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FINAL REPORT

HARBOR SEAL POPULATIONS AND THEIR CONTRIBUTIONS TO FECAL COLIFORM POLLUTION
IN QUILCENE BAY, WASHINGTON

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EXECUTIVE SUMMARY

High fecal coliform concentrations in the northern part of Quilcene Bay have recently been reported and have resulted in the decertification of this area for commercial shellfish growing. The bay also supports a growing population of harbor seals. This study was undertaken to evaluate the potential contribution of harbor seals to the fecal coliform pollution and make recommendations on how to more precisely determine the portion of fecal coliforms contributed by seals. A maximum of 230 harbor seals were counted in Quilcene Bay, primarily concentrated at a log dump on the eastern side of the bay. Seals were present year-round, with no dramatic seasonal patterns in the number of animals. Seal defecation rates were estimated as between 250 to 500 grams per day for a 50 kg seal, with 375 grams the best estimate of average daily fecal production. A variety of bacterial species were identified in 10 fresh seal feces collected from the Dosewallips River Delta (adjacent to Quilcene Bay). *Bacillus* sp. and *E. coli* were the most often found predominant bacterial populations. No *Salmonella* or *Yersinia* were found in enrichment procedures conducted for these two pathogenic bacteria. Fecal coliform concentrations in the 10 harbor seal feces analyzed by Biochem Environmental Services ranged from 4.0×10^6 to 9.2×10^8 coliforms per gram with a geometric mean of 3.1×10^7 . These concentrations are similar to those reported for humans but are higher than for most domestic animals. Ratios of fecal coliforms to fecal streptococcus were much higher than reported for humans or domestic animals. This ratio and some other unique bacterial indicators may be of value in tracing the source of bacteria found in Quilcene Bay. This study indicated that fecal coliform densities in harbor seals are fairly high and given the population size of seals and their defecation rates, seals have the potential to be significant contributors to the high fecal coliform levels in Quilcene Bay.

Recommendations to more accurately determine the proportion of fecal coliform contributed by seals include: 1) increase sample size of seal feces tested, 2) determine portion that dissolves in marine water, 3) determine the portion of seal feces excreted in different parts of Quilcene Bay, 4) use specific bacterial indicators to trace the source of fecal coliforms found in Quilcene Bay waters, 5) test fecal coliform levels at other sites where seals are the only potential source, 6) examine fecal coliform concentrations produced by captive seals.

INTRODUCTION

Commercial harvesting of shellfish in the headwaters of Quilcene Bay has recently been closed due to high fecal coliform levels (Cook 1984, 1985). Since Quilcene Bay supports a large commercial shellfish industry, further closures in other parts of the bay could have serious economic consequences to the local economy. Jefferson County has begun a water quality study of the watershed of Dabob and Quilcene Bays in an attempt to identify sources of pollution in these areas.

Fecal coliforms are found primarily in the intestines and feces of warm-blooded animals (Geldreich 1966). Possible sources of fecal coliform pollution in Quilcene Bay include septic systems, runoff from livestock pastures (especially after rain), stormwater runoff, and contributions from wildlife.

There is a substantial population of harbor seals that inhabit Quilcene Bay and haul out daily on lografts in the northeast part of the bay (Calambokidis et al. 1978, 1979, 1985). The degree to which harbor seals contribute to fecal coliform levels has not been determined previously.

The purposes of this study were to:

- Determine the number of harbor seals seasonally occurring in Quilcene Bay.
- Estimate the amount of feces generated by the seals.
- Determine fecal coliform concentrations in harbor seal scat.
- Evaluate the possible contribution of harbor seals to fecal coliform levels found in Quilcene Bay.
- Make recommendations on how to more precisely determine the proportion of fecal coliform in Quilcene Bay contributed by seals.

METHODS

Censuses

Harbor seals were censused while hauled out on log booms from East Quilcene Bay Road just above the logbooms. Occasionally, counts of seals hauled on oyster rafts in the southeast part of Quilcene Bay (Figure 1) were made from the Quilcene yacht basin (Boat Haven). The site at East Quilcene Road was excellent and provided a close view and high elevation, allowing extremely accurate counts of hauled seals. The Boat Haven site was a relatively poor one due to the distance from the seals and lack of elevation.

Counts were conducted at or near high tide. During each visit, seals were counted every 30 minutes. Information recorded on data sheets included numbers of seals hauled and in the water, number of pups, and weather conditions. Counts were made with binoculars and a 15-60X spotting scope.

Censuses were conducted at least once a month from September 1985 to October 1986. More censuses were made later in this period after funding for this project became available.

Sample collection

Both the Quilcene log rafts and Dosewallips Delta were searched for seal scat for fecal coliform analysis. Dates of collections and samples submitted is given in Table 1. No fresh scats were found on the Quilcene rafts, primarily because scat did not remain on the sloping surfaces of the log booms. Scats were collected at the Dosewallips Delta in the early morning just after a high tide. This time was chosen to minimize the time between defecation and collection and to insure that the scat was likely as cold as possible after defecation (Early morning). Scats were weighed and transported in sterile containers on ice from collection to delivery to the two laboratories. When samples were being delivered to both laboratories, scats were split (without homogenization). Samples were delivered to Jefferson County within 3 hours of collection and to Bio-Chem within 7 hours.

Water samples were collected adjacent the log booms at Quilcene Bay on five occasions for dissolving and diluting scats (Jefferson County tests only). From two to four samples of twelve liters of water were collected in separate cleaned five gallon buckets.

