The Role of Water Quality in Food Safety: Does Water Matter?

International Association for Food Protection

Part 2: What Could Be In Municipal Water?

Monday, April 30 2018, 11:00 a.m. Central Time U.S.

Part 1 gave the basics of EPA rules and what they might mean.

But what could be in the water you get?

Learn what municipal water indicators indicate and whether they predict the presence of microbes that may impact the safety of your product.

Hear from **Dr. Shay Fout**, recently retired from the EPA about what indicators do and do not indicate, from leading researcher Arizona State University's **Dr. Paul Westerhoff** about De facto reuse, how wet weather and variability can impact food safety and the latest on heat resistant microbes from University of Alberta Professor **Norman Neumann** and what they could mean to food processors.

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Atlantium Technologies

Speakers

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Vice Dean for Research and Innovation –
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Does Water Matter?
Part 2: What could be in Municipal Water?

What Do Indicators Really Indicate?

G. Shay Fout
U.S. EPA, National Exposure Research Laboratory, ret.

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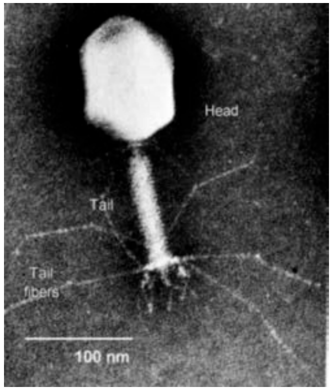
Virus and Indicator Relationships

Outline
- Indicators
- Virus occurrence studies
- Virus and indicator relationships
- Conclusions

Adenovirus

E. coli

Virus and Indicator Relationships



Somatic coliphage
c. 80 nm diameter
350 nm in length

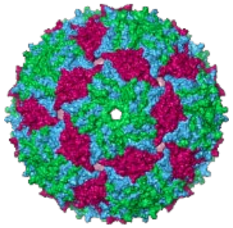
Indicators are microbial agents that indicate whether a pathogen (or just fecal pollution) is present

Perfect Indicators

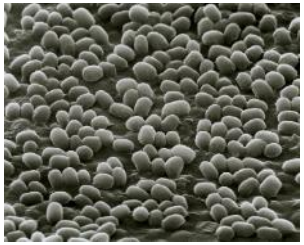
- **Must be present in higher concentration than pathogens**
- **Must always be present when pathogens are present**
- **Must always be absent when pathogens are absent**

There are no perfect indicators for virus occurrence

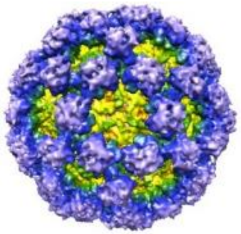
- **Bacterial indicators are always present in human stool while pathogens are only present when people are infected and then normally only for short periods**
- **Bacterial and bacteriophage indicators are excreted from animal as well as human sources, but most viral pathogens of concern are human-specific**
- **In general bacterial indicators die off faster than virus, so while their concentrations are higher than those of viral pathogens close to the source of contamination, the difference in concentrations decreases with time and distance**



F-specific coliphage (MS2)
27 nm



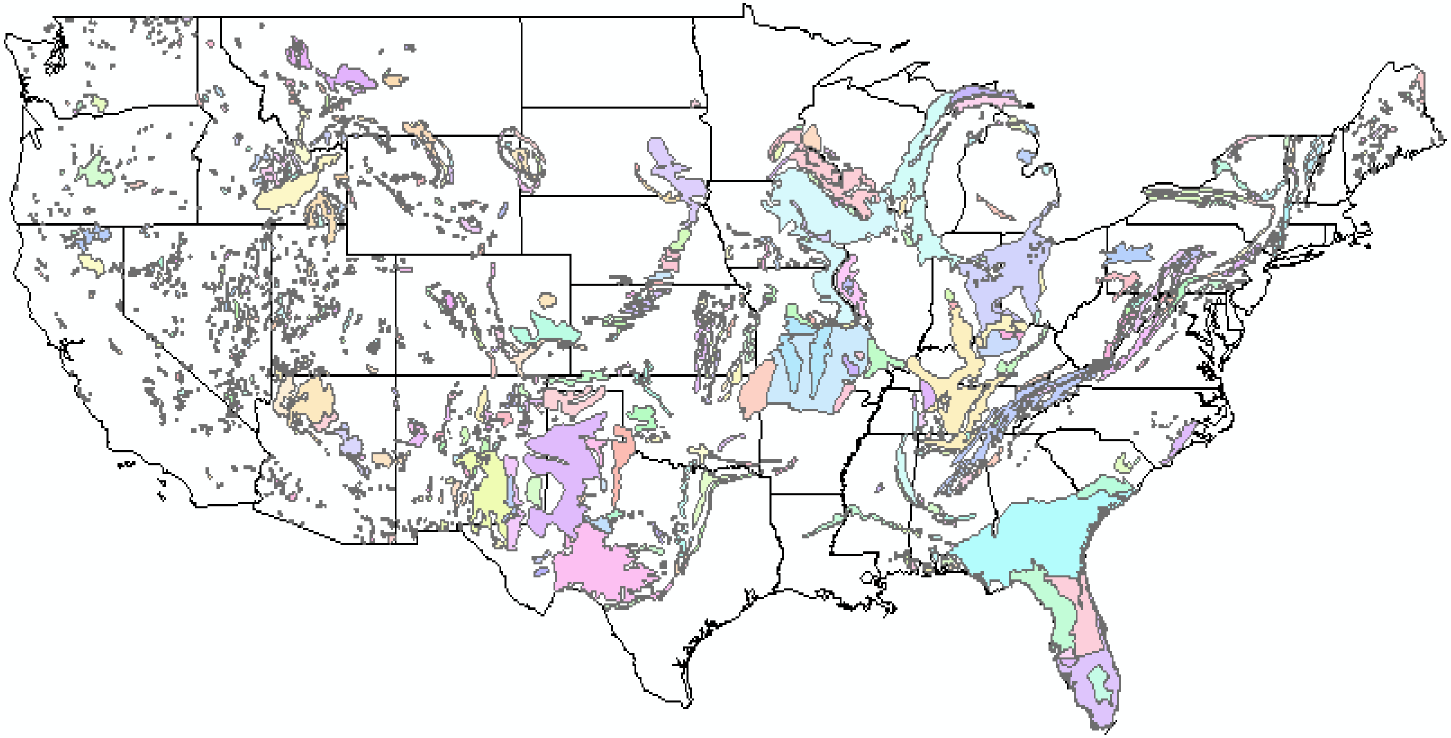
***Bacillus* spores**
c. 840 nm diameter
1,500 nm length



Norovirus
30-38 nm

Virus and Indicator Relationships

Why are indicators less valuable for groundwater?
It depends on the hydrogeology of the aquifer



Limestone and Karst Areas of the US (Tobin and Weary, 2005)

Virus and Indicator Relationships

Virus Occurrence Studies

Study	Number of Wells	Number of Samples	Study Dates
• EPA/AWWARF (US)	30	333	9/92-12/94
• USGS (MO)	182	322	5/97-7/98
• USGS/EPA (MI)	38	169	6/99-7/01
• USGS (PA)	60	60	9/00-2/01
• AWWSC (US)	20	235	3/01-5/02
• UT Knoxville (TN)	4	6	3/04-8/04
• Armand-Frappier (Canada)	36	243	3/04-12/12
• Univ. Rome (Italy)	8	14	6/05-12/05
• Univ. Tokyo (Japan)	46	46	11/05-1/06
• Marshfield Clinic (WI)	36	391	4/06-11/07
• NIER (Korea)	220	383	7/07-12/08
• Iowa DNR (IA)	66	71	3/13-6/13
• EPA (US)	823	1055	7/13-12/15
Totals	**1569**	**3328**	

References:

•Fout et al., 2017. Human virus and microbial indicator occurrence in public-supply groundwater systems: meta-analysis of 12 international studies. Hydrogeology Journal 25:903-917

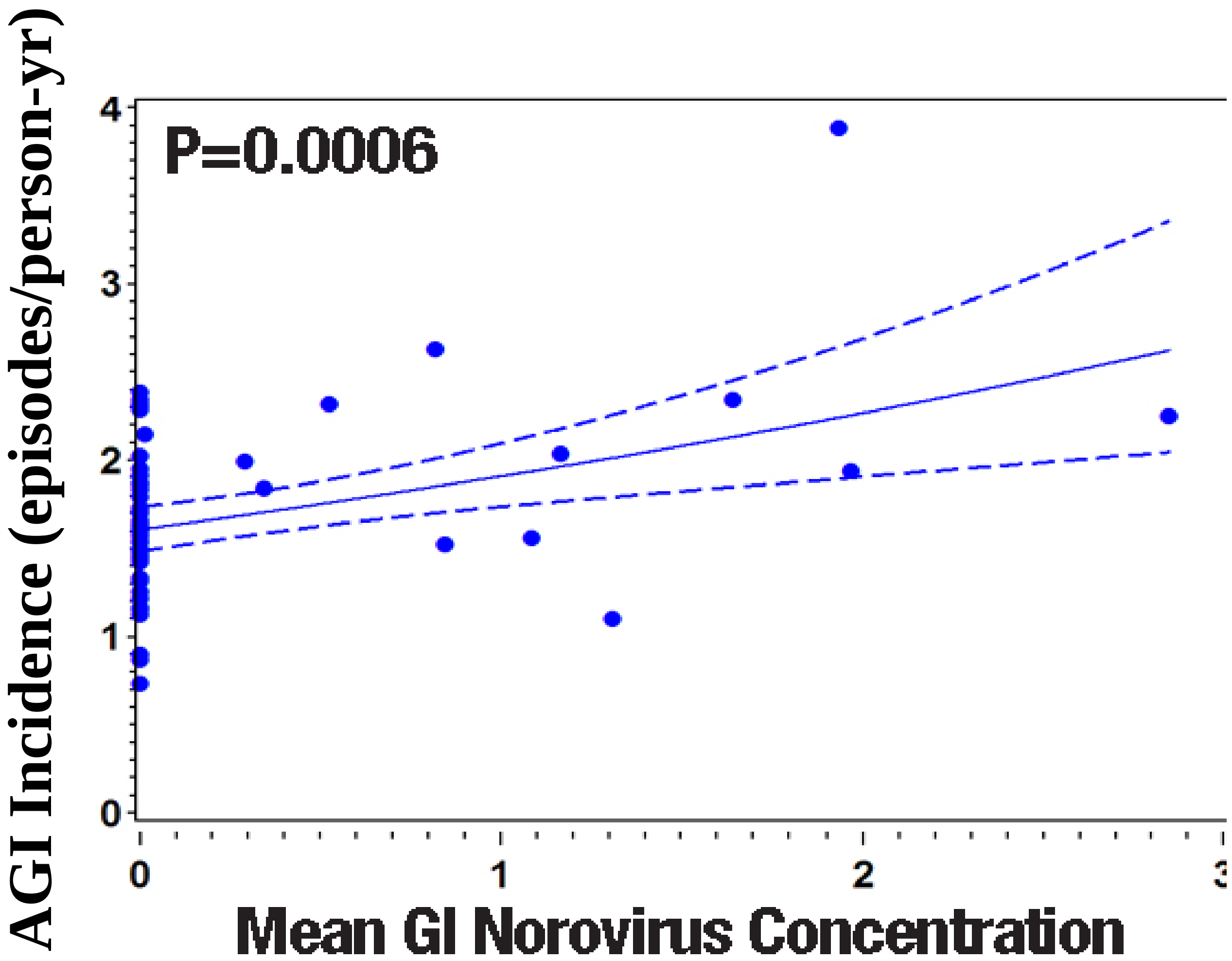
•Fout et al., Virus occurrence in small groundwater public systems located in karstic regions of the U.S. *In preparation*

Virus and Indicator Relationships

Indicator- and Virus-Positive Wells

Indicator/Virus	%	n
Total Coliforms	21	1558
E. coli	6	1558
Enterococci	8	1241
Aerobic spores	39	838
Anaerobic spores	13	50
F-specific coliphage	8	1446
Somatic coliphage	5	1446
Culturable virus	3	1174
PCR-virus	6	1419
Enterovirus	6	1234
Norovirus	8	1250

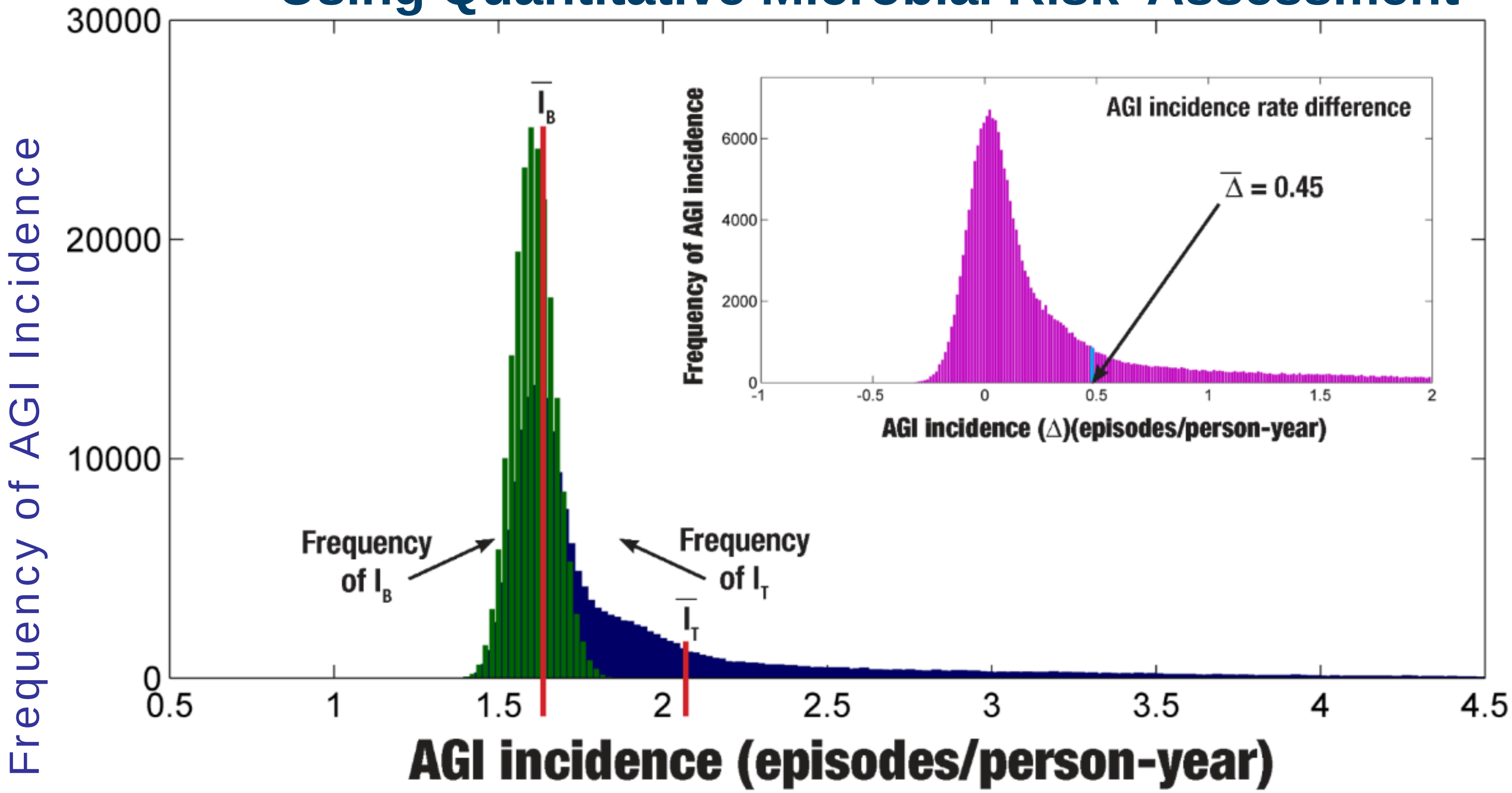
Virus and Indicator Relationships



Borchardt et al. 2012

Virus and Indicator Relationships

Estimating the Fraction of AGI from Drinking Water Using Quantitative Microbial Risk Assessment



- Virus exposure – AGI model: mean concentration GI norovirus, all ages
- 22% of the AGI in the study communities was from virus-contaminated tap water
- For children < 5 yrs, in the spring of 2006, the fraction of AGI from drinking water was **63%**!

