CHAPTER 5

Biological and Ecological Effects

5.1 Introduction

In this chapter, we present our assessment of the biological and ecological effects of anthropogenic semi-volatile organic compounds (SOCs), metals, and fixed nitrogen from atmospheric sources in national parks, preserves, monuments, and wilderness areas of the western United States. We begin with an assessment of the accumulation, magnification, and biological effects of contaminants in WACAP matrices (Section 5.2). We provide evidence for the *bioaccumulation* of SOCs and mercury (Hg) in fish and *biomagnification* of SOCs from the atmosphere to vegetation, and from lake water (and snow) to fish. For fish, abnormal endocrine responses in male fish, such as elevated estrogen-responsive protein, and the appearance of eggs in testes, suggest exposure to contaminants having estrogenic effects. Immune system reactions to stress, e.g., the density of macrophage aggregates (accumulations of immune cells), were also related to contaminant concentrations. These sub-lethal effects on fish could be related to contaminants, but other stressors cannot be ruled out with the current dataset.

Later in the chapter (Section 5.4), we discuss the potential ecological effects of current contaminant concentrations on the food web, including some piscivorous birds and mammals. We compare current contaminant concentrations in fish to EPA contaminant health thresholds to evaluate the potential human health effects of fish consumption for sensitive populations or for subsistence and recreational fishing in the parks.

Parks with enhanced nitrogen deposition are identified and the predictive values of various nitrogen indicators on SOC concentrations in vegetation are explored. Overall, the extent and effects of contamination and perturbation are demonstrated, focusing on the watershed level. The extremely diverse ecosystems at each national park are taken into account through identification of park-specific contaminants associated with measured or potential adverse effects on biological and ecological resources or visitor health.

5.2 Bioaccumulation and Biomagnification

5.2.1 Processes of Bioaccumulation and Biomagnification

The chief concerns regarding anthropogenic contamination of aquatic ecosystems are the processes of bioaccumulation and biomagnification of pollutants and metals. *Bioaccumulation* is the overall increase in contaminants in biota, compared to that of water, through time (Gobas and Morrison, 2000). This phenomenon depends on the rates of contaminant accumulation in the biota and the rates of metabolism of contaminants and eventual excretion, or other loss, from the biota. Chemicals not readily excreted from biota are most subject to bioaccumulation. They are often stored in body fat (lipophilic), and are often bio-active (mimic or stop the natural chemicals that control body systems). This is not to say that only lipophilic compounds bioaccumulate. Many metals bioaccumulate, but they are not necessarily lipophilic. *Biomagnification* is the overall increase in the contaminant concentrations beyond what is stored in food (Gobas and Morrison, 2000). For example, we can observe fairly low concentrations of contaminants in

aquatic vegetation or phytoplankton, the organisms at the lowest trophic levels. The process of biomagnification is the increase in contaminant concentrations that occur in biota that ingest the vegetation, such as snails (*Gastropoda*). Subsequently, contaminants increase again in the fish that eat the snails, and in the birds or mammals (including humans) that eat the fish. Therefore, fairly low input of environmental contaminants to ecosystems can lead to high concentrations in the biota at the upper trophic levels. For clarification, *bioconcentration* is the uptake of chemicals from the water, and *bioavailability* refers only to the truly dissolved, bio-reactive fraction of any given chemical (Gobas and Morrison, 2000).

The relative solubility of chemicals in water or octanol (octanol-water partition) dictates, in part, how readily chemicals bioaccumulate. In general, octanol-water coefficients (K_{ow}) ranging from 4 to 7 have the greatest chance for bioaccumulation (Thomann, 1989). However, bioaccumulation also depends on biological and environmental factors that regulate the uptake and excretion of contaminants in biota (Mackay and Fraser, 2000). For example, toxico-kinetics of individual contaminants and chemical mixtures influence the bioavailability of contaminants to fish (van der Oost et al., 2003). The diet of the fish (food intake) is the main uptake mechanism for bioaccumulative compounds, whereas the metabolism of, and the individual organ sensitivities to, pollutants dictates how or if the contaminants are excreted from the biota. In addition, bioconcentration of contaminants from the water itself is another route of exposure for fishes (Barron, 1990).

5.2.2 Effects of Bioaccumulation and Biomagnification

5.2.2.1 Large Ecosystem Effects of Bioaccumulation

Bioaccumulation can negatively affect the physiological, endocrine, and immune systems of individual organisms exposed to contaminants (van der Oost et al., 2003). However, attributing changes in body systems to chemical concentrations should be done within a multiple stressor framework (Schreck, 2000a, b). That is, contaminants alone might negatively affect biota, but one should consider that additional stressors could be contributing to changes in the body systems.

Larger ecosystem effects occur through biomagnification of chemicals in the food web. That is, piscivorous birds and mammals usually have higher concentrations than fish, and the very top predators (e.g., polar bears), have the highest chemical concentrations (Mackay and Fraser, 2000). The significance of this dynamic is, first, that by the time population and ecosystem changes can be observed, the early biomarker signals either were not observed and measured or were not acknowledged and, second, that environmental contamination has been occurring for a long time (Figure 5-1) (van der Oost et al. 2003).

An example of large ecosystem effects is the eggshell thinning and population reductions that occurred among many bird species during the 1950s as a result of extensive use of the organochlorine insecticide dichlorodiphenyltrichloroethane (DDT). Until population and ecosystem changes were observed, the negative effects of DDT were either unknown or were not observed. Recovery of some bird populations, such as bald eagles, was largely successful, but required 40 years of extensive financial investment and the commitment of personnel from resource management agencies.

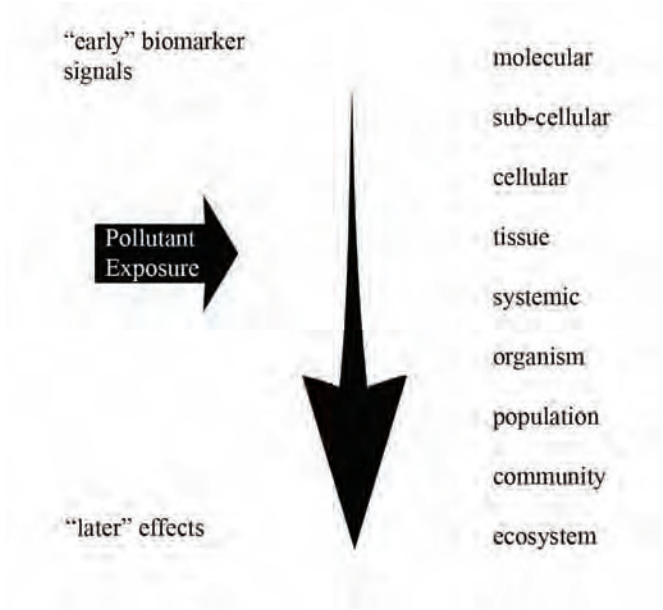


Figure 5-1. Diagram of Increasing Effects of Contaminants, from Individual to Ecosystem Level. Re-drawn from van der Oost et al. (2003).

Long-term concerns about bioaccumulation encompass both organism and ecosystem. Mercury, for example, is a highly persistent natural element and a global pollutant that readily bioaccumulates, biomagnifies, and affects nearly every body system in fish and other vertebrates, including humans. Because Hg is highly persistent, historic emissions (e.g., since the US industrial revolution) of Hg can potentially still be incorporated into the food web by the process of methylation. Furthermore, current emissions of Hg from anthropogenic sources, such as coal-fired power plants in the developed and developing worlds (e.g., the Asian industrial revolution), together with the documented long-range atmospheric transport of contaminants (Jaffe et al., 1999; Wania, 2003; Daly and Wania, 2005), indicate that Hg is likely to have a significant impact on aquatic and other ecosystems in the future.

Contaminants with short half-lives and non-bioaccumulative degradation products pose a lesser concern. Although the bioactivity of those compounds could still be of concern for individual organisms, they do not bioaccumulate and are unlikely to affect the entire food web.

The biota at most risk for adverse effects of bioaccumulation are long-lived, high trophic-level organisms. In the aquatic environments, adverse effects of bioaccumulation are usually observed in piscivorous fish, although insectivorous fish can also bioaccumulate contaminants. In terrestrial environments, birds and mammals that eat fish are susceptible to adverse bioaccumulation effects.

5.2.2.2 Evidence of Bioaccumulation in Fish

Of the many environmental pollutants known to bioaccumulate, Hg is significant because of the numerous and pronounced deleterious effects on fish, wildlife, and humans (Sweet and Zelikoff, 2001). Incorporation of Hg into the food web is highly complex; biota in lakes with similar limnological characteristics can exhibit very different Hg concentrations. For efficient incorporation of Hg into the food web, and for bioaccumulation and biomagnification to occur, Hg must be converted to an organic form by methylation. However, inorganic Hg can be

absorbed across the gut of fish (Hoyle and Handy, 2005). Methyl-Hg is the predominant form found in fish tissues, ranging from 95% to 100% of the entire body burden (Bloom, 1992). Methylation is accomplished in the sediment and wetlands by micro-organisms (Ullrich et al., 2001) and is dependent on lake water pH, total organic carbon, dissolved sulfate (Wiener et al., 2006), temperature, and alkalinity (Ullrich et al., 2001). Mercury exists in three valence states; 0, 1, and 2; Hg^{2+} is the species that is methylated. Methylation of Hg requires a CH_3 donor; methylcobalamin is the most likely donor because it is found under anoxic conditions in micro-organisms and is the only natural compound capable of donating the carbanion (CH_3^-). Data suggest that in bacteria, methylation occurs intra- and extracellularly and that sulfate-reducing bacteria are the principal micro-organisms responsible for carrying out the methylation of inorganic Hg (Ullrich et al., 2001). The actual bio-transformation of Hg^{2+} to methyl-Hg by bacteria is poorly understood. Finally, there is also evidence of abiotic methylation of Hg.

Our observations of Hg accumulation in WACAP fish reflect the highly complex nature of Hg cycling in the environment. In the fish that we captured, Hg was age-dependent in all species up to approximately 15 years of age (Figure 5-2; Appendix 5A). In fish older than 15 years of age, the relationship disappears. In these older fish, Hg concentrations could be related to the trophic status of the fish, as has been demonstrated in other studies (Kidd et al., 1995; Evans et al., 2005). Another possibility is that metabolism and excretion of the Hg could occur, as has been modeled by Trudel and Rasmussen (1997). A third explanation is that Hg might increase steadily, until it eventually reaches toxic levels, and, in conjunction with the likely numerous other stressors, leads to senescence; as a result, only fish with fairly low concentrations of Hg reach old age. In our studies, only lake trout (*Salvelinus namaycush*) reached ages > 20 years, and lake trout were collected only in Alaska. Therefore, it is not known if the observed break-down in the relationship between age and Hg is unique to lake trout or is related to the food webs of which they are a part.

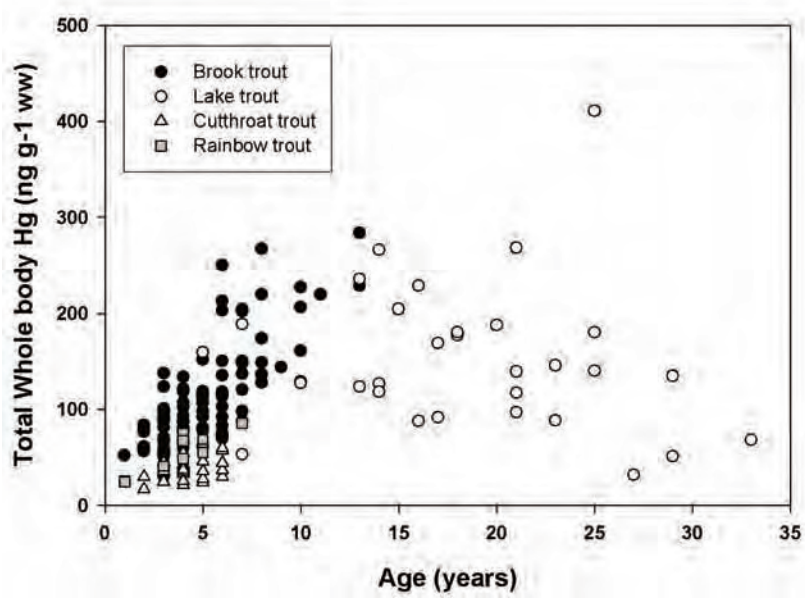


Figure 5-2. Relationship between Fish Age and Total Whole Body Hg in Trout from All Lakes. In fish ≤ 15 years of age, $F_{1,134}$ = 127.36, R^2 = 0.47, P = 1.61 × 10^{-21}.

Similarly, age was an important factor for SOC concentrations in fish, but not as important as the lipid levels of the fish. For most SOCs, percent lipid in the fish was the most reliable single predictor of SOC concentrations (average lake $R^2 = 0.29$), whereas fish age was the second most reliable predictor (average lake $R^2 = 0.22$) (Ackerman, 2007). Like Hg, some correlations for SOCs broke down among the oldest fish.

An important objective of WACAP is to provide the NPS with data on organic and trace element contamination in the western national parks in various environmental compartments. With these data, it is possible to make comparisons of contaminant concentrations in fish from these western parks to contaminant concentrations from studies published in the peer-reviewed literature. Through these comparisons, it is possible to determine the relative level of contamination in similar ecosystems around the world. In addition, data from the literature on highly affected ecosystems provide another perspective on the relative contamination of historically protected ecosystems in the national parks.

Data on organochlorine concentrations from selected publications from the United States, Canada, Africa, Asia, and Europe are compared to WACAP fish concentrations in Table 5-1. As expected, greatly affected areas such as the Ohio River (tributary of the Mississippi River, USA) and the Columbia River (Pacific Northwest, USA) have fish with organochlorine concentrations 10- to 10,000-fold higher than we report in fish from the national parks. It was surprising, however, that dieldrin concentrations were markedly higher in fish from SEKI, ROMO, and GLAC than in farmed Atlantic Salmon (*Salmo salar*) and wild Pacific salmon (*Oncorhynchus* spp.). The lake trout from GAAR and NOAT and brook trout (*S. fontinalis*) from OLYM represented the WACAP fish with lowest overall concentrations of SOCs; concentrations in these fish were generally lower than those reported in these selected studies.

All WACAP fish had lower polychlorinated byphenyl (PCB), hexachlorocyclohexane (HCH), and hexachlorobenzene (HCB) concentrations than the concentrations reported in the literature. However, we acknowledge that cumulative PCB concentrations might have been underestimated in this study because we targeted a limited number of congeners. A surprising finding was that concentrations of DDTs in fish from SEKI and ROMO were higher than those in many fish (including a piscivorous species) from Lake Malawi in East Africa, despite the current use of DDT in Africa for mosquito control.

Mercury concentrations by lake are presented graphically in Section 5.4.1.1; comparisons of fish in this study to concentrations in the peer-reviewed literature are presented in Table 5-2. In general, mercury concentrations in the trout from the parks in this study were lower than those reported for lakes in the Midwest and Northeast United States, including Lake Michigan and Lake Superior. In addition, mercury concentrations were lower in fish at all WACAP parks than in 1-year-old insectivorous yellow perch (*Perca flavescens*) at Voyageurs National Park in Minnesota. The same was true for lake trout and northern pike (*Esox lucius*) in northern lakes (50º N latitude and above) in Canada. But the opposite was the case for Arctic char (*Salvelinus alpinus*), grayling (*Thymallus arcticus*), and brook trout from northern lakes in Canada. Also, mercury concentrations were higher in WACAP lake fish than in brown trout (*Salmo trutta*) from similar mountain and sub-Arctic ecosystems in Europe. Juvenile sturgeon (*Acipenser transmontanus*) in the Columbia River, USA, had lower mercury concentrations than fish from the Arctic, Denali, Olympic, and Mount Rainier National Parks, although a 41-year-old adult female sturgeon had mercury concentrations well above all fish in this study.

Table 5-1. Comparison of Concentrations of Selected Organochlorines (OCs) in Fish from the Literature to Fish from WACAP Parks.

Species Trophic Level	Location	Tissue	Selected Organochlorines (range or mean ± error measure) Units [wet weight (ww) or lipid normalized (lip)] Measured Congeners or Metabolites						(↑ OC > in WACAP, ↓ OC < in WACAP, = OC similar to WACAP); Concentrations reduced 30% for comparisons to muscle							Ref.
			DDTs (D)*	PCBs (P)*	Dieldrin (N)*	Chlordane (C)*	HCH (H)*	HCB (B)*	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Salvelinus fontinalis Insectivorous	Kaweah River, California	Whole fish	40-66 ng/g ww p,p'-DDE	5-8 ng/g ww Sum 98 congener					↓	↓	↓	↓	↓	↓	↓	Datta et al., 1998
Esox lucius Piscivorous	Unspecified lakes, southern Sweden	Muscle	0.13-64 ng/g ww p,p'-DDE	0.32-64 ng/g ww Sum 24 congener					↓	↓	↓	↓	↓	↓	↓	Larsson et al., 1992
Salvelinus namaycush Piscivorous	Peter Lake, Northwest Territories, Canada	Muscle	57.9 ± 148 SD ng/g ww Sum	11.3 ± 23.7 SD ng/g ww PCB153		46.1 ± 112 SD ng/g ww Sum	2.16 ± 4.3 SD ng/g ww Sum		↓	↓	↓	↓	↓	↓	↓	Kidd et al., 1998
Salvelinus alpinus Piscivorous	Peter Lake, Northwest Territories, Canada	Muscle	4.62 ± 1.71 SD ng/g ww Sum	1.3 ± 0.63 SD ng/g ww PCB153		3.45 ± 1.65 SD ng/g ww Sum	1.68 ± 0.8 SD ng/g ww Sum		↓	↓	↑ D ↓ PCH	↓	↓	= D ↓ PCH	↑ D ↓ PCH	Kidd et al., 1998
Ctenopharynx pictus Planktivorous	Lake Malawi, Africa	Whole fish	2 ± 0.9 SD ng/g ww Sum p,p'-						↓	↓	↑	↓	↑	↑	↑	Kidd et al., 2001
Dimidiochromis kiwinge Piscivorous	Lake Malawi, Africa	Whole fish	2.9 ± 1.3 SD ng/g ww Sum p,p'-						↓	↓	↑	↓	↑	↑	↑	Kidd et al., 2001
Engraulicyprus sardella Planktivorous	Lake Malawi, Africa	Whole fish	5.2 ± 3.3 SD ng/g ww Sum p,p'-						↓	↓	↑	↓	↓	↑	↑	Kidd et al., 2001

Table 5-1. Comparison of Concentrations of Selected Organochlorines (OCs) in Fish from the Literature to Fish from WACAP Parks (continued).

Species Trophic Level	Location	Tissue	Selected Organochlorines (range or mean ± error measure) Units [wet weight (ww) or lipid normalized (lip)] Measured Congeners or Metabolites						(↑ OC > in WACAP, ↓ OC < in WACAP, = OC similar to WACAP); Concentrations reduced 30% for comparisons to muscle							Ref.
			DDTs (D)*	PCBs (P)*	Dieldrin (N)*	Chlordane (C)*	HCH (H)*	HCB (B)*	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Labeotropheus fuelleborni Herb-Insectivorous	Lake Malawi, Aftrica	Whole fish	1.2 ± 0.45 SD ng/g ww Sum p,p'-						↓	↓	↑	↓	↑	↑	↑	Kidd et al., 2001
Opsaridium microlepis Piscivorous	Lake Malawi, Africa	Whole fish	34 ± 16 ng/g ww Sum p,p'-						↓	↓	↓	↓	↓	↓	↓	Kidd et al., 2001
Synodontis njassae Insectivorous	Lake Malawi, Africa	Whole fish	58 ± 39 SD ng/g ww Sum p,p'-						↓	↓	↓	↓	↓	↓	↓	Kidd et al., 2001
Salmo salar Plankti-Piscivorous	Scotland fish farm	Muscle		~ 300 ng/g lip Sum	~ 30 ng/g lip				↓	↓	↓	↓	↓	↓P ↑N	↓P↑N	Hamilton et al., 2005
Salmo salar Plankti-Piscivorous	Norway fish farm	Muscle		~ 250 ng/g ww Sum	~ 25 ng/g lip				↓	↓	↓	↓	↓	↓P ↑N	↓P↑N	Hamilton et al., 2005
Salmo salar Plankti-Piscivorous	Chile fish farm	Muscle		~ 150 ng/g lip Sum	~ 10 ng/g lip				↓P =N	↓P = N	↓P↑N	↓	↓	↓P↑N	↓P↑N	Hamilton et al., 2005
Oncorhynchus nerka Plankti-Piscivorous	Southeast Alaska	Muscle		~ 100 ng/g lip Sum	~ 10 ng/g lip				↓P =N	↓P =N	↓P↑N	↓	↓	↓P↑N	↓P↑N	Hamilton et al., 2005

Table 5-1. Comparison of Concentrations of Selected Organochlorines (OCs) in Fish from the Literature to Fish from WACAP Parks (continued).

Species Trophic Level	Location	Tissue	Selected Organochlorines (range or mean ± error measure) Units [wet weight (ww) or lipid normalized (lip)] Measured Congeners or Metabolites						(↑ OC > in WACAP, ↓ OC < in WACAP, = OC similar to WACAP); Concentrations reduced 30% for comparisons to muscle							Ref.
			DDTs (D)*	PCBs (P)*	Dieldrin (N)*	Chlordane (C)*	HCH (H)*	HCB (B)*	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Oncorhynchus tschawytscha Plankti-Piscivorous	Oregon	Muscle		~ 100 ng/g lip Sum	~ 5 ng/g lip				↓P↑N	↓P↑N	↓P↑N	↓	↓P↑N	↓P↑N	↓P↑N	Hamilton et al., 2005
Salmo trutta Insectivorous	Ovre Neadalsvatn Norway	Muscle	0.74 ± 0.31 SD ng/g ww Sum	1.5 ± 0.57 SD ng/g ww Sum			0.28 ± 0.12 SD ng/g ww Sum	0.58 ± 0.21 SD ng/g ww	↑P ↓DHB	↑D ↓PHB	↓PH ↑DB	↓	↑D ↓HB	↑D ↓HB	↑D ↓HB	Vives et al., 2004
Salmo trutta Insectivorous	Ve'ke' Hincovo Slovakia	Muscle	36 ±13 SD ng/g ww Sum	17 ± 3.5 SD ng/g ww Sum			0.91 ± 0.44 SD ng/g ww Sum	0.3 ± 0.11 SD ng/g ww	↓DPH ↑B	↓DPH ↑B	↓DPH ↑B	↓	↓	↓	↓	Vives et al., 2004
Salmo trutta Insectivorous	Redon France / Spain	Muscle	19 ± 13 SD ng/g ww Sum	8.2 ± 4.8 SD ng/g ww Sum			1.6 ± 0.9 SD ng/g ww Sum	0.6 ± 0.36 SD ng/g ww Sum	↓	↓	↓DPH ↑B	↓	↓	↓	↓	Vives et al., 2004
Polyodon spathula Piscivorous	Ohio River USA	Muscle		50 – 3350 ng/g ww Arochlor 1260					↓	↓	↓	↓	↓	↓	↓	Gundersen and Pearson, 1992

Table 5-1. Comparison of Concentrations of Selected Organochlorines (OCs) in Fish from the Literature to Fish from WACAP Parks (continued).

Species Trophic Level	Location	Tissue	Selected Organochlorines (range or mean ± error measure) Units [wet weight (ww) or lipid normalized (lip)] Measured Congeners or Metabolites						(↑ OC > in WACAP, ↓ OC < in WACAP, = OC similar to WACAP); Concentrations reduced 30% for comparisons to muscle							Ref.
			DDTs (D)*	PCBs (P)*	Dieldrin (N)*	Chlordane (C)*	HCH (H)*	HCB (B)*	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Gymnocypris waddellii	Yamdro Lake Tibet	Muscle	~ 2.5 ng/g ww Sum				~ 1 ng/g ww Sum	~ 1 ng/g ww Sum	↓	↓	↑D ↓HB	↓	↑D ↓HB	↑D ↓HB	↑D ↓HB	Yang et al., 2007
Salvelinus fontinalis *Salvelinus confluentus* *Oncorhynchus mykiss* Pisci-Insectivorous	Mountain Lakes in Banff, Jasper, or Yoho National Parks	Muscle	4.5 ± 5.4 SD ng/g ww p,p'-DDE	7.7 ± 10.4 SD ng/g ww Sum 127 congen		61 ± 52 SD ng/g ww gama-chlor-dane			↓	↓	↑D ↓PC	↓	↓	=D ↓PC	↑D ↓PC	Demers et al., 2007
Salvelinus namaycush Piscivorous	Kusawa Lake Northwest Territories Canada	Muscle	26.66 ± 4.15 SD ng/g ww Sum	32.45 ± 3.66 SD ng/g ww Sum		3.01 ± 0.48 SD ng/g ww Sum	0.62 ± 0.08 SD ng/g ww Sum		↓	↓	↓	↓	↓	↓	↓	Ryan et al., 2005
Salvelinus namaycush Piscivorous	Quiet Lake Northwest Territories Canada	Muscle	0.53 ± 0.09 SD ng/g ww Sum	3.51 ± 0.62 SD ng/g ww Sum		0.62 ± 0.12 SD ng/g ww Sum	0.08 ± 0.02 SD ng/g ww Sum		↑DC ↓PH	↑DC ↓PH	↑DC ↓PH	=D ↓PCH	↑D ↓PCH	↑D ↓PCH	↑DC ↓PH	Ryan et al., 2005
Acipenser transmontanus Piscivorous	Columbia River Oregon	Liver	18,700 ± 7300 ng/g SE lip Sum		134 ± .45 SE ng/g lip Sum				↓	↓	↓	↓	↓	↓	↓	Feist et al., 2005

Table 5-1. Comparison of Concentrations of Selected Organochlorines (OCs) in Fish from the Literature to Fish from WACAP Parks (continued).

Species Trophic Level	Location	Tissue	Selected Organochlorines (range or mean ± error measure) Units [wet weight (ww) or lipid normalized (lip)] Measured Congeners or Metabolites						(↑ OC > in WACAP, ↓ OC < in WACAP, = OC similar to WACAP); Concentrations reduced 30% for comparisons to muscle							Ref.
			DDTs (D)*	PCBs (P)*	Dieldrin (N)*	Chlordane (C)*	HCH (H)*	HCB (B)*	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Salvelinus namaycush Piscivorous	Lake Superior, USA, 1990	Whole-fish	180 ± 50 ng/g 95% CI	450 ± 140 ng/g 95% CI Arochlor 1254	40 ± 4 ng/g 95% CI	100 ± 20 95% CI ng/g			↓	↓	↓	↓	↓	↓	↓	De-Vault et al., 1996
Salvelinus namaycush Piscivorous	Lake Huron, USA, 1992	Whole-fish	520 ± 20 ng/g 95% CI	1570 ± 90 ng/g 95% CI Arochlor 1254	60 ± 3 ng/g 95% CI	250 ± 10 ng/g 95% CI			↓	↓	↓	↓	↓	↓	↓	De-Vault et al., 1996
Salvelinus namaycush Piscivorous	Lake Michigan, USA, 1992	Whole-fish	3,490 ± 450 ng/g 95% CI	1,160 ± 180 ng/g 95% CI Arochlor 1254	190 ± 20 ng/g 95% CI	450 ± 30 ng/g 95% CI			↓	↓	↓	↓	↓	↓	↓	De-Vault et al., 1996
Salvelinus namaycush Piscivorous	Lake Ontario, USA, 1992	Whole-fish	2,650 ± 300 ng/g 95% CI	840 ± 120 ng/g 95% CI Arochlor 1254	80 ± 0.9 ng/g 95% CI	170 ± 90 ng/g 95% CI			↓	↓	↓	↓	↓	↓	↓	De-Vault et al., 1996
Sander vitreus Piscivorous	Lake Erie, USA, 1992	Whole-fish	2,200 ± 310 ng/g 95% CI	120 ± 10 ng/g 95% CI Arochlor 1254	30 ± 0.2 ng/g 95% CI	50 ± 0.2 ng/g 95% CI			↓	↓	↓	↓	↓	↓	↓	De-Vault et al., 1996

*In the column headings, letters in ( ) following compound names are codes used in the right side of the table to indicate the compounds.

