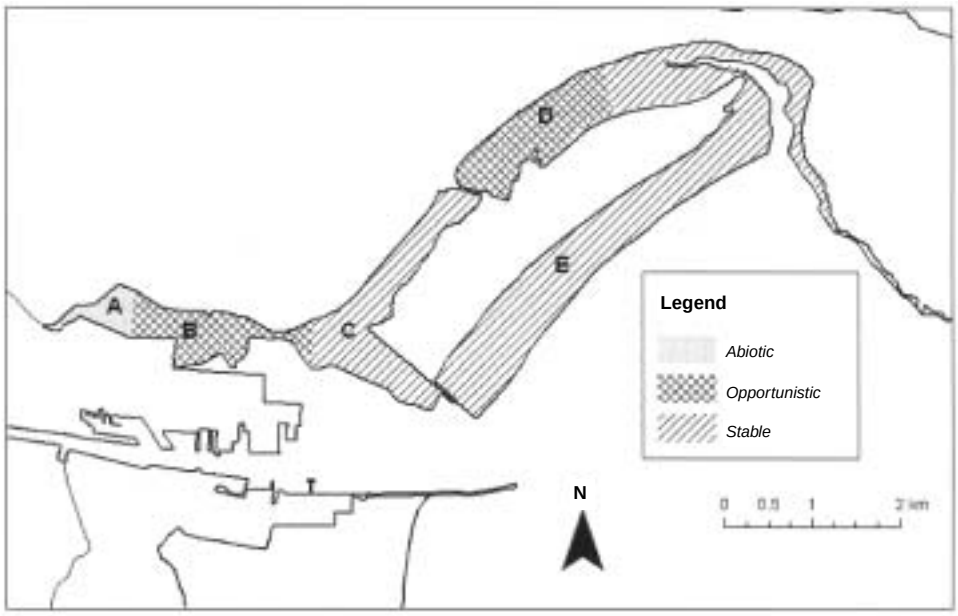

Fig. 2 – Map of BQI zones for North Dublin Bay in 2003.

RESULTS

During the field work, every area of North Dublin Bay was designated as abiotic, opportunistic or stable; using this information a map was drawn dividing North Dublin Bay (Fig. 2). Sites 1 to 4 were designated abiotic (Zone A), sites 5 to 8 and 15 to 16 were designated opportunistic (Zones B and D respectively), sites 9 to 14 and 17 to 24 were designated as stable (Zones C and E respectively). Zone A showed no signs of life and had a very high redox potential discontinuity (RPD) level, indicating that the mud was highly deoxygenated. The opportunistic zones B and D showed signs of life but tended to be dominated by worms and the algae *Ulva* and *Enteromorpha,* indicating eutrophication (*Ulva* has now been reclassified by Hayden e*t al.* (2003) as a member of the *Enteromorpha* genus). The stable zones C and E contained bivalves and longer-living algae such as *Fucus serratus* and *Ascophyllum nodosum* and were often areas of clean sand. The areas of each zone were calculated (Table 1), and the BQI for the total area was calculated. A BQI value of 4.72 was calculated for North Dublin Bay. Biological and chemical results showed North Bull Island to be a much more stable and less polluted area than the Tolka estuary. The concentrations of each contaminant were used to calculate the PLI values for each site and for the whole of the North Bay. An overall PLI of 2.49 was obtained for North Dublin Bay. The mean PLI values for each contaminant indicated that lead was the worst pollutant (3.58), followed by total phosphorus (3.89) and total nitrogen (4.90). Site PLI values indicate the head of the Tolka estuary was the area of most concern. Zone A had the highest concentrations of organic content, organic carbon, total nitrogen, total phosphorus and heavy metals and the lowest average site PLI value of 0.06. The high levels of pollution found at sites 1 to 4 in Zone A explain the near total absence of biota. The Tolka and Wad rivers are the most likely sources of pollution in Zone A. Nitrogen, phosphorus and lead were the only pollutants found in Zone B. The lead pollution at sites 5 and 6 is a particular cause for concern, as these sites are located within a nature reserve. Possible sources of the organic pollution are the Tolka River, the Wad River, the discharge point near sites 5 and 6 and the overflow point near sites 7 and 8. Lead was the only pollutant found in Zones C and E at sites 13, 18, 19, 23 and 24. The high lead level found at site 13 is most likely linked to pollution from the River Nanniken. At sites 15 and 16 in Zone D, total nitrogen was the only pollutant found to be over the threshold limit.

A Pearson Product-Moment correlation table was constructed from the log transformed chemical data in Microsoft Excel (see Table 2). Nitrogen and Organic matter showed the highest correlation, followed by phosphorus and zinc and copper and phosphorus. High correlations between pollutants indicate that the pollutants may come from common sources. The lowest correlation in the Pearson Product-Moment correlation table occurred between manganese and nitrogen. Lead showed the lowest range of correlations (0.286 - 0.478).

Table 1 – Table showing the designated BQI zone types for North Dublin Bay in 2003.

Zone	Area (km^2)	Proportion	Type
A	0.28	0.03	Abiotic
B	0.84	0.11	Opportunistic
C	1.50	0.19	Stable
D	1.19	0.15	Opportunistic
E	4.14	0.52	Stable
Total	**7.95**	**1.00**	

DISCUSSION

Jeffrey *et al.* (1978) completed a detailed survey of the intertidal zone of North Dublin Bay in 1973, very similar to the study area covered in this project although Dollymount Strand was absent from the earlier study. Table 3 compares the mean, range and standard deviations for organic content (OC), nitrogen (N), phosphorus (P), lead (Pb), copper (Cu) and zinc (Zn). The results for Dollymount Strand sites have been omitted from the 2003 values as it was not considered in 1973.

The most significant changes are seen in the nitrogen values; the

maximum level of nitrogen in 2003 is nearly seven times that of the 1973 value. All the maximum, mean and standard deviations are much higher in 2003 with the exception of zinc. The results for the 1973 study were also sieved through a 2mm mesh, so particle size cannot account for these substantial differences. Lead turned out to be of particular concern in 2003, and the levels can be seen to be much higher in 2003. The high levels of nitrogen, phosphorus and organic content in 2003 indicate that organic pollution is more of a problem now than it was in 1973. The decreasing pollution gradient noted in 1973 from the mouth of the Tolka to Sutton still exists 30 years later in 2003.

Wilson (1982) examined the littoral fauna for the whole of Dublin Bay in 1977. Bivalves were found to dominate the standing stock in Wilson's (1982) study. *Cerastoderma edule* was the most abundant bivalve in both 1977 and 2003. Wilson (1982) found the lagoons behind North Bull Island to be the richest in invertebrate biomass. While this was found to be the case in the South Lagoon in 2003, it was not the case in the North Lagoon. There was, however, evidence of biotic life in the North Lagoon (worm casts and feeding birds) and it is possible that the fauna may have been too small (e.g. meiofauna) or too sparse to be found in the sampling process. Shannon-Weiner indices calculated by Wilson (1982) for the whole of the Bay indicated that the inner Tolka basin was the most polluted area in North Dublin Bay. This was still the case in 2003.

The average mean contaminant concentrations from sites 11 to 14 in the South Lagoon (2003) were compared directly with those from the 1987 study in Table 4 (Magennis 1987). Higher levels of nitrogen, iron, copper, lead and manganese were found in 2003. Phosphorus and zinc showed a high correlation in the Pearson-Product Moment correlation table, indicating a common source. As the only two contaminants to decrease over the 16-year period, further weight can be given to the idea of a common source. The increase in nitrogen and corresponding decrease in phosphorus suggests a possible increase in domestic pollution such as sewage and a decrease in agricultural pollution such as fertiliser. The doubling in lead levels since 1987 is not surprising considering that lead was found to be the lowest contaminant PLI in North Dublin Bay. However, it is a cause for concern. The levels of manganese have also nearly doubled since 1987. However, as with lead, the values are still under the threshold limit. While all the pollutants tested for in the South Lagoon in both studies were under the threshold limit, the trends in the South Lagoon may be an indicator of what is happening on a larger scale in North Dublin Bay.

Crisp *et al.* (1974) surveyed the fauna from the mouth of the Tolka estuary to the North Bull Wall. The fauna and flora were rated as they are for the BQI as rare, occasional, frequent, common and abundant. The general conclusion was that the fauna in Dublin Bay indicated a healthy estuary, except near the mouth of the Tolka River (Crisp *et al.* 1974). As far back as 1908 (Letts & Adeney 1908), the Tolka estuary had been identified as the main area of concern in North Dublin Bay. This study shows that the situation is still much the same 100 years later, with the Tolka estuary being identified as the only abiotic area in 2003.

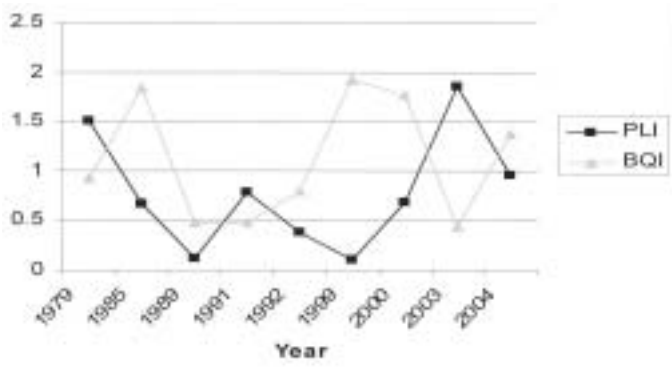

Fig. 3 – Comparison of BQI and PLI for the Tolka Estuary values from 1979 to 2004.

COMPARISON OF BQI AND PLI

BQI and PLI values of 4.72 and 2.49 respectively were obtained for North Dublin Bay. The BQI value is relatively healthy, especially considering the range of rivers and streams entering North Dublin Bay as possible polluters. Only the Shannon, Lee (1979 and 1994), Boyne (1997), Slaney and Baldoyle (1996 and 2003) estuaries obtained higher values in Ireland. The PLI value was only bettered by the Baldoyle (1996 and 2003), Dungarvan, Lee and Shannon estuaries (Wilson 2003). The lowest contaminant PLI value for North Dublin Bay was the phosphorus value obtained at site 1 (1.5×10^{-10}) and the lowest site PLI was 0.01. French and American estuaries showed generally higher BQI and lower PLI values. French estuaries showed a relatively similar ranking to Irish estuaries based on the level of anthropogenic inputs, from the more rural Baie de Veys (BQI =7.65, PLI = 7.37) to the Seine (BQI = 3.87, PLI =0.84) which flows through the conurbations of Paris & Rouen.

Separate PLI and BQI values were calculated for the Tolka estuary for 2003 and values of 0.43 and 1.83 were obtained respectively. These values were compared to data from 1979 to 2004 in Fig. 3 (Wilson, unpublished). The graph shows that the lowest BQI and highest PLI values were in 2003. However the BQI recovers in 2004, probably

Table 2 – Pearson Product-Moment Correlation table for metals, nutrients and organic matter for North Dublin Bay in 2003. Any value over 0.4227 is deemed to be significant at P = 0.05. OM = Organic matter.

	P	***Pb***	***Zn***	***Cu***	***Mn***	***Fe***	***N***	***OM***
P	1.000							
Pb	0.756	1.000						
Zn	0.955	0.752	1.000					
Cu	0.935	0.78	0.902	1.000				
Mn	0.826	0.838	0.859	0.784	1.000			
Fe	0.723	0.653	0.841	0.730	0.82	1.000		
N	0.827	0.677	0.925	0.717	0.830	0.881	1.000	
OM	0.832	0.675	0.945	0.747	0.837	0.904	0.988	1.000

Table 3 Mean, range and standard deviation for variables considered for North Dublin Bay in 1978 and 2003. The LOI values are in %, all other values are in μg g^{-1}.

	1978 (Jeffrey *et al.*)				2003 (This study)			
	Max	*Mean*	*Min*	*S.D.*	*Max*	*Mean*	*Min*	*S.D.*
LOI	11.2	3.967	0.1	2.48	16.36	4.67	1.70	4.48
N	3388.0	733.97	231.0	505.09	23124.70	4234.77	<480.31	6237.10
P	1146.0	270.14	75.0	230.60	2183.76	619.23	163.79	615.74
Pb	72.0	30.09	13.0	9.77	404.37	124.99	<4.94	124.66
Cu	46.4	6.93	0.2	7.07	140.8	27.61	4.85	41.76
Zn	375.0	83.06	10.8	90.57	295.8	75.62	7.60	97.24

because it takes time for the pollutants in the sediment to make their way through the food chain. The graph indicates that the Tolka estuary has been heavily polluted from 1979 to 2004 and there are no real indications that the estuary is improving.

Lead was shown to be the most significant heavy metal contaminant in North Dublin Bay. The maximum concentration recorded in 1973 was below the threshold limit (Jeffrey *et al.* 1978). The Dodder and the Liffey estuaries also showed lead to be a significant pollutant, indicating that lead is a source of concern throughout Dublin Bay (Wilson 2003). These rivers may have contributed to the high lead levels found at Dollymount Strand. Elevated lead levels greater than 50mg/kg (after normalisation for organic content) have also been detected in sediments at Dublin Port, Dún Laoghaire harbour, the Avoca estuary and Bantry harbour (Marine Institute 1999). North Dublin Bay had levels greater than 50mg/kg at seven sites.

The situation in North Dublin Bay has changed little in the past 30 years. The main source of pollution is still the Tolka River, and this is shown by the high levels of pollutants and the absence of fauna and flora in the inner Tolka basin. North Bull Island, with the exception of the North Lagoon, is relatively healthy with a more diverse range of fauna and flora. Both the conditions in the North Lagoon and the lead levels around North Bull Island require further monitoring.

The results of this study show clearly that parts of North Dublin Bay are not of 'good ecological quality' as stipulated under the Water Framework Directive (WFD). Elevated levels of contaminants have been found in the sediments and, along with these, biological quality is significantly reduced. Perhaps more worryingly, the improvements in regard to sewage collection and treatment seem to have had little effect on some inner stations, implying that much more might be required to meet the WFD imperative goal of 'good ecological quality' by the deadline of 2015.

Table 4 – Comparison of mean pollutant concentrations for the South Lagoon in 1987 and 2003. All values except for organic content (%) are in μg g^{-1} DW (Magennis 1987).

Pollutant	1987	2003
Organic Content	1.537	2.2
Nitrogen	424.9	1,106.5
Phosphorus	322.3	239.9
Cadmium	<0.025	<0.025
Chromium	6.0	<0.1
Zinc	62.5	18.7
Copper	4.7	6.2
Iron	3,000	5,237.6
Lead	16.2	37.6
Manganese	86.0	162.0

CONCLUSION

North Dublin Bay is a relatively healthy estuary with a BQI of 4.72 and estuary PLI of 2.49. The BQI value for North Dublin Bay is just below the average value of 4.88 for Irish estuaries and 5.58 for French estuaries. The PLI is just above the average value of 2.19 for Irish estuaries and 2.41 for French estuaries. The inner Tolka basin is the most polluted area in North Dublin Bay. The North Lagoon is also an area of concern. Lead had the lowest contaminant PLI average. Total phosphorus and total nitrogen also had low contaminant PLI averages, indicating domestic organic pollution such as sewage, probably from the rivers and streams entering the Bay. The effect of the new Ringsend sewage treatment plant is unlikely to show an improvement in the sediment of North Dublin Bay for a number of years as the sediment acts as a sink for pollution. The lack of improvement over the past 30 years suggests that a great deal of effort will have to be made to meet the WFD targets by the 2015 deadline. There are major diffuse inputs of contaminants to the system, largely from the River Liffey (Wilson 2005), and these will need to be tackled in addition to the point source efforts with the sewage.

Future work in North Dublin Bay should focus on reducing pollution in the Tolka estuary by locating the sources of pollution to the Tolka River. A similar caution should be entered for the River Liffey, which is the major contributor to the contaminant load on the system (Wilson 2005). The condition of the North Lagoon should also be monitored as further pollution could threaten the abundant winter wild fowl and waders. It is recommended that BQI and PLI indices should be performed for North Dublin Bay every five years. A further study analysing bivalves in North Dublin Bay is recommended in order to examine the affect that high levels of lead may be having on fauna in the area.

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THE BACTERIAL COMMUNITY STRUCTURE OF IRISH SOILS UNDER DIVERSE CROP PLANTATION

Cora G. Carrigg, Siobhán M. Kavanagh, Deirdre Fay and Vincent O'Flaherty

ABSTRACT

Our inability to culture the majority of extant microorganisms has led to a paucity of knowledge in the area of microbial diversity and community structure. To address this, we examined the impact of plant species on soils' microbial community. The bacterial community diversity of 10 loam soils with similar physical and chemical characteristics, under different cropping regimes, was investigated. Soils were sampled in late summer 2003 from areas in the south and east of Ireland cropped by wheat (*Riticum aestivum* L.), barley (*Hordeum vulgare* L.) and potato (*Solanum tuberosum* L.). Bacterial community diversity was investigated using a Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) culture independent molecular approach. A significant relationship between a particular crop-type and the bacterial community structure of the soil was noted.

Key words: soil microbiology; PCR; DGGE; 16S rRNA; crops

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INTRODUCTION

Microorganisms underpin soil health and quality. They play key roles in many processes, including nutrient cycling, organic matter decomposition and energy flow throughout the soil ecosystem (Crecchio *et al.* 2004). Despite this, there is only sparse knowledge of the number and type of species present in different soils and even less on the functions and interactions of these complex microbial communities. Presently, it is thought that only about 0.1-1% of microorganisms present in soil are culturable (Amann *et al.* 1995; Smit *et al.* 2001; Schloss *et al.* 2003) and this impasse has been one of the main factors limiting our knowledge of soil microbial ecology. Publication in 1980 of the first procedure for bacterial DNA isolation from soil (Torsvik 1980) established a molecular approach to the study of soil microbial ecology (Ogram 2000); this was rapidly followed by the introduction of the Polymerase Chain Reaction (PCR) providing one solution to the culturability problem and allowing for improved access to the soil metagenome. An increasing number of techniques (Dahllöf 2002) that enable the study of microbial community dynamics, both temporally and spatially, are being developed, though the heterogeneity of soil brings its own problems when choosing a method of analysis. However, for large-scale or long-term soil microbial diversity monitoring, techniques utilising the ubiquitous 16S rRNA gene such as DGGE (Muyzer *et al.* 1993), Temperature Gradient Gel Electrophoresis (TGGE; Felske *et al.* 1998a), Terminal-Restriction Fragment Length Polymorphism (T-RFLP; Tiedje *et al.* 1999; Blackwood & Paul 2003) and Single-Strand Conformation Polymorphisms (SCCP; Peters *et al.* 2000) have proved to be consistently useful. While these techniques give access to the microbial community at a lower resolution than other monitoring tools available, they are routinely employed in soil monitoring programs as they provide a rapid and reproducible fingerprint of the dominant community members.

In this study we used DGGE to analyse the soil DNA as it is a method that lends itself to the analysis of a large number of samples simultaneously. It can separate individual DNA sequences from complex mixtures based on the melting behaviour of short DNA strands (<500 base pairs), identical in length, in a denaturing gradient. Different DNA sequences will dissociate to single strands at different denaturant concentrations and theoretically each resulting band on the DGGE gel is related to a phylotype seen in the soil microbial community so allowing us to estimate the number of bacterial species present in the sample (Muyzer *et al.* 1993). DGGE also has the added advantage that individual bands can be excised from the gel and sequenced; these sequences can then be used to identify the phylogenetic affiliation of the bacteria matching the individual band in the gel (Rölleke *et al.* 1999).

In the past, very little legal protection was afforded to soil in Ireland; instead it was indirectly guarded through water and air quality policy. However, due to increasing rates of soil degradation, governments across the EU are being required to introduce legislation mitigating this damage (Brogan *et al.* 2002). This new EU policy requires the protection of soil as a natural non-renewable resource and it is to this end that Teagasc, the Irish agricultural research agency, under the auspices of the Environmental Protection Agency, is in the process of establishing the Irish National Soils Archive.

More than 1,000 soil samples from a prescribed 10 × 10 km grid covering ~75% of the country have been collected and subjected to physico-chemical and microbiological analyses. In addition, a process to develop and evaluate a range of soil quality indicators, for use in the long-term monitoring of Irish soils, is ongoing. Microorganisms may be potentially useful as indicators of soil quality due to their rapid response to environmental change but if the potential of microbial soil indicators is to be realised, significant relationships between the microbial composition of soils and key chemical, biotic and abiotic properties should be elucidated.

Presently, it is thought that vegetation type may have a major influence on the microbial composition and diversity of soil (Nüsslein *et al.* 1999; Johnson *et al.* 2003; Bending *et al.* 2004; Crecchio *et al.* 2004). Plant species release a range of exudates into soil; essentially carbon sources that some heterotrophic microorganisms can utilise and others cannot, resulting in the enrichment of certain microbes and the suppression of others. Strong correlations have been shown in the past between vegetation type and microbial

populations (Grayston *et al.* 1997 and references therein; Alvey *et al.* 2003).

The aim of this study was to analyse the underlying bacterial diversity of a number of closely related soil types (Gardiner & Radford 1980) under differing management regimes using a culture independent approach.

MATERIALS AND METHODS

Environmental sampling, DNA extraction and purification, and PCR amplification

Samples were collected at 10 locations in the east and south of Ireland in late summer 2003. At each site, soils were sampled to a depth of 10cm at 5m intervals on a grid measuring 20m x 20m. The 25 cores produced were composited and mixed by shaking inside plastic bags before 25 ~1g samples were removed and added to a DNA stabilisation buffer containing 2.5M guanidine isothiocyanate (Carrigg *et al.* 2004). Community DNA was extracted from the soil using a physical/chemical/enzymatic method (Kavanagh *et al.* 2003) and was subsequently cleaned using the GELase™ Agarose Gel Digesting Preparation enzyme system (Epicentre, U.S.A.). The V3 region of 16S rRNA genes from the soil microbial communities were specifically amplified by PCR as described by Muyzer *et al* (1993) with minor modifications.

