Implications for Reproductive Health in Rockfish (*Sebastes* spp) from Puget Sound Exposed to Polychlorinated Biphenyls

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Introduction and Methods

Rockfish (*Sebastes* spp) are long-lived demersal predators that are distributed widely in Puget Sound marine habitats, usually associated with rocky substrate (Matthews 1989; Matthews 1990). Because of their longevity[1] and high position in the benthic food chain (see Murie 1995), their probability of exposure to persistent bioaccumulative toxins like polychlorinated biphenyls (PCBs) is high. The Puget Sound Ambient Monitoring Program (PSAMP) has monitored the presence and severity of toxic contaminants, including PCBs, in three closely related *Sebastes* species: quillback rockfish (*Sebastes maliger*), brown rockfish (*S. auriculatus*) and copper rockfish (*S. caurinus*) in Puget Sound from 1989 to the present (West & O'Neill 1995; West & O'Neill 1998). These species are ecologically and economically important in the Pacific Northwest (Schmitt and others 1994), and they have been petitioned for protection under the Endangered Species Act (U.S. Department of Commerce 1999) because of declines in their population abundance in Puget Sound over the last three decades.

We estimated exposure of rockfish to PCBs by measuring PCB concentration (as the sum of Aroclors 1254 and 1260) in their skeletal muscle tissue (skin-off fillets). We sampled muscle tissue taken from 115 individual quillback, brown, and copper rockfish in Puget Sound from 1995 to 1998. We subjectively classified three location types from which rockfish were sampled: (1) highly polluted urban and industrialized habitats, termed “urban” locations (Sinclair Inlet and Elliott Bay), (2) areas in Central Puget Sound relatively near to urban areas (termed “near-urban”), and (3) relatively pristine habitats such as the San Juan Islands and Admiralty Inlet, situated far from urban or industrialized areas (termed “non-urban”).

Transfer of lipophilic toxic compounds such as PCBs from mother-to-egg via lipids or yolk have been reported for other fish species (Niimi 1983; Miller 1993; Miller 1994), providing a mechanism for reduction of PCB concentration in females. Related to this, we hypothesized that female rockfish from contaminated habitats should accumulate PCBs at a slower rate than males. In this study we compared accumulation patterns of 115 rockfish to PCBs among three location types (urban, near-urban, and non-urban) and between the sexes, while accounting or adjusting for the effects of variability in fish age.

We also present results of a pilot study investigating potential reproductive impairment of male rockfish (effects) related to their exposure to hormone-mimicking toxics, a group of contaminants that may include some PCBs. We sampled blood serum from eleven male quillback rockfish taken from Elliott Bay in 1998 for the presence of vitellogenin. Vitellogenin is a lipoprotein normally associated with the production of eggs in female fish (DeVlaming et al. 1984), and is normally not found in wild male fish. The presence of vitellogenin in the blood serum of male gonochoristic[2] fish is considered an indicator of exposure to an exogenous estrogenic hormone or hormone-mimicking contaminant (see Lomax and others in press, this conference). When such compounds enter the male body they can function as the reproductive hormone ∃-estradiol, triggering the production of vitellogenin. This process of feminization can cause significant disruption in normal reproductive physiology.

[1] Age of oldest rockfish collected by PSAMP was estimated at 60 years.
[2] Separate sexes – no hermaphroditism

Results and Discussion

Exposure

PCBs (either as Aroclor 1254 or 1260) were detected in 100% of urban rockfish, 97.4% of near-urban rockfish, and 6.7% of non-urban rockfish (West & O'Neill 1998; this study). Rockfish of both sexes exhibited increasing PCB concentrations from non-urban to near-urban to urban conditions (Table 1). In addition, male rockfish exhibited greater PCB concentrations than females in urban and near-urban locations (Table 1). PCBs were rarely detected in either sex from non-urban locations.

Persistent toxics like PCBs can accumulate with age in males of long-lived marine vertebrates such as orcas (Ross and others 2000), pinnipeds (Addison and others 1973, Addison and Smith 1974), and baleen whales (Aguilar and Borrell 1988), although some researchers have shown no relationship between age and PCBs in short-lived marine fishes (von Westernhagen and others 1995) or even a decrease in PCBs with fish age (Pastor and others 1996). Previously, we reported a positive correlation between age and PCBs in male rockfish (West and O’Neill 1998), and therefore treat fish age as an important source of potential variability in PCB concentrations in the groups we tested. Age of rockfish varied among the six groups as well (Table 1). The group with the greatest PCB concentration (urban males) was also the oldest at 18.8 years. Others ranged from 11.7 to 15.8 years.