Table 5-2. Comparison of Concentrations of Total Mercury in Fish from the Literature to Fish from WACAP Parks.

Fish Trophic Level	Location	Tissue	Mercury Species (range or mean ± error measure) Units [wet weight (ww) or dry weight (dw)]	(↑ Hg > in WACAP, ↓ Hg < in WACAP, = Hg similar to WACAP) Concentrations increased for comparisons to muscle following Peterson et al. 2007							Ref.
			Total-Hg	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Sander vitreus Piscivorous	Lakes in North-central Wisconsin	Muscle	120 – 1740 ng/g ww	↓	↓	↓	↓	↓	↓	↓	Wiener et al., 1990
Salmo trutta Insectivorous	Jorisee, Switzerland	Muscle	37 (18 – 56) ng/g ww	↑	↑	↑	↑	↑	↑	↑	Rognerud et al., 2002
Salvelinus alpinusi Piscivorous	Arresjoen, Svalbard Archipelago	Muscle	179 (39 – 441) ng/g ww	↑	=	↓	↑	=	↓	↓	Rognerud et al., 2002
Salmo trutta Insectivorous	Estany Redo, Spain	Muscle	68 (15-158) ng/g ww	↑	↑	↓	↑	↑	↑	↑	Rognerud et al., 2002
Salmo trutta Insectivorous	Stavsvatn, Norway	Muscle	44 (21-79) ng/g ww	↑	↑	↑	↑	↑	↑	↑	Rognerud et al., 2002
Salmo trutta Insectivorous	Gossen-kollesee, Czech Republic	Muscle	25 (16-39) ng/g ww	↑	↑	↑	↑	↑	↑	↑	Rognerud et al., 2002
Salmo trutta Insectivorous	Ovre Neadalsvatn, Norway	Muscle	21 (14-31) ng/g ww	↑	↑	↑	↑	↑	↑	↑	Rognerud et al., 2002
Salvelinus alpinus Insectivorous	Etang d' Aube, France	Muscle	55 (37-78) ng/g ww	↑	↑	=	↑	↑	↑	↑	Rognerud et al., 2002

Table 5-2. Comparison of Concentrations of Total Mercury in Fish from the Literature to Fish from WACAP Parks (continued).

Fish Trophic Level	Location	Tissue	Mercury Species (range or mean ± error measure) Units [wet weight (ww) or dry weight (dw)]	(↑ Hg > in WACAP, ↓ Hg < in WACAP, = Hg similar to WACAP) Concentrations increased for comparisons to muscle following Peterson et al. 2007							Ref.
			Total-Hg	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Salvelinus alpinus Insecti- Piscivorous	Lakes in Northern Canada (1971-2002)	Muscle	115 ± 237 SD ng/g ww	↑	↑	↓	↑	↑	↓	↑	Rognerud et al., 2002
Thymallus arcticus Insectivorous	Lakes in Northern Canada (1971-2002)	Muscle	53 ± 45 SD ng/g ww	↑	↑	=	↑	↑	↑	↑	Lockhart et al., 2005
Salvelinus fontinalis Insectivorous	Lakes in Northern Canada (1971-2002)	Muscle	106 ± 50 SD ng/g ww	↑	↑	↓	↑	↑	↓	↑	Lockhart et al., 2005
Lota lota Piscivorous	Lakes in Northern Canada (1971-2002)	Muscle	210 ± 135 SD ng/g ww	↑	↓	↓	↓	↓	↓	↓	Lockhart et al., 2005
Salvelinus namaycush Piscivorous	Lakes in Northern Canada (1971-2002)	Muscle	384 ± 351 SD ng/g ww	↓	↓	↓	↓	↓	↓	↓	Lockhart et al., 2005
Esox lucius Piscivorous	Lakes in Northern Canada (1971-2002)	Muscle	378 ± 298 SD ng/g ww	↓	↓	↓	↓	↓	↓	↓	Lockhart et al., 2005
Perca flavescens Planktivorous	Voyageurs National Park, USA	Whole fish	182-942 ng/g dw	↓	↓	↓	↓	↓	↓	↓	Wiener et al., 2006

Table 5-2. Comparison of Concentrations of Total Mercury in Fish from the Literature to Fish from WACAP Parks (continued).

Fish Trophic Level	Location	Tissue	Mercury Species (range or mean ± error measure) Units [wet weight (ww) or dry weight (dw)]	(↑ Hg > in WACAP, ↓ Hg < in WACAP, = Hg similar to WACAP) Concentrations increased for comparisons to muscle following Peterson et al. 2007							Ref.
			Total-Hg	GAAR & NOAT	DENA	GLAC	OLYM	MORA	ROMO	SEKI	
Perca flavescens Planktivorous	Northeast USA	Whole fish	230 (<0.50-3180) ng/g ww	↓	↓	↓	↓	↓	↓	↓	Evers et al., 2007
Salvelinus fontinalis Insectivorous	Northeast USA	Whole fish	310 (0.05-2070) ng/g ww	↓	↓	↓	↓	↓	↓	↓	Evers et al., 2007
Juvenile *Acipenser transmontanus* Piscivorous	Columbia River, Oregon	Muscle	170.54 ± 12.67 SE ng/g ww	↑	↑	↓	↑	↑	↓	=	Webb et al., 2006
Adult *Acipenser transmontanus* Piscivorous	Columbia River, Oregon	Muscle	1,094 ng/g ww, *N* = 1, Age = 41 y	↓	↓	↓	↓	↓	↓	↓	Webb et al., 2006
Salvelinus namaycush Piscivorous	Lake Michigan, USA	Whole fish	220 ± 80 SE ng/g (normalization not specified)	↓	↓	↓	↓	↓	↓	↓	Mason and Sullivan, 1997
Sander vitreus Piscivorous	St. Louis River Estuary, Lake Superior, USA (1979-1987)	Not specified	~ 250-1500 ng/g (normalization not specified)	↓	↓	↓	↓	↓	↓	↓	Glass et al., 1990
Esox lucius Piscivorous	St. Louis River Estuary, Lake Superior, USA (1979-1984)	Not specified	~ 250-600 ng/g (normalization not specified)	↓	↓	↓	↓	↓	↓	↓	Glass et al., 1990

Comparison of organochlorines and mercury in WACAP fish (Tables 5-1 and 5-2) with those reported in the literature indicate that contamination of WACAP watersheds by PCBs, hexachlorobenzene, hexachlorocyclohexanes, DDTs, and chlordanes is comparable to or lower than in similar mountain areas in Europe, Canada, and Asia, while dieldrin and PBDE contamination is higher than in similar mountain areas and in Pacific Ocean salmon (Ackerman 2007). In general, organochlorine concentrations are lower in WACAP fish than in other fish reported in the literature, but similar to or higher than values in the literature for mercury. Implications of this finding are unknown and some geographic areas reported in the literature have dramatically higher Hg than that observed for the national parks. The potential risk to consumers from fish in the WACAP parks is presented in Sections 5.4.1 and 5.4.2.

5.2.3 Evidence of Bioaccumulation in Vegetation

Bioaccumulation of SOCs in vegetation over time was observed in a subset of WACAP samples tested for this effect. First- and second-year lodgepole pine (*Pinus contorta*) and white fir (*Abies concolor*) needles from Emerald Lake basin in SEKI were analyzed for pesticides and PCB concentrations. Each pair of samples consisted of one set of branchlets that had been divided, with the terminal bud scars as year markers, into first- and second-year needles before analysis. Concentrations of the current-use pesticides endosulfan (sum of endosulfan 1, endosulfan 2, and the degradation product endosulfan sulfate) and dacthal were 2-3 times higher in second-year compared with first-year lodgepole pine needles (Table 5-3).

Although concentration values for second-year needles were all larger than those for first-year needles (except trifluralin in white fir) (Table 5-3), the small sample number and high variability among field replicates yielded insufficient statistical power to provide evidence of significant differences for the other SOCs. However, when all pairs of measurements for chlorpyrifos, endosulfans, dacthal, HCB, a-HCH, g-HCH, chlordanes, and PCBs were considered together (Table 5-4), there was good evidence that second-year needles had, on average, concentrations of these SOCs about 3 times higher than those in first-year needles and that SOC concentrations are strongly correlated with needle age.

Table 5-3. SOC Concentrations (ng/g lipid) in One- and Two-Year-Old Needles of White Fir (*Abies concolor*) and Lodgepole Pine (*Pinus contorta*) from the Emerald Lake Basin of Sequoia National Park. Nearly every value was larger in year 2 than year 1, but significant differences were demonstrated only for dacthal and endosulfans in pine (t-test, $p < t < 0.05$, equal variances). Statistical power was low because of high variability among field replicates and small sample sizes.

				Abies concolor				*Pinus contorta*		
Group	**SOC**	**Yr**	**N**	**Mean**	**s.d.**	**s.e.**	**N**	**Mean**	**s.d.**	**s.e.**
CUPs	Chlorpyrifos	1	2	**0.0**	0.0	0.0	1	**11.6**		
		2	2	**19.7**	26.1	18.5	2	**20.5**	17.7	12.5
	Dacthal	1	3	**1555**	1066	616	1	**530**		
		2	2	**2007**	1249	883	2	**1474**	1102	780
	Endosulfans	1	3	**2448**	963	556	1	**510**		
		2	2	**7573**	2419	1711	2	**1325**	176	124
	Triallate	1	3	**0.0**	0.0	0.0	1	**0.0**		
		2	2	**0.0**	0.0	0.0	2	**125.3**	177.2	125.3
	Trifluralin	1	2	**10.5**	14.9	10.5	1	**0.0**		

Table 5-3. SOC Concentrations (ng/g lipid) in One- and Two-Year-Old Needles of White Fir (*Abies concolor*) and Lodgepole Pine (*Pinus contorta*) from the Emerald Lake Basin of Sequoia National Park (continued).

			Abies concolor				*Pinus contorta*			
Group	**SOC**	**Yr**	**N**	**Mean**	**s.d.**	**s.e.**	**N**	**Mean**	**s.d.**	**s.e**
		2	1	**0.0**	.	.	2	**19.6**	26.5	18.7
		1	3	**95.2**	86.5	49.9	1	**32.4**		
		2	2	**145.5**	194.7	137.7	2	**38.7**	50.0	35.3
HUPs	Chlordanes	1	2	**64.7**	21.0	14.8	1	**80.9**		
		2	2	**188.5**	27.5	19.4	2	**148.1**	40.5	28.7
	HCB	1	2	**0.0**	0.0	0.0	1	**101.3**		
		2	2	**122.7**	173.5	122.7	2	**210.2**	97.3	68.8
	a-HCH	1	2	**0.0**	0.0	0.0	1	**0.0**		
		2	1	**0.0**	.	.	2	**79.2**	112.0	79.2
	g-HCH	1	3	**72.3**	84.3	48.7	1	**4.9**		
		2	2	**127.3**	41.6	29.4	2	**90.3**	123.6	87.4
PCBs	138, 153, 183, 187	1	3	**72.3**	84.3	48.7	1	**4.9**		

Table 5-4. Paired T-Test Results Comparing SOC Concentrations (ng SOC/g conifer needle lipid) in One- and Two-Year-Old Needles of White Fir (*Abies concolor*) and Lodgepole Pine (*Pinus contorta*)*. When all pairs of measurements were considered, the average SOC concentration was more than 3 times higher in second year than first year needles for both species (Prob > t < 0.05) and SOC concentrations were strongly correlated with needle age (R^2 > 0.93).

Statistic	*Abies concolor*	*Pinus contorta*
Year 2	1186	457
Year 1	360	127
Mean Difference	826	330
Increase (fold)	+3.3	+3.6
Std Error	473.9	167
Upper 95%	1830	709
Lower 95%	-178	-48
N	17	10
Correlation	**0.953**	**0.933**
t-Ratio	1.744	1.973
DF	16	9
Prob > |t|	0.1004	0.08
Prob > t	**0.0502**	**0.04**
Prob < t	0.9498	0.96

*Year 2 and Year 1 = Mean concentrations of the individual SOCs: chlorpyrifos, endosulfans, dacthal, HCB, a-HCH, g-HCH, chlordanes, and PCBs in field replicates of Year 2 and Year 1 conifer needles. Mean difference = average difference in individual SOC concentrations between first and second year needles. Increase (fold) = multiplicative increase in the mean difference between year one and year two. Std Error = standard error of the mean; Upper95% and Lower 95%= 95% confidence intervals around the mean; N = number of measurements; Correlation = correlation between concentrations of individual contaminants and year; t-ratio = t-test statistic; DF = degrees of freedom; Prob > |t| = probability of incorrectly rejecting the null hypothesis that there is no difference in SOC concentrations due to year (two-tailed test); Prob > t = probability of incorrectly rejecting the null hypothesis that year 2 concentrations are not greater than year 1 concentrations (one-tailed test); Prob < t = probability of incorrectly rejecting the null hypothesis that year 1 concentrations are not greater than year 2 concentrations (one-tailed test).

How many years of needles can be found on coniferous trees? Conifer needle retention varies across species and site conditions; maximum needle longevity is optimized by good site and micro-site conditions, including adequate nutrients, light, water, and heat, and low stress from pests, disease, and air pollution (Reich et al., 1994). Needles of most conifers reach at least 3-7 years in age (Li et al., 2006), and in the genus *Pinus*, often reach 15 years, or in the extreme case of the bristle cone pine (*Pinus longaeva*) even up to 45 years (Ewers and Schmid, 1981). Needle longevity generally increases with latitude and elevation, increasing the time that nutrients are resident in trees in less favorable environments and compensating for shorter growing periods in cold temperature (Li et al., 2006; Reich et al., 1996). A relevant example is black spruce (*Picea mariana*), collected for WACAP in DENA, central Alaska. Needle retention of this species varies from 5 to 7 years in its southerly boreal forest range in Quebec, to 13 years in central Alaska, and up to 30 years under subarctic conditions (Lamhamedi and Bernier, 1994).

Whether or not SOC concentrations in conifer needles continue to increase with needle age was not addressed by WACAP. Plant uptake of SOCs occurs primarily from the atmosphere via one of three processes: equilibrium partitioning between the vegetation and the gas phase, kinetically limited gaseous deposition, and wet plus dry particle-bound deposition. Each of these processes depends on different atmospheric concentrations, plant properties, and environmental variables (McLachlan, 1999).

It has been suggested that once the SOCs have been deposited, a two-compartment model for their storage in plant leaves applies (Tolls and McLachlan, 1994; Hauk et al., 1994), accepted also by Simonich and Hites (1995) and Collins et al. (2006). The two-compartment model consists of a fairly small surface compartment with rapid uptake and clearance kinetics (hours), and a larger reservoir compartment with slow chemical migration (months to years). At least some compounds are known to require many months to reach needle concentrations that are in equilibrium with the atmosphere. For example, Douglas-fir (*Pseudtsuga menziesii*) exposed to toluene, ethylbenzene, and xlenes for several years usually equilibrated within 5-6 months (Keymeulen et al., 1993). The composition of the larger reservoir compartment is believed to influence the retention of organic chemicals, but the physiological relationship between the two compartments has not been elucidated. Forces working against eventual equilibration include surface degradation of SOCs, such as the photo-oxidation of endosulfan I and II to endosulfan sulfate (Simonich and Hites, 1995) and seasonal variation in atmospheric concentrations of pollutants (Simonich and Hites, 1994). An interesting follow-up to WACAP would be to determine whether SOC concentrations equilibrate or continue to increase in conifer needles after year 2, especially at high elevations and northerly latitudes where a large percentage of needles are in older age classes.

Other ecologically relevant questions include how much SOC contamination of soils occurs during precipitation (i.e., directly from precipitation but also from the SOC concentration in particulates collected on the surface of needles between precipitation events that wash through the canopy during rain or snowfall) and how needle SOC concentrations change as needles senesce and finally drop to the forest floor. Both pathways are considered to be important sources of soil SOC contamination (Horstmann and McLachlan, 1998; Weiss, 2000; Nizzetto et al., 2006), although quantitative data for the western United States are scarce. Another ecologically important question is whether SOC contributions from litterfall and canopy leachates are sufficiently high, cumulative or long-lasting to adversely affect populations of

arthropod, fungal, or microbial decomposers, or plant life, either as individual contaminants or synergistically. The answers to these questions require more research.

Although concentrations of individual SOCs are fairly small, ranging from ng (pesticides and PCBs) to µg (PAHs) per gram of needles, the total quantity of contaminants absorbed by conifer needles per hectare (ha) can be surprisingly high, especially considering that the exposure is passive. Table 5-5 shows calculation of annual needle production per ha across a wide range of site conditions in coniferous forests from New Mexico to British Columbia, using total above ground annual biomass production and stem:leaf ratios compiled by Hessl et al. (in press) and available on-line at http://ocid.nacse.org/research/ecophys/index.html.

Table 5-5. Estimates of Total SOC Concentrations (mg/ha) in Second-Year Needles from Western North American Coniferous Forests. Annual needle biomass production for each of 31 sites[1], representing xeric-continental to coastal-rain forests, was multiplied by average low and high needle SOC concentrations observed at WACAP parks to estimate per ha SOC concentrations. Needle biomass was more important than needle concentration; maximum estimated total pesticide and PAH concentrations were < 1 and <7 g/ha, respectively at all sites.

				Measure					
	Site Data			***Mean***	***St Dev***	***St Err***	***Median***	***Min***	***Max***
N = 31	Elevation (m)			886	771	267	500	200	2720
	Mean tree age (yrs)			103	91	32	72	22	450
	Carbon Allocation New Stem C: New Leaf C			1.87	0.89	0.31	1.69	0.20	3.32
	Net Above Ground Productivity ($kg/m^2/yr$)			1.55	2.48	0.86	0.84	0.12	10.50
	Annual Needle Production (kg/m^2yr)			0.634	1.156	0.400	0.286	0.100	5.000
	Annual Needle Production (g/ha/yr)			6,344,810	11,556,610	4,004,090	2,861,110	1,000,000	50,000,000
	Parameter	***Level***	***C***	***Mean***	***St Dev***	***St Err***	***Median***	***Min***	***Max***
SOC concentration (mg/ha) in second year needles*	Trifluralin**	high	1.29	0.54	0.98	0.34	0.24	0.08	4.24
	Triallate**	high	8.93	3.72	6.78	2.35	1.68	0.59	29.34
	Chlorpyrifos	low	1.45	0.61	1.1	0.38	0.27	0.1	4.77
		high	7.45	3.11	5.66	1.96	1.4	0.49	24.47
	Dacthal	low	8.76	3.65	6.65	2.3	1.65	0.58	28.78
		high	65.9	27.47	50.04	17.34	12.39	4.33	216.5
	Endosulfans	low	24.5	10.21	18.59	6.44	4.6	1.61	80.43
		high	138	57.7	105.09	36.41	26.02	9.09	454.69
	HCB	low	12.1	5.05	9.19	3.19	2.28	0.8	39.78
		high	12.6	5.23	9.53	3.3	2.36	0.82	41.24
	a-HCH	low	9.78	4.08	7.42	2.57	1.84	0.64	32.11
		high	14.7	6.11	11.13	3.86	2.76	0.96	48.16
	g-HCH	high	9.16	a-HCH	6.95	2.41	1.72	0.6	30.08
	Chlordanes	low	1.31		1	0.35	0.25	0.09	4.32

Table 5-5. Estimates of Total SOC Concentrations (mg/ha) in Second-Year Needles from Western North American Coniferous Forests (continued).

Parameter	*Level*	*C*	*Mean*	*St Dev*	*St Err*	*Median*	*Min*	*Max*
	high	13.8	g-HCH	10.49	3.63	2.6	0.91	45.37
Dieldrin**	high	4.70	1.96	3.56	1.24	0.88	0.31	15.42
DDTs	low	4.51	1.88	3.43	1.19	0.85	0.3	14.82
	high	9.49	3.95	7.2	2.5	1.78	0.62	31.17
CUPs	low		18.7			8.4	3	147.6
	high		92.5			41.7	14.6	729.2
HUPs	low		17.3			7.8	2.7	136.5
	high		26.8			12.1	4.2	211.4
Total Pesticides	low		36			16.3	5.7	284.1
	high		119.4			53.8	18.8	940.7
PCBs	low	1.13	0.47		0.3	0.21	0.07	3.7
	high	1.68	0.7		0.44	0.32	0.11	5.52
PAHs	low	1073	447		282	202	70	3525
	high	20044	8360		5270	3770	1320	65840
Total SOCs	low		483			219	76	3813
	high		8480			3824	1339	66786

[1] Data from the Western Forests Ecophysiology Database (http://ocid.nacse.org/research/ecophys/index.html)

[2] SOC (mg/ha) in 2nd year needles: = C ∗ .0657 ∗ annual needle production ∗ 1 x 10^{-6}, where C = mean conifer needle SOC concentration, ng/g lipid, among the WACAP parks in the lowest or highest group, 0.657 is the mean percentage of lipid in dry needles (converts ng/g lipid to ng/g dw), and Annual Needle Production is the dry weight (dw) of needles produced in one year in g/ha; dividing by 1 million converts SOC units from ng to mg. Values for the constant, C, were calculated from Chapter 4, Table 4-2 and are the mean conifer needle SOC concentrations in parks belonging to lowest and highest groups (i.e., least vs. most contaminated) assigned by the Tukey-Kramer test; parks that were in both highest and lowest groups were included in calculations for both low and high C.

[3]High estimates only are provided for triallate, trifluralin, g-HCH, and dieldrin as these SOCs were detected in only a few parks or concentrations did not differ between parks. Parks for which all samples were below detection limits for an individual SOC were not included in the analysis.

Means for parks in the lowest and highest needle SOC concentrations groups assigned by the Tukey-Kramer park means comparison tests (see Table 4-2 in Chapter 4) were calculated. The total accumulation of SOCs in second-year conifer needles per ha of forest were estimated by multiplying needle SOC concentration (ng/g dw) by the dry weight of needles produced each year (kg/ha) (see Table 5-5). So, in a park exposed to comparatively high concentrations of current use agricultural chemicals, such as SEKI, an estimate for endosulfan concentration in second-year needles might range from 80 to 450 mg/ha of forest, depending upon the productivity of the site, whereas the range for a more remote park might be 1.6 to 9.1 mg/ha. Because this value considers only second-year needles, and 3-7 years of needles are typically present, the total amount in live needles/ha at any point in time would be larger. This quantity obviously could be much lower, as (1) vegetation density varies across the landscape and with elevation from forest to woodland to krummholtz to bare rock and (2) vegetative productivity declines to zero. Productivity (i.e., needle biomass) varies much more across sites than needle SOC concentrations, which were generally less than 8-fold different among the WACAP sites and parks (i.e., from Alaska to Texas). Forest productivity is therefore a more important variable

in predicting the total amount of SOCs removed from the atmosphere and potentially contributed to soils by vegetation.

Estimates of total quantities of historic use compounds accumulated by second-year needles per hectare tended to be lower than estimates of current use pesticides, but within the same order of magnitude (Table 5-5). For example, in the cleanest, most remote sites, a low productivity forest would accumulate about 3.0 and 2.7 mg/ha of current and historic use pesticides, respectively. In contrast, a high productivity forest close to important regional sources of pesticides could accumulate 729 and 211 mg/ha of current and historic use pesticides, respectively. PCBs were about 10-fold lower. Summed PAHs were 10 to 1,000 fold higher, or 70 to 65,840 mg/ha for low productivity remote forests compared with high productivity forests close to sources.

How well are forests scrubbing the air of pesticides relative to the amounts of pesticides applied? There are 125 million hectares of coniferous forest in the western United States and Alaska (US Forest Service, 1997; also see Chapter 1 ecoregion maps), of which 101 million hectares are publicly owned. If the second-year needles on each hectare scrubbed on average 3.65 to 27.5 mg of dacthal or 10.2 to 57.7 mg of endosulfans annually (from Table 5-5), multiplying by 125 million hectares, the total amount scrubbed per year could be between 456 and 3,430 kg of dacthal and 1,280 and 7,210 kg of endosulfans, or at most ~2 % of the reported total national commercial application in 2002 of dacthal and endosulfans, 198,000 and 284,000 kg, respectively (Figure 4-13 in Chapter 4).

Table 5-6. Endosulfans: Per Hectare Comparison of Estimated Annual Endosulfan Accumulation in Second-Year Conifer Needles and Typical 2002 Endosulfan Application Rates of This Pesticide in the Western United States. A hectare of forest can accumulate endosulfans at levels as high as medium regional application rates; total accumulation increases with forest productivity and proximity to sources. Background colors for conifer needle endosulfan accumulation are matched to regional application rates in the lower part of the table.

	Endosulfans Accumulated in Second-Year Conifer Needles (g/ha)*		
Forest location	**Low Productivity Forest**	**Average Productivity Forest**	**High Productivity Forest**
Remote	0.0016	0.010	0.080
Near Source	0.0091	0.058	0.45
2002 US Endosulfan Application Rates**	**(g/ha)****		
Not applied	0		
Lowest	0.0085 to 0.049		
Low	0.05 to 0.15		
Medium	0.16 to 0.55		
Highest	0.55 to 2.19		
Highest	≥ 2.20		

* Estimates from Table 5-1 rows (remote = low SOC concentrations, near source = high SOC concentrations) and columns (low productivity forest = minimum, average productivity forest = average, high productivity forest = maximum). Values in Table 4.1 were multiplied by 1000 to convert from mg/ha to g/ha.

**Average annual use of active ingredient in grams per hectare of agricultural land in county (converted from lbs/square mile) from Figure 4-14 in Chapter 4.

Because most of the national application occurs in the eastern half of the United States, and application in the western United States is uneven in distribution and intensity (see US map in Figure 4-13 in Chapter 4), perhaps a better way of approaching the question is to ask how well forests scrub the air under differing forest productivity and regional application rates. Table 5-6 shows endosulfans as an example.