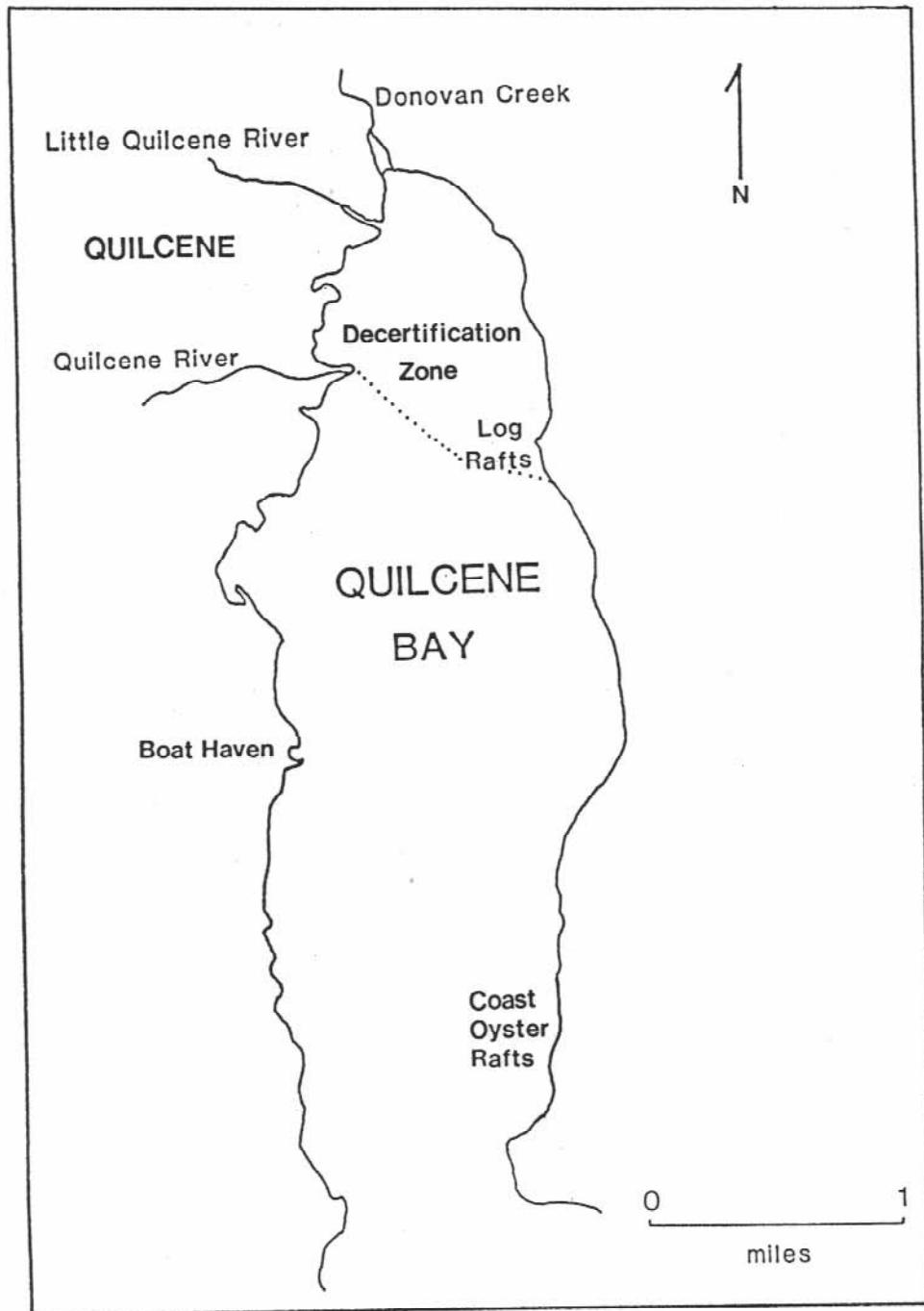


Figure 1. Study area showing log booms and oyster rafts used by seals. Dotted line indicates boundary of decertified area.

Table 1. Dates samples collected for fecal coliform analysis.
 All samples collected from Dosewallips after no scats
 found at Quilcene Bay log dump.

Date	Number collected	Number submitted	
		Bio-Chem	Jeff. Co.
7/23/86	2	0	2
8/19/86	4	4	4
8/27/86	2	0	2
9/3/86	3	3	3
9/15/86	4	3	4
10/8/86	3	0	3
10/20/86	3	0	3
TOTAL	21	10	21

Harbor seal food habits and prey consumption

Seal scats were also examined to determine the food consumed by seals. Scats used for fecal coliform analysis as well as other scats collected previously were screened through nested 0.5 and 2.0 mm screens. Otoliths (bones from the heads of bony fishes that generally are not digested) recovered from the scats were compared to a reference collection at Cascadia Research and the species of fish identified. The length of otoliths was also measured with calipers and the degree of wear or damage scored.

Hake otoliths from 144 fish collected by the Washington Department of Fisheries in Seattle, Washington were measured in a similar fashion as those recovered from seal scat. Data on the fish lengths, weight, sex, and age were provided the Department of Fisheries. Fish lengths and weights were regressed on otolith length to develop equations to predict length and weight of fish from otolith lengths from harbor seal scat.

Sample analyses - Biochem Environmental Services

Ten harbor seal fecal samples were delivered to Biochem Environmental Services, Inc.. These samples were processed for sanitary bacteriology (Total Coliform, Fecal Coliform, Fecal Streptococci) and for the recovery of human pathogenic enteric organisms. Additionally, the predominant aerobic and facultatively anaerobic bacterial populations were characterized.

Samples were delivered to Biochem on: 19 August, 3 September, and 15 September 1986. Samples were processed immediately upon receipt.

Each fecal sample was homogenized by stirring and mashing with sterile wooden applicator sticks, then two-to-four grams of sample were weighed into appropriate volumes of sterile phosphate-buffered water. Decimal dilutions were carried to the ninth logarithmic level.

Total Coliform (TC), Fecal Coliform (FC), and Fecal Streptococci (FS) were assayed using the Most Probable Number (MPN) methods and media as specified in Compendium of Methods for the Microbiological Examination of Foods (Amer. Public Health Assoc., 1976), and Standard Methods for the Examination of Water and Wastewater (Amer. Public Health Assoc., 1985).

Heterotrophic population was assayed by surface plating sample dilutions onto Phenylethanol Agar and Xylose-Lysine-Desoxycholate agar, and incubating at 20-24°C for 48-72 hours. Colonies were counted, and

individual colonies were picked for identification.

Salmonella and Shigella enrichment was performed by weighing one gram of homogenized fecal material into Selenite-F broth, mixing well, and incubation at 35°C for 16-18 hours. The Selenite enrichments were streaked onto enteric isolation media to include Salmonella-Shigella agar, Hektoen agar, and MacConkey agar. These plates were incubated at 35°C for 24 hours. Typical presumptive enteric colonies were picked for identification. Isolates were screened biochemically to identify enteric pathogens.

