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Effects of Time and Watershed Characteristics on the Concentration of *Cryptosporidium* Oocysts in River Water

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Water samples were collected from four locations on two rivers in Washington State and analyzed by membrane filtration-immunofluorescence assay to establish *Cryptosporidium* oocyst concentrations. Sampling locations were selected to evaluate effects of watershed character, from pristine mountain to downstream agricultural, on oocyst concentrations. Samples were collected at six biweekly intervals from late June to early September, with two additional sets of five samples taken on separate days (one set taken at bihourly intervals and one set taken simultaneously). *Cryptosporidium* oocysts were found in 34 of 35 samples at concentrations ranging from about 0.2 to 65 oocysts per liter. Oocyst concentrations were highest early in the sampling period, when they were influenced by postrainfall runoff, and decreased through the summer months. Oocyst concentrations found in ten samples collected on two days (5 samples per day) showed no short-term variations. Oocyst concentrations and oocyst production per square mile (ca. 2.6 km^2) of watershed found in water draining a controlled public water supply watershed were the lowest observed. The concentrations and production rates for drainage from an adjacent, comparable, but uncontrolled watershed were nearly 10 times higher. The concentration and production rates of the downstream area influenced by dairy farming were nearly 10 times higher than rates at the upstream stations. The data showed clearly that oocyst concentrations were consistently observed above the detection limit of the analytical method, about 0.1 oocysts per liter; that oocyst concentrations were continuous as opposed to intermittent; and that watershed character and management affected surface water oocyst concentrations significantly.

Organisms of the genus *Cryptosporidium* are pathogenic agents of diarrhea in both immunogenically intact and immunocompromised humans (10, 14, 19). *Cryptosporidium*-caused respiratory infections have also been reported in humans (13). Outbreaks of cryptosporidiosis have been reported sporadically in day-care centers (2), and community outbreaks attributed to public water supply have recently been reported in both the United States (6, 8) and Europe (3, 21, 22). The disease is normally self-limiting in immunocompetent individuals, but it is potentially life threatening in immunocompromised individuals such as patients who have had organ transplants or cancer chemotherapy or who have the acquired immunodeficiency syndrome (5).

Cryptosporidium sp. has numerous animal hosts, including nearly 40 species of domestic and wild animals (1). The most common species of *Cryptosporidium* to infect mammals is *C. parvum*, which has broad cross-infectivity among many host species (25). Although cryptosporidiosis among humans originally appeared to result only from direct contact with infected animals, it is clear that *Cryptosporidium* can also be transmitted by interpersonal contacts and through water. Analysis of river water samples from the various parts of the United States shows that *Cryptosporidium* sp. should be considered a waterborne organism (7, 11, 12, 16, 20). The *Cryptosporidium* life cycle includes complex sexual and asexual reproductive phases in the intestines of host animals and the formation of environmentally hardy and infectious oocysts that are excreted in the feces (4). These oocysts have been found to be resistant to common chemical disinfectants (18) and require appropriate attention for protection of public water supplies.

This study was undertaken because of the importance of understanding the relation between oocyst concentrations in water and the risk of infection and because a rational approach to risk management, including water quality planning and the design of treatment facilities, is needed. Previous studies have established the presence of *Cryptosporidium* oocysts in rivers in the northwestern United States and California (16), in the Southwest and the Midwest (11, 20), and on the East Coast (8). Thus, the organism should be considered ubiquitous in surface waters.

This work was designed to answer the following questions: At what concentration can *Cryptosporidium* oocysts be found in surface water? Are oocysts present in surface water continuously or intermittently? To what degree do watershed activities and management practices affect oocyst concentrations? The approach used included (i) use of a small-volume membrane filtration-immunofluorescence assay procedure for oocyst recovery; (ii) selection of sampling stations on two rivers that permitted comparison between watersheds and use of three stations along one river to permit comparisons of the effects of a range of watershed activities, from lower human impact upstream to agricultural-runoff effects downstream; and (iii) sampling over long (biweekly) and short (bihourly and simultaneous) time scales in effort to identify potentially intermittent effects.

