Appendix 3

PEDAL2 Faecal Indicator Organisms (FIO) Expert Workshop Report

Lancaster Environment Centre 25th & 26th March 2010

Participants: Phil Haygarth (PMH; LEC), Chris Hodgson (CH; North Wyke Research), Davey Jones (DJ; Bangor), Dave Kay (DK; CREH), Richard Muirhead (RM; AgResearch, NZ), David Oliver (DO; LEC), Trevor Page (TP; LEC), Roger Pickup (RP; SHM, Lancs) Luke Spadavecchia (LS; Defra), Andy Vinten (AV; MLURI), Rob Fish (RF; Exeter), Ting Zhang (TZ; LEC).

1. Background and PEDAL2 FIO aims

The PEDAL2 project includes the following aims relating to FIO (i.e. *E. coli*) delivery from agricultural catchments.

- Estimation of catchment scale *E. coli* burden prior to the hydrological events to be sampled.
- Interpolation of *E. coli* burden between observations using a simple model (Oliver *et al.,* 2010).
- Observation of hydrological event *E. coli* fluxes at the catchment outlets (2 catchments over 2 years) and estimation of seasonal and annual fluxes.
- Estimation of *E. coli* delivery coefficients using the observations above – investigating these at event, seasonal and annual timescales.
- Development of a fuzzy-rule based *E. coli* model (Figure A3.1), including fuzzy rules for modification of *E. coli* delivery given chosen mitigation measures.

The expert workshop evaluated these aims and in particular the catchment and in-stream sampling protocols and proposed modelling structures.

2. Introduction

TP Introduced the PEDAL1 concepts originally developed for phosphorus (P) under the Defra project (PE0113) and explained that the PEDAL2 project seeks to estimate delivery coefficients for FIO losses, rather than export coefficients, such that we can estimate where the highest risks are spatially. The original PEDAL1 project is being extended to cover 3 more catchments under PEDAL2 (WQ0129) and to include delivery of FIOs. The scale of PEDAL2 is the headwater catchment (nominally approx. average catchment size of 1km^2) to minimise the chances of large point sources and in-stream processing; the advantage here being that we are working with the 'true' diffuse signal (although it will of course include agri-point sources such as farmyards and septic tanks in some cases – see below for discussion).This is a deviation from the often-used source-receptor export coefficient approach (where the source is defined by land use and the receptor is, for example, a bathing water. The PEDAL approach was questioned by DK who asked why not keep to the source-receptor link for modelling? TP showed, as an example, that for P, there is a very large range of export coefficients reported in the literature for given land uses and that it may also be the case for FIOs. The PEDAL approach seeks to identify some of the variables that may help to disaggregate these observed ranges: e.g. such as the variation in export coefficient resulting from soil type and topography, which control delivery/transport of P and FIOs to some degree. An important and related question is hence: how are P delivery coefficients related to FIO delivery coefficients?

