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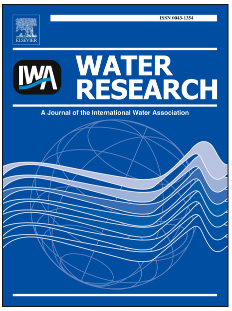

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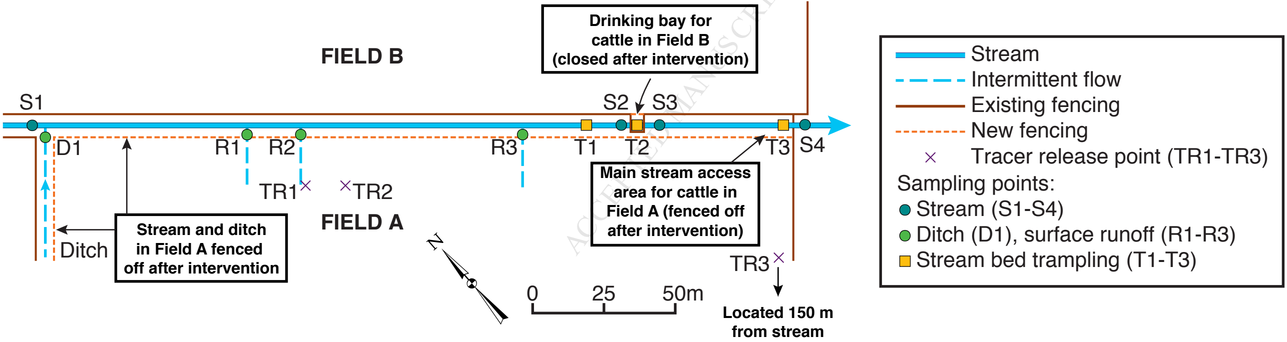
Monitoring of cattle behaviour and sources/input fluxes of FIOs to a stream reach from grazed streamside pastures BEFORE and AFTER installation of additional streambank fencing in the Tamar Demonstration Test Catchment, SW England



Title page

Faecal indicator organism inputs to watercourses from streamside pastures grazed by cattle: before and after implementation of streambank fencing

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Highlights

- Unfenced streamside pastures are a significant FIO pollutant source in catchments.
- Key FIO sources and transmission routes are investigated and quantified.
- Streambank fencing is shown to reduce FIO inputs to streams by *c*. 1–2 $\log_{10}$.
- Empirical data relating to other possible interventions are presented.

Abstract

Faecal indicator organisms (FIOs) are major pollutants in many catchments world-wide, with streamside pastures on livestock farms being potentially significant sources. Hitherto, few empirical studies have quantified FIO fluxes from such areas or investigated streambank fencing (SBF) and other possible mitigation measures. The aim of this two-phase (before/after intervention) study of the effectiveness of SBF was to generate an empirical evidence-base to enable regulatory authorities to make better-informed decisions concerning the implementation of this measure. It was undertaken during the summer bathing season along a 271 m stream reach in the River Tamar catchment, SW England. The study included: cattle distribution surveys; monitoring of changes in *E. coli* (EC) and intestinal enterococci (IE) concentrations and fluxes down the reach and of concentrations in ditch flow and surface runoff; phage tracer studies of surface runoff from pasture land; and experimental streambed trampling to investigate streambed FIO sources. The results show that cattle spend a disproportionately large amount of time in the watercourse/riparian zone along unfenced streams; identify direct defecation to the stream by wading livestock and the release/mobilisation of FIOs from cowpats by surface runoff from the adjacent pastures at times of high flow as key transmission routes; and demonstrate that FIOs become incorporated within streambed sediments, from which they may subsequently be released by trampling. Partial exclusion of cattle through SBF with a drinking bay greatly reduces the

time cattle spend in streams. Total exclusion SBF, with provision of an alternative drinking supply, considerably reduces FIO load inputs to the stream reach, e.g. at times of high flow, which are critical in terms of pollutant fluxes to coastal waters, the mean EC and IE input loads to the reach fell by 0.842 and 2.206 $\log_{10}$, respectively.

Keywords

Pollutant fluxes; Cattle; Streambank fencing; Surface runoff; Streambed sources; Microbial tracer investigations

1. Introduction

Faecal indicator organisms (FIOs) are major pollutants in many catchments world-wide. In the US, for example, FIOs generally exceed all other parameters causing non-compliance under the Federal Water Pollution Control Act (33 U.S.C. 1251-1387; Copeland, 2016). In the UK, substantial reductions in FIO fluxes to coastal waters have been achieved over recent years, through the control and treatment of sewerage-related sources. Despite this, a significant residual loading has remained in many rivers, much of which is derived from livestock-farming activities (Kay *et al*., 2008). In the EU, Article 11 of the Water Framework Directive (WFD; Council of the European Communities (CEC), 2000) requires that a ‘programme of measures’ be adopted to ensure compliance of designated bathing and shellfish waters with use-related water quality standards. The FIO-based microbial standards, now principally *Escherichia coli* (EC) and intestinal enterococci (IE), have become more stringent following implementation of the 2006 Bathing Water Directive (CEC, 2006). There is therefore an urgent need to identify the most effective measures for mitigating FIO losses to watercourses from livestock sources. Newell Price *et al*. (2011) identify a wide range of potential interventions for addressing agriculture-derived pollutants. Unfortunately, the peerreviewed empirical evidence-base for FIOs is limited compared with nutrients and other agricultural pollutants, and the assessments of individual measures available to regulators and the policy community are often based on ‘expert judgement’.

Kay *et al*. (2012) note that because of the high rates of FIO die-off during storage as farmyard manure/slurry and when wastes are disposed of to land, stored wastes are likely to pose a much smaller microbial pollution risk than fresh faeces voided on pastures, especially where grazing livestock have unrestricted access to watercourses. This is supported by recent modelling work which suggests that *c*. 95% of agriculture-derived FIO fluxes in Scottish rivers is derived from grazed pastures (ADAS Consulting Ltd., 2016).

In order to provide foci for field-based research into diffuse pollution and its control, four government-funded Demonstration Test Catchments (DTCs) have been established in the UK. The present project was undertaken along a stream reach in the Tamar DTC in SW England, to investigate the effectiveness of streambank fencing (SBF) in reducing FIO fluxes to watercourses from grazed pastures during the summer bathing season. It was a two-phase study, i.e. before (18 June–9 October 2013) and after intervention (25 August–12 October 2015), comprising: (i) surveys of cattle behaviour in the adjacent fields; (ii) experimental studies of changes in FIO concentrations in the stream as a result of simulated trampling of the stream bed; (iii) use of microbial tracers to investigate the movement of FIOs defecated in different parts of a streamside pasture; (iv) measurement of FIO concentrations in ditch flow and surface runoff from the adjacent pastures; and (v) investigations of FIO concentrations and fluxes at critical points along the stream reach.

2. Materials and methods

2.1. Study site and flow conditions

The study site (Fig. 1) comprises two fields (A and B) and the stream reach that separates them – i.e. between stream ('S') sampling points S1 and S4. Fields A and B have areas of 4.19 and 4.63 ha, respectively, and both are grazed by cattle, cut for silage and receive occasional applications of slurry from spring through to early autumn. The stream reach, which is flanked by a narrow strip of riparian woodland, has a channel length of 271 m and the catchment area draining directly to the reach is 16 ha, which accounts for 10% of the 160 ha catchment upstream of S4. Although both fields are of similar gradient (*c.* 12^o), the lower parts of Field A are more poorly drained. During prolonged rainfall, surface runoff occurs in lower parts of Field A, and in several places it becomes spatially concentrated and discharges to the stream though breaches in the bank. These areas of wetter ground are heavily poached by cattle. Because of the orientation of the reach, the streamside area of Field A receives little shade from the riparian woodland, whereas the lower part of Field B is partially shaded. The numbers of cattle present varied both during and between the two phases of the study, with maxima in Fields A and B of 81 (19.3 ha^{-1}) and 160 (34.6 ha^{-1}), respectively. Towards the end of Phase 2, surface applications of slurry were made to both fields at a rate of *c.* 20 m^3 ha^{-1}, but were found to have no discernible effect upon FIO input loadings to the stream reach.