Denaturing Gradient Gel Electrophoresis

DGGE was performed using the D-Code system (BioRad Laboratories, U.S.A.). Polyacrylamide gels were prepared with denaturing gradients ranging from 35% to 70% (where 100% denaturant contains 7M urea and 40% formamide). Gels were run at 60°C, 60V for 15h and were stained following electrophoresis with Sybr-Gold™ nucleic acid stain (Molecular Probes Inc., U.S.A.), before de-staining in sterile diethyl pyrocarbonate-treated water and visualising the banding patterns on a UV transillumination table. Cluster analysis was carried out on DGGE banding patterns by scoring each band in the profiles as present (1) or absent (0). Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis was performed on the matrix by the MVSP statistical package (Kovach 1999) using Jaccard's coefficient of similarity ($C_J = j/(a+b-j)$ where j is the number of bands in common between lanes A and B, a = the total number of bands in lane A, and b = the total number of bands in lane B.

Sequencing and phylogenetic analysis of DGGE bands

Selected DNA bands were excised from the gel and the DNA eluted and re-amplified as described elsewhere but with minor modifications (Rölleke *et al.* 1996). DNA sequencing was carried out on an ABI 310-3 capillary sequencer (Perkin-Elmer, U.S.A.). Sequences were initially analysed using the BLAST 2.0 program on the NCBI website (www.ncbi.nlm.nih.gov). The highest scoring sequences were recovered from GenBank and further representative sequences were downloaded from the Ribosomal Database Project (RDP) at Michigan State University (http://rdp.cme.msu.edu). Multiple sequence alignments were generated using ClustalX (Thomson *et al.* 1997) and manually edited using Genedoc (www.psc.edu/biomed/genedoc). A phylogenetic tree was constructed using PAUP 4.10b (Swafford 1996) and a neighbour-joining method (Saitou & Nei 1987).

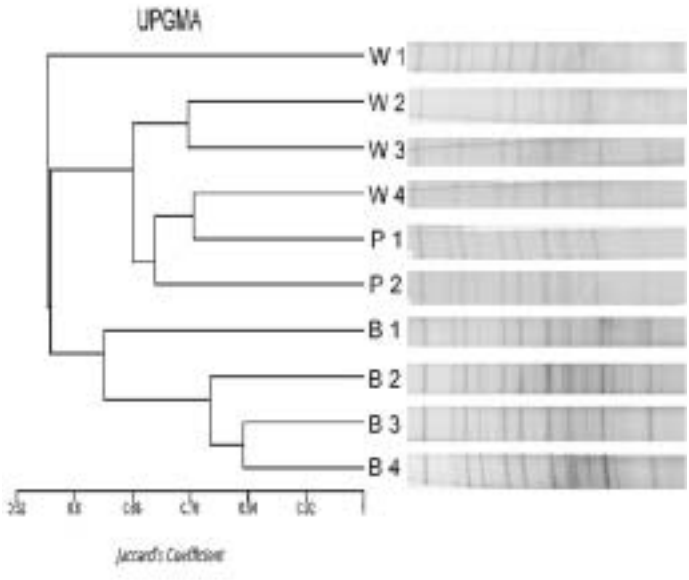

Fig. 1 – DGGE bacterial DNA fingerprints for wheat (W), potato (P) and barley (B) cropped soils. On the left, a cluster dendrogram is shown, representing similarity between the patterns. Profiles were analysed with UPGMA using Jaccard's similarity coefficient.

RESULTS

DNA was extracted from each of the samples using a method developed in our laboratory in NUI, Galway, which has previously been shown to have a lysis efficiency of over 80% across a range of soils (Kavanagh *et al.* 2003). DNA extracted using this method produced bands that were sized at 23kb, as judged by comparison with the size standard, for all of the samples indicating successful extraction of high molecular weight genomic DNA. Due to the presence of high levels of contaminating humic acids in the soils, all samples required purification using the GELase™ Agarose Gel-Digesting Preparation enzyme system (Epicentre, U.S.A.) before successful PCR amplification could be carried out.

DGGE analysis of the soils revealed a highly diverse bacterial community sharing dominant species (12-13 bands or phylotypes) across all soils and with almost uniform distribution of these dominant groups, though with variation in the intensities of the bands associated with some community members (Fig. 1). Microbial species richness (R, indicated by the number of bands present) for barley soils, where numbers of 16S rDNA bands ranged from 38-55, was significantly higher than in the other soil samples studied (Table 1). The potato- and wheat-cropped soils were similar to barley soils with respect to the profile of dominant species, but R values were slightly lower in the potato soils (28-29 bands) than in the wheat soils (29-32 bands; Table 1).

Table 1 – Bacterial richness (R) values of barley, potato and wheat soils studied.

Sample	Barley (1)	Barley (2)	Barley (3)	Barley (4)	Potato (1)	Potato (2)	Wheat (1)	Wheat (2)	Wheat (3)	Wheat (4)
Sample ID	B1	B2	B3	B4	P1	P2	W1	W2	W3	W4
R value	38	55	49	48	29	28	32	32	31	29

Fig. 2 – Phylogenetic relationships among bacterial SSU rRNA genes inferred by neighbour–joining (Saitou & Nei, 1987); DGGE fragments are highlighted. Bootstrap replicates (out of 100 replicate samplings) that supported the branching order are shown at relevant nodes.

For further analysis of DGGE profiles, the presence and absence of bands were scored, resulting in a binary matrix to which Jaccard's coefficient of similarity was applied. The UPGMA algorithm was used to cluster data (Fig. 1) and grouped the soils into three distinct clusters, the first containing all of the barley soils, the second containing the potato soils and all but one of the wheat soils and the third showing a single wheat soil (W 1). As a number of dominant bands present were common to all soils it was the fainter, less dominant bands that distinguished the soil samples. While the barley soils clustered in one group there were substantial differences between them. The potato soils (P 1 and P 2) grouped most closely with one of the wheat soils (W 4) and as part of a cluster with wheat soils W 2 and W 3. The final wheat soil (W 1) grouped separately from all the others, despite sharing many of the major bands with all other soils. In this wheat sample (W 1) some of the more dominant bands disappeared and many new bands appeared, though the total R value (32) was similar to the R values in all other wheat samples.

Two DNA fragments, excised from the same DGGE position in two different soil samples, were sequenced and were found, using BLAST analysis, to be most closely related to an un-cultured *a-Proteobacterium*. Representative species downloaded from the RDP were used to generate phylogenetic trees using the neighbour-joining algorithm (Fig. 2; Saitou & Nei 1987).

DISCUSSION

DNA extraction from soil is a critical step in the fingerprinting of bacterial communities; extracted DNA needs to be of high molecular weight to prevent the formation of chimeras during the PCR reaction, it needs to be representative of the total community present in the soil and sufficiently clean of inhibitors which readily co-extract, e.g. humic acids (Yeates *et al.* 1998). In this case high molecular weight DNA was extracted using a technique which we have found to have a high rate of cell lysis when tested on a number of diverse soil and sediment samples, though substantial amounts of humic acids were also extracted resulting in the inability of the Taq polymerase to amplify the DNA during the PCR (Kavanagh *et al.* 2003). DNA clean-up using the GELase™ Agarose Gel-Digesting Preparation enzyme system (Epicentre, U.S.A.) resulted in DNA that was sufficiently clean for PCR amplification while avoiding major DNA losses, which may have been incurred with other more extensive purifications leading to the loss of rare DNA sequences (Robe *et al.* 2003).

Subsequent DGGE analysis revealed that the bacterial community was dominated by a limited number of phylotypes, present in all samples, despite the variation in vegetation type. Alvey *et al.* (2003) found no dominant indigenous group of bacteria in the bulk soil fraction of West African agricultural soils and suggested this may be due to the sub-optimal growth conditions provided by easily degraded carbon sources. However, the presence of a consortium of well established microorganisms in all soils sampled in this study suggests that this may not be the case in Irish tilled soils. Studies by Gelsomino *et al.* 1999 supported the idea of groups of stable and dominant microorganisms being present in soil even from regions as geographically diverse as Finland and Brazil and other research has shown little seasonal variation in these microbial communities (Felske *et al.* 1998b).

R values were found to be significantly higher in all soils cropped by barley when compared with those cropped by wheat and potato. Perhaps the carbon exudates produced by the barley samples are available to a broader range of microbial species, so enriching a greater part of the microbial community. A Grayston *et al.* (1997) study using the Biolog™ system, found microbial species from soils populated by different plant species produced characteristic carbon source utilisation patterns and that the C utilisation patterns were similar for each crop type even in different soil types.

We found crop type to be strongly associated with barley and wheat soils' bacterial diversity; however, the potato samples, while they did group closely together, still clustered with individual wheat samples. A similar study on a large number of agricultural sites (n=47), using the Intergenic Transcribed Spacer region (ITS) method (Johnson *et al.* 2003), found almond, grape and tomato soils were strongly associated yet cotton and safflower soils did not show the same correlations. Another study on Sudan grass, rape and chickpea found the Sudan grass and rape to have a strong effect on soil microbial community diversity while the chickpea was found to have no such effect (Marschner *et al.* 2001).

Sequence analyses of excised and directly sequenced DGGE bands revealed that they were most closely related to that of an uncultured *a-Proteobacterium* (98%); other studies have commonly found *Proteobacteria* in grassland and tilled soils, with anywhere from 16-50% of the total clones screened being members of this group (Borneman *et al.* 1996; Smit *et al.* 1997; McCaig *et al.* 1999).

A number of studies have found microbial diversity and dynamics to be some of the best indicators of soil health due to their rapid responses to soil perturbation and environmental change, in part

because of their high surface to volume ratio (Nielsen & Winding 2002). Other measurable soil parameters may respond too slowly and not reveal soil degradation until the process is at a much more advanced stage (Li *et al.* 2003). However, as soil chemical and physical factors also bring impacts to bear on soil microbial communities, it would be considered pertinent to couple microbial community analysis with a range of chemical, biochemical and physical soil measurements, such as soil pH, nutrient status, C/N ratios, substrate quality and bulk density, for maximum effectiveness (Nielsen & Winding 2002; Bending *et al.* 2004; De la Iglesia *et al.* 2005).

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INDICATORS AND INDICES DEVELOPMENT: THE APPRAISAL OF SUSTAINABILITY AT SETTLEMENT LEVEL IN IRELAND

John Morrissey, Bernadette O'Regan and Richard Moles

ABSTRACT

Sustainability and Future Settlement Patterns in Ireland (an Environmental Protection Agency, Ireland, funded project) is concerned with studying 80 settlements with the aim of investigating the relationships between settlement size, functionality and geographic location and sustainable development. One aspect of the research, the development of a structured and comprehensive set of indicators and indices of sustainable development, aims to provide a meaningful measure of the level of sustainability of individual settlements or regions. Sustainability indices have been evaluated through derived aggregation of indicators allowing the identification of attributes of settlements preventing, impeding or promoting progress towards sustainability. Analysis in this enables the prioritisation of actions to enhance the sustainability of Irish settlements.

Analyses will attempt to identify linkages between various elements of sustainable development in the context of urban settlements. Final indicator and index presentation will be achieved using GIS, to provide an easily queried and interpreted tool for stakeholders with varying priorities. Results of this study will allow the empirical analysis of settlements and will provide practical recommendations regarding the integration of sustainability goals with Irish settlement planning for the future.

Key words: settlements; sustainability; indicators; indices; questionnaire

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INTRODUCTION

A pilot study entitled *Methodologies for the Estimation of Sustainable Settlement Size (MESSS),* undertaken by the Centre for Environmental Research (CER), University of Limerick (UL), from March to August 2001, drew initial conclusions concerning the relationships between settlement size (measured by population) and sustainable development (quantified through indicators) (Moles *et al.* 2002, pviii; O'Regan *et al.* 2002, p450).

Following the completion of this study, funding was provided by the EPA, under the Environmental Research Technological Development and Innovation (ERTDI) programme to provide further, more comprehensive analysis of the relationships between Irish settlements and sustainable development. The three-year University of Limerick (UL) study titled *Sustainability and Future Settlement Patterns in Ireland* (SFSPI) establishes means of consolidating and reinforcing the strengths of urban and rural areas nationwide by investigating key aspects of sustainable development and spatial planning.

This research will ultimately provide input to the National Spatial Strategy (NSS), a twenty-year planning framework proposed to achieve more equal social, economic and physical development along with balanced population growth in Ireland (NDP 1999a, p45). The NSS addresses the phenomena of urbanisation, an expanding population and trends towards smaller household sizes in Ireland, which are placing a concentration of demands on the State's physical infrastructure and environmental quality (EPA 2000, p13), (CSO 2003a, p19) (Lehane *et al.* 2002, pviii). These trends have manifested in problems such as increased traffic congestion, growth imbalances between and within regions, and housing shortages in urban areas, highlighted by the NDP as issues impeding sustainable development in Ireland (NDP 1999b, p35).

Sustainability and Future Settlement Patterns in Ireland therefore investigates the influence of key factors such as urban size, function, and geographic location (within wider spatial networks) effecting progress towards sustainable development of Irish settlements.

METHODOLOGY

Use of indicators

Indicators are important tools for translating and delivering concise and scientifically credible information (Lehane 1999, p1). Indicators may provide a simplification of complex phenomena (OECD 1997, p15), with successful indicators translating information in a manner that can be readily understood and used by decision makers (Lehane 1999, p1).

Indicators have a valuable role to play in the future of sustainable planning for urban areas. Key urban environmental indicators can help policy makers and the public track sustainability more effectively (Pender *et al.* 2000a, p1).

It may be seen therefore that indicators attempt to quantify aspects of sustainability in order to assess conditions, trends and performance. However, a limitation of indicators is that the whole of human experience cannot easily be measured and rated. Indicators, therefore, may not illustrate the full complexity of the systems or processes they represent (Smeets & Weterings 1999, p6). A degree of simplification is a prerequisite, however, to provide information in a form of practical use to decision-makers and understandable to the community (Kelly & Moles 2002, p891).

Indicator development also requires good quality data, which needs to be updated at regular intervals. This makes indicators particularly dependent on availability of data (Lehane 1999, p1). A lack of data may mean that critical indicators are omitted, or issues of importance are not addressed. Factors of significance may therefore be omitted

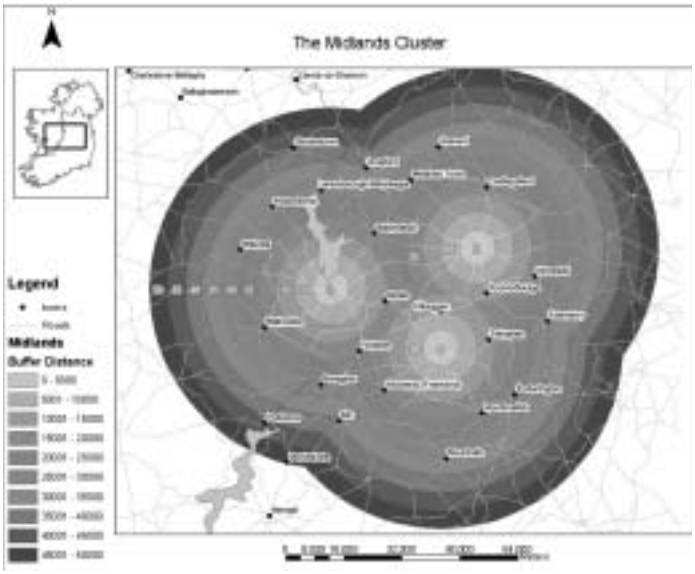


Fig. 1 – Midlands group settlements, with overlaid distance to Gateway buffers.

from the policy formulation process (OECD 2000a, p15). To date, indicators have been developed under all 14 ideal indicator categories.

Data collection and generation

Fourteen ideal indicator categories (see Table 1) were identified by MESSS as the optimal means of assessing sustainability for settlements in an Irish context. These were adopted as a starting point for this research, with data sources previously used in the pilot study investigated for indicator development for each of the study settlements.

Data availability matrices were generated for study settlements, in addition to lists of data gaps for which no known source of information existed. A questionnaire was developed to fill these data gaps, which asked questions designed to generate indicators in each of the 14 categories described in Table 1. Questions covered topics such as travel habits, resource use and waste generation, for example.

Table 1 – List of Ideal Indicator Categories.

Ideal Indicator Category
– Waste
– Water
– Transport
– Energy
– Resource consumption
– Air
– Noise
– Biodiversity
– Education
– Income
– Housing
– Employment
– Health
– Access to Services

The questionnaire was distributed to households in each of the 80 study settlements. Data generation took place in two rounds, from September '02 to March '03, which constituted round one, and from March '04 to June '04, or round two. In total, 8,740 questionnaires were distributed, with 3,399 of these being returned by September '04. This equalled a return rate of 38.89%, highly satisfactory considering time and resource constraints on data generation. Returned data were inputted into an SPSS database for analysis, and summarised to a settlement level. These data were integrated with any collected data to settlement level and a database of candidate indicators was constructed.

Independent variables selected for analysis

In order to investigate the key influences of size, functionality and geographic location on the progress of Irish settlements towards sustainable development, the following were selected as the independent control variables:

- Population size 2002, for the investigation of the influence of population size;
- Index of services, for the influence of functionality;
- Distance from nearest Gateway (km), for the influence of position with the spatial hierarchy and geographical location;
- In addition, the percentage population change from 1996-2002 was identified as an important parameter in identifying settlements where economic and social change was occurring.

Distance to Gateway Zone

Although distance from nearest Gateway settlement does not take into account the full complexity of regional spatial hierarchies, it nevertheless provides a simple, practical and replicable means of investigating spatial and geographical effects on the sustainable development of settlements. Distance in km was calculated for each study settlement using proximity analysis tools in GIS. Settlements were classified according to 10km zonal intervals from central Gateway, with a total of eight classifications being produced.

Index of services

An index of services was adopted as a readily quantifiable and replicable measure of the level of service provision for each particular settlement, with methodologies for development and application of the index primarily been taken from Grove & Huszar 1964; O'Farrell 1968; and O'Farrell 1969). Techniques applied were for the most part based on original methodology applied by W. Christaller, the originator of Central Place Theory in the publication *Central Places in Southern Germany* (1933).

For the level of a specific service present in a particular settlement a simple rating of one, two and three was decided upon for lower order services, intermediate services and services of higher order respectively. This services index score provides a simple and replicable method of describing the role particular settlements have in terms of wider regional functioning.

RESULTS

DEVELOPMENT OF INDICES

A set of aggregated indicators, combined mathematically or through weighting, is termed an index. An index may be used to greatly simplify the information contained in its constituent indicators (Pagina 2000, p2). A single Sustainable Development Index (SDI) has the

Table 2 – Development process of a sustainability index (WGEIO 2002, p15-18).

Index Development Process
1. Selection of variables 2. Transformation of data 3. Weighting of index constituents 4. Valuation of index 5. Presentation and layout of index

benefit of aggregating various measurements into a comprehensive overall index, and demonstrating overarching trends in simple presentations (Pagina 2000, p14). Aggregated indicators may therefore be referred to as "executive summaries of complex realities" (Working Group on Environmental Information and Outlooks 2002, p10). The aggregation of two or more indicators to form an index may be seen as a five-part process (WGEIO 2002, p15-18). This process was followed in the development of a sustainability index for Irish settlements (Table 2).

SELECTION OF SUITABLE INDICATORS

Step one of indicator selection: Indicator variables where data were unavailable for the full complement of settlements remaining were excluded from further analysis.

Step two of indicator selection: From this point, a total of 106 potential indicators were available for selected settlements. To establish which data and variables from these data would be the most suitable to develop indicators of sustainability, statistical tests were carried out on all variables. Tests of normality carried out include Kolmogorov-Smirnov and Shapiro-Wilk, as well as normal probability plots and histograms. Indicators which had shown non-significant tests for Shapiro-Wilk and Kolmogorov-Smirnov, and which had a skewness rating of greater than -1 and less than 1 were considered to have a normal distribution. A total of 79 indicators with normal distributions were deemed suitable for use in final index after this step.

Step three of indicator selection: After the completion of indicator selection step two, indicators were assessed for their sensitivity to the control variables of MVSP grouping, population size (2002), distance to gateway (km) and services index score. Indicators where no sensitivity was displayed for all of the control variables were excluded from any further analysis. From this step of indicator selection, the number of variables deemed suitable for inclusion in the final index was reduced from 79 to 75.

Step four of indicator selection: As already stated, sustainability indicators for decision-making should be representative of key issues of relevance to sustainability and sectoral policy development (Lehane 1999, p1). In order to determine the most effective set of indicators from indicator variables deemed statistically suitable, a further step of indicator characterisation was carried out. The aspect of sustainability being examined by each particular indicator was listed. The most useful set of indicators for the end goal of sustainability assessment were selected. A level of personal judgement was required for this step. Wherever possible, priority was given to indicators where data were considered of better suitability, or indicator was deemed more insightful into state of sustainability. The result of this step was to reduce the final number of suitable indicators to 40 from 75.

Final set of indicators: The culmination of the indicator selection process was 40 indicators, which were subsequently divided into four groups or domains (Table 3). These were Environment, Quality of life, Socio-economic and Transport. These domains cover the key sustainable development bottom-line triumvirate of Environment, Economy and Society, as well as incorporating Quality of Life and Transport aspects, domains which cover issues of immediate concern to the NSS. In this way, sustainable development concerns from the point of view of the major stakeholders (being the NSS and EPA) are addressed. The selected indicators represent the best means of assessing sustainable development for Irish settlements. Indicators follow from the MESSS list of ideal indicators, and cover those identified indicator categories to the furthest possible extent, given data availability, and resources available to the research team, and considering requirements for indicator sensitivity, representativeness and data normality for indicator variables.

TRANSFORMATION AND VALUATION OF DATA FOR INDEX DEVELOPMENT

Transformation of indicator variables

The transformation step is necessary when selected variables do not have the same dimension. Methods of transformation include normalisation, standardisation and distance to policy target analysis of data (WGEIO 2002, p15-18).

