GLOBAL WATER PATHOGEN PROJECT
PART FOUR. MANAGEMENT OF RISK FROM EXCRETA AND WASTEWATER

PERSISTENCE OF PATHOGENS IN SEWAGE AND OTHER WATER TYPES

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Summary

The goal of this chapter is to provide an overview of the literature available on the persistence/ survival of pathogens and indicator organisms in sewage, surface water, groundwater and marine waters. The chapter is based on a scoping review of the literature and includes a summary of the survival of bacteria, viruses, protozoa and indicator organisms under various temperature and light conditions in each of the four water matrices. The data presented herein can be used to understand the survival dynamics of these organisms in aquatic environments and can subsequently be used to inform risk assessment models.

Organism survival/ die-off data are presented and reported as T90, T99, T99.9 or T99.99 values. The T90, T99, T99.9, T99.99 data represent the time in days that it takes for a 1 $\log_{10}$ (T90), 2 $\log_{10}$ (T99), 3 $\log_{10}$ (T99.9) or 4 $\log_{10}$ (T99.00) reduction of the microorganism to be observed.

For example, in sewage, bacterial pathogens such as *Salmonella typhimurium, Enterobacter spp.* and *Streptococcus faecalis* can survive for over 100 days before seeing a 1 $\log_{10}$ reduction. Adenoviruses in secondary and primary effluent have been found to have T99's of up to 58 and 48 days under dark conditions at cold temperatures (4ºC). These die-off rates decrease as temperature increases as well as when the organisms are exposed to a light source.

Temperature, sunlight, DO, DOC, availability of nutrients, and salinity were found to be important environmental conditions to consider when evaluating the persistence of microorganisms in environmental waters. In general, very few data are available on the persistence of pathogens in aquatic environments, Significant gaps remain, particularly on the persistence of protozoa and pathogens found in developing regions of the world.

Persistence of Pathogens in Sewage and Other Water Types

1.0 Introduction

The goal of this chapter is to provide an overview of the literature available on the persistence/ survival of pathogens and indicator organisms in sewage, surface water, groundwater and marine waters. The chapter includes a summary of the survival of bacteria, viruses, protozoa and indicator organisms under various temperature and light conditions in each of the four water matrices. The data presented herein can be used to understand the survival dynamics of these organisms in aquatic environments and can subsequently be used to inform risk assessment models.

In order to capture a large amount of literature in a short timeframe, a scoping literature review was performed in PubMed from February 11 to 18, 2016 using the search terms described in Table 1. The search terms within each column in Table 1 were separated by the Boolean search term "OR" and each column of terms were separated by an "AND". The search was restricted to English articles dating back to 1980 that contained the search terms in either the title or abstract. The initial search returned 1216 articles. Abstracts and titles were screened for relevance. Articles that contained information on the persistence or survival of microorganisms in water matrices were retained for data extraction. Data were extracted from a total of 107 relevant articles of which 42 articles contained specific data on the survival/ persistence of specific microorganisms in different water matrices. Only papers where die-off data were presented as log reductions, T90s or could be readily converted to T90s were retained in the review. These data are discussed and presented in the following sections. Three additional references were added to the review that were published after the initial searching took place. Tables 2 to 16 present a summary of the persistence data recovered during the literature review. The survival/ die-off data in the tables are reported as T90, T99, T99.9 or T99.99 values. The T90 and T99 data represent the time in days that it takes for a 1 $\log_{10}$ (T90) or 2 $\log_{10}$ (T99) reduction of the microorganism to be observed. The T99.9 and T99.99 data represent the time in days that it takes for a 3 $\log_{10}$ or 4 $\log_{10}$ reduction, respectively.

Table 1. Search terms

Microorganism Terms		Persistence Terms	Water Matrices Terms
Helminths Protozoa Viruses Bacteria Pathogen Adenovirus Enterovirus *E. coli* *Shigella* *Salmonella* *Campylobacter* MS2 Cyclospora Toxoplasma *Cryptosporidium* *Giardia* Norovirus	Rotavirus Echovirus Reovirus Gastroenteritis viruses MST Genetic markers Enterococci Feces OR fecal Indicator(s) Indicators and reagents	Persistence Survival Decay	Wastewater Lagoon River Lake Groundwater Seawater Estuary Marine

2.0 Persistence in Sewage and Treated Wastewater

Tables 2 to 4 present a summary of the survival of bacteria, bacteriophages and viruses in wastewater under varying temperatures and light conditions. The data are presented for different types of wastewater including: raw wastewater, primary effluent, secondary effluent, aerated lagoons, wastewater diluted into environmental waters, and wastewater sludge into environmental waters. The survival/ die- off data are reported as T90, T99, T99.9 or T99.99 values. Data were aggregated where possible by temperature range and light source. The studies that looked at persistence in wastewater were performed in the dark, under light or under UVA or UVB radiation.

In the present review, 9 of the 45 articles focused on the survival of indicator organisms or pathogens in wastewater. The majority of the data found in these articles was on the persistence of bacteria (12 types of bacteria) followed by viruses (4 human specific viruses) and then by bacteriophage (2 types of bacteriophages). Bacteriophages are viruses that infect bacteria. No studies were found on the persistence of protozoa in wastewater.

2.1 Bacteria and bacterial indicators

Several studies examined the survival of *Escherichia coli* in untreated wastewater under dark conditions (Table 2). It seems to be most persistent at low temperatures (2 to 6°C), where it is shown to survive for >11 days before seeing a 1 $\log_{10}$ reduction. In secondary wastewater effluent, Mayer et al. (2015) found *E.coli spp.* to be less persistent and observed a 1 $\log_{10}$ die-off after 4 to 11 days. Once temperatures rise to above 21°C, die-off is more rapid and ranged from 1 to 7.1 days to achieve a 1 $\log_{10}$ reduction. These observations were consistent with the results from other studies that showed increased die-off of bacteria (thermotolerant coliforms, *Enterococci*, *Bacteroides*) in wastewaters when temperatures increase above 18.5°C (Table 2). For example, at colder temperatures (5°C) Brooke et al. (2015) observed a 1 $\log_{10}$ die-off of *Bacteroides thetaiotaomicron* only after 9.6 days, however at temperatures ranging from 25 to 43°C, T90s of 1 to 1.8 were reported. This was consistent with findings of the persistence of human specific *Bacteroides* (BacHum-UCD; AllBac) in raw wastewater and secondary effluent, where a 1 $\log_{10}$ reduction was only observed at 5°C after 11 days (Mayer et al. 2015). The same log reduction was observed at 4 days when the temperature was 21°C, further highlighting the importance of temperature on the survival of some bacteria in wastewater (Mayer et al. 2015). In contrast, Mayer et al. (2015) observed no significant difference in die off of *Clostridium perfringens* in raw wastewater and secondary effluent at cold (5°C) and warm (21°C) temperatures. Yeager and Ward (1981) examined the survival of pathogens in sewage sludge at 21°C and found that in liquid sludge (5% solids) the pathogens *Salmonella typhimurium*, *Enterobacter spp.* and *Streptococcus faecalis* and the indicator organism *E.coli spp.* could survive for greater than 100 days before seeing even a 1 $\log_{10}$ die-off.

