

Strong selective pressures

Weak selective pressures

AMR dissemination in the environment

Professor Liz Wellington

WARWICK

THE UNIVERSITY OF WARWICK

Antibiotic resistance in the environment: **soil, sediments, water bodies**

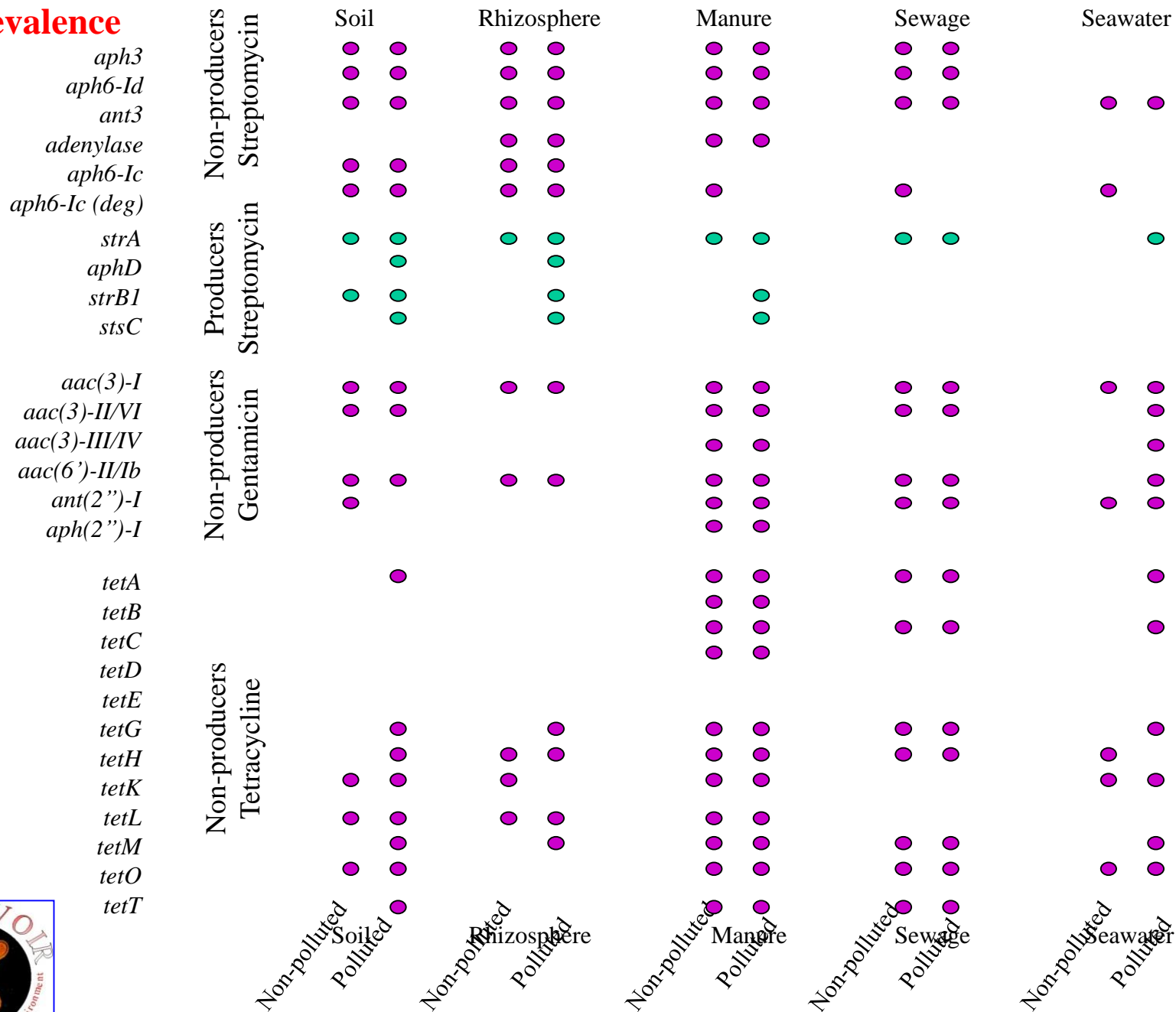
Environment acts as an reservoir for antibiotic resistance genes:

- associated with antibiotic biosynthesis clusters
- in closely related non-producers
- in unrelated non-producers indigenous soil bacteria
- **in unrelated non-producers exotic bacteria = pathogens/commensals added to soil**

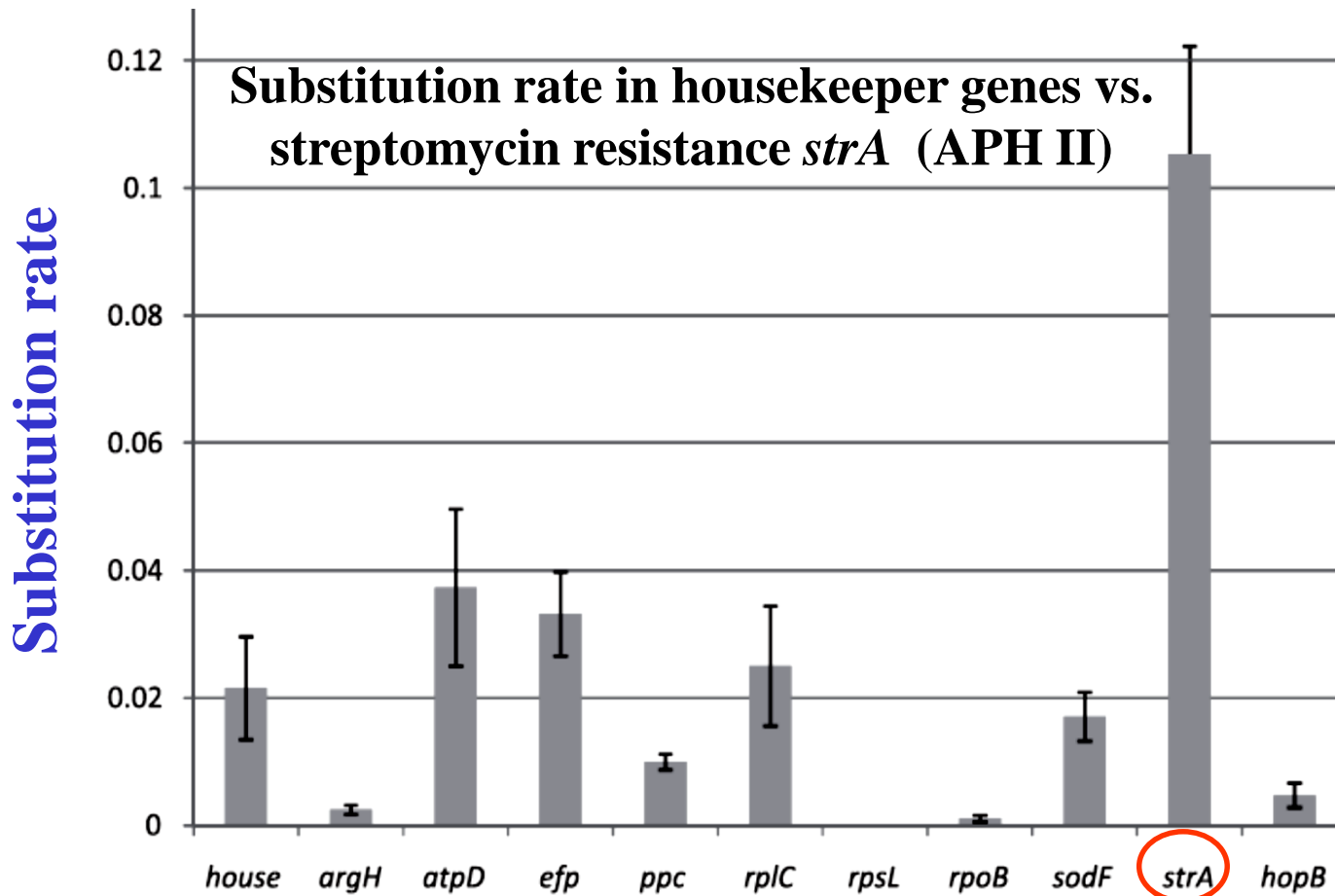
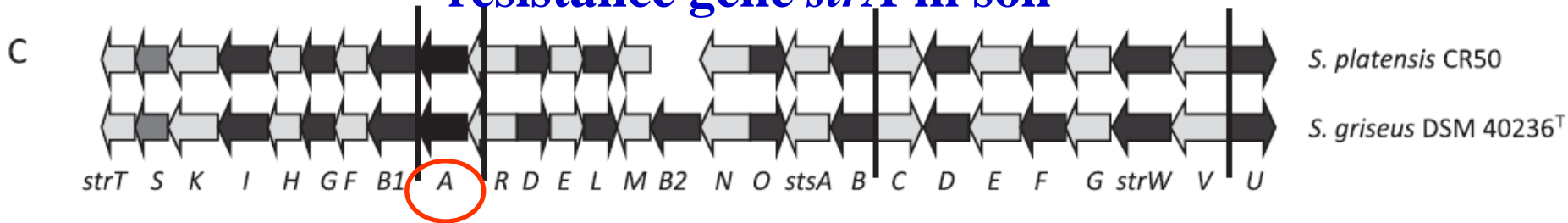
- Potential for selection of resistance -pollution
- HGT of resistance genes- mobilome
- Pathogens can survive in soil
 - Acquire integrons/plasmids
 - Act as source of antibiotic resistance