Sustainability indices were developed for study settlements from the aggregation of selected indicators deemed suitable for use. To this end, methodologies described by Barrera-Roldan & Saldivar-Valdes (2002) were applied to developed indicators. The procedures applied resulted in all indicator values for selected settlements being transformed into a relative score between 0 and 1. To this end, two transformation equations were applied. *Equation A* was applied for indicator variables where high scores were classified as being more sustainable. *Equation B* was applied for indicator variables where low scores were classified as being more sustainable.

Equation A:
Index component score =
1 – ([Optimum Value] – [X] / [Optimum Value] – [Worst Value])

Equation B:
Index component score =
1 – ([X] – [Optimum Value] / [Worst Value] – [Optimum Value])

Valuation of index scores

Valuation of index scores involves comparing index values with predetermined classifications of what constitutes good and poor values (WGEIO 2002, p15-18).

It was decided that optimum values and worst values, as required for data transformation in equations A and B, would be best chosen for variables from existing, real figures, rather than a theoretical optimum, or theoretical worst-case scenario. This further reinforced the fact that developed indices demonstrated scores with respect to other study settlements, and were more sustainable or less sustainable respective to these other settlements. Maximum and minimum values were obtained for all indicators. These values were then used in *Equation A* or *Equation B,* depending on indicator type.

Table 3 – Table of final indicators.

Domain	Indicator
Environment	% Regular Recycling
Environment	Per Capita Annual Volume of Waste
Environment	% Households Connected to Public Sewerage
Environment	Forestry Area (sq km) within 10,000m Radius
Environment	NHA Area (sq km) within 5,000m Radius
Environment	% Interested in Buying Green Energy
Environment	Per Capita Tonnes CO_2 Transport
Environment	Average NO_3 mg/l 2000-2001 in Drinking Water
Environment	Per capita kg CO_2 from ESB
Environment	Level of Wastewater Treatment
Quality of life	% With Health Insurance
Quality of life	Distance from Nearest Hospital
Quality of life	% Involved in Community Activities
Quality of life	% Experience Offensive Odours
Quality of life	% Experiencing Noise Problems
Quality of life	% Satisfaction with Sports Areas
Quality of life	% Satisfaction with Green Spaces
Quality of life	% Workers with 45+ Hours' Employment
Quality of life	Number of GPs per 1,000 Population
Quality of life	Quality of Life Satisfaction
Socio-economic	Services Index
Socio-economic	Population Density Persons/sq km
Socio-economic	Mean Total Annual Income
Socio-economic	% Households in Accommodation, Whole Houses
Socio-economic	% Rented from Local Authority
Socio-economic	% Private Central Heating
Socio-economic	% Primary Education as Highest Level
Socio-economic	% Certificate/Diploma as Highest Level
Socio-economic	House Price Income Ratio
Socio-economic	% Home Internet Access (SAPS & Survey)
Transport	% Relative Car Use
Transport	% Work in Same Town as Residence
Transport	% Households with Two or More Cars
Transport	% Travel Less than 8km to Work
Transport	% Travel Greater than 24km to Work
Transport	% Using Public Transport
Transport	Mean km to Nearest Train Station
Transport	Index of Traffic Flow (km travelled per min)
Transport	Distance to Shops in km
Transport	Distance to Work in km

Weighting and aggregation of indicators

Weighting of index constituent variables involves judging and assigning a value to the relative importance of various components of the index (WGEIO 2002, p15-18).

It was decided that a simple weighting system would be most appropriate for indicator aggregation. The aim was to enable data aggregation to occur in a very simple and transparent manner, with basic weighting techniques. This enables discourse and exchange of ideas and perspectives when index is used by non-technical personnel, with stakeholders from differing backgrounds.

After the data transformation step, all 40 indicators (in four domains), were given a value between 0 and 1 for all study settlements. Indicators in each domain were then summed to give a final score out of 10. By this methodology, Environment, Quality of Life, Socio-economic and Transport indices were developed for the selected study settlements. From these indices a final index of sustainability was developed. This score was calculated by simply summing all four index scores, and multiplying by 2.5 to give a final score out of 100.

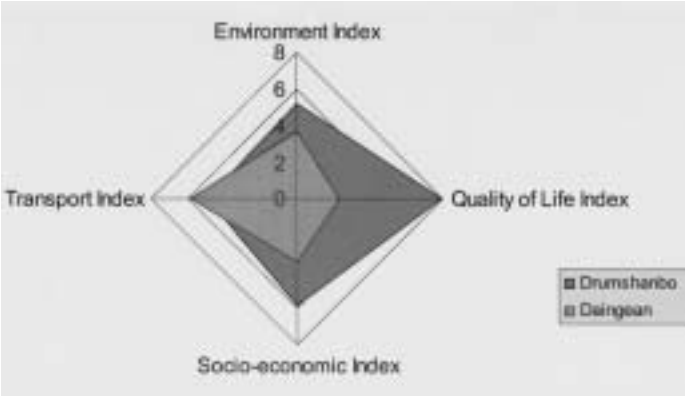


Fig. 2 – "Cobweb" diagram comparing sustainability index scores for two settlements.

Presentation of results and final output

The index scores discussed here form the basis for a detailed and inclusive analysis of the sustainability of settlements. Relationships between settlement index scores and the independent control variables are being analysed through various statistical techniques such as regression and correspondence analysis.

Indices will be presented in easily interpreted graphical format (for example, Fig. 2) enabling criticism and appraisals to be made by stakeholders.

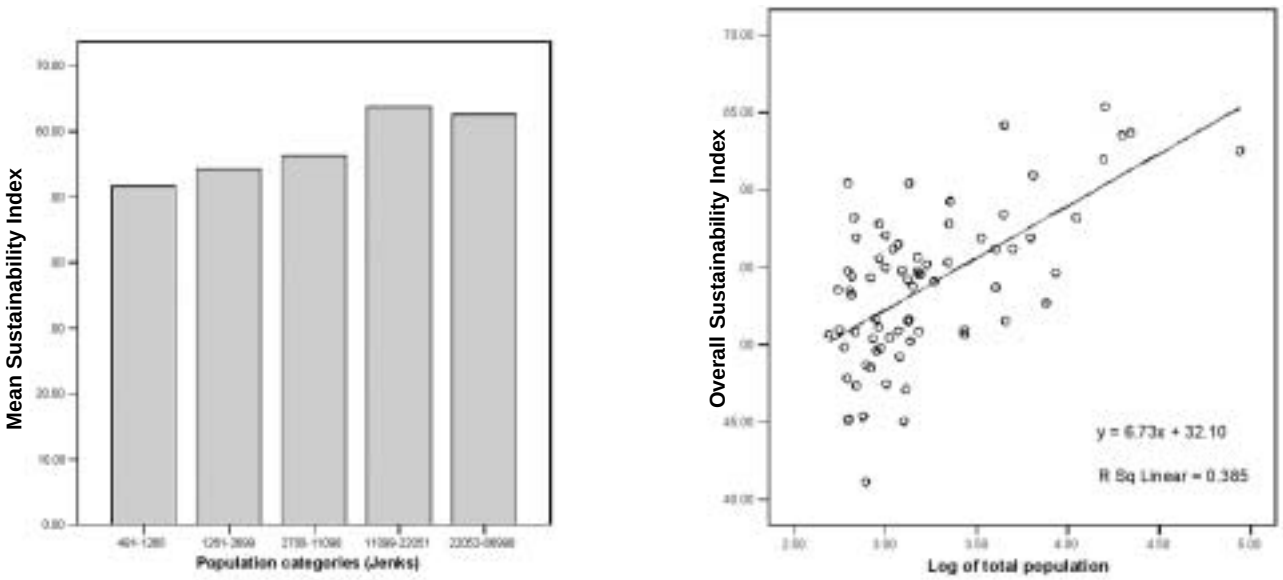
This form of graphical output allows comparisons to be made between settlements with common or contrasting characteristics. By comparing the various elements being used to assess sustainability at settlement level, in the case of this study the four domains of Environment, Transport, Socio-economic and Quality of life, awareness of the trade-offs required to attain a level of sustainable development for settlements can be generated. GIS tools will also be incorporated into final indicator presentation, recognising the important spatial aspect to the work, and further aiding with the presentation of complex data. All output will be inputted into a specifically constructed database, proving a readily accessible tool to stakeholders, with the concise presentation of developed indicators for the selected study settlements.

INITIAL CONCLUSIONS

To date, a total of five indices have been developed for settlements, including an index for each indicator domain, that is Environment, Quality of Life, Socio-economic and Transport. Initial conclusions may be drawn on the observed findings regarding settlement size and these developed indices. Four indices show sensitivity to population size, with a positive linear relationship observed for all four, that is, index value increases with increasing population size. Figure 3 presents the Overall Sustainability Index as an example. The Quality of Life index demonstrates no sensitivity to population size.

Overall sustainability index, by population size

These findings suggest that larger settlements are more sustainable in Environment, Socio-economic and Transport aspects of sustainability, and according to the overall sustainability index developed here, also in



Index	Type	Source	Year
Sustainability index	Aggregated indicator	Various	2000-2004
Analysis shows: Regression analysis P value = 0.000. Straight line fit equation: y = 6.73x + 32.10. $R^2 = 0.385$			

Fig. 3 – Overall sustainability index, by population size.

terms of overall sustainable development. Indices are currently being analysed in terms of settlement population change, distance to Gateway and level of services, with final conclusions pending. Results will provide important provide information for National Spatial Strategy planners, key stakeholders in the research. Forthcoming from analysis will be the identification of attributes of settlements preventing, impeding or promoting progress towards sustainability. The empirical analysis of settlements by these methods will therefore provided practical recommendations regarding the integration of sustainability goals with Irish settlement planning for the future.

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HORMONE MODULATING SUBSTANCES IN THE IRISH MIDLANDS SHANNON CATCHMENT: EXTRACTION, ANALYSIS AND QUANTIFICATION

Antoinette Reid and Jim Roche

ABSTRACT

Many pollutants currently enter the environment through municipal sewage treatment plants, by landfill leachate and by air emissions. Some of these chemicals include hormonally active agents (HAAs) known as endocrine disrupting chemicals (EDCs).Adsorption of these substances to particulate matter and interaction with organic particles will augment the expected levels of HAAs but there will be higher levels present in hydrosoil and sediment in comparison to the bioavailability in surface water. Twenty-three chemical species of known or suspected oestrogenic potential were analysed for in this study. Various extraction techniques were employed leading to simultaneous isolation and enrichment of target analytes, with minimum use of solvent. The overall objective of the research was to analyse matrices with a probability of exposure to endocrine disrupting chemicals and to quantify the environmental levels of these substances in an Irish context. Techniques including high-performance liquid chromatography (HPLC), atomic absorption spectrometry (AAS) and polarography were used in determining the target analytes. Samples were taken and analysed from various locations along the River Shannon including its tributaries. Also influents and effluents from a number of adjacent sewage treatment plants were monitored over a period of time. EDCs were quantified at all the locations tested.

Key words: *endocrine disruptors; extraction; quantitative analysis*

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INTRODUCTION

In recent years, there has been disquiet regarding exposures to low-dose levels of hormonally active agents (HAAs), also known as endocrine disrupting chemicals (EDCs), which may be exerting adverse physiological effects on humans and wildlife populations in terms of endocrinology. Currently, there are many on-going research projects into the reproductive toxicology of these substances. Thus far, there has been a lack of information in terms of the chemical identification and quantification of these species. This study, however, reports on a programme of sampling, extraction and quantification of EDC levels. Presently, more than 50 non-steroidal anthropogenic chemicals are known to mimic the effects of the natural oestrogen, 17 β-oestradiol (Bolz *et al.* 2001). Recent reports indicate that certain metals may also be implicated (Choe *et al.* 2003; Martin *et al.* 2003). Of the 50 suspected EDCs, we have tested for 23 based on their extensive usage and subsequent to considering the Lough Derg/Lough Ree Catchment Monitoring Group Report (McClure Morton 2001). We have chosen to further examine the Shannon Catchment between and including the two upper lakes, and the tributaries along its course. Here we primarily focus on phthalates, a highly potent synthetic oestrogen, certain endocrine disrupting metals (EDMs) and a signal alkylphenol EDC (Fig. 1). The main route of entry into the environment of these substances is likely to be industrial and municipal discharges, landfill leachate, and agricultural effluent, with subsidiary contributions from evaporation, vaporisation and atmospheric deposition.

MATERIALS AND METHODS

The matrices examined included sewage influent, sewage effluent, sewage sludge, soil, river water, river sediment, landfill leachate and the target HAAs included natural and synthetic oestrogens, alkylphenols, phthalate plasticisers and metals. All reagents were of analytical grade and standard solutions of 100 µg/mL were prepared in HPLC grade methanol from which subsequent standard dilutions were made. The following chemicals – dimethyl phthalate (DMP) >99% $C_{10}H_{10}O_4$; dibutyl phthalate (DBP) >98% $C_{16}H_{22}O_4$; phthalic acid bis (2–ethylhexyl ester) (DEHP) 99% $C2_4H_{38}O_4$; bis (3,5,5–trimethylhexyl) phthalate (DINP) $C_{26}H_{42}O_4$; and diisodecyl phthalate (DIDP) >99% $C_{28}H_{46}O_4$; 4-nonylphenol (4-NP) techn.; 17 α-ethinyloestradiol (EE2) and concentrated nitric acid 69% AnalaR® BDH – were purchased from Sigma–Aldrich. HPLC grade methanol, acetonitrile, dichloromethane and ethyl acetate were purchased from Labscan Analytical Ltd. SPE disks from 3M-Empore™ were purchased from JVA Analytical. Standard analytical grade 1 M hydrochloric acid was used for polarography. Stock 100 mg/L metal standards analytical grade (lead, nickel, zinc, cobalt, copper, cadmium, chromium, tin and manganese) in 3 M nitric acid and antimony in 1 M hydrochloric acid were all purchased from Reagecon. Serially diluted solutions of analyte mixtures of the primary stock solutions were carried out as necessary in the appropriate solvent as required on a daily basis. Primary stock solutions were prepared individually from the pure compound at a concentration of 100 mg/L and solutions were stored in amber glass bottles at 4°C, remaining stable for at least eight months. For metals determined by AAS (0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) external standards were used whilst standard additions were used for tin, which required polarographic determination. For the organic compounds, a 5 mg/L standard was used for surface water samples and a 10 mg/L standard was used for effluent and leachate samples.

The sampling process was carried out using an inert stainless steel telescopic sampling rod and sampling cup. Samples were then collected into 2.5 L amber glass bottles, stored in ice and transported to the laboratory immediately. An organic modifier was added to the samples on commencing sample pre-treatment and before the extraction

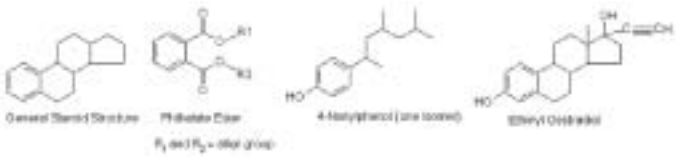

Fig. 1 – Examples of EDC structures.

process was carried out. The chromatograph consisted of a Shimadzu LC-10AD solvent delivery module, a Shimadzu SPD-M6A photodiode array uv-vis detector (nominally set at 226nm), a Shimadzu CBM-10A communication bus module and a Shimadzu FCV-10AL low-pressure gradient delivery system. Both a gradient method using acetonitrile and water and a related isocratic procedure were used during the study. An injection volume of 10 µL and a run time of 40 minutes was used with the gradient, run from 30% to 95% acetonitrile. However, in the isocratic mode, reduced sample volumes were possible. The column used, Pinnacle™ II Phenyl (150 x 2.1 mm, 5 µm) and an equivalent guard column were both purchased from Restek Ireland. The column combined the use of a narrow bore with phenyl-packing material. Peak separations with resolution values of up to 2.85 were achieved for the eleven target analytes using a flow rate of 0.2 mL/min and Table 1 shows the gradient combination used for resolving the mixed solution.

Table 1 – Gradient programme for separation of an 11-compound mix.

Time (mins)	Function	% Solvent B (Acetonitrile)
0.01	B Conc.	30
0.10	B Conc.	30
20.00	B Conc.	95
20.50	B Conc.	95
22.00	B Conc.	30
30.00	Stop	-

For AAS, liquid samples were extracted using solid phase extraction (SPE), whilst solid river sediment samples were dried to constant weight and extracted into 40 mL of 3 M HNO_3. Sample extracts were then analysed at specific wavelengths for each of the test metals using an air/acetylene flame. The linear range was 0–3 mg/L. Polarography was used as an alternative to AAS for the analysis of tin. A standard 1 M HCl solution was used as the electrolyte; the sequence used was anodic stripping with a stir rate of 225 rpm and an electrolysis time of 45 s. The polarograph was run from –800 mV to –200 mV and with tin being identified at –405 mV. For SPE, a three-station vent discharge filtration manifold was assembled and a 47mm extraction disc was placed on top of Kel-F support. The extraction disk was washed and conditioned prior to use. It was imperative that the disk was not allowed to go dry. Meanwhile, water samples were prefiltered using either microfibre glass or nylon filters to remove particulate matter, which tended to block sorbent pores. Strong vacuum was not used as it would pull the analytes through the disk and interaction with the sorbent would not occur. The water sample was then added to the reservoir and under tap vacuum, was filtered as quickly as the vacuum would allow (<<8 mL/min). The solvent rinse was applied followed by the elution process, which was carried out by triplicate additions of 5 mL portions of eluting solvent. Drying down and reconstitution into 200 µL of mobile phase was carried out. The reconstituted sample was vortexed for 30 s. The eluate was transferred into a vial with an insert

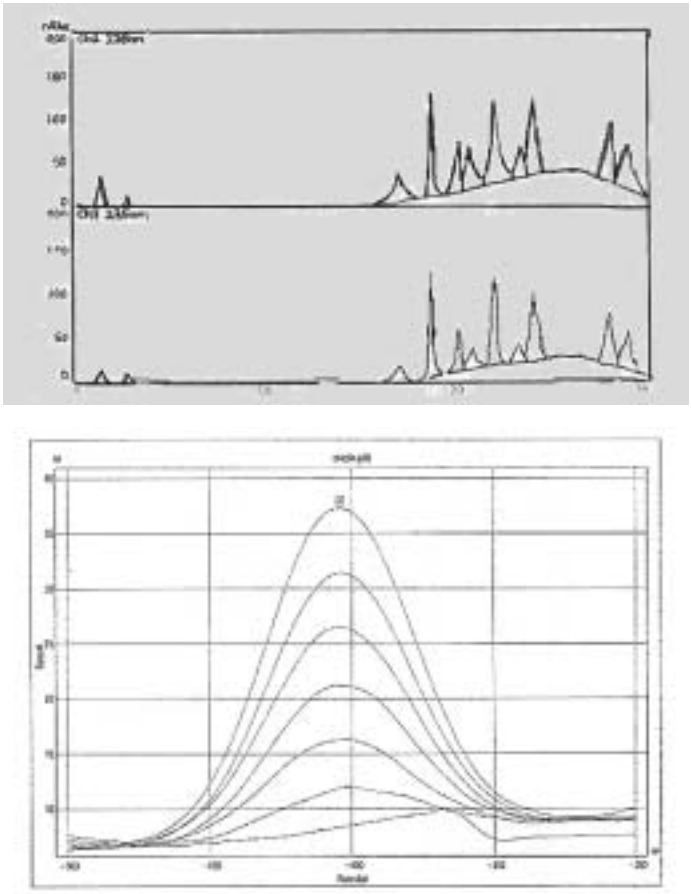

Fig. 2 (a,b) – Gradient HPLC chromatogram of the 11 organic compounds and a polarography curve for tin in extracted sediment.

for subsequent analysis by HPLC or by alternative methods, depending on the nature of the analyte.

RESULTS AND DISCUSSION

The overall objective of the research was to identify matrices with a probability of exposure to endocrine disrupting chemicals and to quantify trace environmental levels of these substances. The results obtained in this study were compared with data in reference material, which examined both the *in vivo* and *in vitro* effects of EDCs and EDMs. Petterson *et al*. (2005) examined bile fluids for EDCs in rainbow trout caged downstream of a sewage effluent pipe, and levels in the bile were comparable to water concentrations with the identified EDCs believed to be responsible for the induction of (VTG) vitellogenin (an oestrogen specific biomarker in fish). In accordance with this, Van Den Belt *et al*. (2004) showed that the oestrogens (natural and synthetic) and NP were all oestrogenic in *in vitro* assays and also resulted in elevated levels of VTG in zebrafish. In relation to metals, Martin *et al*. (2003) claim that treatment of the human breast cancer cell line, known as MCF-7 cells, with divalent metals such as cobalt, copper, nickel, lead, mercury, or tin and chromium (VI) or vanadium (V) stimulated cell proliferation and after six days there was a 2–5 fold increase observed in cell number. These studies indicate that the aforementioned metals can activate the oestrogen receptor, hence introducing metals and metalloids as a new class of non-steroidal environmental oestrogens. Using an oestrogen receptor transcriptional assay and an oestrogen detecting screen, known as an E-screen assay, Choe *et al.* (2004) found high oestrogenicity for cadmium and chromium, whilst lower oestrogenicity was found for cobalt, copper and lead. Bhattacharya (2001) demonstrated the *in vivo* effects of

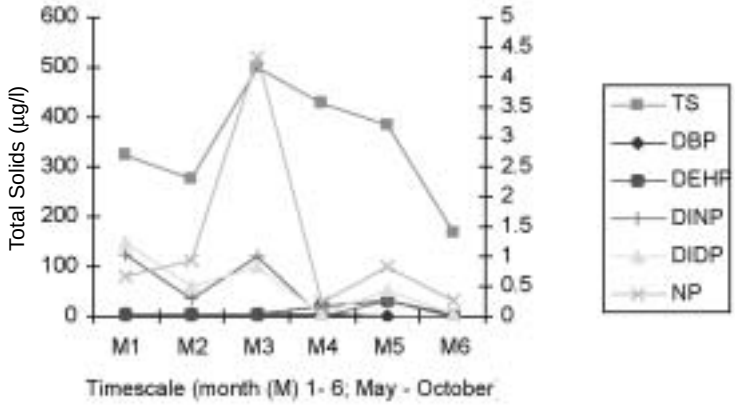

Fig. 3 – a profile of EDC concentration with total solids (suspended and dissolved solids) for a sample location in the Athlone area. One can observe an increase in target analyte concentration when dissolved or suspended solids alter for a sample location. M1 corresponds to May, M2 corresponds with June, and so on as far as October, M6.