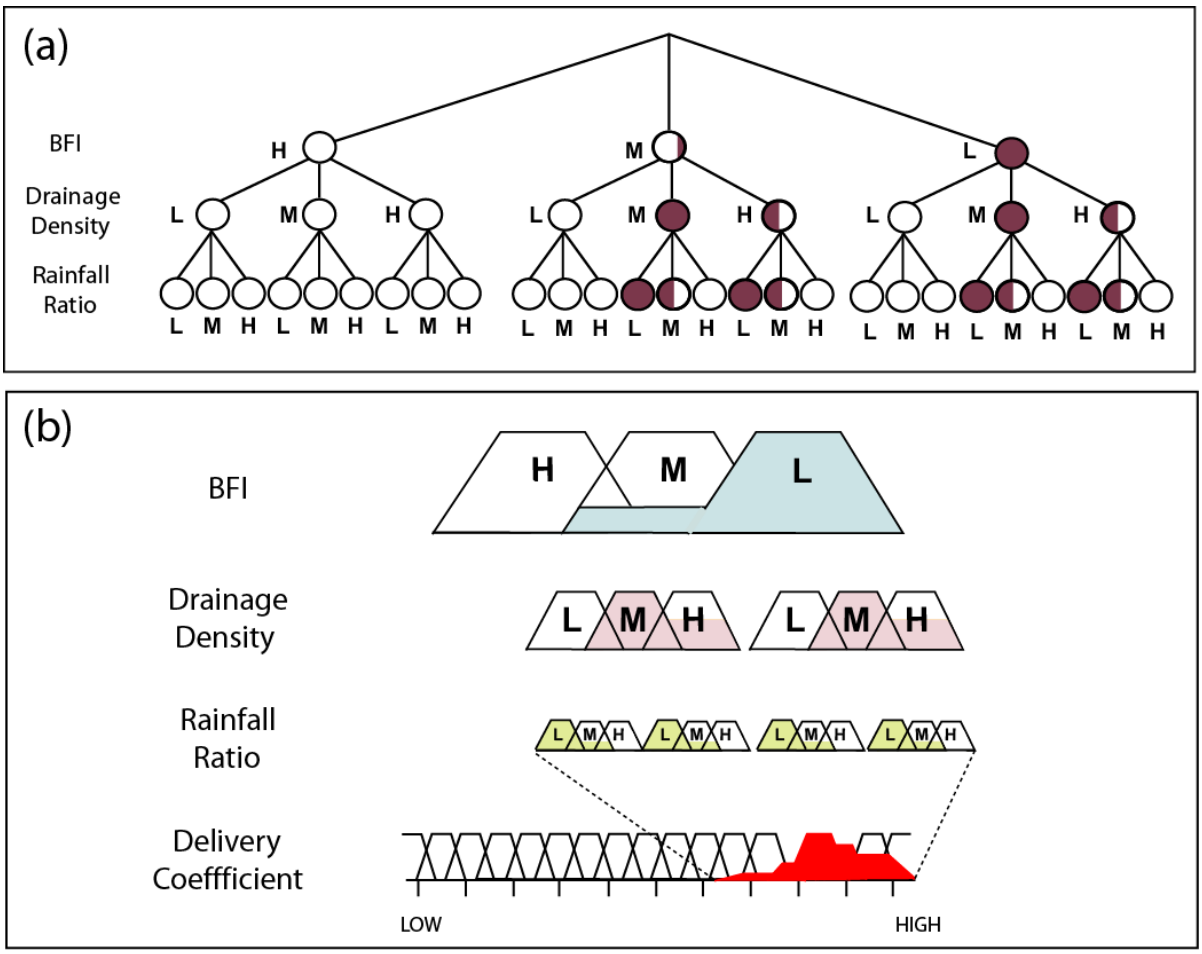
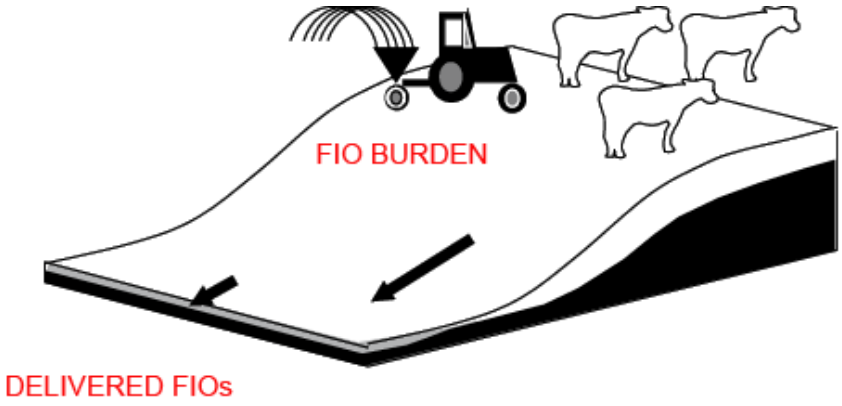


Figure A3.1: Schematic representation of the proposed P model framework. Pane (a) shows how catchments with characteristics (as described by the variables BFI, drainage density & rainfall ratio) are classified through the model (from top to bottom). Where a node is fully shaded then the variable of interest is a full member the fuzzy set, Low (L), Medium (M) or High (H) and where partial members they are shown partially shaded. The fuzzy sets for an example catchment with properties that fall in the BFI (L = full, M = partial), Dd (M = full, H = partial) and Rr (L = full, M = partial) are shown in pane (b) along with the subsequent fuzzy DC range highlighted in red.