Borchardt et al. 2012

Virus and Indicator Relationships

Spearman Rank Order Correlation for Wells (Rho value)				
Indicator	**Culturable virus**	**PCR-virus**	**Enterovirus**	**Norovirus**
Total coliforms	0.3	0.3	0.1	0.3
E. coli	0.3	0.3	0.2	0.2
Enterococci	0.3	0.2	0.2	0.1
Aerobic spores	0.1	0.0**	-0.0**	0.0**
Anaerobic spores	0.1**	0.1**	-0.0**	-0.0**
F-specific coliphage	0.3	0.2	0.2	0.2
Somatic coliphage	0.4	0.3	0.2	0.2
Any indicator	0.2	0.2	0.1*	0.2

Unmarked values are significant at *P* < 0.001; * *P* = 0.01 to 0.05; ** *P* >0.05

Virus and Indicator Relationships

Virus-Indicator Relationships

Sensitivity = the percentage of virus-positive wells the indicator correctly identified as virus-positive

Specificity = the percentage of virus-negative wells the indicator correctly identified as virus-negative

Positive predictive value (PPV) = the percentage of indicator-positive wells that were virus-positive

Negative predictive value (NPV) = the percentage of indicator-negative wells that were virus-negative

Risk Ratio = the increase in odds of finding a virus-positive well when an indicator is present versus when it is absent = PPV-(1-NPV)

Virus and Indicator Relationships

Culturable Virus					
Indicator	Sensitivity	Specificity	PPV	NPV	Risk Ratio
Total coliforms (TC)	64	88	15	98.7	11
E. coli	36	96	24	98.0	12
Enterococci	47	92	15	98.3	9
Aerobic spores	67	61	2	99.4	3
Anaerobic spores	40	68	25	81.3	1**
F-specific coliphage	38	95	21	97.9	10
Somatic coliphage	39	97	31	98.0	16
TC or aerobic spores	75	59	2	99.6	4*

Unmarked values are significant at $P < 0.01$; * $P = 0.01$ to 0.05; ** $P > 0.05$

Virus and Indicator Relationships

PCR-Virus					
Indicator	Sensitivity	Specificity	PPV	NPV	Risk Ratio
Total coliforms (TC)	48	85	35	90	4
E. coli	20	96	49	88	4
Enterococci	30	94	29	93	5
Aerobic spores	41	62	3	97	1**
Anaerobic spores	28	80	85	22	1**
F-specific coliphage	24	95	43	89	4
Somatic coliphage	22	97	51	89	5
TC + aerobic spores	48	59	3	98	1**

Unmarked values are significant at $P < 0.01$; * $P = 0.01$ to 0.05; ** $P > 0.05$

Virus and Indicator Relationships

PCR-Virus (UCMR3 study only)				
	Wells with spores		Wells without spores	
Indicator	Risk Ratio	*P*-value	Risk Ratio	*P*-value
Total coliforms (TC)	0.0	0.69	2.7	0.98
E. coli	0.0	0.98	ND	ND
Enterococci	0.0	0.92	0.0	0.98
F-specific coliphage	0.0	0.97	18.0	0.01
Somatic coliphage	0.0	0.99	0.0	0.99

ND – value could not be determined

Virus and Indicator Relationships

Susceptibility Categories	
Category	**Description**
Total coliform Rule (TCR)	All U.S. wells with >2 health-related TCR violations plus all international wells with >2 likely violations
Hydrogeology	All wells located in karst, fractured bedrock, or gravel/cobble settings
U.S. Groundwater Rule indicators (GWR)	All wells with total coliforms and any of the three GWR-triggered indicators (E. coli, enterococci, or coliphage)

Virus and Indicator Relationships

Ratio of % positive in category/overall % positive (n)		
Category	**Culturable Virus**	**PCR-Virus**
TCR	1.3 (672)	1.2 (148)
Hydrogeology	1.5 (131)	0.9 (65)
GWR	3.9 (59)	1.6 (118)

Virus and Indicator Relationships

Risk Ratios for wells in Susceptibility categories (*P*-value)			
Indicator	**Category**	**Culturable Virus**	**PCR-Virus**
Total coliforms	All	4.5 (0.04)	1.3 (0.04)
	Hydrogeology	3.8 (0.02)	NS
Enterococci	All	4.5 (0.002)	1.0 (0.91)
	Hydrogeology	5.8 (0.01)	NS
	TCR	5.1 (0.02)	4.9 (0.01)
F-Specific coliphage	All	7.7 (0.04)	1.2 (0.3)
	Hydrogeology	8.4 (0.005)	2.2 (0.02)
Somatic coliphage	All	9.1 (<0.001)	1.9 (<0.001)
	TCR	NS	2.8 (0.04)

Values from first 12 studies adjusted for study design; NS – not significant

Virus and Indicator Relationships

Major conclusions

- Human enteric viruses may be found in groundwaters from wells across a wide range of vulnerability assessments
- Indicators are not perfect, but still valuable
- In wells without indicators, viruses are unlikely to be present
- However, indicators are often present when viruses are absent
- And viruses may be present in the absence of indicators
- And viruses in untreated groundwaters used in food processing or restaurants for foods that are not cooked may be a source of foodborne outbreaks

Virus and Indicator Relationships

Acknowledgements:

Authors of the various studies, especially Mark Borchardt, Burney Kieke, Sandhya Parshionikar, Larry Wymer, Michael Jahne, Yury Shtarkman

Does Water Matter?
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De Facto Reuse

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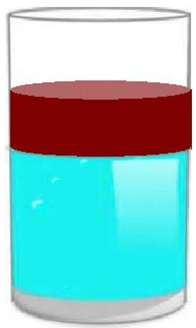
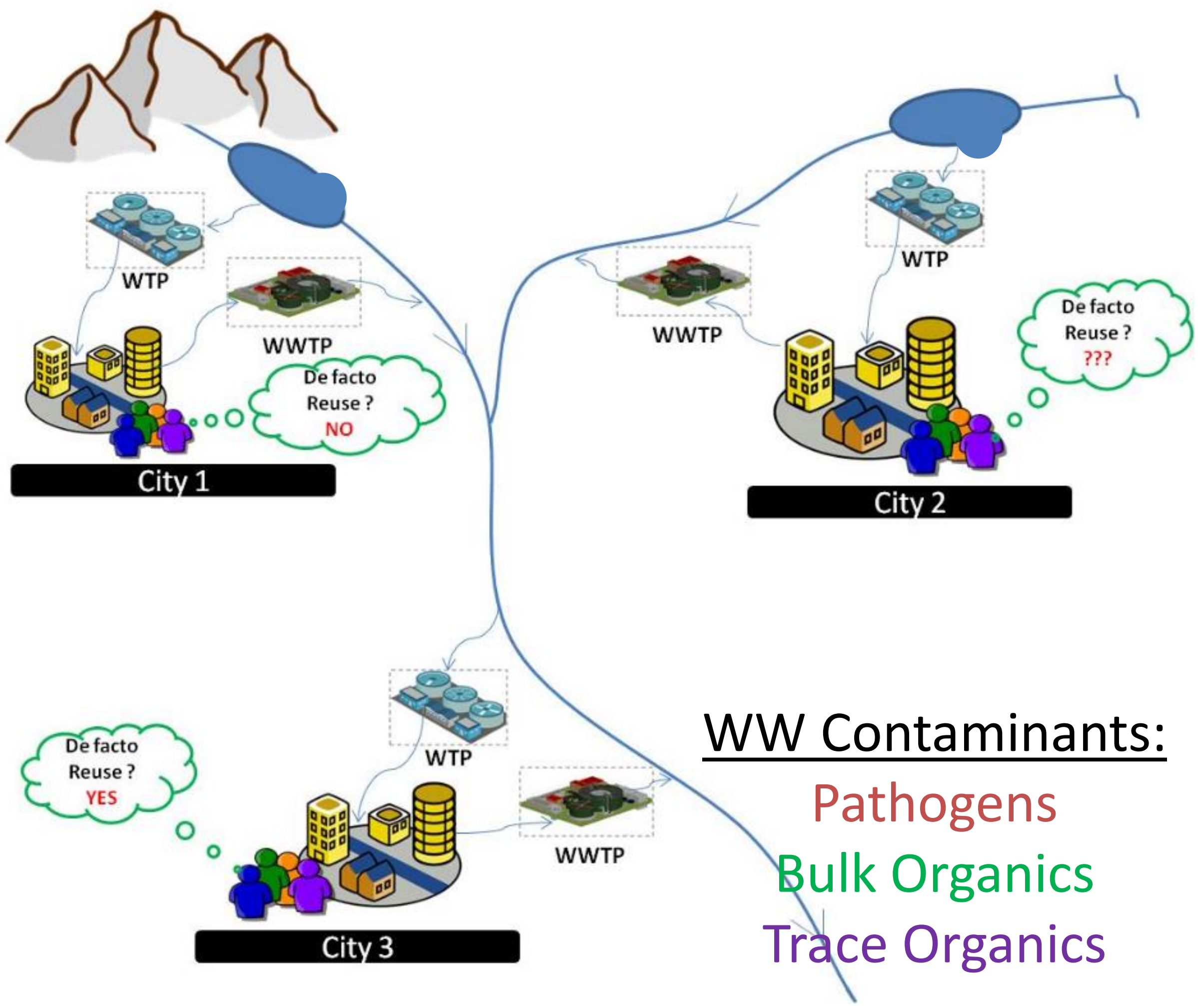
Outline

- **What is de facto reuse (DRF) & Why use it?**
- How did we linked data sources?
- How much wastewater is in rivers?
- How much DFR occurs at DWTPs serving >10k?
  - Spatial considerations
  - Temporal considerations
- Can we validate DFR predictions?
- Implications
  - DWTPs serving <10k vs >10k populations
  - Implications of DFR on DWTP installed treatment processes

Where is drinking water impacted by WW?

DeFacto Reuse is
The unplanned or
incidental presence
of treated
wastewater in a
water supply source





100% - X% = River water
X% = Treated Wastewater
De Facto Reuse = x%

<u>WW Contaminants:</u>

Pathogens
Bulk Organics
Trace Organics

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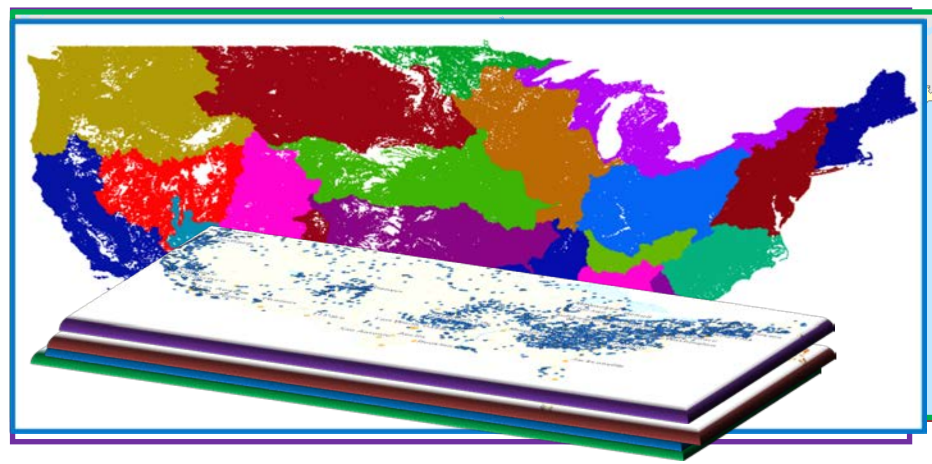
De Facto Reuse Model Development

Base Map: National Atlas of the United States and USGS

Hydrography: USGS National Hydrography Dataset Plus



WWTPs:

- 14,651 data points
- CWNS 2008
- Permit Compliance System used for data mining missing location points

DWTPs:

- 6,330 total active surface water intake points
- 2,056 with population served > 10,000

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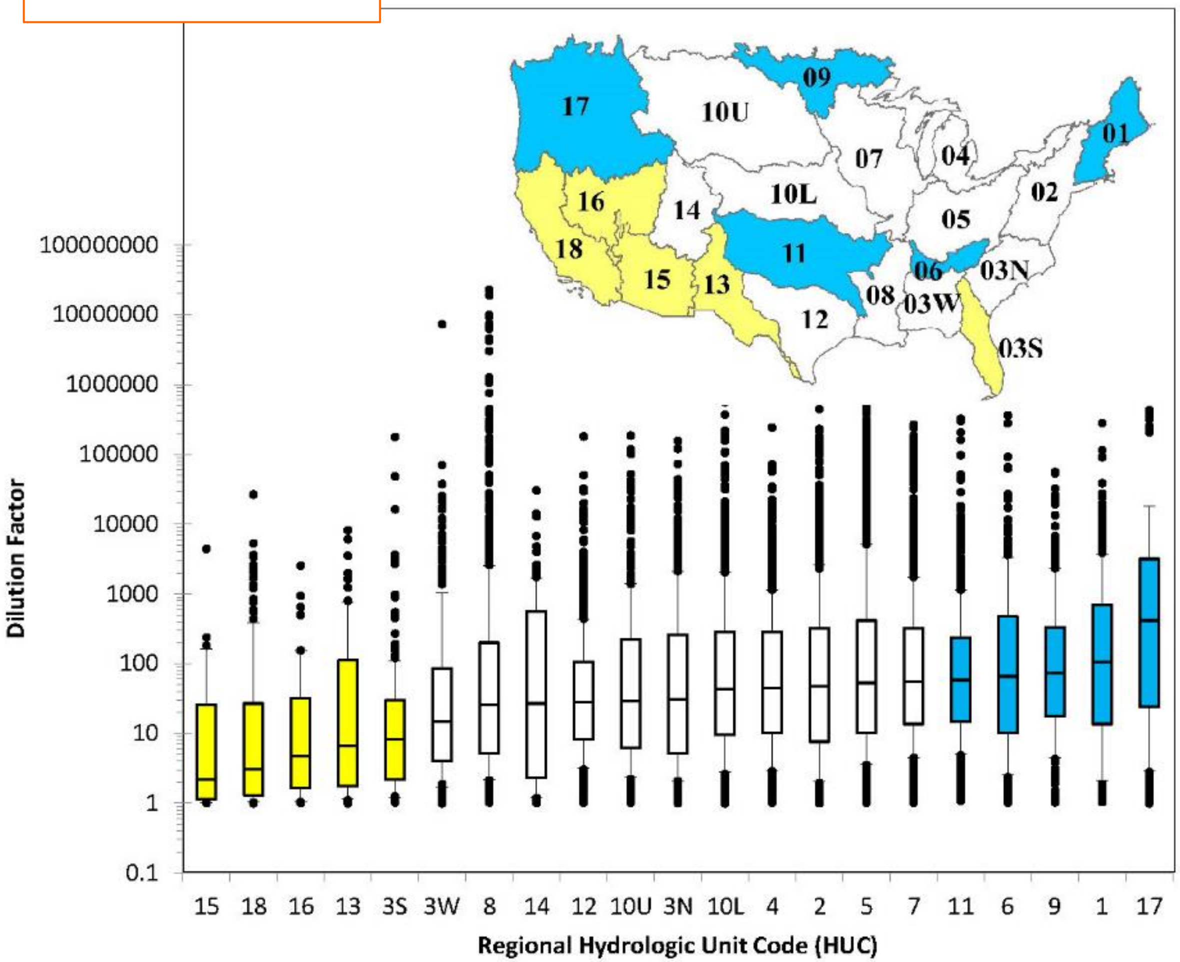
Stream Dilution Factors in Rivers Influence Fish & Discharge Limits

Dilution is the Solution?