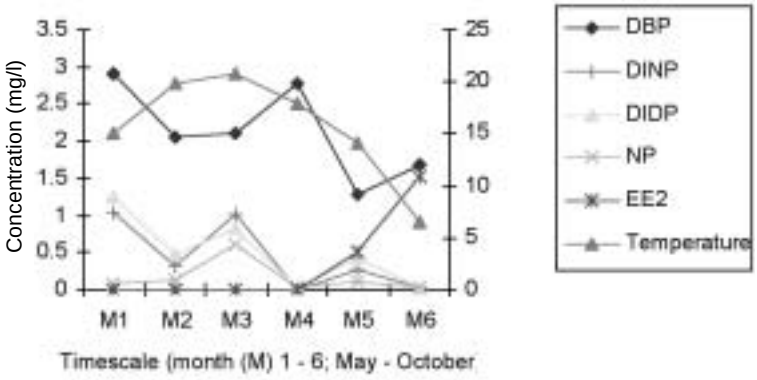

Fig. 4 – a concentration temperature profile of selected EDCs over a continuous six-month period in the Athlone area. Increases in target analyte concentration are observed when temperature alters for a sample location.

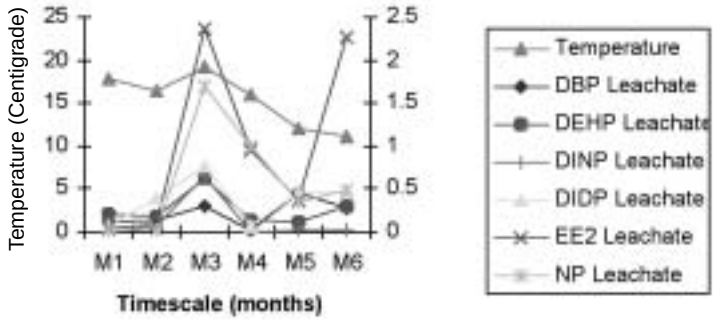

Fig. 5 – this examines leachate of EDCs to a river site in the Athlone area adjacent to an unlined disused landfill, over a period of time, and observed increases in concentration correlates with increases in temperature.

metals in fish in terms of reproduction. These EDCs were also found at relevant concentrations in the Athlone region, indicating the potential for endocrine disruption in fish in the River Shannon.

From the results, we can see that parameters such as temperature, dissolved solids and suspended solids exert a considerable effect on the levels of analyte present. In Fig. 3 we see an increase in concentration when levels of dissolved or suspended solids are elevated and similarly in Fig. 4 we can see the increase in concentration with increasing temperature. In terms of temperature, effects are most pronounced with the more water-soluble compounds such as DBP (Fig. 4). On the other hand lipophilic compounds, such as NP, will sorb onto particulate matter to a greater extent and so when dissolved organic content is elevated, so too will be the NP concentration (Fig. 3). Temperature exerts a similar pattern on the EDC levels in leachate (Fig. 5). However, in this figure we see that there is actually a rise in concentration for the months September and October, which does not follow the trend with temperature. However, this may be explained in terms of the dissolved solids content. For May to October the amount of dissolved solids was 0.218, 0.249, 0.265, 0.289, 0.312 and 0.401 g/L respectively. Whilst the overall total solids content values seen in Fig. 3 shows a decline from August to October, the dissolved solids content increased (suspended solids declined) and high levels of dissolved organic matter can increase the solubility of organic pollutants, so the observed increase in concentration is most probably due to the presence of particulate matter, in spite of declining temperatures. A definitive correlation is not possible, however, as we did record a decrease in rainfall for these three months.

Overall in the Athlone area, concentrations of EDCs in liquid samples over a six-month period varied between 0.03–29.53 µg/L. These values may well be dependent on other environmental parameters as already discussed. In river water, levels between 0.23–4.26 µg/L for each EDC were typical, whereas double these values were observed in leachate and up to 10 times this was found in effluents (unpublished data). The levels of metals found in liquid samples, resulted in values of between 0.015 and 0.602 mg/L. As one would expect, levels up to 100 orders of magnitude greater were found in river sediment (Fig. 6). The levels of EDCs at the landfill location (Fig. 5) are notably affected by temperature but we also detected NP and EE_2. This can be explained in terms of an effluent pipe upstream of the location, which was releasing sewage into the river. The levels of metals in concentrated leachate were compared with leachate downstream of a landfill (both ground and surface water) (Fig. 7). These were also compared with levels found in sewage influent and effluent from a local sewage treatment plant. As expected the levels in concentrated leachate were higher in most cases. There was little difference between ground and surface water. The effluent contained higher levels than the influent, which may be due to pH values. In the tertiary treatment at this particular plant, ferric chloride is used which lowers the pH of the sewage since the secondary sludge from this process is sent back to the aeration tank. Since acidic conditions tend to mobilise metals a higher level of metals would be expected in the effluent. In relation to the overall levels found in the area, concentrations quantified were significant in terms of oestrogenic potential, particularly in sewage effluent and in landfill leachate since both NP and EE_2 are known to be oestrogenic in the ng/L range, not to mention the oestrogenicity of the other target analytes and the possible potential for synergistic activity. We have established levels of EDMs in the Shannon Catchment Region and these are below European intervention levels. We have also noted pH dependence in relation to the concentrations found.

CONCLUSION

The anthropogenic and environmental water cycle are closely bound. Anthropogenic sources include discharge from

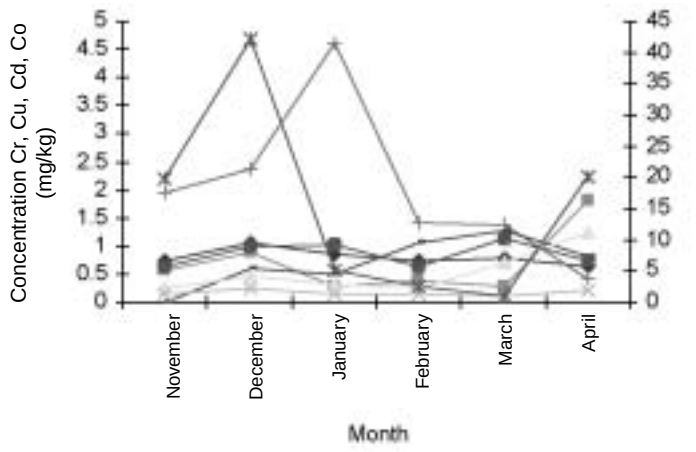

Fig. 6 – this exemplifies the levels of EDMs present in sediment at a sampling location in Athlone. Sediment contains higher levels than liquid matrices.

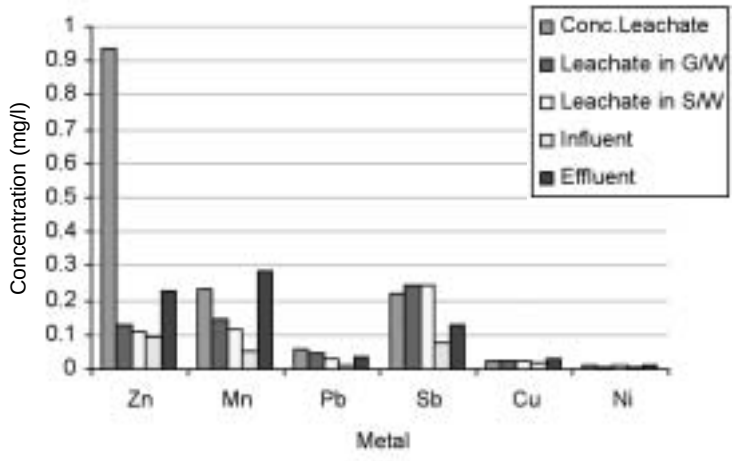

Fig.7 – this compares the levels of metals in concentrated leachate, leachate in both ground and surface water, and in sewage influent and effluent in the Athlone region. Concentrated leachate was found to contain the highest levels of metals. There was little difference observed between surface (S/W) and ground water (G/W), whilst effluent contained higher levels than influent.

households and industry, landfill leachate, effluents and manure from agricultural activities, and discharge from boats, all of which may enter the water table. The environmental water cycle comprises of precipitation leading to runoff, groundwater and subsequently rivers that are often used as potable water sources. Inevitably wastewater, effluents and leachate will penetrate into the environment.

It was observed from our overall sampling that temperature of the water and the amount of dissolved solids present in samples had an effect on the quantity of EDCs found in the sample. An increase in temperature will increase dissolved organic material in the water and hydrophobic molecules will adsorb to this. Even slight increases in the amount of dissolved solids will have a significant impact on the solubility of the more lipophilic analytes. To conclude this study, the levels of endocrine disruptors quantified were relatively low compared with similar studies carried out in Europe and in the UK (Routledge *et al*. 1998). However, some EDCs present even in the ng/L range may provoke reproductive disturbances in riverine fish. For example, EE_2 and NP have been documented to be oestrogenic to fish at levels down to 1 ng/L (Jobling *et al*. 2003) and E_2 has been shown to interfere with the hormone system of various fish species at the level of tens of ng/L (Routledge *et al*. 1998).

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THE EXTENDED MILD SLOPE EQUATION FOR WATER WAVE SIMULATION WITH ENERGY DISSIPATION

Mark Clyne and Thomas Mullarkey

ABSTRACT

A finite element wave propagation model for linear periodic waves in a coastal sea region is developed. Processes simulated by the model include linear shoaling, refraction, full diffraction, total reflection, and breaking of gravity waves on water over arbitrary bathymetry. The method of solution involves complex potential theory. The authors' domain equation is the elliptic-type "extended mild slope equation", which allows wave trains to cross, thereby allowing the formation of amphidromic points. Radiating and absorbing boundaries are modelled using a parabolic equation allowing the passage of energy through the boundary over arbitrary bathymetry. What is unique about this model is that the parabolic equation is used as a boundary condition in Cartesian coordinates given that the equation is designed as a computationally efficient alternative to the elliptic equation in the domain. The mild slope equation, as its name suggests, is designed for use on mildly sloping seabeds; however, the equation has been extended for use over steeper slopes by the addition of terms that describe the slope and curvature of the seabed.

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INTRODUCTION

Propagation of water waves is a phenomenon of great importance to coastal engineering practice. Developing a numerical model that describes all the physical processes involved in wave propagation would be difficult; therefore, some simplifications are made and predominant processes are modelled. Full three-dimensional solutions require excessive computer memory and computational time and are not practical to solve; hence, the dimension of the problem is reduced to two. Berkhoff (1976) develops an elliptic-type mathematical equation, known as the mild-slope equation (abbreviated as MSE hereinafter), to simulate refraction, diffraction and combined refraction-diffraction of water waves around obstacles and over varying bathymetry. Booij (1981) reported favourably on the application of the MSE for quite large slopes of the order 1:3. Examples of more recent programs using the MSE include the work of Xu *et al.* (1996) and Panchang *et al.* (2000). These authors use the elliptic MSE in the domain and develop one form of the parabolic equation in terms of polar and Cartesian coordinates. Xu *et al.* (1996) states "the parabolic approximation is not unique; indeed, there are several ways to derive such approximations and they result in different equations".

Enhancements have been made to the MSE and extended forms of the equations have been developed to incorporate the effects of rapidly varying bathymetry. Kirby (1986) extended the MSE to account for rapid undulations of the depth about a mean level that satisfies the mild slope assumption. Chamberlain & Porter (1995) derive the modified MSE (abbreviated as MMSE hereinafter) incorporating the slope and curvature of the seabed. This form of the MMSE appears frequently throughout the literature up to the present.

Wave breaking is the predominant energy loss process; without it, wave heights tend to infinity at the shoreline. Battjes & Janssen (1978) produce a model to predict the dissipation of energy in random waves breaking on a beach. Zhao *et al.* (2001) examine five wave-breaking formulations including that of Battjes & Janssen (1978) and acquire very favourable results from the model. Booij (1981) derives the necessary additional terms required to incorporate energy dissipation into his version of the parabolic equation and the elliptic equation.

In order to reduce the quantity of data stored when solving the model, a profile storage scheme is employed. Zienkiewicz & Taylor (2000) detail the set up of the profile storage scheme and the algorithms required for the triangular decomposition and solution of the global matrix. Collins (1973) develops an automatic renumbering algorithm that re-sequences nodal numbers to minimize the bandwidth of the global matrix. A highly optimized mesh results from the renumbering of mesh nodes and a solution incorporating the profile storage scheme solves very efficiently.

The elliptic and parabolic equations used in the authors' model are stated below. Results are then presented and discussed and a final conclusion is made.

METHODS

Within the domain, an elliptic mild-slope equation developed by Berkhoff (1976) is taken and extended to account for rapidly varying bathymetry using the slope squared and bottom curvature terms of Chamberlain and Porter (1995). Energy dissipation is also accounted for using the term derived by Booij (1981), giving:-

$$\nabla.(a\nabla\phi)+\kappa^2 a\phi - i\omega_0\gamma\phi + \left\{f_1 g\nabla^2 h + f_2 g(\nabla h)^2\right\}\phi = 0 \quad (1)$$

Symbols used: a is the product of celerity and group velocity, κ is the local wave number, ∇ is a 2-D horizontal differential operator, ϕ is the 2-D spatially varying complex velocity potential, γ is the energy dissipation factor, ω_0 is the wave frequency, g is acceleration due to gravity, and the bottom slope and curvature coefficients are f_1 and f_2 respectively.

By applying the Galerkin finite element method as described by Zienkiewicz and Taylor (2000) to (1), a boundary flux in the form appears and is replaced by the parabolic boundary condition of Booij (1981):-

$$\frac{\delta\phi}{\delta n} = -(a\kappa)^{-1/2}\phi\ \frac{\delta}{\delta n}(a\kappa)^{1/2} + i\kappa\phi +$$

$$iP_2(a\kappa)^{-1}\frac{\delta}{\delta s}\left[a\ \frac{\delta\phi}{\delta s}\right] - P_2\omega_0(a\kappa)^{-1}\gamma\phi \qquad (2)$$

Symbols used: s is tangential to the boundary and perpendicular to n the outward normal, $P_2 = 0.5$ is a constant multiplier originating from a binomial expansion of wave number terms. Absorbing, partially reflecting and radiating boundary conditions are based on (2).

Energy dissipation in the form of wave breaking has a magnitude of γ defined by Zhao *et al.* (2001) based on the theory developed by Battjes and Janssen (1978). Although the breaking model is designed for random waves it can be easily applied to harmonic waves. SWAN as described in Booij *et al.* (1999) is an example of a very powerful and widely used spectral wave model, which uses the Battjes & Janssen (1978) bore based wave breaking model.

RESULTS

Results are presented in 1D and 2D for validation of both the extended terms to the MSE and the breaking criterion used and also to demonstrate the models ability to simulate many important physical processes in the nearshore.

RIPPLE PATCH REFLECTIONS

The first of the examples is a 1D problem that examines the effect of a ripple patch on bottom reflection and how the MMSE captures this feature over the MSE. Waves are propagated over the ripple patch with varying period ranging from 0.7 to 2.3 seconds with a total of 70 program runs for each method, to generate the plot in Fig. 1. A ripple patch ten wavelengths long is used in this example with ripple length of 1m and amplitude of 5cm about a constant depth of 31.3cm. Transmission coefficients T are calculated from the resulting downstream wave heights and reflection coefficients R are calculated from the following:-

$$R = \sqrt{1-T^2} \qquad (3)$$

WAVE BREAKING

In order to verify the model in terms of wave breaking a simple 1D uniform slope is used. Zhao *et al.* (2001) carried out a numerical analysis of waves breaking on a 1 in 20 slope and compared the results obtained with laboratory recorded wave heights. Waves are propagating from right to left in Fig. 2 with a period of 2.29 seconds and incident wave height of 0.202m.

2D BREAKWATER EXAMPLE

To demonstrate the performance of the wave model in 2-D, the following numerical model of an experimental wave tank is chosen. Wave energy is refracting, diffracting, shoaling, reflecting and dissipating due to breaking in this example involving a detached shore parallel breakwater on a 1:50 uniform slope. Symmetry is used in the numerical model with the axis of symmetry passing through the centre of the breakwater at y=4m as can be seen in Fig. 3.

Waves propagate from left to right with an incident wave period of 1.2 seconds and height of 2cm. The breakwater is located on a uniform slope of 1:50 in 6cm of water depth at x=3.025m as seen in Fig. 3. Model boundary conditions consist of a radiation boundary at x=0m, an absorbing boundary at x=6m and the remainder are reflecting. The reflecting boundary at y=0m corresponds to the wall of the experimental wave tank, both sides of the breakwater are reflecting and the symmetry boundary takes the same form as a reflecting boundary.

DISCUSSION

RIPPLE PATCH REFLECTIONS

Fig. 1 shows a clear distinction between the use of the MSE and the MMSE on a ripple patch, especially where resonance occurs at $2k/K=1$, which corresponds to a local water wavelength twice as long as the

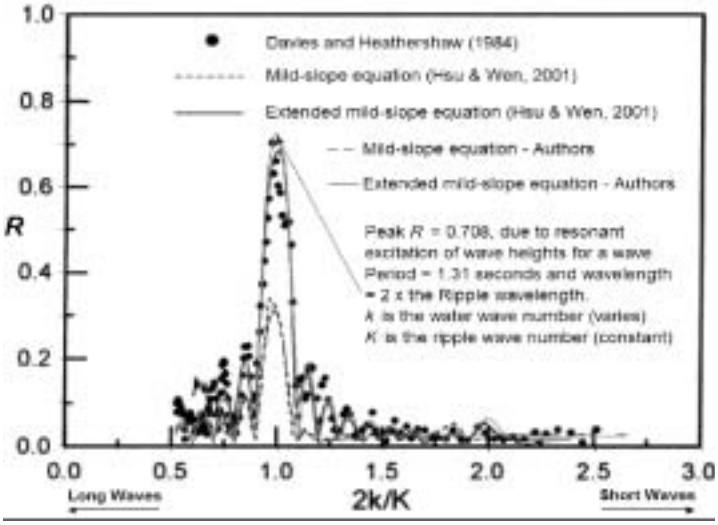

Fig. 1 – Reflection coefficients from a ripple patch for waves ranging in period from 0.7 to 2.3 seconds.

ripple wave length. Symbols used: k is the water wave number for a particular wave period in 31.3cm of water depth, and K is a constant value for the ripple wave number. The authors capture the same features as the experimental and numerical results given in the paper by Hsu & Wen (2001). Clearly the inclusion of the additional bottom slope and curvature terms enhances the models accuracy where rapidly varying bottom features are present.

WAVE BREAKING

Comparisons of the authors' results with those of Zhao *et al.* (2001) are displayed in Fig. 2. The Battjes & Janssen (1978) criteria for wave breaking is used by both the authors and Zhao *et al.* (2001) for the results displayed in Fig. 2. The authors' results are validated by those of Zhao *et al.* (2001) in the form of both numerical and experimental data. Based on the results of this simple example it is clear that the breaking model is working quite well.

2D BREAKWATER EXAMPLE

Inspection of Fig. 3 highlights two key features of a wave incident on a reflecting breakwater. Firstly, upstream of the breakwater a standing wave field is present with an anti-node at breakwater and, secondly, downstream of the breakwater is a shadow zone. Both of these features are clearly seen in the wave height profile plot of Fig. 4, where wave

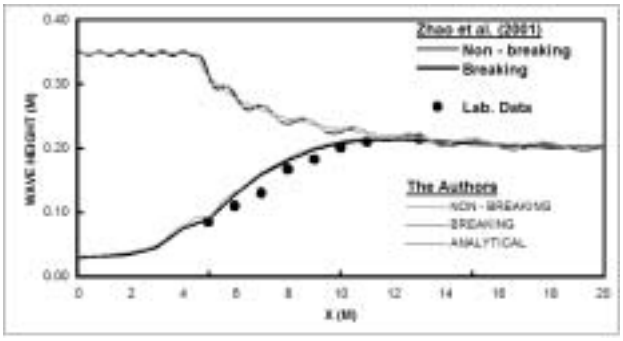

Fig. 2 – Wave height comparisons between results of the authors and Zhao *et al.* (2001) for breaking and non-breaking waves in 1D on a 1 in 20 uniform slope.

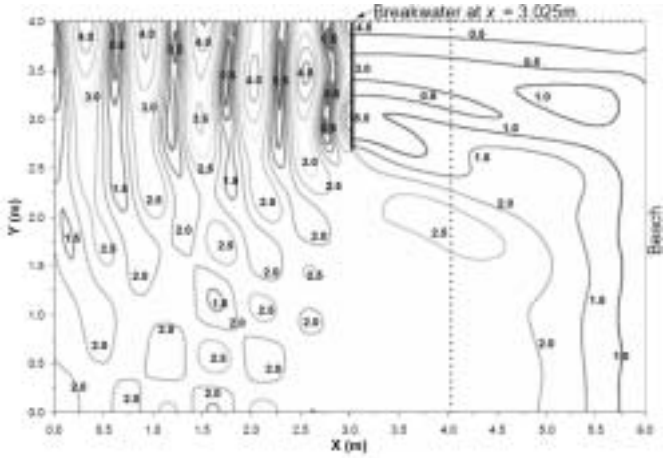

Fig. 3 – Plan view of wave height around a detached breakwater on a 1 in 50 uniform slope.

heights upstream of the breakwater approach twice the incident height at anti-nodes, and much smaller wave heights develop in the protected region downstream of the breakwater. Figs. 4 & 5 show comparisons of wave heights at two sections between the authors' results and those of Watanabe & Maruyama (1986). The comparisons made here are very favourable for the authors' results from their numerical model.