Conditions during Phase 1 were mostly very dry: on occasions the stream was barely flowing (flow recorded nominally as 1.0 l s^{-1}), the maximum flow recorded during low-flow sampling was only 1.4 l s^{-1}, and the maximum flow recorded during high-flow runs was 169 l s^{-1}. By comparison, conditions during Phase 2 were much wetter and the minimum flow in the low-flow runs (16.0 l s^{-1}) and peak flow during high-flow runs (904 l s^{-1}) were correspondingly higher. These differences are taken into account in interpreting the findings.

2.2. Management interventions

In Phase 1, Field A was unfenced along the stream and ditch. SBF was present in Field B, and this included a drinking bay which provided the only water source for livestock Fig. 1). The main interventions undertaken prior to Phase 2 involved:

- erection of SBF along the stream and ditch banks of Field A;
- closing off the drinking bay in Field B; and
- installing a drinking trough in Fields A and B at some distance (150 m and 98 m, respectively) from the stream.

2.3. Field methods

Daily records were made of the number and types of cattle present in each field. In addition, on a number of days when cattle were present, visual observations were made during the daytime of the numbers of cattle present in different locations within the fields: in sections of the stream, in the riparian zone (within 5 m of stream), in the vicinity of the ditch, in the drinking bay, within 5 m of pre-existing and new fencing, etc. Before intervention, recordings were made at 1-h intervals on 26 days (294 and 114 observations, respectively, in Fields A and B), whereas after intervention, which was a shorter, more intensive period of study, recordings were made at 10-min intervals on 4 days (288 and 151 observations, respectively).

A stage board at S4 was used to record water levels during the sampling runs. These were complemented by continuous records from a combination of submerged and atmospheric pressure transducers (Van Essen instruments Divers®). Flow measurement using the ‘velocity area’ method (Environment Agency, 2003), allowed a stage-discharge rating curve to be constructed for flows ≥ 1.4 l s^{-1}. At lower stages, when accurate measurement was precluded (all in the period before intervention), visual assessment of flow in shallower sections of the channel suggested a discharge in the order of 1.0 l s^{-1}. This figure has been assumed for all flows $< 1.4\ \mathrm{l\ s^{-1}}$. Since 10% of the catchment of S4 is associated with land draining to the reach, it has been assumed that flow at the top of the reach (S1) is 90% of that at S4 and increases linearly down the reach in proportion to the straight-line distance from S1 to S4.

The stream was sampled at four locations (S1–S4) to characterise changes in flux along three sections of the reach:

- S1–S2: main section, to which cattle had unrestricted access from Field A before intervention, though cattle rarely visited some sections which are either overgrown and/or have steep banks;
- S2–S3: drinking bay in Field B; and
- S3–S4: lowest section, which includes the main stream-access area in Field A prior to intervention.

In addition, discharge from the ditch was sampled at point D1 and surface runoff from Field A was sampled at three points (R1–R3) where flow occurred though breaches in the stream bank. Stream and ditch samples were taken during each of 22 sampling runs, which encompassed seven sets of ‘Conditions’ (I–VII, Table 1); and runoff at R1–R3 was sampled during two of the high-flow runs after intervention. Details of the sampling regime are presented in Table 1. Samples were taken manually using sterile disposable 150-ml plastic bottles, and refrigerated in dark conditions prior to analysis.

2.4. Simulated trampling of stream bed

The release of FIOs resulting from simulated trampling of the stream bed was investigated at three points: trampling (‘T’) point T1 – an area where cattle access from Field A was limited by steep banks and overgrown vegetation; T2 – within the drinking bay; and T3 – in the main cattle-access area in Field A prior to intervention. The studies were undertaken at low flow when trampling by cattle is likely to have greatest impact upon FIO concentrations in the stream. At each site, a 20-cm wide section of the *c.* 1-2 m wide stream bed was strongly disturbed by trampling for 15 seconds, employing a method similar to kick sampling for invertebrates, with subsequent runs being undertaken at sections located progressively upstream. Stream water sampling downstream of each site was undertaken immediately before trampling and then at 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 min after trampling. Peak fluxes were recorded in the first 3 min following disturbance, and the geometric mean (GM) concentrations recorded in the 1, 2 and 3 min samples have been used to characterise the FIO fluxes following trampling. Stream water was also sampled upstream before and 30 min after trampling to determine background FIO concentrations. 10 trampling experiments were conducted (3, 4 and 3 at T1, T2 and T3, respectively), each characterising different degrees of cattle access/usage.

2.5. Microbial tracer study (after intervention)

The study employed three ‘phage’ tracers (*Enterobacter cloacae* phage (here referred to as ‘*Ent. C* phage’)), MS2 coliphage and *Serratia marcescens* phage (‘*SM* phage’)). The numbers of each phage used were $1.5\text{x}10^{15}$, $7.0\text{x}10^{16}$ and $1.5\text{x}10^{15}$ plaque forming units (pfu), respectively, and each was mixed with 4.5 l of cattle slurry. Three simulated cowpats, each of 1.5 l, were deposited at each release point (TR): TR1 (MS2 coliphage) – 20 m from the stream in an area where spatially concentrated surface runoff had been observed; TR2 (*SM* phage) – 20 m from stream in an area where surface runoff was less likely; and TR3 (*Ent. C* phage) – by the newly installed drinking trough 150 m from the stream. The tracers were released at a time when active surface runoff was occurring following heavy rainfall. Tracer concentrations were monitored from 3 h prior to release for 232 h at S1 (as background control) and S4. Samples were taken manually prior to release. At S4, they were then taken by auto-sampler at 1-h intervals during the first 24 h and thereafter at 4-h intervals. At S1, two samples were taken daily after the release. Pre-release samples at S4, and all samples at S1, contained no *SM* or *Ent. C* phages and had concentrations of MS2 coliphage within natural background levels (maximum, 5.0 pfu ml^{-1}).

2.6. Laboratory analysis

Samples were analysed within 24 h of collection for EC and confirmed IE using standard membrane filtration techniques (Standing Committee of Analysts (SCA), 2009). Analyses were undertaken in triplicate (Fleisher & McFadden, 1980) and resulting concentrations expressed as colony forming units (cfu) 100 ml^{-1}. In the very few cases where concentrations were recorded as below detection limit, the detection limit value has been used. It should be noted that in the streambed trampling studies, some of the FIOs present in the upstream water may have become attached to sediments entrained during disturbance, forming clumps or aggregates, which could reduce colonies counted on the filter plate. The results of these experimental studies therefore need to be interpreted with caution. *Ent. C* phage, MS2 coliphage and *SM* phage were enumerated following the double agar overlay method (Adams, 1959; Havelaar and Hogeboom, 1984; SCA, 2000), and are expressed as pfu ml^{-1}. Further details of the preparation of concentrated phage suspensions and enumeration can be found in Wyer *et al*. (2010).

