Aiko_D_Adell_Zoonoses_QMRA_2016 Comparison of human and southern sea otter (*Enhydra lutris nereis*) health risks for infection with protozoa in nearshore waters

A.D. Adell^{a,b}, G. McBride^c, S. Wuertz^{d,e}, P.A. Conrad^{a,f} and W.A. Smith^{f,*}

^aPathology, Microbiology and Immunology Department, School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, CA 95616, U.S.A.

^bEscuela de Medicina Veterinaria, Facultad de Ecología y Recursos Naturales, Universidad Andres Bello, Republica 440, 8370251 Santiago, Chile

^cNational Institute of Water and Atmospheric Research (NIWA), P.O. Box 11-115, Hillcrest, Hamilton, New Zealand.

^dDepartment of Civil and Environmental Engineering, University of California, Davis, CA, One Shields Avenue, Davis, CA 95616, U.S.A.

^eSingapore Centre for Environmental Life Sciences Engineering (SCELSE) and School of Civil and Environmental Engineering, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551.

^fOne Health Institute, School of Veterinary Medicine, One Shields Avenue, University of California, Davis, CA, 95616.

*Corresponding Author:

One Health Institute, School of Veterinary Medicine, University of California, Davis, One

Shields Avenue, Davis, CA 95616

Tel: 1-530-754 0119

Email: wasmith@ucdavis.edu

Abstract

Cryptosporidium and *Giardia* spp. are waterborne, fecally-transmitted pathogens that cause economic loss due to gastroenteritis and beach closures. We applied quantitative microbial risk assessment (QMRA) to determine the health risks for humans and sea otters due to waterborne exposure of *Cryptosporidium* and *Giardia* spp. when swimming in three types of surface waters: river, stormwater and wastewater effluent during the wet and dry seasons in the central coast of California. This is the first application of QMRA to estimate both the probability of infection in Southern sea otters and the probability of illness in humans, using microbial source tracking (MST) as a variable. Children swimming close to stormwater discharges had an estimated *Cryptosporidium*-associated illness probability that exceeded the accepted U.S. EPA criteria (32 illnesses/1,000 swimmers or 3.2%). Based on the assumption that sea otters are as susceptible as humans to *Cryptosporidium* infection, the infection probabilities were close to 2% and 16% when sea otters were swimming at the end of points of rivers and stormwater discharges, respectively. In the case of *Giardia*, infection probabilities of 11% and 23% were estimated for sea otters swimming at the end of point of wastewater discharges, assuming that sea otters are as susceptible as gerbils and humans, respectively. The results of this QMRA suggest that 1) humans and sea otters are at risk when swimming at outflow sites for rivers,

stormwater and treated wastewater effluent; 2) reduced loads of viable protozoan cysts and oocysts in recreational water can lessen the probability of infection of humans and sea otters; and 3) the risk of infection of humans and sea otters can be reduced with the treatment of wastewater to decrease oocyst and cyst viability before effluent is released into the sea.

Keywords:

Zoonoses, QMRA, Bacteroidales, wastewater, stormwater, recreational water

Nomenclature

Variable	Description
<i>Dih</i>	Pathogen dose ingested by humans while swimming
<i>Diso</i>	Pathogen dose consumed by sea otters while swimming
<i>PC</i>	Pathogen concentration in 10 L from each water source adjusted for the detection test recovery [Oocysts or cysts in 10 L]
<i>DF</i>	The dilution factor
<i>B</i>	Proportion of <i>Bacteroidales</i> (human and ruminant markers) detected in the river samples [%]
<i>VP</i>	Viability proportion of each protozoa in water [%]
<i>SD</i>	Swimming durations for humans [h]
<i>HI</i>	Water ingested per hour of swimming for humans [mL/h] or sea otters [mL/kg]
<i>HI</i>	Water ingested per hour of swimming for sea otters [mL/kg]
<i>BW</i>	Sea otter body weight [kg]
<i>d</i>	Dose given to any individual and not a mean dose
<i>r</i>	Probability that single protozoa could cause infection
<i>Pr_{inf.}</i>	Probability of infection
<i>Pr_{ill}</i>	Probability of illness
<i>r_s</i>	Spearman rank-order correlation coefficient

1. Introduction

Cryptosporidium and *Giardia* spp. are zoonotic waterborne pathogens known to be released to the environment through human and animal feces (Haas et al., 1999; Fayer and Xiao, 2008). These protozoan parasites are transmitted by fecal-oral routes, causing infection and

gastrointestinal disease in humans after ingestion of even small doses of *Cryptosporidium* oocysts or *Giardia* cysts (Rendtorff, 1954; Chappell et al., 2006). Although generally characterized by a self-limiting diarrhea in immunocompetent individuals, disease can be severe and chronic in immunocompromised individuals as well as elderly and infant populations (Navin and Juranek, 1984; Xiao et al., 2004). Several outbreaks of diarrheal disease involving these pathogens in drinking and recreational waters have occurred throughout the U.S. The most significant of these, the Milwaukee outbreak in 1993, was caused by *Cryptosporidium* and affected more than 403,000 people (Mac Kenzie et al., 1994).

Previous studies have shown that protozoan and bacterial pathogens can be transported from land to sea through discharge sources such as stormwater, wastewater effluent, and river outflow in California (Miller et al., 2002; 2006; 2007; 2010; Shapiro et al., 2010). These biologic pollutants of fecal origin are recognized public health risks for humans and may also be negatively impacting the health of wildlife, such as southern sea otters (*Enhydra lutris nereis*) that live in the nearshore coastal ecosystems of California. Wildlife can serve as sentinels for environmental contamination with fecal pathogens such as the zoonotic protozoa *Toxoplasma gondii* (Jessup et al., 2004; Conrad et al., 2005), and this federally-listed threatened species has experienced population recovery largely limited by infectious diseases, with an estimated population of about 2900 otters in 2014 (Thomas and Cole, 1996a; Kreuder et al., 2003; U.S. Geological Survey Web site, 2014). Several pathogens isolated from stranded and dead sea otters may have anthropogenic origins (Thomas and Cole, 1996b; Miller et al., 2002; Kreuder et al., 2003; Conrad et al., 2005) which could be associated with coastal development, wetlands ablation, and coastal wastewater discharge. Baseline data collected from live and dead sea otters along the California coast have confirmed the presence of enteric bacteria, *T. gondii*,

84 *Cryptosporidium*, and *Giardia* infections in sea otters (Miller et al., 2002; Miller et al., 2010;
85 Oates et al., 2012). Additionally, Gaydos et al., (2007) reports *Cryptosporidium* and *Giardia* in
86 16% and 19% of marine foraging river otters, and Méndez-Hermida et al., (2007) reports that
87 *Cryptosporidium* and *Giardia* in 4% and 7% of wild common otters (*Lutra lutra*), respectively.
88 These reports demonstrate that otters can be infected with fecal protozoal pathogens and may
89 contribute to the contamination of surface waters.

90 Reducing the incidence of fecal-origin biologic pollutants in humans and sea otters is a
91 challenge for several reasons. *Cryptosporidium* oocysts and *Giardia* cysts are resistant to
92 chlorine-based treatment and can survive for long periods of time in ambient water (Sattar et al.,
93 1999). Furthermore, they may not be removed by all filtration processes due to their small size
94 (5-10 μm) and a fully effective treatment for cryptosporidiosis in humans remains elusive (Haas
95 et al., 1999; Cacciò and Chalmers, 2016). Thus, a common public health recommendation for
96 reducing the occurrence of these infections and the diarrheal disease they cause is to minimize
97 exposure to contaminated water and food sources.

