EPA 910/9-86-140

United States
Environmental Protection
Agency

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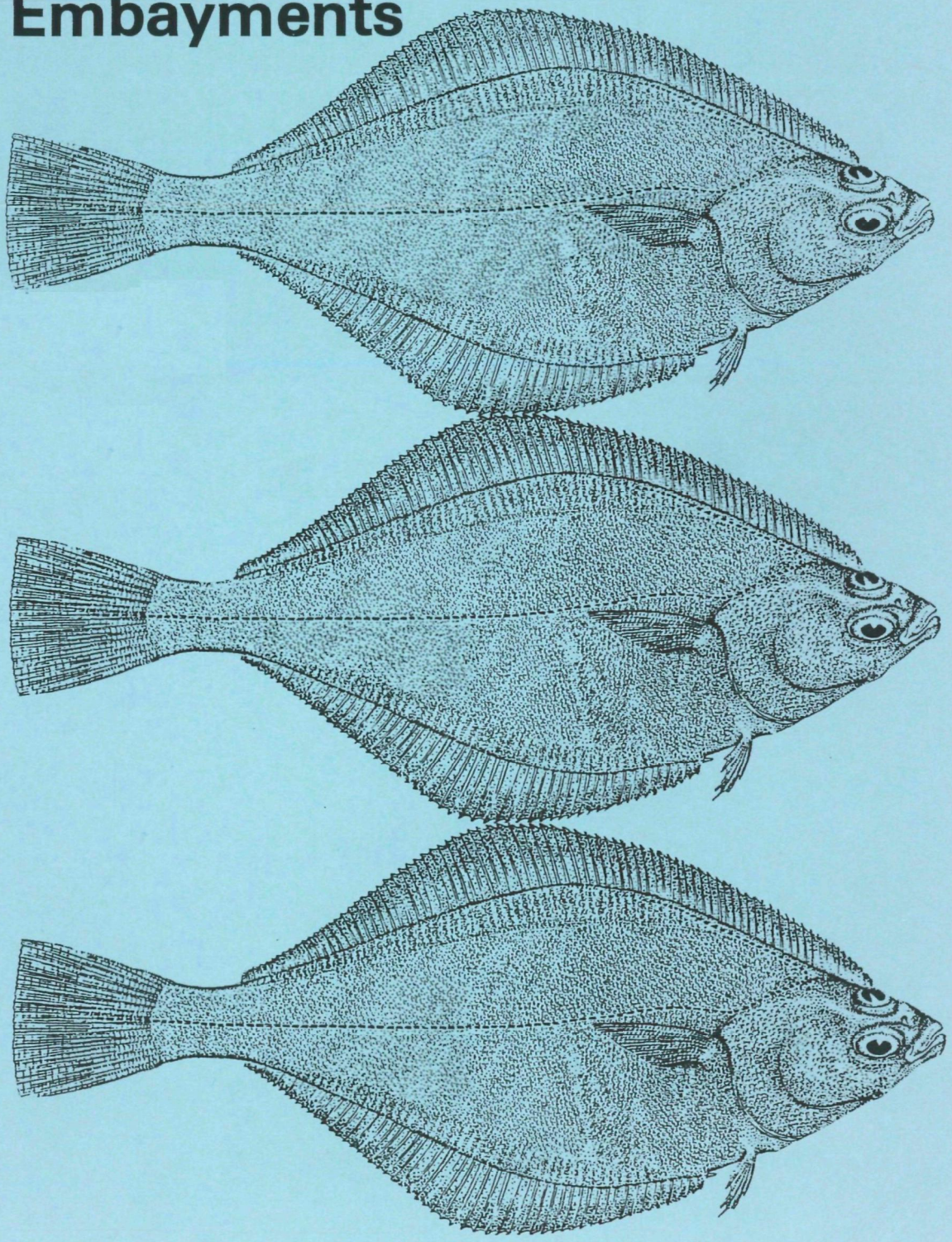
Environmental Services Division

June 1986

EPA

A Comparison Between English Sole Diseases in Commencement Bay and in Selected Nonurban Embayments



EPA-910/9-86-140
June 1986

A COMPARISON BETWEEN ENGLISH SOLE DISEASES IN COMMENCEMENT BAY AND IN SELECTED NONURBAN EMBAYMENTS

by

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SUBMITTED TO:

Environmental Services Division
Region 10
U.S. Environmental Protection Agency
Seattle, Washington 98101

In Partial Fulfillment of
USEPA Interagency Agreement DW13930536-01-1

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CONTENTS

ACKNOWLEDGEMENTS

We wish to thank the following for their participation in this program: W.D. Gronlund, P.K. Emry, W.E. Ames, P.D. Plesha, J.T. Landahl, C.M. Stehr, P.O. Olson, D.R. Craddock, and J.R. Hughes.