Reservoirs of antibiotic resistance genes in diverse environments: survey

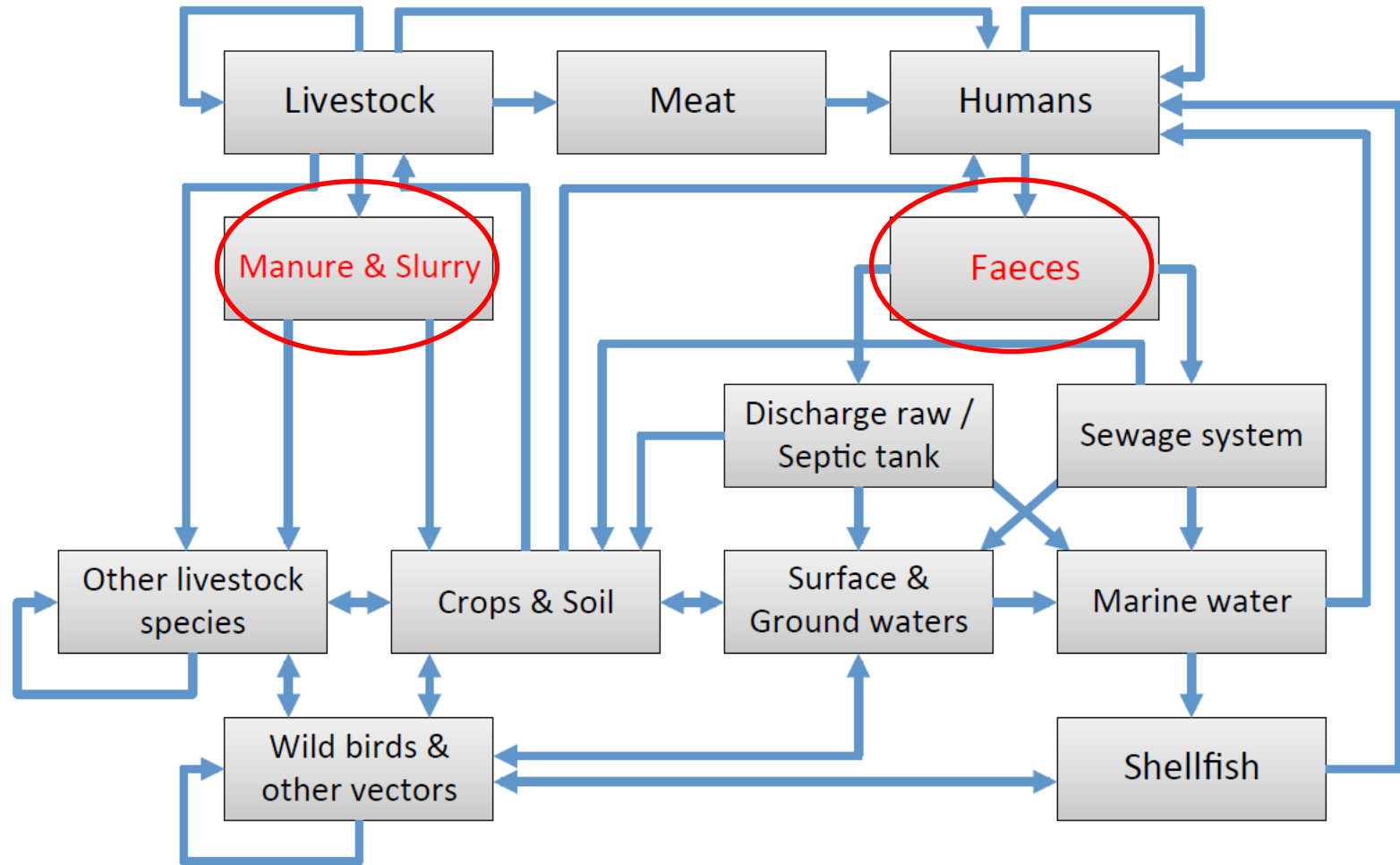
Prevalence



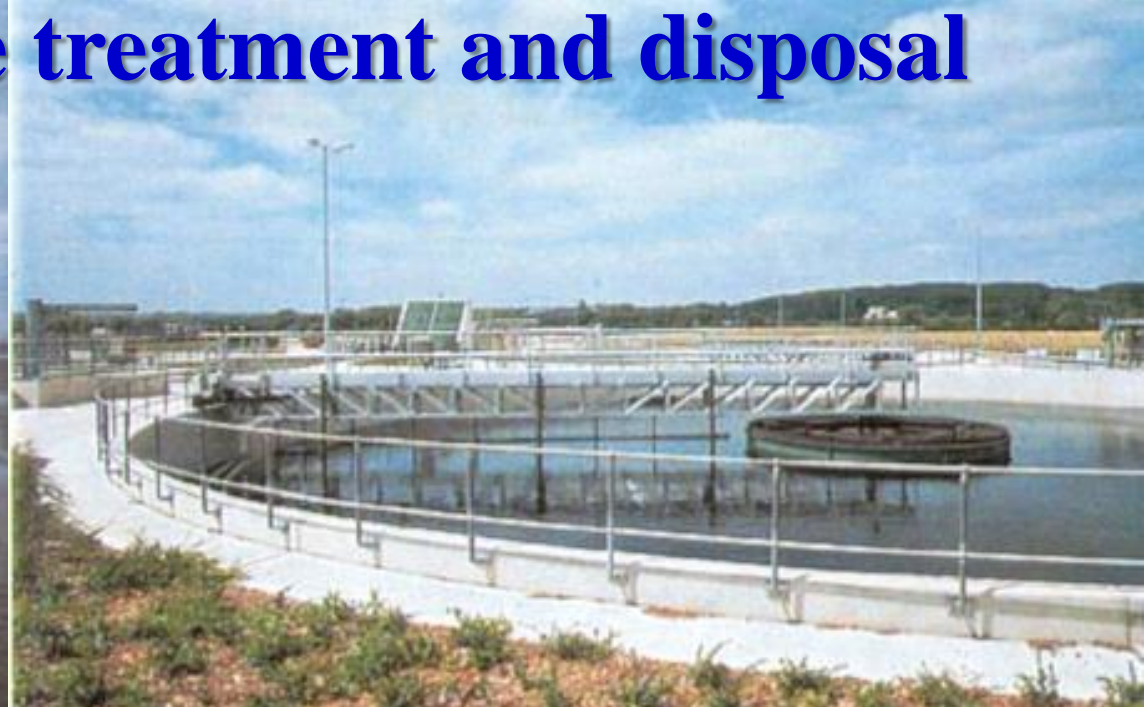
Streptomycin biosynthetic cluster and mobility of resistance gene *strA* in soil



