Supplementary information

**Biofilms in Shower Hoses** 

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**Table S1:** News items concerning *Legionella pneumophila*, all published in the first half of 2017.

Article title	Description	Link	Screenshot Page (After Table S9)
Legionella detected in water systems at two Kane centers (March 10, 2017)	Legionella was detected at two nursing home facilities in Pennsylvania. The centers switched to bottled water and activities like showering were restricted.	http://www.wpxi.com/new s/top-stories/legionella- detected-in-water- systems-at-two-kane- centers/501536863	1 of 16
Ocoee gym being examined after 3 sickened by Legionnaire's disease, officials say (April 19, 2017)	Three people linked to one gym/athletic center in Florida contracted Legionnaire's disease. Testing is underway at the facilty, and before results are returned, the gym closed showers, changed fixtures, and took other measures to reduce risk.	http://www.wftv.com/new s/local/ocoee-gym-being- examined-after-3- sickened-by-legionnaires- disease-officials- say/514286196	2 of 16
Parisian Macao battling Legionnaire's Disease outbreak (April 25, 2017)	A casino in China may have been involved in 3 cases of Legionnaire's disease for elderly visi. Before test results are in, the casino is doing precautionary measures like closing the swimming pools, jacuzzis, and fountains. The article speculated about possible drops in tourism to the casino.	https://calvinayre.com/20 17/04/25/casino/parisian- macao-legionnaires- disease-outbreak/	3 of 16
Legionnaires sickened four people, one fatally, at cancer facility: suit (April 9, 2017)	Several victims in a 2015 outbreak of Legionnaires disease at a cancer lodge (free temporary housing for patients between treatments) in New York are suing the nonprofit which runs the cancer lodge	http://nypost.com/2017/0 4/09/legionnaires- sickened-four-people- one-fatally-at-cancer- facility-suit/	4 of 16
Walton pool and gym reopen almost three months after legionella bug outbreak (February 24, 2017)	A gym/athletic center in the UK involved in a late 2016 Legionella outbreak, with tests of shower water coming back positive for the bacteria, was forced to close for three months in order to modify the water system and eliminate Legionella. The period was hard to estimate and reopening was frequently pushed back.	http://www.clactonandfrin tongazette.co.uk/news/15 115947.Pool_and_gym_r eopen_almost_three_mo nths_after_legionella_bu g_outbreak/	5 of 16
Legionnaires' disease diagnosed in Fresno nursing home patient (January 13, 2017)	A nursing home in California started using bottled water and point-of-use filters for showers, and stopped using ice machines after a resident was diagnosed with Legionnaires'. They also planned to use hyper-chlorination or super-heat treatment.	http://www.fresnobe e.com/news/local/art icle126494379.html	6 of 16

 Table S2: Additional information about shower hoses used in global survey.

Country (# of samples)	Location (Number of samples)	Chlorine use	Notes on water	Estimated minimum age (years)	# people using shower	Household type
				5 8	2 2	house townhouse
	Ghent, East Flanders			2	2	apartment
Belgium	(7)	Chlorinated	Low	2	2	house
(8)		Chiomateu	residuals	2	9	house
				16	4	townhouse
	Loorno Foot			?	4	house
	Laarne, East Flanders (1)			4.5	4	house
				n/a	n/a	apartment
				2	1	apartment
				1	4	apartment
				5	1	apartment
Denmark	Greater Copenhagen area (various	None	None	0.5	1	apartment
(10)	municipalities) (10)	None	None	n/a	n/a	apartment
	maniopanies) (10)			2	5	apartment
				7	4	apartment
				n/a	2	apartment
				1.5	2	apartment
	Kempten, Bavaria (1) Sonthofen, Bavaria (1) Wiggensbach,	None	Emergenc y use only	14	2	apartment
Germany				1.5	2	apartment
(4)				3	2	apartment
	Bavaria (2)			3	2	apartment
				4	2	apartment
	Riga (7)		Residual often fails	5	2	apartment
				4	4	apartment
Latvia (7)		Chlorinated		5	2	apartment
			Oileir iails	0.125	2	apartment
				2	1	apartment
				n/a	n/a	apartment
				2	2.5	apartment
				6.5	3	apartment
	Greater Lisbon area			6.5	3	apartment
Portugal	(various	Chlorinated	Residual used	0.5	2	apartment
(7)	municipalities) (7)		นอฮน	1	3	apartment
				12	4	apartment
				6	3	apartment
				2	2	apartment
0.5515 (4)	No. 10 at 40	Oblested	Limited	3	3	apartment
Serbia (4)	Novi Sad (4)	Chlorinated	residuals	5	3	apartment
				1	2	apartment