MATERIALS AND METHODS

Sample collection. River water samples ranging from 5 to 20 gal (ca. 19 to 76 liters) were collected from three sites along the Snoqualmie River and one location on the Cedar River in the central Cascade Mountains of western Wash-

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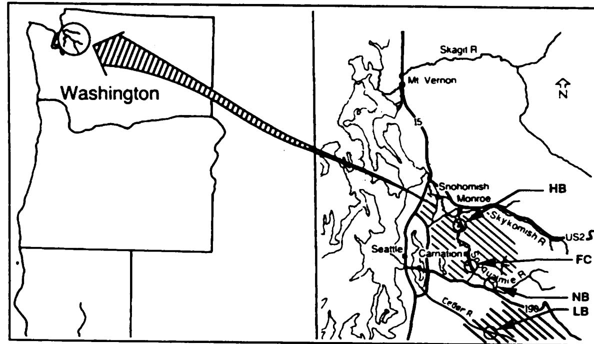

FIG. 1. Sites in Washington State on the Snoqualmie River (NB, FC, HB) and the Cedar River (LB) at which water samples were collected for analysis of *Cryptosporidium* oocyst concentrations.

ington State between 28 June and 2 September 1990 (Fig. 1). The station farthest upstream was located 3 mi (1 mi = 1.609 km) above the community of North Bend (NB) on the Middle Fork of the Snoqualmie River. The watershed above this site was mountainous private and public forests used heavily for logging and recreation. The second station was located about 15 mi downstream at a point about 1 mi downstream from the community of Fall City (FC) on the Snoqualmie River. The North Fork and South Fork of the Snoqualmie River join the Middle Fork between the NB and FC stations. Watershed character tributary to FC is predominantly mountainous forest of the large, relatively remote North Fork drainage. However, runoff from the communities of NB, Snoqualmie (of Twin Peaks fame), and FC and drainage from scattered rural homes enter the river in this reach. The third station was located 20 mi farther downstream, at High Bridge (HB) on the Snoqualmie River 8 mi north of the town of Carnation. Between FC and HB, the Snoqualmie River traverses an agricultural valley about 20 mi long and from 1 to 4 mi wide. Dairy farming is the predominant activity, with over a dozen farms and between 1,000 and 2,000 head of cattle in the area, according to the local U.S. Department of Agriculture agricultural extension agent. Drainage from the communities of Carnation and Duval also enter the river in this reach. A single 20-gal sample was obtained from the South Fork of Snoqualmie 3 mi upstream from NB.

A fourth sampling station was located at the site of the Seattle Water Department municipal water supply intake on the Cedar River at Landsburg (LB). The watershed of the Cedar River tributary to LB is mountainous forestland physically much like the drainages of the North Fork and Middle Fork of the Snoqualmie River. However, the entire 120-mi^2 watershed is owned or controlled through written agreement by the City of Seattle's Water Department. No unsupervised human activity is permitted. Human activities are limited to commercial timber cutting and watershed management, with sanitation carefully controlled. This station served as a convenient reference because of previously published data on *Giardia* spp. (15) and data on coliform bacteria (see Fig. 4.2 in reference 23).

During the 3-month study, weekly to biweekly samples of river water were collected. One set of consecutive bihourly samples was collected from the upper station on the Snoqualmie River over a period of 8 h to examine short-term concentration variations. On a different date at that station, five samples were collected at the same time to assess random variation between individual samples. At each location, samples were obtained at 1 to 3 m from the river bank, where the water was more than 1 m deep. Care was taken during the sampling process to avoid sampling material floating on the surface and to avoid disturbing bottom sediments. Samples were refrigerated within 4 h of collection and processed within 48 h of collection.