The connectivity of potential sources of antibiotic-resistant bacteria



Sewage treatment and disposal



**Application of sewage sludge /biosolids/ manure to land:
what is the impact on antibiotic resistance in soil?**



Occurrence of antibiotics in the natural environment, fish, crops and drinking water from published studies

Antibiotic class	General behaviour	Sewage sludge	River water	Groundwater	Drinking water	Fish	Soil	Crops	Example monitored compounds
Chloramphenicol	impersistent/ mobile	-	√	X	-	-	-	-	-
2,4-diaminopyridines	persistent/ immobile	√	√	X	X	-	√	√	trimethoprim
Fluoroquinolones	persistent/ immobile	√	√	X	X	-	√	-	ciprofloxacin, norfloxacin, ofloxacin
β-lactams	impersistent mobile	-	X	X	X	-	-	-	amoxicillin, cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V
Macrolides	slightly persistent/ slightly mobile	√	√	X	-	-	-	-	azithromycin, clarithromycin, linecomycin, roxithromycin, spiramycin, tylosin
Sulfonamides	persistent/ mobile	√	√	√	X	-	√	√	sulfamethoxazole, sulfadiazine, sulfamerazine, sulfamethazine, sulfapyridine
Tetracyclines	persistent/ immobile	-	√	X	X	√	√	√	chlortetracycline, doxycycline, oxytetracycline, tetracycline

A tick means that it has been monitored for and detected and a cross means that it has been monitored for and not detected. No entry means that no monitoring has been done yet (Alistair Boxall)

Shampoo in the water supply triggers growth of deadly drug-resistant bugs

Household cleaning products are creating a bacterial timebomb in our drains and rivers

by Robin McKie

Science Editor

Fabric softeners, disinfectants, shampoos and other household products are spreading drug-resistant bacteria around Britain, scientists have warned. Detergents used in factories and mills are also increasing the odds that some medicines will no longer be able to combat dangerous diseases.

The warning has been made by Birmingham and Warwick university scientists, who say disinfectants and other products washed into sewers and rivers are triggering the growth of drug-resistant microbes. Soil samples from many areas have been found to contain high levels of bacteria with antibiotic-resistant genes, the scientists have discovered – raising fears that these may have already been picked up by humans.

“Every year, the nation produces 1.5m tonnes of sewage sludge and most of that is spread on farmland,” said Dr William Gaze of Warwick University. That sludge contains antibiotic-resistant bacteria whose growth is triggered by chemicals in detergents, he explained. “In addition, we pump 11bn litres of water from houses and factories into our rivers and estuaries every day, and these are also spreading resistance.”

The study is important because it



hospitals – is not linked to the use of disinfectants.

“Our research shows drug resistance is not confined to hospitals, but is out in the community. It is spreading and all the time it is eroding our ability to control infections. It is extremely worrying,” said Professor Liz Wellington, also of Warwick University.

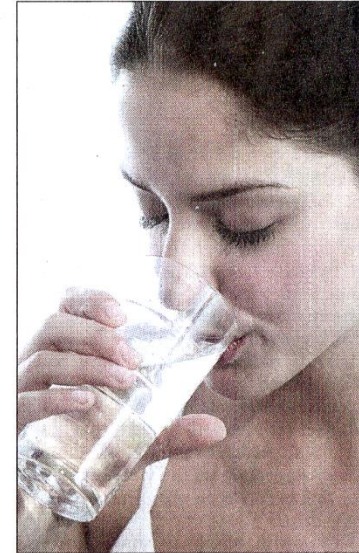
In their study, the scientists looked at quaternary ammonium compounds (QACs) that are used in many household cleaning goods. Every day, huge

ess,” said Gaze. “If other bacteria are killed, those that are resistant to QACs will survive and, without competition, will multiply in vast numbers. However, it turns out that the piece of DNA that confers that resistance also contains genes that confer resistance to antibiotics. In this way, we have created an ideal environment for the emergence of antibiotic-resistant bacteria in our drains and sewers. These microbes are now being spread round the country in river water and in sewage sludge used

techniques similar to those involved in DNA fingerprinting, they then looked for the presence of antibiotic-resistant genes – and found these in high concentrations.

“The inference is clear,” added Gaze. “We are producing sewage and river water that have more and more drug-resistant bacteria in them and that these are now poised to enter the food chain.”

Wellington added: “Once they are in the land, these bacteria will get into the bodies of agricultural workers or peo-



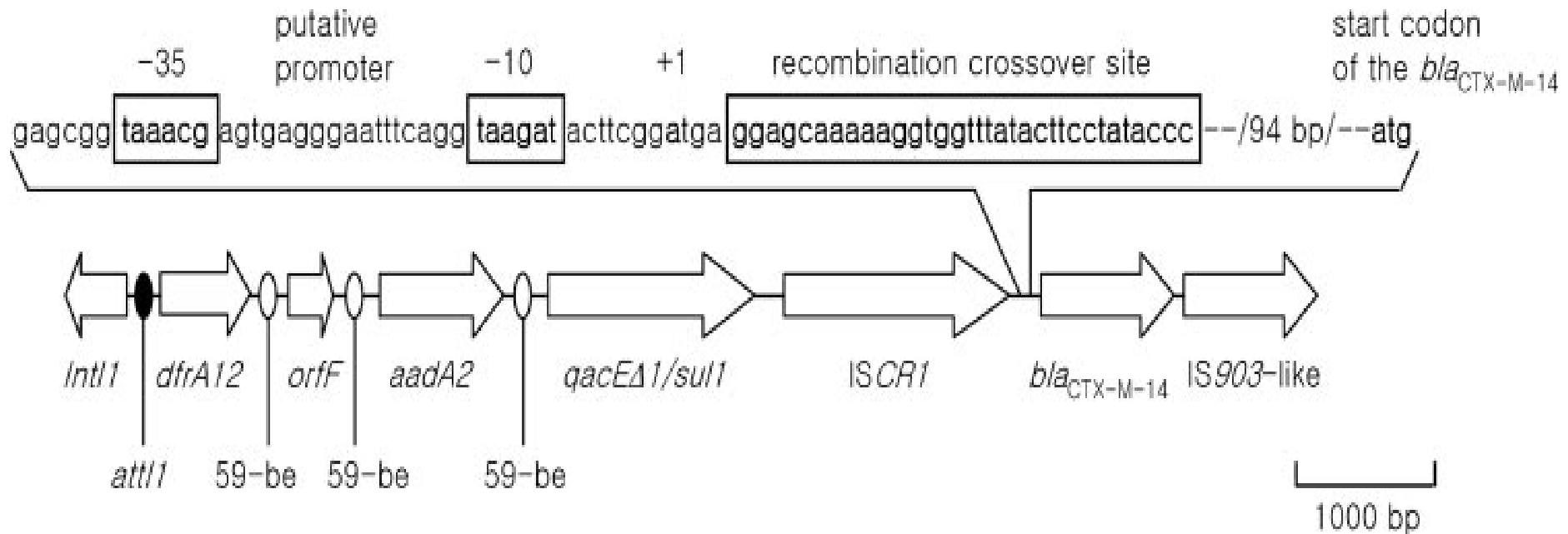
Detergents seen foaming in our rivers, left, are creating a breeding ground for drug-resistant bacteria in Britain's water system.

Environment Agency

– in particular pigs – are also helping to spread drug resistance in the soil. In their tests, the team found samples of pig slurry that possessed high levels of antibiotic-resistant genes, raising fears that strains of resistant bacteria were contaminating the land by another route and could enter the food chain.