(Table S2 continued)

Country (# of samples)	Location (Number of samples)	Chlorine use	Notes on water	Estimated minimum age (years)	# people using shower	Household type
South Africa (3)	Pretoria (3)	None	Private bore holes	35 1 35	2 2 2	house house house
Spain (1)	Bolnueva, Murica (1)	Chlorinated	residual	n/a	n/a	house
				0.5	2	townhouse
				0.75	2	apartment
				2	3	apartment
				0.25	3	apartment
				6	3	apartment
				5	3	apartment
				1	4	apartment
				1.5	1	apartment
	Zürich canton			8	2	apartment
Switzerla	(various		None	8	2	apartment
nd (21)	municipalities) (19)	None		n/a	1	apartment
110 (21)				4	2	apartment
				2	2	apartment
				4	2	apartment
				4	4	apartment
				5	X *	Office *
				5	X *	Office *
				5	X *	Office *
				5	X *	Office *
	Bern canton (1)			5	4	apartment
	Ticino canton (1)			n/a	2	apartment
				n/a	n/a	apartment
	Greater Newcastle			n/a	n/a	apartment
	area (various			2	2	apartment
United	municipalities) (5)			n/a	n/a	apartment
Kingdom		Chlorinated	Residual used	4	1	apartment
(9)			useu	n/a	n/a	apartment
	Greater Glasgow			2	2	apartment
	area (various			2	1	apartment
	municipalities) (4)			3.5	4	apartment
United	Columbus, Ohio (1)			5	3	townhouse
	Carrboro, North Carolina (1)	Chlorinated	Residual	1	4	house
States (4)	Franklin County,		used	16	2	house
	Pennylvania (2)	•		0.75	2	house
* Locker room in an office building. Use-patterns are variable, but as these showers were also used						

<sup>\*</sup> Locker room in an office building. Use-patterns are variable, but as these showers were also used in the controlled study, more information about use patterns is available in Table S6.

Table S3: Elemental Analysis Method Details

Element		nt Target Re isotope r		LOD	Recovery o	
		(m/z)	(1=no gas, 2=H, 3=He)	(ug/l)	Sediment	Rice Flour
Mg	Magnesium	24	2	2	109	72
Ca	Calcium	44	1	9	109	96
Mn	Manganese	55	2	65	102	89
Fe	Iron	56	2	81	96	138
Cu	Copper	65	2	0.5	77	72
Pb	Lead	206	1	0.05	98	