98 This research introduces the use of QMRA to estimate the risks and consequences from
99 exposure of individuals or populations, of animals or humans, to waterborne protozoan parasites.
100 It also combines microbial source tracking (MST) methodology with QMRA. MST focuses on
101 identifying sources of fecal contamination impacting water systems (EPA, 2005). Members of
102 the order *Bacteroidales* are currently the most commonly used source identifiers in water
103 because they have the advantage of demonstrating host specificity and being strictly anaerobic,
104 thus acting as an indicator of recent fecal pollution (Field and Samadpour, 2007; Wang et al.,
105 2010; Wuertz et al., 2011a). Furthermore, *Bacteroidales* markers can provide complementary
106 information to facilitate MST, as they have been demonstrated to have a high sensitivity as an

alternative fecal indicator assay and to show a high discrimination between human and animal fecal sources (Schriewer et al., 2010).

The setting for this study was the central coast of California, where there is stakeholder interest in understanding the risk that fecal pathogen pollution poses to humans and sea otters that utilize nearshore ecosystems. The objectives of this study were to (i) measure the concentration of both *Cryptosporidium* oocysts and *Giardia* cysts in rivers, wastewater effluent and stormwater in the central coast of California, (ii) use the data to conduct a QMRA to determine the health risk for humans and sea otters due to exposure of these fecal protozoa (or hazards) in surface waters; and (iii) evaluate the use of MST with *Bacteroidales* as a variable to provide an estimation of the source of the pathogen contamination and taking into account only those *Cryptosporidium* and *Giardia* species that are most likely to be infectious to humans and to sea otters. The results can provide useful information for the creation of strategies to manage and reduce risk to humans as well as coastal wildlife utilizing fecally contaminated watersheds.

2. Methods

2.1. Sampling

2.1.1. River sampling

Water samples from rivers were obtained from sites along the central California coast from Santa Cruz to the Big Sur area as previously described (Schriewer et al., 2010). Grab samples from rivers at ten coastal locations (Figure 1A) were tested monthly from April 2007 until September 2008. Dry season was categorized from June to November and wet season was

categorized from December to May. Water samples from field collection bottles were combined and mixed in 10-L sterile carboys before protozoan testing.

2.1.2. Stormwater sampling

During the wet season (December to May) a stormwater study focused on three urban communities along the Monterey Bay, and samples were collected three times during each of three storms from a single storm drain in each of three urban communities (Figure 1B). The dates of collection were January, November and December 2008, and January and April 2010. Each storm was to be sampled once per h for 3 h at each of the sites simultaneously. Samples were collected at each site upon confirmation that it was raining enough at all three sites for the water to be sheeting on the road. At some sites a catch-pole was used to obtain a grab sample from the stormwater flow. Each sample was placed in a cooler with double bagged ice while sampling and delivered to the California Department of Fish and Game Marine Wildlife Veterinary Care and Research Center (CDFG-MWVCRC) laboratory.

2.1.3. Wastewater effluent sampling

Samples of wastewater effluent from 4 sites located in the coastal area of central California were collected on May, June, September and December 2007; March, June and September 2008; and March 2011. Grab samples were collected by treatment plant personnel at spigots built into each treatment plant for sample collection. Prior to sampling, all sterile 2 L sample bottles had 1 mL per L of sample of sodium thiosulfate (for dechlorination) added. After collection in the field, samples were placed directly into a cooler with ice and delivered to the CDFG-MWVCRC laboratory for protozoan filtration.

2.2. Protozoa testing

At the CDFG -MWVCRC, 10 L of each water matrix was filtered through an Envirochek filter to concentrate the oocysts and cysts before transport to the UC Davis laboratories for further processing. All samples were collected and filtered on the same day. *Cryptosporidium* and *Giardia* spp. were detected as previously described in Schriewer et al., (2010).

2.3. Recovery efficiencies and detection limits

Spiking experiments were performed using environmental matrix samples to determine method recoveries. For rivers, 10 L water samples from Waddel Creek, Scott Creek, San Lorenzo River, Watsonville Slough and Salinas River were each spiked with 200 oocysts and 200 cysts. The recovery efficiency in stormwater was estimated by spiking 10 L of one stormwater sample with 200 oocysts and 200 cysts. The recovery efficiency in sewage effluent was estimated by spiking 12 samples (10 L each) with 200 oocysts and 200 cysts. Observed concentrations of target pathogens were adjusted after accounting for recoveries for each water matrix using the following equation:

$$\frac{\text{Protozoa concentration detected in 10 L of water sample} \times 100\%}{\text{recovery efficiency (\%)}} \quad (1)$$

The detection limits (*DL*) were determined based on the results of the spiking experiments described above. The *DL* was calculated by dividing 200 oocysts and cysts spiked by the mean number of protozoa recovered after spiking the samples. The *DL* for rivers was 9

oocyst/10 L and 7 cysts/10 L; for stormwater was 29 oocyst/10 L and 34 cysts/10 L; and for wastewater effluent was 4 oocysts/10 L and 13 cysts/10 L, respectively (Table 1).

2.4. Protozoa concentration distribution

The concentrations of *Cryptosporidium* and *Giardia* in 10 L of water were fitted using the @RISK distribution fitting function, however it was not possible to find a good fit by means of best KS statistic due to the presence of numerous non-detects in the data. For data sets with a large number (51 - 80%) of non-detects, Sinha et al., (2006) recommended using bounding methods. Kundu et al., (2013) stipulated that bounded distributions are more realistic because risks are driven from the upper tails of the distributions. The Hockey stick distribution is bounded, that is, it has a minimum and a maximum, and it has been reported as being most suitable for small and right-skewed datasets (Wuertz et al., 2011b). Hence, the concentrations of *Cryptosporidium* and *Giardia* in 10 L of water were fit to the hockey stick distribution (McBride, 2005). Refer to supplementary information file for details on the hockey stick distribution.

2.5. *Bacteroidales*

Microbial source tracking (MST) with *Bacteroidales* (Kildare et al., 2007; Wuertz et al., 2011a) was used to estimate the proportion of protozoa that are more likely to be infective to humans, and also those that might be infective to sea otters, using an indirect approach to identify contributing fecal host source groups. Human-, ruminant- and dog -associated *Bacteroidales* host markers validated using fecal samples from California (Kildare et al., 2007; Silkie et al., 2009; Raith et al., 2013) were detected in the river samples collected in this study as reported in Schriewer et al. (2010). Host-associated markers are any trait used to identify a fecal

source to its host or source species, including targets for host-specific PCR (Field and Samadpour, 2007). Data regarding the percentage of water samples positive for human, cow (currently considered to be a ruminant assay as stated in Raith et al., 2013) and/or dog markers from Schriever et al. (2010) were fitted using the @RISK distribution fitting function (Palisade Corporation, 2013) and the fitted distributions were selected based on chi-square and Kolmogorov-Smirnov (K-S) goodness of fit test. Refer to supplementary information file for details on the *Bacteroidales* distributions and on dose-response and exposure assessment.

2.6. Risk characterization

Risk characterization consists of an estimation of the human and sea otter health risk using a given set of input data (see nomenclature for description of all variables).

2.6.1. Estimation of the dose

The pathogen dose ingested by humans (D_{ih}) while swimming was calculated as follows:

$$D_{ih} = \left(\frac{PC}{10,000 \text{ mL} \times DF} \right) \times B \times VP \times HI \times SD \quad (2)$$

Refer to nomenclature table for description of variables in equation 2

The pathogen dose consumed by sea otters (D_{iso}) while swimming was calculated as follows:

$$D_{iso} = \left(\frac{PC}{10,000 \text{ mL} \times DF} \right) \times B \times VP \times HI \times BW \quad (3)$$

Refer to nomenclature table for description of variables in equation 3.