RM queried how mobilisation was taken into account in the PEDAL2 delivery coefficient and TP responded showing that the delivery coefficient includes mobilisation and transport which implicitly includes the continual and step-wise mobilisation-deposition-remobilisation occurring during transport and that there are no plans explicitly model processes where we have no data to support them. This is conceptualised in Figure A3.2. DK suggested that, as at the moment his models are *black box* (i.e. have no process based representation between land use and concentrations at the receptor) models to be used for prediction they may need some more mechanistic or process based representations (*white box*). TP suggested perhaps somewhere in between may be best ('grey box') and that to a degree PEDAL2 uses a *grey box* model approach as it includes some representations important for describing processes between the source and the receptor. TP went on to say that given the storm-event focus of PEDAL2, we will also obtain delivery coefficients at hydrological event scale, which is a temporal disaggregation of export coefficients.



$$DELIVERY\_COEFF_{FIO} = \frac{DELIVERED\_FIOs}{FIO\_BURDEN}$$

Figure A3.2: FIO delivery coefficient schematic

2.1 Is there a 'true diffuse' signal?

As PEDAL2 sets out to determine, as far as possible, delivery coefficients of a diffuse signal, there was significant discussion on whether or not it was possible to determine a true diffuse signal at catchment scale: it is difficult to separate the diffuse component from agri-point source component. AV suggested that homesteads (hardstandings and septic tanks etc.) were generally thought of as more important for FIO delivery than fields. Homesteads can have roof drainage with similar microbial content to that of river concentrations in terms of enterococci: AV was concerned that the focus is only on landscape sources and does not take into account hardstandings. DK supported the fact that agri-point sources are particularly important indicating that a quick 'back of an envelope' calculation for one catchment suggested that farmyard and roof runoff from 4 farms contributed enough to account for all of the signal observed at downstream beaches. AV similar points relating to relating to the differences seen in FIO concentrations in streams above and below farmsteads.

TP/DO agreed that this could be the case, and to some degree these agri-point sources will be included in the fuzziness of the PEDAL2 delivery coefficients where catchments include farms, but that even if we show that the true diffuse signal is low, then that is a useful result and if we need to (and where data exists) we can model point sources separately within the PEDAL framework.

2.2 Defra aims with respect to FIOs

LS showed that Defra employed a 3 pronged approach to promote/facilitate good land and water management - ***advice, incentives, and Water Protection Zones (WPZ)***. This was shown as a pyramid (regulation sandwich) where the preference is for incentives and advice and WPZ a less preferred last resort (Figure A3.3). Defra are also required to have a multi-objective approach as they cannot focus on FIOs or a single pollutant such that determining which pollutants follow similar delivery patterns is therefore important. Science has a role in providing evidence to set priorities rather than trying to meet preconceived ideas from policy but of course the BWD and SWD need to be met.

In general Defra funded agri-environment schemes incentivise biodiversity/husbandry rather than protection of water quality; there should, however, be some flexibility under the Demonstration Test Catchment (DTC) project to target water quality issues.