Table S4: qPCR method details

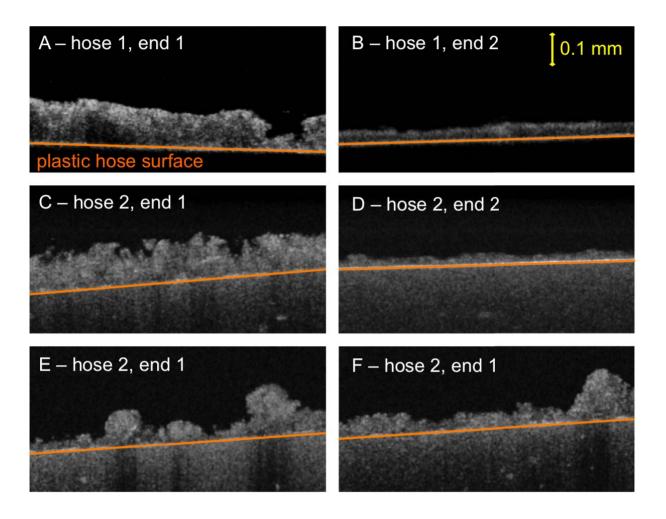
	Reaction Chemistry		Reaction	n timin	g		
Primer/Probe	Sequence	Volume (per 10 uL reaction)	Time (seconds)	Temp ure		Source	
	Legionella pneum	ophila – mip	)				
LmipF (5µM)	AAA GGC ATG CAA GAC GCT ATG	0.5	denatu	ıration		(Wang,	
LmipR (5µM)	GAA ACT TGT TAA GAA CGT CTT TCA TTT G	0.5	120	95	°C	2012)	
Lmip Probe (10µM)	FAM-TGG CGC TCA ATT GCG TTT AAC CGA	0.2	40 cy	cles		(Nazari an,	
water		2.8	15	95	°C	2008)	
Probes supermix	SsoFast Probes Supermix (Bio-Rad)	5	30	60	°C		
Template	,	1					
	Acanthamoeba sp	p. – 18S rRN/	<b>A</b>				
ACF1 (5µM)	CGACCAGCGATTAGGAGACG	0.5	denatu	ıration		(Wang,	
ACR1 (5μM)	CCGACGCCAAGGACGAC	0.5	120	95	°C	2012)	
Taq ACProbe (5µM)	FAM- TGAATACAAAACACCACCATC GGCGC	0.2	40 cy	40 cycles		(Rivière	
water		2.8	15	95	°C	, 2006)	
Probes supermix	SsoFast Probes Supermix (Bio-Rad)	5	30	60	°C		
Template		1					
	Vermamella vermifor	mis – 18S rR	NA				
Hv1227F (5µM)	TTA CGA GGT CAG GAC ACT GT	0.7	denatu	ıration		(Wang,	
Hv1728R (5µM)	GAC CAT CCG GAG TTC TCG	0.7	120	95	°C	2012)	
water		2.1	40 cy	40 cycles		(Kuiper	
Evagreen	EvaGreen Supermix (Bio-Rad)	4.5	8	98	°C	, 2006)	
Template		2	30	72	°C		
Mycobacterium avium – 16S rRNA							
MYCGEN-F (5µM)	AGA GTT TGA TCC TGG CTC AG	0.8	denatu	ıration		(Wang,	
MYCAV-R (5µM)	ACC AGA AGA CAT GCG TCT TG	0.8	120	95	°C	2012)	
water		2.4	40 cy	40 cycles		(Wilton,	
Evagreen	EvaGreen Supermix (Bio-Rad)	5	8	98	°C	1992)	
Template		1	30	68	°C		

Table S5: Details for Illumina sequencing

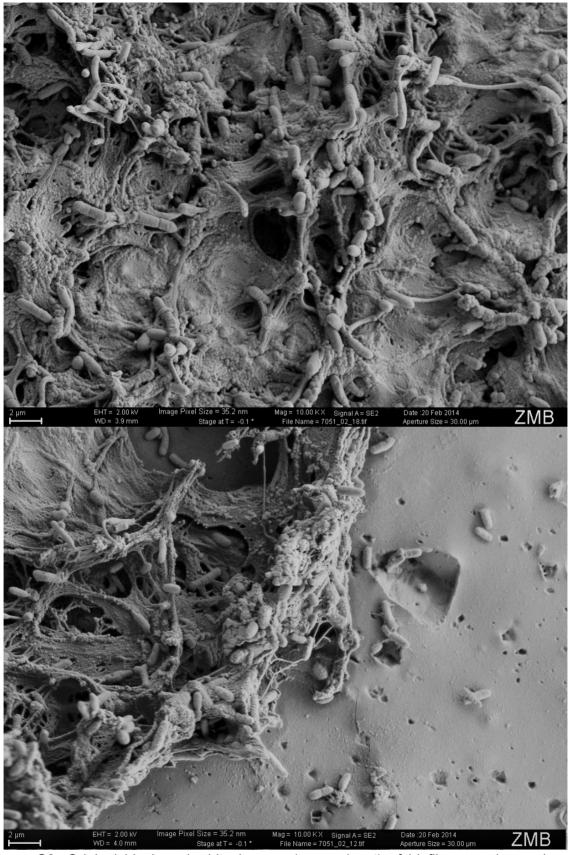
	Primer		Primer Sequence			Reference
ջ		3akt_341F act-0341-b-S	S-17)	CCTAC	GGGNGGCWGCAG	Klindworth, 2013
C Prime		akt_805R act-0785-a- <i>P</i>	x-21)	GACTAC	HVGGGTATCTAATCC	•
Amplicon PC Primers	Nextera adap	oter tail befo	re forward		-GCA-GCG-TCA-GAT- -AAG-AGA-CAG-GA	
A	Nextera adap	ter tail befo	re reverse		-TGG-GCT-CGG-AGA- -TAA-GAG-ACA-GAG	
	Assay	Holding	Cycling Reps	Cycling	Kti/Mix and Reaction Chemistry	Template/Notes
		95 °C		95 °C 0:30	1U KAPA 2G robust HotStart Polymerase (KAPA Biosystems,	2 μL DNA template (0.8-50 ng)
	Amplicon PCR		19 X	54 °C 0:30	Boston, USA),  1 x reaction buffer B, and  0.4 µM of each primer in	Two sets of frame-shifted primer sets were used on each replicate extraction per
PCR Details		5:00		72 °C 0:30	a final volume of 25 μL. Sensoquest Labcycler Basic used.	sample: Sets 0 and 2 for replicate A and sets 1 and 3 for replicate B
PC		95 °C		95 °C 0:30	1 X KAPA HiFi HotStart Ready Mix and 5 μl of	
	Index PCR		10 X	55 °C 0:30	each of the respective  Nextera index primers in a total reaction volume of	Pooled amplicon PCR product
	3:00		72 °C 0:30	50 µl		
		S	tep		System	Protocol
sdi	Purification of Amplicon PCR product				Agencort AMPure beads XP sytem (Beckman	Supplier's protocol
ıal Ste	Purifi	cation of In	dex PCR p	product	Coulter)	
Additional Steps	Quality	Control of	Index PCR	product	Agilent Bioanalyzer	Supplier's protocol
٩	Quantification of Index PCR		product	KAPA library quantification kit	Supplier's protocol	