A hectare of high productivity forest close to sources can absorb endosulfans in amounts that are equivalent to a medium application rate, or ≤ 25% of maximum per hectare application rates. At the other extreme, a hectare of remote, low productivity forest can be expected to absorb a very small total amount of endosulfans relative to a high productivity site near sources, which would still be about 10-20% of the amount applied per hectare in a very low use area but < 0.1% of the amounts applied per hectare in highest use areas. It seems reasonable to conclude that in addition to climatic factors discussed in Chapter 4 (notably temperature and precipitation), the capacity of a forest to scrub endosulfans from the air is a function of forest productivity (amount of leaf area—affected also by species-specific cuticular properties), its proximity to areas where endosulfans are applied, and the application rates in those areas. Pesticides other than endosulfans can be scrubbed by needles to a greater or lesser extent, depending on their physico-chemical properties, such as octanol-air partitioning coefficient (K_{oa}) and air-water portioning coefficient (K_{aw})(Su et al., 2007).

The importance of vegetation in scrubbing the atmosphere of organic contaminants has been discussed by other authors. For example Simonich and Hites (1994) estimated that as much as 24-72% of PAHs emitted in the atmosphere in the northeastern United States are removed by vegetation. More recently, Su et al. (2007) found extraordinarily high deposition velocities of PBDEs, PCBs, and PAHs in boreal and deciduous temperate forests in Canada and Germany. Model calculations suggest that the forest filter effect is most pronounced for SOCs with a log K_{oa} between 7 and 11 and a log K_{aw} > -6 (Wania and McLachlan, 2001). For such chemicals, uptake in forests can notably decrease air concentrations and markedly decrease the long-range transport of some SOCs, for example, to the Arctic (Su and Wania, 2005).

5.2.4 Evidence of Biomagnification

How do SOC concentrations in fish and vegetation compare with those in the media that transport contaminants to ecosystems (i.e., snow, lake water, and air)? The differences can be dramatic, and illustrative of the ability of biological organisms to accumulate molecules from the environment against astoundingly large concentration gradients. Figure 5-3 compares patterns and magnitudes of SOC concentrations in snow, lake water, sediments, lichens, conifer needles, and fish from Emerald Lake (SEKI). SOC compounds are listed in order of increasing K_{ow}, or decreasing polarity and solubility in water. Concentrations of all the media are displayed in picograms (i.e., trillionths of a gram) per gram wet weight, so they can be compared on the same (log) scale. SOC concentrations in XAD resin are not discussed; they were used to indicate relative differences between sites and were not converted to ambient air concentrations. The most striking observation in Figure 5-3 is that concentrations in biota and sediments are very much higher, by 3 to 7 orders of magnitude, than those in snow and, especially, lake water.

Another observation is that patterns of accumulation differed among media and among terrestrial and aquatic environments within the same watershed—evidence of different exposures, accumulation mechanisms, revolatilization rates, and, in the case of biota, the ability to metabolize and actively degrade and eliminate SOCs. For example, compared to sediments, fish had higher

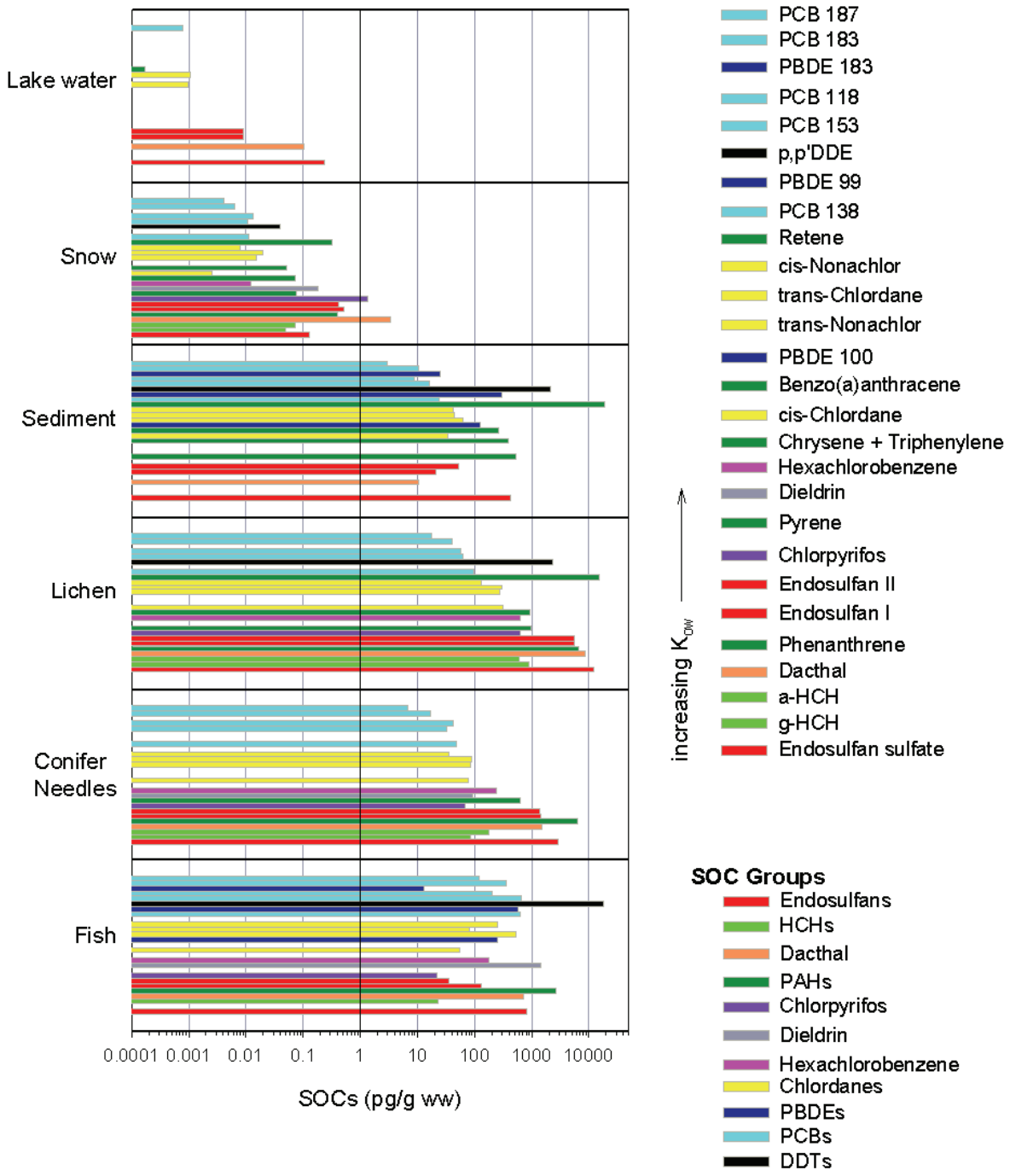


Figure 5-3. Mean SOC Concentrations (pg/g ww) in Lake Water, Snow, Sediments, Lichens, Conifer Needles, and Fish from Emerald Lake (SEKI). SOCs are ordered by increasing K_{ow}, or decreasing polarity and solubility in water, color-coded by group. SOC concentrations were 3 to 7 orders of magnitude higher in sediments and biota relative to snow and water. SOC concentrations in water, snow, and vegetation, but not sediments and fish, generally decreased with decreasing polarity. Compared to vegetation, fish were better accumulators of PCBs and dieldrin and poorer accumulators of PAHs, endosulfans, HCHs, dacthal, and chlorpyrifos. If no data are shown, all samples were below detection limits; PBDEs were measured in sediments and fish only. SOC concentrations (pg/g ww) are on $\log_{10}$ scale.

concentrations of PCBs and most pesticides. Compared to vegetation, fish had higher concentrations of PCBs, dieldrin, and DDTs, but lower concentrations of other pesticides and PAHs.

Fish and sediments concentrated compounds across the K_{ow} spectrum tested, which makes sense because of the complex organic and mineral chemistry of sediments and the presence of both lipids and water in fish and vegetation. In contrast, SOC concentrations in lake water, snow, and vegetation tended to decrease along the K_{ow} gradient, i.e., with decreasing solubility of SOCs in water. Although this is not surprising for snow and lake water, conifer needles and lichens had total lipid concentrations that were similar to those in fish. A possible explanation is that fish are able to accumulate lipophilic substances in fatty internal tissues and organs, whereas lipophilic SOCs absorbed by the waxy cuticles of conifer needles and by the lipid components of lichens might be partially revolatilized into the atmosphere during warm weather. Indeed, age and lipid concentrations were the best predictors of SOC concentrations in WACAP fish (subsection 5.2.2.2), whereas at least some research indicates that SOC concentrations in vegetation can equilibrate with the atmosphere over time (Keymeulen et al., 1993).

One of the air sampling objectives was to determine whether SOC concentrations in XAD resin can be used to predict concentrations in vegetation. All three media (i.e., air, lichens, and conifer needles) were sampled at four WACAP sites in core parks: Wonder Lake (DENA), Snyder and Oldman lakes (GLAC), and Lone Pine Lake (ROMO). Table 5-7 shows Spearman Rho correlations between concentrations of SOCs in XAD resin, conifer needles, and lichens at these sites. As a result of differential absorption abilities of SOCs across media, SOCs in vegetation could not be predicted from concentrations in XAD resin ($R^2 < 0.08$); however, concentrations in conifers and lichens were correlated ($R^2 = 0.63$). Comparison of the patterns of SOC accumulation across the three matrices (Figure 5-4) shows that the XAD resin appears to absorb compounds preferentially according to the K_{ow}, peaking at endosulfan I and then decreasing. Although not useful for predicting SOC concentrations in vegetation, PASDs are still a valuable and simple tool for comparing relative atmospheric concentrations across sites.

Table 5-7. Correlations (R^2 coefficients) between Total Pesticide Concentrations in XAD Resin (pg/g dry XAD), Conifer Needles (ng/g lipid), and Lichens (ng/g lipid) from Wonder, Snyder, Oldman, and Lone Pine Lake Watersheds.

	XAD	Lichens	Conifer Needles
XAD	1.000	0.053	0.073
Lichens	0.053	1.000	0.630
Conifer	0.073	0.630	1.000

5.3 Biological Effects

5.3.1 Effects of Contaminants and the Utility of Biomarkers

Many pollutants (e.g., Hg and especially dioxin) have long been known to be extremely toxic to biota, even at low concentrations. Toxicity occurs as both an acute response and a chronic or delayed response. The acute response, not necessarily applicable to WACAP because most contaminant concentrations are fairly low (0 - 10 ppb), is similar to that of a drug-overdose. Concentrations are elevated to the point of large-scale physiological shutdown. The chronic or

delayed response is more applicable to WACAP, and more interesting biologically, from our perspective. For example, at low concentrations when the chemicals are not acutely toxic, endocrine and immune changes can occur that are measurable in the laboratory with biomarkers. Efforts can then be made to correlate those changes to contaminant concentrations. WACAP does not attempt to establish cause and effect, but looks for patterns that warrant future investigation.

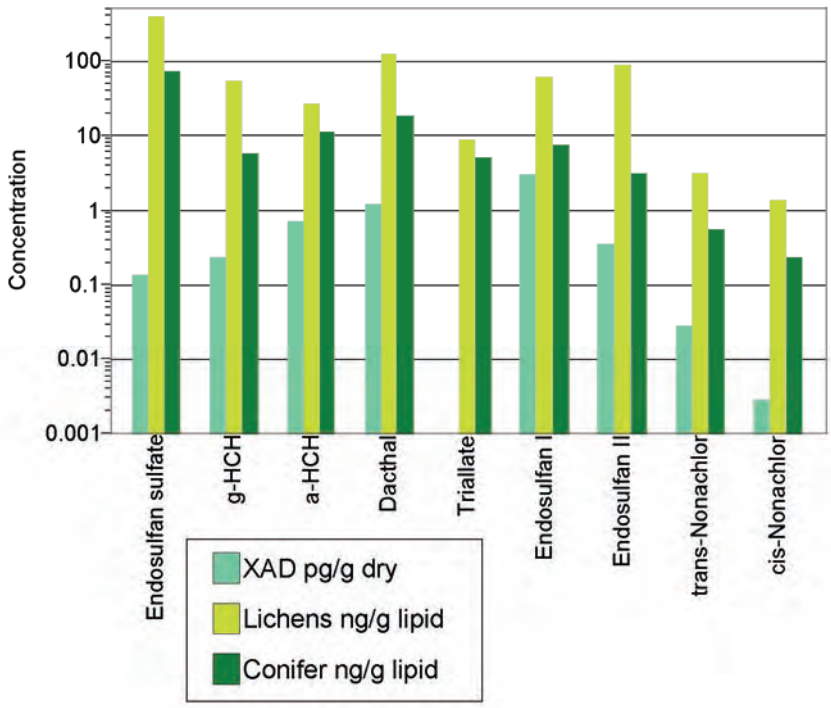


Figure 5-4. Pesticide Concentrations in Dry XAD Resin (used to sample air), Conifer Needles, and Lichens from Oldman Lake (GLAC). Compounds are ordered by increasing K_{ow} or decreasing polarity. Concentrations in vegetation decreased gradually with increasing K_{ow}; concentrations in XAD resin increased to Endosulfan I, then decreased. Differing affinities for SOCs might explain the poor correlations observed between SOC concentrations in XAD resin vs. vegetation (Table 5-7).

Fish are bioindicators of contaminant exposure because they are often top predators of aquatic ecosystems and accumulate organic and metal contaminants, usually via the diet (Thomann, 1989). Salmonids (*Oncorhynchus* spp. and *Salvelinus* spp. in this study), are often the keystone aquatic predators, yet they are prey to birds and mammals (Mackay and Fraser, 2000), where more significant effects of bioaccumulation (because of higher contaminant concentrations) can be observed. Measurement of contaminants in fish thus indicates impact to the part of the food web. Piscivorous animals are likely to have higher contaminant concentrations than the fish and the organisms forming the base of the food web are likely to have lower concentrations.

The potential effects of contaminants on the fish themselves can be determined by identifying changes in fish biomarkers. By extension, negative effects on fish can warn of harm to the ecosystem; therefore, changes in biomarkers are considered an early signal of negative effects (see Figure 5-1) (van der Oost et al., 2003). Fish biomarkers are tools that can be used to determine the relationship between contaminants and impaired health in individual fish (NRC,

1987). Biomarkers fall into three classifications: (1) markers of exposure, (2) markers of effect and, (3) markers of susceptibility (NRC, 1987). The biomarkers used in WACAP are markers of effect because they precede and can predict impaired health (NRC, 1987). The utility of biomarkers hinges on validating them by correlating exposure of chemicals to the change in the biomarker (NRC, 1987). Impaired health, for our purposes, refers to any relationships identified between contaminants and the biomarkers, as changes in biomarkers are considered to be an abnormal response. The difficulty lies in determining what is normal for the animal. Repeated sampling of the same water bodies over long periods of time in an effort to monitor changes in biomarkers was not possible. However, we did obtain samples for reproductive biomarkers for two different years from several of the WACAP sites, which in most cases replicated our initial results. The sampling strategy used by WACAP was unprecedented in geographic scale and ecological variability. As such, this strategy prohibited repeated sampling of the same locations for contaminant concentrations because of the extremely remote nature of the field sites and the time needed to complete the work.

Laboratory studies have documented the deleterious action of environmental pollutants on biota; however, the ecological significance or the significance to overall population health is still largely unknown and is recognized as a limitation on the use of biomarkers (Mills and Chichester, 2005). Results from laboratory studies are often limited by the fact that environmentally irrelevant concentrations of chemicals are often needed in laboratory studies to replicate observations obtained from the field. Recent work, however, has demonstrated that a mixture of environmental estrogens induced an estrogenic response, even though the individual contaminants were below the concentrations needed to induce a response on their own (Rajapakse et al., 2001, 2002). In addition, the "weak" xenoestrogens, dieldrin, endosulfan, and o,p′-DDT, induced estrogenic responses from 10^{-9} to 10^{-12} molar concentrations *in vitro* (Wozniak et al., 2005). In a recent study of trout in mountain lakes of Europe, Garcia-Reyero et al. (2007) found that HCB, PCBs, and DDTs were correlated with estrogenic activity in muscle extracts of the salmonids they examined.

We recognize that extensive laboratory studies testing the same chemicals and mixtures of chemicals identified in the field samples for estrogenic and reproductive effect would be desirable. However these experiments were beyond the scope of WACAP. Instead, we followed the approach used in the USGS Biomonitoring of Environmental Status and Trends Program (Schmitt and Detloff, 2000) and earlier argued by Ham et al. (1997) to use multiple biomarkers for ecotoxicology studies. With these guidelines, researchers can approach the topic of immune or endocrine disruption from a weight of evidence standpoint, by using multiple biomarkers and co-existent contaminant concentrations. To that end, we used macrophage aggregates, plasma vitellogenin (Vtg), 11-ketotestosterone, testosterone, estradiol, and gonad, kidney, liver, spleen, and gill histopathology to look for signs of abnormal changes in fish resulting potentially from contaminant concentrations. Appendix 5A provides a summary of all fish health measurements and biomarker values. In this chapter, we focus on results from macrophage aggregate analysis, plasma Vtg in male fish, and gonad histopathology, and draw upon the remaining biomarkers for supporting evidence when appropriate.

The rationale for choosing the biomarkers we did was guided by the following objectives.

1. Utilize accepted biomarkers that are sufficiently supported by the scientific literature.

2. Use biomarkers that can be attributed to a specific suite of contaminants for which the mechanism of action is established.
3. Use biomarkers that are cost effective, and suitable to current laboratory equipment.
4. Use biomarkers that do not affect other project objectives. For example, because biomarker and SOC analyses were performed on the same fish, it was desirable to remove as little tissue as possible from the animal so as to not dilute the contaminant signatures.
5. Identify numerous endpoints that can be measured in one tissue (e.g., sex steroids and Vtg can be quantified in the blood).

Satisfying these objectives is difficult and we acknowledge that all of our biomarkers do not satisfy every objective. The most important of these objectives were 1 and 4. In terms of number 1, we argue that efforts to develop biomarkers while simultaneously identifying relationships between those biomarkers and contaminants would have been difficult to achieve. Secondly, our foremost project objective was to provide fish intact enough to accurately assay contaminant concentrations. So biomarkers that destroy most of a tissue (e.g., the liver for mixed function oxidase analyses) would violate objective number 4. With these objectives in mind, we provide data on the biomarkers listed (see Appendix 5A for all of our measurements). We report in detail in the following sections on the biomarkers for which we found significant and plausible relationships from which some level of inference could be drawn. In addition, we report on biomarkers deemed to be of interest to the scientific community, the NPS, and the public.

5.3.2 Overview of General Fish Health

An important aspect of WACAP was that SOC and Hg concentrations would be determined in whole fish, as opposed to individual organs (e.g., liver), and that coincident changes in health could be determined in those same fish. With that in mind, a health-based necropsy procedure, similar to that used by Adams et al. (1993) was performed on every fish in the field to identify abnormalities that might or might not be related to contaminant concentrations. As discussed in Chapter 3, numerous tissues also were removed from the fish, successfully preserved in the field, and shipped to the laboratory for further microscopic and analytical procedures to assess the health of the fish. In general, our necropsy procedures did not reveal any gross abnormalities in the fish captured during these studies. However, numerous lake trout from GAAR were infected with nematode worms, later determined to be *Raphidascaris* spp. (Figure 5-5).

The definitive host is the northern pike (*Esox lucius*), one of which was captured as by-catch, so the presence of these parasites was not entirely unexpected. External copepod parasites were found on lake trout from Burial Lake, NOAT, and tapeworms and other unidentified nematodes were also found in Matcharak Lake fish (Figure 5-6). These parasitic infections probably did not result from contaminant concentrations. For complete descriptions of the pathologies identified in the lake trout from the Arctic and the other trout studied in WACAP, see Appendix 5A.

5.3.3 Biomarkers

5.3.3.1 Macrophage Aggregates (MAs)

Macrophage aggregates (MAs) are focal accumulations of pigmented macrophages occurring primarily in hematopoietic and hepatic tissues of fishes and other poikilothermic animals, and are thought to be the primitive analogs to mammalian lymph nodes (Wolke, 1992; Agius and Roberts, 2003). They can also occur in other organs, such as the gonads of fishes captured from

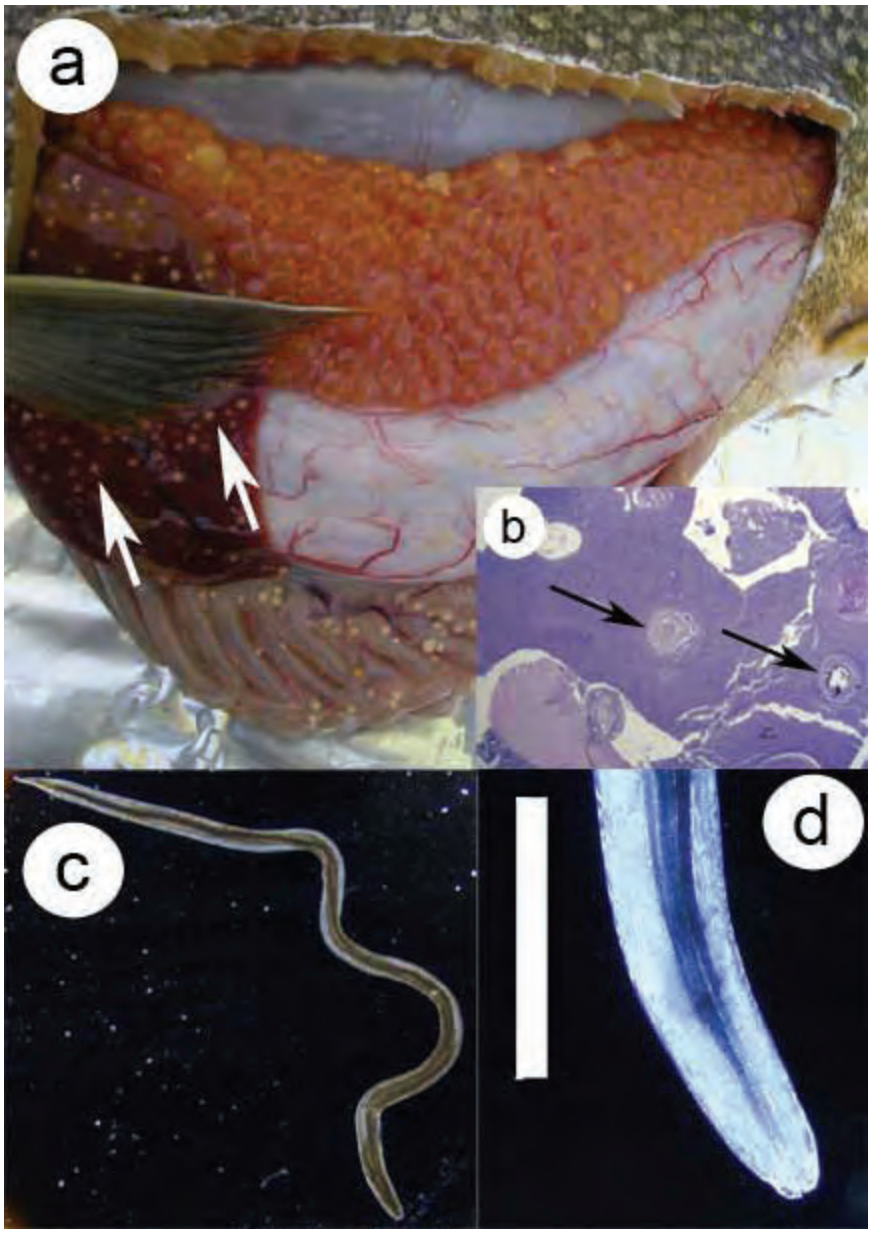


Figure 5-5. Incidental Pathology Affecting Multiple Organs from Multiple Lake Trout at Matcharak Lake (GAAR). Nodules on the liver [arrows in (a)] are encysted larval nematodes shown histologically [arrows in (b)]. Dissection of the nodules revealed numerous *Raphidascaris* spp. (Nematoda) wet-mount preparation shown in phase-contrast, bar = 1 mm (c, d). Hematoxylin and Eosin.

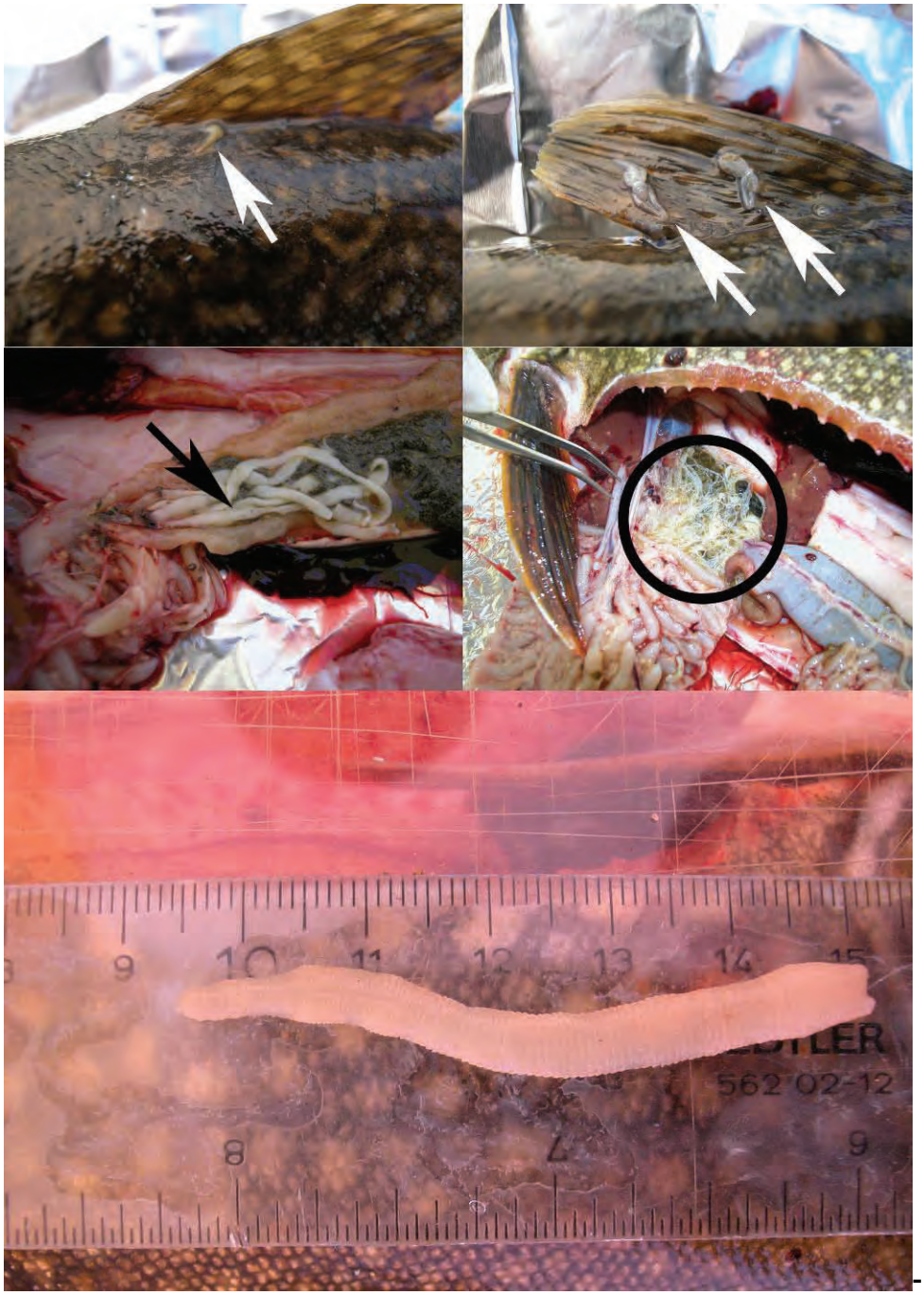


Figure 5-6. External (white arrows) Copepod Parasites and Internal (black arrow, circle) Parasites (tapeworms and roundworms) in Lake Trout from Burial Lake (NOAT) and Matcharak Lake (GAAR), Respectively. Tapeworm on the ruler is representative of the tapeworms denoted by the black arrow.

degraded environments (Blazer, 2002; Patino et al., 2003). Macrophage aggregates are thought to be storage centers for cellular debris (Wolke, 1992; Agius and Roberts, 2003); therefore, toxicants that induce tissue damage are likely to be associated with MAs. In terms of physical appearance, melanin, hemosiderin, and ceroid/lipofuscin are the pigments contained within the MAs, ranging in color from golden to brown to black in slides stained with hematoxylin and eosin (Wolke, 1992; Agius and Roberts, 2003) (Figure 5-7). Increases in pigment content are suggestive of catabolic, infectious, toxic, or otherwise stressful events or exposures (Wolke, 1992; Agius and Roberts, 2003). In a polluted river in Germany, Meinelt et al. (1997) found positive correlations between liver, kidney, and spleen MAs and mercury in individual pike (*Esox lucius*). Handy and Penrice (1993) induced MAs in the rainbow trout kidney by chronic *per os* exposure of 10 mg/kg $HgCl^-$ over 42 days. Although this is a high dose, it establishes the proof of concept that mercuric compounds can induce the formation of MAs in salmonid fishes. This broad application of MAs for assessment of fish and environmental health has been well documented in many fishes.