Yersinia enrichment was performed by weighing one gram of homogenized material into GN broth, mixing well, and incubating at 4°C for 21 days. The enrichment was inoculated to YM agar and MacConkey-Tween agar; incubation was at 20-24°C for 72 hours. Typical presumptive colonies were picked for identification by biochemical screen.

Sample analyses - Jefferson County

Harbor seal samples were also submitted for analysis of fecal coliform concentrations to the water quality laboratory operated by the Jefferson County Planning and Building Department. Enumeration was accomplished using both the MPN (Most Probable Number) and MF (Membrane Filter) techniques in accordance with Standard Methods for the Examination of Water and Wastewater.

The MPN analysis used Laryl Tryptose and EC broths with five tubes per dilution. Five dilutions were used to bracket highly variable results. The original samples were dissolved in seawater of known fecal coliform concentration and subsequent dilutions were dissolved in sterile phosphate buffer solution.

The MF tests were conducted using both MTec agar and MFC broth as the nutrient media. A 2 hour incubation period at 35°C was used for resuscitation of injured bacteria prior immersion at 44.5°C. Dilutions were made for expected yields of 20-80 colonies per plate, with replicates made as possible.

RESULTS AND DISCUSSION

Harbor seal populations - Quilcene Bay

Results of harbor seal censuses are given in Table 2. Harbor seal counts at Quilcene Bay ranged from 0 to a maximum of 230 seals. Seals were primarily found hauled out at log booms on the eastern side of Quilcene Bay. Lower numbers were found intermittently hauled out on oyster rafts at the southeast entrance of Quilcene Bay and these counts are included in Table 2.

Figure 2 shows the monthly high counts of seals counted at Quilcene Bay from September 1985 to October 1986. Monthly high counts were variable and there were no apparent seasonal trends.

Harbor seal populations in Quilcene Bay and the neighboring Dosewallips Delta appear to be increasing. Figure 3 and Table 3 show trends from 1977 to 1986 in the high counts of seals and pups at Quilcene Bay and Dosewallips Delta. Data from previous years was summarized from previous published and unpublished research conducted by Cascadia Research personnel (Calambokidis et al. 1978, 1979, 1985). Counts from 1984 may be underestimated because of the low number of censuses made.

Counts of harbor seals prior to 1977 are not available but interviews with long-time local residents provides some insight. One person stated that prior to the bounty (1923-1960) there may have been about 200 seals in the bay. All four of the people interviewed reported that seal numbers were low during the 1940s and 1950s (as low as 10). One person reported seeing 25-35 seals hauled on logs in the late 1960s. There was one report of 176 seals on the logs in November 1976, a number that closely matches Cascadia's high count of 168 in 1977.

In 1972 harbor seals were legally protected under the Marine Mammal Protection Act. This year probably marks the most likely time when harbor seal populations began growing. Harbor seal populations in other parts of Puget Sound have been growing rapidly in recent years as well. The rate of increase seen in Quilcene Bay appears to be lower than for many other areas in Washington State (Calambokidis et al, 1985).

The overall harbor seal population in Quilcene Bay likely fluctuates with animal movement in and out of the bay. The high count of 230 seals is considered the maximum number of seals present in the bay at any one time.

Table 2. Census results of harbor seals at Quilcene Bay. Mostly taken at log dump on east side of Bay. Counts that include Coast Oyster rafts noted.

Mo.	Date		Time		Haul	High count			High tide Time	Comments
	Day	Year	St.	End		Water	Tot.	Pups		
09	08	1985	1640	1720	39	6	45	6	1424	
09	15	1985	1745	1928	86	7	93	25	1820	
09	22	1985	1330	1502	112	3	115	31	1350	
09	29	1985	1720	1815	95	7	102	21	1807	
10	02	1985	1630	1700	60	12	72	13	1906	
10	11	1985	1600	1700	62	6	68	10	1602	
10	13	1985	1745	1845	85	7	92	14	1653	
10	21	1985	1335	1405	37	0	37	5	1315	
11	10	1985	1345	1415	56	4	60	0	1425	
11	15	1985	1000	1100	84	3	87	9	736	
11	16	1985	916	946	130	3	133	17	835	
11	17	1985	1300	1330	122	3	125	14	937	
11	20	1985	1500	1530	76	5	81	0	1220	
12	08	1985	1300	1400	218	5	223	0	1255	21 on coast rafts
01	26	1986	1600	1630	91	5	96	0	1610	
02	01	1986	930	1000	21	1	22	0	904	
02	03	1986	1122	1400	17	11	28	0	1026	
02	05	1986	1130	1230	0	4	4	0	1221	
02	20	1986	1130	1315	51	5	56	0	1237	
02	24	1986	1600	1630	38	4	42	0	1613	very stormy
02	25	1986	1630	1800	38	15	53	0	1704	
03	08	1986	1430	1500	0	0	0	0	1429	
03	09	1986	1430	1600	99	3	102	0	1550	
03	11	1986	1630	1800	15	5	20	0	1731	
04	06	1986	1400	1500	0	0	0	0	1511	
04	18	1986	1030	1100	0	0	0	0	1033	
04	25	1986	1800	1830	0	17	17	0	1815	
05	09	1986	700	730	225	5	230	0	503	
05	13	1986	630	705	16	77	93	0	654	hailstorm
05	14	1986	700	800	219	4	223	0	736	
05	15	1986	700	800	105	67	172	0	828	
05	22	1986	1830	1900	0	4	4	0	1736	
05	24	1986	2030	2100	103	7	110	0	1923	
06	10	1986	630	730	158	7	165	0	553	
06	11	1986	600	630	0	32	32	0	635	men working boom
07	17	1986	1630	1700	1	25	26	0	1537	
07	23	1986	630	700	7	35	42	0	551	
07	24	1986	610	640	44	2	46	0	654	poor count, fog
07	31	1986	1715	1745	3	11	14	0	1611	
08	06	1986	1745	1850	8	12	20	0	1929	
08	07	1986	2015	2045	0	31	31	0	1950	men working boom
08	13	1986	1415	1445	0	7	7	0	1236	men working boom
08	21	1986	615	645	110	7	117	1	603	
08	26	1986	1130	1200	1	13	14	0	1127	men working boom
08	28	1986	1500	1600	17	13	30	4	1430	
09	04	1986	1715	1845	86	11	97	10	1830	
09	06	1986	1900	2000	90	14	104	18	1911	
09	09	1986	1800	1900	210	5	215	19	2042	
09	11	1986	1155	1530	138	23	161	15	1235	15 on coast rafts
09	14	1986	1500	1610	176	9	185	26	1604	
09	18	1986	1715	1815	190	2	192	33	1811	3 on coast rafts
09	21	1986	1700	1830	179	14	193	19	1924	32 on coast rafts
09	28	1986	1530	1655	178	15	193	15	1527	57 on coast rafts
10	09	1986	1215	1415	184	16	200	17	1107	73 on coast rafts
10	10	1986	1245	1445	172	7	179	8	1226	33 on coast rafts