Sample processing. Processing for oocyst identification and counting closely followed the methods developed by Ongerth and Stibbs (15, 16). Sample volumes ranging from 5 to 20 gal were used. Larger volumes were used initially until establishment of the oocyst concentration range permitted use of smaller samples. Samples were processed in the following order, which was established by expected ascending concentrations: first, a negative control, consisting of 5 gal of distilled water; then, the river water samples (from LB, NB, FC, and HB); and finally, a positive control. The positive controls were prepared by seeding 5 gal of distilled and prefiltered water with *Cryptosporidium* oocysts counted to produce a concentration in the range of 40 to 160 oocysts per liter (Tables 1 and 2). The positive controls were used to estimate the recovery efficiency of oocysts for each batch of samples processed. As previously reported, the recovery efficiencies measured for seeded distilled water and for seeded river water were comparable for the low-turbidity (<1.5 nephelometric turbidity units) rivers in this study (15). Between samples from the individual stations, the filter apparatus was rinsed with distilled water and wiped dry. After the positive control was run, the filter apparatus was washed thoroughly and autoclaved.

Each sample was initially filtered through a 293-mm-diameter polycarbonate membrane with a pore size of 2 μm (Nuclepore, Pleasanton, Calif.) under a vacuum regulated to

TABLE 1. Numbers of *Cryptosporidium* oocysts found by immunofluorescence assay in samples from three sites on the Snoqualmie River and one on the Cedar River in Washington State

Date (mo/day/yr)	No. of oocysts/vol (gal) of sample (concn/liter) at:				Positive control (no./vol [gal])	% Recovery
	NB[a]	FC[a]	HB[a]	LB[b]		
6/28/90	118/15 (6.97)	61/15 (3.6)	1,075/15 (63.5)		31/15	29.8
7/2/90	22/20 (0.84)	14/20 (0.54)	341/20 (13.3)	11/20 (0.42)	763/5	34.3
7/17/90	9/20 (0.46)	21/20 (1.09)	176/10 (18.3)	3/20 (0.15)		25.3
8/2/90	17/20 (1.21)	8/20 (0.56)	47/10 (6.68)	4/20 (0.28)	214/5	18.6
8/7/90	32/20 (1.88)	16/20 (0.94)	38/10 (4.46)	0/20 (0)	318/5	22.5
8/12/90	24/10 (2.93)	11/10 (1.35)	27/10 (3.3)	3/20 (0.18)	421/5	21.6
8/13/90				4/20 (0.24)		

[a] On the Snoqualmie River.
[b] On the Cedar River.

TABLE 2. Numbers of *Cryptosporidium* oocysts found by immunofluorescence assay in samples from NB on the Snoqualmie River

Date (mo/day/yr) and time	Oocysts		% Recovery
	No./vol (gal)	Concn/liter	
8/19/90	790/5		26.9
1430	33/10	3.2	
1630	28/10	2.6	
1830	34/10	2.7	
2030	26/10	2.55	
2230	24/10	2.36	
9/2/90	811/5		29.7
1630	18/10	1.6	
1630	22/10	1.96	
1630	15/10	1.33	
1630	25/10	2.22	
1630	13/10	1.16	

less than 20 in. (1 in. = 2.54 cm) of Hg. Following filtration, the filtrate was discarded and the polycarbonate membrane was placed on a glass plate (35 by 35 cm). The glass plate with the filter on it was positioned on its edge in a rectangularly shaped plastic trough (kitchen drawer organizer; Rubbermaid). The surface of the filter was then rinsed by using a squeeze bottle of distilled and prefiltered water, and the rinse water was collected in the plastic trough. A small rubber squeegee, 4 cm wide, was used to clean the filter surface. The filter was rinsed and squeegeed three times. A total rinse volume of about 100 ml of water was used for each filter. The liquid containing the recovered particles was transferred to 50-ml conical polypropylene centrifuge tubes. The water with particles was centrifuged at 650 × *g* for 15 min and then decanted to 10% of the original volume. Particles in the size and density range of oocysts were further concentrated by resuspending the centrifuged material, layering it on a Percoll density gradient (specific gravity, 1.05 and 1.09) (Sigma Chemical Co., St. Louis, Mo.) in 15-ml centrifuge tubes, and centrifuging it at 650 × *g* for 15 min. Particles at or immediately below the interface of the two density layers were harvested by withdrawing 3 to 4 ml by Pasteur pipette. The recovered particles were deposited on a 13-mm-diameter polycarbonate filter with a 2-μm pore size in an in-line filter holder and rinsed three times with distilled water.