5.3.3.1.1 Data Analysis

Our objectives were to use MAs as potential indicators of age-dependent contaminants, such as Hg, where N = 10-25 per lake, but all contaminants were tested for association with MAs. We also intended to determine if potential among-lake differences in MAs could be associated with differences in contaminants in those lakes. Among-lake comparisons were made by ANOVA or Kruskal Wallis, followed by a Bonferroni post hoc at 95% confidence. Levene's test was used to determine if there was equal variance in MAs between the lakes. Distributions of MAs from the spleen and kidney of WACAP fish for which there was corresponding SOC and Hg data (N = 8-10 fish per lake) are shown, by lake, in Figure 5-8. Fewer data are available for these comparisons because SOCs were determined on a subset of the fish where Hg and biological data were also available. Relationships between contaminants and MAs were made with simple linear or log regression. Arcsine square-root transformations were used on the MA data and log10 transformations on the Hg data. Before we grouped and analyzed all brook trout for contaminant and MA relationships, we determined if potential co-variates (age, sex, maturation state, and condition factor) were different among lakes. Percent area occupied by MAs in the kidney, spleen, and liver was quantified following the method of Schwindt et al. (2006). Liver MAs are reported in Appendix 5A.

5.3.3.1.2 Results and Discussion

The following comparisons were made on trout where Hg, SOC, and biological data were available. In the brook trout (N = 9-10 fish per lake), no significant differences in kidney or spleen MAs among lakes were found, although a significant main effect suggested that overall there were differences among lakes in mean arcsine square root transformed spleen MAs (ANOVA $F_{6,62} = 2.84$, $P = 0.02$), as well as kidney MAs (ANOVA $F_{6,62} = 2.38$, $P = 0.04$). The *Oncorhynchus* spp. (N = 8-10 fish per lake) were analyzed together because different species or subspecies were captured at each lake. Any differences could be confounded by the potential differences between species, but at least the genus is the same. Both spleen ($F_{2,25} = 4.75$, $P = 0.02$) and kidney ($F_{2,25} = 6.84$, $P = 0.004$) MAs were higher in Snyder Lake fish than in Oldman Lake fish, and fish in both GLAC lakes were not different from rainbow trout (*Oncorhynchus mykiss*) at Mills Lake, ROMO. In the lake trout (N = 8-10 fish per lake), arcsine square-root transformed spleen MAs were significantly elevated in Wonder Lake fish compared to fish in Matcharak and Burial lakes ($F_{2,25} = 34.81$, $P = <0.0001$) and higher in Matcharak Lake fish than

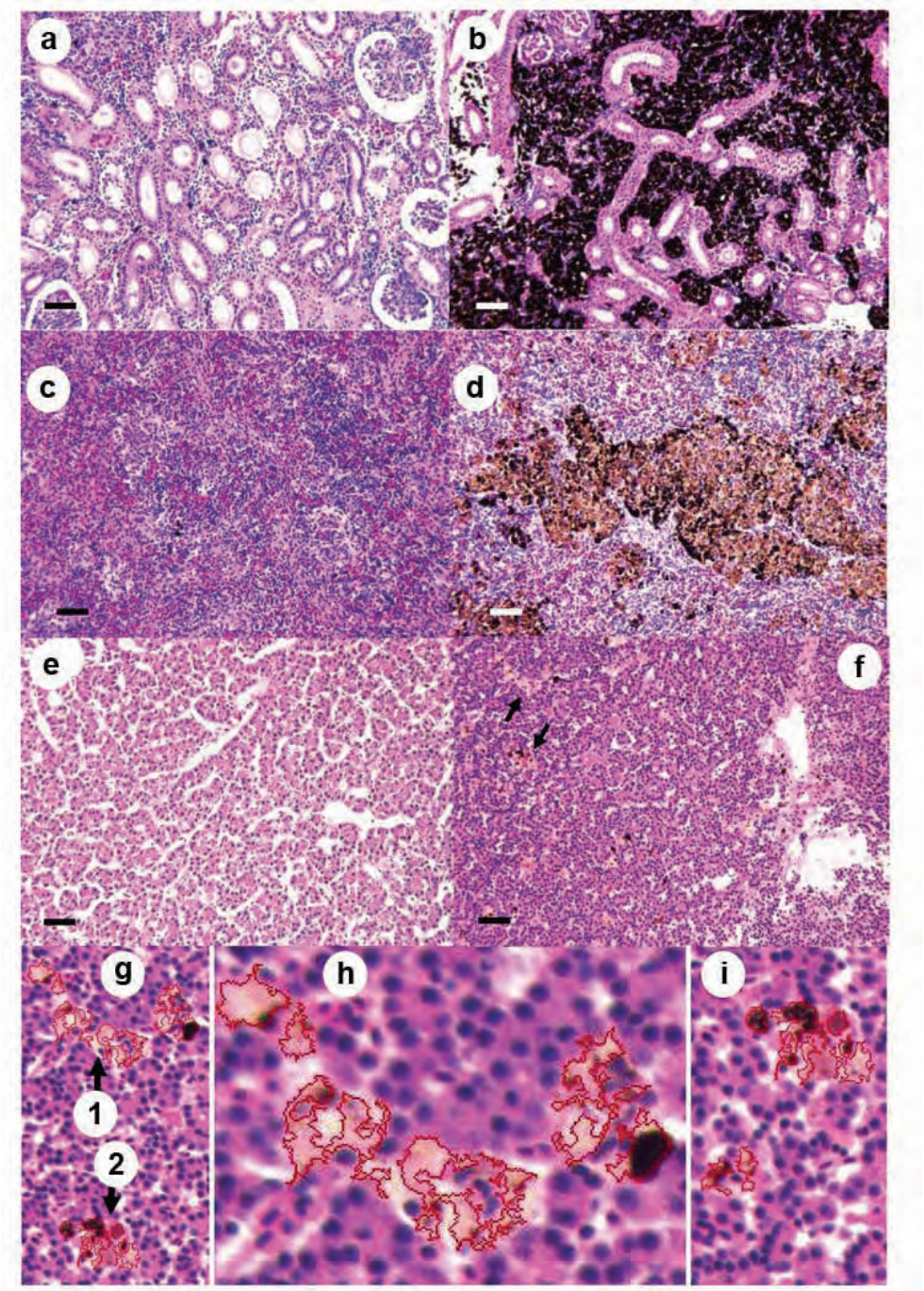


Figure 5-7. Representative Hematoxylin-Eosin Stained Brook Trout Organs Showing the Relative Difference between Fish with Very Few or No Macrophage Aggregates (MAs) and Extensive Accumulations of MAs (a-f) and Outlined High Magnification Hepatic MAs (g-i). Bars = 50 µm; (a) Kidney with a few MAs; (b) Kidney with extensive MAs; (c) Spleen with a few MAs; (d) Spleen with extensive MAs; (e) Liver with no MAs; (f) Liver with extensive MAs; (g) High magnification of liver MAs corresponding to MAs (arrows) in (f); (h) 2X magnification of the MA corresponding to arrow 1 in G; (i) 2x magnification of the MA corresponding to arrow 2 in (g). The outlined areas in (g) through (i) are the computer output of delineated MAs based on pigment selection by the computer program in the liver. Modified from Schwindt et al. (2006).

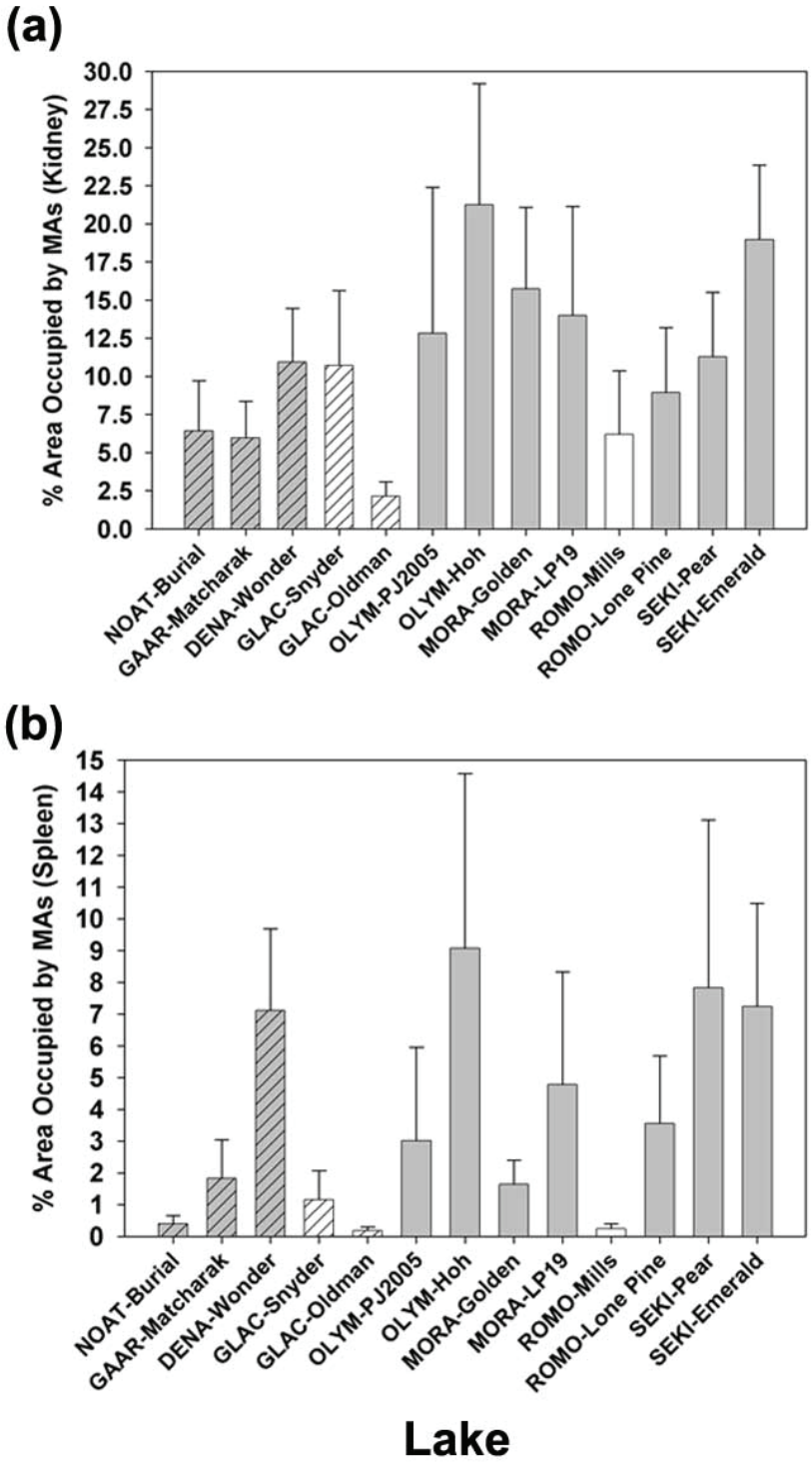


Figure 5-8. Mean Percent, %MAs + 95% Confidence Intervals, for Fish with Corresponding Hg and SOC Data: (a) Spleen, (b) Kidney. Gray bars = brook trout, White bars = rainbow trout, White hatched bars = cutthroat trout, and gray hatched bars = lake trout. *N* = 8-10.

in Burial Lake fish. The significance of these among-lake differences remains to be determined; no contaminant patterns emerged among lakes that could explain differences in the MAs.

In the WACAP lakes with brook trout, mean fish age and condition factor were not significantly different among lakes (ANOVA $p > 0.05$). Comparison of median (because of unequal variance) arcsine square-root transformed spleen MAs in brook trout for which there were biological and Hg data (n = 10-25 fish per lake) yielded a significant main effect (Kruskal Wallis Test Statistic = 14.31, $p = 0.03$), but differences between individual lakes were not detected (Bonferroni). Average Hg ($\log_{10}$ transformed to normalize data) was significantly elevated at LP19 (MORA) and Hoh Lake (OLYM), compared to Lone Pine Lake (ROMO) and Golden Lake (MORA) (ANOVA $F_{6,93} = 4.33$, $p = 0.0007$). The results from these analyses suggest that the observed among-lake differences in Hg were not consistently related to differences in MAs. Furthermore, sex did not appear to affect the regressions because there was no difference in MAs between the sexes ($T_{39female,\ 59male} = 1.12$, $p = 0.26$). Sexual maturation was not different between sites (ANOVA $p > 0.05$, Figure 5-9). Finally, the slopes of the among-lake regression lines were not different for MAs versus age ($F_{1,6} = 0.81$, $p = 0.56$) or MAs versus Hg ($F_{1,6} = 1.43$, $p = 0.21$). This suggests that MAs respond to age and Hg equally among lakes. Based on these results, we did not identify any confounding factors affecting MAs, Hg, or age among lakes. Therefore, we grouped the brook trout data for the following regression analysis.

The following results are based on all brook trout where Hg and biological data are available. Knowing that MAs are correlated with age in these fish (Schwindt et al., 2006), we identified a suite of contaminants thought to be associated with MAs based on their suspected age-dependence. Total whole body Hg was chosen because it was also associated with age, although all contaminants analyzed in WACAP were screened for potential associations to MAs. In our results, Hg was positively associated with spleen MAs and age, with the strongest relationships observed in brook trout ($F_{1,98} = 82.82$, $p < 0.0001$, $R^2 = 0.45$) (Figure 5-10a). In the brook trout, positive relationships were also found between MAs and the ∑PCBs (F1,68 = 26.04, $p < 0.0001$, $R^2 = 0.28$) and the ∑PBDEs ($F_{1,68} = 17.14$, $p = 0.0001$, $R^2 = 0.20$). As mentioned previously, MAs have been associated with pathogenic infections of microbes in fish. However, in our analysis of MAs in fish from WACAP, MAs were not influenced by the presence of parasites, or evidence of other infectious agents (e.g., bacterial kidney disease-like granulomas). That is, MAs were not concentrated around the parasites or granulomas, nor were they more abundant in fish with parasite infestations.

To further delineate the relationship between MAs and Hg, we searched for age-independent associations between MAs and Hg. To do this, we divided our datasets into age classes prior to regression analysis for every species of fish where data were available. The best relationships we found were in the 4- to 6-year-old brook trout with significant age-independent increases in MAs and Hg ($F_{1,46} = 26.27$, $p < 0.0001$, $R^2 = 0.36$) (Figure 5-10b). In the 1 to 3- and 7- to 13-year-old trout, only weak relationships, if any, were identified (see Appendix 5C). Dividing the 7- to 13-year-old classes further did not yield any significant relationships (data not shown). Appendices 5B and 5C contain tables of regression statistics for all permutations performed on the data to search for relationships between Hg, MAs, and age.

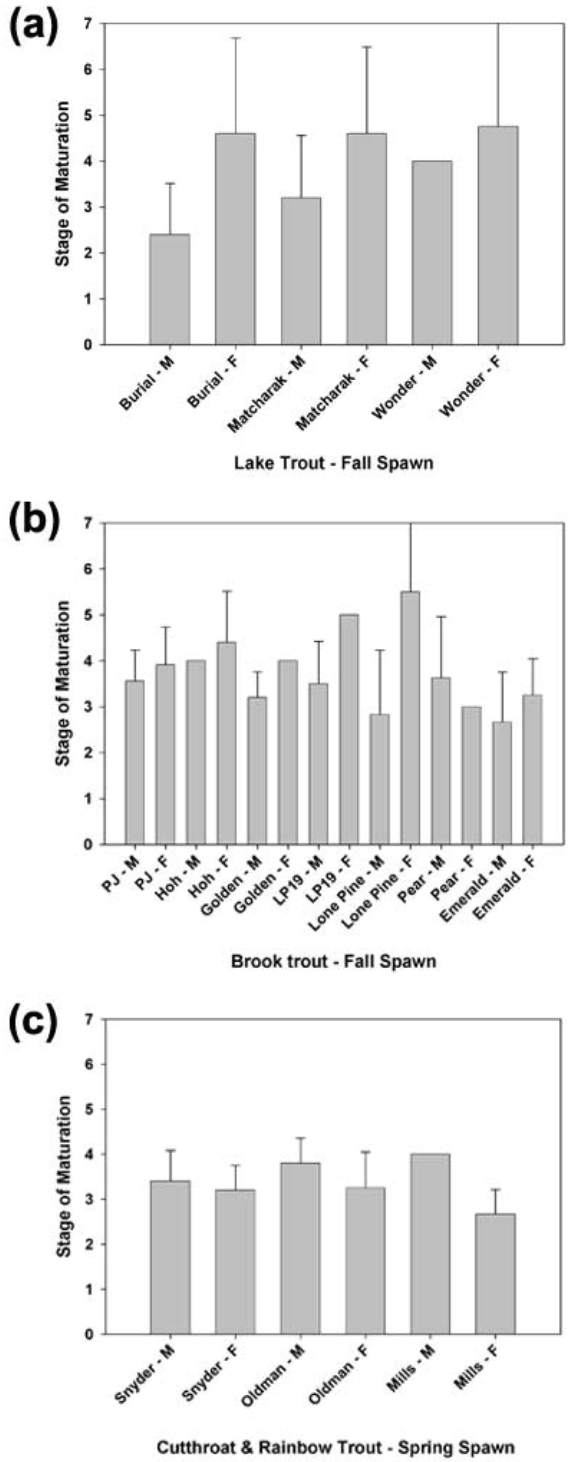


Figure 5-9. Average Stage of Gonad Maturation ± 95% Confidence Intervals in Trout for which Corresponding SOC and Hg Data are also Available. *N* = 2-11; M = male; F = female; (a) lake trout, fall spawn, (b) brook trout, fall spawn, (c) cutthroat and rainbow trout, spring spawn.

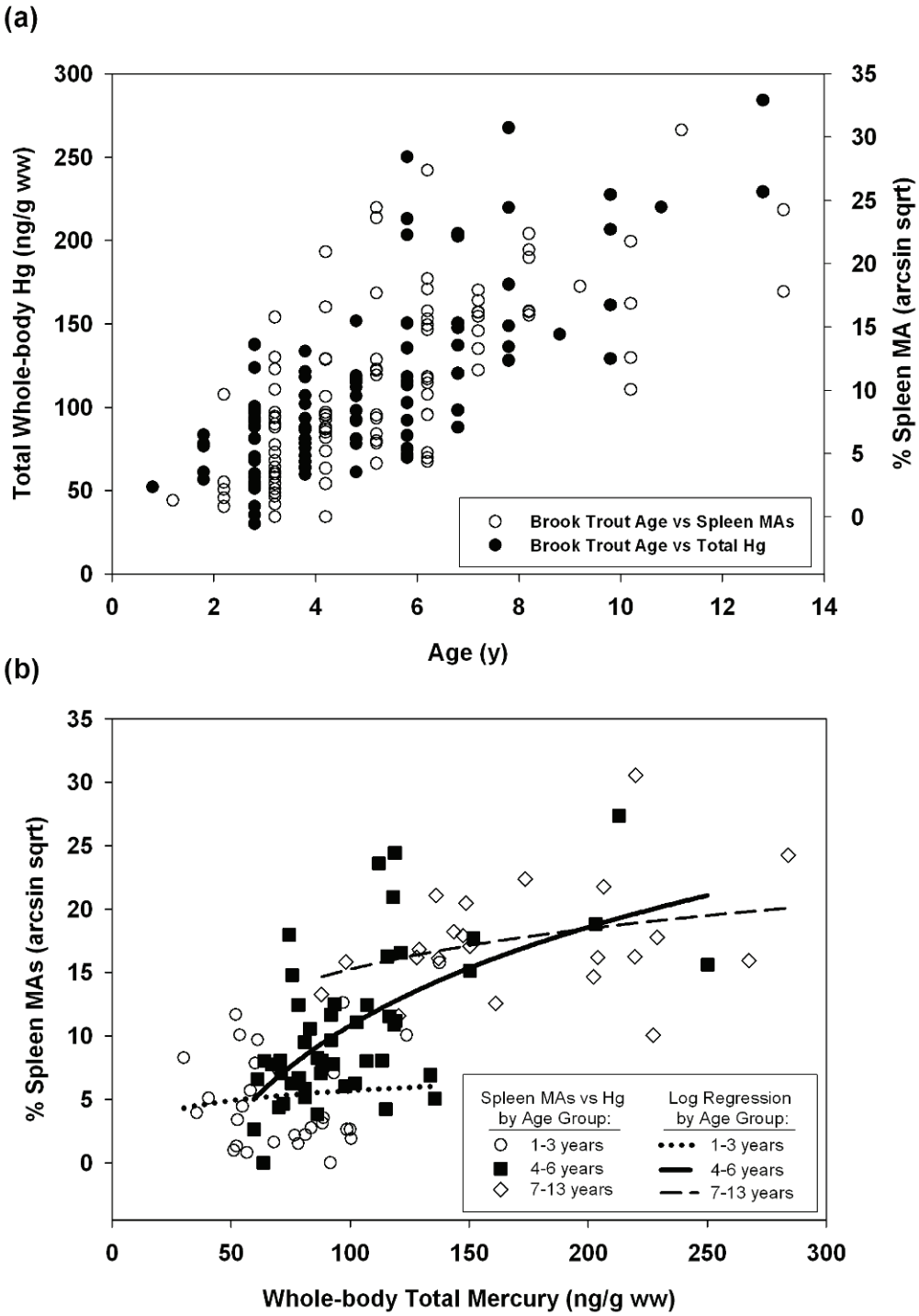


Figure 5-10. Co-Linearity between (a) Splenic MAs, Hg, and Age in Brook Trout, and (b) Log-Linear Relationships between Hg and Brook Trout (*Salvelinus fontinalis*) Splenic MAs. See Appendix 5C for regression statistics. Some data are overlapping and x-axis data in (a) are offset slightly.

Incorporation of Hg varies between life history stages of salmonid fishes (Mathers and Johansen, 1985). Mercury accumulates rapidly during periods of fast growth and increased food consumption (MacCrimmon et al., 1983). Mercury has been associated with MAs (Handy and Penrice, 1993); during periods of fast growth, MAs and Hg increase independently of age, as was observed in our results for 4- to 6-year-old brook trout. Nevertheless, the well-established fact that MAs increase with age has not been explained (for review see Agius and Roberts, 2003). Macrophage aggregates are more likely to result from the bioaccumulation of toxicants, and long-term multiple hits by acute stressors, than merely a side effect of aging and senescence alone. This phenomenon is comparable to the "liver spots" that form on fair-skinned humans. Liver spots (senile lentigines) develop in exposed regions of the skin of older persons because of long-term cumulative effects of UV radiation, not simply because of age itself (Porta, 2002).

Lake trout (from DENA, GAAR, and NOAT) represented a much smaller data set than brook trout, and their environment is considerably different from the lakes of the other trout species. The lake trout were captured from much larger lakes that contained more fish species than lakes in lower 48 states (see Table 5-2 for species). In addition, the lake trout were relatively older, with several individuals > 15 years. Significant relationships between Hg and MAs were found only in lake trout < 20 years old (Appendix 5C). Mercury concentrations decreased in lake trout > 20 years from the Arctic (GAAR and NOAT), yet, as with other fishes, MAs increased with age throughout the entire age structure (Appendix 5C).

Some data suggest a decline in MAs following exposure to contaminants (Payne and Fancey, 1989; Bucke et al., 1992). However, those results might have been confounded by movement of fish in and out of contaminated areas such as polluted bays or estuaries. Lakes in our study, aside from those in Alaska, were relatively small (< 20 km^2 surface area), and thus fish cannot migrate in or out of potentially contaminated areas. Therefore, changes in MAs in relation to contaminants are not confounded by migration and are more likely related to individual responses to the contaminants and/or other stressors. Several explanations, none conclusive, might account for the breakdown of the relationship between of MAs and Hg in older lake trout.

First, eventual sequestration of Hg in the muscle tissue might render Hg less "bioavailable" to the kidney, liver, or spleen, thus MAs decline subsequently. This observation holds, to a certain extent, for the fairly old brook trout as well. In 7- to 13-year-old brook trout, Hg was rather high, but no changes in MAs were observed (Figure 5-10b). Second, Hg is lethal to rainbow trout at exposures of 10-20 µg/g and toxic to fish at 1-5 µg/g (Niimi and Kissoon, 1994). It is conceivable that lake trout with higher Hg concentrations died disproportionately, and that the older fish are represented by those that have experienced less exposure to Hg. Third, ecology changes in food consumption might account for the rapid, non-age-associated changes in contaminant concentrations. To our knowledge, there are no studies that describe the fate of MAs after the stressor has been removed. Therefore, a fourth possibility is that MAs persisted long after the cause had ceased or declined in the old lake trout. Finally, these older fish might be subjected to a different MA-inducing stressor that younger fish that we are not aware of. We observed some interesting relationships when the lakes with lake trout were studied individually. In the lake trout from Wonder Lake, DENA, no relationship was found between MAs and age, thus the entire data set was used to determine positive correlations between MAs and Hg (Appendix 5C). In this case, MAs were significantly correlated with Hg.