Table 1. Mean concentration, sample size (n) and standard deviation (s.d.) of PCBs (sum of Aroclors 1254 and 1260, µg/kg, wet weight) in 115 skinned, skeletal muscle tissue of individual quillback, brown, and copper rockfish sampled by the PSAMP Fish Component, 1995-1998. Mean age estimated in years (age missing for one sample).

		Urban	Near Urban	Non Urban	Combined
Male	Mean	211.84	52.61	4.9	99.9
	N	25	21	19	65
	s.d.	149.60	31.67	2.69	130.5
	mean age	18.8	11.7	14.5	15.2
Female	Mean	93.98	39.33	4.0	54.5
	N	21	18	11	50
	s.d.	52.03	39.37	0.00	54.5
	mean age	15.7	15.8	13.9	15.3
Combined	Mean	158.04	46.48	4.6	80.2
	N	46	39	30	115
	s.d.	129.08	35.58	2.17	106.5
	mean age	17.4	13.5	14.3	15.3

We can account for effects of fish age in our study either by sampling fish of equal ages across the six groups, or by controlling for age statistically, factoring out age effects using analysis of variance (ANOVA) with an age covariate (ANCOVA). Although ANCOVA is the preferred analysis (since it can compare age-adjusted means among all six groups) it also requires the condition of equal slopes in the correlations of PCBs and fish age across the six groups. In our case, PCB concentration (as ln-PCB) was significantly correlated with fish age in only two of the six groups; urban males (linear regression of ln-PCBs by fish age, $p=0.011$, $r^2=0.25$), and near-urban males ($p<0.001$, $r^2=0.58$). This precludes using the ANCOVA for comparing all six groups together, but also suggests that fish age is not an important consideration when comparing PCB concentration in females across location types. This means that our test of PCB concentration across location types can proceed without the confounding effects of variation in fish age for females, but age still needs to be accounted for in males.

The following describes three analyses, using different approaches to incorporate age-effects, depending on the nature of the relationships we observed between age and PCB concentration.

PCBs in Females Across Location Type: As mentioned above, comparison of PCB concentration among location types is not confounded by age, because ages of females were not different among the location types, and there was no significant correlation between age and PCBs. With that, PCB concentration in female rockfish was significantly different between all location types, with urban>near-urban>non-urban (ANOVA of ln-PCB by location type, $p<0.001$, bonferroni *post hoc* pairwise comparison). This pattern suggests that even though PCB concentration did not increase with age in female rockfish, their PCB burden still reflected their environmental condition.

PCBs in Males Across Location Type: In order to test the significance of PCB patterns involving male rockfish, we still need to account for the effects of fish age. This is especially important for the urban and near-urban location types, because it is from these locations where we observed PCBs increasing with fish age (described above). In addition, urban males were significantly older (mean of 18.8 years) than near-urban males (mean age 11.7 years, ANOVA of age by location type, $p=0.014$, bonferroni *post hoc* pairwise test). Ages of urban and near-urban males were not significantly different than non-urban males, so non-urban males were excluded from further analysis. In order to compare PCB concentration between urban and near-urban males, we removed the effects of age by using ANCOVA to adjust their geometric mean PCB concentrations to a common age (15.5 years). With this adjustment, the geometric mean PCB concentration in urban males (136.0 µg/kg) was still significantly greater than that from near-urban males (56.4 µg/kg).

PCBs in Males vs. Females: PCB concentrations in male rockfish were significantly greater that females in both urban ($p<0.001$) and near urban locations ($p=0.049$) and there was no difference in PCBs between the sexes from non-urban locations ($p=0.285$, ANOVA, ln-PCB by sex for each location type). These differences were probably not influenced by age because any age differences we observed between the sexes were not significant for all three location types ($p=0.115$ for urban, $p=0.053$ for near-urban, and $p=0.870$ for non-urban rockfish, ANOVA of age by sex applied separately for each location type).