The immunofluorescence assay procedure was applied to the particles on the 13-mm-diameter filter surface while the particles were in the filter holder. Rabbit antiserum to *Cryptosporidium* spp. prepared as described by Stibbs and Ongerth (24) was used. The antiserum was diluted 1:40 with 0.0175 M phosphate-buffered saline (PBS), pH 7.4, containing 0.1% bovine serum albumin (Sigma). A micropipette was used to fill the inlet chamber in the filter holder with 0.3 ml of rabbit antiserum, and then the chamber was sealed. The filter was then incubated at 37°C for 50 min. Following incubation, the serum was expelled with a syringe and the filter was rinsed three times with 10-ml volumes of PBS. The final volume of PBS was expelled from the filter holder. Next, 0.3 ml of fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin G (Cooper Biochemical Inc., Malvern, Pa.) diluted 1:80 with PBS was introduced into the inlet chamber. The filter holder was sealed and again incubated at 37°C for 50 min. After this second incubation, the filter was washed with PBS three times as described above. Counterstaining was done by introducing 0.3 ml of cold ethidium bromide (0.1% solution) into the inlet chamber, incubating the mixture at 5°C for 5 min, and then giving it a third and final rinse (three times) with 10 ml of PBS. After being stained, the filters were removed from the filter holder and mounted under 20-mm^2 coverslips with elvanol permanent resin mounting medium (9).

A duplicate of one sample (HB, 8/12/90) was split. The immunofluorescence assay procedure was performed on one half by using the procedure described above, and the other half was stained with a commercial murine anti-*Cryptosporidium* monoclonal antibody that does not cross-react with the avian *C. baileyi* (Meridian Diagnostics Inc., Cincinnati, Ohio). The results were comparable in terms of stain color, character, and number of oocysts found on the two filters (12 and 15, respectively).

Oocyst identification and counting. Filters were coded and selected for examination at random to avoid bias in counting. They were examined using epifluorescence microscopy at a magnification of ×400. Oocysts were identified by the following criteria: size, shape, surface features, and stain color (fluorescent apple green). The normal diameter of oocysts ranges from 3 to 5 μm. Oocysts were approximately spherical, although often slightly irregular. Some appeared dented or flattened, and some, having excysted, appeared split open with a pie slice-shaped piece missing. Surface wrinkles or lines characteristically appeared on oocysts. The total surface area of each mounted filter was examined, and each particle fitting the above-described criteria was counted as one oocyst. Candidate objects were reviewed independently by at least one other trained technician experienced with identification of *Cryptosporidium* spp.

Analyses and calculations. The number of oocysts observed in microscopic examination of the entire area of each 13-mm-diameter filter was recorded as number of oocysts per volume of sample. Oocyst concentrations were then calculated from the number found in each sample, the sample volume, and the oocyst recovery efficiency as measured by the positive control run with each batch of samples: oocyst concentration (oocyst/liter) = 100 × [(oocysts counted)/(liters of sample × % recovery efficiency)]. Oocyst production from each sampling station's watershed was calculated in terms of oocysts per mi^2 per day (see Table 5). Data on precipitation, watershed size, and river flow rates were supplied by the U.S. Department of the Interior (see Tables 3 and 4).