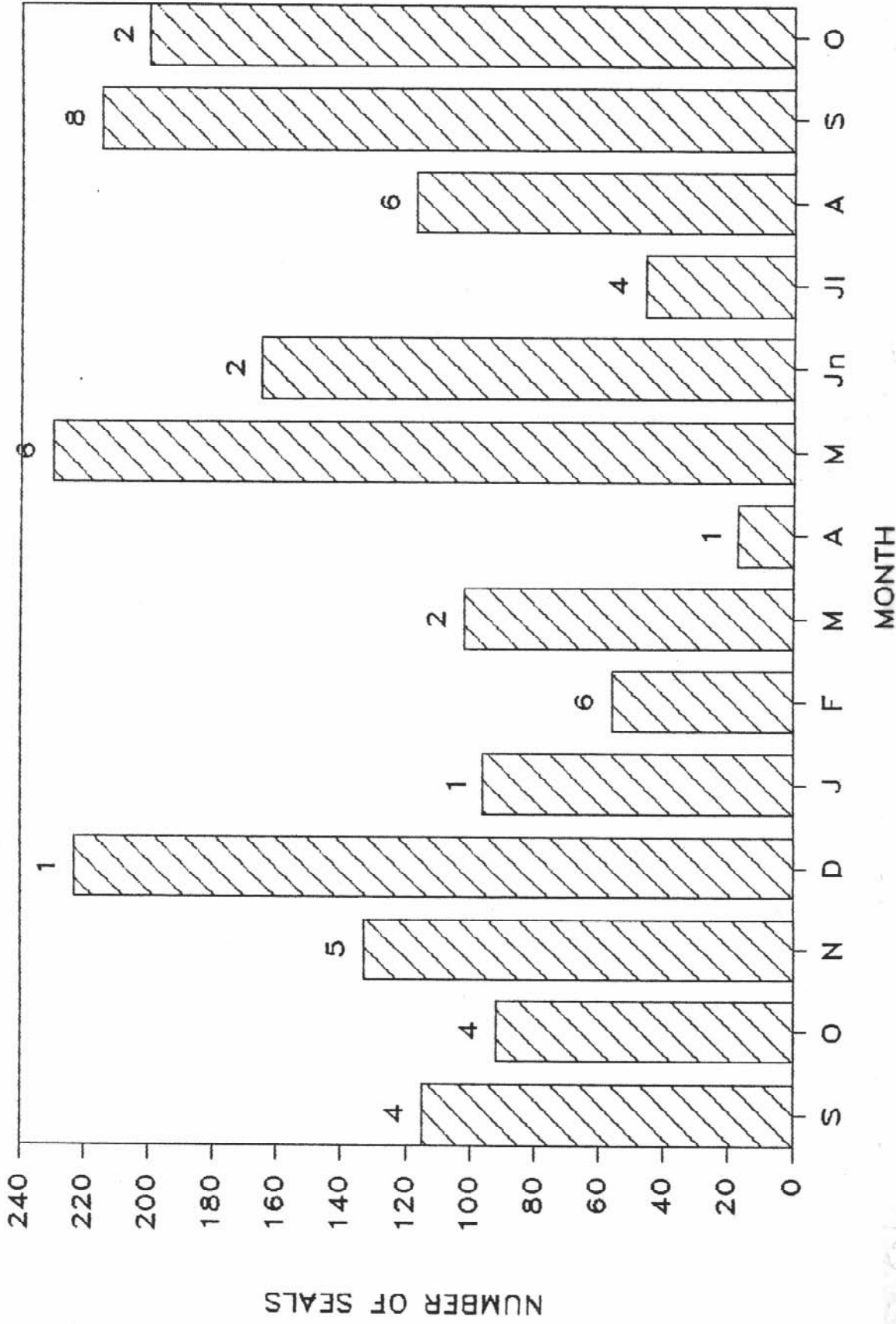


Figure 2. Monthly high counts of seals in Quilcene Bay. Number of days counts were made is listed.