INTRODUCTION

Several studies conducted in Commencement Bay since 1979 (Malins et al. 1980, 1982, 1984) identified a number of pollution-associated diseases in English sole (Parophrys vetulus), rock sole (Lepidopsetta bilineata) and Pacific staghorn sculpin (Leptocottus armatus). The organs of fish containing the greatest numbers of such lesion types were the liver, kidney, and gills. These lesions were not associated with parasites or microorganisms, and were defined as idiopathic (i.e. cause not known). Neoplasms constituted a major type of idiopathic liver lesion. Other major types of idiopathic liver lesions were hyperplasia/foci of cellular alteration [which have been reported to be "preneoplastic" in laboratory rodents (Frith and Ward 1980)], specific degeneration/necrosis, and storage disorders (McCain et al. 1982). Fish with hepatic neoplasms were primarily from the waterways of Commencement Bay (i.e., Hylebos and Sitcum Waterways). No tumor-bearing fish (156 examined) were found in nonurban waters (Case Inlet, Port Madison, Port Susan, and Discovery Bay) during 1979 to 1982; however, samplings conducted in 1983 found tumor-bearing English sole (6.7%, 2 of 30 fish) in Port Madison.

High concentrations of a great variety of chemicals were found in sediments from Commencement Bay (Malins et al. 1982, 1984). For example, the mean concentration of polychlorinated biphenyls (PCBs) in sediment was more than 100 times greater in the Hylebos Waterway than in sediment from the nonurban area of Port Madison. Mean concentrations of aromatic hydrocarbons (AHs) were as much as 40 times greater in parts of the

Hylebos Waterway, compared to nonurban areas. Generally, mean concentrations of sediment-associated metals (e.g., lead) were higher in Commencement Bay than in the nonurban areas. The mean concentrations of cadmium, however, were similar in Commencement Bay and nonurban sediments.

The purpose of the present study was to compare the types and prevalences of idiopathic lesions of the liver previously observed in English sole from Commencement Bay with those from selected nonurban embayments. English sole was selected as the target species because of the high prevalences of pollution-related lesions observed in this species, and because this species is widely distributed throughout Puget Sound and adjacent waters.

METHODS AND MATERIALS

English sole were collected from the RV HAROLD W. STREETER with an otter trawl having a 7.5 m opening, a 10.8 m total length, 3.8 cm mesh in the body of the net, and a 0.64 cm mesh liner in the cod end. Individual trawls were for 5 minutes and covered a distance of approximately 0.2 nautical miles. The results of histopathological examinations of English sole are reported for sole captured near Bellingham Bay, just southwest of Eliza Island; Case Inlet, near Stretch and Reach Islands; and Commencement Bay, several sites including Sitcum and Hylebos Waterways (Figure 1 and Table 1). At each sampling station, up to 30 adult individuals of each target species were collected and placed in holding tanks containing fresh seawater. Only individuals greater than 150 mm in total length (of as broad a size distribution as possible) were selected for necropsy.