RESULTS

In this survey of *Cryptosporidium* oocyst concentrations at five locations on the Snoqualmie and Cedar rivers in forest and farming areas 50 mi east of Seattle in the State of Washington, oocysts were found in 34 of 35 samples collected. Oocyst numbers ranged from 3 to 1,075, corresponding to concentrations ranging from 0.15 to 63.5/liter in samples of from 10 to 20 gal (Table 1). The recovery efficiency of the sampling and analysis procedure ranged from 18.6 to 34.3% (average = 26.2%; standard deviation = 5.5%) (Table 1). Oocyst concentrations decreased over the sampling period from late June to early September (Fig. 2a). Sampling followed a period of moderate precipitation, including a total of 2 in. in May and 3 in. in the first 2 weeks of June. During the sampling period, precipitation included 0.57 in. in the first week of July and 0.61 in. in the third week of August (Table 4). The trend in rainfall was accompanied by decreasing river flow rates (Table 3 and Fig. 2b).

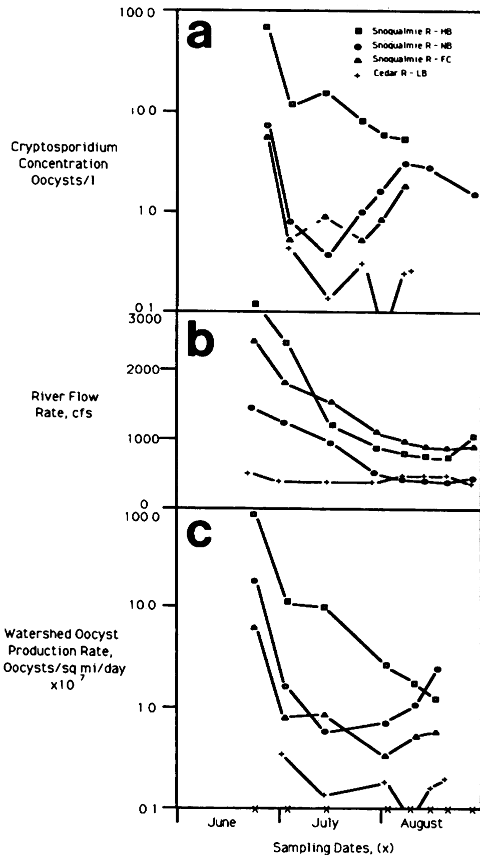

FIG. 2. *Cryptosporidium* oocyst concentration (a), river flow rate (b), and watershed oocyst production rate (c) for eight sampling dates from June to August 1990. cfs, cubic feet per second.

The oocyst concentrations observed at the upper two stations (NB and FC) on the Snoqualmie River were similar, averaging 2.4 and 1.1/liter, respectively (Table 1). The concentration at the downstream station (HB), averaging 18.2/liter, was approximately 10 times higher than concentrations at the two upstream stations. The average oocyst concentration in the Cedar River at LB, 0.2/liter, was nearly 10 times lower than concentrations at the upper two stations on the Snoqualmie River. The average oocyst concentration observed on the Snoqualmie River at HB, 18.3/liter, was nearly 100 times the average concentration observed on the Cedar River at LB, which was 0.2/liter (Table 1).

Distributions of oocyst concentrations were typically skewed, appearing approximately log normal (Fig. 3). The variations in oocyst concentrations at each station, as indicated by the slope of each distribution, were similar and resemble those reported previously for *Giardia* cyst and coliform bacterium concentrations on the Cedar River (15, 23).