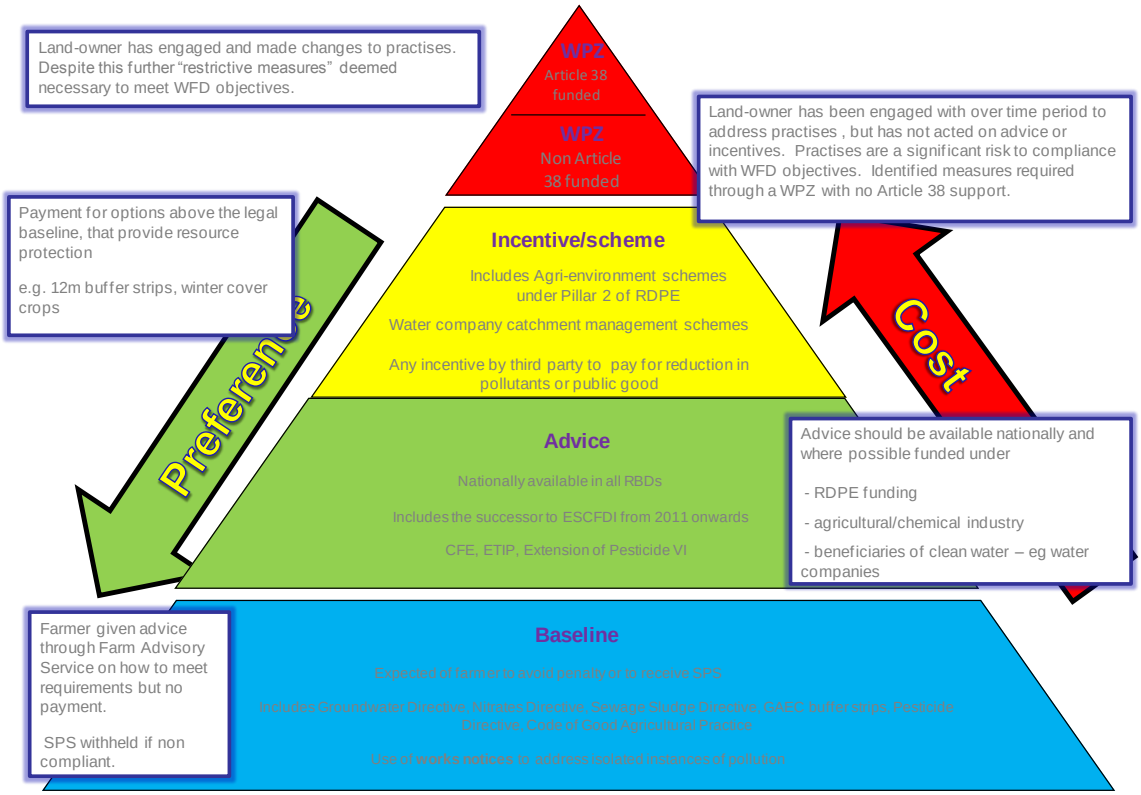


Figure A3.3: The Defra '3 pronged approach' to promote/facilitate good water and land management

3. PEDAL2 Sampling strategy

DO outlined some initial thoughts on a sampling strategy for catchment scale burden and hydrological event sampling (discussed below) and presented arguments for the focus on *E. coli* as the FIO of choice.

3.1 Sampling Burden

DO proposed that we use a stratified random sampling methodology for burden where the stratification is based upon the Critical Source Area (CSA) concept (Figure A3.4). CSAs will be estimated using the catchment visual assessment.

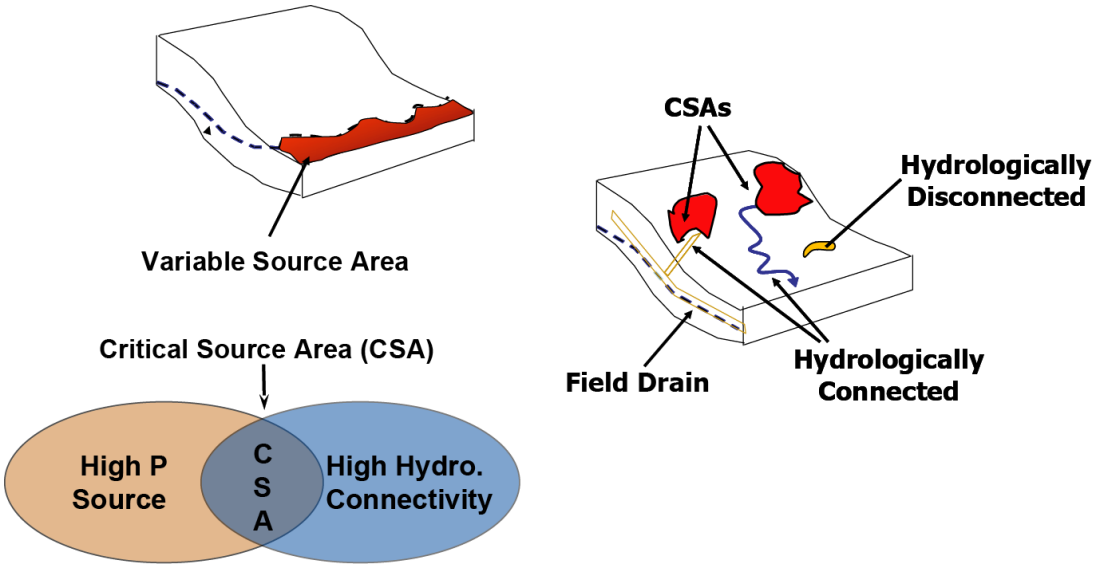


Figure A3.4: The concept of Critical Source Areas

DO communicated the assumption that we are only considering the faecal FIO store rather than the soil store which is normally orders of magnitude lower (e.g. RM reported faecal reservoir ~ 10^8, soil ~ 10^5) during the main period of interest (bathing season): there is always a residual soil store of *E. coli*. Figure A3.5 shows an idealised plot of a temporally stable soil store and a faecal store which is dynamic over time and the resultant total store. DJ confirmed a similar situation with data for *E. coli* O157, highlighting its ability to persist for years, which presents a real problem for *E. coli* O157 contamination of pasture. RP suggested that faecal bacteria have to grow to sustain at that level, so therefore growth must be occurring at some level. RM determined a 1.3 log standard deviation in *E. coli* in faeces; c.f. numbers in soil at 10^5, and this reaffirms the argument for focussing on the faecal reservoir. This argument is strengthened further with respect to storm flows compared to annual averages. RM commented that they observed no relationship between soil level concentration in grazed plots and runoff concentrations for *E. coli*. This does however indicate that the soil store becomes more important in winter, where winter event delivery of FIOs is to be determined.