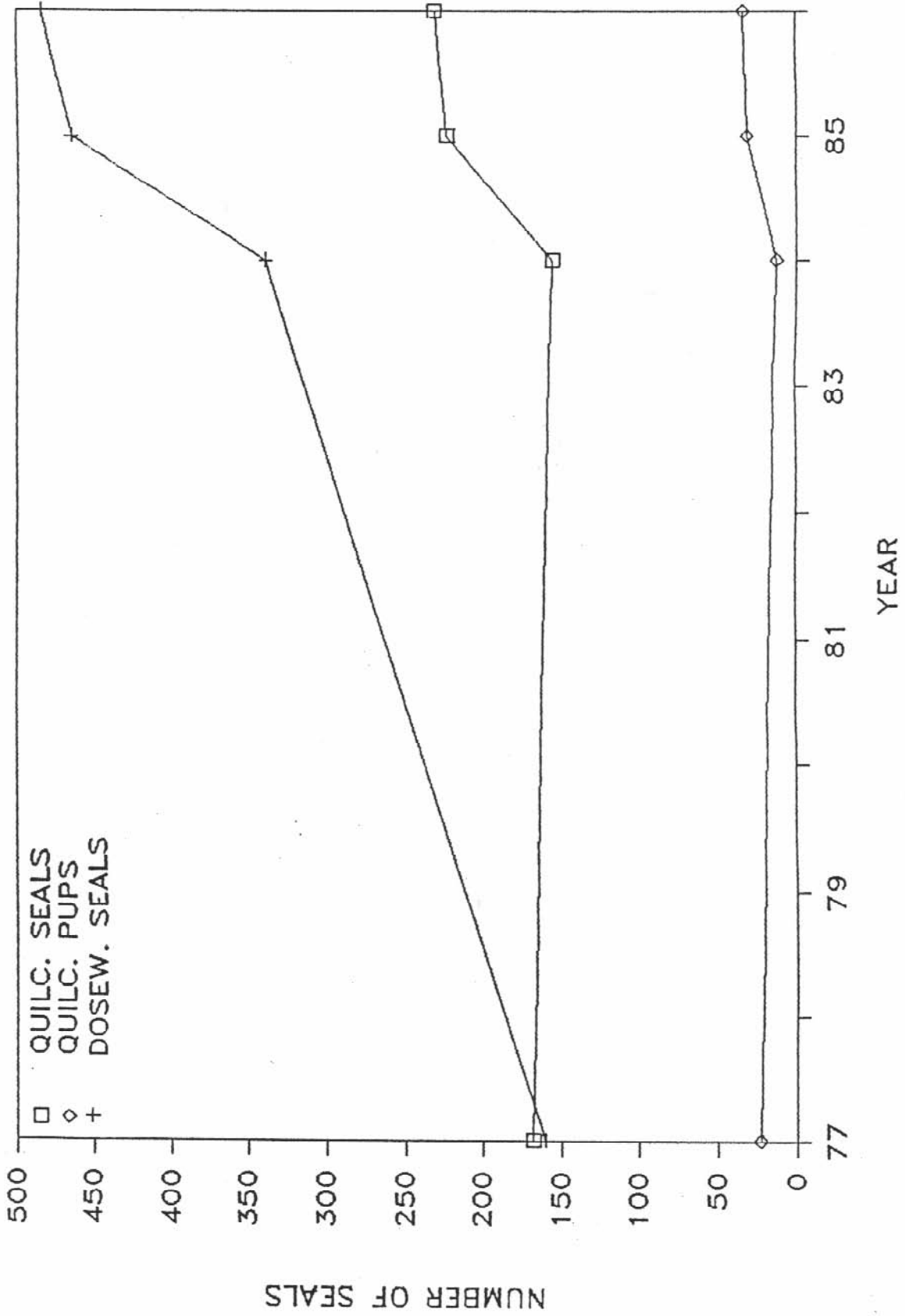


Figure 3. Annual high counts of seals and pups at Quilcene and Dosewallips.

Table 3. Annual high counts of harbor seals in Quilcene Bay made during different years.

Year	Range of dates monit.	Land counts Days	counts Hrs.	No. air counts	High count Number	Date	Max. pups Number	Date
1977	4/18 - 10/28	35	NA	4	168	5/4	23	9/15
1984	6/17 - 10/12	3	1	4	155	7/15	12	9/13
1985	9/8 - 12/8	14	11.5	0	223	12/8	31	9/22
1986	1/26 - 10/10	41	41	0	230	5/9	33	9/18

Scat production

Two techniques are reported here on estimates of total production of fecal material by harbor seals in Quilcene Bay. The simplest method to estimate fecal production is to estimate the number of scats produced on average in a day and multiply by the average scat weight. The average weight of scats collected in this study was 183 grams (Table 4). Harbor seals and other pinnipeds have very rapid rates of digestion with the assimilation to initial defecation period of about 6 hours (Helm 1984). Seals likely engage in at least two feeding bouts a day and would be expected to defecate at least that many times a day. With these assumptions a seal would generate at least 366 g of feces a day. These calculations may underestimate the amount of fecal material produced because not all the feces from one meal may have been recovered.

An alternate way to estimate fecal production is to extrapolate from fish consumption and the assimilation efficiency of seals. Two studies were found reporting the relationship between the amount of food consumed and the fecal material produced. Based on experimental feeding of the closely related Baikal seal (Phoca sibirica), fecal production was estimated as 10% of the weight of food consumed (Pastukhov 1974). Calculations from figures reported by Ashwell-Erickson and Elsner (1981) indicate feces output of 8% to 18% of fish weight for three captive harbor seals (these estimates were based on dry weight of both fish and feces). We used 10% of food weight as the best estimate of fecal weight. Estimated daily fish consumption of harbor seals vary widely with most common estimates between 5 and 10% of body weight (see summary in Ashwell-Erickson and Elsner 1981 and Calambokidis et al. 1984). A mathematical model (Leslie matrix) of a harbor seal population structure was used along with harbor seal weight-age equations provided by Boulva and McLaren (1979) to determine the average weight of a seal in a population growing at 5% per year. The mean weight of a harbor seal was 50 kg based on these calculations. The daily scat production of a typical 50 kg seal can thus be estimated as $50 \text{ kg} \times .05 \text{ to } .10 \text{ (consumption)} \times 0.10 \text{ (fecal/fish weight)} = 250 \text{ to } 500 \text{ g}$. These two calculated rates bracket the estimated fecal production from the first technique described above.

Based on the different estimates of fecal production (minimum of 366 g from the first method and 250-500 g from the second method), we chose 375 g as the best estimate of fecal production for an average 50 kg seal. This estimate is rough and is used only in the absence of a more precise way to estimate fecal production. This figure can be used to estimate the total fecal production of the harbor seals that use Quilcene Bay. The maximum number of 230 seals seen in Quilcene Bay would produce approximately 86 kg

Table 4. Weights of scats, otoliths recovered, and calculation of mean lengths and weights of hake. Sample numbers marked FC are those tested in this study. Only hake otoliths were recovered from these samples. Those marked DO are from previous collections and include only those samples where hake was the only species found.