$$\text{Dilution Factor} = \frac{\overline{Q}_{River}}{\overline{Q}_{Wastewater}}$$



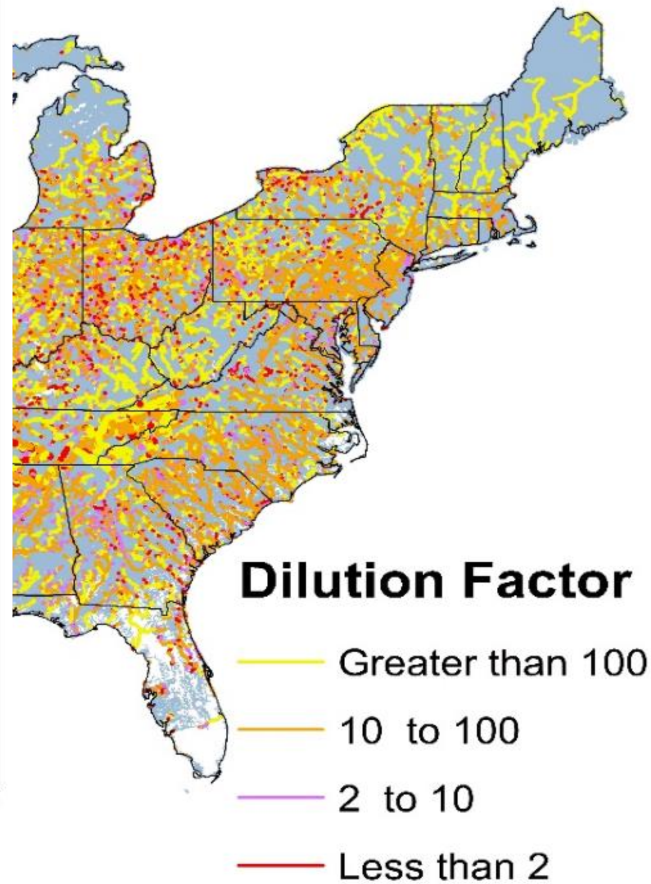
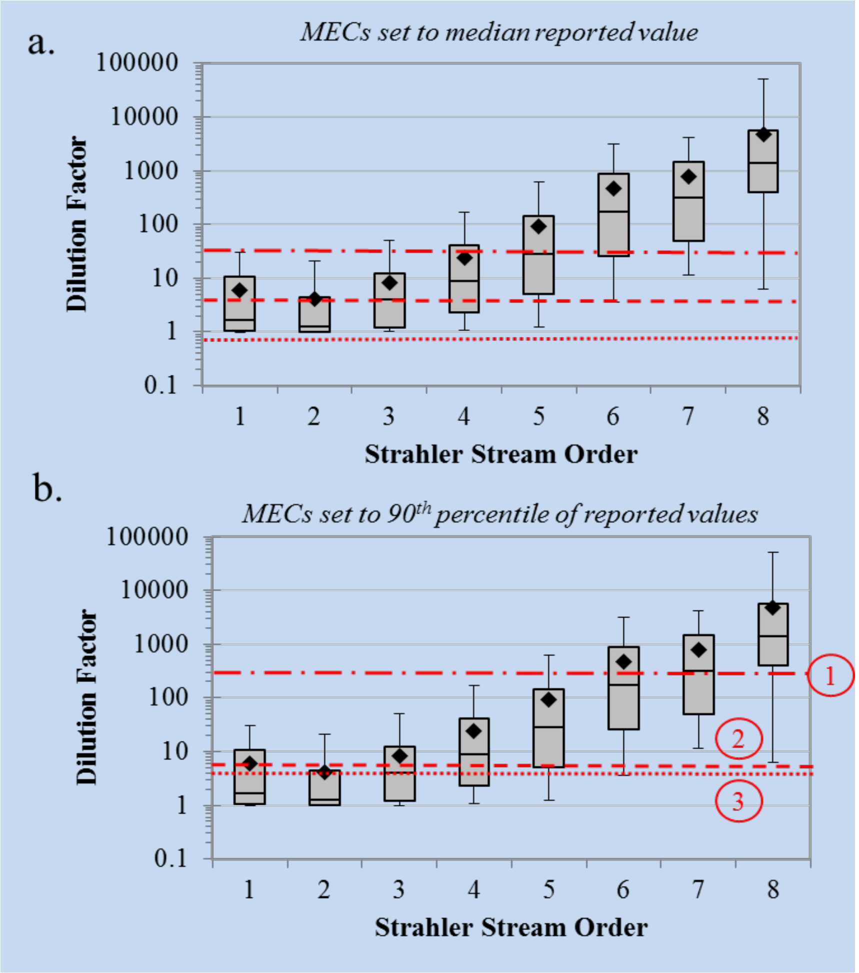


Figure 3. a. Dilution factors under low flow conditions (Q95) with median MEC. b. Dilution factors under low flow conditions (Q95) with 90th percentile MEC. Red lines represent the dilution factors required for (1) 17α-ethinylestradiol, (2) 17β-estradiol, and (3) estrone (labeled from top to bottom) to fall below hazard quotients given a 10-fold safety factor. (. Top and bottom of box= 75th and 25th percentiles, respectively; top and bottom of whisker= 90th and 10th percentiles, respectively; line across inside of box= median (50th percentile). Diamonds represent the average of values within between the 10th and 90th percentiles.



Rice, J. and Westerhoff, P. "US Streams at Low Flow Vulnerable to High Levels of Endocrine Pollutants from Wastewater", Nature Geoscience, 10, 587-591 (2017)

Key Findings

- Wastewater discharges make up >50% of instream flow for over 900 receiving streams
- Dilution factors amongst receiving streams 25th, 50th, and 75th percentile are 8, 43, and 287 respectively (N=14,651)
- Roughly 400 of 1049 reaches are impacted by a HQ value < 10 fold safety factor for all three contaminants under low flow conditions
- Up to a four-magnitude difference between DF's based upon stream orders in the same USGS hydrologic region

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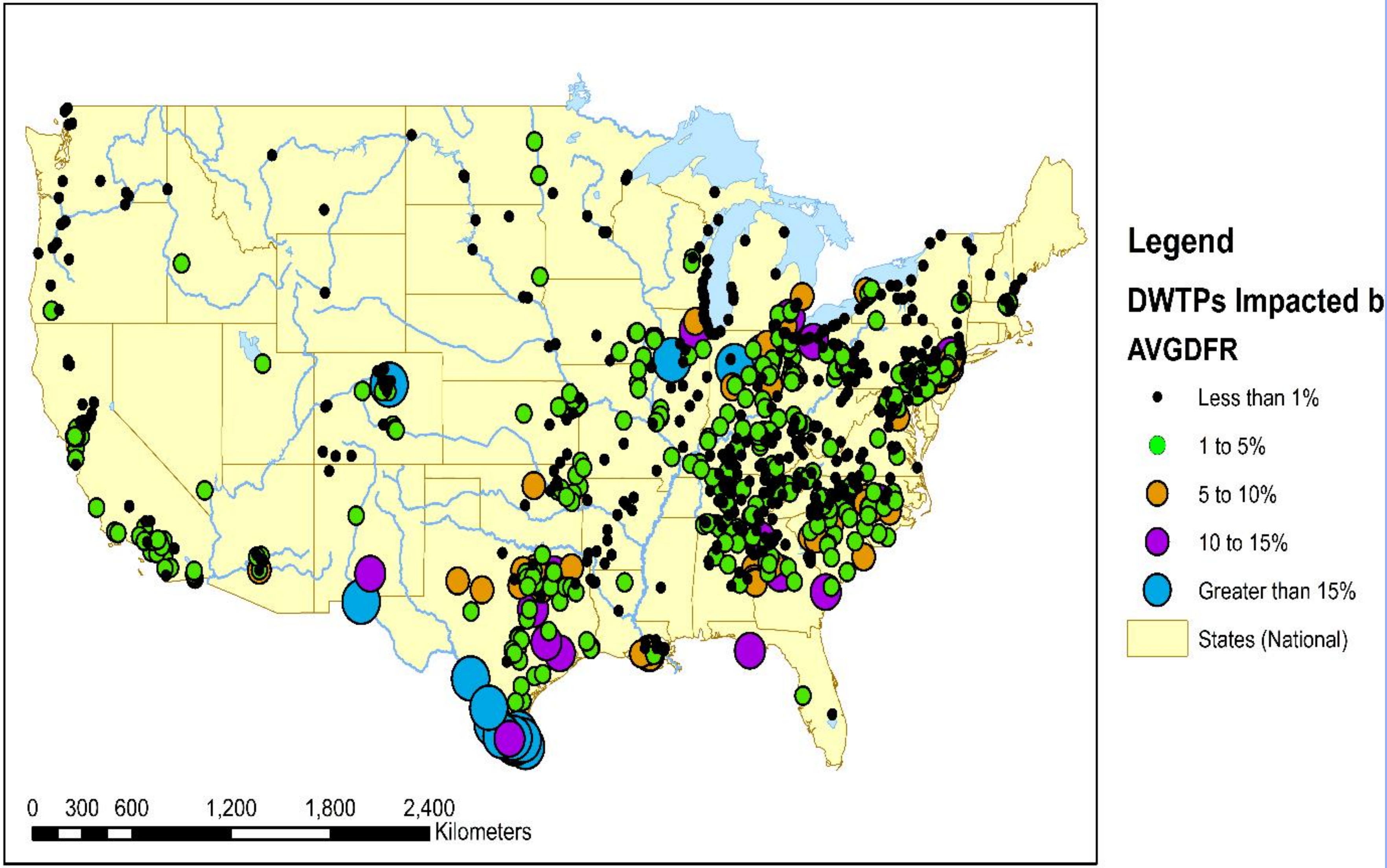
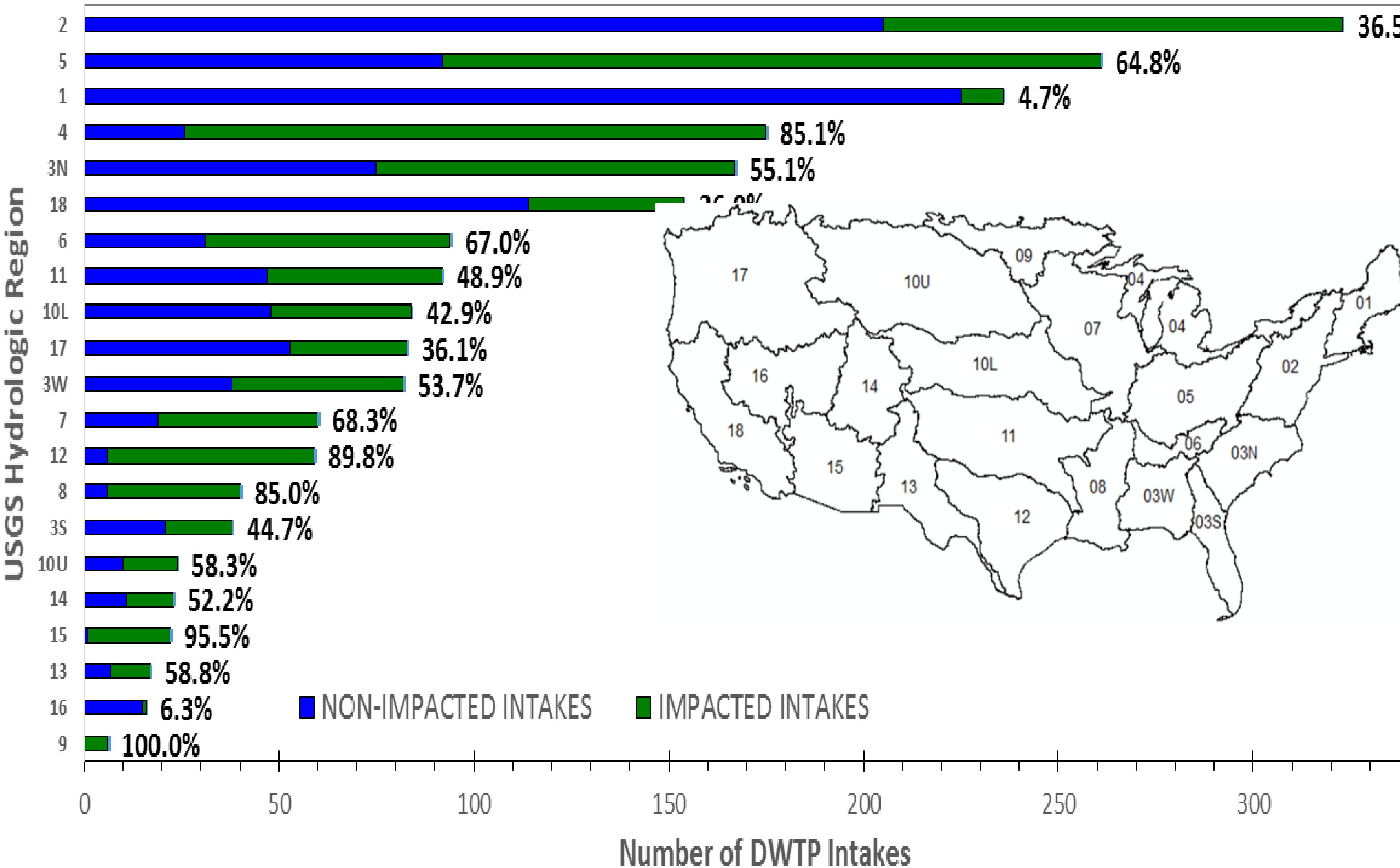
Low Magnitude of De Facto Reuse