“We might think of special measures that will help us control or localise drug-resistant bacteria in hospitals, but the problem is much more widespread than that,” added Wellington. “It is now out

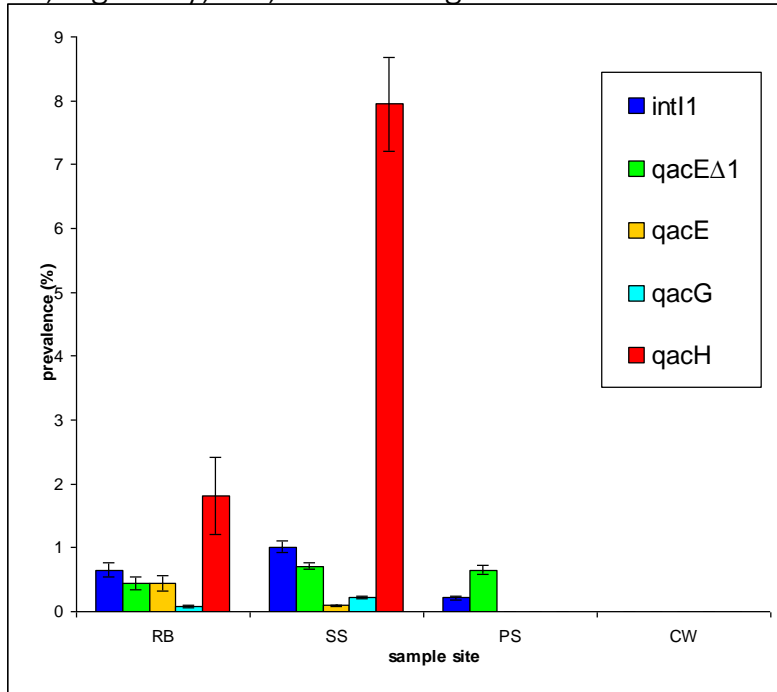
Schematic map of the complex class 1 integron carrying the *bla*_{CTX-M-14} gene on plasmid pAJE0508



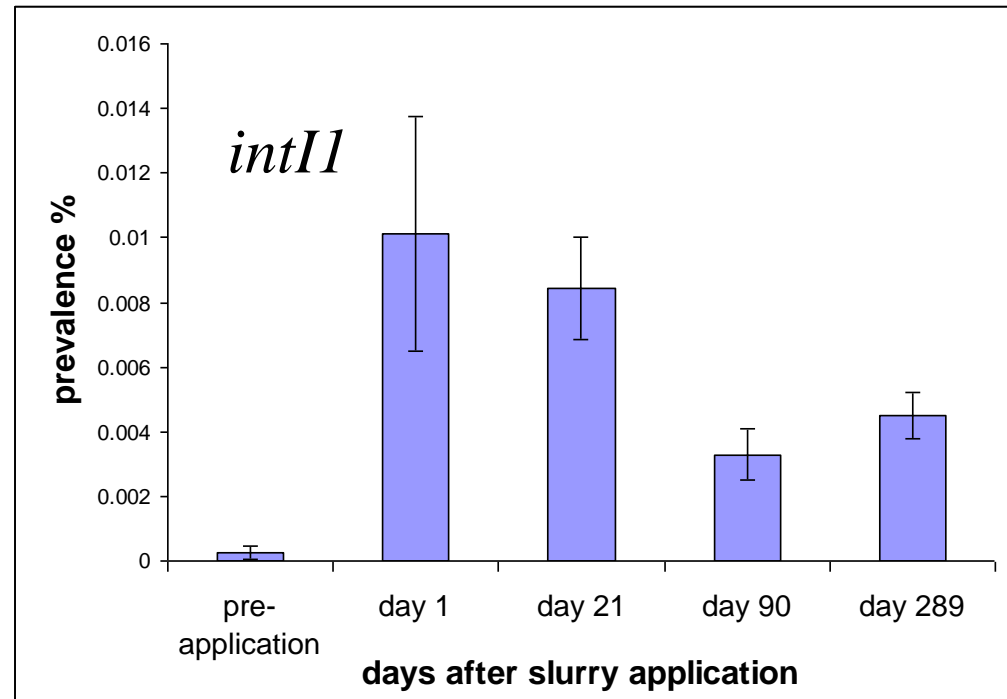
Bae et al., AAC, Aug 2007, 3017-19

Class 1 integron prevalence in sewage sludge, pig slurry and following application to land

RB, Reed bed sediment from textile mill;
SS, Fully digested sewage sludge;
PS, Pig slurry; CW, Fallowed agricultural soil



+ Pig slurry



- **90 million tons animal faecal slurry added to UK soils per year**

Gaze et al., 2011 ISME J ; Bailey-Byrne et al 2011 AEM

Low cost AMR carriage gives selection with very low exposure

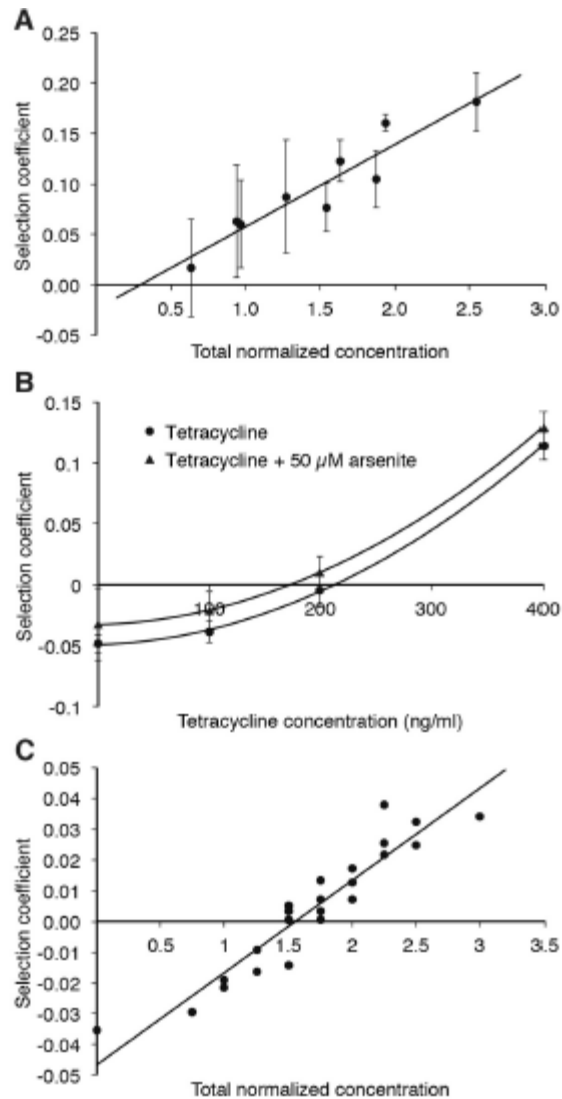


FIG. 2. Selection coefficients as a function of antibiotic and heavy metal

tion, the selective effect was less than the sum of the MICs (Fig. 2C, intercept on x axis is >1 MSC).

MSC is conditional and depends on the fitness cost of resistance. To further elucidate the dependence of MSC on the fitness cost of resistance, three of the resistance cassettes, *tetRA* (tetracycline resistance), *dhfr* (trimethoprim resistance), and the *mph* operon (erythromycin resistance), were transferred from the pUUH239.2 plasmid to the *Escherichia coli* chromosome by genetic recombineering (59). The results of the competitions between these strains and isogenic susceptible strains are shown in Fig. 3. These data demonstrate that the MSC is directly dependent on the fitness cost of the resistance. Thus, when the fitness cost conferred by the rest of the plasmid is eliminated, the selection coefficient curve is shifted upwards, thereby reducing the MSC value. For tetracycline, the MSC value was 1/25 of the MIC_{MSC} (Fig. 3A); for trimethoprim, the value was less than 1/100 (Fig. 3B); and for erythromycin, the value was less than 1/60 (Fig. 3C). These values represent absolute antibiotic concentrations of 30 ng/ml (tetracycline), less than 2 ng/ml (trimethoprim), and less than 200 ng/ml (erythromycin) for the chromosomally carried resistance compared to 45 ng/ml (tetracycline), 33 ng/ml (trimethoprim), and 3,000 ng/ml (erythromycin) when the corresponding resistance was present on the plasmid. As these resistance genes on the chromosome do not confer any significant fitness costs, the MSC values approach zero, making the MSC values for trimethoprim and erythromycin uncertain in this low range.