Rainbow trout were collected only from Mills Lake, ROMO. The age distribution from this population was restricted; most of the fish were between 4 and 6 years of age, thus it was not possible to test other age classes. Nevertheless, the entire dataset was used, and MAs were significantly related to age, as demonstrated in an earlier publication (Schwindt et al., 2006), as well as to Hg (Appendices 5B and 5C) in this lake. Similarly, in the cutthroat trout (*O. clarki lewisi* and *O. clarki bouvieri*) from GLAC, it was not possible to test numerous age classes and MAs were related to both age and Hg (Appendices 5B and 5C).

Mercury is toxic to fish at relatively low concentrations (Niimi and Kissoon, 1994). Exposure of fishes to Hg affects numerous physiological endpoints, including histopathological indicators such as MAs. For example, Handy and Penrice (1993) illustrated numerous histological changes in addition to increased kidney MAs following Hg exposure in the laboratory. Corresponding Hg concentrations in the kidney ranged from approximately 2 to 9 µg/g (Handy and Penrice, 1993), which is within the realm of whole body concentrations found in WACAP. Mercury concentrations in individual organs and muscle tissue in the trout from this study would undoubtedly be much higher, as analysis of whole fish dilutes the Hg sequestered in target organs and skeletal muscle. In blue gourami (*Trichogaster trichopterus*) fed 9 ppb of methyl-Hg and exposed to viral and bacterial pathogens, the hemosiderin bodies, associated with MAs, were infiltrated by the white pulp of the spleen and decreased antibody production was also observed (Roales and Perlmutter, 1980). The reduction in hemosiderin was attributed to the infectious agents, not Hg, and the authors concluded that Hg induced immuno-suppression as indicated by reduced antibody production (Roales and Perlmutter, 1980). In the flounder (*Platichthys flesus*), Pulsford et al. (1992) observed localization of metals in splenic MAs, but no associations were made between the metals and changes in MAs.

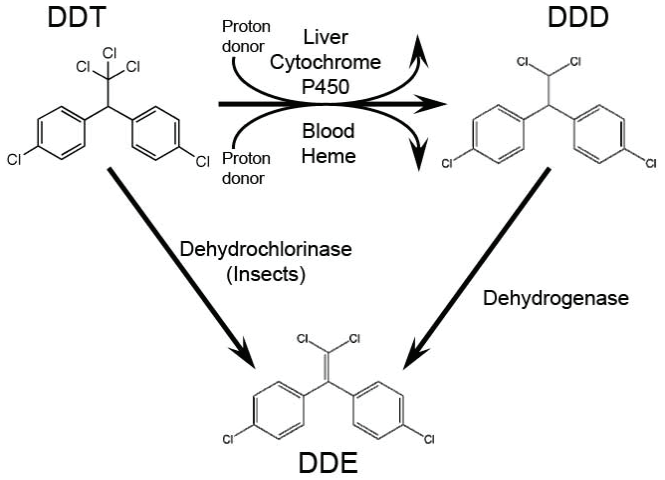
In summary, we demonstrate the association between MAs and Hg for brook trout, given the large geographic area sampled. This suggests that spleen MAs might be ideal sentinels for assessing the incorporation of Hg, or other largely age-dependent pollutants, into the food web. We do not generalize beyond brook trout, however, because of the small sample sizes and the limited geographic areas from which we collected the lake, rainbow, and cutthroat trout. Within lakes (other than those in the Arctic), Hg and age both explained significant amounts of variation in MAs. At present, we cannot explain why MAs were higher in lake trout from Wonder Lake, DENA, compared to the fish from the Arctic lakes (GAAR and NOAT). Nor can we explain why MAs were higher in fish from Snyder Lake, GLAC. We recommend continued Hg monitoring in whole fish. Sampling of fish for MAs could easily be conducted along with Hg sampling.

5.3.3.2 Vitellogenin in the Blood of Male Fish

Vitellogenin is an egg-yolk precursor protein synthesized in the liver of oviparous animals in response to estrogen, or xenoestrogen (Arukwe and Goksoyr, 2003). Male and immature female oviparous animals have the capacity to produce Vtg, but endogenous estrogens are rarely present in sufficient concentrations to induce appreciable amounts of plasma Vtg (Sumpter and Jobling, 1995). Thus, when Vtg is observed in the plasma of male or immature female fish, it suggests exposure and response to a xenoestrogen (Purdom et al., 1994; Jobling et al., 1996, 1998). These features have led to Vtg becoming a biomarker for environmental estrogen exposure and used as such in many studies (e.g., Purdom et al., 1994; Jobling et al., 1996, 1998; Harries et al., 1997; Christiansen et al., 1998; Gronen et al., 1999; Mills et al., 2001, 2003; Zaroogian et al., 2001; Palace et al., 2002; Sepulveda et al., 2002; Kavanagh et al., 2004; Feist et al., 2005).

Metabolism of DDT. It is apparent from these data that certain metabolites of contaminants are present in higher concentrations than the applied or active ingredient. For example, p,p'-DDE, one of the DDT metabolites, is present in higher concentrations in fish than DDT itself. The p,p'- and o,p'- isomers of DDT are metabolized in fish (and in the soil and lake sediment) to DDD and DDE. The most persistent of these is DDE and DDD is found only in very minute concentrations because is it rapidly metabolized.

In fish (and other vertebrates) DDT metabolism occurs in the liver, where many foreign and endogenous compounds are metabolized, as well as the blood. The liver contains the cytochrome P450 (CYP) enzyme family that is responsible for the oxidative metabolism of many substances, including DDT. There are many CYP enzyme sub-families and they reside in microsomes, which are vesicles that break off of cellular organelles. The CYP enzymes degrade DDT by dechlorination and dehydrogenation and eventually DDE (the most persistent metabolite) is broken down to the water-soluble DDA and is excreted from the animal. There is also evidence for non-enzymatic degradations.



Proposed Mechanisms of DDT Metabolism. Redrawn from Kitamura et al. (2002).

Environmental estrogens (xenoestrogens) are artificially produced chemicals that mimic the estrogens produced in animals. The proposed mechanism of action is similar to that of the native 17β-estradiol (E_2). That is, xenoestrogens bind to hormone receptors and either block or initiate the action that would otherwise be controlled by endogenous estrogens. The relative "strength" of xenoestrogens, measured by the concentration needed to displace endogenously produced E_2 from receptors, varies considerably. The birth control hormone, 17α-ethynylestradiol, is about 10 times stronger than E_2, and most other chemicals (e.g., DDT, bisphenol A, alkyphenol ethoxylates) are weakly estrogenic, requiring 10^3 to 10^6 times the E_2 to occupy the receptor (Zava et al., 1997; Kloas et al., 2000). Vitellogenin is the most abundant downstream product of estrogen receptor activation and is specific to E_2 and estrogen-like chemicals. The (in)activation of hormone receptors by anthropogenic chemicals is called endocrine disruption, the consequences of which are currently being debated in the scientific community. Recent evidence suggests that exposure to the birth-control hormone 17α-ethynylestradiol led to a population crash of the fathead minnow (*Pimephales promelas*) (Kidd et al., 2007).

Endocrine disruption is not limited to the actions of xenoestrogens; other hormonal pathways can be disrupted by different chemicals. For example, the brominated flame retardants affect thyroid hormone pathways (Legler and Brouwer, 2003; Jahnke et al., 2004). Polyaromatic hydrocarbons and other aryl-hydrocarbon receptor agonists (such as dioxin) are anti-androgenic (Safe, 1994). The banned organochlorine pesticide DDT (and its metabolites) is a well-known endocrine disruptor (Colburn et al., 1993) that binds to estrogen receptors and initiates cellular changes, such as the synthesis of proteins, such as Vtg. The degradation products of the current-use pesticides endosulfan and methoxychlor are also weakly estrogenic and have been detected in the fish in this study. Although not target analytes in WACAP, detergent additives, plasticizers, birth

control hormones, food additives, phytoestrogens (Kuiper et al., 1998; Bennetau-Pelissero et al., 2004; Fox, 2004), and some personal care products are also weakly estrogenic.

5.3.3.2.1 Data Analysis

Our objectives for the use of blood plasma Vtg in WACAP were to screen male fish for potential response to estrogen-like compounds and relate the Vtg to the contaminant concentrations. We assumed that male fish do not normally produce Vtg because circulating levels of endogenous estrogens are very low (10s to 100s ppt) and are not thought to induce Vtg. For comparison, 10 to 100 times as much E_2 is needed in females to significantly increase Vtg levels. Despite this, all male fish in WACAP were analyzed for E_2 to be sure that endogenous estrogens were not influencing the Vtg levels. Indeed, in our results, male plasma E_2 fell within the above range (Appendix 5A), and was not different than the E_2 in male fish with low, or non-detectable Vtg, indicating that fairly high Vtg levels in some male trout were not caused by endogenous estrogens. Based on the ranges of concentrations in the literature from polluted and reference sites and laboratory studies, we consider Vtg above 1 ppm to be abnormal in male fish. To explore potential relationships between Vtg and suspected or known estrogenic contaminants identified within those same fish, we performed regression analysis on fish for which contaminant concentrations were available and >1 fish exhibited elevated Vtg. Regression analysis could not be performed on the fish from GLAC because only 1 fish from each lake displayed fairly high levels of Vtg and, therefore, one data point would anchor the regression line. Vitellogenin concentrations were determined following the method of Schwindt et al. (2007).

5.3.3.2.2 Results and Discussion

The National Park Service is concerned about contaminant effects (e.g., changes in biomarkers) on populations as well as on individuals. Therefore, we plotted the Vtg concentrations in male fish as a scatterplot and as a mean, so that individual concentrations could be easily visualized with respect to the average (Figure 5-11). Of note were numerous sites from parks in the Rocky Mountains that had appreciably higher levels of Vtg than the other sites, which were at least an order of magnitude lower if not non-detectable (Figure 5-11). Results from our samples obtained in 2003 from ROMO prompted a subsequent study to sample additional waters during the summers of 2005 and 2006, as well as to repeat the work performed in 2003. Additional waters, Sprague Lake in 2005 and Spirit Lake in 2006 (both in ROMO), with male fish displaying fairly high Vtg were discovered, and in 2006, we repeated the findings obtained at Lone Pine Lake in 2003. Spirit Lake is in the same drainage, but upstream from Lone Pine Lake, and does not have campsites, reducing the influence of localized use. Also, two male fish from GLAC and one male fish from Golden Lake, MORA, displayed elevated levels of Vtg (Figure 5-11). In lakes sampled in 2005, Vtg gene expression in the liver was also evaluated. No Vtg gene expression was evident (Biales, pers. comm.); therefore, it appears that the cause of the slightly elevated Vtg we found was probably exposure to something not active around the day of fish collection. Although the sample sizes were very small, significant correlations between known or suspected estrogenic contaminants and Vtg in both lakes sampled at ROMO in 2003 were found (Figure 5-12). Although statistical analysis could not be performed, the fish from Oldman Lake, GLAC, was the only fish in WACAP to have detectable concentrations of o,p′-DDT, as well as the highest concentrations of p,p′-DDT, well-known endocrine disruptors. These results suggest that not only are Vtg levels in certain trout elevated relative to within-lake counterparts, they are also related to concomitant increases in estrogen-like contaminants.

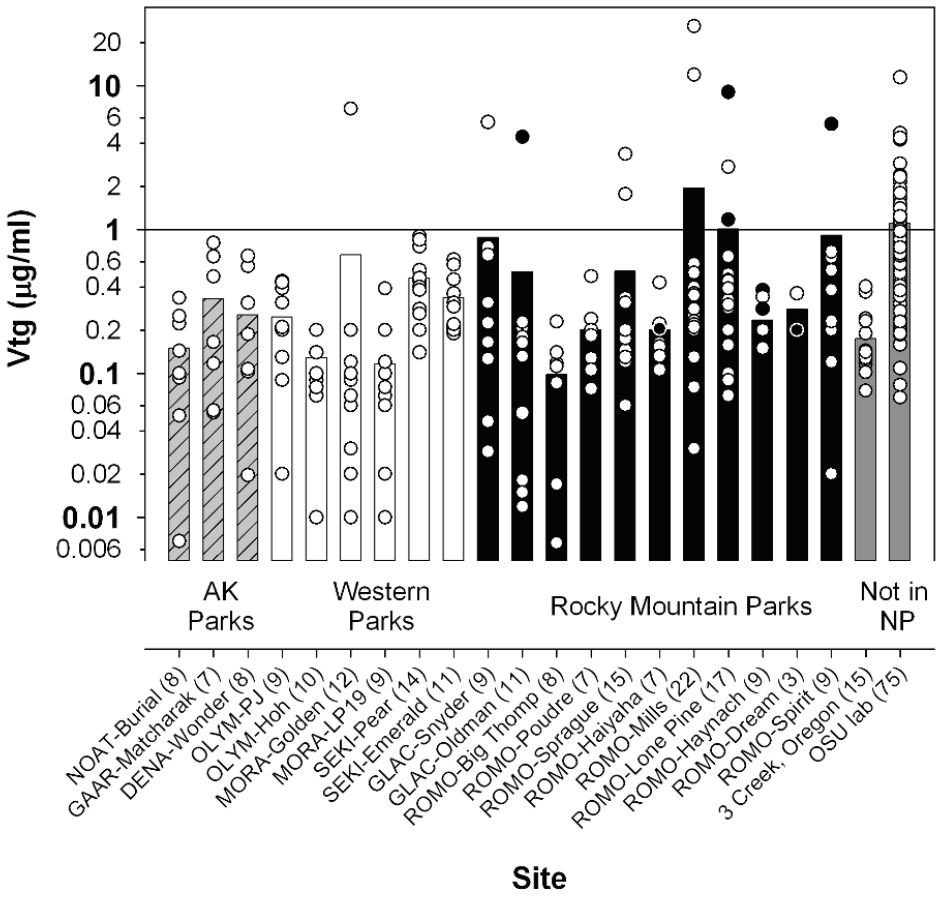


Figure 5-11. Mean Vitellogenin + 95% Confidence Intervals and Concentrations from Individual Male Trout from Lakes or Streams in National Parks in the Western United States and Other Sites. Bars are shaded to denote region. *N* = 3 to 75, depending on location, and is listed for each field site after the site name. Black data points are intersex males. Vtg concentrations determined following Schwindt et al. (2007). Data are plotted on a $\log_{10}$ scale and there is some data overlap.

5.3.3.3 Intersex in Male Fish

Intersex, the presence of both male and female reproductive structures in the same animal, is a commonly used biomarker of estrogen-like chemical exposure in gonochoristic fishes, such as salmonids. Many fishes are natural hermaphrodites. The process is essential to the reproductive life histories of numerous families of fish, and it has been argued that there is a "baseline" level of intersex, even in gonochoristic fishes (Devlin and Nagahama, 2002; Sumpter and Johnson, 2005). Regardless, the underlying assumption that trout, by genetic determination, are phenol-typically male or female implies that any deviation (i.e., intersex) from that is abnormal (Bortone and Davis, 1994). Although this might be true, the difficulty lies in attributing the abnormality to some random genetically or environmentally induced baseline level or to the effect of estrogen-like compounds, as in the case of reports of feminized male fish in the scientific literature (e.g., Jobling et al., 1998; Woodling et al., 2006). Even in the laboratory, consistent induction of intersex with "weak" estrogens is difficult (Carlson et al., 2000).

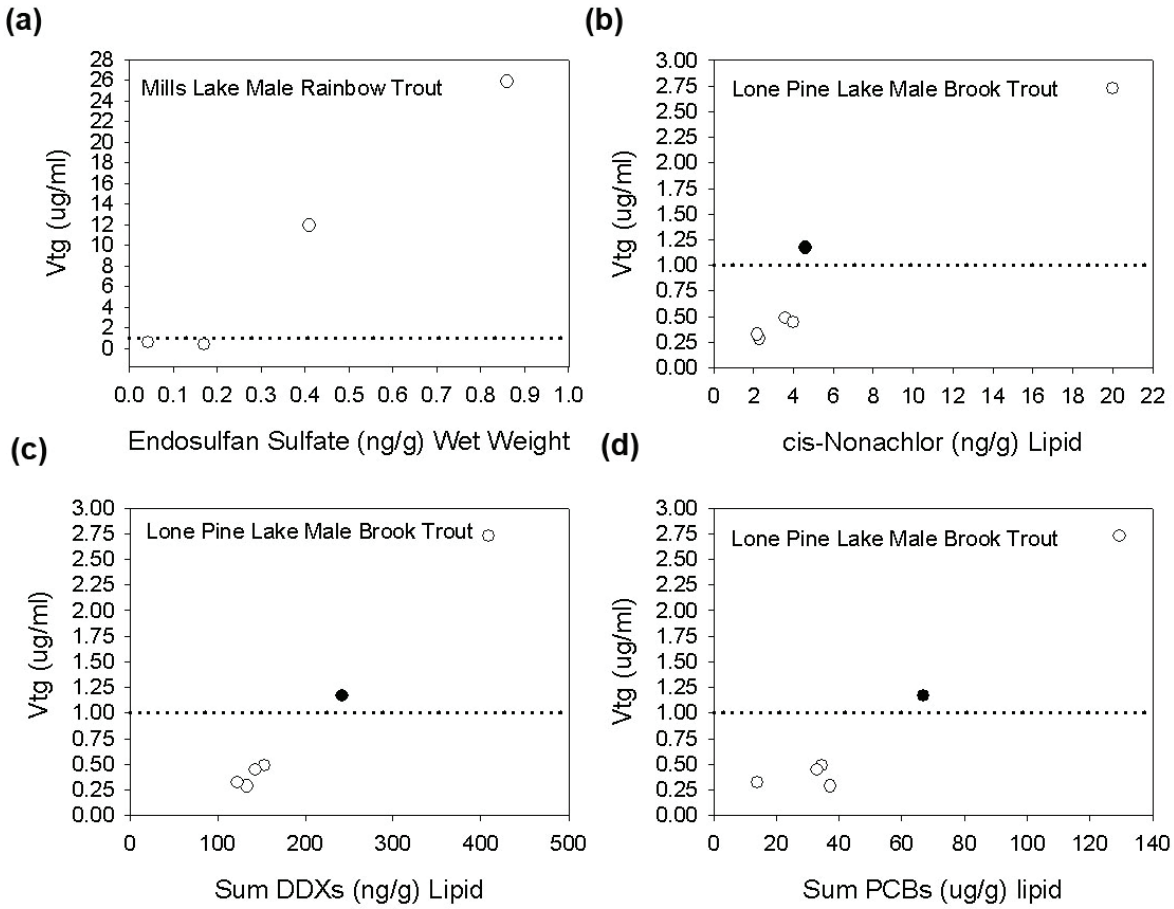


Figure 5-12. Scatterplots Comparing Suspected Endocrine Disruptors and Plasma Vitellogenin (Vtg), a Commonly Used Indicator of Estrogenic Contaminants in Male Trout. For the bottom two graphs, "Sum" indicates that individual contaminant concentrations were added together to arrive at the sum concentration. For DDT, the o,p′- and p,p′- isomers of DDT, DDD, and DDE were summed. For the PCBs, congeners 74, 101, 118, 153, 138, 187, and 183 were summed. The data point in black is an intersex male trout. The dashed line is the quantitation limit for the assay. N = 4 for Mills Lake and N = 6 for Lone Pine Lake. For Mills Lake, Vtg v. Endosulfan sulfate $F_{1,2}$ = 78.38, R^2 = 0.97, P = 0.012. For Lone Pine Lake, Vtg v. cis-Nonachlor $F_{1,4}$ = 77.49, R^2 = 0.95, P = 0.0009. Lone Pine Lake Vtg v. ∑PCBs $F_{1,4}$ = 100.66, R^2 = 0.96, P = 0.0006. Lone Pine Lake Vtg v. ∑DDTs $F_{1,4}$ = 645.33, R^2 = 0.99, P < 0.0001.

Yet, intersex has been used as a biomarker by numerous researchers (Vigano et al., 2001; Gercken and Sordyl, 2002; Kirby et al., 2004) and is considered the most reliable biomarker of reproductive abnormalities. This suggests that intersex should be validated, as well as possible, for study organisms. However, validation of intersex in trout is more difficult than for Vtg. It is a time-consuming endeavor and, to our knowledge, has not been attempted by other researchers. In an effort to validate the use of intersex in trout as a biomarker, we obtained trout from the University of Washington School of Fisheries in Seattle and from the California Academy of Sciences in San Francisco to determine if intersex could be observed in fish captured before the large-scale anthropogenic emission of the endocrine disrupting compounds (pre-1940s). The sites, species, sex ratio, and number intersex for every trout analyzed in these studies are shown in Figure 5-13 and Table 5-8.

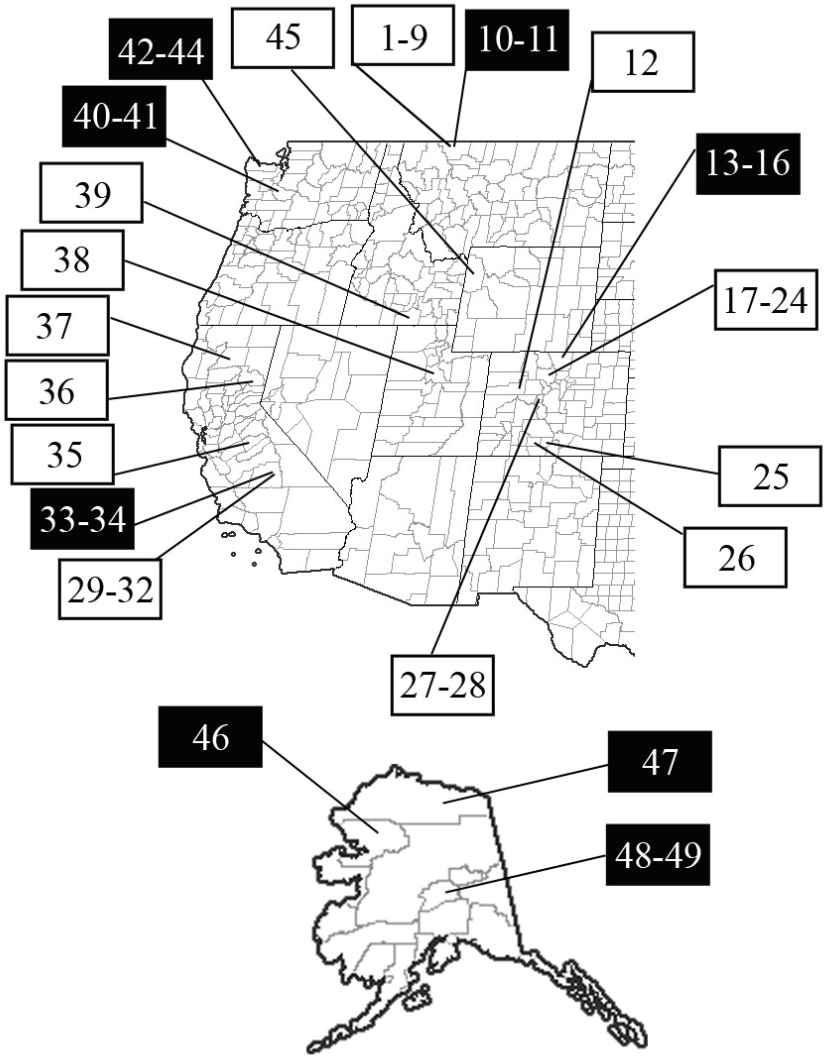


Figure 5-13. Counties or Boroughs (Alaska) Where Museum (white boxes) and/or WACAP (black boxes) Fish Samples Were Collected and Gonads Analyzed for Sex and Intersex. The key to the numbers on the map, and the numbers of male, female, and intersex fish is in Table 5-8.

Table 5-8. Characteristics of Intersex Trout Analyzed from Current and Historic Sampling.

Map #	State	County	Location	Park	Year	Species	M / F	Intersex
1	MT	Glacier	Middle Fork, Flathead River	GLAC	1934	*Oncorhynchus clarki* subsp.	0 / 1	0
2	MT	Glacier	Coal Creek	GLAC	1934	*O. clarki* subsp.	2 / 2	0
3	MT	Glacier	Fish Creek	GLAC	1934	*O. clarki* subsp.	1 / 2	0
4	MT	Glacier	Arrow Lake	GLAC	1934	*O. clarki* subsp.	2 / 0	0
5	MT	Glacier	Lincoln Creek beaver ponds	GLAC	1934	*O. clarki* subsp.	3 / 6	0
6	MT	Glacier	Trout Lake	GLAC	1934	*O. clarki* subsp.	3 / 2	0
7	MT	Glacier	Park Creek	GLAC	1934	*O. clarki* subsp.	1 / 2	0
8	MT	Glacier	Isabel Lake outlet	GLAC	1934	*O. clarki* subsp.	1 / 1	0
9	MT	Glacier	Lower Snyder Lake	GLAC	1934	*O. clarki* subsp.	5 / 2	0
10	MT	Glacier	Lower Snyder Lake	GLAC	2005	*O. clarki lewisi*	9 / 6	0
11	MT	Glacier	Oldman Lake	GLAC	2005	*O. clarki bouvieri*	11 / 4	1 Male
12	CO	Garfield	Trappers Lake		<1871	*O. clarki pleuriticus*	0 / 1	0
13	CO	Grand	Lone Pine Lake	ROMO	2003	*Salvelinus fontinalis*	7 / 8	1 Male
14	CO	Grand	Lone Pine Lake	ROMO	2006	*S. fontinalis*	10 / 20	1 Male
15	CO	Grand	Spirit Lake	ROMO	2006	*S. fontinalis*	9 / 6	1 Male
16	CO	Grand	Haynach Lake	ROMO	2006	*O. clarki bouvieri*	9 / 6	2 Male
17	CO	Larimer	Mills Lake	ROMO	2003	*O. mykiss / clarki*	6 / 9	0
18	CO	Larimer	Mills Lake	ROMO	2006	*O. mykiss / clarki*	16 / 11	0
19	CO	Larimer	North Fork, Big Thompson R.	ROMO	2005	*S. fontinalis*	8 / 7	0
20	CO	Larimer	Lake Haiyaha	ROMO	2005	*O. clarki*	7 / 8	1 Male
21	CO	Larimer	Poudre Lake	ROMO	2005	*S. fontinalis*	7 / 9	0
22	CO	Larimer	Sprague Lake	ROMO	2005	*S. fontinalis*	11 / 5	0
23	CO	Larimer	Sprague Lake	ROMO	2006	*S. fontinalis*	4 / 8	0
24	CO	Larimer	Dream Lake	ROMO	2006	*O. clarki stomias*	3 / 12	1 Male
25	CO	Alamosa	Conejos River		1889	*O. clarki virginalis*	2 / 1	0
26	CO	Rio Grande	Rio Grande		1889	*O. clarki virginalis*	2 / 0	0
27	CO	Lake	Twin Lakes		1889[1]	*O. clarki stomias*	3 / 0	2 Male
28	CO	Lake	Arkansas River		1889	*O. clarki stomias*	2 / 0	0

Table 5-8. Characteristics of Intersex Trout Analyzed from Current and Historic Sampling (continued).