2.6.2. Endpoint

The probability of illness (Pr_{ill}) was adopted as an endpoint for humans. This endpoint was selected because epidemiological studies performed on humans are often based on illness rather than infection, and to harmonize the results obtained in this QMRA with those studies requires that the QMRA be conducted for illness. In this QMRA the probability of infection (Pr_{inf}) was converted to Pr_{ill} using the percent of infections resulting in illness reported by Soller et al. (2010): 50% for *Cryptosporidium* and 45% for *Giardia*. The U.S. EPA 2012 criteria of what is acceptable for recreation is 32 illnesses/1,000 swimmers (or 3.2%) (EPA, 2012), therefore this criterion was considered as the cut-off value to reflect a high Pr_{ill} in humans. The Pr_{inf} was adopted as an endpoint for sea otters as there are no epidemiological studies performed and there is no data regarding the percent of infections resulting in illness for this animal species.

2.6.3. Scenarios

Three scenarios were created in order to estimate the Pr_{inf} of swimming in contaminated surface waters. The worst case scenario (scenario 1) assumed that all the protozoa detected in the water samples were infective ($VP = 100\%$) and that the person or sea otter was swimming in the 'end of point' water source with no dilution factor ($DF = 1:1 = 1$). Scenario 2 replaced the DF from 'end of point' ($DF = 1$) to a dilution of 1:30 ($DF = 30$), keeping the viability of the protozoa constant at 100%. If the worst case scenario for wastewater effluent exceeded the EPA criteria (2012) for how many illnesses are acceptable after a human swims in a recreational water or is

greater than 1% infection is sea otters, then scenario 3 would replace the *VP* for 0.1% (equivalent to a 3-log or 99.9% inactivation), which is the EPA (2006) inactivation requirement for both *Cryptosporidium* and *Giardia* in public water system, while keeping *DF* constant (endpoint or 1:1). These three scenarios were run using the concentration of protozoa detected during the wet season separate from the dry season.

- In order to estimate the risk profile, stochastic models using Monte Carlo simulations were run using Excel add-in @RISK. The simulation consisted of exposing 100 individuals on each exposure occasion to obtain smooth risk profiles for pathogens with low *ID50* (McBride, 2005). The simulation setting was set for 1,000 iterations and 1 simulation; therefore, a total of 10^5 iterations (that is 100 individuals x 1,000 iterations) were run. Monte Carlo ‘random draws’ sampling type and a Fixed Random generator seed (value =1) were chosen as the settings for the analysis. The results provided by this analysis corresponded to the number of illnesses (in the case of humans) or number of infections (in the case of sea otters) for 100 individuals exposed 1,000 times.

Refer to supplementary information file for assumptions.

2.6.4. Estimation of the probability of infection

The simple binomial distribution was used to estimate the Pr_{inf} . The simple binomial:

$$Pr_{inf}(d) = 1 - (1 - r)^d \quad (4)$$

Where d denotes the dose given to any individual and not a mean dose, and r is the probability that a single protozoa could cause infection. This distribution is denoted as a ‘conditional’ dose-

response function (Haas, 2002). The distribution of microorganism surviving (to initiate the infection) is binomial when each of the '*d*' ingested microorganism has an equal and identical probability '*r*' of surviving (Haas, 2002). A QMRA model was built for *Cryptosporidium* and *Giardia* infection risks with various input parameters and the four variables hazard identification, dose-response assessment, exposure assessment, and risk characterization (Figure 2).

2.6.5. Sensitivity Analysis

The sensitivity analysis consisted of running a full simulation at each of a set of possible values for an input, tracking the simulation results at each value, showing how simulation results changed as the input value was changed (Palisade Corporation, 2013). This allows for testing of the sensitivity of an output to the input distribution in a model (Palisade Corporation, 2013). The sensitivity analysis was performed only for scenario 1, and for 1,000 iterations and 21 simulations, using Excel add-ins @RISK. The Spearman rank-order correlation coefficient (r_s) was used to identify the significant variables in the model that are contributing to the uncertainty of the estimation of the health risk. Refer to supplementary information file for details on the Spearman rank-order correlation coefficient.

3. RESULTS

The mean recovery efficiencies in spiked river samples were 12% for *Cryptosporidium* and 15% for *Giardia* (Table 2); in spiked stormwater samples they were 4% for *Cryptosporidium* and 3% for *Giardia*, and in spiked wastewater effluent samples they were 31% for *Cryptosporidium* and 8% for *Giardia* (Table 3). The probabilities of infection of humans and sea

otters by swimming in contaminated water with *Cryptosporidium* and *Giardia* are presented in Tables 4 and 5, respectively.

3.1. Scenario 1: Swimming in water assuming that oocysts and cyst are 100% viable and at the end of point of the contaminated water discharge.

When assuming that the oocysts and cysts detected in the water samples are 100% viable and that humans are swimming at the end of point of the water discharge, the Pr_{ill} to *Cryptosporidium* in children swimming at the end of point of stormwater discharges during the dry season was 5% (Figure 3), which exceeded the EPA (2012) recommendation of 3.2% illness.

In the case of sea otters, when assuming that they are as susceptible to infections as gerbils (ID_{50} was assumed to be 365 for *Cryptosporidium* and 86 for *Giardia*), the Pr_{inf} was 2% when sea otters swam at the end of point of stormwater discharges contaminated with *Cryptosporidium* during the dry season. For *Giardia*, when swimming at the end of point of wastewater effluent discharges during the wet and dry season, the Pr_{inf} was 11%.

When assuming that sea otters are as susceptible to infection as humans (ID_{50} was assumed to be 35 for both *Cryptosporidium* and *Giardia*), the Pr_{inf} to *Cryptosporidium* was 2% when sea otters swam at the end of point of river discharges during the wet season and 16% when sea otters swam at the end of point of stormwater discharges during the dry season (Figure 4). For *Giardia*, when swimming at the end of point of wastewater effluent discharges during the wet and dry season, the Pr_{inf} was 23% (Figures 5A and 5B).

3.2. Scenario 2: Swimming in water assuming that oocysts and cyst are 100% viable and at a dilution factor of 1:30 from the contaminated water discharge.

The Pr_{inf} for humans did not exceed the EPA (2012) recommendation of 3% illness in any of the water matrixes and seasons evaluated. When assuming that sea otters are as susceptible as humans to protozoan infection ($ID_{50} = 35$ oocysts or cysts), the Pr_{inf} for sea otters when swimming in stormwater contaminated with *Cryptosporidium* during the dry season was lower than 1%. The Pr_{inf} for *Giardia* while swimming in wastewater effluent at a dilution factor of 1:30 during the wet season and dry seasons was 1%.

3.3. Scenario 3: Swimming in water assuming that oocysts and cyst are 0.1% viable and at the end of point of the contaminated water discharge.

The Pr_{ill} for humans did not exceed the EPA (2012) recommendation of 3% illness and the Pr_{inf} of sea otters was lower than 1% in all of the water matrixes and seasons.