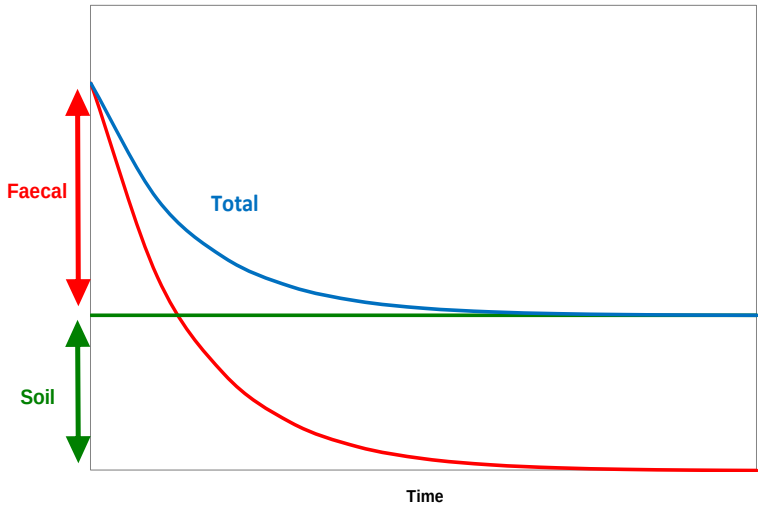


Figure A3.5: Faecal versus soil reservoirs of E. coli on pasture (Muirhead)

The initial plan is to measure catchment-scale burden (prior to hydrological events) approximately 10 times per year and to interpolate where necessary using the model reported by Oliver *et al.*, (2010).

The expert group discussed issues surrounding the concept of determining field-based *E. coli* burden. RP presented data highlighting that persistence studies of faecally-derived microorganisms are often lab-based, with very little field-relevant data actually available (which was backed up by DO's work). Points to bear in mind when using interpolation of the model were made clear – for example RP indicated high variability in terms of persistence profiles. The model developed by DO and TP accounted for variability on die-off coefficients for this very reason. DJ followed with slides presenting data on laboratory scale persistence which again stimulated discussion of lab-scale studies and in this case the lack of accounting for UV effects on persistence.

For the determination of field-based *E. coli* burden the number of samples to be taken is limited ultimately by manpower. However, a possibly more efficient approach was proposed whereby faecal samples are bulked and sub-sampled to determine an area-specific average. The experts commented that we should try to not bulk samples (faeces), but instead back-calculate based on possible sample numbers to get best strategy. DO argued that bulking and sub-sampling is a viable option as we are interested in obtaining the most accurate *average* burden for each area considered (e.g. CSAs and non-CSAs) rather than characterising the spatial variability (TP argued that we were not interested in variability between samples for PEDAL, even though it is interesting and important for other studies). RM commented that we should be aware that bulking implicitly means that we obtain an arithmetic mean (not a geometric mean). DO/TP conceded that the arithmetic/geometric mean argument is valid, but as samples increase the arithmetic mean will converge to the burden value of interest for the project and we anticipate collection of a high number of individual samples to be bulked. RM continued discussion on whether CSAs for FIO were the same as for P, which should be the case for the particulate-associated P fraction to some degree as they share similar transport pathways. Following on from the sampling discussion RM made the important point that we should attempt to estimate the areal coverage of faeces on pasture when extrapolating to obtain the catchment burden.

AV asked if literature studies reported actual burden details, in terms of numbers in faeces, such that a calculation for burden could be made without the need for on-the-ground sampling. DO replied that very little information on *E. coli* numbers in faecal material. RM backed up this comment; for the UK data is limited. AV suggested could still plot PDFs of the data that was available and that it may provide a useful starting point (we can attempt to do this based on work already undertaken by DO, though data is limited).

A number of other important factors which may affect the sampling of burden were also raised:

- Direct in-stream contributions were not within original sampling strategy (e.g. dairy cattle crossings) and there is a lack of data in terms of sheep fording. This is outside the scope for determination of diffuse delivery coefficients but very important for a more holistic model.
- Birds and wildlife were also highlighted as contributors to the burden component.