Table S6: Details for Illumina sequencing data processing

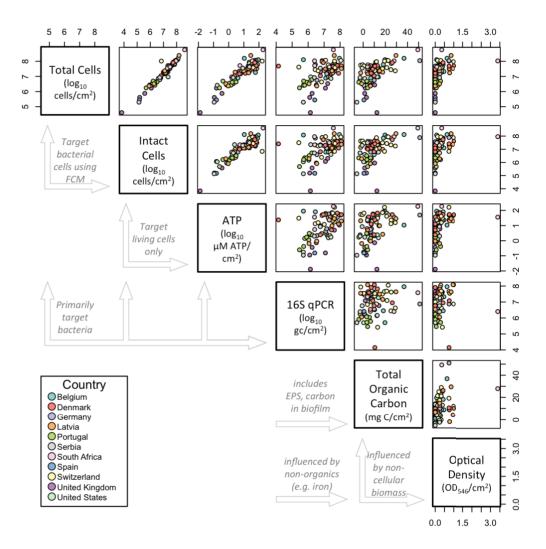
	Step	Algorithm/ Version	Parameters	Citation	
	Quality Control	FastQC v.0.10.1			
			minimum overlap: 40		
	Merge Reads	FLASH v1.2.9	maximum overlap: 100	(Magoc and Salzberg, 2011)	
<u>s</u>			max mismatch density: 0.2		
Bioinformatics Details	Trim adaptor sequences and sort frame shifts	Cutadapt v1.4	error rate: 0	(Martin, 2011)	
oinf			size range: 450-550 bp		
Ä	Quality Filtering	PRINSEQ-lite	minimum mean quality score: 25	(Schmieder and	
	Quanty I Intering	v0.20.4	no ambiguous nucleotides	Edwards, 2011).	
			GC range: 20-80		
			identity cutoff: 97%		
	OTU clustering	usearch v7.0.1090	abundance sorting: 2	(Edgar, 2010)	
			chimera filtering		



**Figure S1:** Optical Coherence Tomagraphy (OCT) images of biofilms from 2 hoses from Belgium (Hose 1) and Switzerland (Hose 2). All are on an equal scale. A and B are from opposite ends of the same hose (Hose 1). C, E, and F are from one 5 cm piece on one end of a hose, while D is from the opposite end of the same hose (Hose 2).



**Figure S2:** Original black and white images (zoomed out) of biofilms on shower hoses, corresponding to Figure1E (top) and 1F (bottom). In Figure1, only particular sections were shown in order to focus attention. Color was also added artificially to highlight key biofilm elements.