TABLE 3. Daily average flow rate of the Snoqualmie and Cedar rivers in Washington State at stations sampled for *Cryptosporidium* oocysts[a]

Date (mo/day/yr)	Flow rate (ft^{3b}/s) at:			
	NB[c]	FC[c]	HB[c]	LB[d]
6/28/90	1,590	2,590	3,250	479
7/2/90	1,230	2,080	2,640	442
7/17/90	792	1,720	1,300	405
8/2/90	394	1,030	944	345
8/7/90	341	908	821	393
8/12/90	298	814	618	458
8/13/90	272	587	819	431
9/2/90	440	899	1,220	335

[a] Data are from reference 27.
[b] 1 ft = 30.48 cm.
[c] On the Snoqualmie River.
[d] On the Cedar River.

TABLE 4. Total recorded rainfall for sampling day and preceding day during *Cryptosporidium* oocyst analysis on the Snoqualmie and Cedar rivers[a]

Date (mo/day/yr)	Precipitation (in.)	Date (mo/day/yr)	Precipitation (in.)
6/27/90	tr	8/6/90	0
6/28/90	0.02	8/7/90	0
7/1/90	tr	8/11/90	0
7/2/90	0.38	8/12/90	0
7/16/90	0	8/18/90	0.08
7/17/90	0	8/19/90	0.03
8/1/90	tr	9/1/90	0.01
8/2/90	0	9/2/90	0

[a] Data are from reference 26.

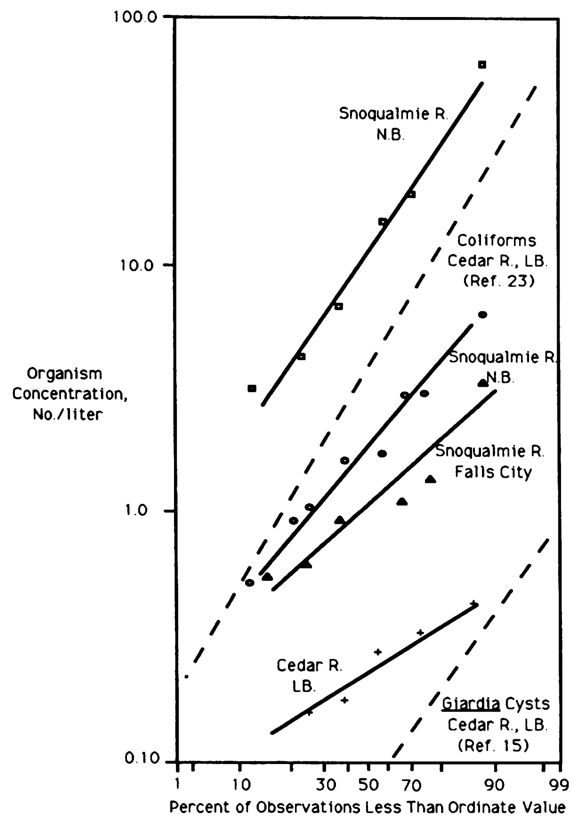

FIG. 3. Cumulative frequency distributions of *Cryptosporidium* oocyst concentration, *Giardia* cyst concentration, and coliform bacterium concentration at sampling stations on the Snoqualmie and Cedar rivers near Seattle.

TABLE 5. Total daily *Cryptosporidium* oocyst production rates for sampling stations on the Snoqualmie and Cedar rivers[a]

Date (mo/day/yr)	Oocyst production rate (10^7 oocysts/mi^2/day) at:			
	NB[b] (154 mi^2)	FC[b] (375 mi^2)	HB[b] (603 mi^2)	LB[c] (120 mi^2)
6/28/90	17.6	6.09	83.7	
7/2/90	1.65	0.73	14.1	0.38
7/17/90	0.59	0.93	12.8	0.13
8/2/90	0.76	0.35	2.79	0.20
8/7/90	1.01	0.51	1.60	ND
8/12/90	1.39	0.54	1.09	0.17
8/13/90				0.21

[a] Data are from reference 27 and were calculated by using oocyst concentrations (Table 1), river flow rate (Table 3), and watershed area.
[b] On the Snoqualmie River.
[c] On the Cedar River. ND, none detected.