3.1.1 Methods for measuring slurry and pats (presentation by CH)

There is a decision to be made regarding the acquisition of slurry samples for *E. coli* enumeration. Critically, should the samples be collected pre-or-post application – PEDAL2 proposes pre-application. For slurry sampled in a tank it should be mixed for a number of hours using a propeller system to make the sample suitably homogeneous. CH reported that farmers had been amenable to this process on a current project with which he was involved. However, this may be difficult to achieve within the PEDAL2 project

and alternative methods for sample acquisition and mixing are currently being discussed based on farmer co-operation and co-ordination with slurry tank filling.

For faeces CH commented it must be decided how to take the sample: homogeneous vs. heterogeneous. For mixing of faecal samples a shaker, vortex or 'stomacher' can be used. Faecal samples collected within the PEDAL2 project will be appropriately mixed to ensure homogeneity of faecal material as best as is practically possible.

There was a brief discussion regarding the use of wet weight versus dry weight for recording FIO content in faecal matrices. RM was in favour of dry weight in support of DOs argument that water content impacts on wet weight values: if a project is looking at changes in population over time it is necessary to use a dry weight approach. Similarly a brief discussion on the use of sterile water versus Ringers solution followed and members of the expert group were in favour of both. Indeed, the take home message here was that it did not matter so long as the methodology remained consistent. Membrane filtration as an approach to FIO enumeration (as used by PEDAL2) was commented on as being suitable for faecal samples because of the levels of dilution involved avoiding the clogging of the membrane filters. Following this, the drop-plate technique was also raised as an alternative, cost-effective approach to cut back on Petri-dish throughput. However, its use depends on the likelihood of whether emerging colonies would be 'spreaders' in terms of their morphology, in which case their presence would invalidate the drop plate methodology.

3.2 Sampling in-stream events

All participants agreed that annual averages are not important and we need to link timescales to the relevant directives. In general, for sampling, this means that sampling hydrological events is appropriate but it was noted that base flow samples should not be ignored: DO confirmed that we will be measuring base flows at low resolution.

A problem put forward for discussion by DO was that of aseptic sampling. This largely stemmed from the use of autosamplers for obtaining water samples for microbial enumeration. A comparative analysis of hand-sampling and autosampler obtained data would prove useful. We might not see a statistical difference between the two approaches. If we have + or – an order of magnitude error in confidence of results does it really matter about autosampler use? For blue-book methods, aseptic hand sampling remains the option of primary choice if logistically possible. It was agreed that sterilisation of bottles was not a major concern with autosamplers, as this could be minimised by correct methodologies; it was more a problem of contamination associated with sample intake, even with multiple autosampler purge cycles. Pumping through the autosampler tubes may break up microbial flocs, but as pointed out by a member of the expert group, that may actually make for a better sample and more representative sample.

There is a problem with sample holding times and a number of participants argued that it is not appropriate to back-calculate based on die-off based on the high uncertainty associated with such approaches. AV commented that if you are explicit about what you did and don't have resources then it is acceptable. PEDAL2 telemetry system will be set up to avoid any use of a back calculation. Rather than put a standard in an autosampler for the duration of the holding time, PEDAL2 will use holding times of less than 24hrs and use a number of cross-comparative sampling campaigns to test for differences in sample acquisition (DK presented Ferguson (1994) study comparing autosamplers with grab sampling in Australia).

There were two other stream sampling points raised:

- Need to use a protected intake tube with autosampler so that sampler doesn't sample stream sediments.

- DJ suggested there are some die-off curves in streams that could be used for integration into a larger model that includes the receptor. DK argued that there are not many data for freshwaters.

4. FIO analytical protocol

DK argued for consistency of methods as different methods of analysis give different statistical distributions: so they cannot be compared. The community should work using the same sampling methods: membrane filtration (MF) technique (as proposed for PEDAL2) being the most appropriate. Aseptic sampling is the best method (as previously discussed), combined with blue book approaches. DK also raised a point regarding the reporting of censored data and counts <100. This highlighted the need for sufficient dilution to ensure numbers on plates are useable and meaningful.

5. Mitigation Measure Effectiveness

RF opened the debate on mitigation effectiveness by speaking to the group about his interest in participatory approaches and risk assessment. He was particularly interested in how to make some working assumptions on the likely effectiveness of mitigation strategies designed to reduce microbial watercourse impairment. This discussion was built into the FIO expert workshop to stimulate debate following the P expert workshop where on Day 1 experts had been reluctant to commit to assigning any kind of ranking on P mitigation strategies. RF stimulated much discussion, and while experts felt uncomfortable about 'scoring' effectiveness and quality of research currently available describing FIO mitigation options this in itself was exactly what the workshop was trying to gauge and document. RF discussion brought to the fore issues relating to the current lack of empirical scale-appropriate mitigation data, but still allowed for initial assessments to be ranked by experts. Discussion followed about how appropriate a single score for each mitigation option really was, and would a fuzzy range of effectiveness not be more appropriate.