Fish to be necropsied were measured for total length (mm), weighed

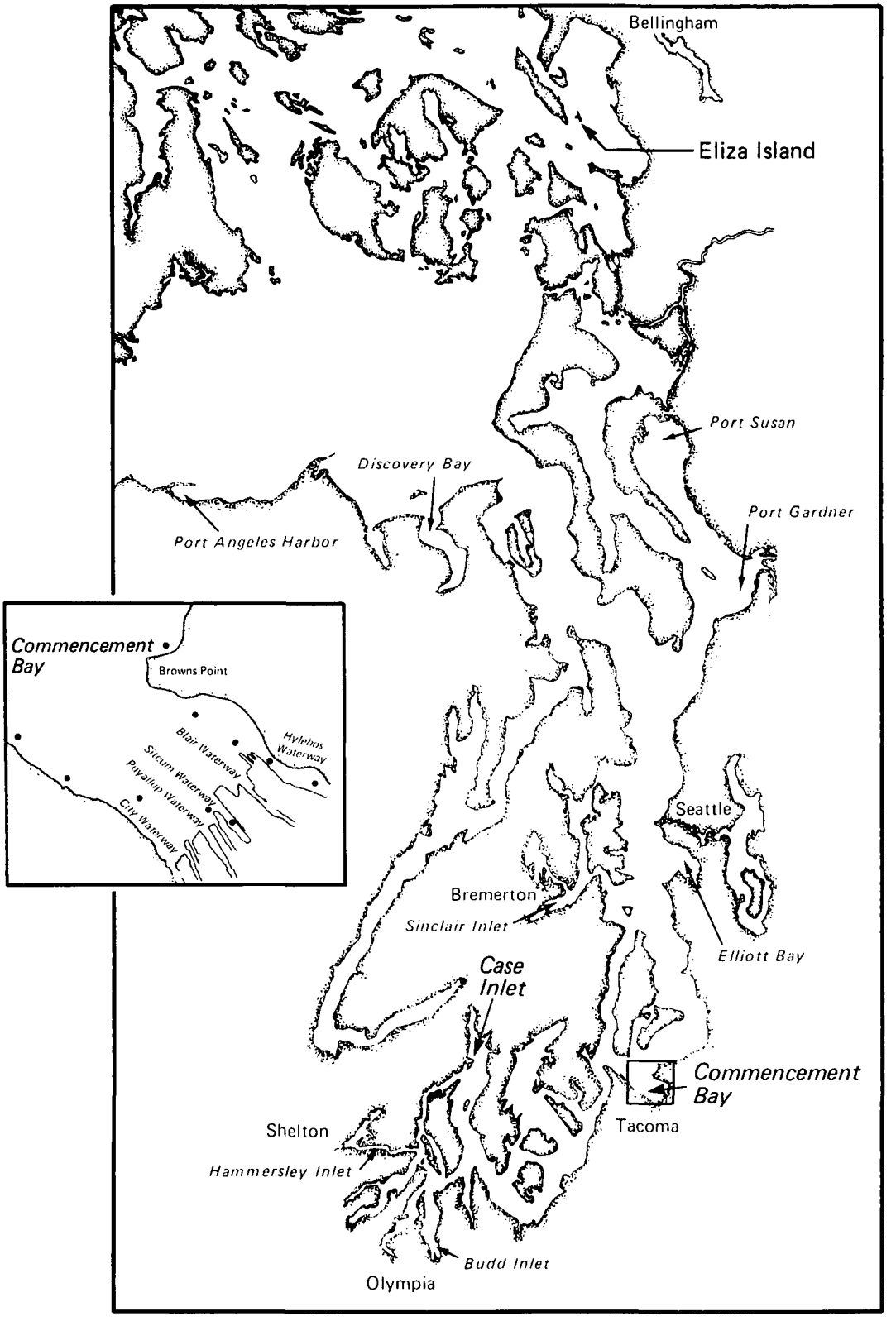

Figure 1. Map showing locations of sampling sites near Eliza Island, in Case Inlet, and in Commencement Bay.

Table 1. Sampling sites, sampling dates and latitude and longitude of the trawl midpoints included in this study.

Eliza Island (4/84)	48°39.1'N	122°33.3'W
Case Inlet (4/84)	47°18.8'N	122°50.3'W
	47°19.5'N	122°47.9'W
	47°20.8'N	122°48.7'W
Case Inlet (4/79)	47°21.2'N	122°47.0'W
	47°18.8'N	122°49.5'W
Sitcum and Hylebos Waterways (4/79)	47°16.2'N	122°22.3'W
	47°16.7'N	122°23.8'W
	47°16.2'N	122°25.0'W
Deepwater Commencement Bay (4/79)	47°17.1'N	122°25.0'W
	47°17.5'N	122°25.2'W
	47°17.3'N	122°28.7'W
	47°18.5'N	122°30.2'W
	47°18.7'N	122°26.1'W
	47°16.7'N	122°27.5'W
	47°16.2'N	122°26.9'W

(g) and assigned an individual field number. The gender was determined and tissues were excised from the liver, kidney, and gill. Any grossly visible lesions were noted, recorded, and tissue specimens were collected. Tissues were placed in labeled cassettes and immersed immediately in Dietrich's fixative (Malins et al. 1980).