CONCLUSION

Inclusion of bed slope and curvature terms to extend the use of the mild-slope equation to more rapidly varying bed features widens the scope of the wave model to better include these features and by doing so improves the accuracy of the model, as can be seen from the results presented here.

Incorporating wave breaking into the model also adds further scope to the model and brings the model closer to predicting realistic waves in nature. Validation of the authors' results against numerical and experimental results suggests that the breaking model is performing well. This is the same wave-breaking model as used in the SWAN spectral wave model of Booij *et al.* (1999).

The use of the elliptic and parabolic forms of the wave equations in the domain and along specific boundaries, respectively, results in a very flexible model capable of simulating refraction, shoaling, diffraction, total reflection and wave breaking very effectively, as demonstrated by the results.

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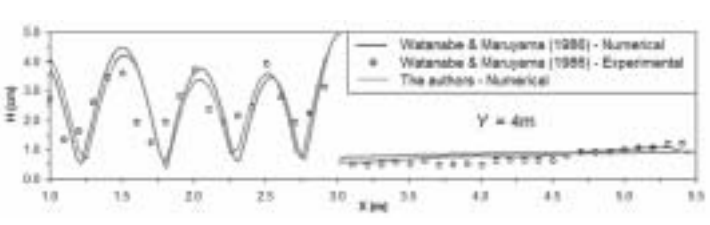

Fig. 4 – Section at y=4m (Fig. 3), the authors' wave heights compared with Watanabe and Maruyama's (1986).

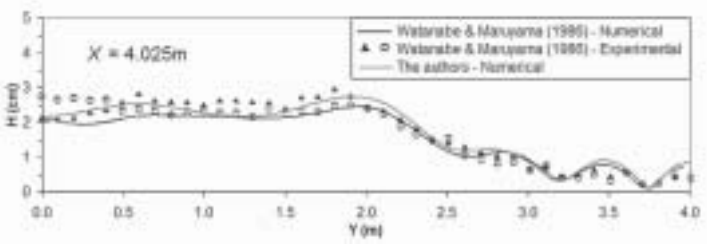

Fig. 5 – Section at x=4.025m (Fig. 3), the authors' wave heights compared with Watanabe and Maruyama's (1986).

INVESTIGATION OF HOMOACETOGENIC BACTERIAL ACTIVITY DURING MESOPHILIC, THERMOPHILIC AND PSYCHROPHILIC ANAEROBIC DIGESTION

Pamela Ryan, Sharon McHugh, Tim Golden and Emer Colleran

ABSTRACT

In this study, the presence and abundance of homoacetogenic bacteria in anaerobic sludge was investigated using conventional, most probable number (MPN) and agar tissue culture flask enumeration techniques. The results obtained show the maintenance of high numbers of these strictly anaerobic bacteria in functioning digesters, despite their much higher K_s values for H_2 than hydrogenophilic methanogens. Batch activity tests on sludge biomass, using H_2/CO_2 as substrate and bromoethanesulfonate (BES) as a specific methanogen inhibitor, revealed that H_2/CO_2 conversion and acetate production commenced only after a lag period of 60-100 h. This finding suggested that the homoacetogen populations of digester sludge may be maintained by heterotrophic growth on sugars or other organic compounds, rather than by autotrophic growth on H_2/CO_2.

Key words: anaerobic digestion; homoacetogenic bacteria; methanogenesis; bromoethanesulphonate (BES)

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INTRODUCTION

Homoacetogens are a diverse group of strictly anaerobic bacteria that generate acetate as the sole end-product of their metabolism. They are capable of autotrophic growth on H_2/CO_2 and of heterotrophic growth on a wide range of sugars, alcohols, methoxylated aromatic compounds and 1C compounds, such as methanol and formate (Li *et al*. 1994). High levels of H_2-utilising acetogens are known to occur within digester sludges and mixed liquors, with numbers in the range of 10^5–10^7 cfu/ml or 10^8-10^{11} MPN/ml (Zeikus 1979; Zhang and Noike 1994). The reason for the maintenance of such high numbers of homoacetogens in digesters is not apparent since, under the low partial pressures of H_2 prevailing in well-functioning digesters, the homoacetogens should be out-competed by hydrogenophilic methanogens, which have a much lower K_s value for H_2. The role played by homoacetogenic bacteria in anaerobic digestion still remains unclear and has not been intensively investigated.

The vast majority of homoacetogens are facultative and can switch from autotrophic to heterotrophic growth and *vice versa*. Enumeration tests using H_2/CO_2 as substrate, therefore, do not necessarily confirm *in-situ* autotrophic growth of homoacetogens. It may, therefore, be the case that homoacetogens are persistent under stable digester operation due to their ability to grow heterotrophically on higher carbon compounds, such as sugars, rather than on their capacity for autotrophic growth on H_2/CO_2.

The objective of the present study was to investigate whether homoacetogens in digester sludges were growing autotrophically or heterotrophically, using batch activity tests developed in the author's laboratory. Enumeration studies were also carried out, using H_2/CO_2 as substrate, to determine the number of homoacetogens capable of autotrophic growth in anaerobic sludge samples removed from a variety of laboratory- and full-scale digesters.

MATERIALS AND METHODS

Source of biomass

Six sludge samples from anaerobic digesters, treating a variety of wastewaters, were used in this study. Sludge A was obtained from a full-scale internal circulation (IC) reactor treating milk-processing wastewater at 37°C (Carbery Milk Products, Ballineen, Co. Cork); sludge B was obtained from a laboratory-scale upflow anaerobic sludge bed (UASB) reactor treating a synthetic volatile fatty acid (VFA) based wastewater (propionate:butyrate:ethanol) at 37°C; sludge C was obtained from a laboratory-scale UASB reactor treating a lactose wastestream at 37°C; sludge D was obtained from a laboratory-scale UASB reactor treating a VFA wastestream at 37°C; sludge E was obtained from a laboratory-scale UASB reactor treating a synthetic glucose/VFA based wastewater under thermophilic conditions (55°C), and sludge F was obtained from a laboratory-scale expanded granular sludge bed (EGSB) reactor treating a synthetic VFA based wastewater under psychrophilic conditions (15°C).

Analytical techniques

Sludge biomass samples were analysed for total suspended solids (TSS) and volatile suspended solids (VSS). The procedure adopted for determination of TSS and VSS was a modification of methods 2540 B and 2540 E (Standard Methods, APHA 2001).

H_2/CO_2 utilising homoacetogen enumeration test media

All media, liquid and agar, were prepared anaerobically using strict anaerobic techniques (Hungate 1969). The enumeration medium contained, per litre of distilled water: NH_4Cl, 1g; KH_2PO_4, 0.33g; K_2HPO_4, 0.45g; $MgSO_4.7H_2O$, 0.1g; yeast extract, 2g; resazurin, 1mg; $NaHCO_3$, 10g; cysteine hydrochloride, 0.5g; $Na_2S.9H_2O$, 0.5g; trace element solution, 20 ml; vitamin solution, 20 ml (DSM

Table 1 – Enumeration of the homoacetogenic populations from a range of granular sludges using MPN and Tissue culture (TC) flask techniques.

Sludge	TC flask count/ml	TC flask count/gVSS	MPN count/ml	MPN count/gVSS
A	4.4 (3.1-5.8)* x 10^7	8.5 (6.0-11.2)* x 10^8	7.9 (2.5-19)* x 10^7	1.5 (0.5-3.6)* x 10^9
B	N.D.	N.D.	1.7 (0.4-4.9) x 10^8	2.3 (0.6-6.8) x 10^9
C	1.8 (1.1-2.6) x 10^6	4.2 (2.6-6.1) x 10^6	1.7 (0.5-4.6) x 10^5	3.7 (1.1-10) x 10^6
D	1.4 (0.8-2.0) x 10^5	3.0 (1.7-4.4) x 10^6	2.0 (0.5-7.0) x 10^4	4.7 (1.2-16.3) x 10^5

* Values in brackets are the 95% confidence limits. N.D.: Not determined.

medium 135). BES was added, when required, to a final concentration of 100mM. Agar media contained 20g Bacteriological No. 1 agar (Oxoid) per litre of distilled water.

Microbial counts

Most probable number (MPN) counts were carried out in 20 ml serum vials (Pierce UK) sealed with gas-tight butyl rubber bungs and crimped in place with one-piece aluminium caps. Ten-fold dilutions (neat to 10^{-8}) were carried out in sterile anaerobic saline solutions containing 8.5g of NaCl per litre. Prior to dilution, all sludge samples were homogenised using a Potter Elvehjem device, within an anaerobic cabinet. Each vial contained 1 ml of sludge dilution and 9 ml of the homoacetogen enumeration test media, described above. Vials were pressurised to 1 atm with a mixture of oxygen-free H_2 and CO_2 (80:20), with five replicate vials per dilution. The vials were incubated for 30 days at 180 rpm on an orbital shaker at 37°C. Vials containing higher levels of acetate than the corresponding blank vials, which underwent parallel incubation, were considered positive.

Enumeration of acetogens on solid media was performed, as described by Braun *et al.* (1979) with some modifications, using tissue culture flasks (250 ml) containing 25 ml of homoacetogen enumeration agar, under an atmosphere of filter sterilised H_2/CO_2. Flasks were incubated upside down for 2 weeks at 37°C. Colonies were counted using a bifocal, low magnification microscope.

Bacterial activity tests

A modified version of the specific methanogenic activity (SMA) test, developed by Reynolds & Colleran (1986) and Concannon *et al.* (1988), and described by Colleran & Pistilli (1994), was used to determine the specific homoacetogenic activity (SHA) of sludge biomass samples. 100 mM BES was included in the test vials, which were set up in triplicate, and also contained sludge biomass and anaerobic buffer. The vials were pressurised with H_2/CO_2 (80:20) to 1 atm and incubated, on orbital shakers (180 rpm), at 37°C (sludges A-D), 55°C (sludge E) and 15°C (sludge F). Pressure decrease in the vials was measured, over time, using a portable pressure transducer (Coates *et al*. 1996). In-vial acetate and % biogas methane concentrations were determined by gas chromatography, as described previously (Colleran *et al.* 1998).

RESULTS AND DISCUSSION

Enumeration of the H_2CO_2 utilising, homoacetogenic bacteria present in four of the test sludges (sludges A-D) revealed high levels of homoacetogens per g VSS (Table 1), correlating with the results of previous studies (Zeikus 1979; Zhang & Noike 1994). MPN and tissue culture counts were found to be in general agreement, within the confidence limits of the enumeration procedures. Sludges A and B were related to each other, as sludge A was used to seed the reactor from which sludge B was removed. Both contained higher levels of homoacetogens capable of autotrophic growth than sludges C and D. Sludges C and D were also related, as the reactors from which they were obtained were inoculated with the same seed sludge (Golden 1996). Higher levels of homoacetogens appear to have been maintained on the lactose-based influent than on the VFA/ethanol influents, perhaps indicating the preferred growth of homoacetogens on sugar substrates.

The specific homoacetogenic activity (SHA), against H_2/CO_2 as substrate, was determined for all sludges in the presence of 100 mM BES. In all cases, a significant lag phase was observed prior to H_2 uptake. Figs. 1(a), 2(a) and 3(a) illustrate the pattern of H_2/CO_2 conversion obtained in the presence and absence of 100mM BES for sludges A, E and F. Stoichiometric conversion of H_2/CO_2 to methane was obtained in the absence of BES within 20-30 hours. By contrast, a lag phase of approximately 60-100 hours prior to H_2/CO_2 conversion was observed in all test vials containing BES. This delayed H_2/CO_2 conversion was associated with acetate accumulation (Figs. 1(b), 2(b) and 3(b)), indicating the involvement of homoacetogenic species. A close correlation was obtained between the theoretical and measured acetate yields in test vials.

All tests were carried out under non-growth conditions. The findings obtained suggest that the delayed onset of acetate accumulation in the presence of BES was due to bacterial adaptation rather than to growth of homoacetogenic bacterial species. When inorganic nutrients were included in test vials, a similar lag phase of 60-100 hours was also observed prior to the onset of homoacetogenesis in vials containing BES (Golden 1996). These initial findings support the hypothesis that homoacetogens may be maintained in high numbers in stable anaerobic digesters, due to their ability to grow heterotrophically on organic compounds, rather than autotrophically on H_2/CO_2.

If true, this alternate role of homoacetogens opens up new opportunities for more efficient and more stable operation of digesters. According to this hypothesis, sugars could be converted to methane in a simple two-step process; i.e. homoacetogenic oxidation of sugars to acetate followed by direct conversion of acetate to methane by the acetoclastic methanogens. This hypothesis supports the finding that more than 70% of the methane produced during stable digester operation is derived from acetate (Smith & Mah 1966; Lettinga 1995; Batstone *et al*. 2002).

Figs. 1(a), 2(a) and 3(a) illustrate the pattern of H_2/CO_2 conversion obtained by representative mesophilic, thermophilic and psychrophilic digester sludges. A lag phase of approximately 60-100 hours prior to H_2/CO_2 depletion in vials containing BES was observed for each sludge at the temperatures tested. This pattern of H_2/CO_2 conversion correlates with the immediate and stoichiometric conversion of H_2/CO_2 to CH_4 in the absence of BES as observed in Figures 1(b), 2(b) and 3(b). This is indicative of lag phase of c.60-100 hour prior to adaptation

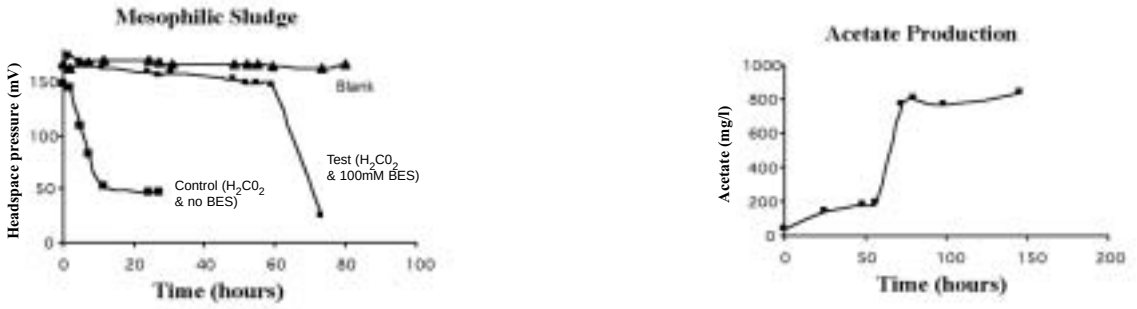

Fig. 1(a) – Headspace pressure decrease using sludge A (mesophilic), in batch activity test vials containing H_2/CO_2 in the presence and absence of 100mM BES. Fig. 1(b) – Measured acetate in-vial production.

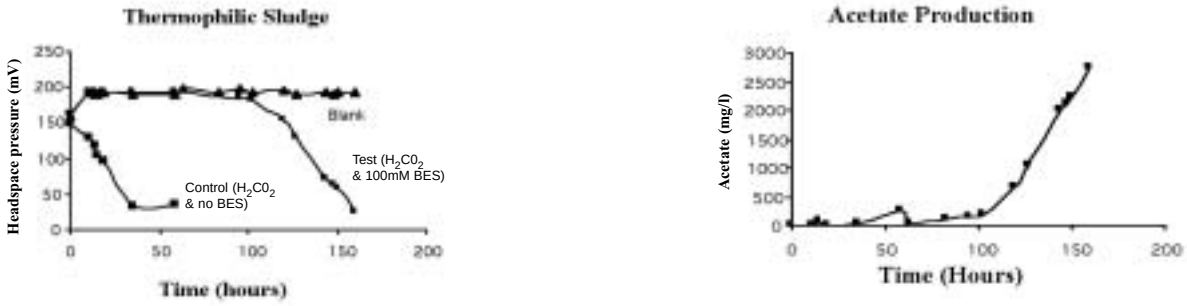

Fig. 2(a) – Headspace pressure decrease using sludge E (thermophilic), in batch activity test vials containing H_2/CO_2 in the presence and absence of 100mM BES. Fig. 2(b) – Measured acetate in-vial production.

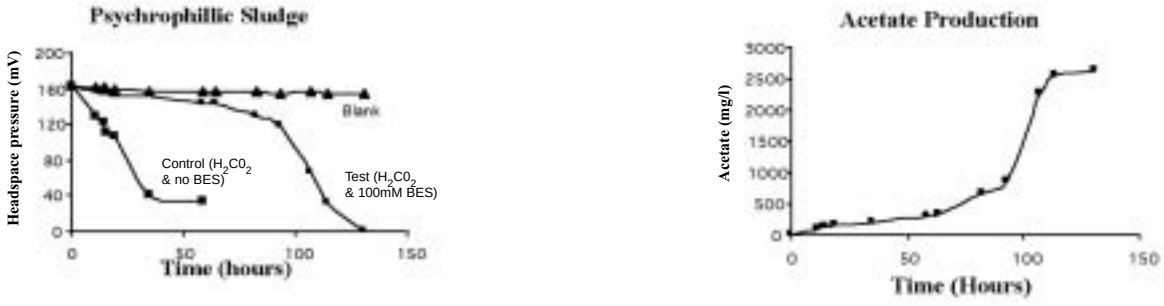

Fig. 3(a) – Headspace pressure decrease using sludge F (psychrophilic), in batch activity test vials containing H_2/CO_2 in the presence and absence of 100mM BES. Fig. 3(b) – Measured acetate in-vial production.

of sludge homoacetogens to autotrophic growth on H_2/CO_2.

Further research is being preformed using pure type strains of homoacetogens to identify genes that may be unique to acetogens during either autotrophic or heterotrophic growth. The application of molecular biology techniques for the study of populations in digester sludges typically utilises genus or species specific primers to amplify the 16S rRNA gene. The homoacetogenic species are an extremely heterogenous group, dispersed among many different genera. Primers based on 16S rRNA gene sequences may not be effective in the study of a group of organisms that are so phylogenetically diverse like the homoacetogens. More recent studies by Leaphart and Lovell (2001) highlighted the feasibility of developing primers for homoacetogenic species based on partial sequences (1,102 of 1680 bp) for the formyltetrahydrofolate synthetase (FTHFS) gene. These FHFTS primers will permit examination of sludge samples to determine the presence, diversity and major physiological features of the homoacetogenic species.

Anaerobic digestion was traditionally used to treat sewage sludge and animal manure, but advances in reactor design and an in-depth insight into the microbiology behind the process has broadened the range of substrates that can be treated. The process has spread to the treatment of food processing wastes, livestock farming residues and a wide range of industrial based wastewaters. Useful products in the form of biogas and biomass can be produced and recovered from anaerobic digestion and anaerobic wastewater systems, hence it is a cost-effective, energy yielding process.

In summary, anaerobic digestion technology offers an attractive alternative for the treatment of several types of wastewaters. Although this technology still has limitations, its practical application will in no doubt increase in the near future because of the economical and environmental advantages (Sekiguchi *et al.* 2001).

CONCLUSION

The results of this study confirmed the presence of high levels of autotrophic, H_2/CO_2-utilising homoacetogens in a range of digester sludges using conventional MPN and agar tissue culture flask enumeration techniques. However, examination of the pattern of H_2/CO_2 uptake in the presence of BES, as a methanogen inhibitor, indicated that a lag-phase of 60-100 h preceded H_2/CO_2 conversion and acetate production. This finding suggests that homoacetogens in digester sludges may be maintained as a result of heterotrophic growth on sugars or other organic compounds and that their enumeration in media containing H_2/CO_2 as the carbon/energy source requires this lengthy period of adaptation from heterotrophic to autotrophic metabolism.

For further improvements in anaerobic biotechnology to occur, we need to expand our knowledge of microbial communities and population dynamics in the anaerobic processes. To acquire this knowledge, detailed molecular surveys of microbial communities in anaerobic sludges are required, together with cultivation of relevant anaerobes.

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EXAMINING MATERIAL FLOWS IN FRESHFORD, CO. KILKENNY: AN ANALYSIS OF HOUSEHOLD TRANSPORT CONSUMPTION

Pauline Ryan, Bernadette O'Regan and Richard Moles

ABSTRACT

This research proposes to advance the pursuit of sustainable development in small Irish settlements by means of exploring the link between materials consumption and sustainability in a small settlement setting. This initiative will facilitate a pattern of development and resource use promoting positive effects on our current supply of natural capital. Natural capital, defined as stocks of natural assets, facilitates our materials consumption, and a criterion for sustainability is now deemed to be non-declining natural capital, thus enabling material flows to be maintained indefinitely (Hinterberger *et al.* 1997, p2; Bartelmus 1999, p159). The consumption component of personal transport has been accounted for in Freshford, Co. Kilkenny. Ecological footprint (EF) analysis was applied as a tool to indicate the sustainability of current transport patterns within the settlement. The results have indicated that reliance on the private car to facilitate the majority of journeys is the primary contributing factor to unsustainable transport patterns in the settlement.

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INTRODUCTION

One of the chief obstacles to sustainable development has been the increasing materials (goods and services) consumption particularly in developed countries (Mega 2000, p228). Sustaining consumption through natural resource extraction and manipulation has resulted in environmental depletion and degradation (Barrera-Roldan & Saldivar-Valdes 2002, p251). Moreover, continuing in this manner is not sustainable as "the limits of nature" are at risk of being exceeded (Spangenberg *et al.* 2002b, p430). Environmental depletion is not only an issue for industry and enterprise, the unsustainable utilisation of materials by households warrants evaluation in order to produce an estimate of the depletion cost relative to household management (Bartelmus 1999, p164*)*. The environmental impacts of household consumption have grown over the last three decades and it is anticipated that they will intensify over the next twenty years (OECD 2002, p12). Promoting sustainable consumption, defined as "the use of goods and services that respond to basic needs and bring a better quality of life, while minimising the use of natural resources, toxic materials and emissions of waste pollutants over the life cycle, so as not to jeopardise the needs of future generations", can assist households in reducing their environmental impact (OECD 2002, p15-16).