2.7. Overview of experimental design and uncertainty

Various papers since 2008 have addressed the sampling design for urban stormwater drains and specifically focused on the uncertainties in estimating chemical and microbial fluxes from urban stormwater infrastructure and surface water streams to receiving waters (e.g. Harmel *et al*., 2006a, 2006b, 2009, 2010, 2016; McCarthy *et al*. 2008, 2012, 2018). The work by Harmel *et al*. was driven by the need to provide guidance on pollutant flux estimates required to inform Total Maximum Daily Load (TMDL) estimates as required under the US Clean Water Act, with the other main activity focus being in Australia, of which the McCarthy papers provide a good exemplar. These teams produced an excellent review paper in 2016 (Harmel *et al*., 2016) which quantified the range of expected uncertainty when deriving bacterial (principally *E. coli*) load estimates in urban surface water drainage (some with sanitary cross connections) and rural streams. This suggested that uncertainties derive from: (i) sampling – e.g. location/depth of an auto-sampler vacuum pipe within the circular storm drain or the sampling location of aseptic hand sampling; (ii) sample storage – e.g. storage of microbial samples for up to 48 h with or without refrigeration; (iii) analytical uncertainties, which can be high for microbial enumerations; (iv) flow measurement uncertainties – e.g. using velocity area methods, particularly at low flows; and finally (v) event sampling uncertainty, including both within and between events.

The present stream-reach study in the UK was not designed or resourced to implement a protocol involving multiple replicate measurements and enumerations which could empirically define uncertainty against these categories. However, we have sought to define and explain the mitigation of uncertainties in this work, as follows: (i) sampling uncertainty was minimised by using best UK practice – i.e. aseptic hand sampling into sterile wide-mouth microbial sample bottles; (ii) sample storage uncertainty was minimised by immediate transfer of all samples to a dark cool box containing melting ice to cool samples quickly (this refrigerated condition was maintained throughout the transport system for which dedicated, directly employed, couriers were used); (iii) analytical uncertainties were minimised by using a laboratory accredited by the UK Accreditation Service (UKAS), which is required for regulatory samples in the UK – this sets out a fully documented AQC system covering sample collection, transport and analysis and requires annual independent inspection of the laboratory systems, with triplicate analyses for FIOs further reducing analytical uncertainty by enhancing enumeration precision; (iv) flows were determined as accurately as possible by using a Sensa RC2 electro-magnetic velocity meter, calibrated to manufacturer's instructions, with the average of three measurements being recorded at each point across the channel profile; and (v) event sampling uncertainties were minimised by through-event sampling at each monitoring site, with more intensive sampling being undertaken during high-flow events.

It is fully accepted that there will be remaining uncertainty in FIO flux measurement in urban and rural catchment systems, and we would not disagree with the total uncertainty estimates provided in Harmel *et al*. (2016, p. 531) for studies involving FIO loadings. With careful attention to AQC procedures and a series of good sampling sites, we consider that the present study has generated data set of "good" quality, for which the likely average level of uncertainty suggested by these authors is ±33 to 34%.

3. Results

3.1. Distribution and behaviour of cattle in Fields A and B

3.1.1. Before intervention (Phase 1)

In Field A, individual cattle spent an average of 3.1% of the time in the stream: 1.7% in vicinity of the main drinking area and 1.4% at various points upstream of S3 (Table 2). A further 11.7% of time was spent in the riparian zone (defined here as being within 5 m of the stream or ditch adjacent to the boundary hedge) and the remaining 85.2% was in the main body of the field.

In Field B, cattle spent 0.8% of time in the drinking bay, which is consistent with the times recorded in the vicinity of the new water troughs in Fields A and B after intervention (1.1 and 0.5%, respectively). This suggests that cattle visit the bay to drink, but do not spend much additional time there. Since Field B is SW facing, the riparian woodland, though fenced off, provides some measure of shade along the edge of the field and cattle spent on average 9.2% of time within 5 m of the fence.

3.1.2. After intervention (Phase 2)

After the stream/ditch in Field A were fenced off and a drinking trough was installed 150 m from stream, cattle spent only an average of 1.1% of time by the trough and 2.7% within 5 m of the SBF, with the remaining 96.2% being spent elsewhere in the field. In Field B, the effect of closing the drinking bay and providing the water trough was that cattle spent a similar time in the vicinity of the trough (0.5%) as they had previously spent in the drinking bay (0.8%). The interventions had no apparent effect on the time spent close to the pre-existing fencing in Field B (10.7%, cf. 9.2% pre-intervention) or in the rest of the field.

3.2. Simulated streambed trampling experiments

The results for several of the trampling studies undertaken following periods when there was no (or very little) cattle activity showed reductions in the FIO fluxes recorded following bed disturbance (Table 3). These findings are counter-intuitive, with the most likely explanation being reduced colony counts during analysis as a result of the attachment of some pre-existing FIOs in the stream water to the entrained sediments (see Section 2.6). If this is the case, then the increases recorded in the remaining trampling runs are likely underestimates of the actual increases in loadings that occurred.

3.2.1. Before intervention

Run 1 was undertaken when there had been no cattle in either field for > 50 d. Even so, increases in EC loadings were recorded at all three locations, with the greatest increase being at T1, which is relatively inaccessible to cattle. These findings show that streambed sediments contain a ‘background’ EC store, which at T1 is likely attributable to sedimentation (and possible growth?) of FIOs derived from upstream sources in a location that is unlikely to have been previously disturbed by cattle trampling for many years. In Run 2, undertaken after cattle had been in Field A for 7 d, very large increases in EC and IE fluxes (4.9×10^6 and 1.6×10^5 cfu s^{-1}, respectively) were recorded at site T3, the main stream access point for cattle. Run 3, which was undertaken solely to investigate the impact of use of the drinking bay, provided evidence of increases in EC and IE, even though cattle had only been present in Field B for 2 d.

3.2.2. After intervention

Run 4 revealed no increases in EC and IE flux derived from bed disturbance at T3, thus demonstrating that SBF has reduced the streambed store in this part of the reach that was previously heavily used by cattle. Run 5 indicates that closing off the drinking bay led to a reduction in the release of EC and IE following disturbance.

3.3. FIO concentrations at the top of the stream reach

The stream has consistently low FIO concentrations at low flow (Table 4a). The *c*. 2 $\log_{10}$ increases in concentration recorded at high flow are typical of catchments with livestock farming (Kay *et al*., 2008) and reflect the greater opportunities for FIO detachment and transport under wet conditions.

3.4. Ditch flow

Before intervention, with cattle spending an average of 2.3% of time in, or within 5 m of, the ditch, the GM EC concentration (Table 4b) exceeded high-flow concentrations at the top of the stream reach by *c*. 1 $\log_{10}$. After the ditch was fenced off, GM EC and IE concentrations decreased by *c*. 1 $\log_{10}$. This likely reflects the elimination of defecation in the ditch and cattle spending less time in the immediate vicinity.

3.5. Surface runoff from grazed pastures

3.5.1. FIO concentrations in field runoff

Spatially concentrated runoff was observed up to a distance of *c*. 25 m from the stream in Field A. Prior to the two sampling runs of runoff at sites R1–3, the field had been quite heavily used by cattle for several weeks, and many fresh cow pats were evident on the lower slopes. Broadly similar GM FIO concentrations were recorded at all three sites over the two events (Table 4c). These GM concentrations are lower than in the unfenced ditch, probably because cattle tended to congregate in the ditch in the partial shade of the adjacent hedge. The GM EC concentrations in the runoff are similar to the stream water at high flow, whereas IE concentrations are *c*. 1 $\log_{10}$ lower.

3.5.2. Tracer investigations of surface runoff

The tracer releases were made at a time when surface runoff was already evident in Field A. Immediately after the release, the rain ceased for *c*. 1 h but there was then a spell of prolonged heavy rain, during which flow at S4 peaked at 297 l s^{-1} (Fig. 2). There was no further rainfall and low-flow conditions then prevailed. Consequently, there was little chance of *Ent. C* phage at TR3 being transported 150 m down Field A, unless some had been carried closer to the stream on the hooves of cattle during the initial period of active surface runoff. In fact, no *Ent. C* phage was detected at S4.