Table 2. Summary of the persistence of pathogenic and indicator bacteria in wastewater or sewage under different temperatures conditions

Bacteria Type[a]	T90 (Days)	Temperature (°C)	Light Source	Wastewater Type	References
Bacteroides thetaiotaomicron	9.6	2 to 6	Dark	10% sewage by volume into river water	Brooks et al., 2015
Bacteroides thetaiotaomicron	1.8	25 to 29	Dark	10% sewage by volume into river water	Brooks et al., 2015
Bacteroides thetaiotaomicron	1	32 to 43	Dark	10% sewage by volume into river water	Brooks et al., 2015
Bifidobacterium adolescentis[b]	3.6 to 3.7	18.5	Dark	1:18% dilution WW into seawater or freshwater	Jeanneau et al., 2012
Clostridium perfringens	>11	5	Dark	Raw Wastewater	Mayer et al., 2015
Clostridium perfringens	>11	5	Dark	Secondary effluent	Mayer et al., 2015
Clostridium perfringens	>11	21	Dark	Raw Wastewater	Mayer et al., 2015
Clostridium perfringens	>11	21	Dark	Secondary effluent	Mayer et al., 2015
E.coli spp.	12.4	2 to 6	Dark	10% sewage by volume into river water	Brooks et al., 2015
E.coli spp.	>11	5	Dark	Raw Wastewater	Mayer et al., 2015
E.coli spp.	4 to 11	5	Dark	Secondary effluent	Mayer et al., 2015
E.coli spp.	1.7 to 5.8	18.5	Dark	1:18% dilution WW into seawater or freshwater	Jeanneau et al., 2012
E.coli spp.	>100	21	NR[c]	moisture content=5% solid sludge	Yeager and Ward, 1981
E.coli spp.	4	21	Dark	Raw Wastewater	Mayer et al., 2015
E.coli spp.	1 to 4	21	Dark	Secondary effluent	Mayer et al., 2015
E.coli spp.	7.1	25 to 29	Dark	10% sewage by volume into river water	Brooks et al., 2015
E.coli spp.	6	32 to 43	Dark	10% sewage by volume into river water	Brooks et al., 2015
Enterobacter spp.	>100	21	NR[c]	moisture content=5% solid sludge	Yeager and Ward, 1981
Enterococci	>28	2 to 6	Dark	10% sewage by volume into river water	Brooks et al., 2015
Enterococci	3.1 to 3.6	18.5	Dark	1:18% dilution WW into seawater or freshwater	Jeanneau et al., 2012
Enterococci	8.4	25 to 29	Dark	10% sewage by volume into river water	Brooks et al., 2015
Enterococci	6.5	32 to 43	Dark	10% sewage by volume into river water	Brooks et al., 2015

Bacteria Type[a]	T90 (Days)	Temperature (°C)	Light Source	Wastewater Type	References
Human specific Bacteroides (HF183)[b]	1.7 to 2.3	18.5	Dark	1:18% dilution WW into seawater or freshwater	Jeanneau et al., 2012
Human specific Bacteroides (BacHum-UCD)[b]	11	5	Dark	Raw Wastewater	Mayer et al., 2015
Human specific Bacteroides (BacHum-UCD)[b]	>11	5	Dark	Secondary effluent	Mayer et al., 2015
Human specific Bacteroides (BacHum-UCD)[b]	4	21	Dark	Secondary effluent	Mayer et al., 2015
Human Specific Bacteroides (AllBac)[b]	> 11	5	Dark	Raw Wastewater	Mayer et al., 2015
Human Specific Bacteroides (AllBac)[b]	>11	5	Dark	Secondary effluent	Mayer et al., 2015
Human Specific Bacteroides (AllBac)[b]	4 to 11	21	Dark	Raw Wastewater	Mayer et al., 2015
Human Specific Bacteroides (AllBac)[b]	4	21	Dark	Secondary effluent	Mayer et al., 2015
Klebsella	50 to 75	21	NR[c]	moisture content=5% solid sludge	Yeager and Ward, 1981
Salmonella Typhimurium	>100	21	NR[c]	moisture content=5% solid sludge	Yeager and Ward, 1981
Streptococcus faecalis	>100	21	NR[c]	moisture content=5% solid sludge	Yeager and Ward, 1981

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]Detected/ monitored using molecular methods; [c]NR: Not Reported

2.2 Viruses and viral indicators

In a variety of wastewaters including: raw sewage, primary effluent, secondary effluent, and aerated lagoons, temperature influenced the survival of bacteriophages and viruses. In experiments by Carratalà et al. (2013), Enriquez (1994), and Skraber et al. (2009), viral persistence and bacteriophage survival consistently decreased at temperatures above 15°C compared to 4°C (Tables 3 and 4). Carratalà et al. (2013) found that adenoviruses were susceptible to UVB radiation at 7°C and that UVA radiation had a larger effect on the persistence of adenovirus at 37°C than 20°C (Table 4). There were no direct comparison experiment of UVA and UVB radiation at the same temperature conditions, therefore it is difficult to compare the UVA and UVB results. Nevertheless, the studies found suggest that UV radiation and temperature are important factors for the persistence of pathogens in different wastewater matrices, particularly in cold environments.

Table 3. Summary of the persistence of bacteriophage in wastewater or sewage under different temperature and light conditions

Bacteriophage Typ[a]	T90 (Days)	Temperature (°C)	Light Source	Wastewater Type	References
F specific RNA phage[b]	1.1 to 5.2	18.5	Dark	1:18% dilition WW into sewater or freshwater	Jeanneau et al., 2012
Infectious F specific phage	8.8	4	Dark	Primary effluent	Skraber et al., 2009
Infectious F specific phage	<2.4	20	Dark	Primary effluent	Skraber et al., 2009
Phages F specific-GIII genome[b]	21.2	4	Dark	Primary effluent	Skraber et al., 2009
Phages F specific-GIII genome[b]	7.2	20	Dark	Primary effluent	Skraber et al., 2009

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]Detected/ monitored using molecular methods

Table 4. Summary of the persistence of viruses in wastewater or sewage under different temperature and light conditions

Virus Type[a]	T90 (Days)	T99 (Days)	T99.9 (Days)	T99.99 (Days)	Temperature (^oC)	Light Source	Wastewater Type	References
Adenovirus 40	NR	RU	NR	NR	4	Dark	Secondary effluent	Enriquez et al., 1994
Adenovirus 40	NR	4P	NR	NR	NR	Dark	Secondary effluent	Enriquez et al., 1994
Adenovirus 40	NR	44	NR	NR	4	Dark	Primary effluent	Enriquez et al., 1994
Adenovirus 40	NR	40	NR	NR	NR	Dark	Primary effluent	Enriquez et al., 1994
Adenovirus 41	NR	4T	NR	NR	4	Dark	Secondary effluent	Enriquez et al., 1994
Adenovirus 41	NR	4R	NR	NR	NR	Dark	Secondary effluent	Enriquez et al., 1994
Adenovirus 41	NR	4U	NR	NR	4	Dark	Primary effluent	Enriquez et al., 1994
Adenovirus 41	NR	4P	NR	NR	NR	Dark	Primary effluent	Enriquez et al., 1994
Human Adenovirus- type 2	0.04	0.0U	0.N4	0.2P	T	UVB Radiation	Raw wastewater	Carratalà et al., 2013
Human Adenovirus- type 2	2.S0	R.4P	U.2S	NN.N0	20	rsA oadiation	Raw wastewater	Carratalà et al., 2013
Human Adenovirus- type 2	0.13	0.31	0.48	0.66	37	Dark	Raw wastewater	Carratalà et al., 2013
Human Adenovirus- type 2	0.12	0.31	0.49	0.69	37	UVA Radiation	Raw wastewater	Carratalà et al., 2013
Norovirus GG1 genome[c]	114.9	NR	NR	NR	4	Dark	Primary effluent	Skraber et al., 2009
Norovirus GG1 genome[c]	22.5	NR	NR	NR	20	Dark	Primary effluent	Skraber et al., 2009
Poliovirus	NR	49	NR	NR	4	Dark	Secondary effluent	Enriquez et al., 1994
Poliovirus	NR	19	NR	NR	15	Dark	Secondary effluent	Enriquez et al., 1994
Poliovirus	NR	36	NR	NR	4	Dark	Primary effluent	Enriquez et al., 1994
Poliovirus	NR	28	NR	NR	15	Dark	Primary effluent	Enriquez et al., 1994

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]NR: Not Reported; [c]Detected/ monitored using molecular methods

3.0 Persistence in Surface Waters

Tables 5, 6, 7, and 8 summarized the findings of the persistence of microorganisms in surface water under varying temperatures and light sources. Survival/ die-off data are reported as T90, T99, T99.9 or T99.99 values. For surface water, all experiments were performed in the dark, under artificial light and under natural sunlight. A couple of studies reported using UVA and UVB radiation specifically.