Sustainable development investigation in Ireland has focused predominantly on large- to medium-sized settlements, specifically through the assignation and development of Gateways and Hubs. The large conurbations of Cork, Limerick, Galway and Waterford (see Fig. 1) have become Gateway cities under the auspices of the 2002 *National Spatial Strategy*. This document sets out the government objective of developing in a sustainable and balanced manner, outlining how Ireland can be spatially structured and developed over the next twenty years in a way that is internationally competitive, socially cohesive and environmentally sustainable (NSS 2002, p10-15). Gateway cities are expected to become engines of national and regional growth. Hubs, strong county towns and large towns located close to Gateways, will support this regional growth and in theory be responsible for disseminating balanced and sustainable growth to smaller settlements and rural Ireland (NSS 2002, pp39 & 50).

Small settlements with populations under 1,500 have not been adequately represented in national sustainable development research to date. More than 550 such settlements exist in Ireland, representing almost 10% of the total population (CSO 2003). The research described here presents empirical findings of the hitherto unexamined materials consumption of small settlement households. Specifically, it provides an account of the current baseline of personal transport metabolism in a small settlement conducted from a bottom-up perspective.

Policies devised to counteract the growing trend of unsustainable materials consumption must be informed by greater understanding of the impact of consumption on ecological services. Ecological footprinting (EF) can be used as a tool to fulfil this requirement. It has the capacity to clarify and make explicit the link between human consumption and nature's ability to sustain humankind demand. The applicability of EF in this research was driven by a number of different functions that EF has to offer in the realm of sustainability research. The focus of community individuals in this research demanded the use of a tool with the ability to simplify and communicate the primary materials consumption data. It was desirable that such a tool would aid in making explicit and communicating to stakeholders the ecological impacts of their current lifestyles. In addition, EF was used in this research to conduct a sustainability assessment of current consumption patterns.

The settlement selected was Freshford Co. Kilkenny (see Figure 1), with a population of 756 in 2002 (CSO 2003). In 2001, the Freshford 2020 Committee was formed and published a *Draft Proposal for a Development Plan in Freshford* in January of that year. In this document it was recognised that Freshford, like other villages and small towns in Ireland, will be facing "dilemmas of growth, pollution and energy sourcing in the next few years" and the committee wished to "offer Freshford a safe, healthy and sustainable future" (Freshford District 2020 2001, p4). An awareness of sustainable development among the settlement residents combined with an appropriate data gathering

Fig. 1 – Map of Ireland displaying location of Freshford and major Irish settlements.

technique resulted in a high level of participation in the study by community members.

METHODOLOGY

This section provides an overview of the methods used to:
– select the materials of relevance for examination in the settlement;
– facilitate the data collection in the settlement;
– conduct the sustainability assessment of the materials consumption;
and it outlines the data categories of personal transport that were gathered from the settlement respondents.

MATERIALS SELECTION

Prior to delivering a materials consumption and metabolism overview of the settlement, particular emphasis was placed on selection of the relevant components of consumption. There is a vast array of materials consumed in any given settlement, but evaluating all these flows was outside the scope of this research. In determining the consumables to be selected for investigation in this settlement, the findings of Spangenberg & Lorek (2002) *(Environmentally sustainable household consumption: from aggregate environmental pressures to priority fields of action)* were considered. Household consumption may be disaggregated into 10 distinct consumption classes comprising more than 95% of household related natural resource consumption (see Table 1). Classes considered significant when determining priority fields of action for households, as consumers, must satisfy two criteria (Spangenberg & Lorek 2002, p134):
– Be environmentally relevant (those clusters activating the most resource flows throughout the product life-cycle);
– The consumers must exert significant influence on the extent of consumption.

Health care, education and social life are chiefly components of state consumption providing services, which are directly or indirectly consumed by households (Spangenberg & Lorek 2002, p135). Households exert limited influence on the frequency and intensity of use they make of these services (*ibid*). For example, a minimum consumption of education is legally required in most countries. In addition, householders have little choice regarding the production of these services. The resource intensity of providing these services is influenced by a series of administrative decisions by public or in some cases private institutions, and not household decisions (*ibid*). These sectors are beyond the reach of consumer influence and as such were regarded as being outside the scope of this study of consumption accounting.

Householder decisions wield substantial influence regarding their respective consumption of each of the seven remaining classes (Spangenberg & Lorek 2002, p135). After due assessment of their causal responsibility towards environmental depletion and degradation, significant variations between the classes emerged. The total resource requirement of three classes, namely housing, food and transport accounted for approximately 70% of material extraction and energy consumption and more than 90% of land use (*ibid*). Elements of these classes and associated material flows were selected for analysis in this research. However, the findings presented in this paper are limited to exploration of small settlement personal transport consumption.

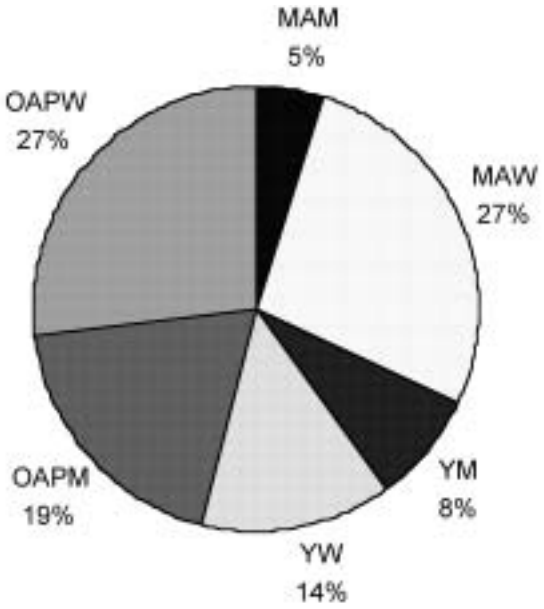
Table 1 – Household consumption classes (Adapted from Spangenberg & Lorek 2002, p134).

No.	Class
1	**Clothing**: textiles for human use
2	**Education**: kindergartens, schools, universities
3	**Food**: food production, cooking
4	**Health care**: hospitals, rehabilitation
5	**Housing**: construction, heating
6	**Hygiene**: washing, disinfecting
7	**Laundry**: cleaning of textiles
8	**Recreation**: leisure activities without the transport involved
9	**Social life**: police, military and other public services
10	**Transport**: commercial transport, commuting and leisure related mobility

SURVEYING

The boundary for the study site encircled the village at each of the speed limit signs on all roads entering the village. There are 258 occupied households located within this village boundary. As data on the relevant materials consumption of these households were not entirely available from government, council or other sources, data were sought directly from the settlement residents by means of surveying. The surveying was conducted on a face-to-face basis. This method can expect to realise a 70–80% response rate (McNeill 1990, p40). A 62% response rate was achieved in Freshford, representing 161 households and a population of 488 (Freshford_161).

Ensuring accurate answers and avoiding guesswork from the respondent required setting the question in an easily measurable context for the householder. For example, in the waste component the question posed directed the respondent to consider wheelie bin capacity. They were not expected to give an estimate of annual tonnage of waste produced. The questions were kept closed where possible, as restricting the number of open questions in the survey avoided the possibility of "over-probing" or influencing responses during the interview and ensured honest and accurate responses (McNeill 1990, p26). At each interview the head(s) of household was requested to complete the survey in an effort to ensure greater precision in the



Legend
W = Woman; M = Man
YW/YM: Young (aged 20–39)
MAW/MAM: Middle-aged (aged 40–59)
OAPW/OAPM: Old-aged Pensioner (aged 60 and upwards)

Fig. 2 – Survey respondents classified by age and gender.

answers. No time constraints were placed on the duration of the interview and in most incidences the time required exceeded 30 minutes. Although the survey was designed to require 15 minutes for completion, securing participation of the householder entailed spending time explaining the aims and objectives of the study and addressing any concerns the householder may have had about the survey. Fig. 2 displays the respondents categorised by age and gender. Females comprised 68% of the total respondents while the greatest number of male respondents was located in the 60^{+} years category.

ECOLOGICAL FOOTPRINTING

Component ecological footprinting, applied in this research, adopts a bottom-up approach to the calculations of the area required to support human consumption (Ryan 2004, p224). Instead of considering the effects of the consumption of raw materials, this approach considers the demand arising from a region's transport, water, energy, etc, requirements (Barrett & Scott 2001, p16; Ryan 2004, p224). It is an additive approach that sums the ecological footprint of all relevant components of a population's resource consumption and waste production (Wackernagel *et al*. 2004, p5-6). Two distinct steps are required to facilitate component-based footprinting (*ibid*):

– Resource accounting: identify all the goods and service and amounts thereof that a population consumes;
– Life Cycle Analysis (LCA) data are used to track the resource requirements of each consumable good or service, from raw material extraction to waste disposal.

Equation 1 describes the calculation used to determine a region's footprint using the component approach (adapted from Barrett *et al.* 2004, pp2-13):

$$EF_r = \sum_{i=1}^{i=n} (D + N) \quad (1)$$

where: EF = total ecological footprint of a region, r
D = direct land use,

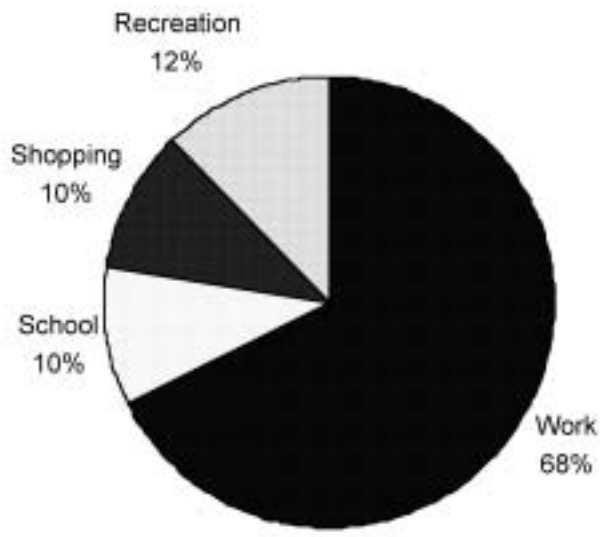


Fig. 3 – Distribution of passenger kilometres.

N = additional land requirement (notional energy land),
I = represents the number of component parts to the footprint.

The component method of ecological footprinting was selected as the calculation method most suited to this research. The reasons for this are twofold. Firstly, the bottom-up nature of data gathering in this research was logically suited to the bottom-up calculation methodology of component footprinting. Secondly, this method is of use in gaining insight into the ecological requirements of activities. With the overriding objective of this research being one of exploring means to advance sustainability in a small settlement, highlighting the community activities prohibiting this progression was paramount. The component calculation methodologies applied in this research have been largely based on work carried out at the Stockholm Environmental Institute–York (SEI-Y) located at the University of York, UK. This research group have applied EF at a number of levels ranging from national level, *An Ecological Footprint of the UK: Providing a Tool to Measure the Sustainability of Local Authorities*, regional level, *A Material Flow Analysis and Ecological Footprint of York* and the *Ecological Footprint of Inverness,* and at the local level, *Sustainability Rating for Homes – The Ecological Footprint Component.*

PERSONAL TRANSPORT

The personal transport footprint investigated the impact of travel by community residents using various modes of transport. Walking and cycling were not included in this analysis as they have a negligible footprint (Barrett *et al.* 2002, p50). In calculating the EF of passenger transport a number of factors required consideration. These included:

– Fuel consumption;
– Energy requirements of manufacturing and maintenance of the vehicle;
– Land area occupied by transport infrastructure;
– Distance travelled;
– Occupancy rate.

The EF of a single passenger kilometre was calculated for each mode and then multiplied by the total number of passenger kilometres per mode to determine an EF value for personal transport by the settlement respondents. This total can be further disaggregated to establish the EF of travel to services such as a) work, b) shopping, c) education and d) recreation.

Table 2 – Total Passenger km by mode.

	Annual Passenger (km)	By Car	By Bus	By Taxi	Car Share
Home to Work	2,150,150	2,150,150	0	0	0
Home to Schoo	314,986	32,595	282,392	0	0
Home to Shops	331,313	283,307	32,094	0	15,912
Home to Recreation	389,197	346,265	0	41,340	1,591
Total	**3,185,646**	**2,812,316**	**314,486**	**41,340**	**17,503**

RESULTS

PERSONAL TRANSPORT

The passenger kilometres (passkms) for 2004 for Freshford_161 residents amounted to 3,185,646 passkms (see Table 2). Travel from home to work contributed 68% of these kilometres (see Fig. 3). Travel to school, shopping and recreation represented an almost equivalent proportion of the remaining passenger kilometres.

Private vehicles and buses were primarily used to provide transport to work, shopping, school and recreational facilities. Other modes of transport include travel by taxi and receiving a lift. It was assumed that the occupancy rate of these latter modes was at least two persons and this assumption was included in the relevant footprint calculations.

ECOLOGICAL FOOTPRINTING

The sum of the personal transport subcomponents represents the overall footprint of personal transport by Freshford_161 residents (see Table 3). Of the total footprint, 137 gha/per capita, the travel to work subcomponent contributed significantly to this total, responsible for a per capita footprint of approximately 103 gha (see Table 3). Contributing factors to this footprint are most likely a) the volume of kilometres related to travel to work, 68% of the total kilometres travelled, b) significant reliance on use of the private car (Table 2 indicates that no other mode of transport is used to provide transport to work), c) low occupancy rates on work journeys, confirmed with field observation, and d) travel to work frequency is greater than that of other travel habits, such as shopping and recreation, etc.

The positive impact of travelling by bus was highlighted in this study of travel habits, particularly in the case of travel to educational services. From Table 2 one can see that total passenger kilometres for each are similar: 314,986 passkms for school and 331,313 passkms for shopping. However, their respective travel by modes contrasted significantly: 283,307 passkms travelled using private vehicles for shopping as opposed to 32,595 passkms travelled using private vehicles for journeys to educational facilities. The remaining passkms for school journeys were travelled using school buses; these buses operate with a high occupancy rate that resulted in the lowest calculated per capita footprint of 3.3 gha (see Table 3).

Table 3 – Overall personal transport footprint results.

Sub-Component	Total Footprint (gha)	Per Capita Footprint (gha)
Travel to Work	50,122	102.7
Travel to Education	1,625	3.3
Travel to Shopping	6,291	12.9
Travel to Recreation	8,655	17.7
Total	**66,693**	**136.6**

DISCUSSION

Sustainability assessment using EF analysis is enabled by the comparison of supply versus demand (the footprint). EF applied to the results of the passkms transport accounting for the settlement comprised the demand share of the comparison, a total per capita demand of 136.6 gha. Supply, or to apply the technical term, biocapacity, currently stands at a global average of 1.8 gha per capita (Loh & Wackernagel 2004, p34). This value corresponds to the equitable amount of renewable natural capital available to the world population in order to facilitate consumption (*ibid*). In cases where demand exceeds supply, remembering that demand is driven by consumption, demand levels can be classified as being unsustainable and contributing to the liquidation of natural capital, thereby hindering its ability to provide satisfactory levels of material flows indefinitely. The footprint of Freshford residents' travel habits as calculated by this study can be classified as being unsustainable and in need of alteration to be steered on a sustainable pathway.

Small settlements offer unique challenges in terms of facilitating the necessary alterations to existing travel habits. The foundation from which to develop sustainable transport strategies is frequently non-existent in these settlements. For example, Freshford is poorly serviced by public transport. A bus leaves the village square just twice daily for Kilkenny city, and other nearby settlements are not serviced. Observations of the bus occupancy rate during surveying periods revealed passenger numbers of less than 10 persons on all occasions. Lack of choice in departure times may be a contributing factor in the poor uptake of public transport in the settlement. Conversely, low passenger numbers, such as those observed, most likely do not encourage transport companies to offer extensions to their services. As a result there appears to be a significant reliance on the use of the private car to facilitate journeys to employment, recreational and shopping services. Combined with an observed occupancy rate of just 1.18 (for travel to work) the large footprint can be classified as being a reflection of unsustainable transport methods.

In addition to transport by bus, other more sustainable methods include walking and cycling. However, it is logical to assume that to increase uptake of these transport modes services such as employment, shopping, recreation and education would have to be located in or very close to Freshford. Survey results indicated that only 19% of workers find employment in Freshford, 45% travel to Kilkenny with the remaining 36% travelling to other locations. The population of Freshford has undergone a 19.6% increase in the period from 1996 to 2002, from 632 to 756 (CSO 2003, p27). The current level of services in Freshford appears to be incapable of wholly supporting the increasing population. The infrastructure in the village has not

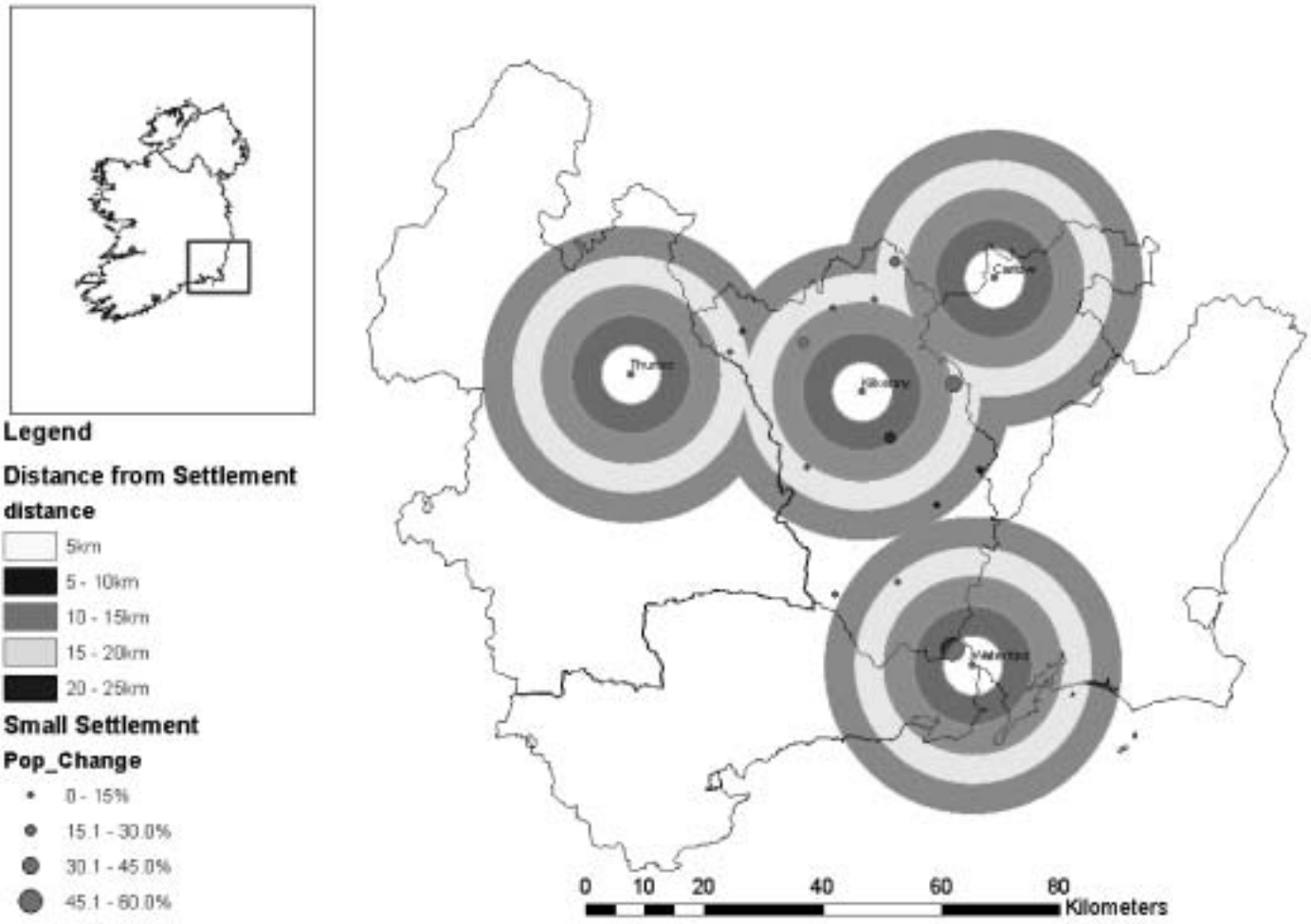


Fig. 4 – Population change of small settlements in relation to proximity to large settlements.

developed in tandem with the population. People are forced, and in some cases perhaps choose, to leave the settlement to avail of educational, shopping, recreational and employment services. While not every service can be catered for within the settlement, such as secondary schooling or a cinema for recreation, emphasis should be placed on existing services and locals encouraged to use these. For example, grocery shops in the settlement may find it difficult to compete with larger supermarkets so instead perhaps their focus should be in offering alternatives, such as organic local products. The high proportion of residents leaving the village on a daily basis is believed to have contributed to an "empty nest syndrome" according to Linda Tallis, Administrator, Freshford 2020 Development Group (27/04/05). Its proximity to Kilkenny makes it an attractive location to live in with the result that a large number of people are commuting to Kilkenny for work, leaving the village in the morning and not returning until evening or night time and as such are not contributing to the vitality of the village on a day to day basis. It is likely that Freshford is not alone in experiencing this phenomenon and this suggests that several small settlements throughout Ireland may have similarly large personal transport footprints. Fig. 4 represents the positive population changes of small settlements within Co. Kilkenny from 1996 to 2002. It is evident that settlements located within a 15km radius of larger settlements experienced the most significant population changes, most notably Slieverue, located 5km from Waterford, which experienced a population increase of 55%. Research such as this reveals the environmental cost of current lifestyles and exposes areas where change is necessary to bring about a reduction in environmental impact. In the national drive towards sustainable development, the personal transport of small settlements is undoubtedly a blackspot. Further research in this area will examine the potential pathways open to small settlements in reversing the unsustainable trend in personal transport.

CONCLUSIONS

The current trends of personal transport in the small settlement of Freshford indicate unsustainable patterns of resource use are facilitating the residents' requirements. Contributing factors include the location of services such as employment, shopping and recreational services and the transport modes with which these services are accessed. The implications of these findings, especially if mirrored in other small settlements nationwide, represent a challenge for policymakers to combat the unsustainable patterns of personal transport emerging from small settlements like Freshford.