In the case of TR1, located where spatially concentrated runoff was active, there was an almost immediate response 175 m downstream at S4 (Fig. 2). The MS2 phage was detected 1 h after release and peaked at 4 and 7 h, thereby demonstrating that faeces can be readily detached from cowpats and transported 20 m or so to a nearby watercourse from parts of pastures with active surface runoff. Thereafter, the concentration fell through to 30 h, which corresponds with the end of the period of high flow, and the progressive reduction in concentration through to 100 h probably reflects a progressively diminishing input from the pasture and depletion of any tracer that had been temporarily retained along the watercourse. A relatively high proportion (20.9%) of the tracer released was 'recovered' at S4 over the 232 h of the study, virtually all of this within the first 24 h, which is a further indication of the significance of such areas of active surface runoff as FIO sources.

TR2, also 20 m from the stream, is in an area with no clear surface runoff or connection with the main surface inputs to the stream. The *SM* phage was first detected 5 h after release, peaked at 6 h and then declined rapidly until 22 h. This response clearly shows some degree of hydrological connectivity between TR2 and the stream under very wet conditions, with the initial delay (cf. TR1) being consistent with slower-moving diffuse runoff. Also, in this case only a relatively small proportion of the tracer (0.3%) was recovered, suggesting that a higher proportion of the simulated cowpats, and their associated FIO pollutant load, remained in the field after the rainfall event.

3.6. Changes in GM EC and IE concentrations down the stream reach

The GM FIO concentrations recorded under the seven sets of conditions investigated (Table 1, henceforth identified as '[I]'-'[VII]') are presented in Fig. 3. Since the land area contributing to the stream reach accounts for only 10% of the catchment upstream of S4, the changes in FIO concentrations down the reach are generally quite small. The notable exception is at low flow before intervention when cattle were present in Field A [II] and spending an average of 3.1% of time in the stream. Under this condition, GM EC and IE concentrations increased down the reach by 2.428 and 1.258 $\log_{10}$, respectively, with the greatest increases occurring from S3–S4 through the main drinking area in Field A. It should be noted that these increases were recorded under particularly low flow conditions in Phase 1, when overall FIO fluxes were small and the sunny conditions and clear/shallow stream flow would favour die-off through exposure to UV light. They therefore represent a very minor contribution to overall FIO loadings that the Tamar catchment delivers to coastal waters. Increases in concentration were also recorded at low flow through the drinking bay (S2–S3) when cattle were using the drinking bay in Field B [III]. Otherwise, the downstream changes in concentration are small. Some reductions are recorded, which are likely attributable to die-off, sedimentation of particle-attached FIOs and/or ‘dilution’ by inputs of water (surface runoff, soil throughflow, etc.) with lower concentrations than the stream.

3.7. Changes in EC and IE input loadings to the stream reach and assessment of effectiveness of interventions

The average contribution that the stream reach makes to EC and IE loadings within the Tamar catchment under the various conditions, evaluated as the change in flux from S1 to S4 (expressed as cfu s^{-1}), together with the percentage inputs derived from the main cattle access points in Fields A and B before intervention, are presented in Table 5. Clearly, variations in the magnitude of the FIO flux changes recorded are partly attributable to differences in the volumes of flow between the relatively dry conditions of Phase 1 and the much wetter conditions of Phase 2. Summary data for the key impacts investigated are presented in Table 6.

3.7.1. Before intervention/Low flow: Impact of cattle in Field A (no cattle in Field B)

With no cattle present in Field A [I], the overall change in mean EC load down the reach is quite small. The main drinking area accounts for 77% of the increase, which is perhaps attributable to the release of residual FIOs from streambed sediments. In the case of IE, a very small overall reduction in load is recorded. The marked increases in FIO concentrations recorded when cattle were present [II] are reflected in increases in mean FIO fluxes down the reach, with 73% and 82%, respectively, of the increases in EC and IE being derived from the main drinking area. These results demonstrate the impact of cattle upon the FIO loadings from an unfenced pasture, with the average EC and IE inputs along the reach increasing by 2.363 $\log_{10}$ and 4.653 $\log_{10}$, respectively (Table 6). These large increases in loads highlight the impact that cattle access to streams can have upon microbial pollutant loadings at low flow, presumably as a result of defecation in the stream, the washing off of organisms attached to the legs of animals, and disturbance of streambed sediments through trampling.

3.7.2. Before intervention/Low flow: Impact of cattle using drinking bay in Field B

Small increases in EC and IE loads were recorded along the 16 m stretch through the bay [III]. Assuming the FIOs are entirely derived from within the bay, then the EC and IE flux increases through the bay are 3.7×10^4 and 1.9×10^3 cfu s^{-1}. Assuming, further, that other FIO inputs to the stream from cattle in Field B would be negligible along the 271 m reach at low flow, then the EC and IE inputs from the bay are 1.608 and 1.374 $\log_{10}$ less than those recorded at low flow when cattle had unrestricted access to the stream from Field A [II]. On the basis of these results, cattle have a smaller impact in a pasture with SBF and associated drinking bay than unfenced pastures. However, the magnitude of the differences does need to be interpreted with some caution, as cattle had only been using the bay for 1 or 2 d at the time of sampling.

3.7.3. Before intervention/High flow: Impact of cattle in Field A

Only one sampling run was undertaken at high flow with no cattle in Field A [IV], and the discharge was lower than the remaining high-flow runs. EC fluxes through the reach increased, whereas for IE a small loss was recorded. This reduction is difficult to explain, since at high flow there will be limited opportunity for die-off as the deeper and more turbid waters are transmitted quite rapidly down the reach, and seems unlikely to be unrepresentative.

In the two high-flow runs undertaken with cattle in Field A [V], there are marked increases in the mean EC and IE fluxes down the reach (Table 5), with smaller proportions of the increases (46% and 52%, respectively) being derived from the main drinking area compared with at low flow. This demonstrates that streamside grazed pastures are significant pollutant sources at high flow, with FIOs being detached from cowpats and transported by rainsplash and surface runoff/ditch flow (Table 4b/c) as surface flow extends headwards into the catchment. Defecation within the riparian zone also represents a potentially significant source, as a result of rising water levels and localised streamside runoff, particularly in areas poached by cattle trampling. In the case of EC, the presence of cattle increases the load inputs to the reach by 1.571 $\log_{10}$ cfu s^{-1} (Table 6).

3.7.4. After intervention/Low flow: Impact of SBF

The mean changes in EC and IE loadings down the reach [VI] are far less than those recorded at low flow with cattle accessing the stream [II]. The differences recorded equate to reductions of 2.523 $\log_{10}$ and 1.000 $\log_{10}$, respectively (Table 6). What does need to be borne in mind, however, is that the low flows sampled in Phase 1 [II] were recorded as having a nominal discharge of 1.0 l s^{-1}, whereas in the low-flow runs of Phase 2 [VI] the flow was consistently higher (range, 16.0–27.5 l s^{-1}). It is likely therefore that, had flow in the two phases been similar, then the reductions in EC and IE loads as a result of the SBF would have been *c.* 3.5 $\log_{10}$ and 2 $\log_{10}$, respectively.