Tissue specimens preserved in fixative for a minimum of 48 hours were dehydrated and paraffin-infiltrated with an automated tissue processor, embedded in paraffin and sectioned at a thickness of 5-6 um. Sections were routinely stained with Mayer's hematoxylin and eosin-phloxine (AFIP 1968). All slides were examined by light microscopy employing a "blind" system, in which the examining histopathologist had information only on the species, length, weight, sex and description of grossly visible lesions, with no knowledge of the location from which the fish was collected. Diagnoses were then performed using the lesion categories described in Malins et al. (1982). The diagnostic information was coded on National Ocean Data Center (NODC) File type 13, and placed on computer disk along with information on other biological characteristics of each fish. Data management and lesion prevalence analyses were performed utilizing Minitab version 81.1 (Ryan et al. 1981) and SPSS version 9.1 (Nie et al. 1975) on a Burroughs 7800 computer.

For comparisons of lesion prevalences, the specimens were first classified into 5 size classes (adjusted for gender) roughly corresponding to age classes 1+, 2+, 3+, 4+ and $\geq$5+. The size class limits were determined from size distributions of each age-class by gender of English sole from a variety of sites in Puget Sound (n=1004). In order to minimize the influence of seasonal differences in liver,

kidney and gill lesion prevalences, only lesion prevalence data for fish collected in the spring were compared. Lesion prevalence data was then computed for 5 sites (sampling month and year in parentheses): (1) Eliza Island (March 1984); (2) Case Inlet (March 1984); (3) Case Inlet (April 1979); (4) combined Sitcum and Hylebos Waterways (April 1979); and (5) combined deeper water Commencement Bay sites (April 1979). Comparisons were made by computation of the G-value from the natural logarithms of the lesion frequencies and comparison with appropriate chi-square values. Since 4 multiple tests were required for each organ (i.e., 4 major lesion categories were considered for each organ), the critical p-value was adjusted to 0.0125 for all G-tests to reduce Type I error.

RESULTS AND DISCUSSION

Although the original design of this study called for collecting English sole from four nonurban embayments in or near Puget Sound, (Sequim Bay, Dabob Bay, Samish Bay and Case Inlet), sole were not collected from three of these areas (Sequim Bay, Samish Bay and Dabob Bay) due to a scarcity or absence of adult English sole. Malins et al. (1982) demonstrated that English sole less than 2 years old had significantly ($p \leq 0.05$) lower prevalences of idiopathic liver diseases than fish over 2 years old. For example, liver neoplasms were not detected in males less than 2 years old or in females less than 1 year old. Consequently, our protocol called for sampling English sole >150 mm, and fish from these year classes were absent in these areas. In order to partially compensate for this problem, an additional reference station near Eliza Island (west of Bellingham Bay, Station No. 04015) was sampled, where a sufficient number of adult English sole were collected.

Ten stations in Commencement Bay (Figure 1) were sampled for diseased English sole in April of 1979 (Malins et al. 1982, 1984). Seven of these stations were located around the periphery of the bay at depths between 20 and 150 m. Three of these stations, two in the Hylebos Waterway and the other in the Sitcum Waterway, were located between 10 and 15 m in depth.

Comparisons in this report were made between the prevalence data for the major types of idiopathic liver, kidney, and gill lesions observed in the nonurban embayments sampled in the present study and the prevalence data for those lesions found in Commencement Bay since they were the only organs which displayed significant idiopathic lesions. Data comparisons by site were made for each size class, as well as for all size classes combined.

The only type of hepatic lesions found in sole from near Eliza Island were storage disorders (Table 2). This condition was found in sole from Case Inlet and the Commencement Bay sites, and was previously found in several bottom fish species from both urban and nonurban areas of Puget Sound (Malins, et al. 1984). Storage disorders can be caused by a variety of factors, many of which are unrelated to pollution (e.g. diet, reproductive stage).