In summary, there was a clear trend of increasing PCB concentration with degree of urbanization for both male and female rockfish that was unrelated to fish age. In addition PCBs increased with age in males but not females, supporting the notion that female rockfish possess the means to depurate PCBs. We suggest the most likely means of this depuration is transfer of PCBs to larvae during reproduction (as hypothesized above). Males do not have this mechanism, so PCBs continue to accumulate in their bodies throughout their lives. This disparity further suggests a need for different approaches when evaluating the potential effects of exposure to lipophilic toxics like PCBs. The most logical and efficient approach would focus on groups that experience the greatest exposure (e.g., urban male rockfish), and the most likely and easiest-observed indicators of adverse effects.

PCBs were rarely detected (average detection limit of 4.6 µg/kg) in males or females from non-urban areas, suggesting that PCB exposure of non-urban rockfish was minimal.

Effects

There are no rockfish-specific PCB threshold data available to determine whether the concentrations we observed are likely to adversely affect rockfish health. However, a recent review of PCB dose-response studies of salmonids (Meador, 2000) indicates that concentrations we commonly observed in rockfish exceeded concentrations shown to cause adverse sublethal effects[3] in trout, salmon, and char. Meador (2000) reported a threshold of 140 µg/kg PCBs (whole-body wet weight), “above which juvenile salmon would be expected to exhibit adverse sublethal effects from accumulated PCBs.” Nineteen of our 115 rockfish (16.5%) exceeded this threshold, while more than half of urban males exceeded the threshold.

At least four issues arise when applying dose-response thresholds from the Meador (2000) study to rockfish that prompt discussion.

(1) PCB concentrations measured in salmonids were from whole bodies, while we sampled skin-off rockfish muscle tissue. However, based on comparisons of filet and whole body from lake trout and salmon (Amrhein and others 1999), we argue that PCB concentrations in muscle tissue will underestimate the whole body condition.

(2) Some of the effects-endpoints included in the Meador (2000) study are specific to salmonids and therefore are not applicable to rockfish. However, there is no reason to think that the majority of these endpoints (e.g., growth, reproduction, mortality, enzyme activity, immunodeficiencies, and enzyme effects) would not apply to other fish species challenged with PCB exposure.

(3) Lipid concentrations in salmonids from the Meador (2000) studies (mean 7.5%) were roughly eight times the average lipid concentration of our rockfish (mean 0.94%). Because PCBs are lipophilic, they may partition unevenly throughout the body. However, the commonly used ratio approach of lipid normalization to factor out presumed lipid effects can result in spurious conclusions (Hebert & Keenleyside 1995). The difference in lipid concentrations and tissue-type is a problem that remains difficult to resolve. The ANCOVA approach advocated by Hebert and Keenleyside works well, however their approach requires demonstrating a clear model of the relationship between PCBs and lipids for all groups, which we lack in both salmonids from the Meador (2000) report as well as from our data. However, one can argue that because rockfish muscle is leaner than salmonid tissue, fewer total PCB molecules are partitioned in lipids, and therefore more available in tissues or organs to cause damage.

(4) Life histories and ecology of salmonids and rockfish are different. Certainly these differences could cause increased or decreased susceptibility to PCB dosing, which is a fertile field for further study.

Vitellogenesis in Male Rockfish

We detected vitellogenin in two of 11 male rockfish in 1998 from Elliott Bay, one of our most highly contaminated locations. This, combined with similar results for English sole in Puget Sound presented by Lomax and others in this conference, suggests that fish living in urban or industrialized conditions are exposed to exogenous estrogens or estrogen mimics. Further, as mentioned previously, presence of vitellogenin in males is evidence that reproductive function of these males has likely been altered. It should be noted that an alternative cause for the presence of vitellogenin in male fish is the natural condition of intersex, or hermaphroditism. However, rockfish are generally considered to be gonochoristic (i.e., single sex (Moser 1967a; Moser 1967b), which would make natural vitellogenesis in males unlikely. We determined the sex of our fish visually in the field with a gross examination of gonads. It is possible that rockfish have naturally occurring ovarian tissue not recognizable in a gross examination. Gonads from the 11 fish examined in this study were confirmed as testes by histology, and we plan to compare gonad conditions of urban rockfish with rockfish sampled from unpolluted habitats.

The PCB exposure we observed in rockfish may or may not be related to our observations of vitellogenin in male rockfish. Aroclors were not measured in these fish samples, however total PCBs as measured by a high performance liquid chromatography technique (Krahn and others 1994) were relatively low in these two samples (not detailed in this report). Indeed there are conflicting data on the estrogenicity of various PCB congeners and their metabolites (Gierthy and others 1997; Ramamoorthy and others 1999; Kester and others 2000). This suggests that although rockfish in Elliott Bay were exposed to an exogenous source of estrogen or estrogen-mimic, PCBs were likely not the cause of vitellogenesis.