3.7.5. After intervention/High flow: Impact of SBF

The overall changes in EC and IE fluxes down the reach after intervention [VII] show reductions of 0.842 $\log_{10}$ and 2.206 $\log_{10}$, respectively, compared with when cattle were present at high flow before intervention [V]. The flows recorded in the high-flow sampling in Phase 1 (peak discharge, 169 l s^{-1}) were also substantially lower than in two of the Phase 2 runs, which had peaks of 307 and 904 l s^{-1}. Had discharges been similar, then the reductions in EC and IE inputs to the reach as a result of the SBF would likely have been greater.

4. Discussion

4.1. Magnitude of FIO inputs to watercourses/riparian zones from cattle in unfenced pastures

Where unfenced watercourses provide the sole or main source of drinking water, then livestock inevitably spend time in the water/riparian zone, e.g. cattle typically spend 0.5–0.8% of time drinking (Bond *et al*., 2014; Sheffield *et al*., 1997). There is, however, evidence from the present study and others (Bagshaw, 2002; Bond *et al*., 2012; Sheffield *et al*., 1997;

United States Environmental Protection Agency, 2006) that cattle spend a disproportionately large amount of time in the watercourse (typically, 1–6%) and riparian zones (typically, 1–4%). Moreover, cattle also tend to defecate more frequently in watercourses than in the riparian zone and adjacent pastures (Bagshaw, 2002; Bond *et al*., 2014; Gary *et al*., 1983 – the mean of these studies being 5.7%). Defecation to watercourses clearly represents a very potent FIO pollutant source since the ‘fresh’ load enters the water with no opportunity for die-off (cf. defecation on land surfaces). A recent synthesis of 13 data sets has given a GM EC (including faecal coliform) burdens for mature cattle of $2.2\text{x}10^{10}$ cfu d^{-1} (Centre for Research into Environment & Health (CREH), 2017). Based on these data, the estimated EC input by cattle to unfenced watercourses is $1.3\text{x}10^{9}$ cfu animal^{-1} d^{-1}.

4.2. Impact of SBF with drinking bays

Much existing SBF in the UK has been erected for stock management purposes and/or to reduce bank erosion and suspended sediment loadings, rather than FIO loadings. In many cases the SBF includes drinking bays. In the present study cattle were found to spend only 0.8% of time in the bay – visiting to drink, but not spending much additional time there. This figure compares with the unfenced side of the stream reach where cattle spent an average of 12.5% of time in the stream/riparian zone. While the limited data from the streambed trampling studies and two low-flow stream sampling runs indicate the bay to be a source of FIOs, SBF with a bay has undoubtedly reduced the magnitude of FIO loadings compared with unfenced pastures.

4.3. Effects of preventing access to streams upon cattle behaviour

The amount of time spent in the drinking bay of Field B (0.8%) before intervention and by the drinking troughs of Field A and B (1.1 and 0.5%, respectively) after intervention provide a measure of cattle's drinking needs. Since cattle in Field A spent 3.1% of time in the stream prior to SBF, they were clearly spending more time there than was necessary for drinking. Pre-SBF they also spent 11.7% of time in the riparian zone, whereas after intervention only 2.7% of time was spent within 5 m of the new SBF. Apart from being the only drinking water source, it would seem that the stream and riparian zone are attractive to cattle on summer days, presumably as a result of the cool stream water and shade afforded by the streambank vegetation. The interventions in Field A have thus not only eliminated cattle defecation in the watercourse and any faeces carried from the field being washed from their hooves and lower legs, but have also prevented defecation in the riparian zone and likely reduced defecation rates on the pasture close to the stream. In view of the runoff from the lower parts of Field A, these interventions could be significant in reducing FIO transmission to the stream under wet conditions.

SBF with a drinking bay substantially reduces the average time individual cattle spend in the stream and riparian zone compared with unfenced streams, thereby reducing defecation in these critical source areas. Closure of the drinking bay and provision of a water trough, located away from the stream, did not affect the time (*c.* 10%) that cattle spend close to the SBF, in the partial shade provided by the riparian woodland – which suggests that shade influences cattle behaviour on summer days.

4.4. Effectiveness of SBF in reducing FIO inputs to streams from grazed pastures

The present study has shown that stock exclusion through SBF considerably reduces EC and IE inputs to the stream reach under both low- and high-flow conditions. Times of high flow are critically important in terms of catchment FIO fluxes and their impact on coastal waters (Kay *et al.*, 2008). During high flows, EC and IE load inputs to the 271 m reach from Field A (i.e. along one bank of the stream) were reduced by 0.842 and 2.206 $\log_{10}$, respectively, as a result of SBF, and the reductions would likely have been greater if flows during high-flow sampling runs in Phases 1 and 2 been of similar magnitude. The only other UK studies of the impact of SBF on FIOs, both at a catchment scale, were undertaken in the Brighouse Bay (Kay *et al*., 2007) and Sandyhills catchments (Kay *et al*., 2005), Scotland. From the combined results of these studies it is estimated that in catchments with limited pre-existing SBF, complete fencing of streamside pastures would reduce high-flow presumptive EC and presumptive enterococci loadings by 1.019 and 1.421 $\log_{10}$, respectively (CREH, 2017). These figures suggest that SBF alone can reduce load inputs by at least 1 $\log_{10}$ under high-flow conditions.

4.5. Potential benefits of combining SBF with riparian vegetated buffer strips and grass swales

The results demonstrate that surface runoff and ditch flow are significant routes by which faecally contaminated water is transmitted to the stream under wet conditions. Vegetated buffer strips (VBSs) and grass swales have been found to be effective in attenuating FIO fluxes in diffuse runoff and channelised flow by *c*. 1 $\log_{10}$ (Kay *et al*., 2012). Creating riparian (R)VBSs by erecting fencing at a distance of say 5–10 m from a watercourse will have the dual benefits of preventing livestock defecation in the riparian/streamside zone and of attenuating FIO fluxes in diffuse runoff from adjacent grazed pastures (Collins *et al*., 2007). Reducing fluxes in spatially concentrated field runoff is more problematic. It may be possible to eliminate much of this flow by minor ‘reprofiling’ of the base of slopes during establishment of RVBSs, e.g. by filling in any micro-channels on slopes and breaches in stream banks. Development of a dense grass sward in critical parts of a RVBS and along ditches (to create grass swales) will further augment the SBF and fencing off of ditches. Clearly, implementing such measures would be more costly than conventional

SBF, in part because of the substantial strips of pasture land that would be taken out of production.

4.6. Attracting cattle away from unfenced streams

Whilst cattle visit the watercourse/riparian zone to drink, the present study has shown that, even under UK summer conditions, they tend to favour these areas, presumably attracted by the cooling water and the shade afforded the riparian vegetation. Attracting livestock away from unfenced watercourses therefore represents a possible means of reducing the time they spend in, or close to, the water. Most obviously this could be achieved through the provision of an alternative drinking source(s) well away from the riparian zone, ideally with some measure of shade (either from existing trees/hedges or a constructed shelter). Indeed, consideration should be given to creating more extensive shaded areas within pastures, e.g. by allowing sections of hedges to grow taller. Several US studies have shown that cattle spend less time in watercourses where alternative drinking sources are provided (Byers *et al*., 2005; Franklin *et al*., 2009). The only known reporting on this in the UK are qualitative observations on a dairy farm in Scotland which suggest that where cattle have access to both a stream and water trough, they prefer to drink from the trough (McGechan *et al*., 2008).

5. Conclusions

- Where present, pastures grazed by cattle contribute significantly to the FIO loadings discharged to coastal waters from rural catchments during the summer bathing season, especially where streamside pastures are unfenced and at times of high flow.
- Even under summer conditions in the UK, cattle spend a disproportionately large amount of time in the watercourse and riparian zone, presumably attracted by the cool water and shade provided by bankside trees and shrubs.