Oocyst concentrations found in five consecutive 10-gal samples taken at 2-h intervals from the Snoqualmie River NB station on the afternoon of 19 August 1990 ranged from 2.4 to 3.2/liter (average = 2.6/liter; standard deviation = 0.32) (Table 2). Oocyst concentrations found in five consecutive 10-gal samples taken one right after the other from the same NB station on the afternoon of 2 September 1990 ranged from 1.2 to 2.2/liter = (average 1.65/liter; standard deviation = 0.44).

The total oocyst production rates of the watershed areas tributary to the four sampling stations were calculated for comparison to similar data reported elsewhere (15) and for use in assessing the general scale of contributing sources (Table 5). Oocyst production was lowest from the Cedar River watershed, averaging about 0.2×10^7 oocysts per mi^2 per day. Production from the upper watershed on the Snoqualmie River was 10 times higher, averaging 2.7×10^7 oocysts per mi^2 per day. Production from the entire Snoqualmie River watershed to HB averaged 1.9×10^8 oocysts per mi^2 per day, nearly 10 times higher than production from the upper watershed. If the increase in production from FC to HB is calculated only for the increment in watershed area between the two stations (228 mi^2), the corresponding production rate was 3.1×10^8 oocysts per mi^2 per day. At the beginning of the sampling period, in late June following a period of moderate rainfall with correspondingly higher runoff, the peak production rate for this agricultural area of the lower watershed was 1.4×10^9 oocysts per mi^2 per day.

DISCUSSION

The data presented here on the concentration of *Cryptosporidium* oocysts in river water samples should be examined and considered with care. No similar data collected elsewhere by others are available for comparison. Data on *Cryptosporidium* found in surface water by others have been reported without information on the recovery efficiency of the method, thus permitting no estimation of concentration. Our experience suggests that the recovery efficiency of the "yarn wound" filter method may be significantly lower than that for the method reported here (15). Concentrations reported by the yarn wound method may thus be significantly underestimated.

Oocyst concentrations found in 34 of 35 Snoqualmie and Cedar River samples were above the limit of detection of the method, which ranged from 0.04 to 0.14 oocysts per liter (1 in 26 to 1 in 7 liters) depending on the sample volume (19 to 76 liters) and the recovery efficiency (18.6 to 34.3%). The highest concentrations (10 to 60/liter) were observed at the location farthest downstream (below numerous dairy farms) and early in the sampling period, when runoff influence was greatest. Lowest concentrations (0.15 to 0.45/liter) were observed at the sampling location on the Cedar River, representing discharge from a large (120-mi^2), well-managed public water supply watershed.

The consistent appearance of oocysts in samples processed during the 3-month study period (Fig. 2A) clearly describes a continuous (as opposed to intermittent) presence of this organism in water. Cumulative probability plots of oocyst concentrations for each station (Fig. 3) show approximately log-normal distributions, reinforcing the apparent continuity of oocyst concentrations. Comparisons of the oocyst concentration distributions with coliform bacterium distributions (23) and *Giardia* cyst concentrations reported previously (15) (Fig. 3), both on the Cedar River at LB, show a clear similarity. Overall, the data presented here suggest that short-term variations in individual oocyst sources had little effect on oocyst concentrations seen in the surface source, resulting in a relatively slowly changing profile of oocyst concentration at a single station over time (Fig. 2A) as opposed to a widely varying or even intermittent pattern.

In an effort to understand the continuity of the oocyst concentrations, it is useful to consider the origins of these concentrations in the watersheds. The oocysts found in these two rivers originate in upland watershed areas among wild-animal populations consisting of hundreds of individuals per square mile (total watershed areas tributary to the four stations in this study ranged from 120 to 600 mi^2). Animal feces containing the organisms are deposited and find their way into surface tributaries directly or via runoff. Rapid mixing in the streams results in concentrations that were observed to be virtually homogeneous when viewed over short time scales (hours). This is illustrated by oocyst concentrations in the five samples taken over an 8-h period and in the five samples that were taken one after the other from water flowing past the NB sampling point over a period of 15 to 20 min (Table 2). Clearly, the presence of major oocyst sources such as dairy farms (17) or wastewater treatment plant discharges (20) can affect the variation of concentrations over time (Fig. 2A) but only to the extent that these concentrations vary independently of factors influencing variations in other distributed sources.