Map #	State	County	Location	Park	Year	Species	M / F	Intersex
29	CA	Tulare	Volcano Creek		1891	*O. mykiss aguabonita*	1 / 0	0
30	CA	Tulare	Golden Trout Creek		1893[1]	*O. mykiss aguabonita*	7 / 1	0
31	CA	Tulare	Golden Trout Creek		1904	*O. mykiss aguabonita*	2 / 0	0
32	CA	Tulare	Cottonwood Lakes		1912	*O. mykiss aguabonita*	0 / 2	0
33	CA	Tulare	Emerald Lake	SEKI	2003	*S. fontinalis*	11 / 5	0
34	CA	Tulare	Pear Lake	SEKI	2003	*S. fontinalis*	14 / 3	0
35	CA	Mariposa	near Merced Lake	YOSE	1921	*S. fontinalis*	1 / 1	0
36	CA	Plumas	Gold Lake		1899	*O. mykiss*	1 / 0	0
37	CA	Shasta	Fall River		1898	*O. clarki*	1 / 1	0
38	UT	Utah	Provo River		1889[1]	*O. clarki virginalis*	1 / 1	0
39	ID	Cassia	Cottonwood Creek		1894	*O. clarki lewisi*	0 / 1	0
40	WA	Pierce	LP19	MORA	2005	*S. fontinalis*	9 / 6	0
41	WA	Pierce	Golden Lake	MORA	2005	*S. fontinalis*	12 / 3	0
42	WA	Clallum	PJ Lake	OLYM	2003	*S. fontinalis*	4 / 11	0
43	WA	Clallum	PJ Lake	OLYM	2005	*S. fontinalis*	5 / 10	0
44	WA	Clallum	Hoh Lake	OLYM	2005	*S. fontinalis*	10 / 5	0
45	WY	Teton	Pacific Creek		1891	*O. clarki*	2 / 0	0
46	AK	NW Arctic	Matcharak Lake	GAAR	2004	*S. namaycush*	7 / 8	0
47	AK	North Slope	Burial Lake	NOAT	2004	*S. namaycush*	8 / 7	0
48	AK	Denali	McLeod Lake	DENA	2004	*Lota lota*	2 / 0	0
49	AK	Denali	Wonder Lake	DENA	2004	*S. namaycush*	8 / 7	0

[1]Collection date is inferred from information associated with the specimens.

5.3.3.3.1 Data Analysis

Our objectives were to identify abnormalities in the gonads of male and female fish. In the event that abnormalities were identified, we searched for possible hypothesis-generating relationships to aid in interpreting the significance of the abnormalities. To find the intersex fish, evidenced by ova-testis, or other abnormalities, we scanned the entire gonad section, beginning with 100× total magnification, by compound light microscopy. When oocytes were visualized in the testis, we increased the magnification to 400× to confirm and capture digital images. In some instances, 25× or 50× total magnifications were sufficient to observe the maturing ova present in the testis. To determine the extent of testicular abnormalities, we developed a grading system, similar to that developed by Jobling et al. (1998), for the degree of abnormality observed in the male gonads. The testes observed in these studies are characterized as the following: (1) normal testis, (2) poorly developed testis for the size of the fish (i.e., does not show signs of reproductive maturity), (3) normally developing testis with primary or perinucleolar oocytes, and (4) poorly developed or degenerative testis with perinucleolar oocytes and/or vitellogenic oocytes (Figure 5-14). The numbers of fish we observed in each category, separated by geographic region and current or historic sampling, are listed in Table 5-9. The proportion of current and historic sites with intersex fish in the Rocky Mountains was compared with Fisher's Exact Test and the results are reported in Table 5-10.

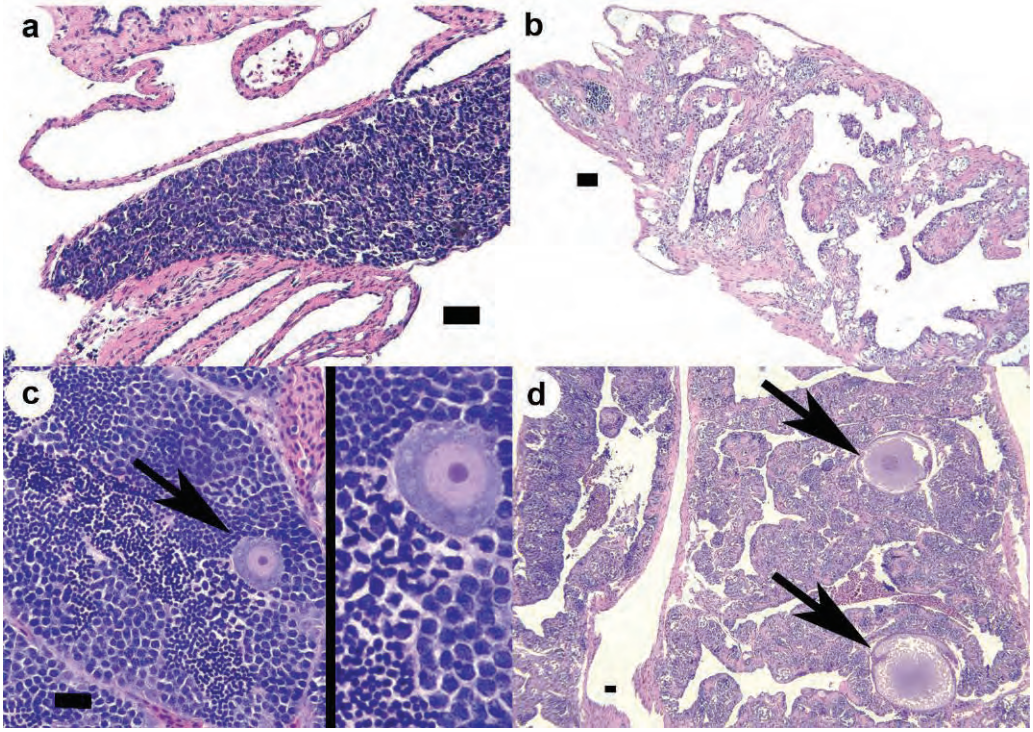


Figure 5-14. Categories of Relative Gonad Abnormality: (a) Normal immature testis, (b) Poorly developed testis or degenerative testis, for the size of fish, (c) Normally developing testis with primary or perinucleolar oocytes (inset is the magnified oocyte at the arrow), and (d) Poorly developed testis with perinucleolar oocytes and/or vitellogenic oocytes (arrows). Hematoxylin and Eosin; bars = 50 µm.

Table 5-9. Categorization of Trout Testes by Abnormality, Geographic Region, and Current or Historic Sampling.

Region	Sample	Total Males	Testis Category			
			a	b	c	d
Rocky Mountains	Current	117	107	2	5	3
	Historic	30	28	0	2	0
Sierra Nevada	Current	25	25	0	0	0
	Historic	12	11	0	0	0
Olympics/Cascades	Current	40	40	0	0	0
	Historic	1	1	0	0	0
Denali, Alaska	Current	10	10	0	0	0
	Historic	0				
Arctic Alaska	Current	15	15	0	0	0
	Historic	0				

Table 5-10. Comparison of Sites with Intersex Fish from the Rocky Mountains.

Dates	Intersex	Not Intersex	Proportion	*p* (Fisher's Exact Test)
1871-1934	1	13	0.07	
2003-2006	6	5	0.54	0.0213

5.3.3.3.2 Results and Discussion

In the historic samples, we dissected a total of 85 trout, finding 42 males, 28 females, 2 intersex males (counted as male), and 15 fish specimens for which we could not identify sex because of the poor quality of the specimen. Of note, we obtained westslope cutthroat trout samples from the University of Washington captured in 1934 from Lower Snyder Lake, the same lake we sampled from GLAC in 2005. In both the current and the museum samples, intersex trout from this lake were not found (Figure 5-13; Table 5-8). In the current samples, 207 total male fish were sampled and 8 of 117 male fish in the Rocky Mountains were intersex. The within-lakes frequencies ranged from 9% to 33%, and 50% of these intersex fish also produced elevated concentrations of Vtg.

We found two historic samples collected in the late 1800s from Twin Lakes, Colorado, in the Rocky Mountains that were intersex (Figure 5-15). This lake is quite close to ROMO. It is also in the vicinity of where extensive heavy metal mining took place in the 1800s. This is the earliest known intersex trout, to our knowledge, and a noteworthy finding in and of itself. We are unaware of the work showing that metals can lead to intersex among teleosts fishes, but there is evidence that cadmium has estrogenic properties (Johnson et al., 2003). There is also evidence that elevated concentrations of some heavy metals such as cadmium can cause testicular injury (Sangalang and O'Halloran, 1972, 1973). Our observations of intersex male trout in very old specimens, long before the manufacture of organic contaminants, warrants a more extensive investigation into the depth and breadth of this phenomenon, albeit it is beyond the scope of WACAP. Reeder et al. (2005) analyzed the gonads of 814 cricket frogs (*Acris crepitans*) dating from 1852 to 2001 and found that the frequency of intersex fluctuated with relative anthropogenic input to the environment.

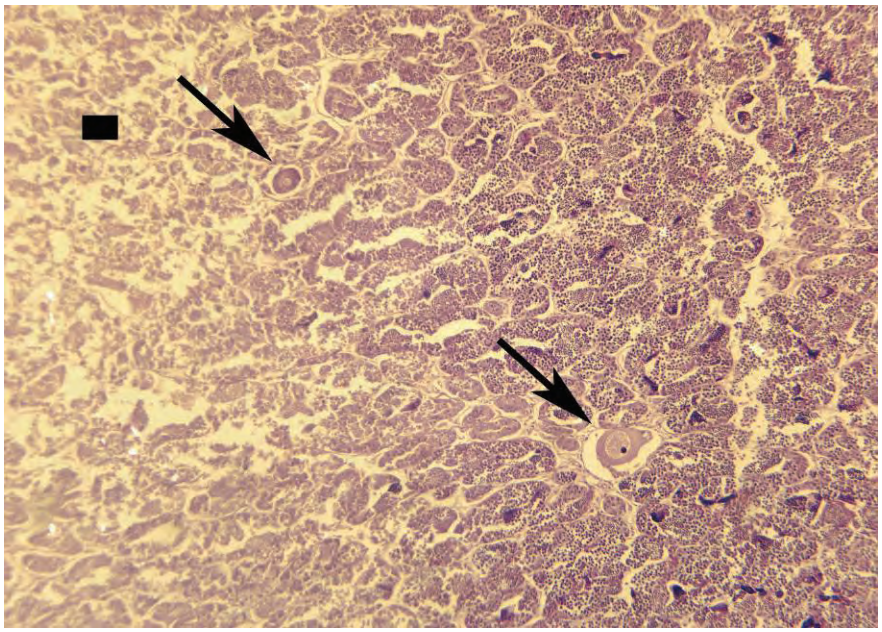


Figure 5-15. Intersex Male Greenback Cutthroat Trout from Twin Lakes, Colorado, Captured in the Late 1800s. Primary oocytes (arrows) are surrounded by normally developing testis, indicating category c gonad abnormality (see Figure 5-14). Hematoxylin and Eosin; bar = 50 µm.

While the frequency of intersex trout we found across the West and in Alaska is quite low, it appears concentrated in the Rockies where the sites with intersex fish today is significantly greater in number than in the past (Table 5-9). The severity of abnormalities observed in the current samples is also greater (Table 5-9). All three intersex trout from Lone Pine and Spirit Lakes displayed category 4 gonad abnormalities, and had low androgen and estrogen levels and elevated levels of Vtg during the time of year when the other trout were nearing sexual maturity. Based on this information, we argue that these individuals are incapable of reproduction, whatever the cause. Identifying the cause of these observations is impossible with the current dataset. If similar numbers of fish from the museums were sampled, then the possibility of finding similar numbers of category 4 testis abnormalities is definitely possible.

The intersex fish at Lone Pine Lake in 2003 (Figure 5-16a-c) had the second highest concentrations of p,p′-DDT and 10 to 100 times the dieldrin, both documented endocrine disruptors, of the other fish analyzed in WACAP. This finding implies that contaminants are at least influencing the reproductive health of that individual. Furthermore, that similar levels of disruption were found at Lone Pine Lake in 2003 and 2006 indicates that the observed abnormalities are not transient phenomena. The consequences of 1 in 7 and 1 in 10 males in 2003 and 2006, respectively, not reproducing, if indeed they cannot reproduce, are unknown. Long-term monitoring is needed to determine if the population is stable. The intersex fish at Oldman Lake, GLAC, was category 3 (Figure 5-16d), had elevated Vtg levels, and had the highest concentrations of chlordanes (*cis, trans, oxy* and nonachlors), the dioxin-like PCB 118, and was the only fish in the study with detectable concentrations of o,p'-DDT, also a confirmed xenoestogen. This fish also had normal levels of plasma androgens and, aside from being intersex, had normally developing gonads.

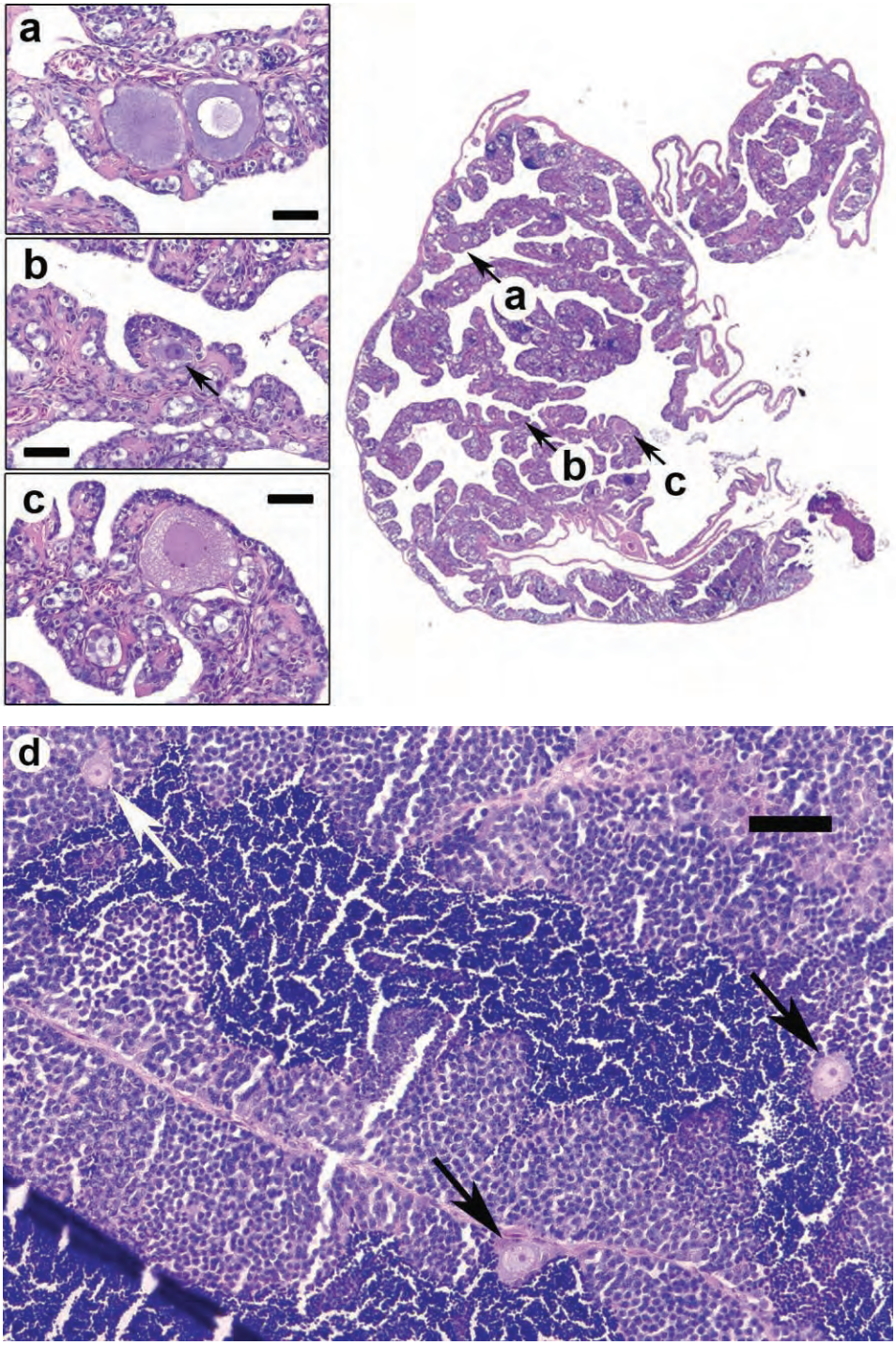


Figure 5-16. Intersex Male Trout from Lone Pine Lake, ROMO (a–c) and Oldman Lake, GLAC (d). The insets (a–c, 400x), areas denoted by the corresponding letters and arrows on the low magnification image (composite, 50x), depict perinucleolar oocytes surrounded by a poorly developed testis. In (d) primary oocytes (arrows) are surrounded by normally developing testis (composite, 400x). Hematoxylin and Eosin; bars = 50 µm.

In summary, the use of intersex as biomarker for endocrine or reproductive disruption is not perfect; however, our studies signify the importance establishing a baseline to which future sampling can be compared. Intersex had not been observed, to our knowledge, in trout from ecologically protected areas. In the scientific literature, increased frequencies or severity of intersex is highly suggestive, if not indicative, of reproductive dysfunction (Devlin and Nagahama, 2002), whatever the cause. Our recommendations are to continue monitoring lakes in the Rocky Mountains, including ROMO and GLAC, for Vtg, sex steroids, and gonad abnormalities. In addition, we recommend that sampling be expanded to include Grand Teton and Yellowstone National Parks, as they are roughly mid-way between ROMO and GLAC. Other parks in the Rocky Mountains could consider initiating similar monitoring programs. These future efforts, in coordination with expanded sampling of museum specimens, could help provide resource managers with the information necessary to determine if biomarkers of reproductive endocrine disruption are changing through time.

5.3.3.4 Interpretation and Integrated Analysis: Emphasizing Park and Regional Differences in Biomarkers and Contaminants

Our analysis of biomarker responses and their relationship to contaminant concentrations focused on effects on trout in general. That is, we identified relationships between MAs and Hg in brook trout from all the parks. However, we have not explained among-lake differences in MAs, for the lake trout and *Oncorhynchus* spp. and how they might relate to contaminant concentrations. The question that has not been addressed is: Are MAs higher in fish with higher contaminant concentrations? At present we cannot answer this question because no readily apparent statistical relationships emerged (because of small sample sizes). In other analyses, we have related contaminants suspected to have estrogenic action to biomarkers that respond to estrogenic contaminants. One question that arises from these analyses is: Why is the evidence for endocrine disruption (Vtg and intersex male fish) confined to parks in the Rocky Mountains? The following paragraphs are intended to address this question as best possible, considering the small sample sizes and subtle among-park and regional differences.

5.3.3.4.1 Biomarkers Related to Reproductive Disruption

Vitellogenin persists in the blood for about 2 weeks following an acute induction (Schultz et al., 2001), so the window of detectability is limited for this biomarker. For Emerald and Pear Lakes (SEKI), where some contaminant concentrations were higher than those of Lone Pine Lake (ROMO) and Golden Lake (MORA) (Figure 5-17), the absence of Vtg could be related to the absence of estrogenic contaminants in the blood. Without mobilization of contaminants from fat, or ingestion of contaminated food, it is unlikely that elevated Vtg would be observed, despite higher contaminant concentrations in SEKI. So, one reason for not observing elevated Vtg in SEKI is that the fish were sampled outside the window of sensitivity to the contaminants or the biomarker. The lakes with *Oncorhynchus* spp. (Mills Lake, ROMO, and both lakes in GLAC) all had fish with elevated levels of Vtg. Our analysis of contaminant sums by class (e.g., the chlordanes) revealed subtle differences in mean concentrations (generally higher at Oldman Lake, GLAC), but Vtg levels in the rainbow trout (from Mills Lake, ROMO) were generally higher than in the other species. This could be a result of the species-specific sensitivity to the chemicals, including the contaminants measured in WACAP, as well as those not measured. An alternative explanation is that the polyclonal antibody used in the assay was generated against rainbow trout Vtg (see Schwindt et al., 2007), so these antibodies might recognize more epitopes on the rainbow trout Vtg than on the Vtg in the other salmonids.

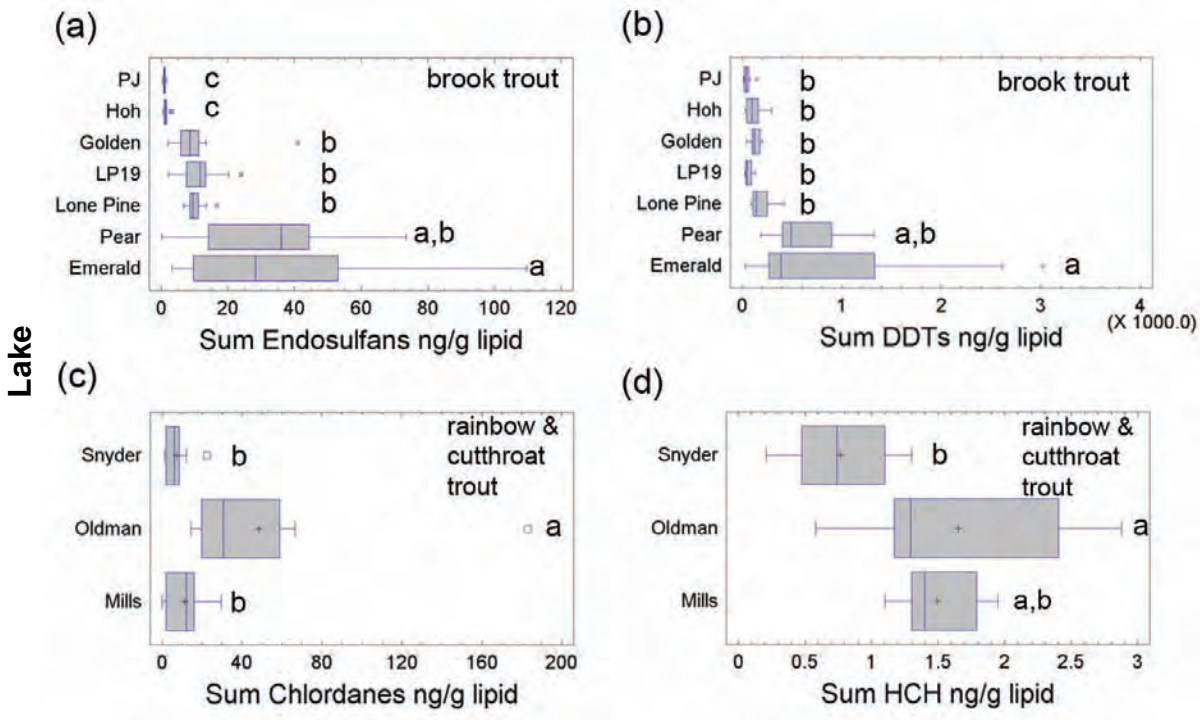


Figure 5-17. Box and Whisker Plots of Select Groups of Organochlorines Analyzed by Brook Trout (a,b) or *Oncorhynchus* spp. (c,d). Median sum endosulfans (a) and DDTs (b) are compared by Kruskal Wallis because of unequal variance. Sum chlordanes (c) and HCHs (d) are compared by ANOVA followed by a Bonferroni *post hoc* at 95% confidence. Different letters denote significantly different medians or means at $p < 0.05$. Boxes are the middle 50% of the data divided by the median (line); the "+" symbol represents the mean. Whiskers are the range of data and individual points are outliers. Sum endosulfans = endosulfan I, II, and sulfate. Sum DDTs = o,p′- and p,p′- isomers of DDT, DDD, and DDE. Sum chlordanes = *cis*, *trans*, *oxy*-chlordane, and nonachlor. Sum HCH = a-, d-, and g-HCH. Data below the detection limits are represented as ½ the EDL.

Alternative explanations for the presence of intersex fish and elevated Vtg include the presence of estrogens from human birth control, perhaps from humans swimming in the lakes. It is not known if the numbers of humans on birth control that would urinate in the lakes during a possible backcountry swim could excrete a quantity of estrogenic substance sufficient to be endocrine disrupting. Other sources of estrogenic substances could be from horses and wildlife that frequent the lakes, but several of these lakes in which intersex fish are present are not accessible to horses or mules. In addition, the unknowns mentioned for human excretions would apply to wildlife. One other possibility is that some lakes might contain fish that have fed on artificial baits introduced by anglers that contain endocrine disrupting substances to a sufficient amount. However, attributing the endocrine disruption to sources other than airborne contaminants appears considerably more speculative. In addition, the elevated Vtg evident in our laboratory population of trout is probably attributable to the presence of estrogenic substances known to be present in artificial fish feeds.

The intersex condition was found only in parks in the Rocky Mountains and the limited number of intersex fish prevents establishing statistical relationships with contaminants. However, dieldrin was much higher in the intersex brook trout from Lone Pine Lake, ROMO and o,p′-DDT was detectable only in the intersex Yellowstone cutthroat trout from Oldman Lake, GLAC. It

could be purely coincidental that these estrogenic contaminants were higher in the intersex fish. At present there is no robust scientific explanation for the regional confinement of the intersex condition to the Rocky Mountains; however, our data suggest that the incidence is increasing. Perhaps if more fish were sampled from the lakes in SEKI, MORA, or OLYM, or if more lakes were sampled in SEKI or other parks in the Sierra Nevada, intersex fish would be found. The percentages of intersex fish we found in ROMO (9 to 33%) were fairly high, compared to those reported in the literature (range from 1% to 0.0002%). Therefore, it could simply be a matter of sampling enough fish from the other parks to increase the probability of finding intersex fish.

Six of 11 water bodies in ROMO had intersex fish. This is a remarkable occurrence that is biologically fascinating, and potentially alarming to resource managers and stakeholders. The established implications in the literature for high frequencies of intersex, is that endocrine or reproductive disruption is occurring. Whether these observations are caused by contaminants is impossible to say with the current dataset. We attempted to use a weight of evidence approach to establish some credible evidence for endocrine disruption (or lack of disruption). Our data show that some intersex fish also produce Vtg, have underdeveloped testis, and low sex steroids at a time of year when cohorts are nearing reproductive maturity. We even provide evidence that Vtg is related to some organochlorine concentrations. One would be hard pressed to find such compelling evidence for reproductive disruption based on observational data in the literature. To our knowledge, there is a paucity of studies in which contaminants and reproductive biomarkers have been measured in the same fish. Regardless, without experimental spiking of lakes in national parks with contaminants, measuring the contaminants in the fish, and finding statistically significant changes in the proportion of intersex fish, there is no way to attribute the intersex condition to contaminants, or anything for that matter. Intersex can be caused by genetic mutations, parasites, abrupt temperature changes, hormonal abnormalities, and contaminants. The fact that our observations occurred in two different genera, in three different species, and in allopatric populations reduces the possibility that endogenous factors (mutations and hormonal abnormalities) are at fault. Abrupt temperature changes are unlikely in these lakes and parasites were not found in the intersex fish (see Appendix 5A).