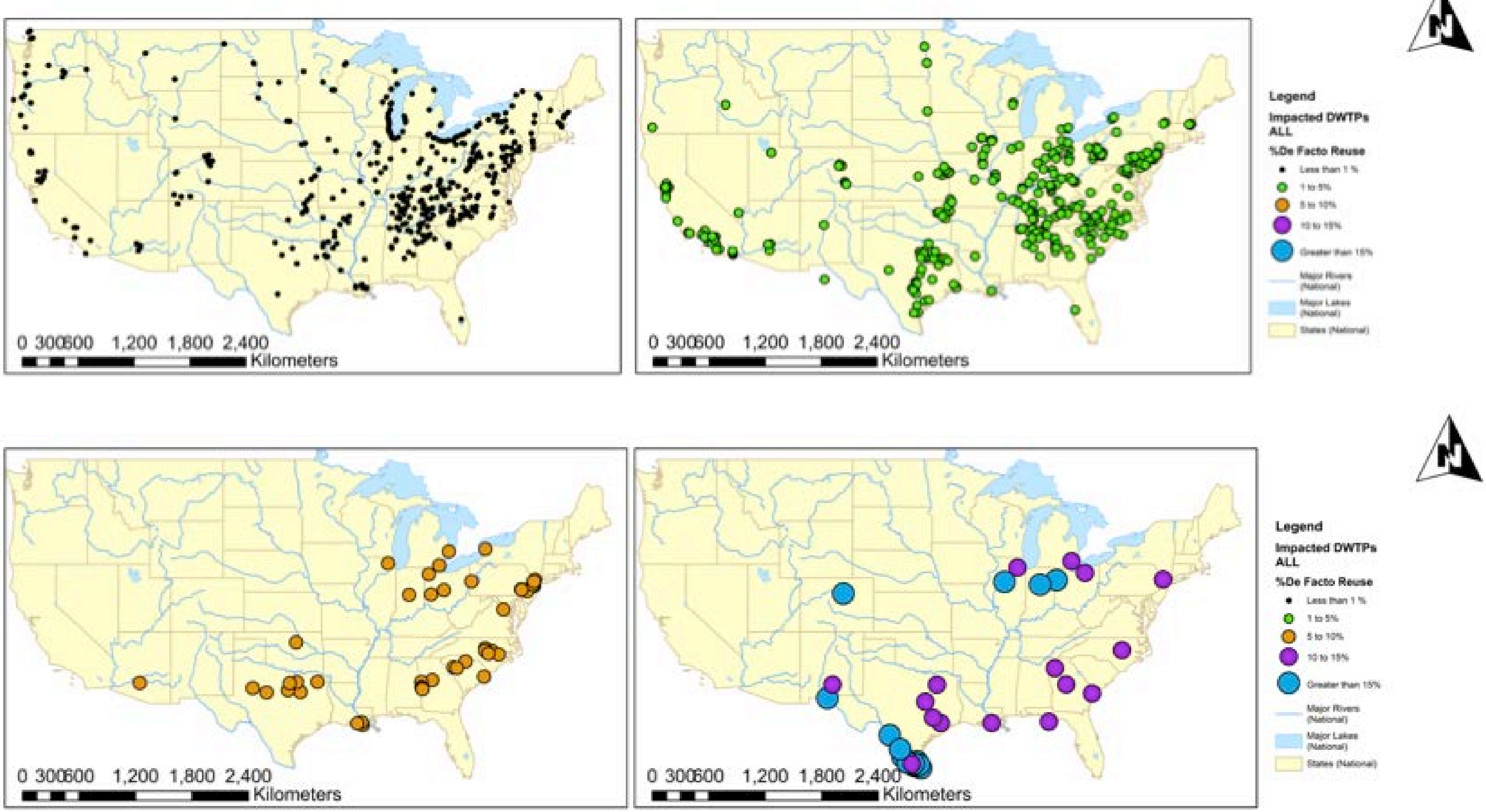


National Occurrence: Average Flow Conditions

High Occurrence Frequency of De Facto Reuse



National Occurrence: Average Flow Conditions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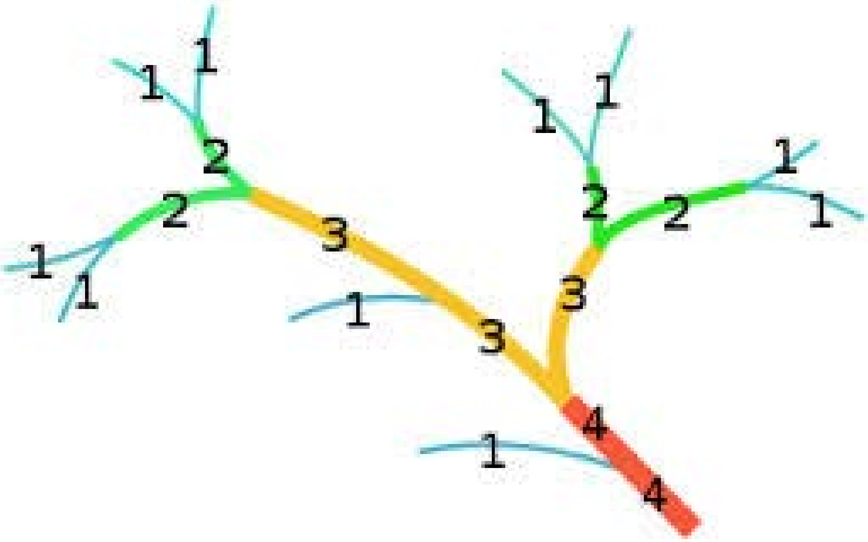
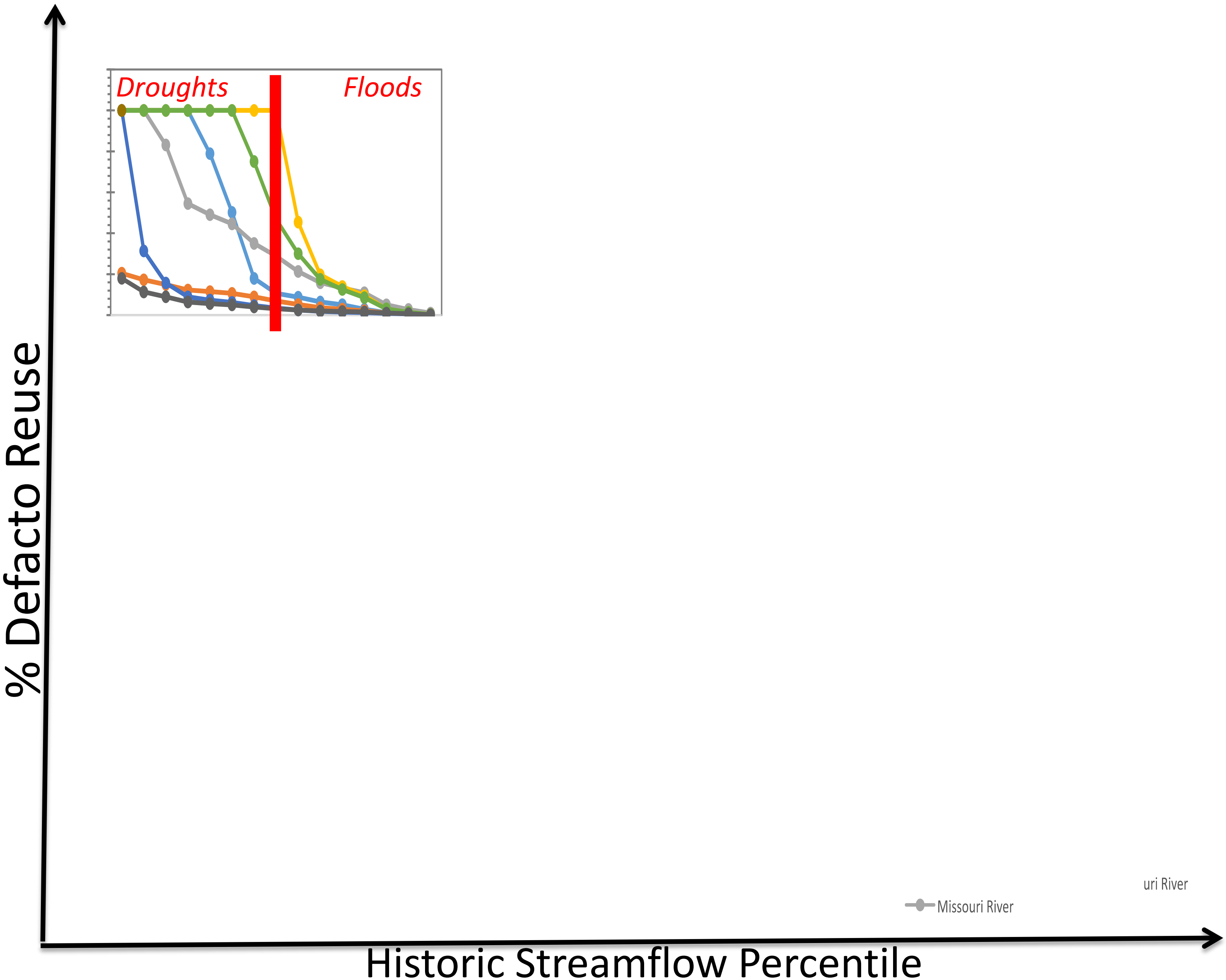
Kilometers
0 300600 1,200 1,800 2,400
Kilometers
Legend
Impacted DWTPs ALL
%De Facto Reuse
Less than 1 %
1 to 5%
5 to 10%
10 to 15%
Greater than 15%
Major Rivers (National)
Major Lakes (National)
States (National)
0 300600 1,200 1,800 2,400
Kilometers
0 300600 1,200 1,800 2,400
Kilometers
Legend
Impacted DWTPs ALL
%De Facto Reuse
Less than 1 %
1 to 5%
5 to 10%
10 to 15%
Greater than 15%
Major Rivers (National)
Major Lakes (National)
States (National)

Influence of Droughts & Floods

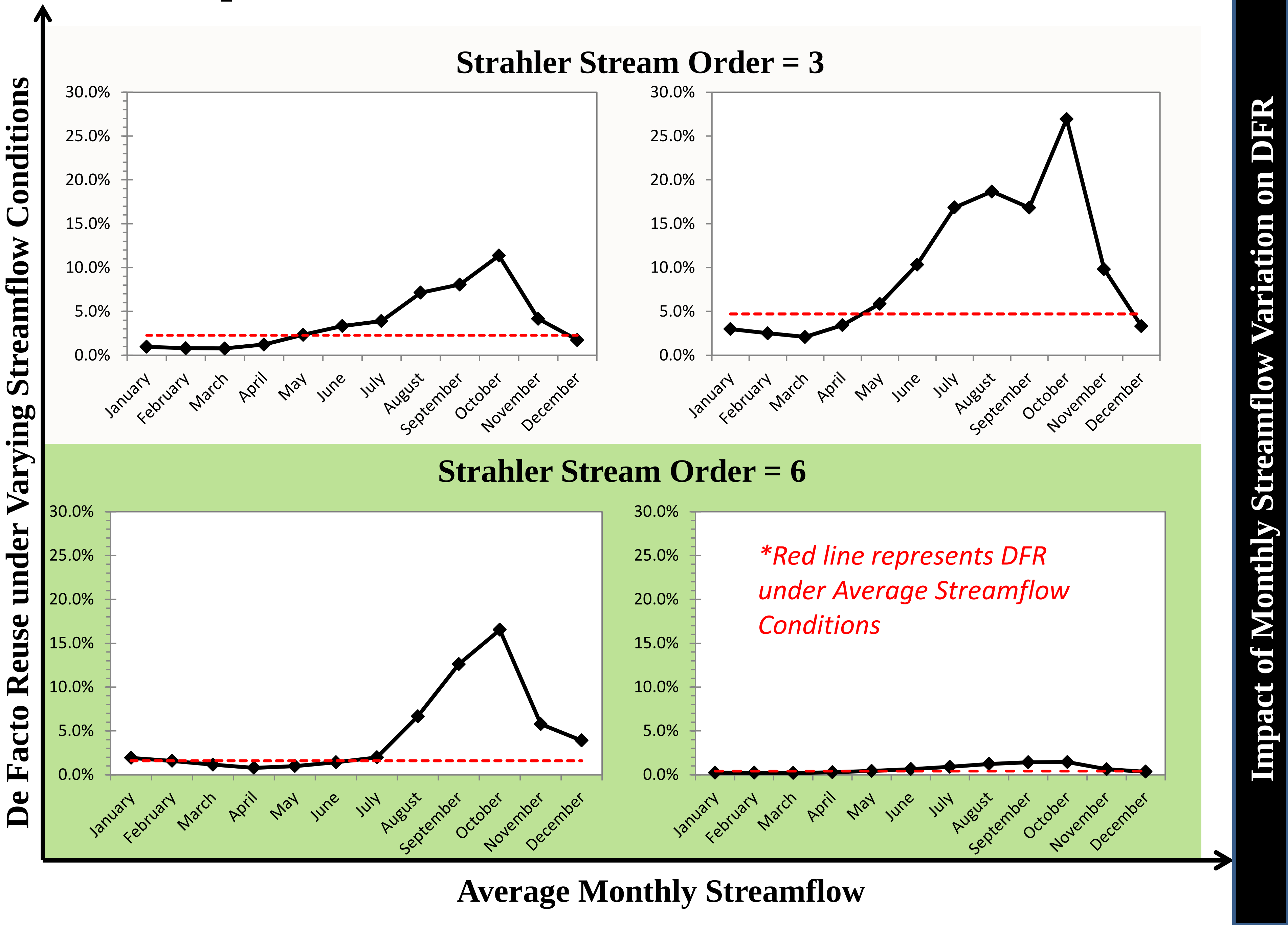


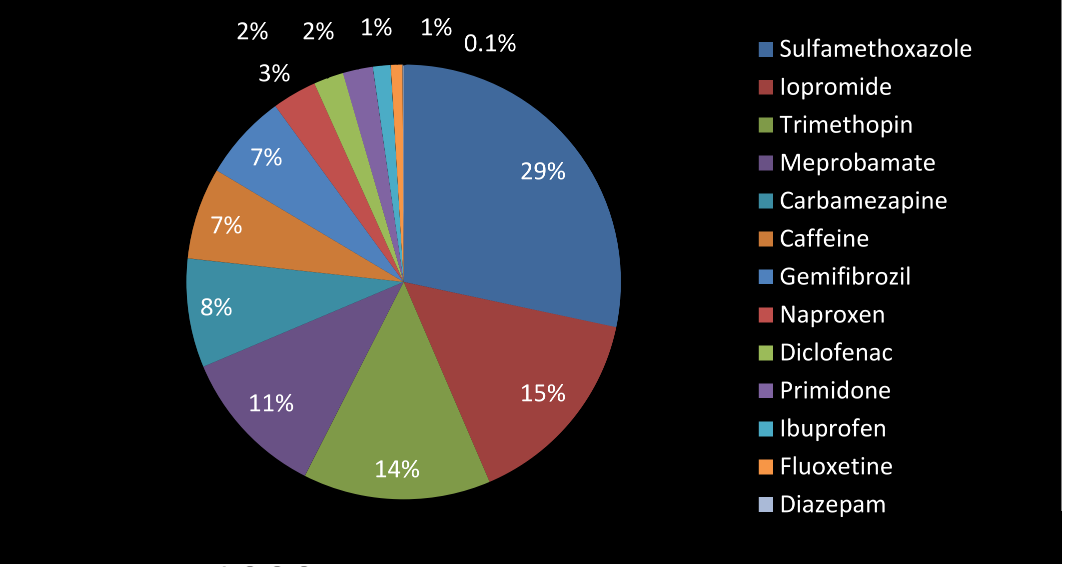
Strahler Stream Order

Stream Order & Streamflow Variations



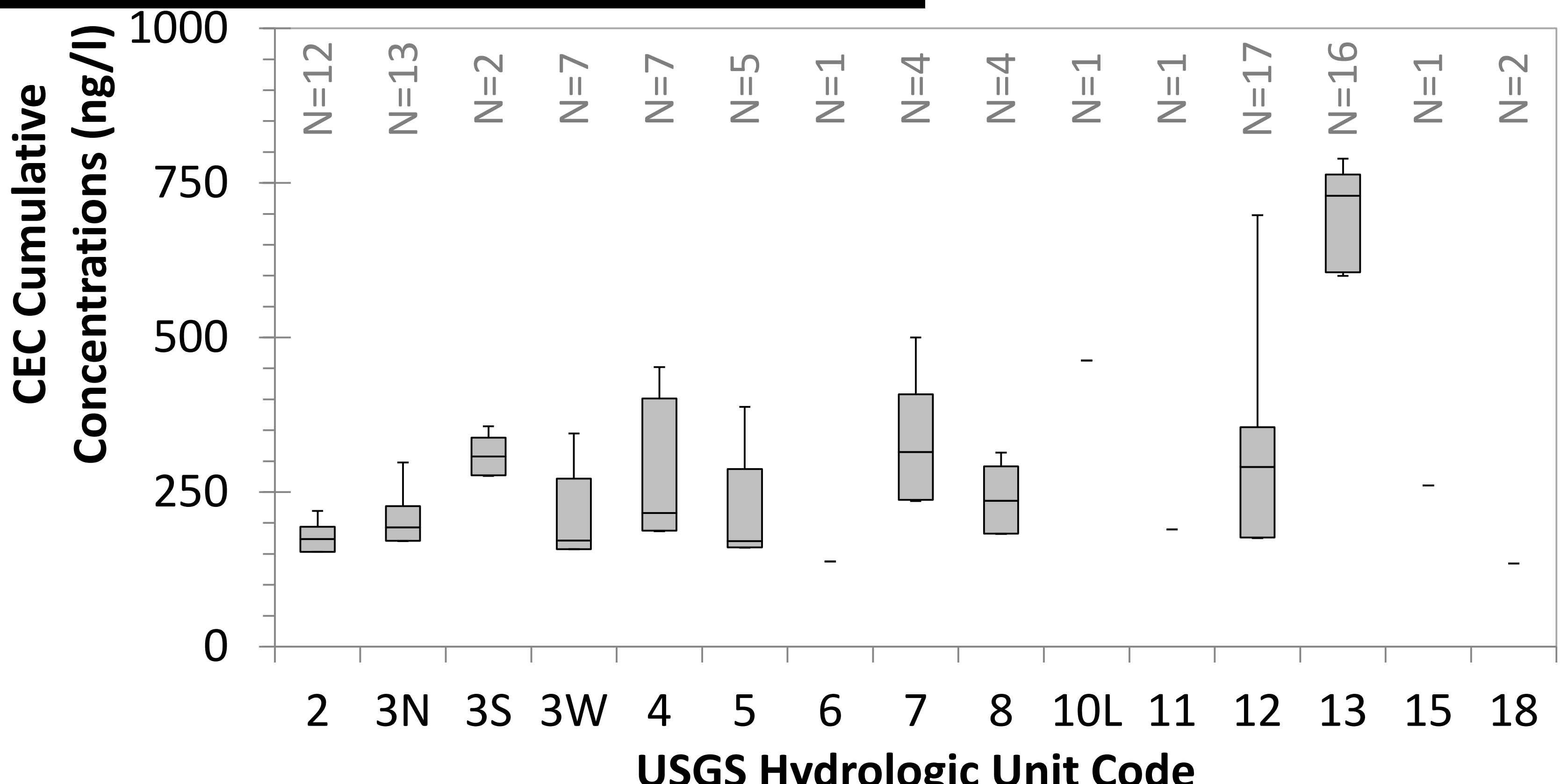
Impacts of Seasonal Streamflow on De Facto Reuse