The differences in oocyst concentrations observed between the four sampling stations (Fig. 2 and 3) appeared relatable to differences in potential oocyst sources in watershed areas tributary to the respective stations. The character of watershed areas of the Middle and South forks of the Snoqualmie River tributary to the uppermost station, near NB, and of the Cedar River at LB were similar. They were forested and mountainous, typical of the central western Cascade Range. The three watersheds were adjacent from north to south and of similar size (80 to 150 mi^2). Although animal habitat was virtually indistinguishable in the three watersheds, human and associated domestic-animal activities differed markedly. The Snoqualmie Middle Fork drainage above our sampling site contained only scattered rural residences. It was heavily used for logging and recreation, including hiking, camping, and kayaking. It had no paved public roads. The Snoqualmie South Fork drainage had the State of Washington's major east-west interstate highway corridor (I-90) and was heavily used for recreation, with commercial timber uses comparable to those of the North Fork. The Cedar River watershed tributary to LB was the

watershed for the City of Seattle Water Department and was closed to the public. The intensity of commercial timber activities was comparable to that of the Snoqualmie River drainages to the north, but sanitation was carefully monitored by the water department.

The reach of the Snoqualmie River between the Middle Fork sampling station, NB, and the next station downstream, FC, incorporates flow from the North and South forks and contributions from three communities (NB, Snoqualmie, and FC) and from a dispersed rural population totaling about 5,000 individuals. Immediately below FC, the Snoqualmie River meanders through a broad, flat, agricultural valley in which dairy farming is a major activity. Dairy farms line the river along most of the 17 mi from FC to the last sampling station at HB. This reach of the river is also used for fishing, swimming, and boating (kayaking and canoeing) by the nearby urban population of the Seattle area (about 1.2×10^6 people).

The differences in watershed uses between physically similar watershed areas of the upper Snoqualmie River (NB) and Cedar River (LB) are reflected in an approximately fivefold difference in oocyst concentration and overall oocyst production rate (Fig. 2A and C). The watershed control activities of the Seattle Water Department limit only the human population. Wild-animal populations are not managed, and, given restrictions on the human population, populations of the larger game animals (deer, elk, bear, and coyote) are likely to be higher than in the open watersheds to the north. Accordingly, lower oocyst concentrations in the Cedar River were likely attributable to the watershed management practice prohibiting unsupervised human activity.

The nearly 10-fold difference in oocyst concentrations between the upper stations on the Snoqualmie River and the lower station at HB (Fig. 2A and C) appeared strongly related to the domestic-animal-related (dairy farming) sources in the lower reach. The reach also included two sizable communities, Carnation and Duval, although neither had direct sewage discharge to the river.

The data presented here with the discussion above led to the following conclusions. (i) *Cryptosporidium* oocysts are present in river water of both inhabited and uninhabited areas at concentrations above the ability of the method used here for detection, i.e., about 0.05 to 0.15 oocysts per liter. (ii) Oocyst concentrations in watersheds of appreciable size were continuous as opposed to intermittent. (iii) Seasonal factors, including runoff of land drainage, may affect oocyst concentrations by 10-fold, with concentrations in drier periods being significantly lower than those in wetter periods. (iv) The character and intensity of both human and domestic animal activities in a watershed may affect oocyst concentrations in the surface water by as much as 10- to 15-fold. (v) Public water supply watershed management practices of limiting human activity may reduce oocyst concentrations by as much as fivefold.

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