In the present review, 21 of the 45 articles focused on the survival of indicator organisms or pathogens in surface water supplies. The majority of the data found in these articles were on the persistence of bacteria and bacteriophages in surface water (13 types of bacteriophages; 10 bacteria), followed by viruses (6 virus types). Similar to the groundwater, only 1 article presented data on the persistence of protozoa.

3.1 Bacteria and bacterial indicators

In general, bacteria seem to persist for shorter periods of time (T99s ranging from 1.5 to 69.5 days) in surface waters (Table 5) than in groundwater (T99s ranging from 2.85 to 119 days) (Table 9). Temperature appears to have a moderate effect on bacterial persistence of indicator organisms such as *E. coli spp.*, fecal coliforms, and fecal enterococci. For example, at temperatures ranging from 13 to 30°C, T90s for fecal coliforms ranged from between 3.6 to 3.9 days. For fecal enterococci, T90s ranged from 1 to 1.9 days for temperatures between 5 to 17°C. Interestingly, once temperatures rise to above 22°C, Balleste and Blanch (2010) reported T90s as high as 4.42 to 5.25 days for fecal *enteroccoci*. In contrast, persistence of *E. coli spp.* in wastewater seems to be longer at temperatures around 15°C (T99: 3.01 to 5.65) compared to 25°C (T99: 2.18) (Table 3).

Table 5. Summary of the persistence of pathogenic and indicator bacteria in surface waters under different temperature and light conditions

Bacteria Type[a]	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	Comments	References
Animal Specific *Bacteroides* CF193 DNA[c]	NR[b]	2.75	15	Sunlight	NR	Liang et al., 2012
Animal Specific *Bacteroides* CF193 RNA[c]	NR	2.12	15	Sunlight	NR	Liang et al., 2012
Bacteroides spp. [d]	1.27 to 1.77	NR	5 to 10	Sunlight	NR	Balleste and Blanch, 2010
Bacteroides spp. [d]	1.15	NR	13 to 15	Sunlight	NR	Balleste and Blanch, 2010
Bacteroides spp. [d]	0.83 to 0.92	2.55	22 to 30	Sunlight	NR	Balleste and Blanch, 2010; Dick et al., 2010
Bacteroides spp. [d]	NR	2.73	15	Dark	1% WW into freshwater	Dick et al., 2010
Bacteroides spp. [d]	NR	3.28 to 4.44	25	Dark	NR	Dick et al., 2010
Bacteroides thetaiotaomicron [d]	0.32 to 0.84	NR	5 to 10	Sunlight	NR	Balleste and Blanch, 2010
Bacteroides thetaiotaomicron [d]	0.65 to 2.91	NR	13 to 15	Sunlight	NR	Balleste and Blanch, 2010
Bacteroides thetaiotaomicron [d]	0.75 to 2.2	NR	22 to 30	Sunlight	NR	Balleste and Blanch, 2010
Bacteroides fragilis [d]	1 to 8.2	NR	5 to 10	Sunlight	River water & sterile river water	Balleste and Blanch, 2010
Bacteroides fragilis [d]	0.82 to 5.41	NR	13 to 15	Sunlight	River water & sterile river water	Balleste and Blanch, 2010
Bacteroides fragilis [d]	0.48 to 2.2	NR	22 to 30	Sunlight	River water & sterile river water	Balleste and Blanch, 2010
Bacteroidales pig 1 bac[c]	22	NR	4	Dark	NR	Marti et al., 2011
Bacteroidales pig 1 bac[c]	10.3	NR	20	Dark	NR	Marti et al., 2011
Bacteroidales pig 2 bac[c]	19.3	NR	4	Dark	NR	Marti et al., 2011
Bacteroidales pig 2 bac[c]	1.9	NR	20	Dark	NR	Marti et al., 2011
Bovine *E.coli*	NR	8.32	15	Nat. light cycle	bovine feces into freshwater	Liang et al., 2012
Campylobacter	4.08	NR	4	Dark	NR	Rodríguez and Araujo, 2012
Campylobacter	2.0 to 7.0	NR	10 to 11	Dark	NR	Rodríguez and Araujo, 2012; Schang et al. 2016
Campylobacter	2.3 to 3.8	6.3 to 8.7	10 to 15	Nat. Light cycle	50 to 100 NTU, in stormwater wetland	Meng et al. 2016
Campylobacter	0.91 to 1.65	NR	20	Dark	NR	Rodríguez and Araujo, 2012

Bacteria Type[a]	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	Comments	References
Campylobacter	0.92	NR	30	Dark	NR	Rodríguez and Araujo, 2012
Campylobacter	0.46	NR	37	Dark	NR	Rodríguez and Araujo, 2012
Campylobacter	0.77	NR	6.8	Sunlight	NR	Rodríguez and Araujo, 2012
Campylobacter	0.692	NR	14.1	Sunlight	NR	Rodríguez and Araujo, 2012
Campylobacter	0.429	NR	22.3	Sunlight	NR	Rodríguez and Araujo, 2012
Campylobacter	2.54	NR	7.4	Shade	NR	Rodríguez and Araujo, 2012
Campylobacter	0.9	NR	21.5	Shade	NR	Rodríguez and Araujo, 2012
Enterola-Enteroccoccal 23S rRNA gene[c]	>3 to <5	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterola-Enteroccoccal 23S rRNA gene[c]	>3 to <5	NR	14 to 15	Sunlight[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterola-Enteroccoccal 23S rRNA gene[c]	>3 to <5	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterola-Enteroccoccal 23S rRNA gene[c]	>3 to <5	NR	14 to 15	Dark[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterococci	<3	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterococci	<3	NR	14 to 15	Sunlight[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterococci	<3	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterococci	<5	NR	14 to 15	Dark[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Enterotoxigenic *E. coli*	NR	69.5	21	Nat. light cycle	NR	Lothigius et al., 2010
E.coli O157:H7	NR	2.8 to 10.6	10	Dark	River	Avery et al., 2008
E.coli O157:H7	NR	9 to 19.4	10	Dark	Lake	Avery et al., 2008
E. coli spp.	NR	>43	4	Dark	NR	Marti et al., 2011
E. coli spp.	2.2	3.01 to 5.65	15	Sunlight	NR	Ahmed et al. 2014; Dick et al., 2010; Liang et al., 2012
E. coli spp.	7.2 to 14.7	15.5 to 29.6	10 to 15	Nat. Light cycle	50 to 100 NTU, in stormwater wetland	Meng et al. 2016
E. coli spp.	<7		20 to 30	Nat. light cycle	NR	Schang et al. 2016
E. coli spp.	NR	2.02	25	Dark	NR	Dick et al., 2010
E. coli spp.	NR	2.18	25	Sunlight	NR	Dick et al., 2010
E. coli spp.	>3 to < 5	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
E. coli spp.	>3 to < 5	NR	14 to 15	Sunlight[d]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
E. coli spp.	<5	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
E. coli spp.	<5	NR	14 to 15	Dark[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Fecal Coliforms	4.6 to 5.2	NR	5 to 10	Sunlight	NR	Balleste and Blanch, 2010