Sample	Number otoliths	Scat wt. (g)	Tot. fish wt. (g)	Ave. fish wt. (g)	Ave. fish length (cm)
DO-19	64	396	2458	76.8	21.9
DO-20	57	257	2044	71.7	21.3
DO-24	24	247	1350	113	25.7
DO-25	26	184	964	74.2	21.1
DO-27	3	266	114	76.1	23.1
DO-28	16	365	523	65.3	21.2
DO-30	8	424	423	106	25.4
DO-31	35	365	1273	72.7	21.9
FC-3	16	197	520	65	21.4
FC-5	23	346	902	78.4	22.9
FC-6	30	233	1153	76.8	22.8
FC-7	6	335	189	63.1	21.2
FC-8	10	107	381	76.3	22.8
FC-10	2	257	101	101	25.5
FC-13	4	193	166	82.9	23.8
FC-14	21	260	469	44.6	17.5
FC-15	32	171	1056	66	21.3
FC-17	2	180	43	43.2	18.2
FC-18	2	71	82	81.8	23.7
FC-20	4	192	229	115	26.6
FC-21	2	254	85	85	24
Mean	13.3	183	501	77.9	22.5
Total	387	5300	14525	1634.9	473.3

No otoliths were found in samples FC-9, FC-11, FC-16, FC-19, DO-21, DO-22, DO-26, and DO-32.

(57-115 kg) per day of feces.

Ashwell-Erickson and Elsner (1981) reported that prey consumption and assimilation rates may vary depending on prey. The only prey identified in scats collected during this study were of Pacific hake. Table 4 lists the average lengths and weights of hake present in the samples. These were calculated from regressions of fish length and weight on otolith length of hake sampled by the Department of Fisheries. Seals ate almost exclusively young 1 and 2 year-old hake.

Bacterial speciation

Biochem Environmental Services determined species of bacteria present in 10 harbor seal feces samples. Identification of the predominant bacterial populations and qualitative data for pathogen detection are presented in Table 5. The predominant bacterial population, i.e., those organisms comprising the upper 10% of the population included *Bacillus* species, *Escherichia coli*, *Klebsiella*, *Edwardsiella*, *Streptococcus*, *Staphylococcus*, and *Micrococcus*. The two types of *Streptococci* recovered were unusual in that one was a beta-hemolytic pyogenic form and the other a non-hemolytic form not fitting Sherman criteria.

Three types of *E. coli* were found. The conventional form conforming to typical biochemical patterns, a more rare anaerogenic form, and a type exhibiting an unusual carbohydrate utilization pattern: positive in lactose, mannitol, and rhamnose while negative in sucrose, salicin, and dulcitol.

No human enteric pathogens were isolated from either the *Salmonella* or *Yersinia* enrichment procedures. Organisms recovered included species of *Proteus*, *Citrobacter*, *Pseudomonas*, and *Aeromonas*.

Salmonella is the pathogen of primary concern because of its association with most cases of disease related to shellfish consumption. Fecal coliform concentrations serve only as indicators of the possible presence of pathogens such as *Salmonella*. Though no *Salmonella* were recovered from the 10 samples analyzed by Biochem Environmental Services this does not necessarily indicate harbor seals are not a potential source of these pathogens. The occurrence of *Salmonella* in the feces of humans and other animals has been summarized by Olivieri (1982). Only one percent of humans in Europe and North America were found to excrete *Salmonella*. The percent of animals tested that were excreting *Salmonella* was often higher than for humans but less than 20% of the individual animals tested.

Table 5. Predominant bacterial populations identified from harbor seal samples and organisms recovered by pathogenic enteric enrichment procedures for Salmonella and Yersinia. No Salmonella or Yersinia were found, however, other organisms identified during each test are listed. Analyses conducted by Biochem Environmental Services.

Sample ID	Predominant bacterial popul.	Organisms found from enrichment procedures Salmon. procedure	Yersinia procedure
CRC-FC-3	<u>Bacillus subtilis</u> <u>B. megaterium</u> <u>E. coli</u> <u>Klebsiella oxytoca</u>	<u>Escherichia coli</u> Proteus sp.	Aeromonas sp. Pseudomonas sp.
CRC-FC-4	Bacillus sp. <u>E. coli</u>	<u>E. coli</u> Proteus sp.	<u>Aeromonas hydrophila</u> Pseudomonas sp.
CRC-FC-5	Bacillus sp.	Citrobacter sp. Proteus sp.	Aeromonas sp. Pseudomonas sp.
CRC-FC-9	<u>E. coli</u> <u>E. coli</u> (anaerogenic) Staphylococcus sp. Streptococcus spp. ¹	<u>E. coli</u> <u>Prot. rettgeri</u> <u>Prot. vulgaris</u>	Aeromonas sp. <u>Prot. rettgeri</u> Pseudomonas sp.
CRC-FC-10	Bacillus sp. <u>E. coli</u> <u>E. coli</u> ²	Aeromonas sp. <u>E. coli</u>	Aeromonas sp. Pseudomonas sp.
CRC-FC-11	Bacillus sp. <u>B. cereus</u> <u>B. lichenformis</u> <u>Micrococcus luteus</u> Staphylococcus sp.	<u>Alteromonas putrifaciens</u> <u>E. coli</u> Moraxella sp. <u>Prot. rettgeri</u> <u>Prot. vulgaris</u>	Aeromonas sp. Pseudomonas sp.
CRC-FC-13	Bacillus sp. <u>E. coli</u> ²	<u>E. coli</u> Proteus sp.	Aeromonas sp. Pseudomonas sp.
CRC-FC-14	Bacillus sp. <u>E. coli</u> ²	<u>E. coli</u> Proteus sp.	<u>Prot. rettgeri</u> Pseudomonas sp.
CRC-FC-15	<u>E. coli</u> ²	<u>E. coli</u> <u>Prot. rettgeri</u> Aeromonas sp. Enterobacter sp.	Aeromonas sp. Pseudomonas sp.

¹ beta-hemolytic pyogenic streptococcus and non-hemolytic streptococcus not fitting Sherman criteria.

² E. coli showing unusual carbohydrate pattern.

We know of no reports that have identified Salmonella from the tissues or feces of harbor seals. Salmonella was not among the various bacteria found in tissue samples and swabs from dead or dying harbor seals in Washington State (Calambokidis et al. 1985). Salmonella has been found in the tissues of other pinniped species (Keyes 1965, Anderson et al. 1979, Stroud and Roelke 1980) and is likely present in some portion of the harbor seal population.

Calambokidis et al. (1985) report bacteria identified from the tissues of 50 dead or dying harbor seals in Washington State. Many of the bacteria found in harbor seal tissues were also identified in the feces examined in this study. These include E. coli, Streptococcus sp., Proteus sp., Pseudomonas sp., and Enterobacter sp. Other species identified in harbor seal tissues but not recovered in the feces include Corynebacterium sp., Pasteurella sp., and Acinetobacter sp.