- The principal FIO transmission routes identified in the present study are direct defecation to the stream, and the release/mobilisation of FIOs from cowpats to the stream at times of high flow as a result rising water levels in the riparian zone, headward extension of ditch flow and surface runoff from adjacent pastures. Some FIOs become incorporated within streambed sediments, from which they may be released by disturbance by cattle trampling or under more turbulent flow conditions.
- Partial exclusion of cattle through SBF with a drinking bay greatly reduces the average time cattle spend in the water, and there is evidence of a consequent reduction in FIO load inputs along the reach at low flow.
- Total exclusion of cattle from streams (SBF with alternative drinking supply) is shown to reduce EC and IE inputs along the stream reach by *c*. 1–2 $\log_{10}$.
- Further reductions might be achieved by attenuating FIO fluxes in surface runoff and ditch flow by augmenting SBF with RVBSs and grass swales.

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TABLES

Table 1. Sets of conditions (I–VII) investigated in stream reach (Sites S1–S4), ditch (D1) and surface runoff (R1–R3) sampling runs and details of the sampling regime

Condition		Sampling runs (#)	Sites sampled[a]
(a) Before intervention			
I	Low flow/No cattle	5	S1–S4
II	Low flow/Cattle in Field A	4	S1–S4
III	Low flow/Cattle in Field B using drinking bay	2	S1–S4
IV	High flow/No cattle	1	S1–S4
V	High flow/Cattle in Field A	2	S1–S4, D1
(b) After intervention			
VI	Low flow/Cattle frequently in Field A, infrequently in B	5	S1–S4
VII	High flow/Cattle frequently in Field A, infrequently in B	3[b]	S1–S4, D1, R1-R3

[a] Sampling regime: during each low-flow sampling run, 6 samples were taken at each site at *c*. 1-h intervals; and in the high-flow runs typically ≥ 12 samples were taken at *c*. 0.5-h intervals

[b] D1 and R1–R3 were only sampled in the first 2 (of the 3) high-flow runs

Table 2. Average % of time cattle spend in particular locations within the fields during daytime

Location in field	Before intervention	After intervention
FIELD A[a]		
In stream: Upstream of S3	1.4	No access
In stream: From S3–S4 (inc. main access area in Field A)	1.7	No access
In riparian zone[b]: Upstream of S3	5.8	n.a.[c]
In riparian zone[b]: S3–S4 (inc. main access area in Field A)	3.6	n.a.
In or within 5 m of ditch	2.3	n.a.
Within heavily poached area by new drinking trough	n.a.	1.1
Within 5 m of new SBF	n.a.	2.7
Elsewhere	85.2	96.2
FIELD B[a]		
In drinking bay	0.8	No access
Within heavily poached area by new drinking trough	n.a.	0.5
Within 5 m of pre-existing SBF	9.2	10.7
Elsewhere	90.0	88.8

[a] Based on the following numbers of observations made before/after intervention: Field A 294/288; Field B 114/151

[b] Riparian zone: within 5 m of stream

[c] n.a. = not applicable

Table 3. Geometric mean FIO fluxes in background stream waters and after simulated trampling at sites T1–T3 and the changes resulting from trampling

Run[a]	Before or After intervention/Cattle activity in Fields A and B	EC flux (cfu s^{-1}): Background[b]	After trampling[c]	Change after trampling[d]	IE flux (cfu s^{-1}): Background[b]	After trampling[c]	Change after trampling[d]
T1: Section of stream relatively inaccessible to cattle from Field A							
1	Before/No cattle in A or B for > 50 d	4.7x10^{3}	1.1x10^{5}	1.1x10^{5} (2240)	1.4x10^{3}	1.4x10^{3}	0 (0)
2	Before/55 cattle in A for 7 d	1.4x10^{5}	2.4x10^{5}	1.0x10^{5} (71)	5.0x10^{4}	4.5x10^{4}	-5.0x10^{3} (-10)
4	After/SBF present for *c*. 1 y	9.4x10^{4}	1.7x10^{5}	7.6x10^{4} (81)	1.6x10^{4}	2.5x10^{4}	9.0x10^{3} (56)
T2: Drinking bay in Field B							
1	Before/No cattle in B for > 50 d	1.2x10^{4}	1.4x10^{4}	2.2x10^{3} (17)	1.2x10^{3}	1.1x10^{3}	-1.0x10^{2} (-8)
2	Before/No cattle in B for > 95 d	3.0x10^{5}	6.0x10^{5}	3.0x10^{5} (100)	8.4x10^{4}	4.8x10^{4}	-3.6x10^{4} (-43)
3	Before/35 cattle in B for 2 d	3.2x10^{4}	2.0x10^{5}	1.7x10^{5} (525)	3.2x10^{3}	1.4x10^{4}	1.1x10^{4} (338)
5	After/No cattle access for 34 days	6.0x10^{4}	5.8x10^{4}	-2.0x10^{3} (-3)	1.8x10^{4}	2.3x10^{4}	5.0x10^{3} (28)
T3: Main drinking area in Field A							
1	Before/No cattle in A for > 50 d	8.6x10^{3}	6.3x10^{4}	5.4x10^{4} (633)	1.3x10^{3}	2.0x10^{3}	7.0x10^{2} (54)
2	Before/55 cattle in A for 7 d	5.3x10^{5}	5.4x10^{6}	4.9x10^{6} (919)	8.9x10^{4}	2.5x10^{5}	1.6x10^{5} (181)
4	After/SBF present for *c*. 1 y	1.1x10^{5}	9.0x10^{4}	-2.0x10^{4} (-18)	2.6x10^{4}	1.8x10^{4}	-8.0x10^{3} (-31)

[a] Five separate simulation runs were conducted; the first two covered all three sites, whereas 3-5 targeted only one or two sites

[b] Based on geometric mean (GM) fluxes recorded upstream of trampling location immediately before trampling and 30 min after trampling

[c] Based on GM fluxes recorded downstream of trampling 1, 2 and 3 min after trampling

[d] Difference between the GM downstream and background fluxes; % changes shown in parentheses; -ve values indicate a recorded reduction in flux

Table 4. Range and geometric mean of EC and IE concentrations recorded at the top of the reach over both phases of the study (low- and high-flow conditions); and in ditch flow and surface runoff in Field A when cattle present during individual sampling runs

		EC (cfu 100 ml^{-1}):			IE (cfu 100 ml^{-1}):		
	n[a]	Minmum	Maximum	Geom mean	Minmum	Maximum	Geom mean
(a) Stream at top of reach (Site S1)							
Low-flow	16	2.0x10^{2}	1.7x10^{3}	5.3x10^{2}	3.7x10^{1}	4.3x10^{2}	1.4x10^{2}
High-flow	6	1.7x10^{4}	1.2x10^{5}	4.6x10^{4}	1.9x10^{3}	8.6x10^{4}	1.6x10^{4}
(b) Ditch flow (Site D1) at times of active flow (high-flow condtions) when cattle present in Field A							
Before: Ditch unfenced	2	1.4x10^{5}	9.6x10^{5}	3.6x10^{5}	2.9 x10^{4}	6.8x10^{4}	4.5x10^{4}
After: Ditch fenced off	2	5.2x10^{4}	6.1x10^{4}	5.6x10^{4}	2.2x10^{3}	8.1x10^{3}	4.3x10^{3}
(c) Surface runoff (Sites R1-R3) at times of active flow (high-flow condtions) when when cattle present in Field A							
R1	2	3.4x10^{4}	5.0x10^{4}	4.1x10^{4}	1.3x10^{3}	6.7x10^{3}	2.9x10^{3}
R2	2	5.2x10^{4}	6.1x10^{4}	5.7x10^{4}	1.0x10^{3}	5.8x10^{3}	2.4x10^{3}
R3	2	6.8x10^{4}	1.2x10^{5}	9.1x10^{4}	9.8x10^{2}	6.1x10^{3}	2.4x10^{3}