3.4. Sensitivity Analysis

We performed a sensitivity analysis of the relative importance of the stochastic variables that affect the predicted pathogen doses for humans and sea otters: protozoa concentration (oocysts or cysts/10 L), *Bacteroidales* (% of *Bacteroidales* markers detected in the river samples), swimming duration for humans (hours), water ingested while swimming (mL/h for humans and mL/kg for sea otters), and sea otter body weight (kg). The median Spearman rank correlation coefficient (r_s) obtained from each scenario and variable distribution is shown on Table 6. For humans and sea otters, the most significant variables were protozoa concentration (median $r_s = 0.4$ and 0.55 for *Cryptosporidium* and *Giardia* scenarios, respectively) and *Bacteroidales* (median $r_s = 0.4$ and 0.48 for *Cryptosporidium* and *Giardia* scenarios, respectively). Water ingested per hours of swimming (median $r_s = 0.29$ and 0.31 for

Cryptosporidium and *Giardia* scenarios, respectively), swimming duration (median $r_s = 0.29$ and 0.38 for *Cryptosporidium* and *Giardia* scenarios, respectively), and sea otter body weights (median $r_s = 0.12$ and 0.17 for *Cryptosporidium* and *Giardia* scenarios, respectively), were somewhat less important.

4. Discussion

The probability of illness for humans and the probability of infection for sea otters were estimated when swimming in different water matrices contaminated with *Cryptosporidium* oocysts and *Giardia* cysts. Stormwater and wastewater effluent discharges are contributed the most to health risks for humans and sea otters of infection with *Cryptosporidium* and *Giardia*.

QMRA studies in drinking and other water sources contaminated with *Cryptosporidium* and *Giardia* (Rose et al., 1991; Pouillot et al, 2004; Cummins et al, 2010) assumed that all the *Cryptosporidium* and *Giardia* detected in the water samples were genotypes or assemblages that were infective, leading to an overestimation of infection risk (Regli et al., 1991; Rose et al., 1991). In the present study, the risk of infection was estimated by including in the model only those *Cryptosporidium* and *Giardia* (oo)cysts that are infective to humans and sea otters, thus avoiding an overestimation of the risk caused by non-infectious species present in the water. In this regard, microbial source tracking (MST), a method that identifies sources of fecal contamination impacting water systems (EPA, 2005), was incorporated into the model as a variable. Validated *Bacteroidales* markers were used in the model as MST source identifiers because they provide complementary information and exhibit both high sensitivity as an alternative fecal indicator assay and excellent discriminatory power between human and relevant animal fecal sources (Schriewer et al., 2010). Therefore, the presence of host-associated

Bacteroidales markers could be associated with the presence of *Cryptosporidium* and *Giardia*, which are shed by animal host species. Among all the described *Cryptosporidium* spp. and *Giardia* spp., only *C. parvum*, *C. hominis*, and *G. duodenalis* assemblages A and B have been reported to be infectious to humans (Okhuysen et al., 1999; Chappell et al., 2006; Caccio et al., 2005), while *C. parvum* and *G. duodenalis* assemblages A, B and D have been reported to be infectious to other marine mammals (Deng et al., 2000; Dixon et al., 2008; Gaydos et al., 2008) and therefore could be infectious to sea otters. For the present QMRA, the prevalence of human, ruminant and dog markers was included in the model, allowing for an estimation of the source of the pathogen contamination and taking into account only those *Cryptosporidium* and *Giardia* species that are most likely to be infectious to humans and to sea otters; thus providing more realistic results. Furthermore, based on the sensitivity analysis, the contribution of *Bacteroidales* to the uncertainty of the estimation of the health risk is greater than the contribution of other variables in the model, suggesting that *Bacteroidales* is a variable that should be considered when designing QMRA models.

Another challenge that can arise in QMRA analysis is the underestimation of the exposure by not taking into account the non-detections (Teunis and Havelaar, 2002). Samples having zero oocysts and cysts counts are frequent, however, this does not indicate that there are no organisms present in the sample, only that they were not detected (Teunis and Havelaar, 2002). This situation occurs when there are low concentrations of oocysts and cysts in water and the detection methods used to detect these protozoa in water have recovery efficiencies lower than 100% (Teunis and Havelaar, 2002). Furthermore, the efficiency of the detection method varies with conditions of the water sample, such as turbidity (Teunis and Havelaar, 2002). The non-detection of oocysts and cysts in the samples results in an underestimation of the exposure

and therefore of the risk. Kundu et al., (2013) tested three statistical methods to account for non-detects (substituting non-detects with zeros, with sample detection limits or with half detection limits) and found that tested variables had less of an effect on the risk when the latter two substitution methods were employed. As there were many non-detects for the wastewater effluent, river, and stormwater samples, the non-detects were replaced by the detection limit in order to create a more realistic scenario. Petterson et al. (2007) estimated stochastic oocyst and cyst concentrations by using three approaches: 1) not considering recovery efficiency, 2) using sample recovery as an independent random variable when data were limited and 3) adjusting each result for a concurrently derived estimated recovery. The results of that study showed that not considering recovery efficiency caused an underestimation of the health risk. Therefore, site specific recovery efficiency should be incorporated into source water concentrations whenever possible when inferring health risks to consumers.

The dose response curve for *Cryptosporidium* in humans was based on the study of Teunis (2009) where the probability that a single oocyst could cause infection was inferred by interpreting the data on the infectivity of several *C. parvum* isolates. Notably, they did not include the *C. hominis*, which is infectious to humans, in that study; therefore, this species is not represented in the dose response curve. Similarly, the dose response curve for *Giardia* was based on the study by Rendtorff (1954) in which the data were derived using one *G. duodenalis* strain and a relatively small sample population (Rose et al., 1991) which did not take into consideration the variety of other *G. duodenalis* strains and assemblages. Despite these limitations, Rendtorff (1954) is the only *Giardia* experimental infection study done in human volunteers. Due to the human health implications involved, there are no experimental infective studies done in children or immunosuppressed human volunteers, which are the susceptible populations for disease. For

this reason, in this study it is assumed that all the humans exposed are healthy and, therefore, immunocompetent individuals. Furthermore, the same dose response curve was used for adults and children, even though children are more susceptible to illness than adults. This situation has been recognized in part by doubling the amount of water ingested by children while swimming.

There are no published experimental infection studies with either *Giardia* or *Cryptosporidium* in sea otters or any other members of the mustelids family; therefore, the dose response curve and *ID50* were based on infective experimental studies done in related mammalian hosts that have shown similar infection patterns to sea otters for the fecal protozoan *T. gondii* (Dabritz et al., 2007): mice (Ernest et al., 1986) and gerbils (Finch et al., 1993; Li et al., 2007) for *Cryptosporidium* and *Giardia*, respectively. Ernest et al. (1986) infected immunocompetent, newborn mice with known doses of *C. parvum* oocysts and the infectivity was determined by means of oocysts shedding. A meta-analysis reported by Adell et al. (2012) showed that young and adult animals did not differ significantly regarding infectivity with *C. parvum* based on oocyst shedding. The probability that a single *Giardia* cyst could cause infection was obtained by fitting the “simple exponential model” to infections in gerbils as reported by Finch et al., (1993) and Li et al., (2007). In both studies, immunocompetent Mongolian gerbils (*Meriones unguiculatus*) were infected with known doses of *G. duodenalis* cysts and the infectivity was determined by means of cyst shedding. Finch et al. (1993) infected adult animals, while Li et al., (2007) infected weanling animals. Pooling data from different studies has the advantage of enhancing the confidence in inferring behavior of different strains, if the same organism has the same dose response in different animal species, confidence in extrapolating to new species (e.g. sea otters) is increased. This also increases the statistical precision with which the dose response relationship is estimated (Haas et al., 1999). Despite this

advantage, it would be interesting to analyze the data with the dose response curve and *ID50* for mustelids if that data becomes available. Furthermore, accounting for uncertainty in dose-response generally causes a decrease in predicted risks (Wuertz et al, 2011), however, in this study; uncertainty was not incorporated in the dose response curve which may have lead to a more conservative (higher) estimate of risks.