Fecal coliform concentrations

Quantitative results for the sanitary bacteriological procedures (TC,FC,FS) and Heterotrophic Plate Count conducted by Biochem Environmental Services are presented in Table 6. Results of analyses conducted by Jefferson County are given in Table 7. Six samples were analyzed by both laboratories, though not always using the same methods (Table 8). The three samples analyzed by MPN by both labs vary by up to a factor of three. Though this is not close agreement, differences between laboratories is much smaller than the variations between different samples and is also smaller than the typical order of magnitude confidence limits inherent to these procedures. The three samples tested by MFC by Jefferson County did not agree as well with the MPN results of these samples conducted by Biochem, with Biochem's MPN results over an order of magnitude higher than the MF results for two of the three samples. Differences between MPN and MF procedures have been examined by El-Shaarawi and Pipes (1982), who conclude that the two techniques are generally comparable, though MPN has an inherent positive bias.

The MPN results of the 10 samples analyzed by Biochem provide the largest and most reliable dataset on fecal coliform concentrations in harbor seal feces. We therefore made these figures the basis for all calculations used in this report. Fecal coliform concentrations per gram in these 10 samples ranged widely from 4.0×10^6 to 9.2×10^8 . The most common measures of central tendency in bacterial counts are the median and the geometric mean, 2.7×10^7 and 3.1×10^7 , respectively, for the 10 samples.

Table 6. Densities of total coliform, fecal coliform, and fecal streptococcus in harbor seal feces analyzed by Biochem Environmental Services.

BioChem ID	Cascadia ID	Heterotrophic Plate Count	MPN / gram		
			Total Coliform	Fecal Coliform	Fecal Streptococci
C0498	CRC-FC-3D	1.2×10^7	7.9×10^6	4.9×10^6	4.3×10^4
C0499	CRC-FC-4D	3.5×10^7	9.2×10^7	9.2×10^7	9.3×10^3
C0500	CRC-FC-5D	3.6×10^8	5.4×10^8	5.4×10^8	2.3×10^4
C0501	CRC-FC-6D	1.2×10^7	2.2×10^7	2.2×10^7	9.3×10^4
C0569	CRC-FC-9	1.3×10^8	4.9×10^7	4.0×10^6	1.5×10^4
C0570	CRC-FC-10	2.0×10^6	4.9×10^6	4.9×10^6	4.3×10^3
C0571	CRC-FC-11	6.7×10^8	9.2×10^8	9.2×10^8	1.2×10^3
C0597	CRC-FC-13D	5.7×10^7	3.3×10^7	3.3×10^7	4.3×10^3
C0598	CRC-FC-14D	2.8×10^7	3.3×10^7	3.3×10^7	4.3×10^2
C0599	CRC-FC-15D	2.0×10^8	1.3×10^7	7.9×10^6	4.3×10^3

Table 7. Results of analyses conducted by Jefferson County. Samples CRC-FC-7 to CRC-FC-21 were analyzed at dilutions to maximize accuracy in the range of 10^6 to 10^8 fecal coliforms per gram. Densities outside this range are of lower accuracy.

Sample #	MPN	Method		Comments
		MFC	MF-MTec	
CRC-FC-1	>2,400	-	-	
CRC-FC-2	"	-	-	
CRC-FC-3	"	-	-	
CRC-FC-4	"	1.6×10^6	1.3×10^6	
CRC-FC-5	"	-	-	
CRC-FC-6	"	-	-	
CRC-FC-7	-	$<10^5$	-	
CRC-FC-8	-	"	-	
CRC-FC-10	-	1.3×10^6	-	
CRC-FC-11	-	7.0×10^7	-	
CRC-FC-12	2.6×10^7	-	-	
CRC-FC-13	1.1×10^7	-	-	
CRC-FC-14	1.6×10^7	-	-	
CRC-FC-15	2.0×10^7	-	-	
CRC-FC-16	4.8×10^4	-	-	
CRC-FC-17	4.8×10^4	-	-	
CRC-FC-18	9.5×10^7	-	-	
CRC-FC-19	-	-	2.2×10^7	
CRC-FC-20	$<10^5$	-	3.2×10^4	Possibly not fresh
CRC-FC-21	$<10^5$	-	6.0×10^4	Possibly not fresh

Table 8. Comparison of fecal coliform concentrations determined by different methods. Only samples tested by multiple methods shown. All concentrations are in millions per gram of sample.

Sample #	Bio-Chem MPN (mil./g)	Jefferson County MPN (mil./g)	MFC (mil./g)
CRC-FC-4	92	-	1.6
CRC-FC-10	4.9	-	1.3
CRC-FC-11	920	-	70
CRC-FC-13	33	11	-
CRC-FC-14	33	16	-
CRC-FC-15	7.9	20	-

Comparison of seal fecal coliform concentrations to other animals

Comparison of densities of fecal coliforms in harbor seal scat can be compared to values available from other species. Geldreich (1976) and Mara and Oragui (1981) list fecal coliform concentrations in the feces of humans and a variety of wild and domestic animals (Table 9). The ranges of concentrations for the same species vary greatly (up to five orders of magnitude) and the median values reported by Geldreich (1976) are often an order of magnitude different from the geometric mean values for the same species reported by Mara and Oragui (1981). Despite these variations some general comparisons can be made between other species and seals. The geometric mean concentration we found in seals (3.1×10^7) is lower than the geometric mean concentration reported for humans by Mara and Oragui (1981) but higher than the median value reported by Geldreich (1976). Fecal coliform concentrations of seals were generally higher than for most domestic animals. Fecal coliform concentrations in dogs, cats, pigs, and ducks tended to be similar or higher than in seals.