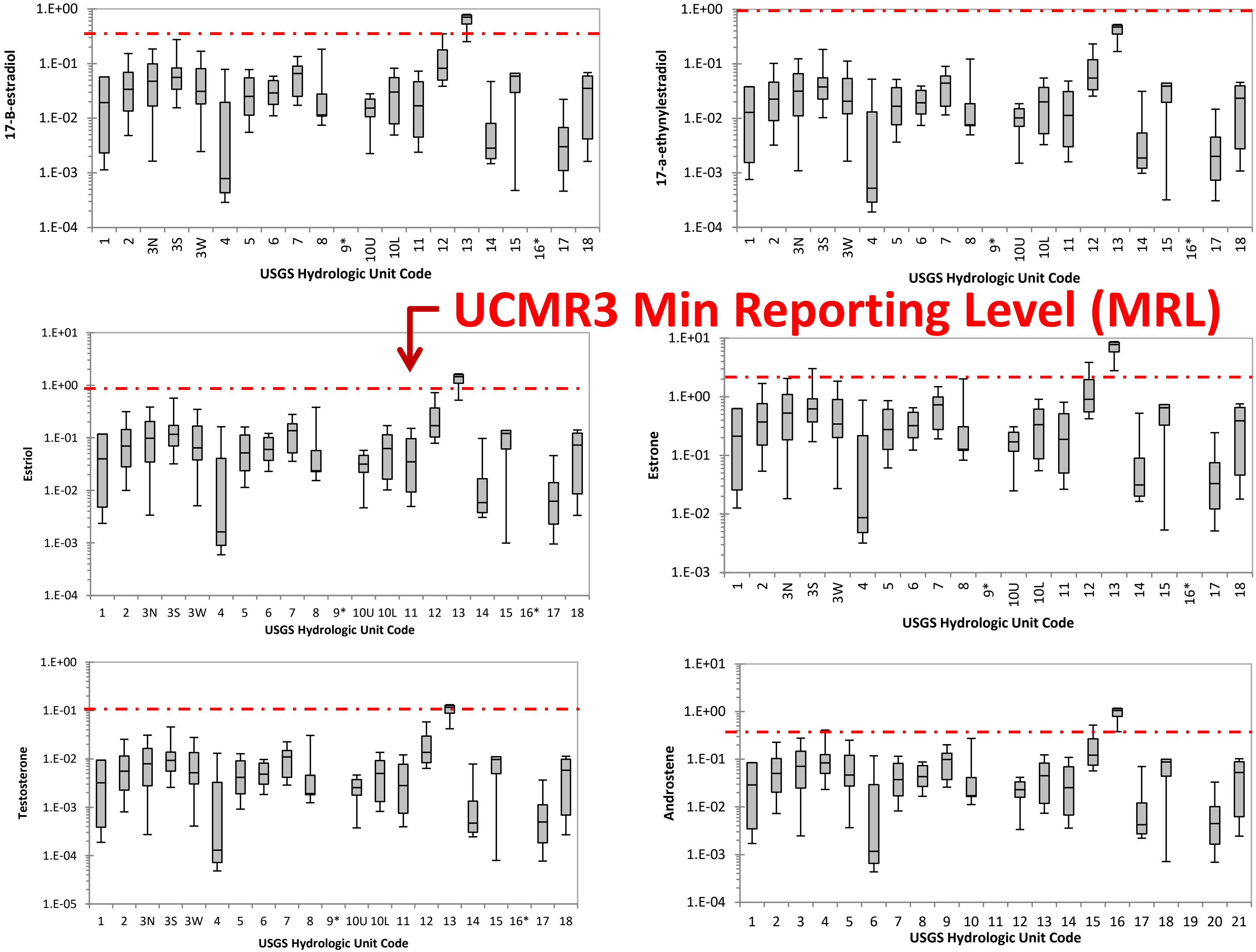


2011 NRC Report Suggested:

DWTPs with ≥ 5% DFR received higher levels of CECs than planned reuse schemes

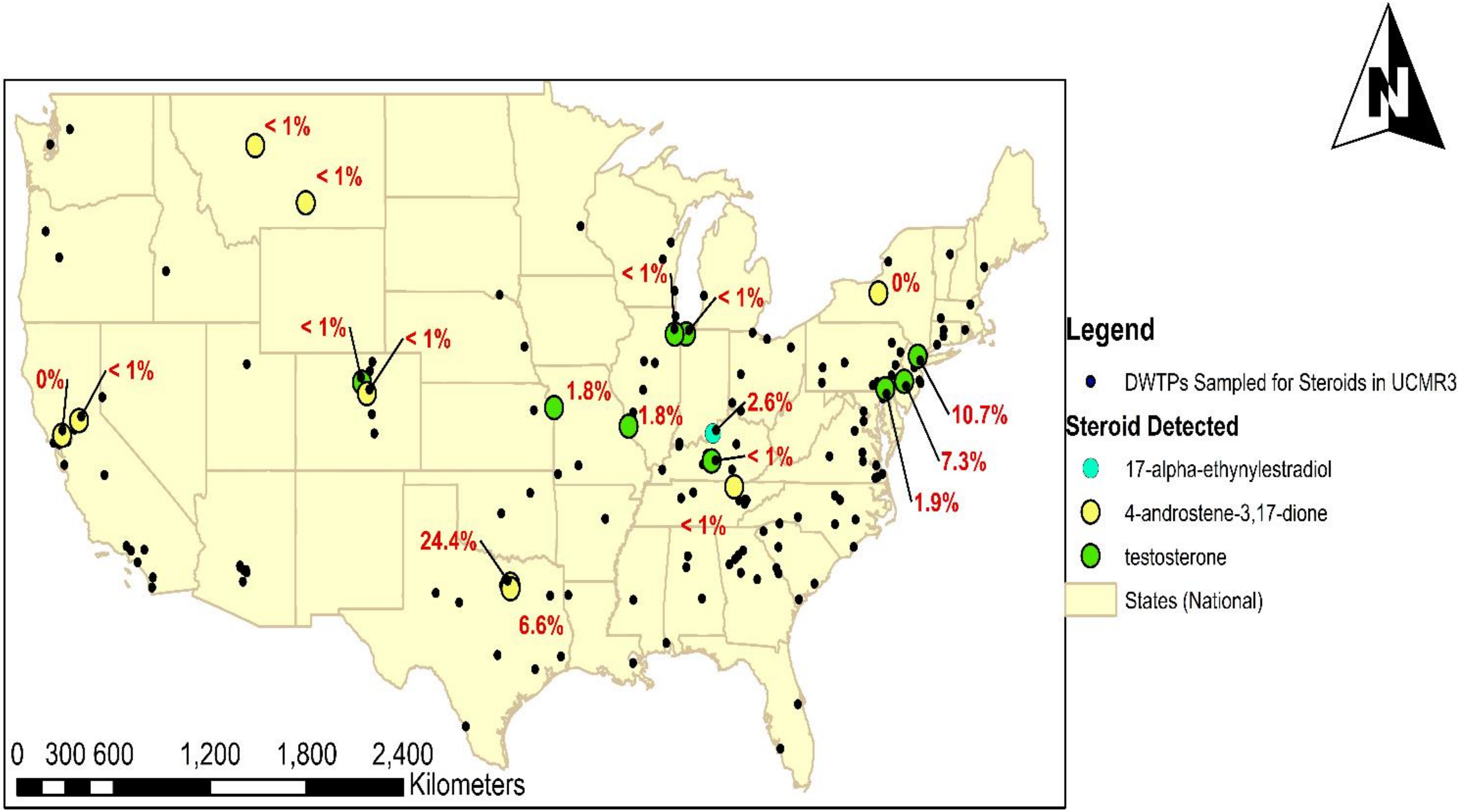


Estimates for Steroids (ng/L) From UCMR3

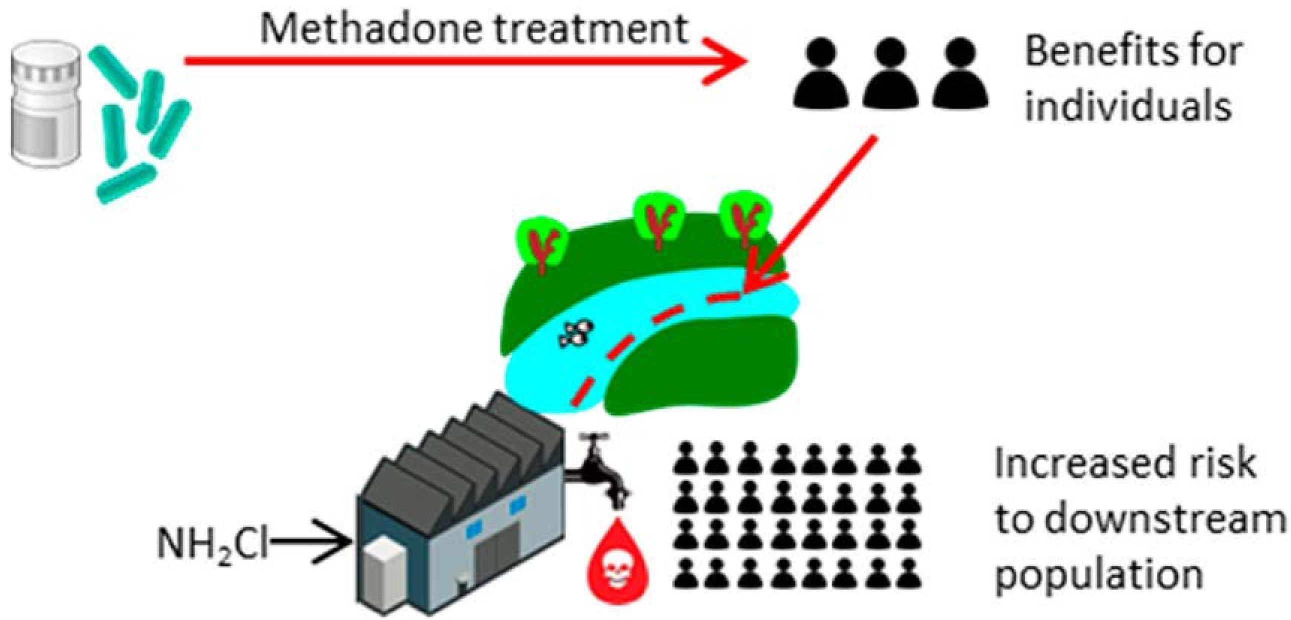


Legend: top and bottom of box= 75th and 25th percentiles respectively; top and bottom of whisker = 90th and 10th percentiles respectively; line across inside of box= median(50th percentile).

Comparison of Model Predicted "HITS" vs Observed in UCMR3 for Steroid



Disinfection Impacts

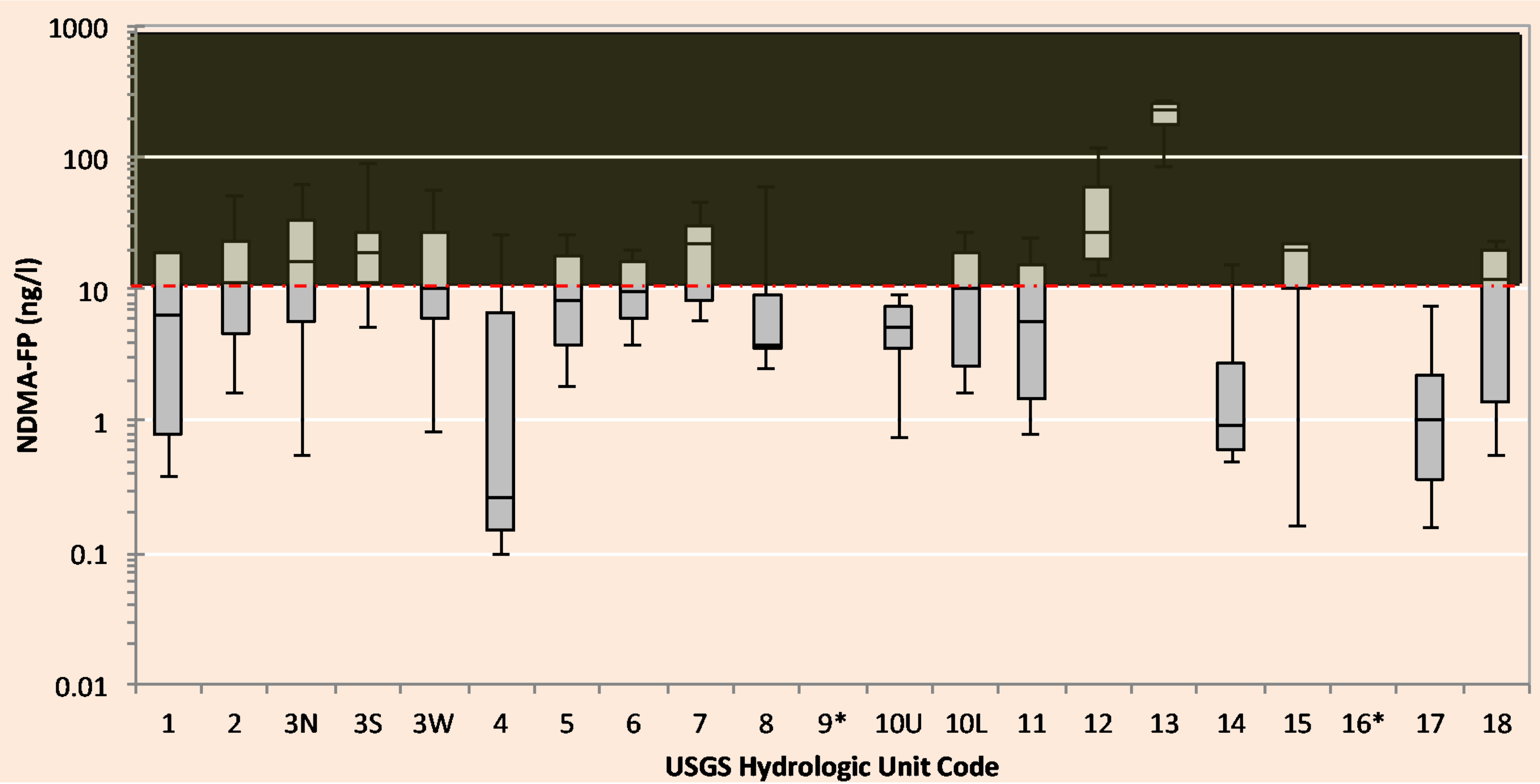


Chloramination is practiced at WTPs serving water to >50% of the US Population

Chloramines react with Wastewater Organics to form DBPs (Nitrosodimethylamine – NDMA)

Wastewater effluents contain antibacterial resistant organics & little is known about *chlorine* resistance

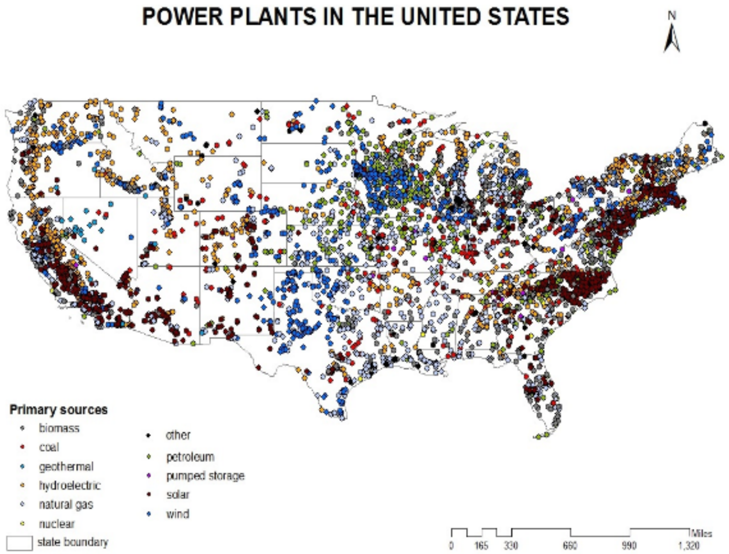
Predictions of NDMA precursors from wastewater at DWTPs



Red line represents the CA Notification Level (10 ng/l)

Summary of key points

- Big data & GIS allows us unprecedented opportunities to understand spatial and temporal impacts of wastewater on our water supplies
- There is a high frequency, but low magnitude, of de facto reuse
- Communities on smaller streams are more susceptible to wastewater impacts
- Next we hope to include industrial and agricultural discharges
- WTPs with *de facto* reuse have lower treatment goals than planned reuse projects (e.g., RO)



Supporting references & Acknowledgements

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American Water Works Association

EEC-1449500
DEB-1637590
SES-1462086

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De facto
Reuse
Incidence in our
Nations
Consumable
Supply

Does Water Matter?
Part 2: What could be in Municipal Water?