Taking into account the strengths and limitations of this QMRA, the model estimated that the *Pr_{ill}* of children when swimming end of point of stormwater discharges during the dry season exceeded the accepted EPA criteria of a maximum 32 illness/1000 population (3.2 %) from *Cryptosporidium*. The average annual reported cryptosporidiosis incidence rate in humans in California between 2006 and 2008 was 0.8 per 100,000 populations (CDC, 2010), while during 2001 and 2008 there were 18.4 cases per 100,000 population among children 1 to 4 years of age and 7.3 cases per 100,000 among children 5 to 14 years of age (CDPH, 2011). The annual incidence rate reported by CDC is lower than the probability of illness predicted by this QMRA; however, epidemiological data are known to be underestimated because people do not always present to a primary care facility for diarrhea and the etiology of cases are often not investigated or reported. Furthermore, cases contracted from swimming often disperse widely from the site of exposure and therefore, are less likely to be identified as part of an outbreak (Fayer, 2008). Based on the results of this QMRA model, it would be advisable to prevent children from swimming in coastal areas that have a stormwater discharge close by.

In our study, the risk of infection to *Cryptosporidium* was estimated in sea otters when swimming at the end of point of river discharges during the wet season, assuming that the sea otters are as susceptible as humans to *Cryptosporidium* infection. Rivers receive the rainfall-initiated runoff from urban, suburban and rural landscapes, and wastewater outfalls, which can

carry oocysts and cysts (Fayer et al, 2004), and sea otters can be found swimming close to river discharges, making it possible for this species to be infected with *Cryptosporidium* and *Giardia*.

The Pr_{ill} in humans and the Pr_{inf} in sea otters were reduced to levels lower or equal to 1%, when a dilution factor of 1:30 was included in the wastewater effluent scenario, indicating that having the wastewater effluent discharges far away from where humans and sea otter swim may reduce the infection of humans and this marine mammal. Wastewater effluent has a deep ocean outfall that extends between one and two miles offshore, where sea otters may swim. The Pr_{inf} of sea otters was reduced to levels $<0.04\%$ when the viability of protozoa followed the EPA recommendations for public water systems; therefore the results of this QMRA suggests that applying disinfection based on UV light or other methods to wastewater effluent could almost eliminate the Pr_{inf} of children, sea otters and other marine mammals.

According to the results of the sensitivity analysis, protozoan concentration was one of the variables that had the most influence on an estimation of the probability of infection. Therefore, the risk of infection of both humans and sea otters could be reduced by reducing the load of protozoal pathogens being discharged through rivers. Shapiro et al., (2010) and Hogan et al., (2013) conducted transport studies to evaluate if the loss of estuarine wetlands is contributing to an increased flux of oocysts and cysts into coastal waters. Shapiro et al., (2010) found that more *Toxoplasma* surrogates were recovered from unvegetated mudflat habitats, which represent degraded wetlands, and Hogan et al., (2013) found that the presence of vegetation was a significant factor for removal of *Cryptosporidium*, *Giardia* and *Toxoplasma* surrogates in a unidirectional transport wetland system; hence having more wetlands between the river discharge and the sea may be a natural method to reduce the load of protozoan parasites in river and reduce the probability of infection of sea otters.

5. Conclusions

- The estimated probability of illness with *Cryptosporidium* in children when swimming at the end of point of stormwater discharges during the dry season exceeded the reported EPA criteria of 3% illness in this analysis.
- The highest probability of infection with *Cryptosporidium* and *Giardia* for sea otters was when they swim in stormwater discharges during the dry season and at the end of point of wastewater effluent discharges during both the dry and wet season, respectively.
- The reduced risk of infection after dilution factor (1:30) or UV light treatment to decrease viability indicated that discharging wastewater effluent discharges far away from where swimming areas or and treating wastewater effluent with UV light may reduce the infection of humans and this marine mammals.
- Inclusion of *Bacteroidales* in QMRA models can control for overestimation of the risk caused by non-infectious *Cryptosporidium* and *Giardia* isolates in the water.
- Efforts focused on reduction of *Cryptosporidium* oocyst and *Giardia* cyst loads in stormwater and wastewater effluent discharges would contributed most significantly to decreasing the health risks for humans and sea otters from these parasites.

Acknowledgements

We acknowledge the financial support received from the “Presidente de la República” Chilean scholarship, the Central Coast Long-term Environmental Assessment Network (CCLEAN) (grant #: 06-076-553) from the California State Water Board to the City of Watsonville, the National Science Foundation (NSF) Ecology of Infectious Disease Grant

496 Program (grant #: OCE-1065990), UC Davis Graduate Student Support Program (GSSP), UC
497 Davis Graduate group in Comparative Pathology Block Grant and the 6th QMRA Summer
498 Institute Funding (CAMRA, Michigan State University). Thanks are due to Dr. Tim Tinker for
499 providing the sea otter body weight dataset, and to Dr. Kaitlyn Hanley and Dr. Arti Kundu for
500 comments and suggestions.
501

6. References

- Adell, A.D., Miller, W.A., Harvey, D.J., Vanwormer, E., Wuertz, S., Conrad, P.A., 2012, Individual subject meta-analysis of parameters for *Cryptosporidium parvum* shedding and diarrhoea in animal experimental models. *Epidemiol Infect*, 1-17.
- Caccio, S.M., Thompson, R.C., McLauchlin, J., Smith, H.V., 2005. Unravelling *Cryptosporidium* and *Giardia* epidemiology. *Trends Parasitol* 21, 430-437.
- Cacciò S.M., Chalmers R.M. 2016., 2016, Human cryptosporidiosis in Europe. *Clin Microbiol Infect* 22, 471-80. doi: 10.1016/j.cmi.2016.04.021. Epub 2016 May 10.
- CDC, 2010. Morbidity and Mortality Weekly Report. Cryptosporidiosis Surveillance - United States, 2006– 2008 and Giardiasis Surveillance - United States, 2006–2008. *MMWR Morb Mortal Wkly Rep* 59(SS06);1-14.
- CDPH, 2011. Epidemiologic summaries of selected general communicable diseases in California, 2001-2008.
- Chappell, C.L., Okhuysen, P.C., Langer-Curry, R., Widmer, G., Akiyoshi, D.E., Tanriverdi, S., Tzipori, S., 2006, *Cryptosporidium hominis*: experimental challenge of healthy adults. *Am J Trop Med Hyg* 75, 851-857.