DISCUSSION

In this study, we investigated the selective effect of four clinically relevant antibiotics as well as two heavy metals on the maintenance of the ESBL plasmid pUUH239.2. This plasmid confers resistance not only to β -lactams but also to aminoglycosides, tetracycline, trimethoprim, sulfonamides, and erythromycin, as well as biocides such as quaternary ammonium compounds and the heavy metals copper, silver, and arsenic. Results show that selection of the multidrug resistance plasmid can occur at concentrations far below the MIC of a susceptible strain and that this general pattern is observed for many different classes of antibiotics and heavy metals. To exclude the possibility that any genetic changes might occur in the plasmid or chromosome during the course of the competition experiments (40 generations of growth), we performed whole-genome sequencing (WGS) of 12 independent lineages of plasmid-carrying bacteria that were grown for 40 generations in the presence of low levels of the different antibiotics and

Adaptive Landscapes of Resistance Genes Change as Antibiotic Concentrations Change

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Associate Editor: Arndt von Haeseler

Abstract

Most studies on the evolution of antibiotic resistance are focused on selection for resistance at lethal antibiotic concentrations, which has allowed the detection of mutant strains that show strong phenotypic traits. However, solely focusing on lethal concentrations of antibiotics narrowly limits our perspective of antibiotic resistance evolution. New high-resolution competition assays have shown that resistant bacteria are selected at relatively low concentrations of antibiotics. This finding is important because sublethal concentrations of antibiotics are found widely in patients undergoing antibiotic therapies, and in nonmedical conditions such as wastewater treatment plants, and food and water used in agriculture and farming. To understand the impacts of sublethal concentrations on selection, we measured 30 adaptive landscapes for a set of TEM β -lactamases containing all combinations of the four amino acid substitutions that exist in TEM-50 for 15 β -lactam antibiotics at multiple concentrations. We found that there are many evolutionary pathways within this collection of landscapes that lead to nearly every TEM-genotype that we studied. While it is known that the pathways change depending on the type of β -lactam, this study demonstrates that the landscapes including fitness optima also change dramatically as the concentrations of antibiotics change. Based on these results we conclude that the presence of multiple concentrations of β -lactams in an environment result in many different adaptive landscapes through which pathways to nearly every genotype are available. Ultimately this may increase the diversity of genotypes in microbial populations.

Key words: adaptive landscapes, antibiotic resistance, antibiotic concentrations, β -lactam, inhibitor resistant, epistasis, genotype-by-environment interactions.

Introduction

Bacteria are routinely exposed to a broad range of antibiotics that are present in a wide spectrum of concentrations due to their differential accessibility in the tissues of patients undergoing antibiotic therapy and in the environment outside the human body from the breakdown of antibiotics and their presence in agricultural runoff, wastewater, and food (Gullberg et al. 2011). This occurrence has been heavily documented (Gustafson 1991; Wegener 2003). For example, an-

of antibiotic use (Gustafson 1991; Wegener 2003). Considering the extensive use of antibiotics in clinical and agricultural environments, it is not surprising to find evidence that sublethal concentrations of antibiotics are important selective pressures acting upon bacteria (Blazquez et al. 2012; Hughs and Andersson 2012). There is abundant evidence that sublethal concentrations of antibiotics in the environment contribute to the increased frequency of antibiotic resistance mutations among microbial popu-

Waste water treatment plants as a reservoir for antibiotic resistance

Waste Water treatment plants
Hotspot for Horizontal Gene Transfer
(HGT) as waste received from
various sources

Little is known about the impacts
of effluent further downstream in
the river or the possible role of
co-selection of antibiotic resistant
determinants via quaternary
ammonium compounds (QACs)
(Gaze *et al.*, AAC 2005, ISMEJ
2011)



The risk of consuming 3GC resistant coliforms equal to or greater than the dose needed for colonization can be calculated using the inverse cumulative Poisson distribution

$$P = 1 - e^{-\lambda} \sum_{i=100}^k \frac{\lambda^i}{i!}$$

P = probability of being colonized by a 3GC resistant coliform. λ = average number of 3GC coliforms consumed, which is equal to number of 3GC coliforms multiplied by the amount of water consumed (ml). $i = 100$, the number of coliforms needed for colonization.

The volume of water consumed for > 99% probability of transient colonization of a 3GC resistant coliform at minimum levels of sediment disturbance was 12.5 ml downstream and 58 ml upstream, and under high levels of sediment disturbance, will decrease to 1.3 ml downstream and 5.8 ml upstream.

Children swimming (37 ml of water consumed on average) downstream of treatment plants have a $P > 99\%$ chance of being transiently colonized by a 3GC resistant coliform.

Upstream of the WWTP, even under high levels of sediment disturbance, only swimming carried risks of colonization by 3GC resistant coliforms.

ARTICLE

Received 18 Mar 2015 | Accepted 21 Aug 2015 | Published 30 Sep 2015

DOI: 10.1038/ncomms9452

OPEN

Limited dissemination of the wastewater treatment plant core resistome

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& Morten O.A. Sommer¹

Horizontal gene transfer is a major contributor to the evolution of bacterial genomes and can facilitate the dissemination of antibiotic resistance genes between environmental reservoirs and potential pathogens. Wastewater treatment plants (WWTPs) are believed to play a central role in the dissemination of antibiotic resistance genes. However, the contribution of the dominant members of the WWTP resistome to resistance in human pathogens remains poorly understood. Here we use a combination of metagenomic functional selections and comprehensive metagenomic sequencing to uncover the dominant genes of the WWTP resistome. We find that this core resistome is unique to the WWTP environment, with <10% of the resistance genes found outside the WWTP environment. Our data highlight that, despite an abundance of functional resistance genes within WWTPs, only few genes are found in other environments, suggesting that the overall dissemination of the WWTP resistome is comparable to that of the soil resistome.

ORIGINAL ARTICLE

Quantitative and qualitative impact of hospital effluent on dissemination of the integron pool

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There is increasing evidence that human activity, and especially the resulting effluent, has a major role in the dissemination of bacterial antibiotic-resistance determinants in the environment. Hospitals are the major antibiotic consumers and thus facilitate the spread of antibiotic resistance. Questions are increasingly being raised about the management of hospital effluents, but their involvement in antibiotic-resistance dissemination has never been assessed. Integrons are a paradigm of genetic transfer between the environmental resistome and both commensal and pathogenic bacteria. In order to assess the impact of hospital activities on antibiotic-resistance dissemination in the environment, we monitored integrons and their gene cassettes in hospital effluents, and their release in the environment. We found that bacterial communities present in a hospital effluent contained a high proportion of integrons. In terms of both their gene cassette diversity and gene cassette arrays, the urban effluent and municipal wastewater treatment plant (WWTP) influent were most similar, whereas the hospital effluent and recirculation sludge exhibited very specific patterns. We found that anthropogenic activities led to the release of abundant integrons and antibiotic-resistance gene cassettes, but we observed no specific impact of hospital activities on the receiving environment. Furthermore, although the WWTP did not reduce the normalized integron copy number, it reduced the diversity of gene cassette arrays contained in the raw wastewater, underlining the effect of the biological treatment on the anthropogenic integron pool arriving at the WWTP.

The ISME Journal (2014) 8, 768–777; doi:10.1038/ismej.2013.189; published online 24 October 2013

Subject Category: Microbial population and community ecology

Keywords: antibiotic resistance; effluent; hospital; integron; wastewater treatment plant

Combined sewer overflows Campaign

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regularly dispose of untreated sewage, even during times of low rainfall or none at all.

During the 2015 bathing season our Safer Seas Service reported on 926 sewer overflow events across the English beaches in the service. Only 11% of these discharges could have been monitored by the Environment Agency, leaving 821 discharges to impact the environment unchecked.