The prevalences of hepatic neoplasms (3.3%) and foci of cellular alteration FCA (3.3%) in sole from Case Inlet (Table 2) were not significantly different from the prevalences of neoplasms (3.6%) and FCA (14.3%) found in sole from Sitcum and Hylebos Waterways (3.6%). However, only one individual from Case Inlet had both the liver neoplasm and the FCA. None of these liver lesions were observed in sole from Case Inlet in either April of 1979 (Table 1) or during other sampling

Table 2. Hepatic lesion prevalences and numbers of examined livers by size class and by site for English sole collected in March and April of 1979 and 1984. Size classes 1 through 5 approximately correspond to age classes 1+, 2+, 3+, 4+, and ≥5+, respectively. The G-test was used to determine differences in lesion prevalences among the sites.

	SIZE CLASS					
	1	2	3	4	5	All size classes combined
Number of livers examined						
Eliza Island (1984)	8	19	1	0	0	28
Case Inlet (1984)	6	15	3	2	4	30
Case Inlet (1979)	0	1	0	0	8	9
Sitcum-Hylebos Waterways (1979)	2	2	5	6	13	28
Deep Commencement Bay (1979)	2	5	7	14	22	50
Neoplasms						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	25.0	3.3
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	16.7	0	3.6
Deep Commencement Bay (1979)	0	0	0	0	0	0
Foci of cellular alteration						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	25.0	3.3
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterwats (1979)	0	0	0	33.3	15.4	14.3
Deep Commencement Bay (1979)	0	0	0	0	0	0
Specific degeneration/necrosis						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	0	0
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	33.3	23.1	17.9[H]
Deep Commencement Bay (1979)	0	0	0	0	4.6	2.0
Storage disorders						
Eliza Island (1984)	12.5	5.3	0			7.1
Case Inlet (1984)	16.7	0	0	0	25.0	6.7
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	16.7	7.7	7.1
Deep Commencement Bay (1979)	0	0	0	0	0	0

"H" indicates significantly higher lesion prevalence as determined by G-test, critical p=0.0125

periods of 1979 (n=34). It is difficult to evaluate temporal changes in lesion prevalences in Case Inlet fish, due to the low prevalence values and the relatively small sample sizes.

The absence of specific degenerative lesions of the liver in sole from Case Inlet and near Eliza Island contrasted with the significantly higher prevalence (17.9%) in sole from Sitcum and Hylebos Waterways (Table 2). In previous Puget Sound studies, this lesion type was detected almost exclusively in bottomfish from urban embayments (Malins et al. 1984). Lesions morphologically similar to this lesion type have been induced in mammals and fish exposed to a variety of toxic chemicals under laboratory conditions.

Renal depositional disorders were not found among the fish examined in this study, although these conditions have been found in English sole in other studies (Malins et al. 1982). Degenerative and necrotic kidney lesions and renal inflammations were found at both non-urban and Commencement Bay sites at statistically uniform prevalences (Table 3). Renal proliferative disorders were characterized by high prevalences at the non-urban sites sampled in 1984, and low prevalences at the 1979 sampling of the Commencement Bay sites and Case Inlet. The significance of this result is not clear and such a pattern has not yet been previously reported (Malins et al. 1980, 1982). Possibly a seasonal factor may have a role in the etiology of this lesion since Rhodes et al. (In Preparation) have observed higher prevalences of this condition in sole captured in the summer and fall than sole captured in winter and spring.

Table 3. Renal lesion prevalences and numbers of examined kidneys by size class and by site for English sole collected in March and April of 1979 and 1984. Size classes 1 through 5 approximately correspond to age classes 1+, 2+, 3+. 4+. and ≥5+, respectively. The G-test was used to determine differences in lesion prevalences among the sites.