DK raised the point about how do mitigations feed into farm support systems? This is critical, and requires incentives and agri-environment schemes. This highlights that we do need a model dealing with more localised burden and delivery, rather than just source to receptor. DK suggested that mitigation measures that reduce FIOs by 60-80% might not be enough and that win-win scenarios do not ensure mitigation maintenance over time. LS advised that Defra do not have any specific priorities for which mitigation measures are to be employed.

The PEDAL2 model will include fuzzy representations of the effects of mitigation measures on burden and delivery coefficients (see Figure A3.6). Most of the issues behind implementing these given the generally sparse data on effectiveness are reported in the PEDAL2 P expert workshop report (e.g. see the examples ranges of effectiveness provided for P by TP in Figure A3.7). DJ raised the concern that mitigation that works for one problem doesn't necessarily have benefit for another. An example was provided: enhancing lapwing populations through root crop stripping under HLS also completely devastated the pasture giving the potential for massive loading of FIOs to watercourses and soil erosion. DJ showed an interesting plot of mitigation measures effectiveness vs. practicality from work undertaken by a wider RELU[1] team project with which he is involved (note that this was for *E. coli* O157).

[1] http://www.relu.ac.uk/about/

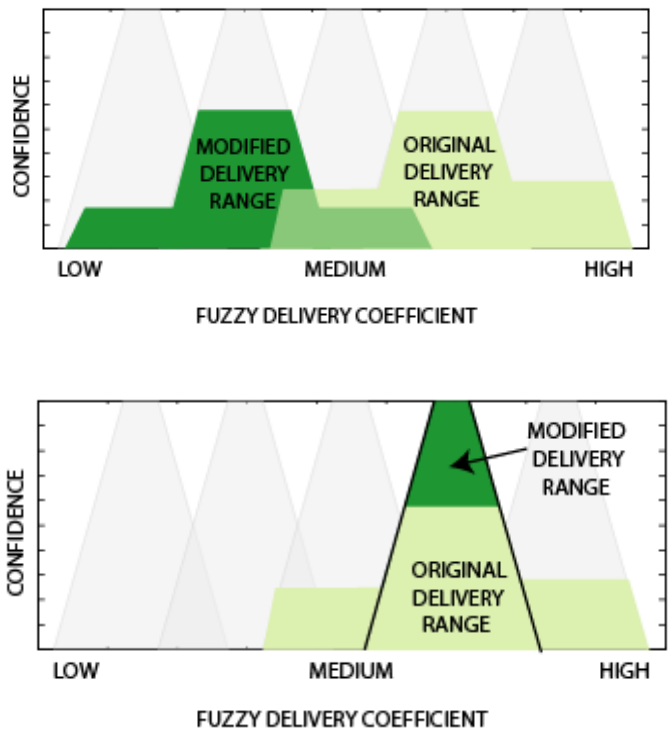


Figure A3.6: An example of the modification of a fuzzy delivery coefficient range where (a) the distribution has been shifted along its axis and (b) the shape of the distribution has been modified.

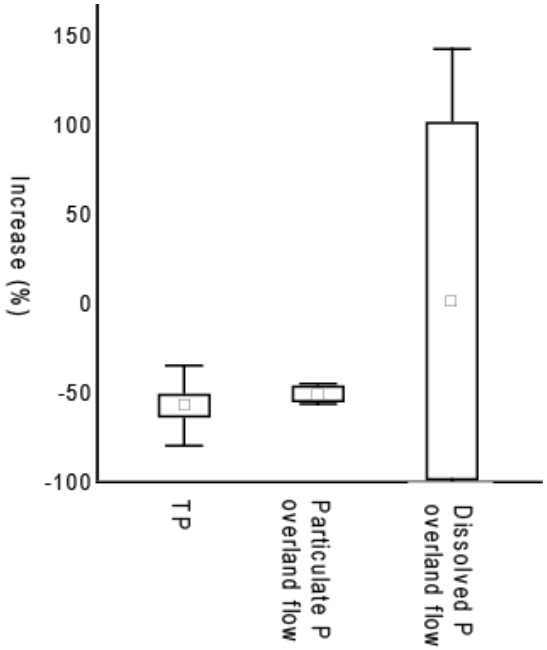


Figure A3.7: Percent P reduction (-) or increase (+) from riparian buffer zones when compared to control plots – adapted from Stevens and Quinton (2009).