525 Conrad, P.A., Miller, M.A., Kreuder, C., James, E.R., Mazet, J., Dabritz, H., Jessup, D.A.,
 526 Gulland, F., Grigg, M.E., 2005, Transmission of *Toxoplasma*: clues from the study of sea otters
 527 as sentinels of *Toxoplasma gondii* flow into the marine environment. Int J Parasitol 35, 1155-
 528 1168.
 529
 530 Cummins, E., Kennedy, R., Cormican, M., 2010, Quantitative risk assessment of
 531 *Cryptosporidium* in tap water in Ireland. Sci Total Environ 408, 740-753.
 532
 533 [Dabritz, H.A.](#), [Miller, M.A.](#), [Packham, A.E.](#), [Rejmanek, D.](#), [Leutenegger, C.M.](#), [Gardner, I.A.](#),
 534 [Atwill, E.R.](#), [Conrad, P.A.](#), 2007, Experimental infection of *Peromyscus californicus* with
 535 *Toxoplasma gondii*. J Parasitol, 93, 1360-4.
 536
 537 Deng, M.Q., Peterson, R.P., Cliver, D.O., 2000, First findings of *Cryptosporidium* and *Giardia*
 538 in California sea lions (*Zalophus californianus*). J Parasitol 86, 490-494.
 539
 540 Dixon, B.R., Parrington, L.J., Parenteau, M., Leclair, D., Santin, M., Fayer, R., 2008, *Giardia*
 541 *duodenalis* and *Cryptosporidium* spp. in the intestinal contents of ringed seals (*Phoca hispida*)
 542 and bearded seals (*Erignathus barbatus*) in Nunavik, Quebec, Canada. J Parasitol 94, 1161-1163.
 543
 544 EPA 2005. Microbial source tracking guide document. EPA/600-R-05-064.
 545
 546 EPA 2006. Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface
 547 water treatment rule. EPA 815-R-06-007.

548

549 EPA 2012. Recreational water quality criteria. Office of Water 820-F-12-058.

550

551 Ernest, J.A., Blagburn, B.L., Lindsay, D.S., Current, W.L., 1986, Infection dynamics of

552 *Cryptosporidium parvum* (Apicomplexa: Cryptosporiidae) in neonatal mice (*Mus musculus*). J

553 Parasitol 72, 796-798.

554

555 Fayer, R., Dubey, J.P., Lindsay, D.S., 2004, Zoonotic protozoa: from land to sea. Trends

556 Parasitol 20, 531-536.

557

558 Fayer, R, Xiao, L. (2008). *Cryptosporidium* and Cryptosporidiosis. 2nd edition. Taylor & Francis

559 Group, Boca Raton, Florida.

560

561 Field, K.G., Samadpour, M., 2007, Fecal source tracking, the indicator paradigm, and managing

562 water quality. Water Res 41, 3517-3538.

563

564 Finch, G.R., Black, E.K., Labatiuk, C.W., Gyurek, L., Belosevic, M., 1993, Comparison of

565 *Giardia lamblia* and *Giardia muris* cyst inactivation by ozone. Appl Environ Microbiol 59,

566 3674-3680.

567 Gaydos, J.K., Miller W.A., Gilardi, K.V., Melli, A., Schwantje, H., Engelstoft, C., Fritz, H.,

568 Conrad, P.A., 2007, *Cryptosporidium* and *Giardia* in marine-foraging river otters (*Lontra*

569 *canadensis*) from the Puget Sound Georgia Basin ecosystem. J Parasitol 93, 198-202.

570 Gaydos, J.K., Miller, W.A., Johnson, C., Zornetzer, H., Melli, A., Packham, A., Jeffries, S.J.,
 571 Lance, M.M., Conrad, P.A., 2008, Novel and canine genotypes of *Giardia duodenalis* in harbor
 572 seals (*Phocavitulina richardsi*). J Parasitol 94, 1264-1268.
 573
 574 Haas C.N., Rose J.B., Gerba C.P., 1999. Quantitative Microbial Risk Assessment. New York,
 575 John Wiley & Sons.
 576
 577 Haas, C.N., 2002, Conditional dose-response relationships for microorganisms: Development
 578 and application. Risk Anal 22, 455-463.
 579
 580 Hogan, J.N., Daniels, M.E., Watson, F.G., Oates, S.C., Miller, M.A., Conrad, P.A., Shapiro, K.,
 581 Hardin, D., Dominik, C., Melli, A., Jessup, D.A., Miller, W.A., 2013, Hydrologic and Vegetative
 582 Removal of *Cryptosporidium parvum*, *Giardia lamblia*, and *Toxoplasma gondii* Surrogate
 583 Microspheres in Coastal Wetlands. Appl Environ Microbiol 79, 1859-1865.
 584
 585 Jessup, D., Miller, M., Ames, J., Harris, M., Kreuder, C., Conrad, P., Mazet, J., 2004, Southern
 586 sea otters as a sentinel for marine ecosystem health. EcoHealth 1, 239e245.
 587
 588 Kildare, B.J., Leutenegger, C.M, McSwain, B.S., Bambic, D.G., Rajal, V.B., Wuertz, S., 2007,
 589 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-
 590 specific fecal Bacteroidales: A Bayesian approach. Water Research 41, 3701-3715.
 591

592 Kreuder, C., Miller, M.A., Jessup, D.A., Lowenstine, L.J., Harris, M.D., Ames, J.A., Carpenter,
 593 T.E., Conrad, P.A., Mazet, J.A., 2003, Patterns of mortality in southern sea otters
 594 (*Enhydra lutris nereis*) from 1998-2001. J Wildl Dis 39, 495-509.
 595
 596 Kundu, A., McBride, G., Wuertz, S., 2013, Adenovirus-associated health risks for recreational
 597 activities in a multi-use coastal watershed based on site-specific quantitative microbial risk
 598 assessment. Water Research 47, 6309-6325.
 599
 600 Li, D., Craik, S.A., Smith, D.W., Belosevic, M., 2007, Comparison of levels of inactivation of
 601 two isolates of *Giardia lamblia* cysts by UV light. Appl Environ Microbiol 73, 2218-2223
 602
 603 Mac Kenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A., Peterson, D.E.,
 604 Kazmierczak, J.J., Addiss, D.G., Fox, K.R., Rose, J.B., et al., 1994, A massive outbreak in
 605 Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. N Engl J
 606 Med 331, 161-167.
 607
 608 McBride, G.B., 2005, Using statistical methods for water quality management. Issues, problems
 609 and solutions. John Wiley & Sons, Hoboken, New Jersey.
 610
 611 Méndez-Hermida F., Gómez-Couso H., Romero-Suances R., Ares-Mazás E. *Cryptosporidium*
 612 and *Giardia* in wild otters (*Lutra lutra*). Vet Parasitol. 2007 Mar 15;144(1-2):153-6.
 613

614 Miller, M.A., Gardner, I.A., Kreuder, C., Paradies, D.M., Worcester, K.R., Jessup, D.A., Dodd,
615 E., Harris, M.D., Ames, J.A., Packham, A.E., Conrad, P.A., 2002, Coastal freshwater runoff is a
616 risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). Int J
617 Parasitol 32, 997-1006.

618

619 Miller, M.A., Byrne, B.A., Jang, S.S., Dodd, E.M., Dorfmeier, E., Harris, M.D., Ames, J.,
620 Paradies, D., Worcester, K., Jessup, D.A., Miller, W.A., 2010, Enteric bacterial pathogen
621 detection in southern sea otters (*Enhydra lutris nereis*) is associated with coastal urbanization and
622 freshwater runoff. Vet Res 41, 1.

623

624 Miller, W.A., Miller, M.A., Gardner, I.A., Atwill, E.R., Byrne, B.A., Jang, S., Harris, M., Ames,
625 J., Jessup, D., Paradies, D., Worcester, K., Melli, A., Conrad, P.A., 2006, *Salmonella* spp., *Vibrio*
626 spp., *Clostridium perfringens*, and *Plesiomonas shigelloides* in marine and freshwater
627 invertebrates from coastal California ecosystems. MicrobEcol 52, 198-206.

628

629 Miller, W.A., Lewis, D.J., Lennox, M., Pereira, M.G., Tate, K.W., Conrad, P.A., Atwill, E.R.,
630 2007, Climate and on-farm risk factors associated with *Giardia duodenalis* cysts in storm runoff
631 from California coastal dairies. Appl Environ Microbiol 73, 6972-6979.