	SIZE CLASS					
	1	2	3	4	5	All size classes combined
Number of kidneys examined						
Eliza Island (1984)	8	17	1	0	0	26
Case Inlet (1984)	6	15	3	2	4	30
Case Inlet (1979)	0	1	0	0	7	8
Sitcum-Hylebos Waterways (1979)	2	2	5	6	12	27
Deep Commencement Bay (1979)	2	5	7	11	20	45
Depositional disorders						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	0	0
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0
Deep Commencement Bay (1979)	0	0	0	0	0	0
Degeneration/necrosis						
Eliza Island (1984)	0	5.9	0			3.8
Case Inlet (1984)	0	13.3	0	0	0	6.7
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	20.0	33.3	8.3	14.8
Deep Commencement Bay (1979)	0	0	0	0	15.0	6.7
Proliferative disorders						
Eliza Island (1984)	25.0	35.3	0			30.8[H]
Case Inlet (1984)	33.3	20.0	0	0	25.0	20.0
Case Inlet (1979)		0			14.3	0
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0
Deep Commencement Bay (1979)	0	0	0	0	0	0[L]
Inflammations						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	0	0
Case Inlet (1979)		0			0	
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0
Deep Commencement Bay (1979)	0	0	0	0	5.0	2.2

"H" indicates significantly higher lesion prevalence as determined by G-test, critical p=0.0125

"L" indicates significantly lower lesion prevalence as determined by G-test, critical p=0.0125

With the exception of idopathic respiratory inflammations, prevalences of gill lesions were statistically uniform among the sites (Table 4). The prevalence of inflammatory conditions were found to be significantly higher among sole from the non-urban sites and lower among sole from Commencement Bay ($p < 0.0125$). Again, a possible role of season may be involved in lesion prevalence variations.

No statistically significant differences in the prevalences of any of the liver, kidney or gill lesions were found among the size classes for any of the sampling sites; however, no fish in the first 3 size classes had hepatic neoplasm, FCA, or specific degeneration/necrosis. This suggests that differences in prevalence among the sites are not attributable to differential lesion prevalences among the size classes of sole examined.

In conclusion, the number and prevalences of different types of idiopathic liver lesions were generally greater in English sole from Commencement Bay compared to the nonurban areas sampled in this study. Nevertheless, the prevalences of these lesions in Commencement Bay sole are relatively low compared to other polluted areas ("hot spots") in Puget Sound [e.g., the Duwamish Waterway (Malins et al. 1984), Eagle Harbor (Malins et al. 1985), and Mukilteo (Malins et al. 1986)]. In contrast, the prevalences of a renal and a respiratory lesion were greater at the non-urban sites. These types of results underscore the necessity of clarifying lesion etiology if lesions are to be used as indicators of envionmental quality.

Table 4. Respiratory lesion prevalences and numbers of examined gills by size class and by site for English sole collected in March and April of 1979 and 1984. Size classes 1 through 5 approximately correspond to age classes 1+, 2+, 3+, 4+, and >5+, respectively. The G-test was used to determine differences in lesion prevalences among the sites.

	SIZE CLASS					
	1	2	3	4	5	All size classes combined
Number of gills examined						
Eliza Island (1984)	8	17	1	0	0	26
Case Inlet (1984)	6	15	3	2	4	30
Case Inlet (1979)	0	1	0	0	6	7
Sitcum-Hylebos Waterways (1979)	1	2	4	4	10	21
Deep Commencement Bay (1979)	1	3	6	12	17	39
Degeneration/necrosis						
Eliza Island (1984)	0	0	0			0
Case Inlet (1984)	0	0	0	0	0	0
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0
Deep Commencement Bay (1979)	0	0	0	0	0	0
Proliferative disorders						
Eliza Island (1984)	12.5	29.4	0			23.1
Case Inlet (1984)	0	6.7	0	0	0	3.3
Case Inlet (1979)		0			50.0	42.9
Sitcum-Hylebos Waterways (1979)	100	0	0	25.0	60.0	38.1
Deep Commencement Bay (1979)	0	0	0	8.3	17.6	10.3
Vascular disorders						
Eliza Island (1984)	12.5	23.5	0			19.2
Case Inlet (1984)	16.7	0	0	0	25.0	6.7
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0
Deep Commencement Bay (1979)	0	0	0	0	0	0
Inflammations						
Eliza Island (1984)	75.0	58.8	0			61.5^{H}
Case Inlet (1984)	66.7	60.0	33.3	50.0	50.0	56.7^{H}
Case Inlet (1979)		0			0	0
Sitcum-Hylebos Waterways (1979)	0	0	0	0	0	0^{L}
Deep Commencement Bay (1979)	0	0	0	0	0	0^{L}

"H" indicates significantly higher lesion prevalence as determined by G-test, critical p=0.0125

"L" indicates significantly lower lesion prevalence as determined by G-test, critical p=0.0125

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