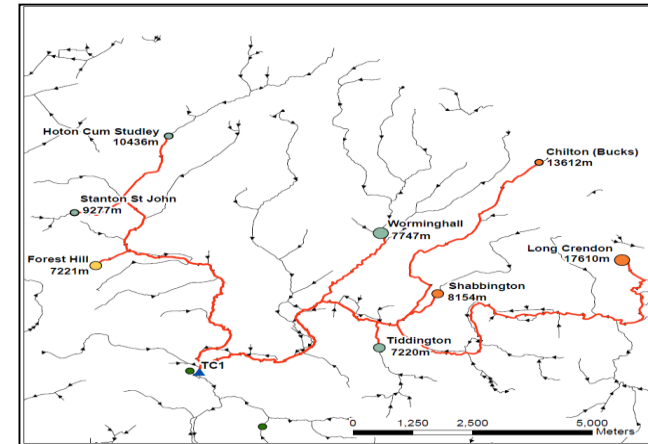
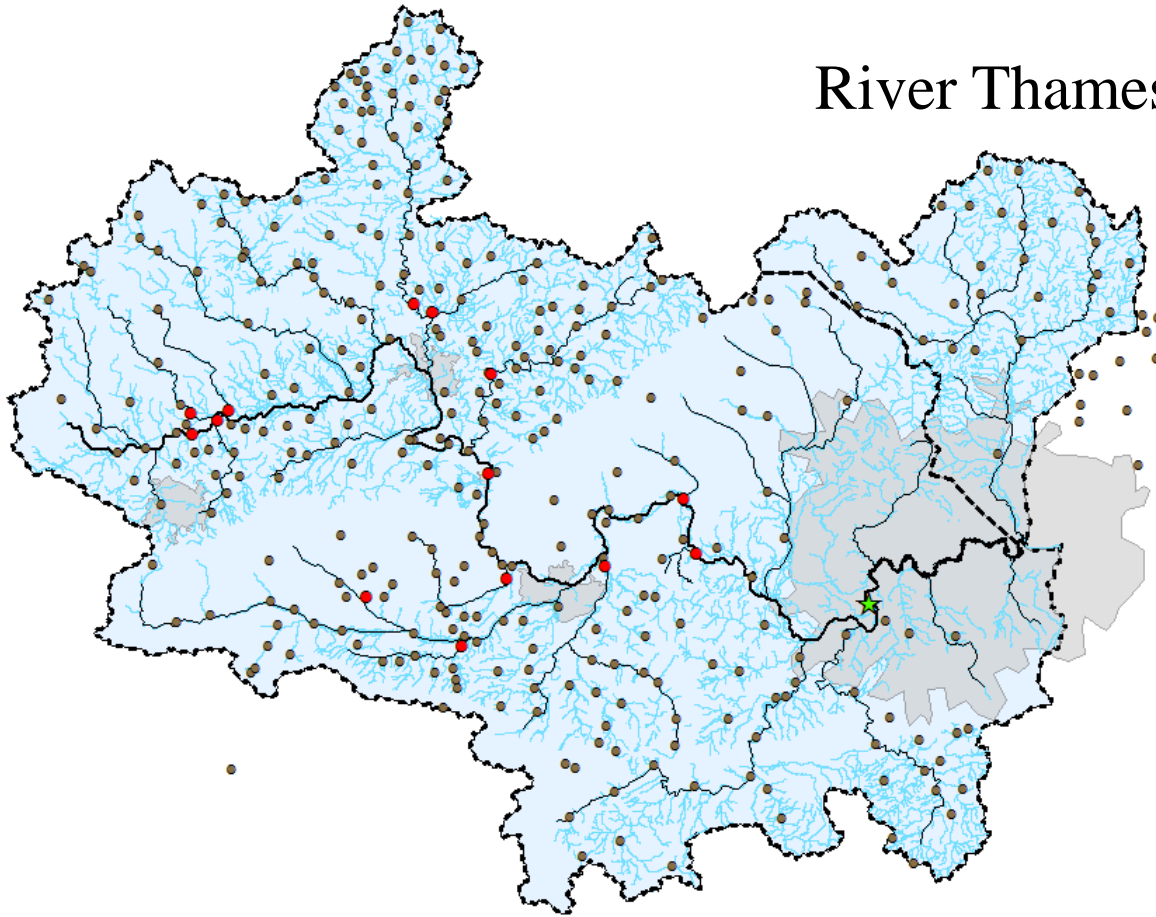
Surfers Against Sewage sits on a European Commission expert panel reviewing the bathing water directive, Defra's Cleaner Seas Forum and various regional Bathing Water Liaison Groups.

You can help us [lobby the Secretary of State for the Environment](#) calling for more action on the pollution impacting our beaches. We are calling for more urgent action to address on-going sewage pollution at our



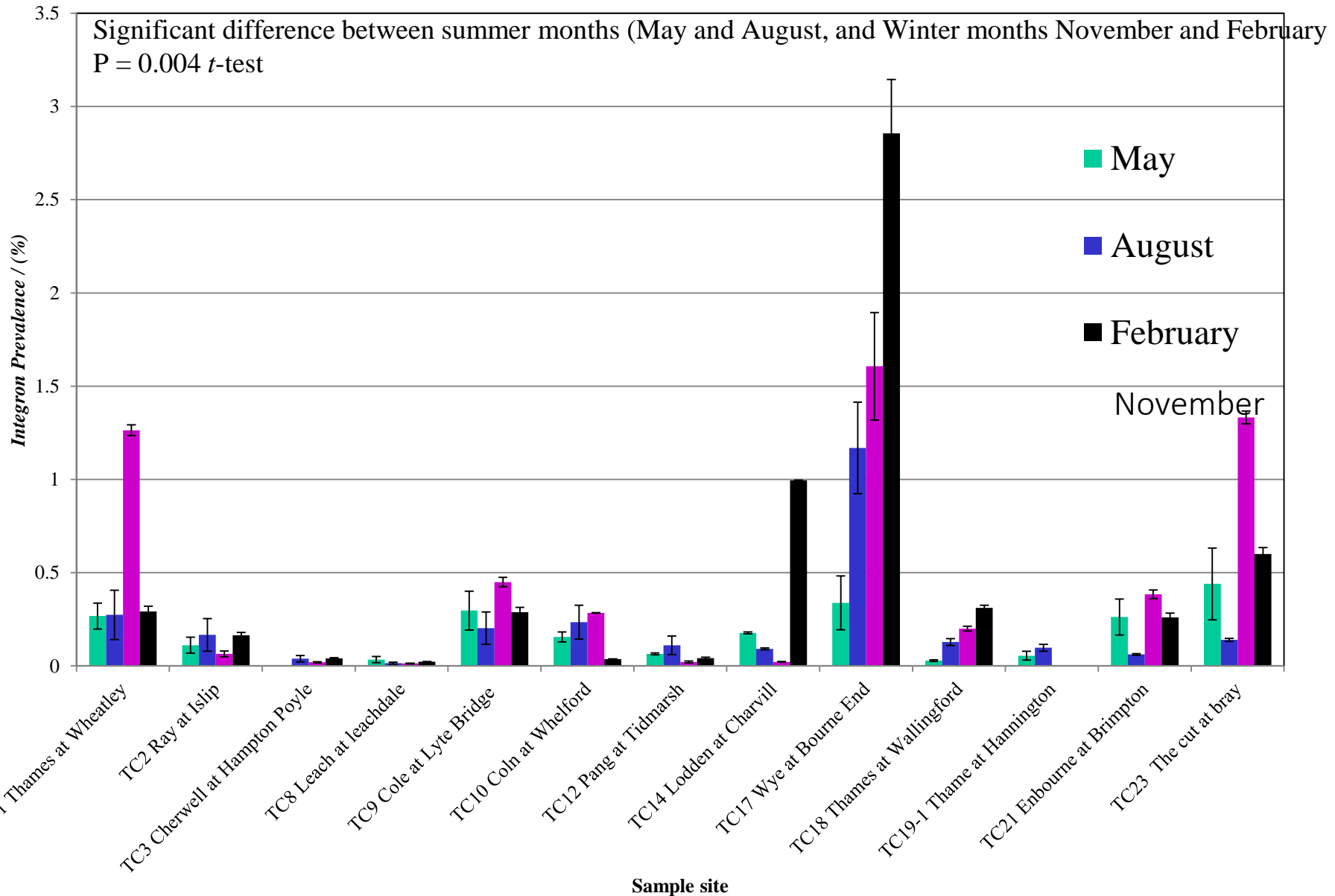
Contribution of WWTP effluent to integron levels in a whole river system