International Association for Food Protection

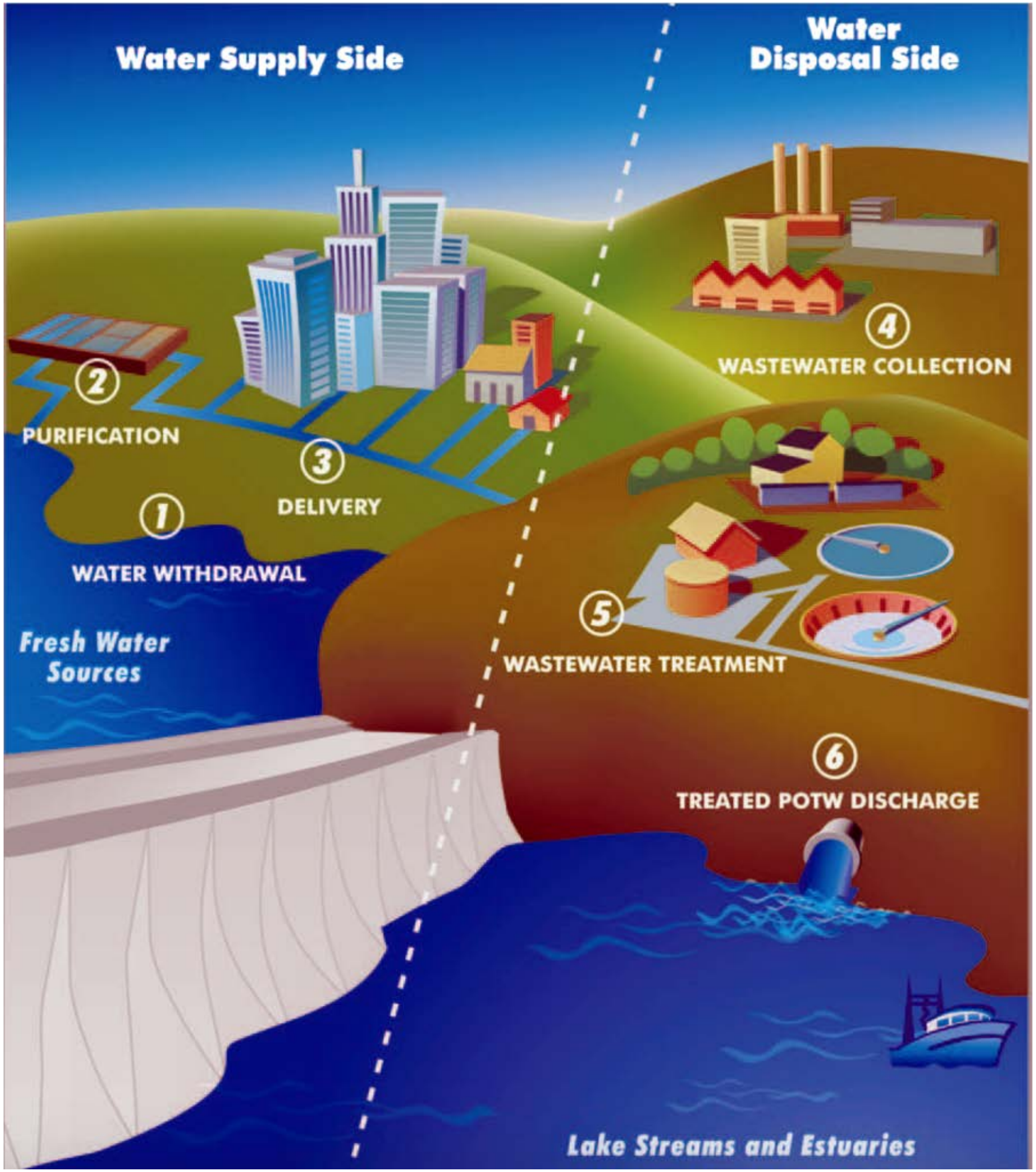
Resistant Microbes and VBNC - What Might They Mean to Food Processors?

Norman Neumann
Professor
School of Public Health
University of Alberta

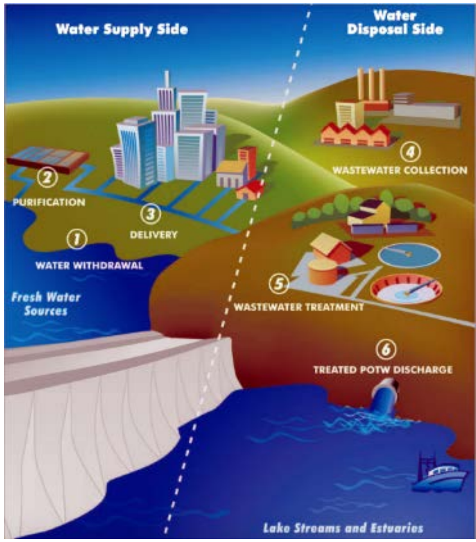
Sponsored by **IAFP's Water Safety and Quality PDG**
&
Atlantium Technologies

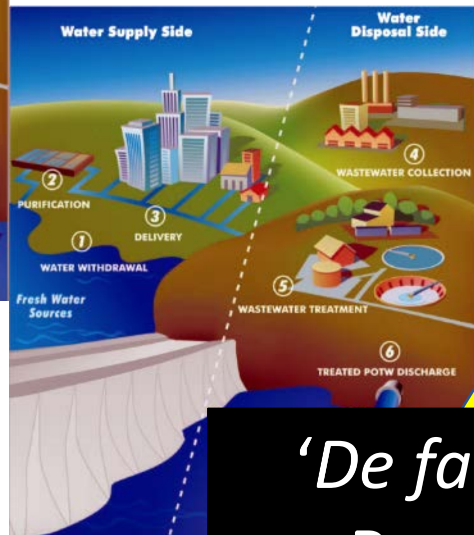
I hope to convince you that:

- Stress resistance is common in bacteria.
- Bacteria evolved adaptive stress mechanisms long before humans came onto the scene!
- Humans have simply ‘facilitated’ the natural selection and evolution of extreme resistance.
- We need a ‘re-awakening’ of our research agenda to ensure better food/water safety practices.

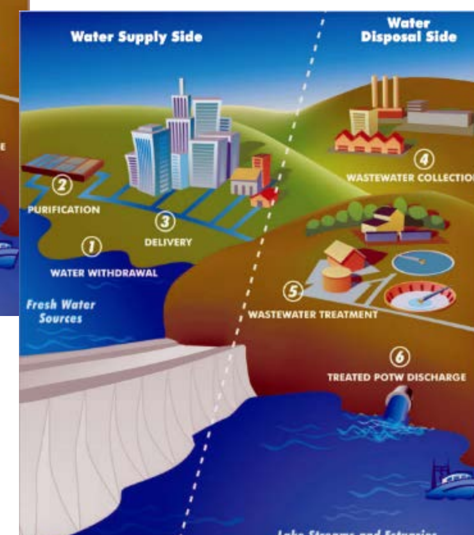


The Urban Water Cycle





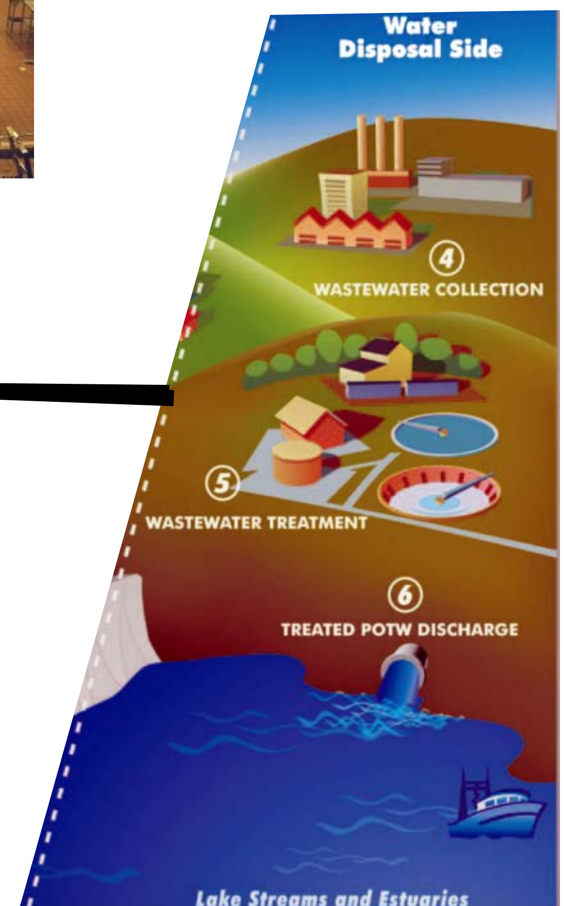
‘*De facto*’
Reuse

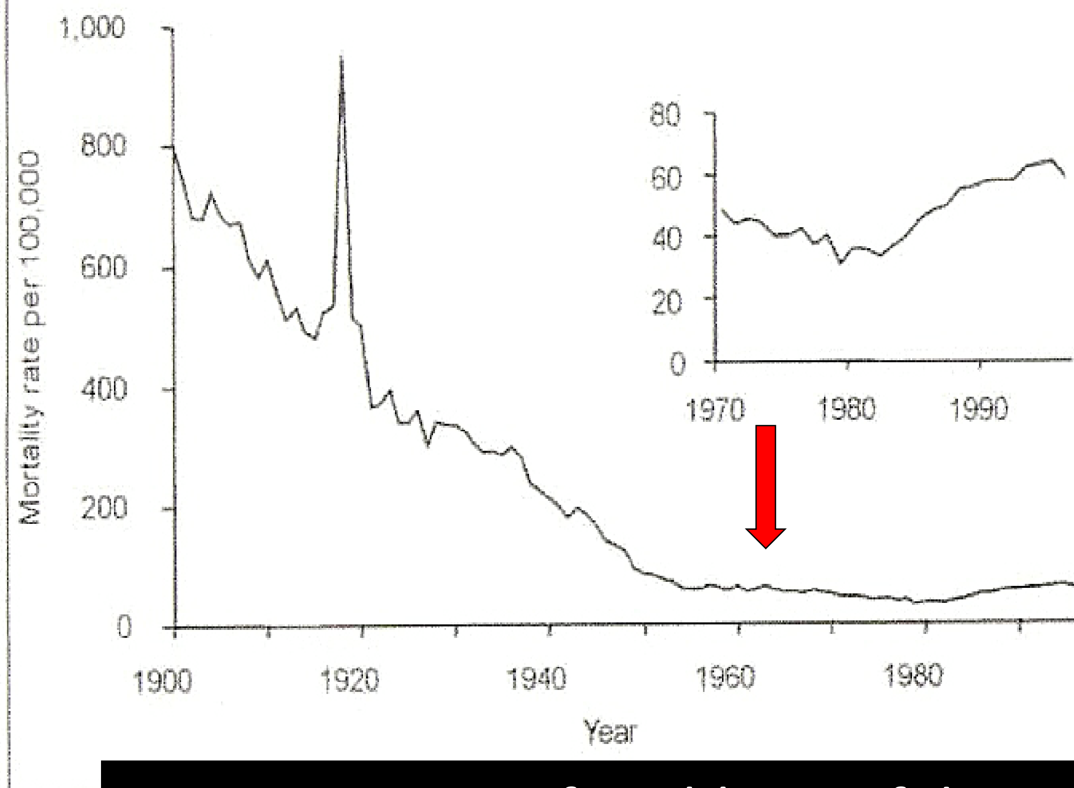


Could our water disposal practices be facilitating the emergence of pathogen resistance in the food-water nexus?

???

???





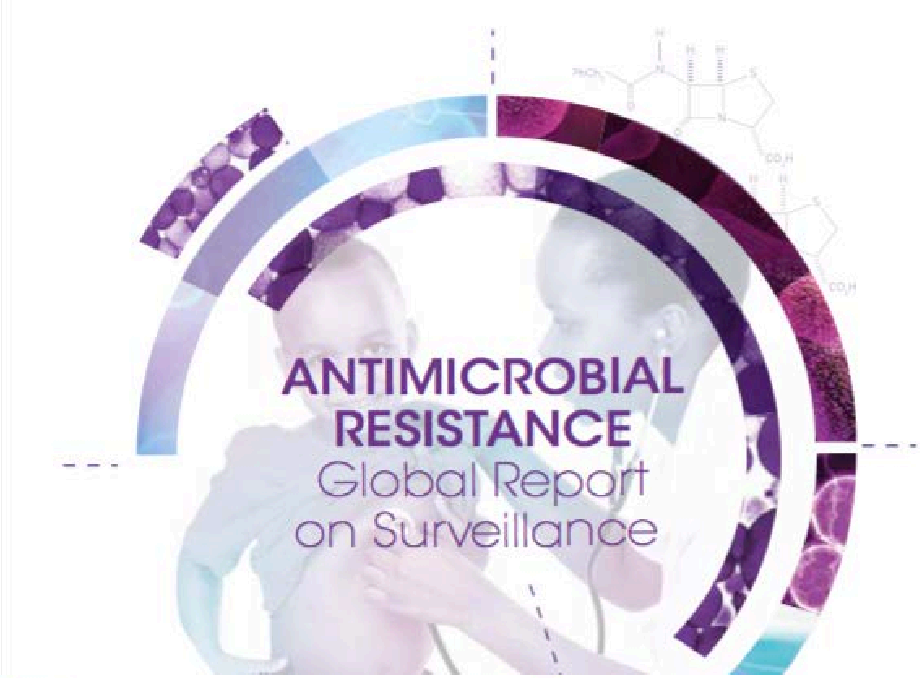
Hughes, J.M. 2001. Emerging Infectious Diseases: A CDC Perspective. *Emerging Infectious Diseases*, 17: 494-496.

Let's start…by going back IN TIME to the early 1960's…

Science was comfortable, confident ….and <u>complacent</u> !!!

- "We can look forward with confidence to a considerable degree of freedom from infectious diseases at a time not too far in the future. Indeed…it seems reasonable to anticipate that within some measurable time...all major infections will have disappeared". (T. Aidan Cockburn [1963] in his book the *Evolution and Eradication of Infectious Diseases* as quoted by Merrill Singer in the book*, Anthropology of Infectious Diseases* [Page 157], Left Coast Press, 2015).