Bacteria Type[a]	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	Comments	References
Fecal Coliforms	3.9	NR	13 to 15	Sunlight	NR	Balleste and Blanch, 2010
Fecal Coliforms	3.6 to 3.7	NR	22 to 30	Sunlight	NR	Balleste and Blanch, 2010
Fecal Enteroccocci	>43	NR	4	Dark	NR	Marti et al., 2011
Fecal Enteroccocci	1 to 1.25	NR	5 to 10	Sunlight	NR	Balleste and Blanch, 2010
Fecal Enteroccocci	2.3	NR	13 to 15	Sunlight	NR	Balleste and Blanch, 2010
Fecal Enteroccocci	1.9	NR	14 to 17	Sunlight	NR	Ahmed et al. 2014
Fecal Enteroccocci	4.42 to 5.25	NR	22 to 30	Sunlight	NR	Balleste and Blanch, 2010
GenBac3 *Bacteroidales* 16s RNA gene[c]	>3 to <5	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
GenBac3 *Bacteroidales* 16s RNA gene[c]	>3 to <5	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
GenBac3 *Bacteroidales* 16s RNA gene[c]	>3 to <5	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
GenBac3 *Bacteroidales* 16s RNA gene[c]	>3 to <5	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Human *E. coli*	NR	5.65	16	Nat. light cycle	human feces into freshwater	Liang et al., 2012
Human Specific *Bacteroides* HF183 DNA[c]	3.5	2.72	14 to 18	Sunlight	Dilute sewage in freshwater; human or bovine feces into freshwater	Ahmed et al. 2014 ; Liang et al., 2012
Human Specific *Bacteroides* HF183[c]	1.7 to 2.3	2.53	15 to 20	Dark	NR	Dick et al., 2010; Jeanneau et al., 2012
Human Specific *Bacteroides* HF183[c]	NR	1.71 to 2.78	25	Dark	NR	Dick et al., 2010
Human Specific *Bacteroides* HF183 RNA[c]	NR	2.08	15	Sunlight	NR	Liang et al., 2012
Human Specific *Bacteroidales* HF183- 16s RNA gene[c]	>3	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Human Specific *Bacteroidales* HF183- 16s RNA gene[c]	>3	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Human Specific *Bacteroidales* HF183- 16s RNA gene[c]	>3	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Human Specific *Bacteroidales* HF183- 16s RNA gene[c]	>3	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Human Specific *Bacteroides* –BacHum[c]	NR	2.35	15	Dark	NR	Dick et al., 2010
Human Specific *Bacteroides* –BacHum[c]	NR	1.74 to 3.03	25	Dark	NR	Dick et al., 2010
Human Specific *Bacteroides* –BacHum[c]	NR	1.54	25	Light source	NR	Dick et al., 2010
HumM2- *Bacteroidales*-like putative s factor[c]	>3	NR	14 to 15	Sunlight	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014

Bacteria Type[a]	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	Comments	References
HumM2-*Bacteroidales*-like putative s factor[c]	>3	NR	14 to 15	Sunlight[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
HumM2-*Bacteroidales*-like putative s factor[c]	>3	NR	14 to 15	Dark	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
HumM2-*Bacteroidales*-like putative s factor[c]	>3	NR	14 to 15	Dark[e]	River water & primary effluent (1:1 ratio)	Korajkic et al. 2014
Salmonella	47.9 to 95.8	NR	15	Dark	NR	Boehm et al., 2012
Salmonella	0.015 to 0.025	NR	15	Light	NR	Boehm et al., 2012

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]NR: Not Reported; [c]Detected/ monitored using molecular methods; [d]Although these organisms are frequently detected via molecular methods, in this case the organism was cultured; [e] In presence of indigenous river microbiota

In studies by Liang et al. (2012) and Lothigius et al. (2010), bovine, human, and pathogenic strains of *E. coli spp.* all persisted longer than generic *E.coli* spp in the experiments conducted. This is an important observation, as *E. coli spp.* is frequently used as an indicator of human excreta and may not be an appropriate surrogate for looking at the survival of specific strains of *E.coli,* particularly those that may be human pathogens. Lothigius et al. (2010) report that Enterotoxigenic *E. coli* (ETEC) had a T99 of 69.5 days at 21°C under natural sunlight conditions. In a similar study at 25°C, *E. coli spp.* had a T99 of only 2.18 days (Dick et al. 2010).

Rodríguez and Araujo (2012) found that *Campylobacter* persists longer at lower temperatures (4 to 10°C), than at higher temperatures (>20°C). This was also consistent with the findings of Schang et al., (2016). The persistence further increased when the organism was studied under dark conditions and decreased when subjected to sunlight. The T90s of *Campylobacter* ranged from 4.08 days under cold (4°C) dark conditions to as little as 0.43 days when exposed to sunlight and when temperatures reached 22.3°C (Table 5). A T90 of 0.46 days was also achieved in dark conditions when temperatures rose to 37°C (Rodríguez and Araujo, 2012).

In work by Boehm et al. (2012), *Salmonella spp.* was shown to be extremely sensitive to sunlight. Under dark conditions at 15°C, *Salmonella spp.* was shown to survive from between 47.9 to 95.8 days before seeing a 1 $\log_{10}$ die-off. Under the same temperature conditions but under sunlight, the organism showed a 1 $\log_{10}$ die-off within a matter of hours (Table 5).

Several studies have been conducted on the survival and persistence of *Bacteroides* species by either culture or molecular techniques. Balleste and Blanch (2010) reported that *B. fragilis* die-off (by culture) was affected by high temperatures and the presence of other environmental predators. They studied the effect of environmental predators by comparing the persistence of *B. fragilis* in sterile river water and non-sterile river water. *B. fragilis* survived for longer in the sterilized water compared to the natural river water (Balleste and Blanch 2010). They also observed the lowest T90 values for *B. fragilis* when temperature and DO concentrations were the highest (Balleste and Blanch, 2010). In contrast, they found that *B. thetaiotaomicron* and environmental *Bacteroides spp.* were more affected by the concentration of DO in the water and could survive longer when DO concentrations were lower and temperatures were higher. In addition, they reported that environmental *Bacteroides spp.* (by culture) died off more rapidly than indicator organisms such as fecal coliforms and fecal enterococci, thus suggesting that the presence of *Bacteroides spp.* in surface water supplies could be a good indicator of recent fecal contamination. Finally, when using molecular techniques, it was found in the same experiments that temperature significantly affected the detection of *Bacteroides* DNA in the water samples. In summer months, they were only detected occasionally, therefore potentially producing false negative results. These findings seem to be consistent with those of Dick et al. (2010) and Liang et al. (2012) who reported similar die-off times to achieve 2 log removal (using molecular detection methods) as the die-off times reported by Balleste and Blanch (2010) to achieve only a 1 $\log_{10}$ reduction (using culture-based detection methods). The DNA seems to degrade more rapidly than culturable organisms.

In a recent study by Korajikic et al. (2014) that looked at four genetic human-associated MST markers (Enterola, GenBac3, HF183, HumM2), they found that the organisms' RNA persisted for between 3 to 5 days in river water. Interestingly, they found little difference between the persistence of the genetic material under different environmental conditions (sunlight, dark, in the presence and absence of indigenous river microbiota) (Korajikic et al., 2014). In contrast, in the same study they observed a more rapid die-off of culturable enterococci (T90 < 3 days) for all environmental conditions except the die-off was less rapid in the dark (T90 >3 days to < 5 days). *E.coli* persisted longer than *enteroccocci* and under all environmental conditions studied took greater than 5 days for a 1 $\log_{10}$ die-off to be observed (Korajikic et al., 2014). These data presented herein raise an important question of whether

organism survival should be evaluated using molecular techniques.

3.2 Protozoa and protozoan indicators

Only two studies were found that examined the persistence of protozoa in surface water *(*Ives et al., 2007; Sidhu et al., 2015) (Table 6). These studies examined the survival of *Cryptosporidium* under dark conditions at various temperatures. The organism seems to be extremely sensitive to temperature. It was shown to survive for greater than 200 days at 5°C, whereas a 2 $\log_{10}$ reduction was achieved in 10 to 11 days at 30°C (Ives et al., 2007). Although no studies were found on the persistence of the organism in sunlight conditions, it has been established that *Cryptosporidium* can be inactivated by UV disinfection (Morita et al., 2002), therefore it is expected that under sunlight conditions, die-off would be more rapid in the environment. More data is needed on the survival of *Cryptosporidium* and *Giardia* in surface waters under a variety of environmental conditions.