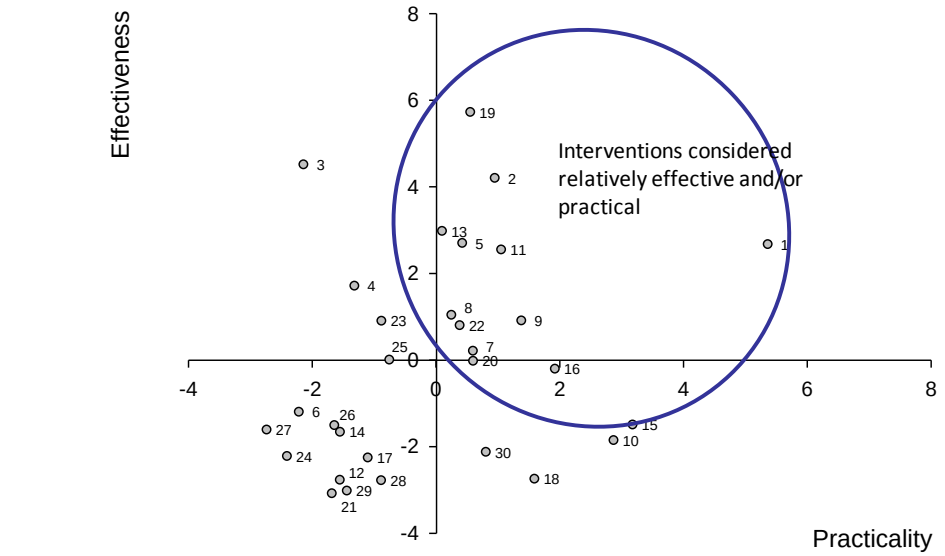


Fig A3.8: Assessing mitigation effectiveness and practicality (Cross et al., 2012)

PH presented work from the user manual and showed that manual options 39-44 are delivery specific mitigation measures. Of course, source mitigation measures also impact on delivery. For FIOs we need to look at the *Manure, Animal* and *Connectivity Plans* in the user manual. The *Soil Plan* was considered ineffective for FIOs which once again backed-up the strategy to focus on the faecal reservoir over the soil store. DK warned that, although FIO work in the user manual is qualitative, the numbers are expressed as a percentage and can end up taking on a life of their own. This is dangerous, and we need to communicate the quality of our scientific understanding and outputs. AV pointed out that ponds and wetlands do not always work very well for storm events, unless they have large capacity (residence time) and can become a source of FIOs over time. There can also be significant leakage issues with ponds

6. Conclusions

The main points that the PEDAL2 team took from the workshop and will incorporate into the project are listed below:

- Although source-receptor models may provide more accurate estimates of microbial pollution for regulatory purposes, PEDAL2 provides an important tool for identifying diffuse hotspots of delivery for diffuse FIOs.
- Agri-point sources may need to be modelled as a separate component (not explicitly part of PEDAL2).
- Analysis of spatial similarities between P and FIO delivery coefficients fits well with Defra's multi objective goals.
- Defra have no particular priorities for which mitigation measures to include in PEDAL2.
- PEDAL2 will use a stratified random sampling methodology for burden where the stratification is based upon the Critical Source Area (CSA) concept (specific sampling strategy TBC – w.r.t. constraints of number of samples that can be analysed).
- Bulking burden samples for the area of interest is valid where a high number of faecal pats are to be sampled – the areal coverage of faecal needs to be assessed.

- Assumption that the faecal store is primary during the grazing season is valid but the soil store may be important at other times.
- Direct deposition to streams is an issue where catchments are unfenced and this will fall outside of any sampling strategy (as do birds and wildlife).
- Slurry samples are to be collected pre application for ease in conjunction with the farmer who will know how many loads are applied.
- The event-focused in-stream sampling strategy was supported by all as annual averaged were seen as unimportant.
- Aseptic sampling is the best methodology but where autosamplers are to be used bottles can be sterilised but the sampling tube cannot: high numbers of purge cycles are beneficial.
- Stream water holding times must be kept under 24hrs.
- Membrane filtration approach to FIO enumeration (as used by PEDAL2) was commented on as being the most suitable.
- The effectiveness of mitigation measures at catchment scales is poorly known – PEDAL2 will need to use the few studies available and expert opinion to determine fuzzy ranges of likely effectiveness to be included in the model as fuzzy rules, which can be updated as new information becomes available.

References

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