- It is alleged that a couple of years later the Surgeon General of the U.S., Dr. William Stewart, said "It is time to close the book on infectious diseases". (Merrill Singer in the book *Anthropology of Infectious Diseases* [Page 157], Left Coast Press, 2015).



Fast forward to 2014....

Our 'saviour' in 1963 became our 'demon' in 2014.

"Pride goeth before destruction, and a haughty spirit before the fall."
Proverbs 16:18

Foreword

Are you comfortable, confident, and complacent with your food/water safety practices??

Antimicrobial resistance (AMR) within a wide range of infectious agents is a growing public health threat of broad concern to countries and multiple sectors. Increasingly, governments around the world are beginning to pay attention to a problem so serious that it threatens the achievements of modern medicine. A post-antibiotic era—in which common infections and minor injuries can kill—far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century.

What about the evolution of water-treatment resistant microbes?

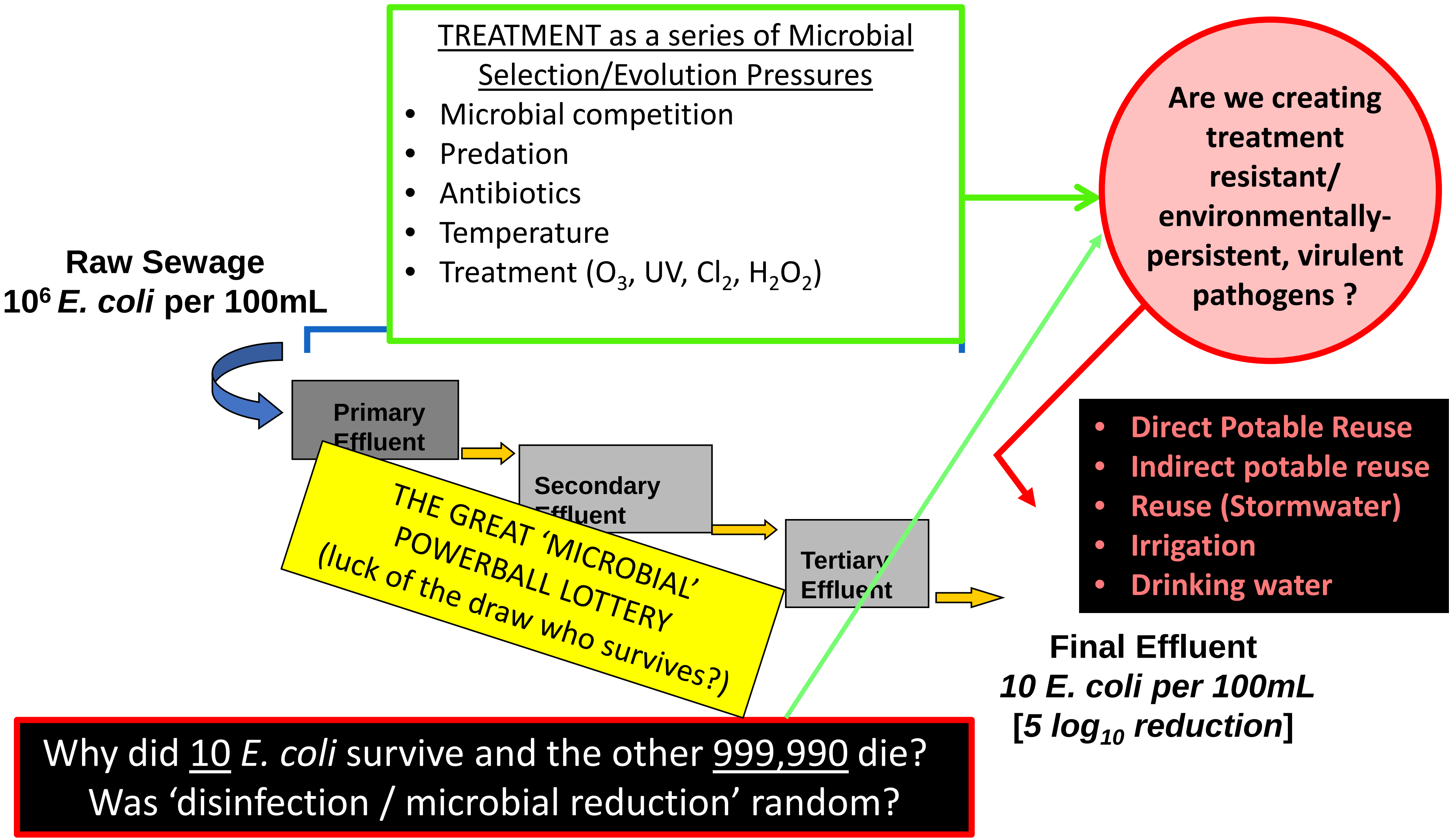
- Like antibiotic resistance, evolutionary selection for treatment resistance has been going on for a very long time….millions/billions of years!!!

- Examples
  - The mammalian immune system uses reactive chlorine (e.g., HOCl), reactive oxyen (H_2O_2, $O_2^{\cdot}$, $OH^{\cdot}$) and reactive nitrogen (peroxynitrite [$OONO^{\cdot}$], nitric oxide [$NO^{\cdot}$]) as a defenses against microbes.
    - Microbes have evolved a number of strategies to deal with these 'toxic' molecules
  - Many microbes need to survive in an environment until the next host comes along to infect....
    - solar radiation (polychromatic UV)
    - dessication
    - osmotic pressure
    - temperature
    - predation
    - microbial competition

They already have the tools in the toolbox!!

...microbes have had a long time to think about these 'disinfection' problems...and...they have 'invented' diverse and remarkable solutions!

AN ENGINEER's VS. AN EVOLUTIONARY MICROBIOLOGIST's Perspective on Wastewater Treatment



INACTIVATION OR KILLING OF MICROBES IS NOT RANDOM IN A WASTEWATER TREATMENT PROCESS

We do not choose who lives and who dies from treatment !
Nature decides !

The <u>10</u> *E. coli* survived…not because they were lucky…but…..
because they had
MICROBIAL KEVLAR™
(i.e., they were wearing bullet proof vests)!!!!!!

Some *E. coli* strains have evolved to live and survive in wastewater !

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

CrossMark click for updates

Evidence of Naturalized Stress-Tolerant Strains of *Escherichia coli* in Municipal Wastewater Treatment Plants

Shuai Zhi,[a] Graham Banting,[a] Qiaozhi Li,[a] Thomas A. Edge,[b] Edward Topp,[c] Mykola Sokurenko,[a] Candis Scott,[a] Shannon Braithwaite,[a] Norma J. Ruecker,[d] Yutaka Yasui,[a] Tim McAllister,[e] Linda Chui,[f] Norman F. Neumann[a,g]

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Journal of Environmental Engineering and Science

Stress resistance in naturalised waste water *E. coli* strains
Zhi, Banting, Ruecker and Neumann

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Paper 1600021
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Keywords: environment/waste management & disposal/water supply

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Stress resistance in naturalised waste water *E. coli* strains

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The authors recently demonstrated that naturalised strains of *Escherichia coli* exist in municipal waste water, characterised by (*a*) biomarker patterns in intergenic regions distinct from human and animal *E. coli* strains and (*b*) an insertion element (*IS30*) located in the *uspC–flhDC* intergenic region of the genome. Remarkably, these strains are naturally adapted to survival and growth in waste water and differentially survive the treatment process. The authors sought to explore the adaptive mechanisms used by these strains for survival. A serial stress experiment (nutrient deprivation and osmotic stress followed by chlorine treatment) was performed and survival was measured using culture. Waste water strains were shown to be approximately 100 times more resistant to chlorine treatment than a wild-type human faecal strain. Naturalised waste water strains were also more robust at producing biofilms – an adaptive strategy for surviving environmental stressors. Since biofilm formation has been linked to increased motility, the authors examined the expression of the flagellar regulator gene, *flhDC*, under serial stress conditions. Chlorine was a potent inducer of *flhDC* expression in waste water strains. The results demonstrate that waste water strains possess adaptive genotypic/phenotypic properties related to their survival in waste water and challenge the understanding of treatment reduction based on *E. coli* as an indicator of treatment performance.

Stress-induced Chlorine Resistance in Wastewater Naturalized *E. coli* strains

Table 2. Survival of human and naturalised waste water *E. coli* strains after nutrient deprivation/osmotic stress and chlorine treatment

E. coli strains	*E. coli* source	Control	Treatment			
			Nutrient deprivation/osmotic stress[a]		Chlorine treatment[b]	
		E. coli numbers after 24 h culture in TSB:[c] ml^{-1}	*E. coli* numbers after osmotic stress:[c] ml^{-1}	$\log_{10}$ reduction after osmotic stress[c]	*E. coli* numbers after chlorine treatment:[c] ml^{-1}	$\log_{10}$ reduction after chlorine treatment[c]
H51						$4{\cdot}1 \pm 0{\cdot}3^{e}$
H54						$2{\cdot}1 \pm 0{\cdot}10^{e,f}$
WW10						$2{\cdot}1 \pm 0{\cdot}03^{e,f}$
WW63	Waste water	$9{\cdot}3 \pm 1{\cdot}5 \times 10^{8}$	$8{\cdot}8 \pm 1{\cdot}0 \times 10^{8}$	$-0{\cdot}01 \pm 0{\cdot}10^{d}$	$5{\cdot}6 \pm 0{\cdot}91 \times 10^{6}$	$2{\cdot}2 \pm 0{\cdot}13^{e,f}$

Wastewater strains were ~100 times more resistant to chlorine than some fecal and lab strains, as well as better biofilm producers!

[a] Nutrient deprivation/osmotic shock performed by diluting TSB cultures to 1:10 in distilled water and incubating for 24 h at room temperature
[b] Cells treated with 0·3–0·5 ppm residual-free chlorine for 5 min
[c] *E. coli* concentrations and $\log_{10}$ reductions are presented as mean ± standard error ($n = 3$)
[d] No signif … on/osmotic stress treat…
[e] Significan … eatment
[f] Significan … lised RpoS stress respo…

These wastewater strains were originally isolated in the lab by treating raw sewage with a ~5 $\log_{10}$ microbicidal treatment with chlorine (bleach)!

APPLIED MICROBIOLOGY
Vol. 12, No. 1, p. 1–6 January, 1964

Killing of Chlorine-Resistant Bacteria by Chlorine-Bromine Solutions

H. FARKAS-HIMSLEY[1]

Department of Bacteriology, Hebrew University—Hadassah Medical School, Jerusalem, Israel

Received for publication 10 May 1963

ALLEN, L. A., AND E. BROOKS. 1949. Destruction of bacteria in sewage and other liquids by chlorine and by cyanoge nchloride. J. Hyg. **47**:320–335.

BOGOLYUBOV, K. K. 1947. Secondary appearance of *B. coli* in chlorinated water. Gigiena i Sanit. **12**:33–36.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1998, p. 4658–4662 Vol. 64, No. 12
0099-2240/98/$04.00+0

Effects of Starvation on Physiological Activity and Chlorine Disinfection Resistance in *Escherichia coli* O157:H7

JOHN T. LISLE,[1] SUSAN C. BROADAWAY,[1] ANNETTE M. PRESCOTT,[2] BARRY H. PYLE,[1] COLIN FRICKER,[2] AND GORDON A. MCFETERS[1]*

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Received 23 April 1998/Accepted 14 September 1998

Science of the Total Environment 458–460 (2013) 169–175

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Characterization and identification of a chlorine-resistant bacterium, *Sphingomonas* TS001, from a model drinking water distribution system

Wenjun Sun [a,b,*], Wenjun Liu [a], Lifeng Cui [a], Minglu Zhang [a], Bei Wang [c]

[a] School of Environment, Tsinghua University, Beijing 100084, China
[b] Department of Chemical and Biochemical Engineering, Western University, London N6K 3K7, Canada
[c] School of Engineering, University of Guelph, Guelph N1G 2W1, Canada

HIGHLIGHTS

"…this strain was very resistant to chlorine, and 4 mg L^{-1} of chlorine with 240 min retention time provided only approximately 5% viability reduction…"

Sphingomonas
Chlorine resistant
UV disinfection

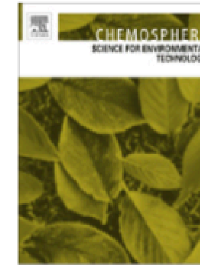
tion (99.9%) was obtained for UV fluencies of 40 mJ cm^{-2}. A high chlorine-resistant and UV sensitive bacterium, *Sphingomonas* TS001, was documented for the first time.

Chemosphere 90 (2013) 2247–2253

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere



Effect of chlorination and ultraviolet disinfection on tetA-mediated tetracycline resistance of *Escherichia coli*

Jing-Jing Huang [a,c], Hong-Ying Hu [a,b,*], Yin-Hu Wu [a], Bin Wei [a], Yun Lu [a]

[a] *State Key Joint Laboratory of Environmental Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, PR China*
[b] *State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (MARC), Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, PR China*
[c] *China Power Engineering Consulting Group Corporation, Beijing 100120, PR China*

- Tetracycline-resistant *E. coli* showed tolerance to chlorine at high doses.
- Chlorination with a high dose shifted tetracycline-resistant *E. coli* to become even more tolerant to tetracycline.

Mutagenesis vol. 19 no. 5 pp. 349–354, 2004

Divergent adaptation of *Escherichia coli* to cyclic ultraviolet light exposures

David Alcántara-Díaz[1], Matilde Breña-Valle and Jorge Serment-Guerrero

Departamento de Biología, Instituto Nacional de Investigaciones Nucleares, PO Box 18-1027, CP 11801, México DF, México

Experimental design

- SINGLE strain of *E. coli* (PQ30) exposed to increasing UVC …. for 80 generations!
- ~ final irradiating natural selection dose was 640 J/m^2

Findings

- 5 $\log_{10}$ difference in susceptibility (100,000X more resistant)!
- Vertical heredity potential– parent to progeny
- Does NOT include horizontal gene transfer potential

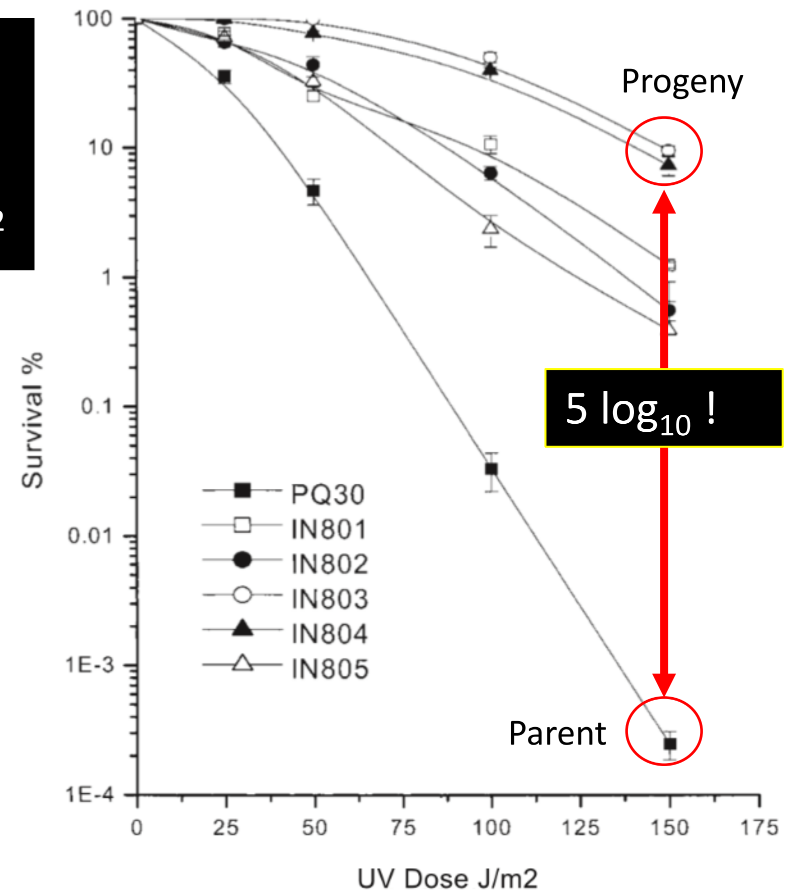
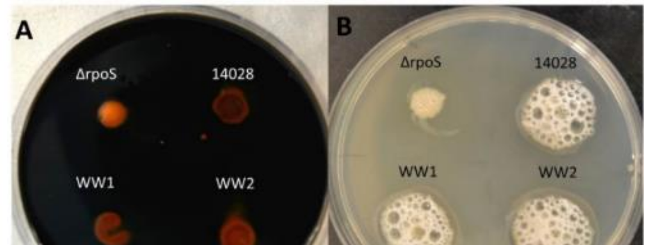


Fig. 2. Dose–responses to UV light of wild-type *E.coli* PQ30 and UV-resistant derivatives isolated after 80 UV irradiation cycles. See Figure 1 for details.