**Figure S3:** Correlation between various measures of biofilm concentration. All scatter-plot points represent values for individual shower hose samples, colored by country of sample origin. Some factors (Total cells, intact cells, ATP, and 16S qPCR gc) are transformed as indicated due to the wide spread of values.

## Further discussion about biofilm concentration measures:

- TCC, ICC, and ATP had strong correlations with one another. These all target primarily bacteria. ICC and ATP both target living biomass.
- qPCR did not correlate well with TCC in this study. This could be because the efficiency of extra processing steps (e.g., DNA extraction) was affected unequally by inorganic deposits that varied unequally across biofilms (e.g., iron).
- TOC was likely influenced by both cells and extracellular polymeric substance (EPS) production. It may have captured some aspects of 'sliminess', which is otherwise difficult to quantify.
- OD did not correlate well with TCC. While cells likely contributed to OD, other factors contributed to the color, and thus the OD of the suspended biofilms. For example,

red color can be caused by inorganic (iron deposition) and organic (*Meiothermus* bacteria). Hardness deposition (calcium, magnesium) likely contributed with opaque white particles. OD did correlate with several metals (Spearman's  $\rho$  for lead:0.68, iron:0.67, copper:0.86, calcium:0.40, and manganese:0.51).

#### **Methods (related to Figure S3)**

## Intact cells (ICC) analysis for biofilm suspensions

Measurement of ICC was the same as for TCC, except for the stain used. Propidium iodide (PI; 30 mM) was mixed with the SYBR® Green I working solution to a final PI concentration of 0.3 mM. 200  $\mu$ L was stained with SGPI at 10  $\mu$ L/mL. Samples were preheated to 35 °C (3 min), then incubated with stain in the dark for 10 min at 35 °C before measurement. Flow cytometric measurements were performed, as described previously, using a BD Accuri C6® flow cytometer (BD Accuri cytometers, Belgium). Data analysis was performed using the BD Accuri CFlow® software, following the procedure described previously (Prest *et al.*, 2013) to calculate ICC.

### Adenosine tri-phosphate (ATP) analysis for biofilm suspensions

Total ATP was determined using the BacTiter-Glo™ reagent (Promega Corporation, Madison, WI, USA) and a luminometer (Glomax, Turner Biosystems, Sunnyvale, CA, USA) as described elsewhere (Hammes *et al.*, 2010). A biofilm suspension sample (100 µL) and the ATP reagent (100 µL) were warmed to 38 °C simultaneously in separate sterile Eppendorf tubes. The sample and the reagent were combined and then the luminescence was measured after 20 second reaction time at 38 °C. The data were collected as relative light units (RLU) and converted to ATP (nM) by means of a calibration curve made with a known ATP standard (Promega). ATP was measured in triplicate, and the relative standard deviation among technical replicates was below 4%.

#### 16S qPCR

Quantification of the 16S gene were completed as previously described (Proctor *et al.*, 2016). Briefly, the primers Bact349F/Bact806R and probe Bac516F (Takai and Horikoshi, 2000) and were performed using LightCycler 480 Probes Master hot start reaction mix (Roche). Either 100-fold or 1000-fold sample dilutions were used. qPCR reactions were performed on a LightCycler 480-II (Roche) and analyzed using the LightCycler 480 ver. 1.5.1 software (Roche).

#### **Total organic carbon (TOC)**

TOC concentration was determined by thermal oxidation to  $CO_2$  and infrared detection with the non-purgeable organic carbon method according to EN 1484 (TOC-V<sub>CPH</sub>, Shimadzu, Kyoto, Japan). All samples were diluted tenfold with a purified water with TOC < 5  $\mu$ g/mL organic carbon.

#### **Optical Density**

Optical density of biofilm suspensions was measured on an Uvikon 930 spectrophotometer (Kontron Instruments, Germany).