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[3] These effects included increased enzyme activity, altered thyroid hormones, decreased LC50 to toxics, increased thyroid activity, increased nephrosis and hepatocytes, increased fin erosion and altered liver lipids, decreased growth, and decreased vitellogenin production in females.

The PCB results detailed in this report, combined with PSAMP results on other contaminants in rockfish and other species illustrate a pattern exposure of urban fish in Puget Sound to a variety of contaminants, including mercury, organo-chlorinated pesticides, and polycyclic aromatic hydrocarbons like benzo(a)pyrene, naphthalene, and phenanthrene (Puget Sound Water Quality Authority 1992; Puget Sound Water Quality Authority 1993; O'Neill and others 1995; O'Neill and others 1998; Puget Sound Water Quality Action Team 1998; Puget Sound Water Quality Action Team 2000); also see (O'Neill & West, in press, this conference). Some of these contaminants, such as o,p'-DDTs (Donohoe & Curtis 1996; Christiansen and others 2000), as well as others associated with urban runoff or sewage not monitored in fish by PSAMP, have been implicated in vitellogenesis in male fish. In our study, PCBs may well be considered an indicator of exposure to other such contaminants.

We propose that further research be conducted into reproductive effects associated with exposure of male rockfish in Puget Sound's urban habitats to exogenous estrogen-mimics. In addition, although not tested in this study, reproduction in female fish may be disrupted under these contaminant-conditions (Johnson and others 1988; Johnson & Casillas 1991; Johnson and others 1991; Ashfield and others 1998; Celius & Walther 1998; Metcalfe and others 2000), and the transfer of lipophilic toxics may adversely affect the health of their offspring (Metcalfe and others 2000). Because the latter effects occur at a sensitive stage of development in the life cycle, we also propose investigation of this pathway for potential adverse effects.

Literature Cited

Addison, R. F., S. R. Kerr, J. Dale, and D. E. Sergeant. 1973. Variation of organochlorine residue levels with age in Gulf of St Lawrence harp seals (*Pagophilus groenlandicus*). Journal of the Fisheries Research Board of Canada **30**:595-600.

Addison, R. F., and T. G. Smith. 1974. Organochlorine residue levels in Arctic ringed seals: variation with age and sex. Oikos **25**:335-337.

Aguilar, A., and A. Borrell. 1988. Age- and sex-related changes in organochlorine levels in fin whales (*Balaenoptera physalus*) from the eastern North Atlantic. Marine Environmental Research **25**:195-211.

Amrhein, J. F., C. A. Stow, and C. Wible. 1999. Whole-fish versus filet polychlorinated-biphenyl concentrations: an analysis using classification and regression tree models. Environmental Toxicology and Chemistry **18**:1817-1823.

Ashfield, L. A., T. G. Pottinger, and J. P. Sumpter. 1998. Exposure of female juvenile rainbow trout to alkylphenolic compounds results in modifications to growth and ovosomatic index. Environmental Toxicology and Chemistry **17**:679-686.

Celius, T., and B. T. Walther. 1998. Differential sensitivity of zonagenesis and vitellogenesis in Atlantic salmon (*Salmo salar* L) to DDT pesticides. Journal of Experimental Zoology **281**:346-353.

Christiansen, L., K. Pedersen, S. Pedersen, B. Korsgaard, and P. Bjerregaard. 2000. *In vivo* comparison of xenoestrogens using rainbow trout vitellogenin induction as a screening system. Environmental Toxicology and Chemistry **19**:1867-1874.

DeVlaming, V., R. FitzGerald, G. Delahunty, J. J. Cech, Jr., K. Selman, and M. Barkley. 1984. Dynamics of oocyte development and related changes in serum estradiol-17b, yolk precursor, and lipid levels in the teleostean fish (*Leptocottus armatus)*. Comp. Biochem. Physiol. **77A**:599 -610.

Donohoe, R. M., and L. R. Curtis. 1996. Estrogenic activity of chlordecone, o,p'-DDT and o,p'-DDE in juvenile rainbow trout: Induction of vitellogenesis and interaction with hepatic estrogen binding sites. Aquatic Toxicology **36**:31-52.