Finding intersex fish in the museum samples, prior to the manufacture of the organochlorines and other synthetic chemicals was indeed surprising. These results call to attention the possibility of some level of intersex in trout that is not associated with organic pollutants. The area around Twin Lakes was subjected to extensive ore mining. Perhaps metal contamination of waters resulting from natural or mining induced acid rock drainage was at fault. Or, the stocking of fish might have resulted in hybridization that induced a genetic abnormality (Metcalf et al., 2007). Clearly, more sampling of the museum specimens is necessary. Focusing on acquiring not only more fish from each water body, but from more water bodies in the western and northeastern parts of the USA is needed before definitive conclusions can be drawn.

In summary, there is strong inference that our results regarding intersex and Vtg in some populations in the Rocky Mountain parks can better be explained by endocrine disruption related to contaminants than by other potential causes. We also believe that it is the more responsible explanation from the perspective of conservation biology. The consequences to park resources would not be negatively affected if further investigation found this contention to be wrong. However, consequences could be negative if we do not take this contention seriously and it is indeed true, as we think.

5.3.3.5 Contaminant (SOCs and Metals) Accumulation in Moose and Potential Biological Effects on Moose in the Parks

The WACAP Research Plan included analysis of moose tissue samples for SOCs and metals in an attempt to make an explicit linkage between the Alaskan food web that WACAP was exploring for contaminants and the humans that use this food web for obtaining subsistence foods. Moose was chosen because Alaska NPS experts advised WACAP scientists that this subsistence animal was considered one of the most widely distributed and used terrestrial game animal in Alaska. Moreover, the range of an individual moose is generally small, compared to that of caribou, the other subsistence animal considered. The smaller range of the moose would tie the results of contaminant analysis to a smaller area of the Alaskan landscape. We intended to obtain moose muscle and liver samples from subsistence hunters in or near the three core WACAP sites in Alaska over a 3-year period. We allocated a total of 35 SOC and metal samples for this effort. We hoped to obtain at least three moose samples per year from each park for 3 years. At the time of the peer review, the reviewers recognized this component of WACAP as an attempt to link the more rigorous WACAP science effort to the life of the subsistence human. However, the peer review panel suggested that we not try to integrate this effort into the main WACAP science effort focused on explicit objectives and having a rigorous, controlled sampling design and schedule. Rather, understanding the interest in making the connection to the human component of the food web, they suggested we continue this as a minor effort in WACAP.

We worked through NPS personnel at DENA, NOAT, and GAAR toward the goal of obtaining moose samples from the three core Alaskan parks. We prepared information packets, tissue sub-sampling materials, and packaging and mailing items for the NPS contacts to give to potential moose hunters. In total, during the 3-year effort, we obtained tissue samples from only three animals, all collected in DENA. Two samples were obtained in 2004 and one in 2005. The moose samples were collected and delivered to the WACAP laboratory, generally following the simple procedures we outlined in a flyer distributed to subsistence hunters. Samples received at the WACAP laboratories arrived frozen solid and wrapped, and were accompanied by meta data regarding the location of the animal when it was harvested.

5.3.3.5.1 SOCs in Moose Tissue

The moose tissue analyses for SOCs revealed that few of the target compounds were detected in the tissues and, when present, the concentrations of the compounds were generally quite low (Table 5-11). Many of the compounds were below detection limits for the analysis, or were flagged for various reasons. As a general rule, when sample concentrations approach the detections limits for an analytical procedure, the number of flags tends to increase.

Fifteen SOC compounds were detected at least once in either muscle or liver tissue of the three moose. With three moose sampled, that means that we could have detected the presence of these 15 compounds 45 times for each of the 2 tissue groups, liver and muscle. For liver, 12 out of a possible 45 samples contained detectable concentrations of SOCs. In muscle, we detected SOCs in half that number, 6 of a possible 45 samples. One of the animals had a fairly high concentration of p,p′-DDD in its liver, at 340 ng/g lipid. On the other hand, many of the concentrations of detected SOCs were very low.

The generally low detection frequencies and the absence of any major patterns among SOC compound groups, among individual moose, or between moose tissue types suggest that moose for which we obtained samples were not biomagnifying SOCs to a level of concern at this time.

Table 5-11. SOC Concentrations in Moose Meat and Liver.

		Fish Tissue					
SOC	**Unit**	**Liver**	**Liver**	**Liver**	**Meat**	**Meat**	**Meat**
Endosulfans	ng/g wet wt	BDL	BDL	BDL	BDL	BDL	BDL
PCB 153	ng/g lipid	DF	0.025	DF	DF	0.48	DF
	ng/g wet wt	DF	0.0062	DF	DF	0.0037	DF
TFLN	ng/g lipid	X	X	X	BDL	0.62	X
	ng/g wet wt	X	X	X	BDL	0.0048	X
HCB	ng/g lipid	DF	0.72	DF	DF	X	DF
	ng/g wet wt	DF	0.18	DF	DF	X	DF
CLPYR	ng/g lipid	DF	BDL	BDL	1.2	BDL	BDL
	ng/g wet wt	DF	BDL	BDL	0.15	BDL	BDL
Dieldrin	ng/g wet wt	BDL	BDL	BDL	BDL	BDL	BDL
ACE	ng/g lipid	56	BDL	44	X	BDL	BDL
	ng/g wet wt	6.9	BDL	11	X	BDL	BDL
FLO	ng/g lipid	X	2	X	X	X	BDL
	ng/g wet wt	X	0.51	X	X	X	BDL
FLA	ng/g lipid	X	BDL	X	BDL	7.2	X
	ng/g wet wt	X	BDL	X	BDL	0.055	X
PYR	ng/g lipid	BDL	BDL	1.2	BDL	BDL	BDL
	ng/g wet wt	BDL	BDL	0.32	BDL	BDL	BDL
pp-DDD	ng/g lipid	340	BDL	32	BDL	BDL	BDL
	ng/g wet wt	42	BDL	8.4	BDL	BDL	BDL
	FLAG	b					
op-DDT	ng/g lipid	BDL	BDL	BDL	630	BDL	BDL
	ng/g wet wt	BDL	BDL	BDL	76	BDL	BDL
pp-DDT	ng/g lipid	DF	BDL	2.4	BDL	BDL	BDL
	ng/g wet wt	DF	BDL	0.63	BDL	BDL	BDL
MXCLR	ng/g lipid	DF	3.2	BDL	18	BDL	BDL
	ng/g wet wt	DF	0.8	BDL	2.2	BDL	BDL
CHR/TRI	ng/g lipid	BDL	BDL	0.46	BDL	BDL	BDL
	ng/g wet wt	BDL	BDL	0.12	BDL	BDL	BDL
B[b]F	ng/g lipid	BDL	BDL	1	BDL	BDL	BDL
	ng/g wet wt	BDL	BDL	0.26	BDL	BDL	BDL

SOC names: PCB = polychlorinated biphenyl; TLFN = trifluralin; HCB = hexachlorobenze; CLPYR = chlorpyrifos; ACE = Acenaphthene; FLO = fluorene; FLA = fluoranthane; PYR = pyrene; MXCLR = methoxychlor; CHR/TRI = Chrysene + Triphene; B[b]F = Benzo(b)fluoranthene

Flags: BDL = below detection limit; DF = detected, but flagged as not meeting QA Objectives; X = no value reported, lab blank > 33% of sample value; b = value is above the calibration range

However, this screening was based on only three animals taken from one national park (DENA). Notably, moose are herbivores, located quite low on the food web, and would not be expected to demonstrate biomagnification to the same degree as a predator.

5.3.3.5.2 Metals in Moose tissue

Moose liver and meat metal concentrations for the three animals sampled are provided in Table 5-12 for seven metals: Cadmium (Cd), Copper (Cu), Nickel (Ni), Lead (Pb), Vanadium (V), Zinc (Zn), and Mercury (Hg).

To consider the three WACAP moose samples from DENA in context with other moose sampled from North America, we present the mean and standard deviation for the WACAP moose liver and muscle tissue samples along with literature citations from Canada (Yukon), the Coville River area of Alaska, and "Other Alaska Killed Moose" (Gamberg et al., 2005; O'Hara et al., 2001) in Table 5-11.

We compared WACAP moose liver sample values to Cd, Cu, and Zn concentrations from the previously published studies. For Cd, Cu, and Zn, the mean WACAP moose sample concentrations were always lower. However, these differences were significantly different only at the 0.05 level for Zn in liver from Coville, Alaska. Although these general findings are good news for subsistence users of moose, these very low Cu concentrations in moose have been determined to adversely affect Fe adsorption mobilization, transformation, and incorporation into hemoglobin (Owen, 1982; Suttle, 1991). The WACAP mean moose liver Cu concentrations of 9.4 ± 4.9 µg/g ww were considered to be in the deficient range (from 5 to < 10 ppm ww) for Cu by Frank et al. (1994). Two of the three WACAP moose tissue samples analyzed in this study fell within this deficient range for Cu. This finding might be of interest to DENA wildlife biologists.

For the sparse data available from other studies for metals in moose tissue, the WACAP (DENA) moose tissue sample values were (1) lower than all values reported for Cd and Cu and (2) similar in Zn concentrations to the Coville (Alaska) moose but much less than the mean Zn concentrations for other Alaska moose (Table 5-11). However, as with liver samples, in only one instance was there a significant difference ($P \leq 0.05$) and that was for Cd in muscle from the Coville, Alaska, study.

5.4 Ecological Effects

5.4.1 Mercury

Mercury is a persistent bioaccumulating and biomagnifying non-essential metal. It is present in three forms in the atmosphere (elemental, reactive gaseous, and particulate), but it must be methylated to an organic form for efficient incorporation into the food web. Methylation is accomplished in the sediment or water column of a water body by microbial organisms (Ullrich et al., 2001). Source apportionment of Hg is difficult, because it does not degrade and is a global pollutant. Current thinking is that most Hg entering the national parks is via atmospheric deposition from local, regional, and trans-Pacific sources (Keeler et l., 2006; Wiener et al., 2006). It is estimated that up to 75% of the Hg entering the atmosphere is from anthropogenic sources (Nriagu, 1990) such as combustion; steel, iron, coke, and lime production; smelting; petroleum refining; and mercury cell chlor-alkali production (Keeler et al., 1995; Landis et al., 2004). Methyl-mercury affects the brain and nervous system, reproductive system, and immune system and is not readily excreted from animals. The concentrations of whole-body total Hg in WACAP

Table 5-12. Metal Concentrations in Moose Meat and Liver (µg/g wet weight).

Samples	Date Collected	Hg ng/g ww	Cd µg/g ww	Cu µg/g ww	Ni µg/g ww	Pb µg/g ww	V µg/g ww	Zn µg/g ww
Liver								
WACAP	9/13/2004	11.88	1.7	8.2	0.65	0.023	0.004	13
WACAP	9/27/2004	32.66	8.3	14.8	0.16	0.017	0.006	40
WACAP	9/23/2005	6.88	1.2	5.2	0.009	0.012	0.048	16
WACAP Mean	Mean ± s.d.	17.14 ± 13.67	3.8 ± 4.0	9.4 ± 4.9	0.27 ± 0.33	0.017 ± 0.006	0.019 ±0.025	23 ± 15
Yukon[1]	Mean ± s.d.	-	4.94 ± 3.52	40.3 ± 47.7	-	0.1 ±0.63	-	34.9 ±36.0
Coville R. Alaska[2]	Mean ± s.d.	-	3.13 ± 2.5	9.8 ± 12.7	-	-	-	66.5 ±58.1*
Other Alaska Killed Moose[2]	Mean	-	11.9	103.9	-	-	-	73.3
Meat								
WACAP	9/13/2004	25.82	0.010	1.7	0.004	0.008	0.003	69
WACAP	9/27/2004	4.56	0.022	0.8	0.019	0.029	0.001	47
WACAP	9/23/2005	2.06	0.006	1.3	0.080	0.011	0.030	71
WACAP Mean	Mean ± s.d.	10.81 ± 13.06	0.013 ± 0.008	1.3 ± 0.4	0.13 ±0.15	0.016 ± 0.011	0.011 ±0.016	62 ± 14
Yukon[1]	Mean ± s.d.	-	0.03 ±0.03	1.48 ± 0.53	-	0.03 ± 0.09	-	51.7 ± 27.9
Coville R. Alaska[2]	Mean ± s.d.	-	1.78 ±1.69*	-	-	-	-	
Other Alaska Killed Moose[2]	Mean ± s.d.	-	-	5.53	-	-	-	183.3

*Significant differences (P ≤0.05) between WACAP moose samples and the other studies based on the T-test

All concentrations based on wet weights.

Metals were analyzed on freeze-dried tissue, and Hg was analyzed on liquid nitrogen ground tissue, not dried.

Moose tissue collected on same date are from the same animal.

[1]Gamberg et al., 2005.

[2]O'Hara et al., 2001.

fish are shown in Figure 5-18. At numerous sites, mean lake concentrations were above contaminant health thresholds for piscivorous biota. The contaminant health threshold for humans is 300 ng/g wet weight (USEPA, 2001), and is based on methyl-Hg in the fillet for a general population of adults with 70 kg body weight and 0.0175 kg fish intake per day (approximately the same intake rate used for determining consumption thresholds for recreational fishers). We converted the threshold value to 185 ng/g whole-body total Hg based on (1) 95-100% of the Hg in fish being methyl-Hg (Bloom, 1992), and (2) conversion from fillet to whole-body basis by the formula [log (fillet biopsy Hg) 0.2545 + 1.0623 log (whole-fish Hg)] developed by Peterson et al. (2007).

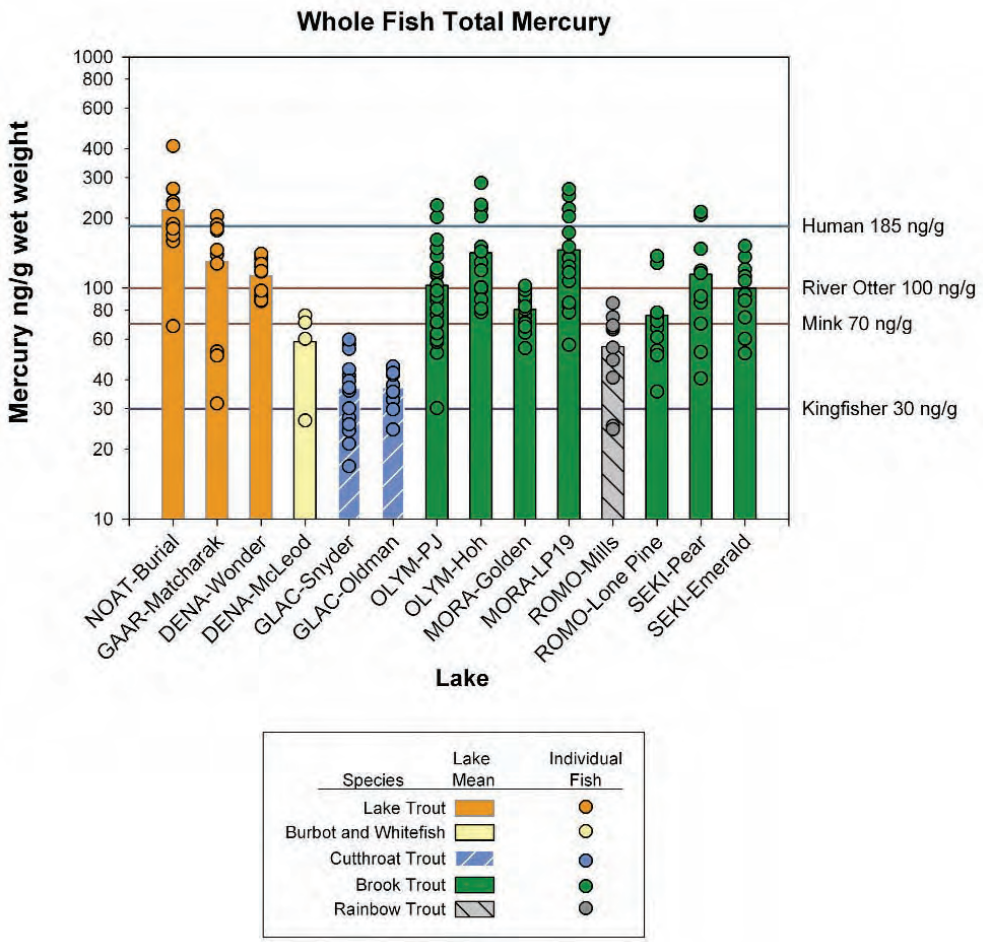


Figure 5-18. Fish Whole-Body Lake Mean (bars) and Individual Fish (symbols) Total Mercury and Contaminant Health Thresholds for Various Biota. The mean ng/g total Hg in fish at NOAT exceeds the human contaminant health threshold; the ng/g total Hg in some fish at GAAR (Matcharak), OLYM (PJ, Hoh), MORA (LP19), and SEKI (Pear) exceeds the human contaminant health threshold. The mean ng/g Hg concentration in fish at all parks exceeds the kingfisher contaminant threshold, and the mean at seven lakes exceeds all wildlife thresholds—NOAT (Burial), GAAR (Matcharak), DENA (Wonder), OLYM (PJ, Hoh), MORA (LP19), and SEKI (Pear). The human threshold is 300 ng/g wet weight (USEPA, 2001), and is based on methyl-Hg in the fillet for a general population of adults with 70 kg body weight and 0.0175 kg fish intake per day. 95-100% of Hg in fish is methyl-Hg (Bloom, 1992), and 300 ng/g in the fillet is equivalent to 185 ng/g ww whole body methyl-Hg (Peterson et al., 2007). Contaminant health thresholds in piscivorous animals (wildlife) are based on 100% fish in the diet for whole body total Hg, as determined by Lazorchak et al. (2003). Data are plotted on a $\log_{10}$ scale; the y-axis starts at 10 ng/g.

5.4.2 Selected SOCs with Contaminant Health Thresholds for Piscivorous Biota

Potential risk to piscivorous biota from fish consumption is of concern. Lazorchak et al. (2003) developed SOC fish contaminant health thresholds for mink (*Mustela vison*), river otter (*Lutra canadensis*), and belted kingfisher (*Ceryle alcyon*) in the mid-Atlantic States, USA, and we used these values to identify national parks that contained fish with contaminant concentrations above the various criteria. Although the criteria were developed for the mid-Atlantic states, mink, otter, and kingfishers inhabit nearly all the field sites in this study (Table 5-13). As indicated by Lazorchak et al. (2003), deleterious effects on the wildlife can vary, because effects are contingent upon numerous other factors. Individual differences in responses to contaminants depend on sex, reproductive strategy and status, exposure to other stressors, and the overall health of the animal.

5.4.2.1 PCBs

Polychlorinated biphenyls were used for insulation and cooling of electrical transformers and capacitors, among many other uses. Being ring-structures, similar to cholesterols and steroid hormones, PCBs are highly bio-active inducing birth-defects, reproductive failure, liver damage, and tumors. Like mercury, PCBs are a global pollutant, are environmentally persistent, and they bioaccumulate and biomagnify in the food web. Because of the toxic nature of PCBs, legislation in the United States banned production and use in 1979; however, it is estimated that 82 million kg of PCBs remain in various forms. PCBs arrive at the national parks via regional and long range atmospheric transport (Eisler, 1986). The concentrations of the sum of PCBs in WACAP fish are shown in Figure 5-19; concentrations in all fish were below contaminant health thresholds for piscivorous biota.

5.4.2.2 DDT and Metabolites

DDT is an organochlorine insecticide and is similar to the PCBs in that it is bioactive because of the ring-structure. Also, as for the PCBs, legislation stopped the large-scale use of DDT in the United States in 1972. However, DDT is still used in the developing world. It is one of the few chemicals that effectively reduce numbers of *Anopheles gambiae*, the mosquitoes that carry malaria. In fact, the World Health Organization recently advocated that DDT be “painted” on the inside of dwellings in Africa to help curb the spread of malaria. DDT was used extensively in the United States from the mid-1940s to the early 1960s as an insecticide, after which Rachel Carson publicized the negative effects of DDT, and other organochlorines, on birds in the book, *Silent Spring*. Henceforth p,p′-DDT, the isomer o,p′-DDT, and the metabolites, o,p′-DDE and p,p′-DDE, have received extensive testing for various biological effects in the scientific literature. The effects range from acute toxicity to reproductive or developmental abnormalities, and endocrine and immune disruption. The concentrations of the sum of DDTs in WACAP fish are shown in Figure 5-20. The mean DDT concentration at Oldman Lake (GLAC) and the concentrations in some individual fish at Pear and Emerald Lakes (SEKI) are above contaminant health thresholds for piscivorous birds.

Table 5-13. Species Represented in Each Guild of the Loop Analysis.

Guild	Species in Each Guild		
Detritus	Not Specified		
Invertebrates	Numerous		
Fish	Salvelinus fontinalis OLYM, MORA, ROMO, SEKI	*Oncorhynchus mykiss* ROMO	*O. clarki* subsp. GLAC
	Thymallus arcticus NOAT, GAAR, DENA	*Cottus* spp. NOAT, DENA, MORA	Gasterosteidae spp. NOAT, GAAR
	Prosopium cylindraceum NOAT, GAAR, DENA		
	S. namaycush NOAT, GAAR, DENA	*Esox lucius* GAAR	*Lota lota* DENA
Mammals	*Lutra canadensis* NOAT, GLAC, MORA, ROMO	*Mustela vison* DENA, GLAC, OLYM, MORA	*Ondatra zibethicus* DENA, GLAC
	Sorex palustris OLYM, MORA	*Spilogale putorius* OLYM	*Mustela erminea* MORA
Birds	*Ceryle alcyon* DENA, GLAC, OLYM, MORA, ROMO, SEKI	*Anas* spp. NOAT, DENA, OLYM, MORA, ROMO	Scolopacidae spp. OLYM, ROMO
	Gavia spp. NOAT, GAAR, DENA, GLAC	Podicipedidae spp. GAAR, DENA, MORA,	*Mergus* spp. GAAR, OLYM, MORA, ROMO
	Larus spp. NOAT, GAAR, DENA	*Sterna paradisaea* NOAT, GAAR, DENA	*Pandion haliaetus* DENA, GLAC, OLYM, MORA, ROMO
	Ardea herodias GLAC, OLYM, MORA, SEKI	Strigidae spp. OLYM, MORA	*Haliaeetus leucocephalus* GLAC, OLYM, MORA, ROMO
	Aquila chrysaetos MORA, SEKI	*Buteo regalis* MORA	*Falco* spp. ROMO, SEKI
Aquatic Birds	*Cinclus mexicanus* DENA, OLYM, ROMO		

Notes:
NOAT = Noatak National Preserve
GAAR = Gates of the Arctic National Park and Preserve
DENA = Denali National Park and Preserve
GLAC = Glacier National Park
OLYM = Olympic National Park
MORA = Mount Rainier National Park
ROMO = Rocky Mountain National Park
SEKI = Sequoia and Kings Canyon National Parks

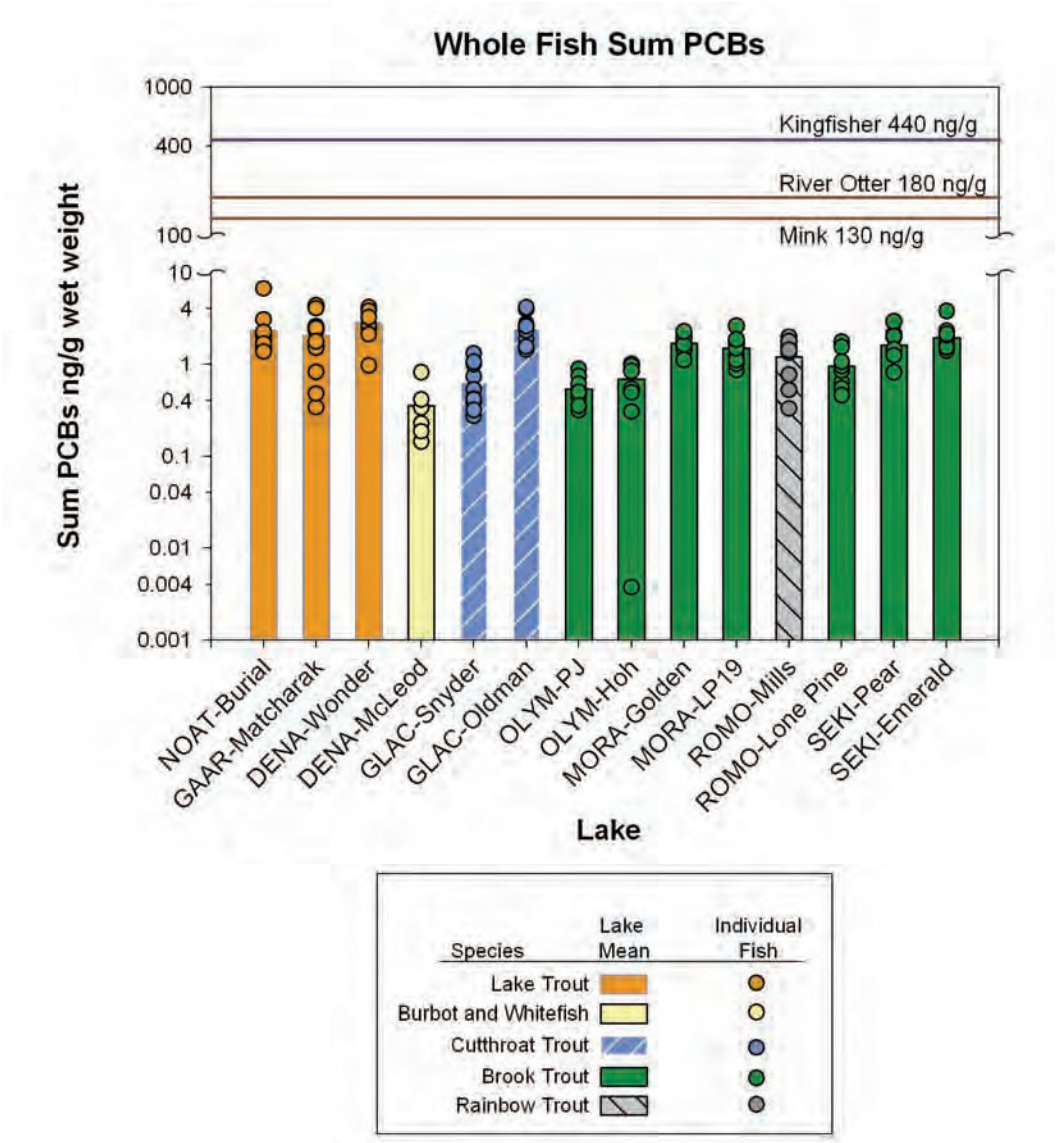


Figure 5-19. Fish Whole-Body Lake Mean (bars) and Individual Fish (symbols) Sum PCB Concentrations, with Contaminant Health Thresholds for Various Wildlife. *N* = 10, except *N* = 6 for McLeod Lake. Contaminant health thresholds in piscivorous animals are based on 100% fish in the diet for whole body total PCBs as determined by Lazorchak et al. (2003). Data are plotted on a $\log_{10}$ scale and below detection limit values are reported as ½ the EDL.