River Thames catchment area:

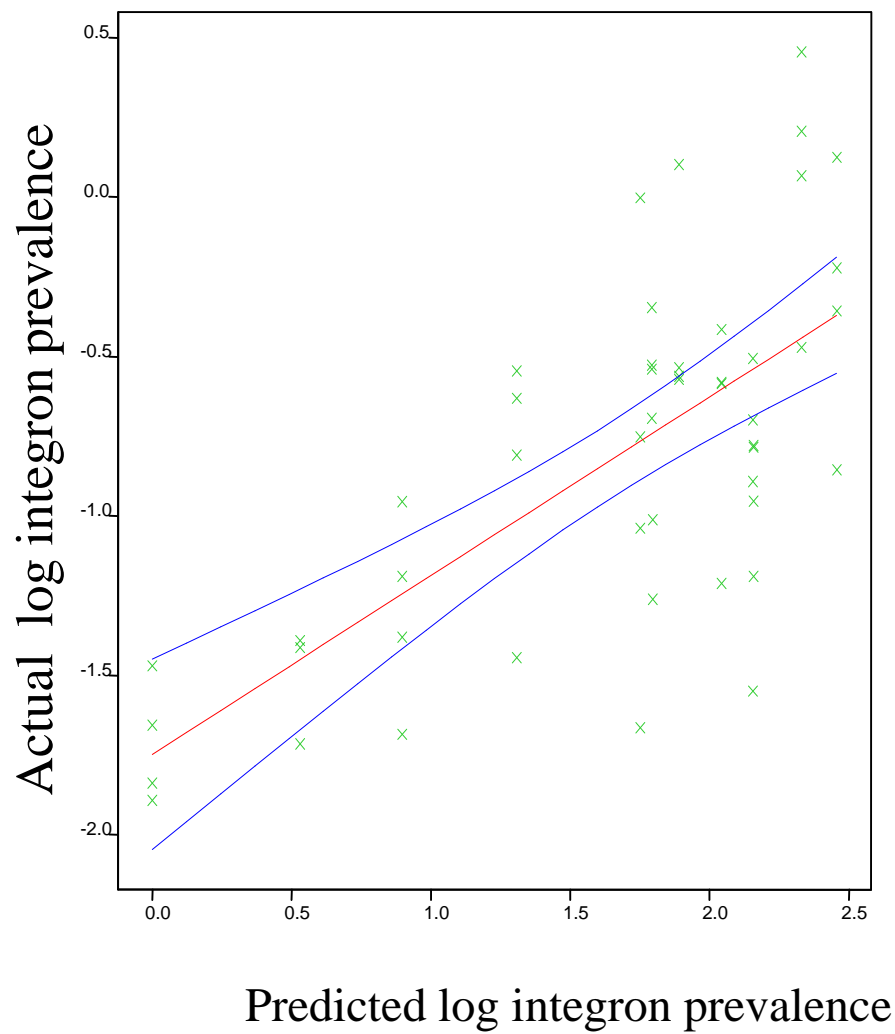


Collaboration with Wallingford CEH, meta-data available
13 sites samples every 3 months for a year: analysed for integron prevalence and 3GC resistance counts