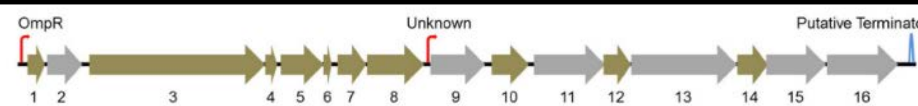
Additional characteristics of wastewater *E. coli* strains

Generalized stress response (*rpoS*), universal stress response (*usp*)



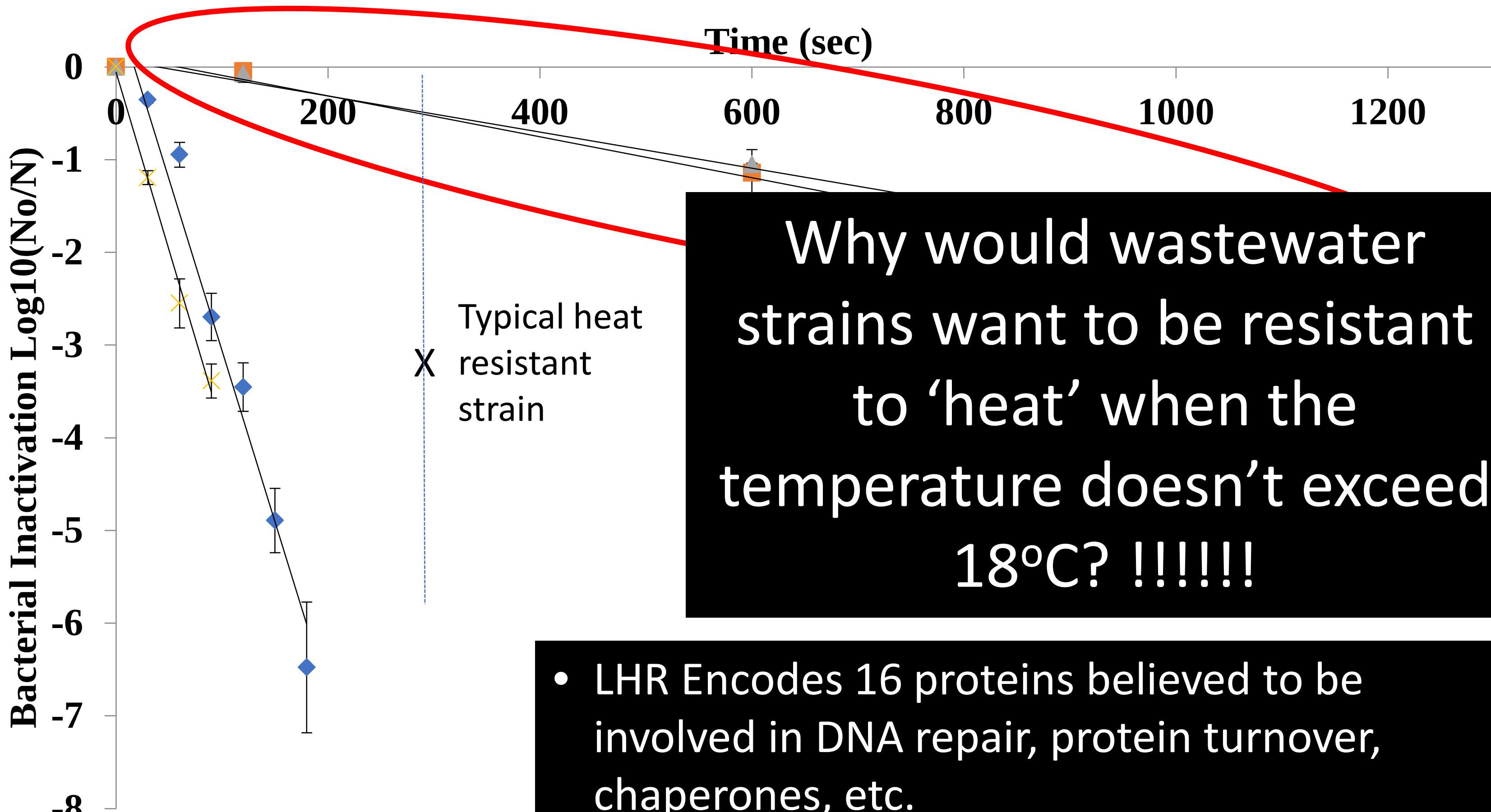
Our wastewater *E. coli* also show a resistance phenotype to UV !

Presence of a Heat Resistant Genomic Island (Locus of Heat Resistance)



- **Originally described in *Klebsiella* heat/disinfection treatment tolerant strains in hospitals. Recently found in *E. coli* by Mercer et al., (2015). Can withstand 60ºC for 5 minutes**

Heat tolerance of Wastewater Naturalized *E. coli* strains (60ºC)



Why would wastewater strains want to be resistant to ‘heat’ when the temperature doesn’t exceed 18ºC? !!!!!!

- LHR Encodes 16 proteins believed to be involved in DNA repair, protein turnover, chaperones, etc.
- LHR probably not a good name for this locus….

Courtesy of Dr. Nicholas Ashbolt and Michael Gro

Other Examples of Heat Resistance

- We have isolated strains of:
  - *E. coli* that can survive temperatures reaching >55^{o}C (maximum temp. of 55^{o}C) for 8 days, and potentially persist in a viable-but-non culturable (VBNC) state for >30 days!
  - *Salmonella* that can survive > 55^{o}C for 13 days and persist in a VBNC state for >30 days!

Extremely Heat Resistant *E. coli* and *Salmonella*…ioriginating from sewage treatment plants (biosolids)!

Are we seeing co-evolutionary selection between virulence and treatment-resistance in *E. coli* as a result of our engineering practices?

<u>WHERE'S THE EVIDENCE</u> ?

Virulence and plasmidic resistance determinants of *Escherichia coli* isolated from municipal and hospital wastewater treatment plants

Vera Calhau, Catarina Mendes, Angelina Pena, Nuno Mendonça and Gabriela Jorge Da Silva

ABSTRACT

"WWTPs contribute to the dissemination of virulent and resistant bacteria in water ecosystems, constituting an environmental and public health risk."

aac(6′)-Ib-cr. Aminoglycoside resistance and multidrug-resistant phenotypes were also detected. PAI IV$_{536}$, PAI II$_{CFT073}$, PAI II$_{536}$ and PAI I$_{CFT073}$, and uropathogenic genes *iut*A, *papAH* and *sfa/foc* were detected. With regard to the clinical ST131 clone, it carried *bla*$_{CTX\text{-}M\text{-}15}$, *bla*$_{TEM\text{-}type}$, *qnrS* and *aac(6′)-Ib-cr*; IncF and IncP plasmids, and virulence factors PAI IV$_{536}$, PAI I$_{CFT073}$, PAI II$_{CFT073}$, *iutA, sfa/foc* and *papAH* were identified in the effluent of a hospital plant. WWTPs contribute to the dissemination of virulent and resistant bacteria in water ecosystems, constituting an environmental and public health risk.

Portugal

Key words | *Escherichia coli*, phylogeny, plasmidic resistance determinants, virulence factors, WWTP

WATER RESEARCH 47 (2013) 6670–6679

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Survival of *Escherichia coli* in two sewage treatment plants using UV irradiation and chlorination for disinfection

E.M. Anastasi [a,d], T.D. Wohlsen [b], H.M. Stratton [c,d], M. Katouli [a,d,*]

[a] Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC 4558, Queensland, Australia
[b] Unitywater, Maroochydore Sewage Treatment Plant, Maroochydore, Australia
[c] School of Biomolecular and Physical Sciences, Griffith University, Nathan Campus, Queensland, Australia
[d] Smart Water Research Centre, Griffith University, Gold Coast Campus, Queensland, Australia

”Strains surviving UV irradiation were…carrying virulence genes associated with urinary pathogenic E. coli (UPEC) and intestinal pathogenic *E. coli* (IPEC).”

“Our data suggest that some *E. coli* strains have a better ability to survive sewage treatment plants utilizing chlorination and UV irradiation for disinfection.”

Applied and Environmental Microbiology, Sept. 2010, p. 5882–5886 Vol. 76, No. 17
0099-2240/10/$12.00 doi:10.1128/AEM.00141-10

Prevalence and Persistence of *Escherichia coli* Strains with Uropathogenic Virulence Characteristics in Sewage Treatment Plants▽

E. M. Anastasi,[1] B. Matthews,[2] A. Gundogdu,[1] T. L. Vollmerhausen,[1] N. L. Ramos,[1] H. Stratton,[2] W. Ahmed,[3] and M. Katouli[1]*

Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC 4558, Queensland, Australia[1]; School of Biomolecular and Physical Sciences, Griffith University, Nathan 4111, Queensland, Australia[2]; and Department of Environment and Resource Management, 80 Meiers Road, Indooroopilly, Brisbane 4068, Queensland, Australia[3]

Received 20 January 2010/Accepted 29 June 2010

"Our results indicate that certain…UPEC strains can survive the treatment processes of sewage treatment plants."

strains. Of these, 120 (76.4%) strains belonged to seven persistent C-BPTs and were found in all four STPs. Our results indicate that certain clonal groups of *E. coli* with virulence characteristics of uropathogenic strains can survive the treatment processes of STPs. These strains were common to all STPs and constituted the highest proportion of the strains in different treatment tanks of each STP.

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ORIGINAL RESEARCH

Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa

Martins A. Adefisoye[1,2] & Anthony I. Okoh[1,2]

[1]SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Alice 5700, South Africa
[2]Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, South Africa

Keywords

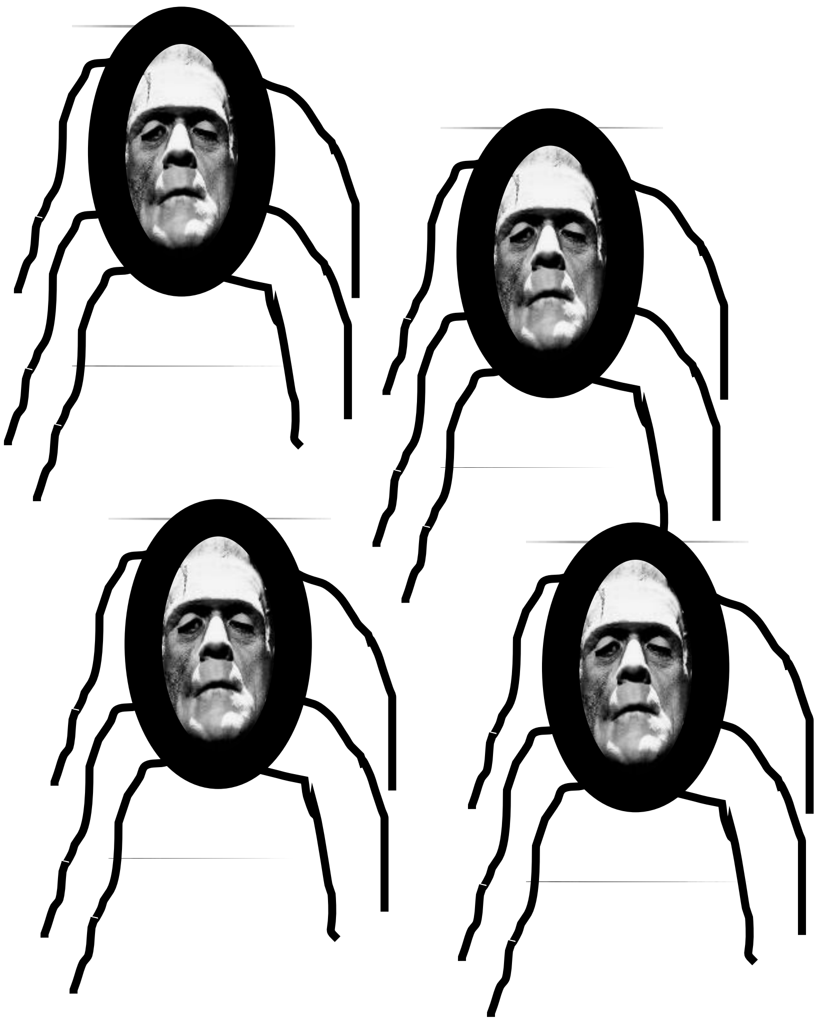
Abstract

"Molecular characterization revealed five pathotypes...: ETEC (1.4%), EPEC (7.6%), EAEC (7.6%), NMEC (14.8%) and UPEC (41.7%)."

"We conclude that municipal wastewater effluents are important reservoirs for dissemination of potentially pathogenic *E. coli* (and possibly other pathogens)....."

and antibiotic resistance genes in the aquatic milieu of the Eastern Cape and a risk to public health.

Microbial Frankensteins



Question #1:

Are we actually 'creating' new MICROBIAL MONSTERS for our industry ?

Question #2:

If we are creating these problems then what are the solutions ?

- More chlorine?
- More ozone?
- More UV?
- More heat?
- More dessication?
- More sanitizers?
- More disinfectants?
- More additives?

Or will this lead to more resistance?

Implications for Food Processors

- Resistant bacteria are part of nature…don't assume they're not a problem in you facility.
  - Are you complacent or diligent?
- These principles apply to all microbes, including foodborne pathogens (*Salmonella*, *Campylobacter*, *Arcobacter*, *Listeria*, etc.)
  - Evolutionary principles govern all living organisms (i.e., survival of the fittest)
    - Antibiotic-resistance, vaccines, pesticide resistance (mosquitoes, molluscs), clinical resistance (viruses, bacteria, parasites, worms)
- Don't rely on a single barrier for food safety. Multi-barrier approach to HACCP programs needed.

The Role of Water Quality in Food Safety: Does Water Matter?

International Association for Food Protection®

Part 3: Does Water Quality Matter To My Food Company?

Monday, June 4, 2018, Noon, Eastern Daylight Time U.S.

Part 1 gave the basics of EPA rules and how time lags might impact food processors.

Part 2 described what could be in the compliant Safe Drinking water you get.

In Part 3, learn what to do about it!

University of Arizona's **Dr. Chuck Gerba** explains the basics of Quantitative Microbial Risk Assessment (QMRA) and determining your risk profile, including what information you need to evaluate your risk and where to get it;

Dr. Vince Hill of the CDC explains why we don't hear much about the nexus between water and food contamination;

Will Daniels, President, Produce Division, IEH Laboratories will advise on Measures you can take if your water isn't as safe as your business requires.

Sponsored by **IAFP's**
Water Safety and Quality PDG
&
Atlantium Technologies

Speakers

Vincent Hill,
Chief, Waterborne Disease Prevention Branch –
Division of Foodborne, Waterborne and Environmental Diseases, (CDC)

Dr. Chuck Gerba,
Professor
University of Arizona

William C. Daniels,
President, Produce Division
IEH Laboratories & Consulting Group

Moderator

Phyllis Butler Posy,
Chair - Water Quality Safety PDG
Vice President of Strategic and Regulatory Affairs
Atlantium Technologies