Gierthy, J. F., K. F. Arcaro, and M. Floyd. 1997. Assessment of PCB estrogenicity in a human breast cancer cell line. Chemosphere **34**:1495-1505.

Hebert, C. E., and K. A. Keenleyside. 1995. To normalize or not to normalize? Fat is the question. Environmental Toxicology and Chemistry **14**:801-807.

Johnson, L. L., and E. Casillas. 1991. The use of plasma parameters to predict ovarian maturation sage in English sole *Parophrys vetulus* Girard. Journal of Experimental Marine Biology and Ecology **151**:257-270.

Johnson, L. L., E. Casillas, T. K. .Collier, B. B. McClain, and U. Varanasi. 1988. Contaminant effects on ovarian development in English sole *(Parophrys vetulus)* from Puget Sound, Washington. Canadian Journal of Fisheries and Aquatic Sciences **45**:2133-2146.

Johnson, L. L., E. Casillas, M. S. Myers, L. D. Rhodes, and O. P. Olson. 1991. Patterns of oocyte development and related changes in plasma 17-b estradiol, vitellogenin, and plasma chemistry in English sole *Parophrys vetulus* Girard. Journal of Experimental Marine Biology and Ecology:161-185.

Kester, M. S., S. Bulduk, D. Tibboel, W. Meinl, H. Glatt, C. N. Falany, M. W. Coughtrie, A. Bergman, S. H. Safe, G. G. Kuiper, A. G. Schuur, A. Brouwer, and T. J. Visser. 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology **141**:1897-1900.

Krahn, M. M., G. M. Ylitalo, J. Buzitis, C. A. Sloan, D. T. Boyd, S.-L. Chan, and U. Varanasi. 1994. Screening for planar chlorobiphenyl congeners in tissues of marine biota by high performance liquid chromatography with photodiode array detection. Chemosphere **29**:117-139.

Lomax, D. P., L. L. Johnson, W. T. Roubal, J. E. West, and S. M. O'Neill. in press. Should we be concerned about estrogenic compounds in Puget Sound Waterways? Adult male english sole collected near urban areas may provide the answer (poster). *in* Puget Sound Research 20001. Puget Sound Water Quality Action Team, Meydenbauer Center, Bellevue, WA.

Matthews, K. R. 1989. A comparative study of habitat use by young-of-the-year, subadult, and adult rockfishes on four habitat types in central Puget Sound. Fisheries Bulletin **88**:223-239.

Matthews, K. R. 1990. An experimental study of the habitat preferences and movement patterns of copper, quillback and brown rockfishes (*Sebastes* spp.). Environmental Biology of Fishes **29**:161-178.

Meador, J. P. 2000. An analysis in support of tissue and sediment based threshold concentration of polychlorinated biphenyls (PCBs) to protect juvenile salmonids listed by the Endangered Species Act. "white paper" Environmental Conservation Division, Northwest Fisheries Science Center National Marine Fisheries Service, NOAA, Seattle, WA.

Metcalfe, T. L., C. D. Metcalfe, Y. Kiparissis, A. J. Niimi, C. M. Foran, and W. H. Benson. 2000. Gonadal development and endocrine responses in Japanese medaka (*Oryzias latipes*) exposed to o,p'-DDT in water or through maternal transfer. Environmental Toxicology and Chemistry **19**:1893-1900.

Miller, M. A. 1993. Maternal transfer of organochlorine compounds in salmonines to their eggs. Canadian Journal of Fisheries and Aquatic Sciences **50**:1405-1413.

Miller, M. A. 1994. Organochlorine concentration dynamics in Lake Michigan chinook salmon (*Oncorhynchus tshawytscha*). Archives of Environmental Contamination and Toxicology **27**:367-374.

Moser, H. G. 1967a. Reproduction and development of *Sebastodes paucispinis* and comparison with other rockfishes off southern California. Copeia **4**:773-797.

Moser, H. G. 1967b. Seasonal histological changes in the gonads of *Sebastodes paucispinis* (Ayres), an ovoviparous teleost (family scorpaenidae). Journal of Morphology **123**:329-353.

Murie, D. J. 1995. Comparative feeding ecology of two sympatric rockfish cogeners, *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish). Marine Biology **124**:341-341.

Niimi, A. J. 1983. Biological and toxicological effects of environmental contaminant in fish and their eggs. Canadian Journal of Fisheries and Aquatic Sciences **40**:306-312.