Concentrations of fecal streptococcus

Concentrations of fecal streptococcus were also determined for all 10 samples analyzed by Biochem. Concentrations per gram ranged from 4.3×10^2 to 9.3×10^4 . The fecal coliform to fecal streptococcus ratio ranged from 114 to 767,000. The fecal coliform to fecal streptococcus ratio has been used as an indicator of the potential source of fecal contamination since domestic animals and humans have different ratios of fecal coliform to streptococcus (Geldreich and Kenner 1969, Flachem 1975). A high ratio of fecal coliform to streptococcus is considered more typical of human feces than those of most domestic animals (Geldreich and Kenner 1969, Geldreich 1976, Mara and Oragui 1981). This ratio in seals overlaps but is generally higher than the ratio reported for humans.

The fecal coliform to fecal streptococcus ratio could serve as a potential indicator of the source of fecal pollution in Quilcene Bay if this data were gathered from freshwater and marine samples. The usefulness of this ratio as an indicator, however, is limited by the differential die-off rates of fecal coliform and fecal streptococcus which alters the ratio of the two over time.

Fecal coliform contribution by harbor seals

Total fecal production of harbor seals in Quilcene Bay was estimated above at 86 kg per day (57 to 115 kg per day). The geometric mean fecal coliform density in harbor seals was 3.1×10^7 fecal coliforms per gram.

Table 9. Fecal coliform concentrations per gram found in the species of humans and various animals as reported in the literature.

Fecal source	Geldreich (1976)		Mara and Oragui (1981)			
	# samples	Median	# samples	Geometric mean	Min	Max
Human	43	1.3×10^7	18	6.3×10^7	1.3×10^5	9.0×10^9
Cattle	11	2.3×10^5	8	1.0×10^6	1.5×10^5	6.5×10^6
Sheep	10	1.6×10^7	7	2.7×10^6	1.8×10^5	5.6×10^7
Pig	11	3.3×10^6	8	3.9×10^7	4.9×10^6	6.0×10^8
Horse	-	1.3×10^4	5	1.0×10^3	6.3×10^2	3.4×10^3
Duck	8	3.3×10^7	5	2.0×10^7	8.8×10^6	4.9×10^7
Chicken	10	1.3×10^6	9	6.3×10^6	3.7×10^6	1.5×10^7
Turkey	10	2.9×10^5	6	7.9×10^8	2.6×10^8	2.0×10^9
Goose	-	-	3	6.3×10^3	9.7×10^2	6.6×10^4
Cat	19	7.9×10^6	5	6.3×10^7	8.9×10^4	2.6×10^9
Dog	24	2.3×10^7	5	5.0×10^8	4.1×10^6	4.3×10^9
Rabbit	14	2.0×10^1	4	1.0×10^4	2.8×10^3	4.9×10^4
Mouse	7	3.3×10^5	4	6.3×10^6	4.7×10^6	1.0×10^7
Rat	2	1.8×10^5	4	1.6×10^5	5.6×10^4	6.3×10^5
Gull	-	-	6	6.3×10^3	1.7×10^2	2.7×10^5
Chipmunk	3	1.5×10^5	-	-	-	-
Elk	32	5.1×10^3	-	-	-	-
Robin	-	2.5×10^4	-	-	-	-
English sparrow	-	2.5×10^4	-	-	-	-
Starling	-	1.0×10^4	-	-	-	-
Red-winged blackbird	-	9.0×10^3	-	-	-	-
Pigeon	-	1.0×10^4	-	-	-	-

This would translate to 2.7×10^{12} (1.8 to 3.6×10^{12}) fecal coliforms per day generated by the harbor seals that use Quilcene Bay. This figure represents the theoretical maximum contribution of harbor seals.

Two primary factors would serve to reduce the actual contribution of harbor seals to the fecal coliform levels found in the water column in Quilcene Bay. First, the seals that use Quilcene Bay for hauling out likely do not spend all their time inside Quilcene Bay and therefore only a portion of the fecal contribution estimated above is excreted inside the Bay. Second, only a portion of the fecal material excreted by harbor seals would likely become dissolved in the water column. Significant portions of fecal material would be expected to immediately settle out and not dissolve in the water column. The unknown magnitude of these factors confounds comparisons between inputs from seals and those from other sources.

The distribution of fecal coliform pollution in Quilcene Bay can be compared to the likely distribution of seals in the bay. The northeastern portion of Quilcene Bay was the area decertified because of high fecal coliform levels (Figure 1). Harbor seals were usually seen when they were hauled out at the log dump at the boundary of the decertified area, though some harbor seals were seen in the water in the decertified portion of the bay. The primary food source of harbor seals in Hood Canal, Pacific hake, is likely not abundant in the extremely shallow northern end of Quilcene Bay. Most of the defecation of seals, therefore, likely occurs outside or at the edge of the decertified area and would only be carried into the area by currents.

Comparison to other areas

Several other locations in Puget Sound have large seal concentrations and water quality problems. The situation in Henderson Inlet is somewhat analogous to Quilcene Bay. Up to several hundred seals haul out in Henderson Inlet near the mouth of the inlet. Portions of Henderson Inlet have been decertified due to high fecal coliform concentrations. The portion of Henderson Inlet with the lowest water quality, however, is near the head of the inlet far from the area of highest seal concentration. A study of the causes of water quality problems in Henderson Inlet concluded that urban stormwater runoff, poor animal keeping/pasture management practices, and failing or inadequate on-site sewage systems were responsible for the fecal coliform contamination (Taylor 1984). The issue of potential contributions by seals was not addressed in the study.

Other studies of fecal pollution in Washington State have considered

the potential contributions of wildlife to fecal coliform levels. A study of pollution in Capital Lake at the southern end of Puget Sound concluded waterfowl were a major contributor to the high fecal concentrations found in the Lake (CH2M Hill 1978). Determan et al. (1985), in a study of Minter Bay and Burley Lagoon, Washington, another area with a high winter concentrations of waterfowl, came to the opposite conclusion that waterfowl likely did not significantly contribute to fecal coliform pollution.

CONCLUSIONS

- Up to 230 harbor seals haul out on log booms and oyster rafts in Quilcene Bay.
- Calculated estimates of the fecal production of a typical harbor seal range between 250 and 500 grams per day with 375 grams used for calculations in this study.
- A variety of bacterial species were identified in 10 harbor seal fecal samples, though the pathogens Salmonella or Yersinia were not found in any samples.
- Geometric mean fecal coliform concentrations in 10 seal fecal samples was 3.1×10^7 per gram. This concentration is in the same range as for humans but is higher than most domestic animals.

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