632

633 Navin, T.R., Juranek, D.D., 1984, Cryptosporidiosis: clinical, epidemiologic, and parasitologic
634 review. Rev Infect Dis 6, 313-327.

635

636 Oates, S.C., Miller, M.A., Hardin, D., Conrad, P.A., Melli, A., Jessup, D.A., Dominik, C., Roug,
 637 A., Tinker, M.T., Miller, W.A., 2012, Prevalence, environmental loading, and molecular
 638 characterization of *Cryptosporidium* and *Giardia* isolates from domestic and wild animals along
 639 the Central California Coast. Appl Environ Microbiol 78, 8762-8772.
 640
 641 Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R., DuPont, H.L., 1999, Virulence of
 642 three distinct *Cryptosporidium parvum* isolates for healthy adults. J Infect Dis 180, 1275-1281.
 643
 644 Palisade Corporation, 2013. Risk analysis and simulation add-in for microsoft excel. pp. 299,
 645 610. Accessed May 17, 2013 at
 646 http://www.palisade.com/downloads/manuals/612/EN/RISK6_EN.pdf
 647
 648 Petterson, S.R., Signor, R.S., Ashbolt, N.J., 2007, Incorporating method recovery uncertainties in
 649 stochastic estimates of raw water protozoan concentrations for QMRA. J Water Health 5 Suppl
 650 1, 51-65.
 651
 652 Pouillot, R., Beaudreau, P., Denis, J.B., Derouin, F., 2004, A quantitative risk assessment of
 653 waterborne cryptosporidiosis in France using second-order Monte Carlo simulation. Risk Anal
 654 24, 1-17.
 655
 656 Raith, M.R., Kelty, C.A., Griffith, J.F., Schriewer, A., Wuertz, S., Mieszkina, S., Gourmelon, M.,
 657 Reischer, G.H., Farnleitner, A.H., Ervin, J.S., Holden, P.A., Ebentier, D.L., Jay, J.A., Wang, D.,
 658 Boehm, A.B., Aw, T.G., Rose, J.B., Balleste, E.W., Meijer, G., Sivaganesan, M., Shanks, O.

2013, Comparison of PCR and quantitative real-time PCR methods for the characterization of ruminant and cattle fecal pollution sources. *Water Res.* 47, 6921-6928.

Rendtorff, R.C., 1954, The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *Am J Hyg* 59, 209-220.

Regli, S., Rose, J.B., Haas, C.N., Gerba, C.P., 1991, Modeling the risk from *Giardia* and viruses in drinking water. *Am Water Works Assoc* 83, 76-84.

Rose, J.B. and Gerba C.P., 1991. Use of risk assessment for development of microbial standards. *Water Sci. Tech.* 24(2):29–34. In: World Health Organization, 2012, *Animal Waste, Water Quality and Human Health*. Edited by Dufour, A., Bartram, J., Bos., R., Gannon, V., Chapter 10, p. 393. Accessed June 22, 2016 at http://www.who.int/water_sanitation_health/publications/2012/ch10.pdf.

Rose, J.B., Haas, C.N., Regli, S., 1991, Risk assessment and control of waterborne giardiasis. *Am J Public Health* 81, 709-713.

Sattar, S.A., Chauret, C., Springthorpe, V.S., Battigellu, D., Abbaszadegan, M., LeChevallier, M., 1999, *Giardia* cysts and *Cryptosporidium* oocysts survival in watersheds and factors affecting inactivation. AWWA Research foundation and American Water Works Association, Ontario, Canada.

682 Schriewer, A., Miller, W.A., Byrne, B.A., Miller, M.A., Oates, S., Conrad, P.A., Hardin, D.,
 683 Yang, H.H., Chouicha, N., Melli, A., Jessup, D., Dominik, C., Wuertz, S., 2010, Presence of
 684 *Bacteroidales* as a predictor of pathogens in surface waters of the central California coast. Appl
 685 Environ Microbiol 76, 5802-5814.
 686
 687 Silkie, S., Nelson, K.L., 2009, Concentrations of host-specific and generic fecal markers
 688 measured by quantitative PCR in raw sewage and fresh animal feces. Water Research 43, 4860-
 689 4871.
 690
 691 Shapiro, K., Conrad, P.A., Mazet, J.A., Wallender, W.W., Miller, W.A., Largier, J.L., 2010,
 692 Effect of estuarine wetland degradation on transport of *Toxoplasma gondii* surrogates from land
 693 to sea. Appl Environ Microbiol 76, 6821-6828.
 694
 695 Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J.E., Ashbolt, N.J., 2010, Estimated human
 696 health risks from exposure to recreational waters impacted by human and non-human sources of
 697 faecal contamination. Water Res 44, 4674-4691.
 698
 699 Teunis, P. F. M., 2009. Uncertainty in dose response from the perspective of microbial risk.
 700 Chapter 6 in R. M. Cooke (ed.), Uncertainty Modeling in Dose Response. Wiley, Hoboken, NJ.
 701 In: World Health Organization, 2012, Animal Waste, Water Quality and Human Health. Edited
 702 by Dufour, A., Bartram, J., Bos., R., Gannon, V., Chapter 10, p. 393. Accessed June 22, 2016 at
 703 http://www.who.int/water_sanitation_health/publications/2012/ch10.pdf.
 704

705 Teunis, P.F.M., Havelaar, A.H., 2002, Risk assessment for protozoan parasites. International
 706 Biodeterioration& Biodegradation 50, 185-193.
 707
 708 Thomas, N.J., Cole, R. (1996a). The risk of disease and threats to the wild population.
 709 Endangered Species Update Special Issue: conservation and management of the southern sea
 710 otter, Endangered Species Update 13, 23-27.
 711
 712 Thomas, N.J., Cole, R. 1996b. Biology and status of the southern sea otter: the risk of disease
 713 and threats to the wild population. Endangered Species Update 13: 23-27.
 714
 715 U.S. Geological Survey Web site. 2014 Slowly Swimming Towards Recovery, California's Sea
 716 Otter Numbers Holding Steady. Available at:
 717 [http://www.usgs.gov/blogs/features/usgs_top_story/slowly-swimming-towards-recovery-](http://www.usgs.gov/blogs/features/usgs_top_story/slowly-swimming-towards-recovery-californias-sea-otter-numbers-holding-steady/)
 718 [californias-sea-otter-numbers-holding-steady/](http://www.usgs.gov/blogs/features/usgs_top_story/slowly-swimming-towards-recovery-californias-sea-otter-numbers-holding-steady/). Accessed Mar 1, 2015.
 719
 720 Xiao, L., Fayer, R., Ryan, U., Upton, S.J., 2004, *Cryptosporidium* taxonomy: recent advances
 721 and implications for public health. ClinMicrobiol Rev 17, 72-97.
 722
 723 Wang, D., Silkie, S.S., Nelson, K.L., Wuertz, S., 2010, Estimating true human and animal host
 724 source contribution in quantitative microbial source tracking using the Monte Carlo method.
 725 Water Res 44, 4760-4775.
 726

727 Wuertz, S., Wang, D., Reischer G., Farnleitner, A. 2011a. Library-independent bacterial source
728 tracking methods, In *Microbial Source Tracking: Methods, Applications, and Case Studies*,
729 Hagedorn, C., Blanch, A., Harwood, V. J. (Eds.), Springer New York, Dordrecht, Heidelberg,
730 London, pp. 75-126.
731
732 Wuertz, S., Miller, W., Bambic, D., McBride, G., 2011b. Quantification of Pathogens and
733 Sources of Microbial Indicators for QMRA in Recreational Waters. IWA Publishing, ISBN
734 9781843395430, p. 200

- 1 Table 1. Mean recovery and detection limits (DL) of spiked *Cryptosporidium* oocysts and
- 2 *Giardia* cysts from water samples collected in coastal central California.