**Table S7:** Water quality and use patterns for 15 samples in the controlled study

						V	/ater C	uality		
		Hous	ehold use patterns	Incoming (Cold) water					Ho t♭	
	Tiousenoid use patterns incoming (				ng (Cold) water					
Sample	Number of People	Uses per week	Notes	Nitrogen	Phosphorous	Magnesium	Calcium	Total Cells	% Intact cells	% Intact Cells
				mg/ L	μg/L	mg/ L	mg/ L	cells/mL	%	%
1	3	14		4.7	7.8	2.5	5.00	2.44E+06	46	62
2	1	4		0.9	7.3	7.5	49.2	2.55E+05	40	63
3	locker room	8	*same room as 6 often stagnant on weekend	2.56	6.7	11.5	71.0	7.72E+05	42	64
4	locker room	20	*same room as 5 often stagnant on weekend	3.14	4.9	12.4	76.1	1.77E+06	45	55
5	locker room	30	*same room as 4 often stagnant on weekend	3.14	4.9	12.4	76.1	1.77E+06	34	55
6	locker room	9	*same room as 3 often stagnant on weekend	2.56	6.7	11.5	71.0	7.72E+05	38	58
7	2	2	**same household as 14	1.0	6.8	9.6	56.5	1.85E+05	55	52
8	3	15		3.68	3.0	1.3	7.19	2.70E+04	70	73
9	5	16		1.0	3.0	8.0	50.7	1.59E+05	63	73
10	4	21		0.92	3.9	7.9	51.0	6.07E+04	61	76
11	2	14		6.0	3.0	9.6	32.4	2.53E+05	57	75
12	2	17. 5	often stagnant on weekend	1.11	6.9	8.5	53.7	7.27E+04	63	77
13	2	9.5	Typically only used for high temperature cleaning	2.30	7.2	13.1	113. 9	8.10E+04	72	77
14	2	14	**same household as 7	1.0	6.8	9.6	56.5	1.85E+05	47	62
15	2	11		0.84	9.4	6.6	47.6	1.69E+05	54	51

<sup>\*</sup> Hoses 3 – 6 were from two locker rooms (men's, women's) in the same building.

<sup>\*\*</sup> Hoses 7 and 14 were from two bathrooms in the same apartment.

b Hot water samples as described in methods.

# Water Quality Methods (related to Table S7)

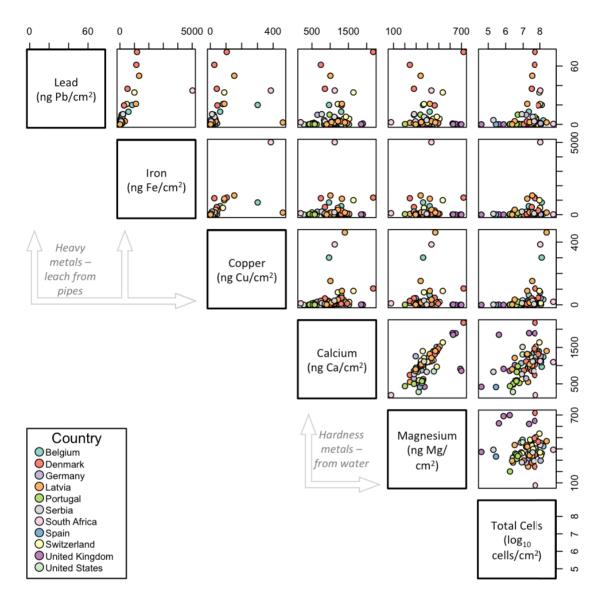
Water quality parameters were quantified by the AuA laboratory of Eawag, Switzerland. Briefly, nitrogen was measured via chemiluminescence utilizing Shimadzu TOC-L CSH. Phosphorus (Total-P) was measured after chemical digestion with potassium peroxide in the autoclave (Truttnauer/Systec 2540 EL) at 121°C followed by the spectrophotometric determination of ortho-phosphate after the reaction to phosphorus-molybdenum-blue-complex. Total-P was measured on a Spektrophotometer Varian Cary 50 Bio. Magnesium and calcium were measured using Ion chromatography (Column: Metrohm C6 – 250/4.0) with a Metrohm 930 Compact Flex. Intact cells were measured as described with Figure S3. The percentage is determined by dividing the intact cell concentration by the total cell concentration.

**Table S8:** Correlation values between use patterns and calculated weekly doses and biofilm concentration. Calculated weekly loads were derived by multiplying the number of uses per week by water quality concentrations (nitrogen, phosphorous, magnesium, calcium or total cells in the cold flowing water, (Table S7)) and the volume inside a shower hose. For all, N=15 for the 15 biofilm samples. Uses per week alone (e.g., without multiplying by a water quality parameter) had the strongest correlation with biofilm TCC, while other water quality measurements had only moderate correlations. That is to say, no single nutrient or load was controlling the system with stronger predictive power than frequency of alone. It could be either a combination of nutrients/cells or some other aspect of frequency of use that controls the biofilm TCC.