Table 6. Summary of the persistence of the protozoan Cryptosporidium parvum in fresh surface waters under different temperatures conditions in the dark

T90 (Days)	T99 (Days)	Temperature (^{o}C)	References
NR[a]	>200	5	Ives et al., 2007
38[b,c] to 86[d]	30 to 45[d]	20 to 25	Ives et al., 2007; Sidhu et al. 2015
NR	10 to 11	30	Ives et al., 2007

[a]NR: Not Reported; [b]Sidhu and Toze, 2012; [c]Sidhu et al. 2015; [d]Ives et al., 2007

3.3 Viruses and viral indicators

Long and Sobsey (2004) conducted an extensive study on the survival of several types of bacteriophages in surface water under dark conditions at both 4°C and 20°C (Table 7). All the bacteriophage strains studied were sensitive to temperature; all of them survived longer at 4°C than at 20°C. The T99s at 4°C ranged from 7.3 days to 250 days, whereas, the T99s at 20°C only ranged from 1.7 to 35 days. These observations were consistent for poliovirus and human adenoviruses, whose survival decreased with increasing temperatures and also decreased further when exposed to sunlight or UVA/ UVB radiation (Table 8). It is difficult to compare the bacteriophage data with the virus data as all the bacteriophage experiments were conducted in the dark, whereas most of the virus experiments were performed under sunlight or under UV radiation. In one case, an experiment was done with human adenoviruses under dark conditions and Rigotto et al. (2011) showed that it could survive over 301 days at 19°C (Table 8). This survival surpasses the most persistent bacteriophage survival by nearly 10-fold under the same temperature and light conditions, thus suggesting that bacteriophage would not be a good surrogate organism for adenoviruses. Adenoviruses do seem to be affected by temperature, and are less persistent at lower temperatures (10°C) and higher temperatures (37°C) than moderate temperatures (19°C)(Table 8).

Table 7. Summary of the persistence of bacteriophage in fresh surface waters under different temperatures conditions in the dark

Type of Phage	RNA or DNA	T99 (Days)	Temperature (°C)
f1	DNA	70	4
fd	DNA	57	4
M13	DNA	220	4
OW	DNA	33	4
SD	DNA	20	4
ZJ/2	DNA	76	4
Dm3	RNA	8.3	4
Go1	RNA	250	4
Ms2	RNA	240	4
SG1	RNA	171	4
SG4	RNA	8.3	4
SG42	RNA	7.3	4
Sp2	RNA	51.5	4
f1	DNA	10	20
fd	DNA	7.4	20
M13	DNA	35	20
OW	DNA	5.4	20
SD	DNA	3.2	20
ZJ/2	DNA	8.8	20
Go1	RNA	25	20
Ms2	RNA	29	20
SG1	RNA	23	20
SG4	RNA	1.7	20
SG42	RNA	1.6	20
Sp2	RNA	12	20

Source: Long and Sobsey, 2004

Table 8. Summary of the persistence of viruses in fresh surface waters under different temperature and light conditions

Virus Type[a]	T90 (Days)	T99 (Days)	T99.9 (Days)	T99.99 (Days)	Temperature (^{O}C)	Light Source	References
Feline calicivirus	4.2 to 7.1[c]	8.3 to 14.3[c]	12.5 to 21.4[c]	16.7 to 28.6[c]	4	Dark	Bae and Schwab, 2008
Feline calicivirus	1.9 to 3.8[c]	3.7 to 7.7[c]	5.6 to 11.5[c]	7.4 to 15.4[c]	25	Dark	Bae and Schwab, 2008
Human Adenovirus-type 2	0.03	0.07	0.17	0.20	7	UVB Radiation	Carratalà et al., 2013
Human Adenovirus	30 to 40	NR	NR	NR	10	Dark	Rigotto et al., 2011
Human Adenovirus-qPCR[b]	NR	13	NR	NR	14 to 18	Sunlight	Ahmed et al.,2014
Human Adenovirus	161	NR	NR	301	19	Dark	Rigotto et al., 2011
Human Adenovirus-type 2	0.57	1.26	1.96	2.66	20	UVA Radiation	Carratalà et al., 2013
Human Adenovirus-type 2	0.45	0.79	1.10	1.42	37	Dark	Carratalà et al., 2013
Human Adenovirus-type 2	0.29	0.57	0.85	1.14	37	UVA Radiation	Carratalà et al., 2013
Human Adenovirus	NR	NR	NR	5	37	Dark	Rigotto et al., 2011
Murine norovirus	4.5 to 10[c]	9.1 to 20[c]	13.6 to 30[c]	18.2 to 40[c]	25	Dark	Bae and Schwab, 2008
Tulane virus	NR	NR	NR	28	4	Dark	Arthur and Gibson, 2015

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods;[b]Detected/ monitored using molecular methods; [c]Calculated from $\log_{10}$/ day reduction values reported in the paper; [d]NR: Not Reported

4.0 Persistence in Groundwater

Tables 9,10,11, and 12 summarized the findings of the persistence of microorganisms in groundwater under varying temperatures. Survival/ die-off data are reported as T90 or T99 values. For groundwater, all experiments were performed in the dark.

Of the 45 articles retained in the review, 15 focused on the survival of indicator organisms or pathogens in groundwater supplies. Most of the data found in these articles was on the persistence of viruses and bacteriophages in groundwater (16 types of viruses/ bacteriophages). Only 3 articles presented data on protozoa and 4 articles presented data on bacterial pathogens or indicator organisms.

4.1 Bacteria and bacterial indicators

Data on the persistence of *Campylobacter jejuni*, *Salmonella spp.*, *Salmonella typhimurium*, and indicator bacteria such as coliforms, *E.coli spp.,* or *Enterococci* are reported in Table 9. Of the organisms studied, *E.coli spp.* was the most persistent at low temperatures (0 to 4°C). In experiments at low temperatures (0 to 4 °C), *E.coli* was found to have a T99 of 91 to 119 days. In the same experiment *Campylobacter jejuni* had a T99 of only 15 to 21 days. In contrast, in another study at 20 to 25°C, *E.coli spp.* was found to have a T90 of 0.025 to 24.39 days, while, *Salmonella typhimurium* took between 1 to 18.51 days to have the same die-off. These data highlighted that using *E.coli* spp. as a representative indicator organism for understanding of persistence of bacterial pathogens in groundwater supplies will need to be specific to the bacterial pathogen under study. These results suggest that *E.coli* may be representative of *Salmonella* in groundwater, however not of *C. jejuni*. John and Rose (2005) highlighted that increased temperature can contribute to the inactivation of bacteria in groundwater, however, this issue is more complex as some coliform bacteria have shown to thrive and replicate in waters of higher temperatures if sufficient nutrients are available. For example, the presence of competing organisms, nutrient availability, and the presence of other compounds in the water may all be temperature dependent and therefore temperature on its own is not the only factor to consider when examining bacterial persistence. More research is needed on the survival of bacterial pathogens under different environmental conditions in groundwater.

In addition to temperature, Cook and Bolster (2007) examined the influence of dissolved organic carbon (DOC) and dissolved total nitrogen on the persistence of *C. jejuni* and *E.coli*. They found that *C. jejuni* survived the longest when the DOC was the highest (4.0 mg/L). Interestingly, *E.coli* survived longer in experiments where the DOC was the lowest, but the total dissolved nitrogen was the highest.

Dissolved oxygen (DO) is another environmental factor that can affect the inactivation of organisms in groundwater supplies (John and Rose, 2005). Data on DO are lacking, however research by Gordon and Toze (2003) suggest that *E.coli* inactivation was slightly reduced under anaerobic conditions compared to aerobic conditions.