O'Neill, S. M., and J. E. West. in press. Toxic contaminants in Pacific herring (*Clupea pallasi*) from Puget Sound, Washington. *in* Puget Sound Research 20001. Puget Sound Water Quality Action Team, Meydenbauer Center, Bellevue, WA.

O'Neill, S. M., J. E. West, and J. C. Hoeman. 1998. Spatial trends in the concentration of polychlorinated biphenyls (PCBs) in chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in Puget Sound and factors affecting PCB accumulation: results from the Puget Sound Ambient Monitoring Program. Pages 948 *in* R. Strickland, editor. Puget Sound Research '98 Proceedings. Puget Sound Water Quality Action Team, Seattle, WA.

O'Neill, S. M., J. E. West, and S. Quinnell. 1995. Contaminant monitoring in fish: overview of the Puget Sound Ambient Monitoring Program Fish Task. Pages 1038 *in* E. Robichaud, editor. Puget Sound Research '95 Proceedings. Puget Sound Water Quality Authority, PO Box 40900, Olympia, WA 98504-0900, Meydenbauer Center , Bellevue, Washington.

Pastor, D., J. Boix, V. Fernandez, and J. Albaiges. 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus*, *Mugil cephalus* and *Dicentrarcus labrax*). Marine Pollution Bulletin **32**:257-262.

Puget Sound Water Quality Action Team. 1998. 1998 Puget Sound Update: sixth report of the Puget Sound Ambient Monitoring Program. Annual Report Puget Sound Water Quality Action Team, Olympia, WA.

Puget Sound Water Quality Action Team. 2000. 2000 Puget Sound Update: seventh report of the Puget Sound Ambient Monitoring Program. Annual Report Puget Sound Water Quality Action Team, Olympia, WA.

Puget Sound Water Quality Authority. 1992. 1992 Puget Sound Update: Third report of the Puget Sound Ambient Monitoring Program. Annual Report Puget Sound Water Quality Authority, Olympia, WA.

Puget Sound Water Quality Authority. 1993. 1993 Puget Sound Update: fourth report of the Puget Sound Ambient Monitoring Program. Annual Report Puget Sound Water Quality Authority, Olympia, WA.

Ramamoorthy, K., M. S. Gupta, G. Sun, A. McDougal, and S. H. Safe. 1999. 3,3'4,4'-Tetrachlorobiphenyl exhibits antiestrogenic and antitumorigenic activity in the rodent uterus and mammary cells and in human breast cancer cells. Carcinogenesis **20**:115-123.

Ross, P. S., G. M. Ellis, M. G. Ikonomou, L. G. Barrett-Lennard, and R. F. Addison. 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: effects of age, sex and dietary preference. Marine Pollution Bulletin **40**:504-515.

Schmitt, C., J. Schweigert, and T. P. Quinn. 1994. Anthropogenic influences on fish populations of the Georgia Basin. Pages 390 *in* R. C. H. Wilson, R. J. Beamish, F. Aitkins, and J. Bell, editors. Review of the marine environment and biota of Strait of Georgia, Puget Sound and Juan de Fuca Strait: Proceedings of the BC/Washington Symposium on the Marine Environment, Jan 13 & 14, 1994. Can. Tech. Rep. Fish. Aquat. Sci.

U.S. Department of Commerce. 1999. Listing endangered and threatened species and designating critical habitat: petition to list eighteen species of marine fishes in Puget Sound, Washington. **Federal Register Vol. 64**:30337-33040.

von Westernhagen, H., P. Cameron, D. Janssen, and M. Kerstan. 1995. Age and size dependent chlorinated hydrocarbon concentrations in marine teleosts. Marine Pollution Bulletin **10**:655-659.

West, J. E., and S. M. O'Neill. 1995. Accumulation of mercury and polychlorinated biphenyls in quillback rockfish (*Sebastes maliger*) from Puget Sound Washington. Pages 1038 *in* E. Robichaud, editor. Puget Sound Research '95 Proceedings. Puget Sound Water Quality Authority, Bellevue, WA.

West, J. E., and S. M. O'Neill. 1998. Persistent pollutants and factors affecting their accumulation in rockfishes (*Sebastes* spp) from Puget Sound, Washington. Pages 948 *in* R. Strickland, editor. Puget Sound Research '98 Proceedings. Puget Sound Water Quality Action Team, Seattle, WA.