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THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON PROMOTING DISINFECTANT-MEDIATED RESISTANCE TO ANTIBIOTICS IN *PSEUDOMONAS AERUGINOSA*

Paul H. McCay and Gerard T.A. Fleming

ABSTRACT

There is increasing concern that the ever-increasing use of disinfectants in domiciliary, industrial and clinical settings may promote cross-resistance to antibiotics in common pathogens such as *Pseudomonas aeruginosa*. As such, the misuse or overuse of biocides may select for disinfectant and antibiotic-resistant microorganisms. In this study *Pseudomonas aeruginosa* PAO1 was grown in chemostat cultures to which the disinfectant Benzalkonium chloride (BKC) was added at increasing concentrations. Long-term cultures (>720h) were carried out in complex (CM) and minimal (MM: glucose or magnesium-limited) medium. BKC-adapted strains were selected in the magnesium-limited chemostat which showed resistance to 700mg l^{-1} BKC. The original strain had an intrinsic resistance of <12-15mg l^{-1} for BKC. Chemostats which employed CM exhibited populations with lesser degrees of adaptation (300–500mg l^{-1}) BKC. There was an overall decrease in the level of resistance to fluroquinolone antibiotics (ciprofloxacin and levofloxacin) in chemostats operated with CM and BKC selection pressure. Sensitivity to tobramycin evolved in chemostat populations (CM) in response to BKC selection pressure. No significant changes in antibiotic or BKC resistance were found for populations unchallenged with the biocide. Limitation by glucose or magnesium with MM did not elicit significant changes in antibiotic resistance profiles.

Key words: *Pseudomonas aeruginosa*; chemostat culture; benzalkonium chloride; cross-resistance; disinfectant; antibiotic

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INTRODUCTION

Bacterial resistance to disinfectants is currently topical in the scientific literature. The increase in use of biocides in domiciliary, industrial and clinical settings has led to concern regarding the development of bacterial strains showing increased resistance to key disinfectants (Loughlin *et al*. 2002). It has recently been argued that bacteria which acquire increased tolerance for disinfectants may also exhibit elevated resistance to antibiotics (Fraise 2002). The premise underlying this assumption is that both disinfectant and antibiotic resistances are often mediated by analogous cell-mediated mechanisms (Gilbert *et al.* 2002).

Do biocides co-promote resistance to antibiotics? Strains showing resistance to biocides are traditionally generated using simple batch cultures with disinfectant selection pressures. Findings are often inconclusive (Joynson *et al*. 2002; Loughlin *et al.* 2002).

In this study, *Pseudomonas aeruginosa* was used as a model system to determine if selection for increased tolerance to the disinfectant Benzalkonium chloride (BKC) was concomitant with the evolution of widespread antibiotic resistance (Akimitsu *et al.* 1999). It has been shown that nutrient availability will affect the ability of a bacterial culture to develop resistance to antibiotics or disinfectant. Complex medium (CM) or minimal medium (MM) were employed throughout the chemostat operation.

MATERIALS AND METHODS

Bacterial Strains, Media and Growth Conditions

Pseudomonas aeruginosa PA01 was obtained from the National Collections of Industrial Food and Marine Bacteria (NCIMB). PAO1 was routinely grown in nutrient broth (Oxoid: Oxoid Ltd, Basingstoke, Hampshire, UK), a complex medium (CM) whose limiting nutrient is unknown or M9 minimal medium (Molecular Cloning 2nd Edition, Cold Spring Laboratory 1982), as a minimal medium (MM). Cultures were grown as shake flask cultures (100ml in 500ml Erlenmeyer flasks) with shaking (140 rpm) at 37°C. M9 was routinely supplemented with 60g l^{-1} carbon (in the form of glucose) or 30g l^{-1} magnesium (in the form of $MgSO_4$) to achieve differing limiting nutrients (Goldberg and Er-el 1981).

Three sets of antibiotics susceptibility discs (Oxoid Ltd., Basingstoke, Hampshire, England) were chosen for this study: Levofloxacin, Ciprofloxacin and Tobramycin. Antibiogram analysis, Total Viable Counts (TVCs) and Minimum Inhibitory Concentrations (MICs) were performed following National Committee for Clinical Laboratory Science (NCCLS) guidelines using antibiotic susceptibility discs (Oxoid Ltd., Basingstoke, Hampshire, England). Benzalkonium chloride (BKC: Sigma Aldrich) was dissolved in deionised water and filtered through a 0.2µm filter (Millipore) before use.

Selective and non-selective chemostat culture

The test organism was grown in continuous (chemostat) cultures (described previously by Fleming *et al*. 1988) at sub-maximal growth rates using nutrient-rich media, CM, and nutrient-poor media, MM. Conditions in the chemostat reflect those of the natural environment to a greater extent than simple batch cultures (Fleming *et al.* 2002).

Chemostats were challenged with increasing levels of BKC (0 up to 700 mg l^{-1}) or were operated without selection pressure. Culture populations were allowed to evolve in the reactors over periods of 25–57 days. Samples taken from the chemostats at intervals were tested for their levels of resistance to BKC and antibiotics. Reactors were operated at a dilution rate of 0.04 hr^{-1} for at least 700h. Chemostats populations were routinely sampled on a daily basis. Total

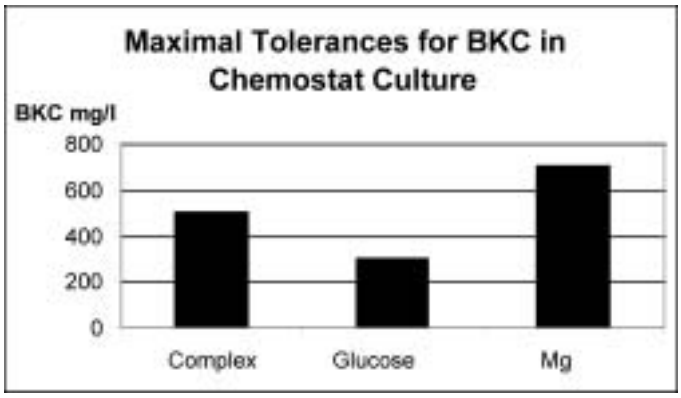

Fig. 1 – Maximal Tolerance for BKC in Chemostat Culture. PAO1 was grown in CM or MM (glucose or carbon limited) at a dilution rate of 0.04 hr^{-1}.

Viable Counts, antibiogram analysis and MICs for BKC and antibiotics were performed on these samples.

RESULTS

Evolution of Resistance to BKC

The maximal tolerances for BKC by chemostat populations at the termination of the cultures are shown in Fig. 1. The original strain had an intrinsic tolerance of <12mg l^{-1} BKC. Chemostat culture without BKC selection pressure did not yield BKC-adapted populations (not shown).

Evolution of Resistance Profiles to Antibiotics

BKC-mediated cross-resistance to fluroquinolone (levofloxacin and ciprofloxacin) and tobramycin (cell wall-active antibiotic) was examined for chemostat-derived populations grown with or without BKC selection pressures. Results are shown in Figs. 2 and 3. Zone of inhibition size (in mm) was used as an index of the degree of cross-adaptation to antibiotics.

It is evident from Figs. 2 and 3 that populations evolved in the BKC-supplemented chemostats show altered degrees of resistance to antibiotics. Reactors operated without BKC did not show significant differences in antibiogram patterns from that of the original strain (not shown).

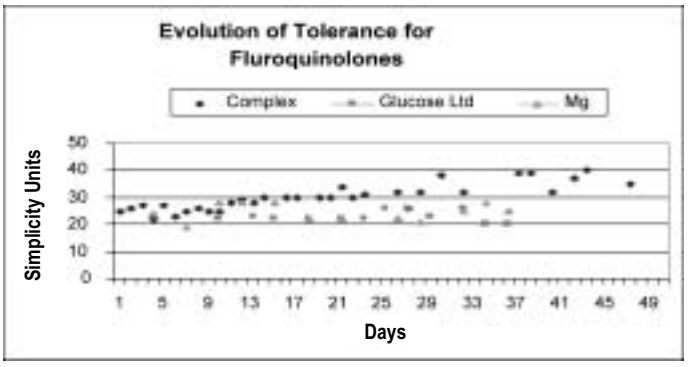

Fig. 2 – Zones of Inhibition from Chemostat samples to fluroquinolone antibiotics.

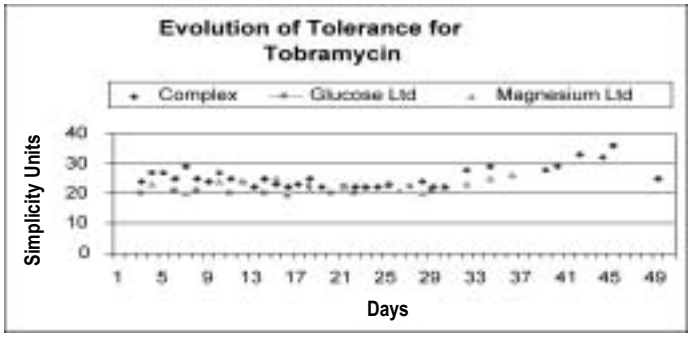

Fig. 3 – Zones of Inhibition from Chemostat Samples to tobramycin.

DISCUSSION

The controversy surrounding disinfectant-mediated cross-resistance to antibiotics has not been tested in culture systems that mimic the natural environment. Growth in chemostats is always substrate-limited and sub-maximal. Furthermore, bacteria will evolve resistance in chemostat culture in response to a selection agent such as a biocide (Dykhuizen and Dean 2004). Simple serial batch-culture studies employing complex have shown that resistant populations of PA01 could evolve in response to BKC selection pressure (Loughlin *et al.* 2002; Joynson *et al.* 2002) Increased levels of resistance some 10-15 fold were reported in that study. Results presented in Fig. 1 demonstrate the evolutionary capacity of PA01, whereby populations evolved towards the end of the chemostat fermentations which possessed increased levels of resistance compared with the original strain. The magnitude of the increase was greatest for magnesium-limited cultures, 700mg l^{-1}, some 30-35 fold increase in its MIC compared to the original strain of <20mg l^{-1}. It is known that magnesium is essential for maintaining cell wall integrity (Vaara 1992). Reducing magnesium availability is believed to disrupt the cell wall and thus potentates the action of the disinfectant through increased permeability. Commercial preparations of BKC (e.g. Dettol® fresh) include EDTA for this purpose. Results presented here clearly demonstrate that PA01 not only survives with increasing levels of BKC but population could adapt to a resistant phenotype under magnesium-limiting conditions. The magnitude of developed resistance was smallest in the glucose-limited culture (Figure 1). This culture was energy-limited and there was possibly less cellular ATP to run efflux pumps that are known to play a role in excluding BKC from the cells (which normally give rise to resistance characteristics).

It would appear from Figs. 2 and 3 that selection for BKC-resistant clones can yield populations with altered antibiotic susceptibilities. Interestingly, there was an overall decrease in the level of antibiotic resistance when PA01 was grown in MM. The evolution of resistance to fluroquinolones or tobramycin in BKC-rich environments appears neither to be energetically efficient or of competitive advantage to mutants of PA01 when grown in complex medium. The evolution of resistance to BKC does not seem to be concomitant with increased resistance to the antibiotics chosen for this study.

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VARIATIONS IN THE SOIL NUTRIENT PROPERTIES OF TEMPORARY LIMESTONE LAKES (TURLOUGHS) LOCATED WITHIN TWO CONTRASTING CATCHMENT AREAS IN THE WEST OF IRELAND

Sarah Kimberley and Stephen Waldren

ABSTRACT

Turlough basins are geological depressions in karst regions that are periodically inundated in response to the local groundwater system and lack a surface outflow. Ireland possesses the greatest global density of turlough basins. This restricted range and their ecological interest led to designation as a priority habitat under the Annex I of the EU Habitats Directive (1992). This study addresses the current paucity of information relating to turlough nutrient dynamics by focusing on hydrologically linked basins within two catchment systems of contrasting trophic status. Soil nutrient characteristics were described within eight turlough basins, four located in the Coole-Garryland Complex, Co. Galway, and four located in the East Burren Complex, Co. Clare. Results indicated that there is a high degree of variation in soil nutrient status between the two catchment areas. Peat soils were identified as being dominant in the East Burren Complex whereas gley soils were dominant in Coole-Garryland Complex. The nutrient variables delineating basins from the two catchments were pH, total P, total N, total K, total Fe and total Mg. An alkaline pH, elevated nitrogen levels and distinctly low potassium levels were associated with the peats, whereas the gley soils had more acidic properties and elevated iron concentrations. Variations in the soil nutrient properties of turloughs located within the two contrasting catchment areas are significant and would be expected to impact on the establishment of different vegetation types.

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INTRODUCTION

Turloughs or temporary limestone lakes may be described as geological depressions in karst areas, which flood periodically and lack a surface outflow (Goodwillie 1992). Turlough ecosystem function requires an infrequently occurring combination of low-lying limestone and wet climate, and the greatest global density of this habitat type is found in central and western Ireland (Coxon 1987).

This restricted geographic range has led to turlough designation as a priority habitat under Annex 1 of the EU Habitats Directive. Turlough vegetation is a focus of ecological interest and comprises distinctive zones reflecting the varying water levels, where wetland plant species often occur in close proximity to species-rich, calcareous grassland. Goodwillie (2001) highlighted a lack of comprehensive, consolidated soil nutrient information necessary for understanding soil-plant community relationships and for monitoring changes in turlough nutrient loading. This information is fundamental for the adequate protection and management of this priority habitat as previous research has shown that plant community diversity in such temporary wetlands is commonly limited by nutrient availability (Verhoven *et al*. 1996).

Turloughs are highly variable in relation to area, depth, groundwater connections and inundation patterns. Basins range in area from 10ha to 280ha and each basin has a unique topography and associated flooding regime. Consequently, turlough soils are complex and highly variable in their origin and distribution (MacGowran 1985; Coxon 1986) and this variability encourages the establishment of different vegetation types having different requirements and tolerances for nutrient availability, draining properties and presence of toxic substances (Goodwillie 1992). The main turlough soil types noted are organic rendzinas, marl, peat, river silts, gleys, non-calcareous drift and calcareous drift (MacGowran 1985).

Stimulated by the considerable challenges this natural variation poses for habitat management, efforts have been made to group turloughs according to their hydrological patterns and physical features. Turloughs are pulsing systems (Junk *et al.* 1989), where flooding is the primary regulator of productivity. Catchment areas are useful subdivisions of physiographic regions that group turloughs linked by hydrological processes (Robertson *et al.* 1999). The majority of the 102 turloughs designated as SACs (Special Areas of Conservation) and NHAs (Natural Heritage Areas) in Ireland occur across 14 river catchment areas. Such a catchment-based approach allows basins under similar environmental influences and nutrient loading to be grouped together (Table 1.).

The important hydrological factors, other than water-level fluctuations, influencing turloughs include the chemical characteristics and nutrient content of the floodwater, each of which are related to catchment size, type and land-use. The main importance of catchment area is that it controls the volume and quality of water passing through a turlough basin (Goodwillie 2001). Hard-water inputs to turloughs may result in the deposition of carbonates (Coxon 1994), the presence of which in soil can negatively influence plant phosphorus availability (Tisdale *et al*. 1990), resulting in a naturally oligotrophic environment. Turlough trophic status is often influenced by land management practices beyond the basin boundary and eutrophication of a site can result from anthropogenically enriched floodwater (Goodwillie 2001).

This highlights the fact that turlough soil type is influenced by a number of catchment factors. Bedrock, basin type and flooding regime are major factors influencing

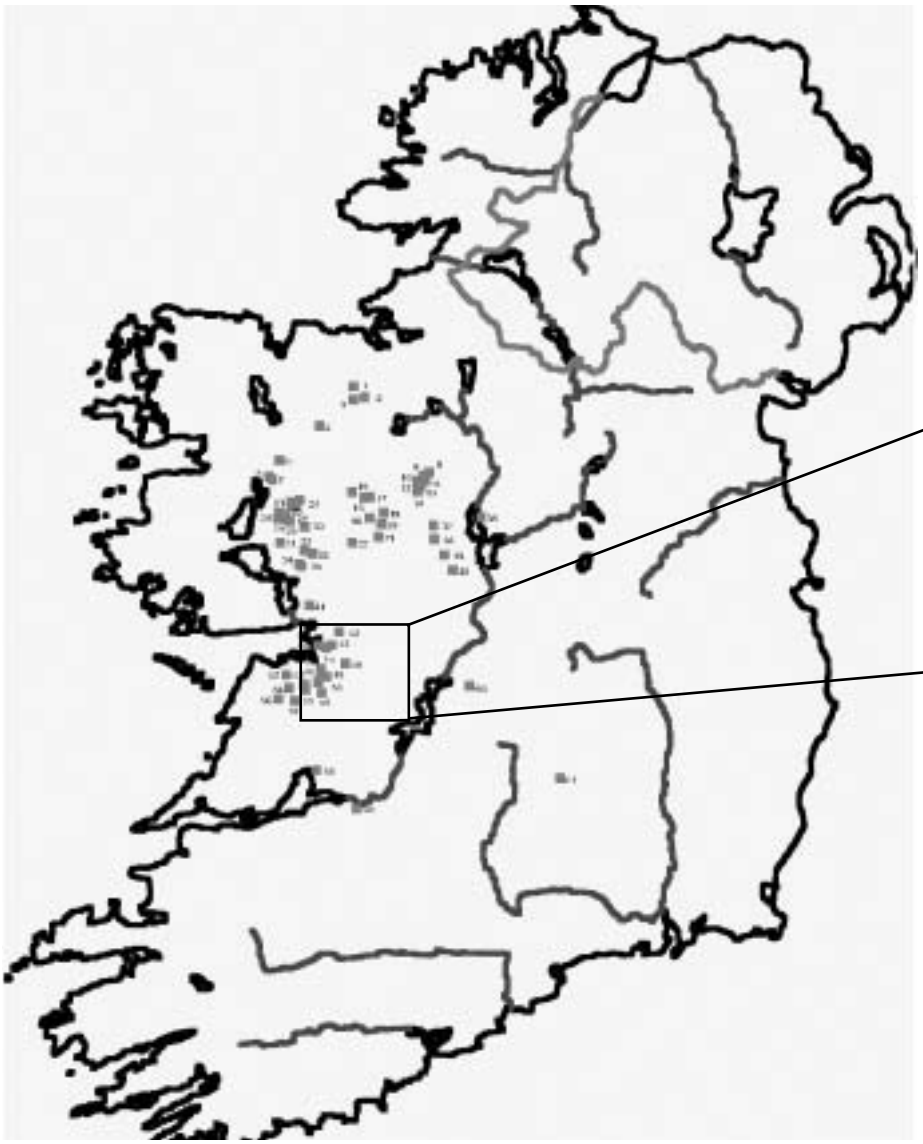

Fig. 1 – Map showing the national distribution of turloughs greater than 10 ha (Coxon, 1986) and the location of study sites. Irish Grid References are also provided.

the general types and combinations of soils that will develop in a basin. The aim of this study is to assess the variations in basic soil nutrient characteristics of soil types associated with two catchment areas under contrasting incidence of nutrient loading and soil development. Such soil nutrient investigations are potentially a tracking device for monitoring changes in turlough trophic status and provide an initial step in assessing whether soil nutrient variation is a significant driver of turlough vegetation diversity.

SITE DESCRIPTIONS

Site selection

Two alternative complexes of hydrologically linked basins with SAC status were selected based on their location within catchment areas of contrasting trophic condition.

East Burren Complex, Co. Clare (SAC Site Code 1926)

This SAC covers 18,800 hectares of the Burren region in north Clare and a small part of southwest Galway (Fig. 1). The area encompasses a complete range of limestone habitats that include the largest expanse of limestone pavement in the country, and includes one of the most important and extensive examples of a low-nutrient wetland system (Burrenbeo 2005). There are eight turloughs within the site and the four basins chosen for this study occur within the Fergus River system catchment with an area of 348ha (Goodwillie 1992). Turloughs from this catchment have significant amounts of bedrock exposed in the basin, while marl accumulation is common either in the present or in the past (Coxon 1986). The turloughs within this catchment are oligotrophic as the basins themselves are very lightly grazed and land-use within the surrounding catchment area is moderate. Goodwillie (1992) noted that Knockaunroe is a basin of international importance and is likely to be extremely sensitive to nutrient enrichment (unpublished data EPA Turlough Ecology Working Group 2004). The basins in this catchment are fed almost entirely by nutrient-poor, alkaline groundwater where nutrients are held in less-available form.

According to the General Soil Map (Gardiner & Radford 1980) the parent material of the East Burren Complex is composed mainly of limestone. The principal soil type is rendzinas with outcropping rock and the associated soil types are lithosols and shallow brown earths.

Coole-Garryland Complex, Co. Galway (SAC Site Code 252)

This SAC is situated in a low-lying karstic limestone area west of Gort, County Galway (Fig. 1). The estimated catchment area is 32,437ha and the turloughs in this catchment occur on the Gort River system. It contains a series of hydrologically linked turloughs, which are fed by springs and a partly submerged river, surrounded by woodland, pasture

Table 1 – Classification of turloughs according to inflow type and catchment type (Goodwillie 2003).

Type I Fluctuations in level large, >5m		% of active sites
1A Riverine turlough with large throughput of water	– catchment mixed	8
1B	– catchment limestone	4
Type II fluctuations in level, moderate (2-5m)		
2A Basin retaining water, i.e. partly below summer watertable	– on drift	18
2B	– on rock	11
2C	– with widespread peat development	6
2D Basin drying out, above summer watertable	– on drift – eutrophic	26
2E	– on drift – mesotrophic	19
2F	– on rock – oligotrophic	8

and limestone heath (Burrenbeo 2005). The basins located within this catchment are generally mesotrophic or eutrophic as they are often moderately to heavily grazed, surrounded by improved pasture and receive iron-rich waters from the Slieve Aughty mountains (Goodwillie 2001). Coole and Caherglassaun are of international importance and are likely to be moderately sensitive to enrichment (unpublished data EPA Turlough Ecology Working Group 2004). Both basins lie in the bed of an underground river and receive a high load of suspended matter and dissolved nutrients (Goodwillie 2001). According to the General Soil Map (Gardiner & Radford 1980) parent material of this catchment area is limestone till, shallow in places. The principal soil types are shallow brown earths and rendzinas and the associated soil types are grey brown podzolics, gleys and peats.