Table 9. Summary of the persistence of bacteria and indicator bacteria in groundwater under different temperatures conditions in the dark

Bacteria Type[a]	T90 (Days)	T99 (Days)	Temperature (°C)	References
Campylobacter jejuni	NR	15 to 21	0 to 4	Cook and Bolster, 2007
Coliform	20[b]	40[b]	0 to 10	John and Rose, 2005
Coliform	10[b]	20[b]	15 to 20	John and Rose, 2005
Coliform	10[b]	20[b]	21 to 37	John and Rose, 2005
E. coli spp.	NR	91 to 119	0 to 4	Cook and Bolster, 2007
E. coli spp.	1.1 to 1.4	NR	10 to 19	Gordon and Toze, 2003
E. coli spp.	1 to 24.39	NR	20 to 25	Sidhu et al., 2015; Sidhu and Toze, 2012
Enterococci	10[b]	20[b]	3 to 22	John and Rose, 2005
Enterococcus Fecalis	1 to 9.17	NR	20 to 25	Sidhu et al., 2015; Sidhu and Toze, 2012
Salmonellla spp	1.43 to 10[b]	2.85 to 20[b]	10 to 22	John and Rose, 2005
Salmonella Typhimurium	1 to 18.51	NR	20 to 25	Sidhu et al., 2015; Sidhu and Toze, 2012

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]Calculated from $\log_{10}$/ day reduction values reported in the paper; [c]NR: Not Reported

4.2 Protozoa and protozoan indicators

Three studies have examined the persistence of *Cryptosporidium* in groundwater. At low temperatures it can take > 200 days for a 2 $\log_{10}$ removal (Table 10). Between 20 to 25°C, a die-off of 2 $\log_{10}$ was reported between 48 and > 200 days. *Cryptosporidium* seems to be less persistent in warmer temperatures (26 to 36°C) as Ives et al. (2007) reported a 2 $\log_{10}$ die off in 17 to 18 days. These elevated temperatures are less likely to be observed in environmental groundwater except in the case of hot springs. Consequently, in groundwater supplies used for drinking water, *Cryptosporidium* may persist for extremely long periods of time. The author was unable to find any data on the survival of *Giardia* in groundwater. This is a significant research gap that needs to be filled.

Table 10. Summary of the persistence of the protozoan Cryptosporidium parvum in groundwater under different temperatures conditions in the dark

T90 (Days)	T99 (Days)	Temperature (°C)	References
NR[a]	>200	5 to 9	Ives et al., 2007
31[b] to 120[c]	48[d] to >200[d]	20 to 25	Ives et al., 2007; Sidhu et al., 2015; Sidhu and Toze, 2012
NR	17 to 18	30	Ives et al., 2007

[a]NR: Not Reported; [b]Sidhu and Toze, 2012; [c] Sidhu et al., 2015; [d]Ives et al. 2007

4.3 Viruses and viral indicators

In general, this review found that bacteriophages (Table 11) are less persistent in groundwater than viruses (Table 12), suggesting that bacteriophage may not be an appropriate surrogate organism for studying the survival of viruses in groundwater. For instance, in a study of GA bacteriophage (an RNA bacteriophage) in groundwater, it was found that at 4°C it took 19.9 days to have a 1 $\log_{10}$ reduction (Ogorzaly et al., 2010). At the same temperature, other viruses such as adenovirus or rotavirus were shown to persist for 131.6 days and between 34 to 200 days, respectively, before experiencing a 1 $\log_{10}$ reduction. Lopman et al. (2012) reported that noroviruses can survive up to 2 months in groundwater supplies and Seitz et al. (2011) found them to be still infectious after 60 days. Consequently, in these cases, using GA bacteriophage data to simulate persistence of pathogenic RNA viruses could significant underestimate the survival and subsequent public health risk associated with the persistence of viruses in groundwater supplies.

Table 11. Summary of the persistence of bacteriophage in groundwater under different temperatures conditions in the dark

Bacteriophage Type[a]	T90 (Days)	Temperature (°C)	References
Coliphage	10 to 33.3[b]	4 to 30	John and Rose, 2005
GA	19.9	0 to 4	Ogorzaly et al., 2010
GA	1.2	20 to 25	Ogorzaly et al., 2010
MS2	23.4[c] to 37[d]	0 to 4	Bae and Schwab, 2008[e]; Yates et al. 1985; Ogorzaly et al., 2010
MS2	1[f] to 33[d]	10 to 19	Gordon and Toze, 2003; Yates et al. 1985
MS2	1.4[c] to 3[d]	20 to 25	Bae and Schwab, 2008[e]; Yates et al. 1985
MS2	2.7[f] to 8.2[f]	26 to 36	Bae and Schwab, 2008[e]; Gordon and Toze, 2003

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]Calculated from $\log_{10}$/ day reduction values reported in the paper; [c] Ogorzaly et al., 2010; [d] Yates et al. 1985; [e] Bae and Schwab, 2008 report results as $\log_{10}$/ day reductions ; calculated values for T90s fall within the ranges reported in the table; [f] Gordon and Toze, 2003

Table 12. Summary of the persistence of viruses in groundwater under different temperatures conditions in the dark

Virus type	T90 (days)	Temperature (°C)	References
Adenovirus[n]	131.6[b] to > 160[c]	4	Enriquez et al., 1994; Ogorzaly et al., 2010; Rigotto et al., 2011
Adenovirus	160[c]	10[c] to 19[c]	Rigotto et al., 2011
Adenovirus[o]	NR	15	Enriquez et al., 1994
Adenovirus	35.6[b] to >200[e]	20[b,e] to 25[e]	Ogorzaly et al., 2010; Sidhu et al., 2015
Adenovirus[p]	NR	23[d]	Enriquez et al., 1994
Coxsackievirus	16.7[g]	0 to 20	John and Rose, 2005
Coxsackievirus	7.6[e,g] to 10.5[h]	12[e] to 15[h]	Charles et al., 2009; Gordon and Toze, 2003
Coxsackievirus	17.3[g,i] to 169[f]	20[g] to 25[e]	John and Rose, 2005; Sidhu et al., 2015
Coxsackievirus	10.2	28	Gordon and Toze, 2003
Coxsackievirus	10[g]	25 to 30	John and Rose, 2005
Echovirus	10[g]	10 to 20	John and Rose, 2005
Echovirus	5.4[g] to 19.6[g]	12 to 13	Yates et al. 1985
Echovirus	10[g]	20 to 25	John and Rose, 2005
Echovirus	1.6[g] to 5.3[g]	23[i]	Yates et al. 1985
Feline calicivirus	8.3[g] to 12.5[g]	4	Bae and Schwab, 2008
Feline calicivirus	5.6[g] to 11.1[g]	20 to 25	Bae and Schwab, 2008
Hepatitis A	100[g]	0 to 10	John and Rose, 2005
Hepatitis A	33.3[g]	20 to 30	John and Rose, 2005
Norovirus[l]	50[g]	4	Bae and Schwab, 2008
Norovirus[k,l]	5[g,j] to 1266[m]	20[m] to 25[j]	Bae and Schwab, 2008; Seitz et al., 2011
Poliovirus	50[g]	0 to 10	John and Rose, 2005
Poliovirus	12.5[g] to 50[g]	4	Bae and Schwab, 2008
Poliovirus	13.7[g]	12	Charles et al., 2009
Poliovirus	11.1[g] to 10[g]	11 to 20	John and Rose, 2005
Poliovirus	10.8 [g] to 28.6[g]	12 to 18	Yates et al. 1985
Poliovirus	5	15	Gordon and Toze, 2003
Poliovirus	5[g,i] to 11.1[g,j]	21[i] to 25[i]	John and Rose, 2005; Bae and Schwab, 2008
Poliovirus	1.5 [g] to 2.8 [g]	23	Yates et al. 1985
Poliovirus	2.5[g]	26 to 36	John and Rose, 2005
Poliovirus	1	28	Gordon and Toze, 2003
PRD-1	50[g]	0 to 10	John and Rose, 2005
PRD-1	10[g]	21 to 25	John and Rose, 2005
Rotavirus	2.5[g]	3 to 15	John and Rose, 2005
Rotavirus	34 to > 200	22 to 23	Sidhu et al., 2015
Tulane virus	2.6[g]	2 to 6	Arthur and Gibson, 2015