[a] Number of sampling runs for which data are available; details of sampling regime are reported in Table 1

Table 5. Change in mean fluxes of EC and IE (cfu s^{-1}) down the stream reach (from S1 to S4) under different sets of conditions, based on the arithmetic means of the fluxes recorded in individual sampling runs, and the % of the flux change attributable to inputs from the main cattle access points to stream: Field A (S3 to S4) and the drinking bay in Field B (S2 to S3) before intervention

Condition: Description[a]	EC: Mean flux change (cfu s^{-1})[b]	EC: % input from main stream access points[c]	IE: Mean flux change (cfu s^{-1})[b]	IE: % input from main stream access points[c]
Before intervention				
I: LF/No cattle (5)[d]	6.5×10^3	77 (A)	-1.3×10^2	100 (A)
II: LF/Cattle in A (4)	1.5×10^6	73 (A)	4.5×10^4	82 (A)
III: LF/Cattle in B (2)[e]	2.1×10^3	100 (B)	9.1×10^2	100 (B)
IV: HF/No cattle (1)[f]	1.1×10^6	20 (A)	-2.2×10^5	100 (A)
V: HF/Cattle in A (2)	4.1×10^7	46 (A)	9.8×10^6	52 (A)
After intervention				
VI: LF/Cattle in A (5)	4.5×10^3		4.5×10^3	
VII: HF/Cattle in A (3)	5.9×10^6		6.1×10^4	

[a] Flow: HF = High flow, LF = Low flow; A and B refer to Fields A and B

[b] Negative values indicate a reduction in load

[c] (A) = S3 to S4 in Field A, (B) = Drinking Bay in Field B; values of 100 indicate that the inputs from these access points exceed the total recorded for the reach

[d] Figures in parentheses show number of sampling runs (details of sampling regime are presented in Table 1)

[e] The actual EC and IE flux changes through the drinking bay are 3.7×10^4 and 1.9×10^3 cfu s^{-1}, respectively

[f] These figures must be regarded with caution since based on a single sampling run; the reduction in EC flux, based on a single sampling run, is not considered to be representative (see text)

Table 6. Summary of key impacts investigated on EC and IE fluxes (cfu s^{-1}) down the stream reach (S1 to S4): the flux differences presented are derived as the flux of first condition identified minus that of the second (as presented in Table 5)

Flow	Conditions compared	EC flux difference (cfu s^{-1})[a]	IE flux difference (cfu s^{-1})[a]
(a) Before intervention: Impact of cattle in Field A (cf. no cattle)			
Low	[II] Cattle in A – [I] No cattle	1.5×10^6 (2.363)	4.5×10^4 (4.653)[b]
High	[V] Cattle in A – [IV] No cattle	4.0×10^7 (1.571)[c]	n.d.[d]
(b) Before intervention: Impact of cattle using drinking bay in Field B (cf. cattle in unfenced Field A)			
Low	[III] Cattle in B – [II] Cattle in A	-1.5×10^6 (-1.608)	-4.3×10^4 (-1.374)
(c) Impact of streambank fencing in Field A			
Low	[VI] Fenced – [II] Unfenced	-1.5×10^6 (-2.523)	-4.1×10^4 (-1.000)
High	[VII] Fenced – [V] Unfenced	-3.5×10^7 (-0.842)	-9.7×10^6 (-2.206)

[a] +ve and –ve values indicate increases and reductions in fluxes, respectively; figures in parentheses present the $\log_{10}$ change in flux, derived as difference between the $\log_{10}$ fluxes recorded for the two sets of conditions

[b] Indicative only: a value of 1 has been inserted to replace the –ve flux changes recorded in the first set of conditions

[c] These figures must be regarded with caution since only one sampling run was undertaken for condition IV

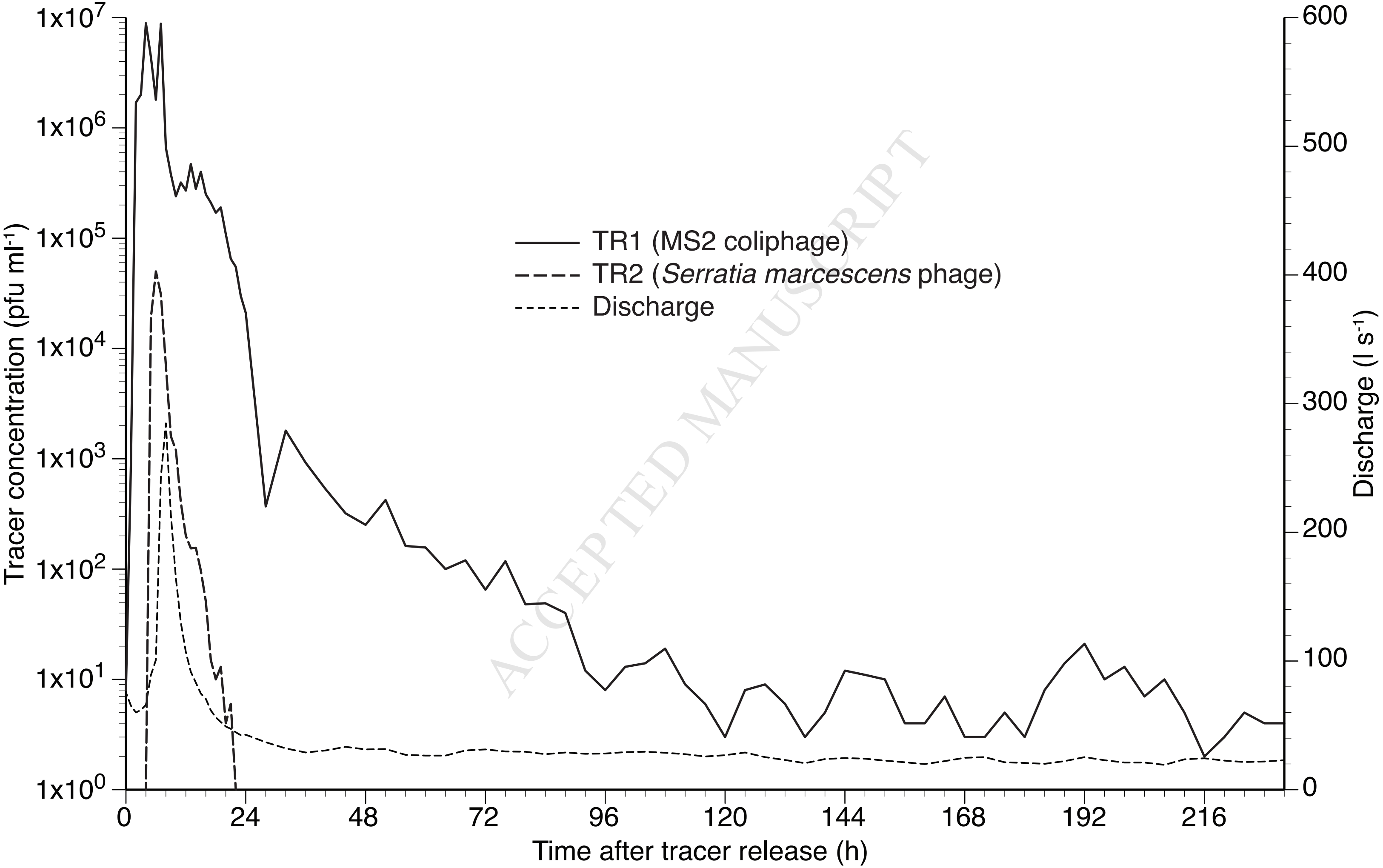
[d] n.d. = not determined as data considered to be unrepresentative (see Table 5)