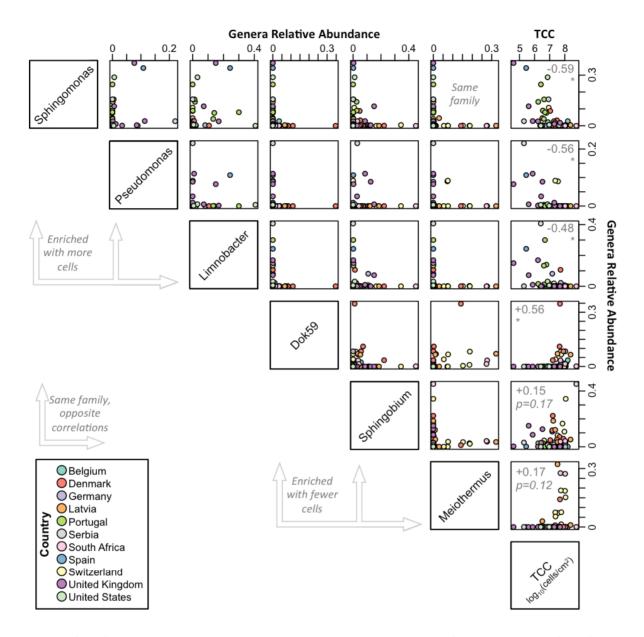
Correlation with biofilm total cell			(Pearson's test)		
COI	concentration [log <sub>10</sub> (cells/cm2)]			р	
Uses		use/week	0.70	0.004	
	Total nitrogen	mg/week	0.61	0.02	
/ load	Total phosphorous	μg/week	0.52	0.04	
Calculated weekly load	Magnesium	mg/week	0.58	0.02	
ated v	Calcium	mg/week	0.62	0.01	
Calcul	Total cells	log <sub>10</sub> (cells)/week	0.52	0.04	



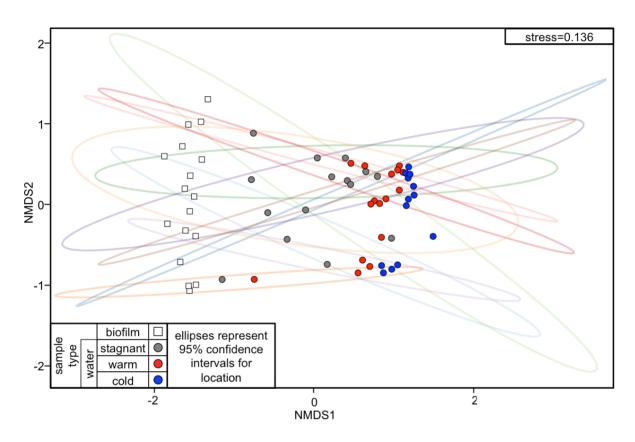
**Figure S4:** Photo from outside of a shower hose, with metal partially removed (left), and the inside of a shower hose with a particularly red/orange biofilm (right). On the left, biofilm formed yellow spots, resembling bacterial colonies on agar. On the right, the biofilm had a rough texture and deep red/brown/orange color.



**Figure S5:** Concentrations of metals (lead, iron, copper, calcium, and magnesium) and biofilm total cell concentration (biofilm TCC), with their correlations. Points represent individual hoses, colored by country. None of the metals correlated well with biofilm TCC. There were many non-detects for lead, iron and copper, but when present, there tended to be positive relationships between these metals – i.e., these metals precipitated together in biofilms. Calcium and magnesium were present in all biofilms, and had a positive relationship with each other. While the first three metals likely originate from up-stream pipes in the distribution system, calcium and magnesium likely originate from hardness in the water.



**Figure S6:** Correlational analyses between relative abundance of genera (sum of all OTUs identified within that genera) and biofilm total cell concentration (TCC) (log10 transformed). Points represent individual hoses, colored by country. Spearman rank correlations noted for correlations with biofilm TCC, with (\*) indicating p<0.001. These are the three most significant positive and negative correlations from the top 10 most abundant genera, but these do not necessarily represent the strongest correlations. For example, a significant positive correlation was found between *Legionella* and biofilm TCC (Spearman's  $\rho$ =0.36, p<0.001). This analysis focused on the top 10 most abundant genera because more frequent non-detects with less abundant OTUs likely affected results strongly. Notably, two genera from within the same family (*Sphingomonas* and *Sphingobium*) were both amongst the top 10 most abundant genera, but had opposite correlations.