NR: Not Reported; [a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b] Ogorzaly et al., 2010; [c] Rigotto et al., 2011; [d]Enriquez et al., 1994 [experiments done in tap water, from a well water source (absent of chlorine)]; [e]Charles et al., 2009; [f] Sidhu et al., 2015; [g] Calculated from $\log_{10}$/ day reduction values reported in the paper; [h]Gordon and Toze, 2003; [i] John and Rose, 2005; [j] Bae and Schwab, 2008 [k] Infectious after 60 days; [l] Molecular methods used; [m]Seitz et al., 2011; [n]T99 of 92[d] to 304[d]; [o] T99 of 87[d] to 124[d]; [p] T99 of 16[d] to 84[d]

In a comprehensive review conducted by John and Rose (2005), it was found that virus inactivation occurs more rapidly at higher temperatures (> 20°C) which is consistent with what was found in the present review (Table 12). Gordon and Toze (2003) reported that inactivation of poliovirus and coxsackievirus was much slower in anaerobic groundwater compared to aerobic conditions, whereas MS2 bacteriophage was inactivated more quickly in anaerobic conditions. It is possible that DO levels may be linked to the presence of other native organisms in the environment and thus could affect the persistence of viruses (John and Rose, 2005). The influence of DO on virus survival still needs further investigation.

5.0 Persistence in Marine Waters

Tables 13,14,15, and 16 summarized the findings of the survival of bacteria, protozoa, bacteriophage and viruses in brackish or saltwater under varying temperatures and light conditions. Survival/ die-off data are reported as T90, T99, or T99.9 values. These studies were performed in the dark, under light, and/or under natural sunlight conditions. In addition, some studies looked at the effect of UVA and UVB radiation specifically on organism survival.

In the present review, 16 of the 45 articles focused on the survival of indicator organisms or pathogens in saltwater. The majority of the data found in these articles was on the persistence of bacteria in saltwater (10 types of bacteria) followed by viruses (4 virus types) and then bacteriophage (1 type). Similar to the groundwater and surface water sections in this chapter, only 1 article was found on the persistence of protozoa.

5.1 Bacteria and bacterial indicators

From the literature presented in Tables 9 and 13, it appears that bacteria are less persistent in saltwater than surface fresh waters. Lothigius et al. (2010) reported that the survival of ETEC was significant affected by salinity in their experiments. A T99 of 8.75 days was observed in saline water compared to a T99 of 69.5 days in surface water. Chandran et al. (2013) reported that the persistence of *Salmonella* and *E.coli* was largely unaffected in brackish/ saline water until concentrations reached 25 ppt (parts per thousand)(Table 13). Seawater is approximately 35 ppt, therefore, suggesting that these organisms would not survive for very long in seawater but could survive in brackish waters. These findings are in line with Boehm et al. (2012) who reported a T90 of 0.017 to 0.025 for *Salmonella* in saltwater under sunlight conditions. Ahmed et al. (2014) conducted experiments in both freshwater and saltwater microcosms, they found that the fecal indicators *enterococci*, *E.coli*, and the microbial source tracking marker (MST) human *Bacteroides* (HF183) had a slightly faster inactivation rate in saltwater than freshwater (although not statistically significant). The *Bacteroides* marker had a similar decay rate as the fecal indicator organisms suggesting that it might be a reasonable indicator of recent fecal contamination (Ahmed et al., 2014). In contrast, Walters et al. (2009) reported that the human specific *Bacteroidales* marker persisted much longer (>18 days) than the culturable enterococci (5 days).

Table 13. Summary of the persistence of bacteria and indicator bacteria in saltwater or brackish water under different temperature and light conditions

Bacteria Type	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	References
Bacteriodales human marker[b]	1.77	NR	17	Light	Walters et al. 2009
Bacteriodales human marker[b]	8.72	NR	17	Dark	Walters et al. 2009
Bacteriodes HF183 DNA[b]	2.7	NR	14 to 18	Sunlight	Ahmed et al., 2014
Catellicoccus marimammalium marker	0.56[d]	NR	8 to 12	Dark	Brown and Boehm, 2015
Clostridium perfringens	>1.3[d]	NR	22 to 24	Dark	Zhang et al. 2015
E.coli spp.	9 to 10.7	14	-2 to 0	Dark	Smith et al., 1994
E.coli spp.	1.7	NR	16.8	Sunlight	Ahmed et al., 2014
E.coli spp.	0.68 to 16.7[d]	NR	22 to 24	Dark	Zhang et al. 2015

Bacteria Type	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	References
E.coli spp.	NR	16 to 22	20 to 30	Nat. light cycle	Chandran et al., 2013
E. faecalis	NR	8 to 35	-2 to 0	Dark	Smith et al., 1994
Enterotoxigenic E. coli	NR	8.75	21	Nat. light cycle	Lothigius et al., 2010
Fecal enterococci	0.06 to 0.07[d]	NR	8 to 12	Nat. light cycle	Brown and Boehm, 2015
Fecal enterococci	0.27 to 0.40[d]	NR	8 to 12	Dark	Brown and Boehm, 2015
Fecal enterococci	1.04 to 1.d	NR	14 to 18	Sunlight	Ahmed et al., 2014; Walters et al. 2009
Fecal enterococci	1.32 to 5.56[d]	NR	22 to 24	Dark	Zhang et al. 2015
Fecal enterococci	0.28 to 1.46	NR	16 to 20	UV Radiation	Kay et al., 2005
Fecal enterococci	0.63 to 2.54	NR	16 to 20	Dark	Kay et al., 2005; Walters et al. 2009
Fecal enterococci (qPCR)[b]	0.19 to 0.44[d]	NR	8 to 12	Nat. light cycle	Brown and Boehm, 2015
Fecal enterococci (qPCR) [b]	> 0.4[d,e]	NR	8 to 12	Dark	Brown and Boehm, 2015
Fecal coliforms	0.88 to 2	NR	22 to 26	Fluorescent	Fujioka et al., 1981
Fecal coliforms	0.02 to 0.06	NR	22 to 26	Sunlight	Fujioka et al., 1981
Fecal streptococci	1.5 to 3.5	NR	22 to 26	Fluorescent	Fujioka et al., 1981
Fecal streptococci	0.04 to 0.13	NR	22 to 26	Sunlight	Fujioka et al., 1981
Salmonella typhimurium	4 to 11	17 to 22	-2 to 0	Dark	Smith et al., 1994
Salmonella enterica	0.02 to 0.03	NR	15	Light	Boehm et al., 2012
Salmonella enterica	47.9	NR	15	Dark	Boehm et al., 2012
Salmonella paratyphi	NR	15 to 16	20 to 30	Nat. light cycle	Chandran et al., 2013

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b] Detected/ monitored using molecular methods; [c]NR: Not Reported; [d]Calculated from $\log_{10}$/ day reduction values reported in the paper; [e]Study also reported zero die-off ($\log_{10}$/ day reduction = 0)

Importantly, both Walters et al. (2009) and Ahmed et al. (2014) noted that human enteroviruses and adenoviruses, respectively, survived much longer than the bacteria in the seawater microcosm experiments. These findings highlight that when evaluating public health risk, it is important to rely on multiple microbial indicators, not only bacteria or traditional fecal indicator organisms. Similar to surface water, bacteria in saltwater are affected by sunlight and persist longer under dark conditions (Tables 9 and 13).

5.2 Protozoa and protozoan indicators

Similar to groundwater and surface water, very few data were found regarding the survival of protozoa in saltwater. In a study by Sidhu et al., (2015), it was found that *Cryptosporidium* oocysts survived significantly longer in brackish groundwater supplies (T90: 56 to 120 days) (Table 14) than non-brackish groundwater (T90: 38 days) at 22 to 23°C (Table 10).