Captions for figures

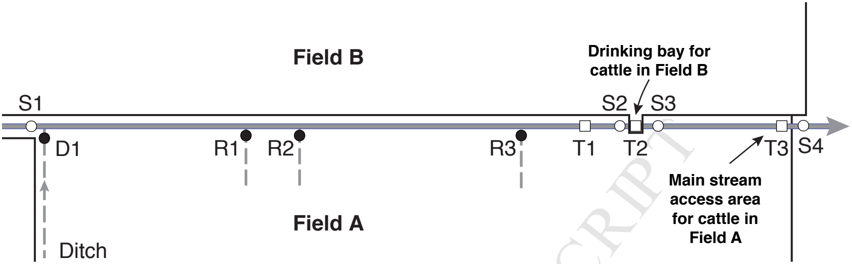
Fig. 1. Schematic plan of the study site before and after intervention

Fig. 2. Stream discharge and concentrations of the two tracers released at points TR1 and TR2 recorded at the bottom of the stream reach (S4) after release in the 'labelled' cow pats

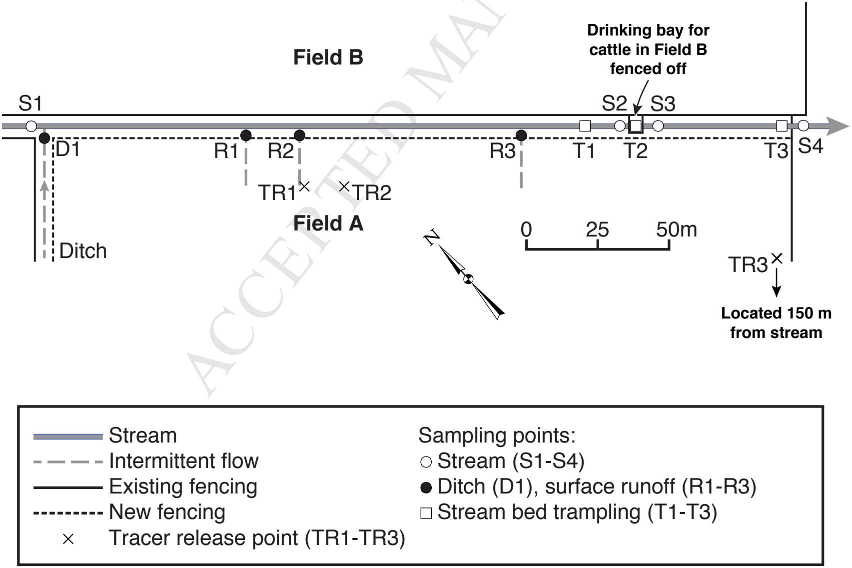
Fig. 3. Variations in GM EC and IE concentrations down the stream reach from sampling points S1–S4 under different sets of conditions (key: Before[B] or After[A] intervention/Low flow[L] or High flow[H]/Cattle status: Absent[0], Field A[FA], Field B[FB] or Field B infrequently[fb]): I = B/L/0, II = B/L/FA, III = B/L/FB, IV = B/H/0, V = B/H/FA, VI = A/L/FA+fb, VII = A/H/FA+fb (details in Table 1)


1x10^7
1x10^6
1x10^5
1x10^4
1x10^3
1x10^2
1x10^1
1x10^0
Tracer concentration (pfu ml-1)
600
500
400
300
200
100
0
Discharge (l s-1)
0
24
48
72
96
120
144
168
192
216
Time after tracer release (h)
TR1 (MS2 coliphage)
TR2 (*Serratia marcescens* phage)
Discharge

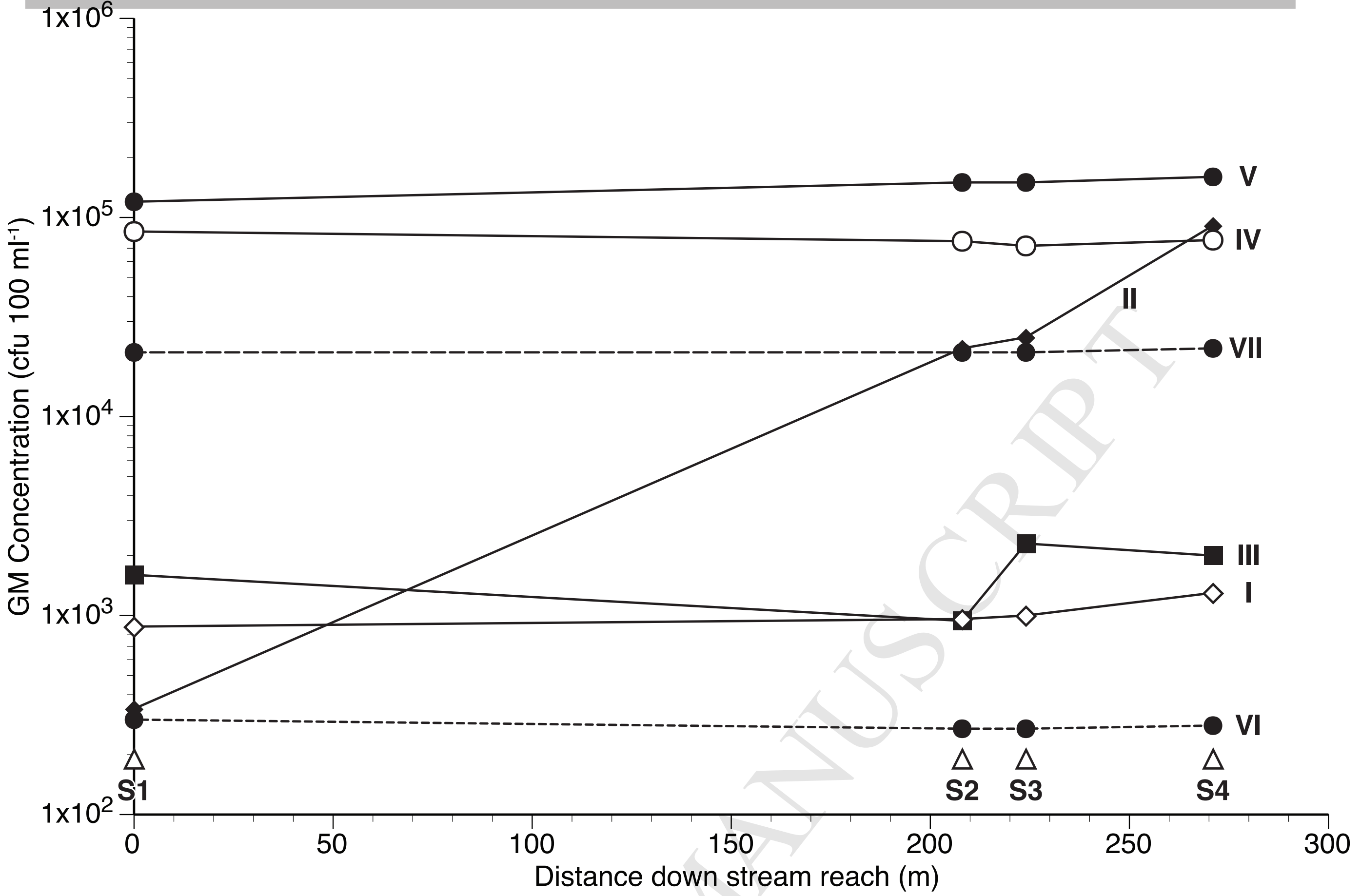
(a) Before intervention



(b) After intervention



(a) *Escherichia coli*



(b) Intestinal enterococci