Integron prevalence

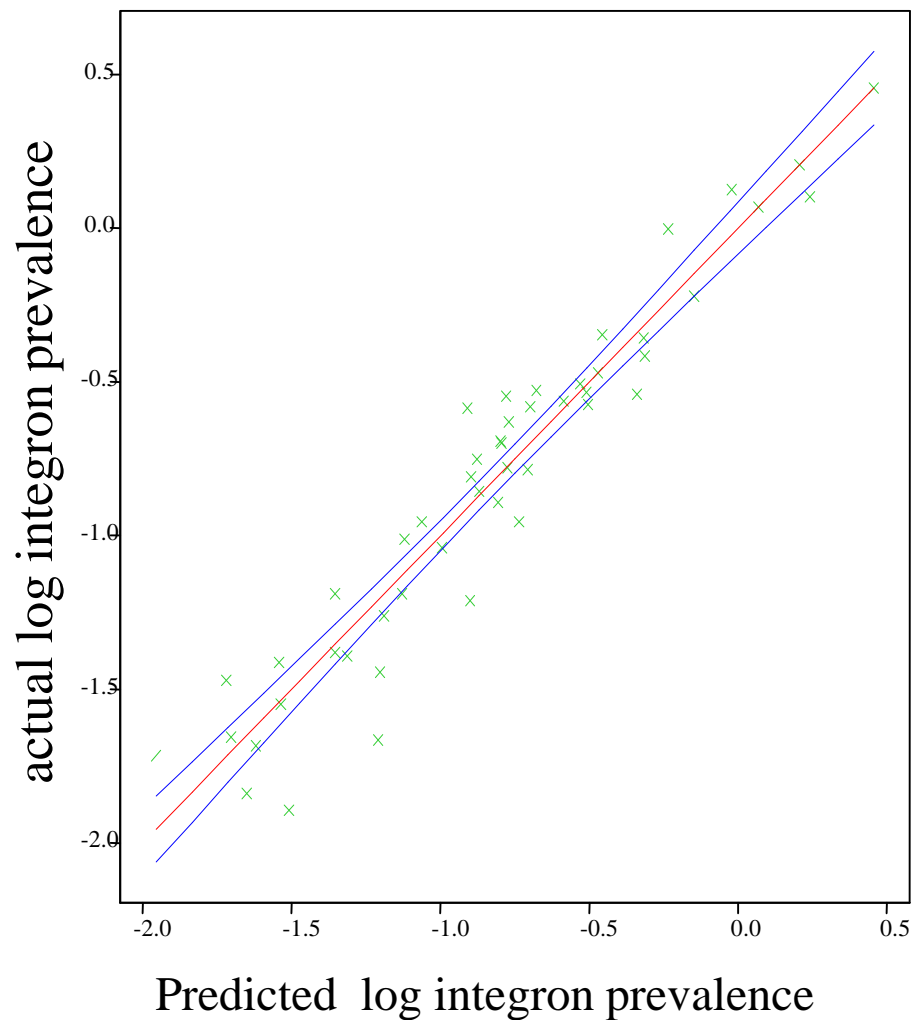


Output WWTP only



Explained 49 % of variance:
 R^2 adjusted \rightarrow (0.49) $P < 0.01$

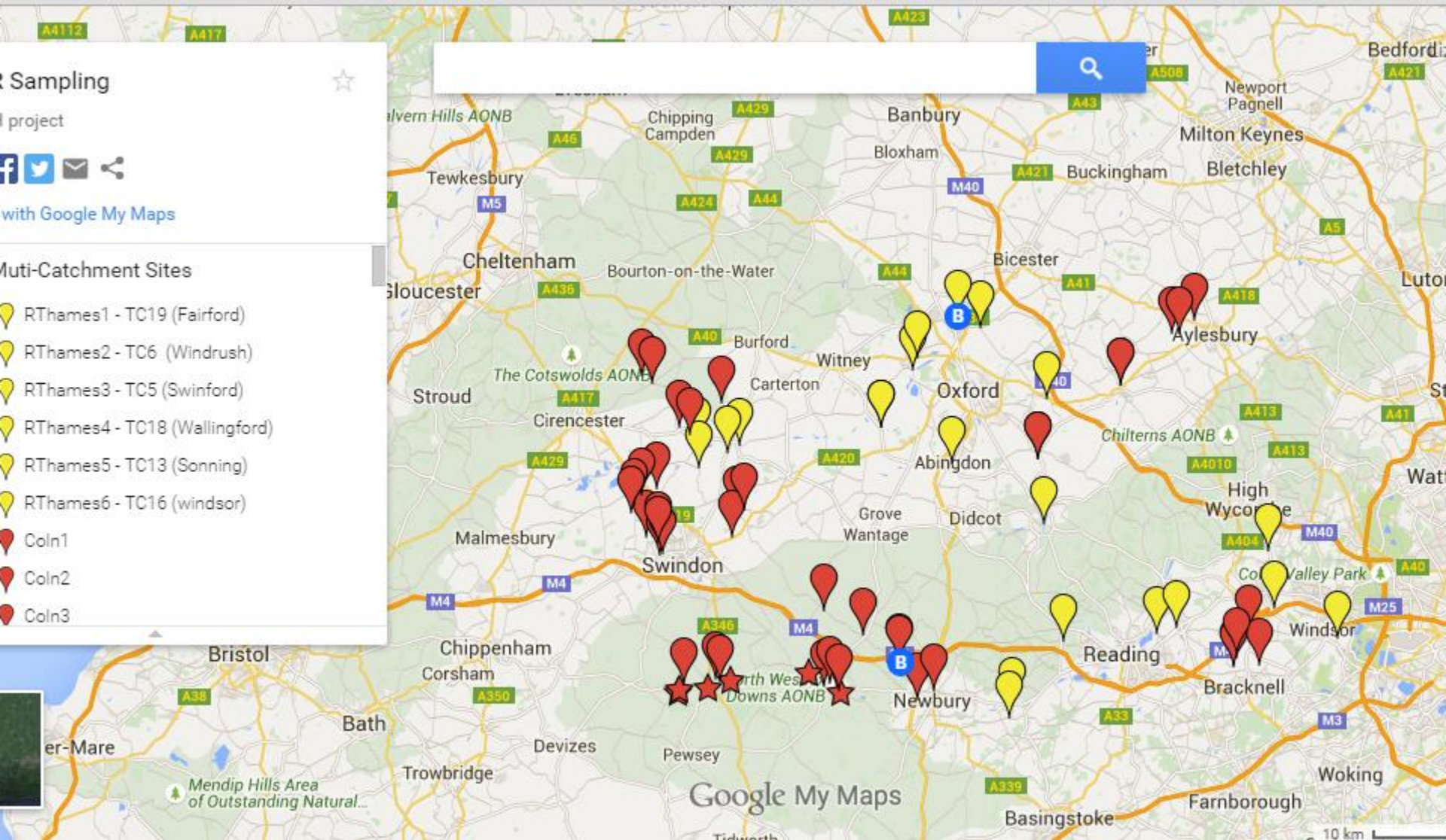
All metadata included



Explained 82.9 % of variance :
 R^2 adj (0.83) $P < 0.01$

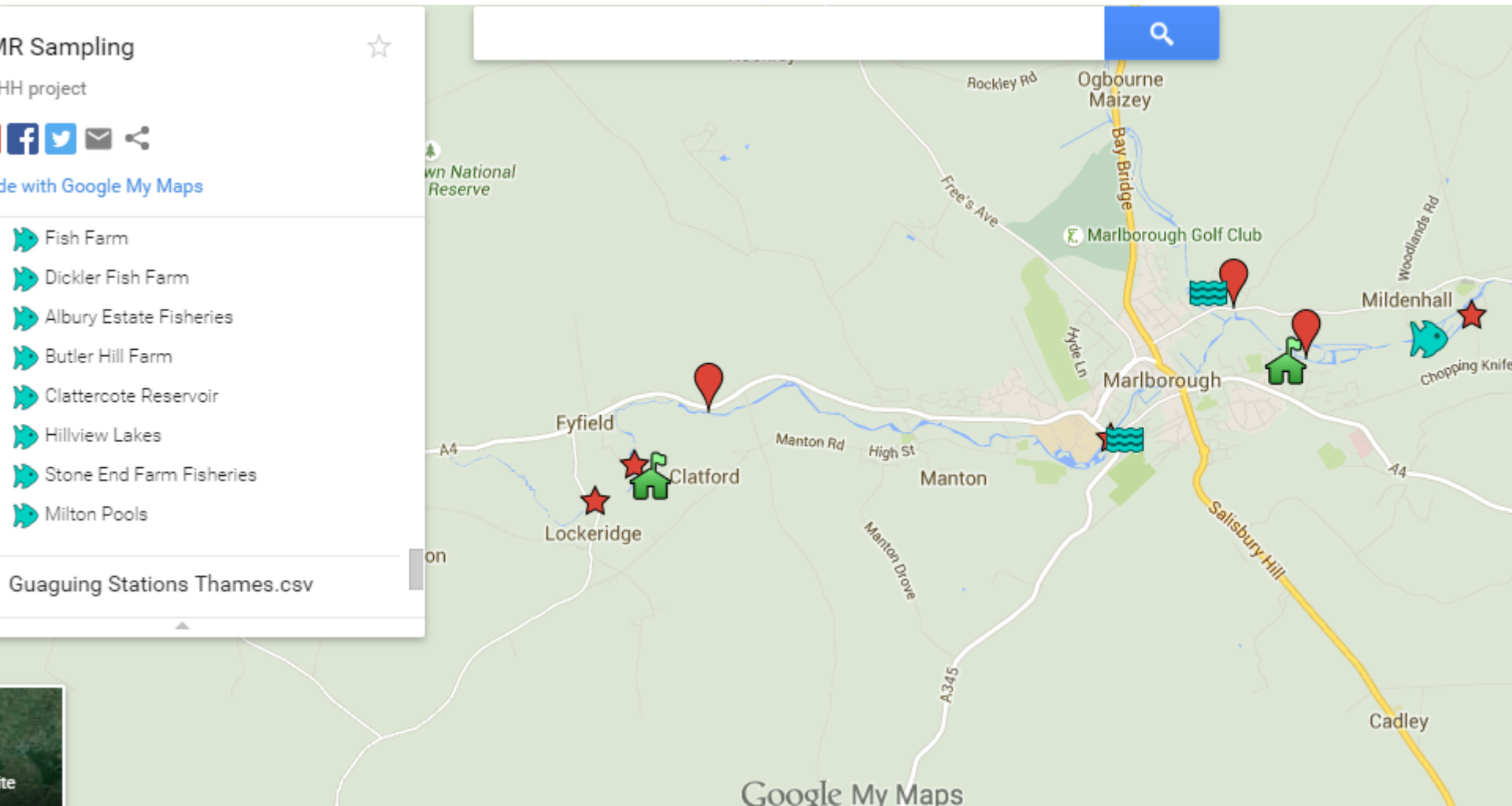
Amos et al., 2015 ISME J

New sampling campaign 2015-2017 Thames Catchment



New Campaign

Small scale intensive sampling, planktonic, sediment, direct and indirect



WWTPs, Monitoring stations and fishfarms

Acknowledgements

Past:

William Gaze
Greg Amos
Lihong Zhang
Kathy Byrn-Bailey
Paris Laskaris
Leo Calvo-Bado
Helen Green

Present:

Gemma Hill
Hayley King
Jennie Holden
Severine Rangama
Chiara Borsetto

Rothamsted Research

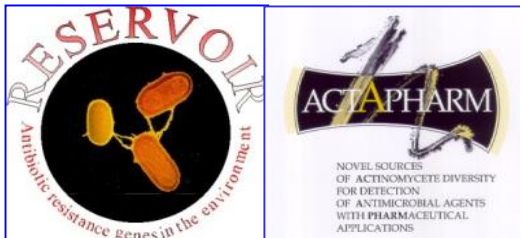
Andrew Mead

CEH Wallingford

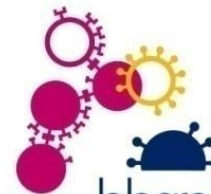
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