Table 14. Summary of the persistence of the protozoan Cryptosporidium parvum in saltwater or brackish water between 20-25°C in the dark

Protozoa Type	T90 (Days)	Temperature (°C)	Light Source	References
Cryptosporidium	56 to 120	20 to 25	Dark	Sidhu et al. 2015

5.3 Viruses and viral indicators

Flannery et al. (2013) compared the persistence of FRNA bacteriophage GA via culture and quantitative reverse transcription PCR (RT-qPCR) in seawater experiments (Table 15). They found that RT-qPCR significantly overestimates the survival of infectious bacteriophage, and therefore conclude that using RT-qPCR to estimate organism survival is inappropriate (Flannery et al. 2013). In addition, they examined the persistence of norovirus GI & GII in seawater and found very little difference in their survival at 9 to 11°C under either sunlight or dark conditions (Table 16). Once temperatures reached 16 to 18°C, norovirus inactivation was more rapid in sunlight (T90: 0.85 to 0.9) than in the dark (T90: 1.71 to 2.49). In a study by Seitz et al. (2011), it was found that norovirus could persist for extremely long times in groundwater at 20 to 25°C (T90: 1266 days). These results suggest that norovirus may be affected by salinity and persist for shorter periods of time in saltwater than freshwater sources, however more data are needed.

Table 15. Summary of the persistence of bacteriophage in saltwater or brackish water under different temperatures and light conditions

Bacteriophage Type	T90 (Days)	Temperature (°C)	Light Source	References
FRNA phage GA (culture)	0.63	9 to 11	Dark	Flannery et al., 2013
FRNA phage GA (culture)	0.17	9 to 11	Sunlight	Flannery et al., 2013
FRNA phage GA (culture)	0.22 to 2.10	16 to 18	Dark	Flannery et al., 2013
FRNA phage GA (culture)	0.01	16 to 18	Sunlight	Flannery et al., 2013
FRNA phage GA (qPCR)[b]	3.96	9 to 11	Dark	Flannery et al., 2013
FRNA phage GA (qPCR)[b]	3.25	9 to 11	Sunlight	Flannery et al., 2013
FRNA phage GA (qPCR)[b]	2.1	16 to 18	Dark	Flannery et al., 2013
FRNA phage GA (qPCR)[b]	0.69	16 to 18	Sunlight	Flannery et al., 2013

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b] Detected/ monitored using molecular techniques

Table 16. Summary of the persistence of pathogenic and indicator organisms in saltwater or brackish water under different temperatures conditions

Specific Organism/ Pathogen	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	References
Enterovirus	>14	NR	17	Dark	Walters et al. 2009
Enterovirus	> 8	NR	17	Sunlight	Walters et al. 2009
Enterovirus (genome)[b]	16.4	NR	17	Dark	Walters et al. 2009
Enterovirus (genome) [b]	15.7	NR	17	Sunlight	Walters et al. 2009
Human Adenovirus-type 2	0.04	0.08	7	UVB Radiation	Carratalà et al., 2013

Specific Organism/ Pathogen	T90 (Days)	T99 (Days)	Temperature (°C)	Light Source	References
Human Adenovirus-qPCR[b]	9.40	NR	13 to 18	Sunlight	Ahmed et al., 2014
Human Adenovirus 40 & 41		77 to 85	15	Dark	Enriquez et al., 1994
Human Adenovirus-type 2	0.28	0.64	20	UVA Radiation	Carratalà et al., 2013
Human Adenovirus-type 2	0.34	0.67	37	Dark	Carratalà et al., 2013
Human Adenovirus-type 2	0.23	0.57	37	UVA Radiation	Carratalà et al., 2013
Norovirus GI by qPCR[c]	3.58	NR	9 to 11	Dark	Flannery et al., 2013
Norovirus GI by qPCR[c]	3.72	NR	9 to 11	Sunlight	Flannery et al., 2013
Norovirus GI by qPCR[c]	2.49	NR	16 to 18	Dark	Flannery et al., 2013
Norovirus GI by qPCR[c]	0.90	NR	16 to 18	Sunlight	Flannery et al., 2013
Norovirus GII by qPCR[c]	4.23	NR	9 to 11	Dark	Flannery et al., 2013
Norovirus GII by qPCR[c]	3.50	NR	9 to 11	Sunlight	Flannery et al., 2013
Norovirus GII by qPCR[c]	1.71	NR	16 to 18	Dark	Flannery et al., 2013
Norovirus GII by qPCR[c]	0.85	NR	16 to 18	Sunlight	Flannery et al., 2013
Poliovirus	NR	18	15	Dark	Enriquez et al., 1994
Poliovirus (naked genome)[c]	32.4	NR	17	Dark	Walters et al. 2009
Poliovirus (naked genome)[c]	22.3	NR	17	Sunlight	Walters et al. 2009

[a]Unless specified otherwise, all data presented are from cultured organisms and were not monitored using molecular methods; [b]NR: Not Reported; [c]Infectious after 60 days

In seawater microcosms exposed to sunlight, Walters et al. (2009) studied the survival of enteroviruses via culture and through molecular methods. Infectious enteroviruses persisted for 8 days and the genetic enterovirus marker persisted for 28 days. Data are lacking on the survival of enteroviruses in surface water, therefore we are unable to assess the effect salinity may have on their persistence in environmental waters. It is clear that enteroviruses can persist longer in dark conditions than under sunlight (Table 16).

In studies of human adenovirus, there was no difference in the survival of adenoviruses between surface water, wastewater and seawater exposed to UVB radiation at 7°C (Carratalà et al., 2013). In the same study at 20°C under UVA radiation, adenoviruses decayed twice as fast in seawater (T90: 6.66) compared to surface water (T90: 13.96). Ahmed et al. (2014) also found that adenoviruses persisted longer in freshwater than saltwater at temperatures between 13 to 18°C under sunlight conditions.

6.0 Data Gaps

Temperature, sunlight, DO, DOC, availability of nutrients, and salinity were found to be important environmental conditions to consider when evaluating the persistence of microorganisms in environmental waters. This review was performed using a systematic methodology, however, it may not have included all the literature that exists on organism persistence in water matrices. Based on the literature recovered, very few data are available on the persistence of pathogens in aquatic environments.

Table 17 highlights the most significant data gaps in terms of organisms that should be examined for each water source as well as relevant water quality and environmental conditions that have been understudied to date. In general, there remain significant gaps in the literature on the

persistence of pathogens in water matrices, particularly for protozoa. The review did not recover any persistence data on *Giardia* in water matrices and only a few of papers on the persistence of *Cryptosporidium*. Additionally, there is a significant lack of data from developing regions of the world. Most of the literature recovered in this review originated from more developed regions of the world. Consequently, the persistence of relevant microorganisms in developing country contexts such as *Vibrio cholera*, rotavirus and helminths have been largely unexplored. The present review recovered no data on the survival of helminths in environmental waters.

Table 17. Significant data gaps in our knowledge on the persistence of organisms in environmental matrices

Water Matrices	Significant Data Gaps				
	Bacteria	**Protozoa**	**Viruses**	**Water Quality**	**Environmental Conditions**
Groundwater	*Campylobacter* *Enterotoxigenic E.coli* *Vibrio cholera*	*Giardia,* *Cryptosporidium*	enterovirus	DO DOC Nutrients	NR
Surface water	NR	*Giardia,* *Cryptosporidium*	enterovirus hepatitis A norovirus rotavirus	DO DOC Turbidity Nutrients	NR
Saltwater	*Campylobacter* *Enterotoxigenic E.coli* *Vibrio cholera*	*Giardia,* *Cryptosporidium*	hepatitis A rotavirus	Salinity	Cold (<8°C) Warm (> 20°C)
Wastewater	*Campylobacter* *Salmonella* *Enterotoxigenic E.coli* *Vibrio cholera*	*Giardia,* *Cryptosporidium*	enterovirus hepatitis A norovirus rotavirus	Lagoons Turbidity	Cold (< 5°C) Warm (> 20°C) Sunlight

NR: Not Reported

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