**Figure S7:** NMDS representing Bray-Curtis dissimilarities between samples in the controlled study. Samples consist of 15 biofilms, and matching stagnant water, warm running water, and cold running water. Ellipses represent 95% confidence intervals for each household. These ellipses are generally narrow across an NMDS2 range, but extend widely through NMDS1 to capture all sample types. Household explained 41% of microbiome variation (Adonis). Thus, while there are similarities between sample types (symbol type and color), there are some strong similarities in a household. This could, for example, be due to drift into the household (cold flowing water) selecting downstream microbiomes.

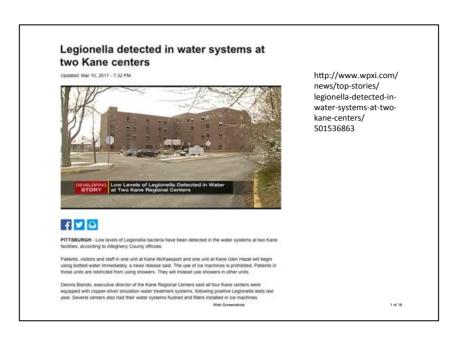
**Table S9:** Products available for addressing biofilms in shower hoses, including quarterly replacement systems and single-use shower hoses.

	Product name	Description	Link	Screenshot Page (After Table S9)
nent	TSafe® Replaceme nt Hand and Wall Showers	Shower hoses and heads made with antibacterial coatings. With four different colors, they can be replaced quarterly, or taken off for cleaning.	https://www.grahamasset management.co.uk/Data baseDocs/nav_6024781_ _replacement_shower_br ochure_oct_2016.pdf	7 of 16
Quarterly Replacement	Dupal L8 Shower ®	The company replaces antimicrobial shower heads and hoses quarterly, and collects them for recycling. The system uses four different colors to keep track of replacement.	https://www.antibacterials hower.co.uk/	9 of 16
	Challis Ag+® Antimicrobi al shower hoses	Made with anti-microbial plastics, and four different colors to keep track of replacement. The company delivers new hoses and collects old ones for recycling.	http://www.alchallis.com/ enviroment/environmenta I_policy.php	12 of 16
Single-use	Steri-Spray Steri- Cleanse Showering Attachment	Flexible single-use hose which easily connects to Steri-Spray shower systems. Designed to be used once to limit patient-to-patient contamination, it also ensures that biofilm does not grow.	http://www.steri- spray.com/shower- attachment/	15 of 16

#### References

- Hammes F, Goldschmidt F, Vital M, Wang Y, Egli T. (2010). Measurement and interpretation of microbial adenosine tri-phosphate (ATP) in aquatic environments. *Water Res* **44**: 3915–3923.
- Prest EI, Hammes F, Kötzsch S, van Loosdrecht MCM, Vrouwenvelder JS. (2013). Monitoring microbiological changes in drinking water systems using a fast and reproducible flow cytometric method. *Water Res* **47**: 7131–42.
- Proctor CR, Gächter M, Kötzsch S, Rölli F, Sigrist R, Walser J-C, *et al.* (2016). Biofilms in shower hoses choice of pipe material influences bacterial growth and communities. *Environ Sci Water Res Technol*. e-pub ahead of print, doi: 10.1039/C6EW00016A.
- Takai K, Horikoshi K. (2000). Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. *Appl Environ Microbiol* **66**: 5066–72.

Web screenshots (referred to in Table S1 and Table S9).







https://calvinayre.com/ 2017/04/25/casino/parisianmacao-legionnaires-diseaseoutbreak/





http://nypost.com/2017/04/09/legionnairessickened-four-people-one-fatally-at-cancerfacility-suit/

# Walton pool and gym reopen almost three months after legionella bug outbreak





http:// www.clactonandfrintongazett e.co.uk/news/ 15115947.Pool\_and\_gym\_re open\_almost\_three\_months\_ after\_legionella\_bug\_outbrea k/

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http://www.fresnobee.com/news/local/article126494379.html



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Veb-Screenshots

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