MATERIALS AND METHODS

Sampling

Sample collection was carried out during the turlough drained-period between April and August 2003. Soils were collected from each basin along obvious flooding gradients and from the main vegetation zones. At each sampling point, approximately 15 cores were taken to a depth of 10cm from a $1m^2$ quadrat and bulked to provide a representative, composite sample. Where soil was collected, vegetation was recorded using DOMIN scores (Kent & Coker 1992). Samples returned to the Centre for the Environment, TCD Laboratories, were oven-dried at 35°C for 24 hours, ground, sieved (2mm mesh) and stored at room temperature.

Nutrient analyses

All samples were analysed for desorpable phosphorus (P_{feo}), total nitrogen (N_t), total phosphorus (P_t), total potassium (K_t), total magnesium (Mg_t), total iron (Fe_t) and total calcium Ca_t. Phosphorus desorption to solution was determined by the iron-oxide paper strip test (Menon *et al*. 1988), using a solution soil ratio of 40:1 with 0.01 M $CaCl_2$ and one paper strip over a run time of 16 hours.

N_t was measured using a Leco elemental analyser CNS 1000. P_t, K_t, Mg_t and Fe_t were analysed following nitric acid digestion using a MDS 2000 microwave. P_t was determined colorimetrically by a phosphomolybdate blue method (Murphy & Riley 1962). K_t, Mg_t and Fe_t were analysed for by flame atomic absorption on a Perkin Elmer Spectrometer (model 3100).

Table 2 – Principal soil types in the East Burren Complex and the Coole-Garryland complex.

Soil type	*% OM*	*East Burren*	*Coole-Garryland*
Organic rendzinas, podzolics	<12%	n=2	n=1
Gleys	12.1-20%	n=4	n=20
Peaty gleys, peaty podzols	20.1-30%	n=6	n=9
Peats	>30%	n=16	n=1

Non-nutrient analyses

Organic matter (OM) was measured as a percentage weight loss following ignition at 550°C. Carbonate content was estimated as a percentage weight loss following further ignition at 1000°C. The bulk density of the samples was estimated from % organic matter by loss on ignition using the method of Jeffrey (1970). The pH of soils was estimated with a glass-calomel electrode. Soil colour was determined using Munsell Soil Colour Charts. Estimates of soil texture were made using hand textural analyses (Ball 1986). Broad soil groups were determined using colour, texture and organic matter content (Finch 1971; Daly *et al.* 2001).

Data treatment

Laboratory analysis of samples was carried out in duplicate and an average value calculated. All soil nutrient data were expressed in mg l^{-1} soil using the estimated soil bulk densities. Statistical analysis was carried out on soil data collated from both catchments in Datadesk® 6.0 using one-way nested analysis of variance, with turloughs or soil type nested within catchment. The data were transformed for normality when necessary. The significance of soil nutrient differences are noted by symbols ** and *** for significant differences of $P < 0.01$, 0.001 respectively. Eigen values for the PCA analysis are reported in brackets.

RESULTS

To examine the differences in soil nutrient status between the two catchment areas, the soil data were subdivided into four broad soil groups, according to organic matter content and principal soil type. Peats (n=16) are the most common soil type in turloughs from the East Burren Complex whereas Gley (n=20) soils dominate in turloughs from the Coole-Garryland Complex (Table 2.). In general the vegetation types associated with peats in the East Burren Complex are sedge fen and species-poor grassland whereas species-rich grassland is generally associated with the gleys of the Coole-Garryland Complex. These peats and gley soils were investigated for differences in soil nutrient status (Table 3).

Summary statistics of the soil nutrient results for both the peats and gley soils are presented in Table 3. ANOVA indicated that some of the soil characteristics associated with each of the principal soil types showed statistically significant differences. The pH of the peats from the East Burren Complex ranged from neutral to strongly alkaline with

Table 3 – Summary statistics of soil nutrient characteristics and analysis of variance for the most common soil types in the Burren Lowlands (Peats) and the Gort/Garryland Complex (Gleys) (ns = not significant, ** = P<0.01, *** = P<0.001)**.**

	pH	*% OM*	*% $CaCO_3$*	*P_t*	*P_{feo}*	*N_t*	*K_t*	*Fe_t*	*Mg_t*
Peats (n = 16) East Burren Complex									
	***	***	*ns*	**	*ns*	***	***	***	***
Mean	7.7	50.3	9.8	308	11.6	6779	814	2364	565
Median	7.6	38.9	5.3	257	11.1	6919	780	1859	572
Min	7.0	30.3	4.1	73	0.25	4178	45	690	90
Max	8.5	86.4	36.9	642	17.8	8363	2589	5050	1083
Gleys (n = 20) Coole-Garryland									
Mean	6.4	15.9	5.8	466	13.6	4646	5575	18141	2429
Median	6.3	15.2	5.8	431	15.5	4523	5248	17262	2444
Min	5.5	12.4	3.3	307	2.9	2424	1444	8210	714
Max	7.8	26.6	10.3	725	25.5	6503	9050	30535	5139

a mean of 7.7 (moderately alkaline). In contrast, the pH of the gleys from the Coole-Garryland Complex ranged from moderately acid to moderately alkaline with a mean of 6.4 (slightly acid) (Finch 1971). Significant differences were not noted between the calcium carbonate (% $CaCO_3$) content of the soils but a wide range of values in the Burren were noted (4.1%–39.9%) indicating that some areas of the Burren have elevated carbonate levels. Generally, the elevated levels of $CaCO_3$ were associated with soils with a pH above 8.

The P_t status of Peat soils from the East Burren Complex ranged from 73-642 mg l^{-1} with a mean of 308 mg l^{-1}. Significantly higher concentrations were noted in the Coole-Garryland Complex where P_t values ranged from 307-725 mg l^{-1} with a mean of 466 mg l^{-1}. Significantly higher concentrations of N_t were noted in the Peats of the East Burren Complex whereas significantly higher concentrations of Fe_t, K_t and Mg_t were recorded in the Gley soils of the Coole-Garryland complex. P_{feo} values in the East Burren Complex ranged from 0.25 to 17.8 mg l^{-1} and were of a similar magnitude as P_{feo} values in the Coole-Garryland complex.

PCA ordination of all soil nutrient variables is presented in Fig. 2. The main aim of this analysis was to determine any consistent differences between and within catchments. The triangles and diamonds represent oligotrophic basins from the East Burren Complex clustering to the right whereas the more mesotrophic basins from the Coole-Garryland Complex are clustered to the left on Axis 1. Total iron (Fe_t) (*-0.424*), total potassium (K_t) (*-0.401*), total magnesium (Mg_t) (*-0.390*) and total phosphorus (P_t) (*-0.327*) are the main soil nutrient properties delineating basins from contrasting catchments along Axis 1. ANOVA indicated that Fe_t (p=0.0013), K_t (p=0.0035) and Mg_t (p=0.0001) show highly significant between catchment variation (Table 3).

The outlying basins (denoted by*) in Fig. 2, located within the Coole-Garryland complex, represent sampling points located within the lowest basin areas in Hawkhill, Coole and Caherglassaun. This suggests that the basin floors of the turloughs located within this catchment, which remain flooded for longer periods, have a distinctly different soil nutrient status than the rest of the basin area. PCA analysis showed that within-catchment variation along Axis 2 is primarily influenced by pH (*-0.434*), % $CaCO_3$ (*-0.507*) and Ca_t (*-0.479*), most notably in the East Burren Complex.

DISCUSSION

The results from this work indicate that turlough soils from both catchments are generally organic rather than mineral, most likely due to the fact that the basins are flooded for approximately six months of the year. The decomposition of organic matter in a submerged soil proceeds at a slower rate than in a well-drained soil (Ponnamperuma 1972) leading to the accumulation of plant residues (Degens 1965). The physiographic nature of the basin type in both catchments leads to the differences in the occurrence of soil type. Soils with elevated levels of organic matter predominate in the East Burren Complex as basins within this catchment tend to be flat, shallow and remain wet, facilitating the development of peaty soils. Turlough basins in the Coole-Garryland Complex have a more undulating topography and tend to dry out completely during the summer months, leading to the development of gley soil types (Goodwillie 1992).

Peat soils are generally acidic but the pH range associated with the peats was noted as between 7.0-8.5. This alkaline pH range indicates that they are more similar to fen peats, which are formed under the influence of base-rich ground water. They are composed mainly of the remains of reeds, sedges and other semi-aquatic plants (Finch 1971).

Within-catchment variation in the East Burren Complex was likely to be driven by pH fluctuations (Figure 2). The wide ranges of pH and $CaCO_3$ levels result from the accumulation of gastropods and

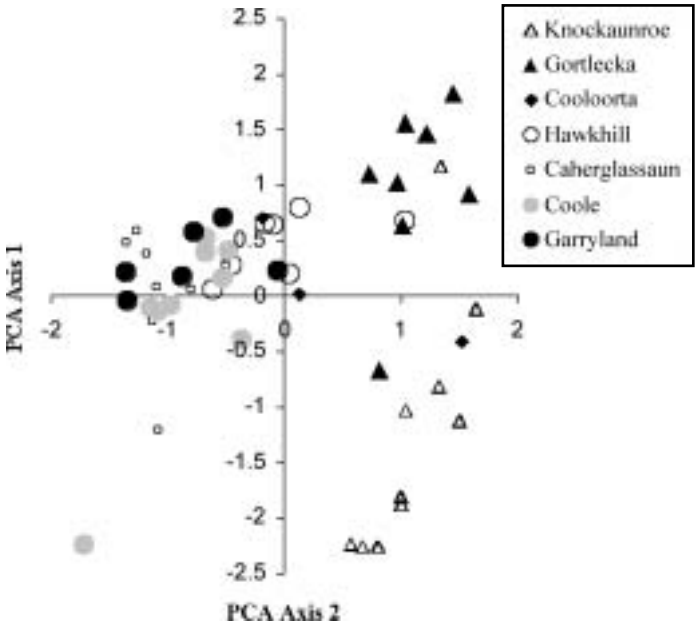

Fig. 2 – PCA ordination of soil data generated from soil samples collected from both catchments. 55.3 % of variation in the data set is accounted for by Axis 1 (34.9) and Axis 2 (20.4).

freshwater molluscs associated with calcium-rich lakes and carbonate deposition from inflowing alkaline groundwater (Coxon 1994).

The gleys of the Coole-Garryland Complex are referred to as ground-water gleys as the gley condition results from a high water-table (Finch 1971). The Coole-Garryland gleys have a similar pH range to the gley soil series – alkaline parent material phase recorded by the National Soil Survey of Ireland (1971). The range of $CaCO_3$ levels in these soils is much narrower than that in the peats, indicating that shell marl accumulation and carbonate deposition are not characteristic of this soil type.

The results for total phosphorus from both catchments were compared with data (Daly *et al.* 2001) on the soil phosphate content of various Irish grassland soil types. The range of P_t and P_{feo} values of the turlough peat soils are similar to the ranges noted by Daly *et al*. (2001) for the same soil type, which are comparatively low when regarded in the context of other general Irish soil types. The ranges for P_t and P_{feo} noted in the turlough gley group are much lower than those recorded for Irish gleys in general. The same authors also noted that high organic matter soils have lower P sorption capacities than mineral soils and are vulnerable to environmentally significant P losses. This information suggests that the phosphorus status of both turlough soil types is low, indicating that they may be experiencing considerable P deficiency and that P may be severely limiting to the sward by restricted availability.

The significantly higher concentrations of total nitrogen in the peat soils is in agreement with previous research as it has been shown that the accumulation of soil nitrogen closely follows that of soil organic matter (Haynes 1986). Generally, the range of soil nitrogen values are very similar to Burren grassland soils (O'Donovan 1987). It must be noted however that the studies conducted by O'Donovan (1987) indicated that the levels of total nitrogen fluctuated markedly with seasonal changes.

Typical values of total potassium were noted in the gley soil type whereas extremely low values were noted in the peat soils. Keddy (2000) noted that potassium may be a limiting nutrient in wetland environments as, being a highly mobile ion (Grootjans *et al.* 1986), it may be washed out of these systems. As the peats remain wet during the summer period, the low potassium levels may indicate that losses of this cation occur via leaching.

The elevated iron levels in the Coole-Garryland Complex are most likely due to the fact that basins within this catchment receive iron-rich waters from the Slieve Aughty mountains and are drift-based rather than rock-based, leading to the development of iron-rich soils.

In summary, the turlough basins located within each of the contrasting catchment areas have different geomorphological features, which have led to the development of two distinctive soil types. The variations identified by this study in soil nutrient properties associated with the two soil types are likely to impact on the vegetation types which develop in the two catchment areas.

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TROPHIC STATUS AND PHOSPHORUS DYNAMICS IN EXPERIMENTAL CUTAWAY PEATLAND LAKES

Tara Higgins and Emer Colleran

ABSTRACT

Lake creation represents an increasingly important post-harvesting land-use option for cutaway peatlands in Ireland. The current research assesses the trophic status of four experimental cutaway lakes in Co. Offaly, with particular focus on phosphorus dynamics. Results indicated that many cutaway lakes exhibit elevated total phosphorus levels and, concurrently, support high phytoplankton growth rates. Abiotic and biotic processes involved in the regeneration of inorganic phosphate from refractory organic phosphorus compounds were found to play an important role in maintaining a constant supply of available phosphorus to phytoplankton communities in cutaway lakes.

Key words: cutaway peatland, phosphate regeneration, UV labile phosphate, phosphatase enzymes

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INTRODUCTION

By 2030, over 80,000 hectares of industrially-milled peatland will be redundant in Ireland. Bord na Móna estimates that more than 50% of this will be designated as non-commercial semi-natural wilderness areas, encompassing 15,000-20,000 hectares of shallow lakes and wetlands (Egan 1999). A series of experimental lakes has already been created within a 2,000-hectare cutaway site in Co. Offaly, called the Lough Boora Parklands. The current research sought to investigate the trophic status of four of these cutaway lakes and to understand the complex physico-chemical and biological processes operating in these essentially artificial ecosystems. A particular focus of the research was to evaluate the occurrence and significance in cutaway lakes of alternative abiotic and biotic mechanisms involved in the regeneration of bioavailable phosphate from the dissolved organic phosphorus pool.

MATERIALS AND METHODS

Four selected cutaway lakes, Finnamore, Tumduff, Turraun and Clongawny, were sampled over the three-year period between August 2001 and September 2004, at two-week intervals for the first year and monthly thereafter. For detailed descriptions of the study lakes, see Higgins & Colleran (2004) and Higgins (2005). The chlorophyll *a* concentration of near surface (1m) water samples from each lake was determined using the spectrophotometic method described by Burnison (1980), involving acetone-DMSO (1:1v/v) extraction. Concentrations of various phosphorus fractions were determined using the procedure of Murphy & Riley (1962), involving persulphate digestion for total phosphorus (TP) and total soluble phosphorus (TSP) analyses. Filtered water samples (GF/C filter, 1.2 µm pore size) were used for the measurement of soluble reactive phosphorus (SRP) and TSP, while unfiltered samples were used for TP determination.

On seven sampling occasions during 2004-05 (2/06/04, 30/06/04, 14/07/04, 6/08/04, 26/08/04, 28/09/04 and 26/01/05), filtered water samples from each cutaway lake were analysed for their response to two alternative phosphate regeneration mechanisms: UV-induced phosphate release and enzymatically hydrolysable phosphate release. UV-induced phosphate (P-UV) release was measured using modifications of the methods described by Francko & Heath (1982) and Cotner & Heath (1990). Duplicate samples from each lake were irradiated for 4h using UV radiation of similar strength to mild solar radiation in Ireland (0.9 mW cm^{-2} at 365nm). The difference between SRP concentrations in the UV irradiated samples and equivalent dark controls was considered as the P-UV fraction of the soluble phosphorus pool. The enzymatically hydrolysable phosphorus fraction (P-EH) was determined on cutaway lake samples using the method described by Chrost *et al.* (1986). One litre of filtered (GF/C) water samples from each lake were placed in 1-litre sterile flasks, to which was added 10 ml of 1.0 M tris buffer and 50 ml of pure chloroform. Samples were shaken well, to ensure sterility, and incubated at 25°C for five days to promote the action of existing extracellular phosphatase enzymes. After incubation, samples were analysed immediately for SRP. According to this method, any increase in SRP after incubation, termed P-EH, is due to free microbially-produced dissolved phosphatases (Bradford & Peters 1987; Chrost *et al.* 1986).

RESULTS

The data presented in Table 1 highlight the contrasting trophic statuses of the four cutaway peatland lakes. Both Finnamore and Tumduff were mesotrophic lakes, Turraun was a meso-eutrophic lake, while Clongawny, was a eutrophic-hypereutrophic lake. Mean SRP levels in the four lakes were consistently very low (<1.6µg l^{-1}), while soluble organic phosphorus (SOP) were moderately high. The very large TP pool in Clongawny was dominated by particulate phosphorus, indicating a high concentration of planktonic organisms in this lake.

Table 2 presents the results of the two phosphate regeneration mechanisms investigated. These data indicate that the phosphate regeneration mechanisms studied consistently resulted in a mean increase in SRP concentrations in the four study lakes. UV light produced the smaller change in SRP, stimulating a mean increase in SRP of 0.9µg l^{-1} across all lake samples. Release of UV-labile phosphorus was highest in Turraun (1.1µg l^{-1}) and lowest in Clongawny (0.6µg l^{-1}). The cutaway lake samples showed a greater release of SRP in response to enzymatic hydrolysis, which resulted in a mean increase in SRP concentrations of 2.6µg l^{-1}. Release of P-EH was greatest in Tumduff (3.0µg l^{-1}) and lowest in Clongawny (2.1µg l^{-1}).

Table 1 – Phosphorus and chlorophyll concentrations in the cutaway lake samples. Values shown are mean (n=54), ±standard error of the mean. (TP-total phosphorus; SRP-soluble reactive phosphorus; SOP-soluble organic phosphorus; PP-particulate phosphorus).

	Finnamore	Tumduff	Turraun	Clongawny
Chlorophyll μg l^{-1}	5.2±0.4	3.3±0.2	12.7±1.4	52.5±6.2
TP μg l^{-1}	12.2±0.5	15.6±0.5	26.7±1.5	26.7±1.5
SRP μg l^{-1}	1.9±0.2	1.6±0.2	2.3±0.2	2.7±0.3
SOP[1] μg l^{-1}	5.2±0.3	8.2±0.4	9.0±0.4	7.4±0.6
PP[2] μg l^{-1}	5.2±0.3	6.0±0.5	14.5±1.4	28.9±3.1
Trophic classification[3]	Mestrophic	Mestrophic	Mestrophic -eutrophic	Eutrophic-hypereutrophic

[1]SOP: TSP-SRP; [2]PP: TP-TSP (where TSP: total soluble phosphorus).
[3]Based on the OECD Lake Classification Scheme (Vollenweider and Kerekes 1982).

DISCUSSION

Variations in the nutrient characteristics of the four cutaway lakes largely reflected contrasting land-uses in the catchment areas. Finnamore and Tumduff were mesotrophic lakes, reflecting their isolation from external phosphorus inputs and subsequently low chorophyll *a* levels. Elevated summer phosphorus levels in Turraun, accompanied by large seasonal peaks in chlorophyll *a*, classified this lake on the boundary of the mesotrophic and eutrophic categories. The fourth study lake, Clongawny, was categorised as an eutrophic-hypereutrophic lake, a consequence of phosphorus fertilizer runoff from adjacent coniferous forestry plantations (Higgins *et al.* in press). Cutaway peatlands are highly vulnerable to nutrient leaching and the subsequent development of sudden and sustained phytoplankton blooms, due to a severe paucity of recolonist vegetation at these sites, the naturally poor chelating capacity of peat and the widespread susceptibility of industrially milled peatlands to runoff and erosion.

The phosphate regeneration experiments indicated that inorganic phosphate can potentially be released from the large dissolved organic P pool in cutaway lakes, by way of two alternative regeneration mechanisms: UV-induced cleavage, and hydrolysis by biologically produced phosphatase enzymes. The release of phosphate by enzymatic hydrolysis was found to be particularly important in the cutaway study lakes, supplementing SRP levels by 238-334%. Contrary to other reports (Jones 1972; McGarrigle & Kilmartin 1992) the occurrence of UV and enzyme-mediated phosphate regeneration did not vary significantly according to the trophic status of the cutaway lakes. Although their occurrence is often regarded as being indicative of a phosphate-deficient waterbody (Vrba *et al.* 1993), phosphate regeneration mechanisms are by no means irrelevant in productive waterbodies. Low SRP concentrations commonly occur in the photic zones of eutrophic and mesotrophic lakes during the growing season. A significant ($p<0.05$) inverse relationship between ambient SRP levels and P-EH release in Clongawny water samples (T. Higgins, unpublished data) suggests that enzymes are produced adaptively in this eutrophic-hypereutrophic lake in response to periodic depletions of SRP. It appears that the regeneration of SRP from the large internal SOP pools may significantly account for the development and prevalence of large phytoplankton populations in cutaway lakes, by maintaining a constant supply of available phosphorus in these systems.

Table 2 – Phosphate release in the four cutaway peatland lakes by the two P-regeneration mechanisms (P-UV: UV-labile phosphate; P-EH: enzymatically hydrolysable phosphate). Values shown are the mean of seven sampling dates during 2004-2005. Mean percentage changes in SRP in response to the P-regeneration treatments are shown in parenthesis.

	P-UV μg l^{-1}	**P-EH** μg l^{-1}
Finnamore	1.0 (54%)	2.5 (238%)
Tumduff	1.0 (70%)	3.0 (321%)
Turraun	1.1 (68%)	2.9 (257%)
Clongawny	0.6 (151%)	2.1 (334%)
All lakes	**0.9 (121%)**	**2.6 (362%)**

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