Water Matrix	Spike	Mean recovery		DL	
		# Oocysts	# Cysts	# Oocysts	# Cysts
River water	200	23	30	9	7
Stormwater	200	7	6	29	34
Wastewater effluent	200	63	23	4	13

- 3
- 4

- 1 Table 2. Percent recovery of spiked protozoal oocysts and cysts from river waters in the
- 2 Monterey Bay area of California.

Site	Collection Date	Recovery %	
		<i>Cryptosporidium</i>	<i>Giardia</i>
Waddell Creek	5/22/2007	2%	1%
Scott Creek	6/11/2009	26%	15%
San Lorenzo River	7/9/2007	8%	0%
Watsonville Slough	9/4/2007	18%	30%
Salinas River	12/4/2007	5%	30%
Mean		12%	15%

3

1 Table 3. Percent recovery of spiked protozoal oocysts and cysts from wastewater effluent water
 2 in the central coast of California.

Collection Date	Recovery %	
	<i>Cryptosporidium</i>	<i>Giardia</i>
3/1/2011	8%	18%
3/1/2011	18%	4%
3/1/2011	51%	5%
3/7/2011	65%	14%
3/2/2011	17%	14%
3/2/2011	16%	1%
3/2/2011	5%	2%
3/8/2011	36%	13%
3/3/2011	21%	4%
3/3/2011	43%	6%
3/3/2011	12%	2%
3/9/2011	87%	15%
3/1/2011	8%	18%
Mean Recovery	31%	8%

3

Table 4. Probability of illness in humans as a result of swimming in water contaminated with *Cryptosporidium* and *Giardia* spp.

Matrix	Season ^a	Scenario ^b	Probability of illness (%)			
			<i>Cryptosporidium</i>		<i>Giardia</i>	
			Adult	Children	Adult	Children
River	Wet	1	0.1	0.3	0.01	0.03
		2	ND ^c	ND ^c	ND ^c	ND ^c
	Dry	1	0.01	0.02	0.01	0.01
		2	ND ^c	ND ^c	ND ^c	ND ^c
Stormwater	Wet	1	0.05	0.1	0.1	0.1
		2	ND ^c	ND ^c	ND ^c	ND ^c
	Dry	1	2.6	5	0.03	0.1
		2	0.2	0.2	ND ^c	ND ^c
Wastewater effluent	Wet	1	0.02	0.1	1.3	2.6
		2	ND ^c	ND ^c	ND ^c	0.1
		3	ND ^c	ND ^c	ND ^c	0.003
	Dry	1	0.1	0.1	1.3	2.5
		2	ND ^c	ND ^c	ND ^c	0.1
		3	ND ^c	ND ^c	ND ^c	0.003

^aWet season was categorized from December to May and dry season was categorized from June to November.

^bScenario 1: Swimming in water assuming that oocysts and cyst are 100% viable and at the end of point of the contaminated water discharge; Scenario 2: Swimming in water assuming that oocysts and cyst are 100% viable and at a dilution factor of 1:30 from the contaminated water discharge; and Scenario 3: Swimming in water assuming that oocysts and cyst are 0.1% viable and at the end of point of the contaminated water discharge.

^c Not done as the probability of illness is below the EPA (2009) recommendations of 3% illness.

Table 5. Probability of infection in sea otters as a result of swimming in water contaminated with *Cryptosporidium* and *Giardia* spp.

Matrix	Season ^a	Scenario ^b	Probability of infection (%)			
			<i>Cryptosporidium</i>		<i>Giardia</i>	
			<i>ID</i> 50 = 363	<i>ID</i> 50 = 35	<i>ID</i> 50 = 86	<i>ID</i> 50 = 35
River	Wet	1	0.3	2	0.03	0.2
		2	ND ^c	0.1	ND ^c	ND ^c
	Dry	1	0.02	0.2	0.01	0.04
		2	ND ^c	ND ^c	ND ^c	ND ^c
Stormwater	Wet	1	0.1	1	0.5	1
		2	ND ^c	0.04	ND ^c	0.04
	Dry	1	2	16	0.3	0.8
		2	0.1	0.7	ND ^c	ND ^c
Wastewater effluent	Wet	1	0.1	0.5	11	23
		2	ND ^c	ND ^c	0.5	1
		3	ND ^c	ND ^c	0.01	0.03
	Dry	1	0.2	1	11	23
		2	ND ^c	0.1	0.4	1
		3	ND ^c	0.002	0.01	0.03

^aWet season was categorized from December to May and dry season was categorized from June to November

6 ^bScenario 1: Swimming in water assuming that oocysts and cyst are 100% viable and at the end
7 of point of the contaminated water discharge; Scenario 2: Swimming in water assuming that
8 oocysts and cyst are 100% viable and at a dilution factor of 1:30 from the contaminated water
9 discharge; and Scenario 3: Swimming in water assuming that oocysts and cyst are 0.1% viable
10 and at the end of point of the contaminated water discharge.
11 ^c Not done as the probability of infection lower than 1% of infection

Table 6. Median Spearman's rank order correlation (r_s) resulting from the sensitivity analysis of variables affecting predicted *Cryptosporidium* and *Giardia* doses scenarios

Variable	Median Spearman Rank Correlation (r_s)	
	<i>Cryptosporidium</i> Scenarios	<i>Giardia</i> Scenarios
Protozoa concentration ^a	0.4	0.55
<i>Bacteroidales</i> ^b	0.4	0.48
Water ingested while swimming ^c	0.29	0.31
Swimming duration for humans ^d	0.29	0.38
Sea otter body weight ^e	0.12	0.17

^a Oocysts or cysts/10 L

^b % of *Bacteroidales* markers detected in the river samples

^c mL/h for humans and mL/kg for sea otters

^d hours

^e kg

Figure 1. Locations of sampling sites along the central California coast, with (A) river water collection sites and (B) stormwater collection sites. Map created using Quantum GIS version (QGIS) 1.8-Lisboa open source software (<http://qgis.osgeo.org>).

*DFA: Direct Immune Fluorescence, IMS: immune magnetic separation

Figure 2. Schematic representation of each step of the QMRA model for *Cryptosporidium* oocyst and *Giardia* cyst infection.

Figure 3. Monte Carlo simulation results for children swimming in stormwater during the dry season, assuming that *Cryptosporidium* oocysts are 100% viable and at the end of point of the contaminated water discharge (Scenario 1).

Figure 4. Monte Carlo simulation results for sea otter swimming in stormwater during the dry season (Scenario 1); assuming that *Cryptosporidium* oocysts are 100% viable at the end of point of the contaminated water discharge and sea otters are as susceptible to *Cryptosporidium* infection as humans

Figure 5. Monte Carlo simulation results for Scenario 1; (A) Sea otter swimming in wastewater during the wet season, assuming that *Giardia* cysts are 100% viable, at the end of point of the contaminated water discharge and sea otters are as susceptible to *Giardia* infection as humans; (B) Sea otter swimming in

wastewater during the dry season, assuming that *Giardia* cysts are 100% viable, at the end of point of the contaminated water discharge and sea otters are as susceptible to *Giardia* infection as humans.