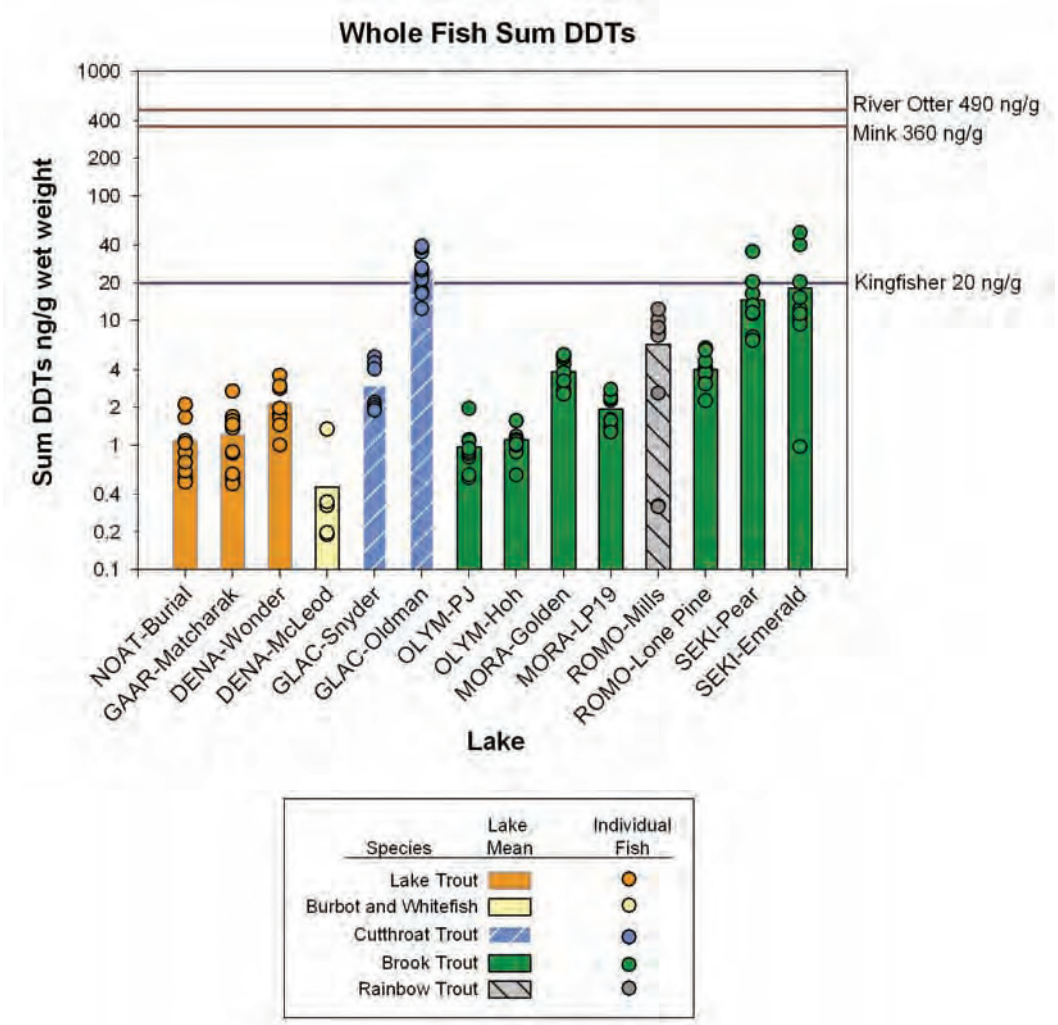


Figure 5-20. Fish Whole-Body Lake Mean (bars) and Individual Fish (symbols) Sum DDT Concentrations (DDT, DDD, and DDE), with Contaminant Health Thresholds for Various Wildlife. *N* = 10, except *N* = 6 for McLeod Lake. Contaminant health thresholds in piscivorous animals are based on 100% fish in the diet for whole body total DDTs as determined by Lazorchak et al. (2003). Data are plotted on a $\log_{10}$ scale. All lake means (bars) were derived from the sums of DDTs in individual fish in each lake; more than 50% of the DDT forms used in the sums were below detection limits and were replaced with values equal to ½ the estimated detection limit. The form of DDT most frequently detected in the fish was p,p′-DDE.

5.4.2.3 Chlordanes (cis- and trans-chlordane, and cis- and trans-nonachlor)

Chlordane is a broad use pesticide. The technical mixture contained some or all of the additional chemicals listed in the previous subsection. Chlordane is an organochlorine, like DDT, and concerns about potential carcinogenic effects led to banning chlordane in 1983. Chlordane in wildlife is highest in areas where the pesticide has been used to control underground termites. Highly lipophilic, chlordane accumulates in the fatty tissues of animals such as the gonads, liver, and brain. Chlordane differs from Hg, PCBs, and DDT in that it does not readily bioaccumulate, but high concentrations have been found in top marine predators.

Chlordane is transported to the national parks in the atmosphere and is widespread in the environment. It is a suspected carcinogen and endocrine disruptor (Eisler, 1990). Concentrations of the sum of chlordanes in WACAP fish are shown in Figure 5-21; the concentration sum in one fish in Oldman Lake (GLAC) was above contaminant health thresholds for piscivorous birds. The concentrations of chlordane are low enough to protect piscivorous mammals.

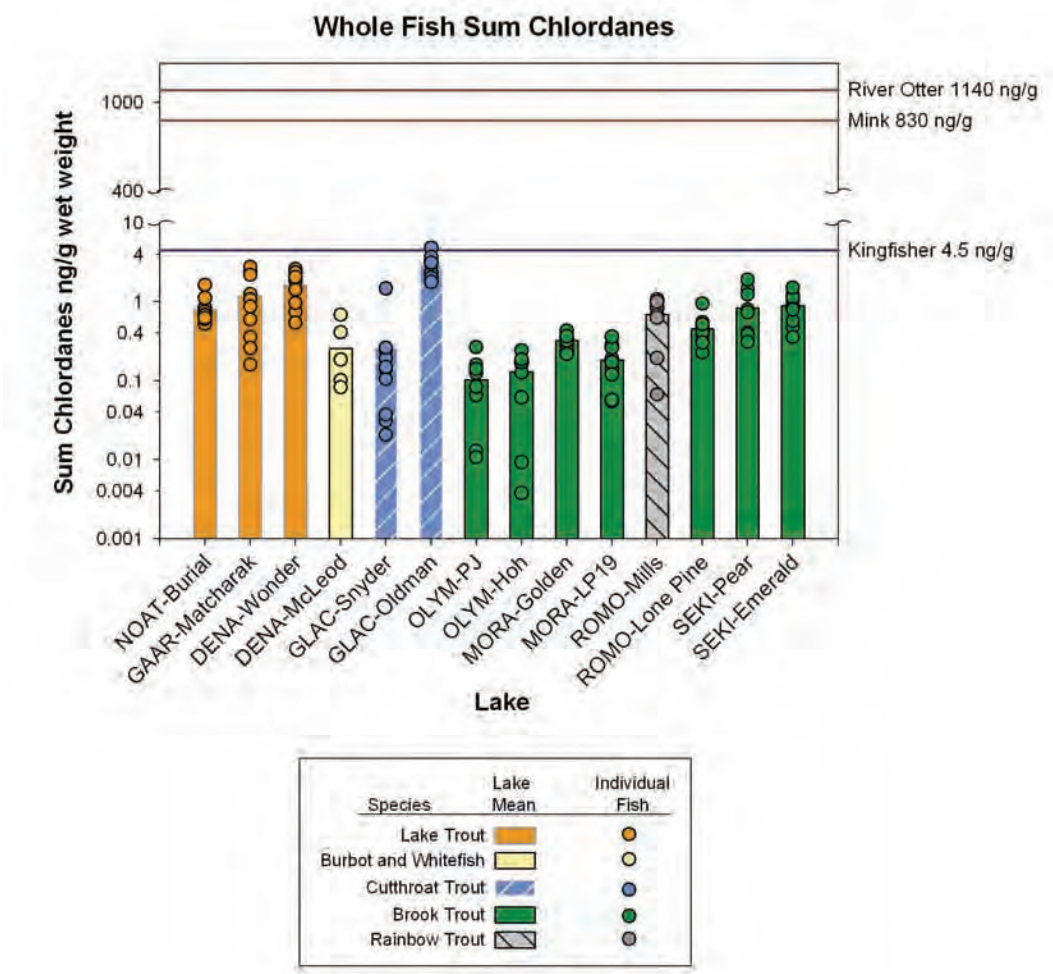


Figure 5-21. Fish Whole-Body Lake Mean (bars) and Individual Fish (symbols) Sum Chlordane Concentrations, with Contaminant Health Thresholds for Various Wildlife. *N* = 10, except *N* = 6 for McLeod Lake. Contaminant health thresholds in piscivorous animals are based on 100% fish in the diet as determined by Lazorchak et al. (2003). Data are plotted on a $\log_{10}$ scale and below detection limit values are reported as ½ the EDL.

5.4.2.4 Dieldrin

Dieldrin is a breakdown product of the organochlorine pesticide aldrin, but once dieldrin itself proved to be an effective pesticide, it was produced along with aldrin. Dieldrin was produced in Denver, Colorado, from 1952 to 1973 (Walden Research Division of Abcor, 1975). In the United States, it was banned for agricultural use in 1974 and for most uses in 1987. Perhaps not coincidentally, some of the highest dieldrin concentrations in fish in this study were found in Rocky Mountain National Park, less than 160 km from Denver, where it was once manufactured. Like the PCBs, DDT, and chlordane, the aldrin/dieldrin/endrin family of pesticides are members of

the so called "dirty dozen," a group of persistent bioaccumulative chemicals that have been largely banned from production and use. The bioaccumulation of dieldrin has been observed in piscivorous and non-piscivorous birds, and hatchling success is diminished in the former that have been exposed to dieldrin. The maternal transfer of dieldrin to fish eggs has also been documented. Acutely toxic, dieldrin is carcinogenic and is a suspected endocrine disruptor, with potential developmental and reproductive effects (Jorgenson, 2001). The concentrations of dieldrin in fish are shown in Figure 5-22; concentrations are low enough to protect piscivorous wildlife.

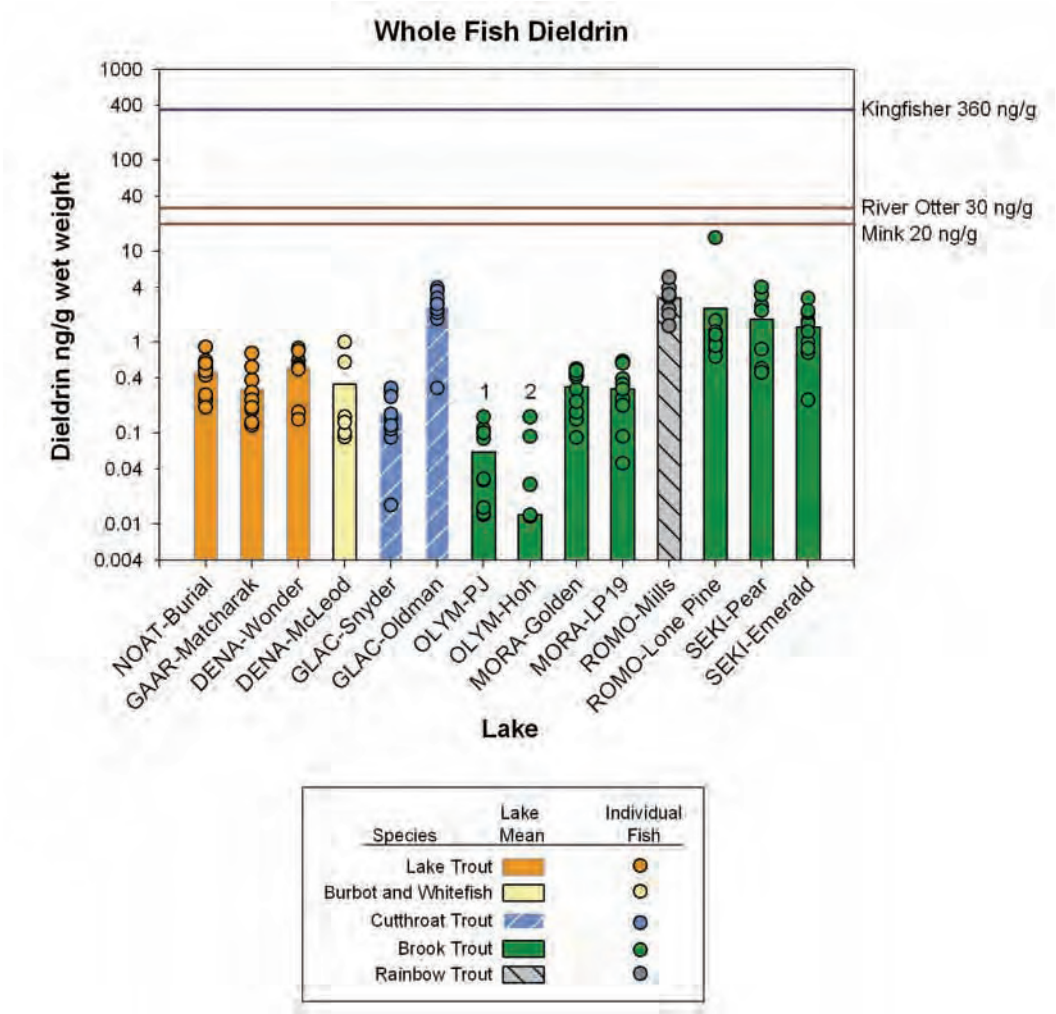


Figure 5-22. Fish Whole-Body Lake Mean (bars) and Individual Fish (symbols) Sum Dieldrin Concentrations, with Contaminant Health Thresholds for Various Wildlife. *N* = 10, except *N* = 6 for McLeod Lake. Contaminant health thresholds in piscivorous animals are based on 100% fish in the diet as determined by Lazorchak et al. (2003). Data are plotted on a $\log_{10}$ scale and below detection limit values are reported as ½ the EDL. "1" indicates that dieldrin was detected in 50-70% of the samples (PJ Lake, OLYM); "2" indicates that dieldrin was detected in < 50% of the samples; the mean value on the graph is ½ the EDL (Hoh Lake, OLYM).

5.4.3 Human Health Risks from Consumption of SOCs in Fish

A formal human risk assessment for the consumption of SOCs in fish was beyond the scope and resources of WACAP. Instead, we used USEPA's (2000) "Guidance for Assessing Chemical Contaminant Data for use in Fish Advisories" to adjust contaminant health thresholds for recreational and subsistence fishers who eat fish only from WACAP lakes. The contaminant health threshold is the point at which a 70-kg person who consumes 17.5 g of fish per day (recreational fishing) or 142 g/day (subsistence fishing) would increase the lifetime risk of developing cancer by 1 in 100,000 for carcinogenic contaminants, or measurably increase the risk of chronic conditions from toxic contaminants. Concentrations of SOCs in individual fish and the average concentration (by WACAP lake) were then compared with the USEPA contaminant health thresholds (Figures 5-23 through 5-27). It is assumed that recreational fishers, but not subsistence fishers, reduce their contaminant exposure 32% by trimming and cooking their fish. Specifically, cancer slope factors and reference doses for the 13 SOCs that were detected in > 50% of WACAP fish were obtained from the EPA-ORD Integrated Risk Information System database (2007) and forward-multiplied to estimate individual contaminant concentrations in fish tissue sufficient to exceed EPA contaminant health thresholds for humans eating fish.

A total of 13 SOCs in 136 fish from the 14 WACAP lakes were compared with calculated contaminant health thresholds (Figures 5-23 through 5-27). Concentrations in fish ranged from <0.23 pg/g to 120 ng/g, and averaged 0.55 ng/g wet weight. Most contaminant concentrations in fish from the WACAP lakes fell below contaminant health thresholds calculated for recreational and subsistence fishing. However, over half (77 of 136) of the individual fish from 11 of the 14 lakes analyzed carried concentrations exceeding subsistence fishing contaminant health thresholds for dieldrin (Figure 5-23) and/or p,p′-DDE (Figure 5-25).

WACAP Adjustments for Contaminant Health Thresholds (Fish Consumption)

Consumption of fish offers many health benefits but can also increase exposure to environmental pollutants. Some pollutants are carcinogenic, teratogenic, and/or mutagenic; therefore, impaired health resulting from the consumption of contaminated foods is of concern. The EPA and other agencies (e.g., the World Health Organization) develop benchmarks for the consumption of fish in amounts that should not raise the risk of developing cancer or other chronic conditions. In this report, we call these benchmarks *contaminant health thresholds*. Their use is intended to provide an estimate of potential health risk resulting from the consumption of fish from lakes in the national parks we studied. The contaminant health thresholds do not consider the beneficial aspects of eating fish.

Different populations of humans consume fish at different rates; therefore, contaminant health thresholds are different for recreational and subsistence fishing. The values are calculated for 70-kg adults. For recreational fishing, it is assumed that 2.3 8-ounce fillets are consumed every month for a lifetime; for subsistence fishing, it is assumed that 19 8-ounce meals of whole fish are consumed every month. Based on these estimated amounts of fish consumed, the contaminant health thresholds are concentrations of contaminant exposure that would raise the risk of cancer above 1:100,000 (0.001%).

We adjusted the recreational fishing contaminant health threshold values upwards 32% to account for the estimated amount of chemical lost from fresh, whole fish during filleting and cooking. By making these adjustments, we were able to compare the benchmark, developed for cooked fillets, to our data, gathered from whole uncooked fish. In all the graphs, the concentrations were not adjusted; only the thresholds were adjusted to account for the difference between whole fish and filleted cooked fish. In the subsistence fishing scenario, no adjustment was made—per the EPA (2000) recommendation. Raw, whole-fish concentrations were compared with reference doses and cancer risk threshold values.

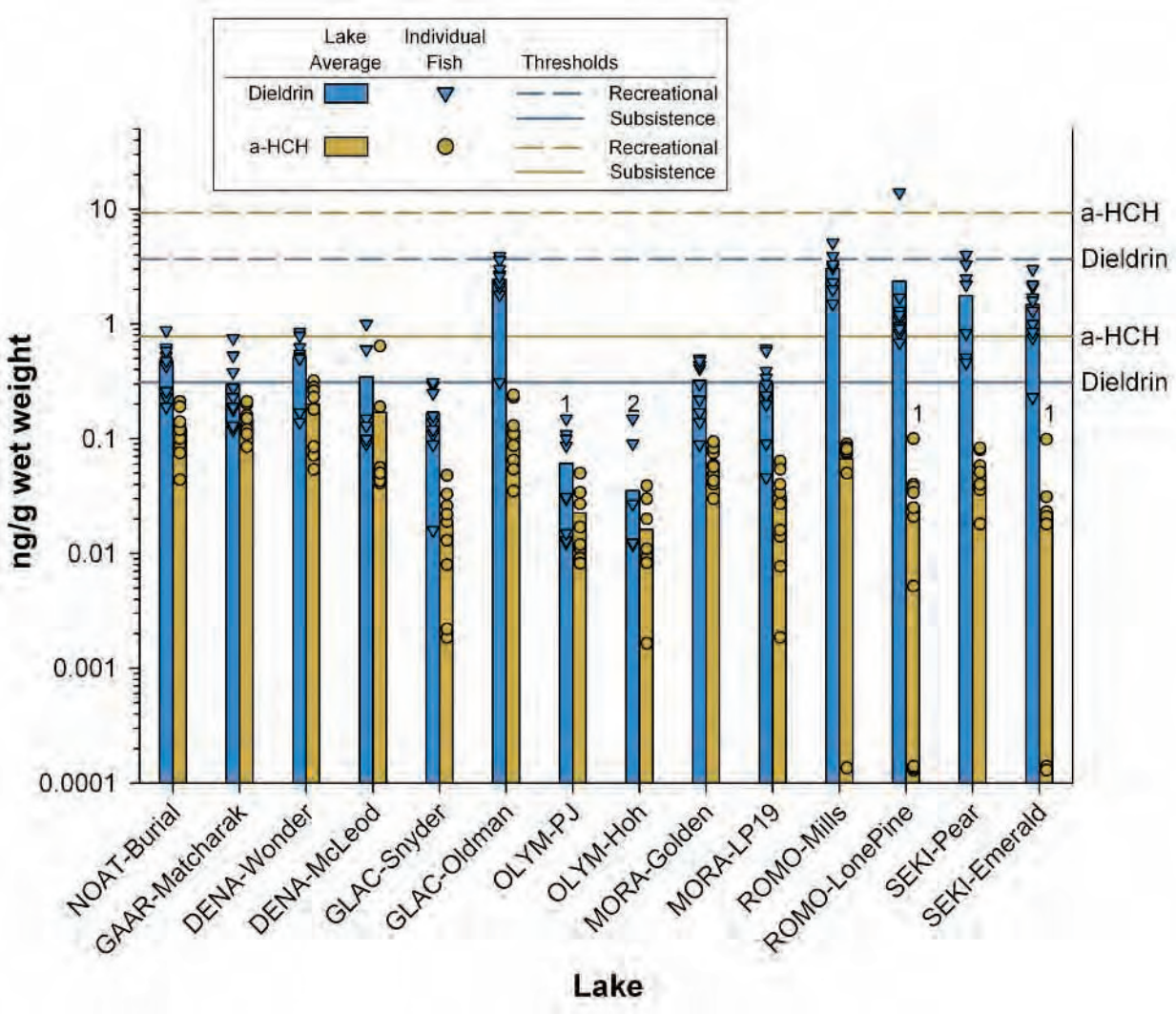


Figure 5-23. Concentrations of Historic-Use Pesticides for Dieldrin and a-HCH in Individual Fish (symbols) and Lake Average Fish (bars) Compared to Contaminant Health Thresholds for Cancer for Fish Consumption for Recreational and Subsistence Fishers (USEPA, 2000). Data are plotted on a log scale; below detection limit values are reported as ½ the EDL. Some fish from SEKI, ROMO, and GLAC exceed contaminant health thresholds for dieldrin for recreational fishing. The lake average concentration of fish from SEKI, ROMO, Golden Lake (MORA), Oldman Lake (GLAC), DENA, and NOAT, and some fish from GAAR and LP19 (MORA), exceed contaminant health thresholds for dieldrin for subsistence fishing. Exceedances imply that a lifetime consumption can increase the risk of developing cancer by more than 1 in 100,000. If no label is present at the top of a bar, the component was detected in at least 70% of the samples. “1” indicates the analyte was detected in 50–70% of the samples; “2” indicates the analyte was detected in less than 50% of the samples.

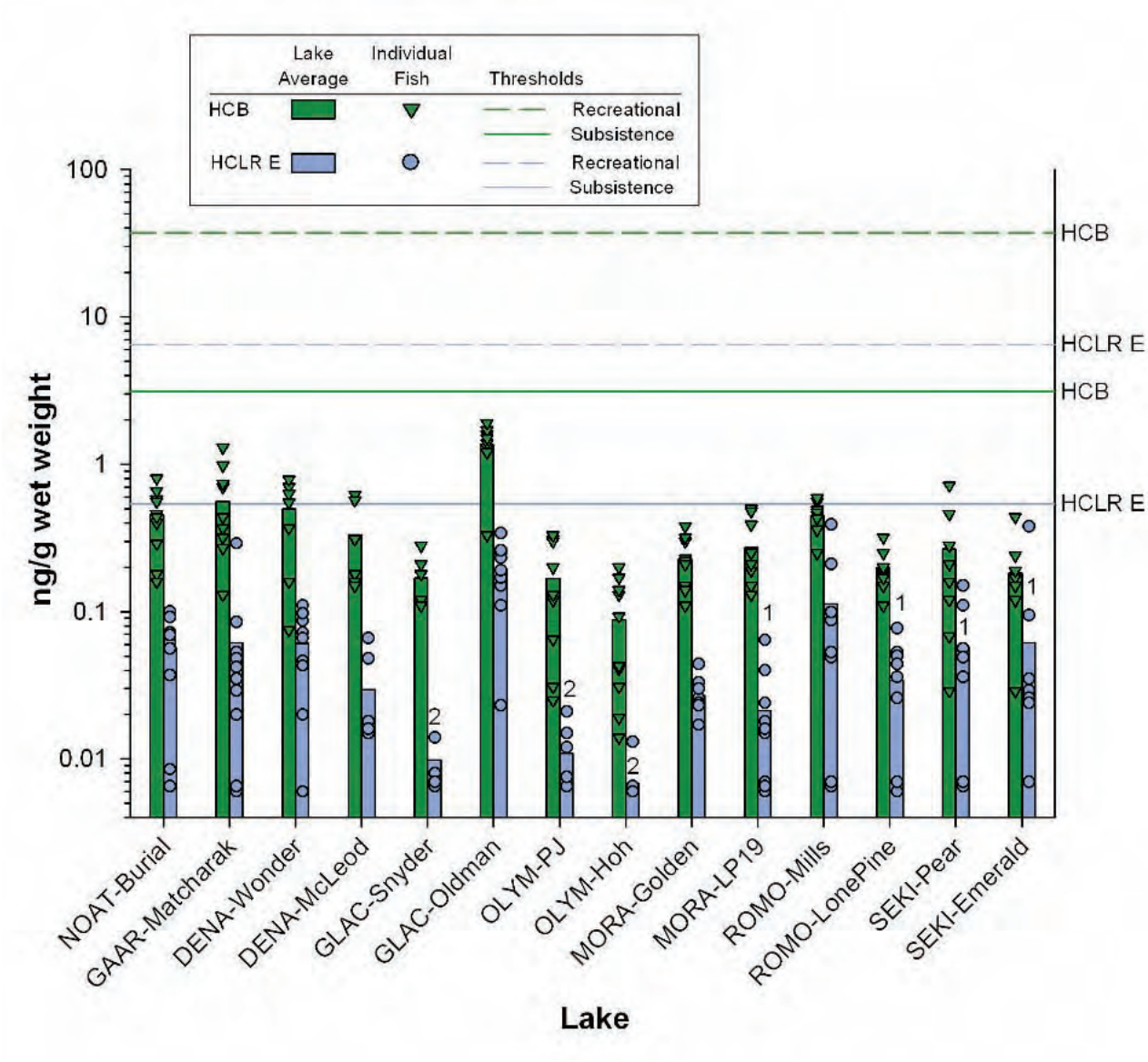


Figure 5-24. Concentrations of Historic-Use Pesticides for Hexachlorobenzene (HCB) and Heptachlor Epoxide (HCLR E) in Individual Fish (symbols) and Lake Average Fish (bars) Compared to Contaminant Health Thresholds for Cancer for Fish Consumption for Recreational and Subsistence Fishers (USEPA, 2000). Data are plotted on a log scale; below detection limit values are reported as ½ the EDL. Concentrations of all compounds were below contaminant health thresholds at all lakes. If no label is present at the top of a bar, the component was detected in at least 70% of the samples. “1” indicates the analyte was detected in 50–70% of the samples; “2” indicates the analyte was detected in less than 50% of the samples.

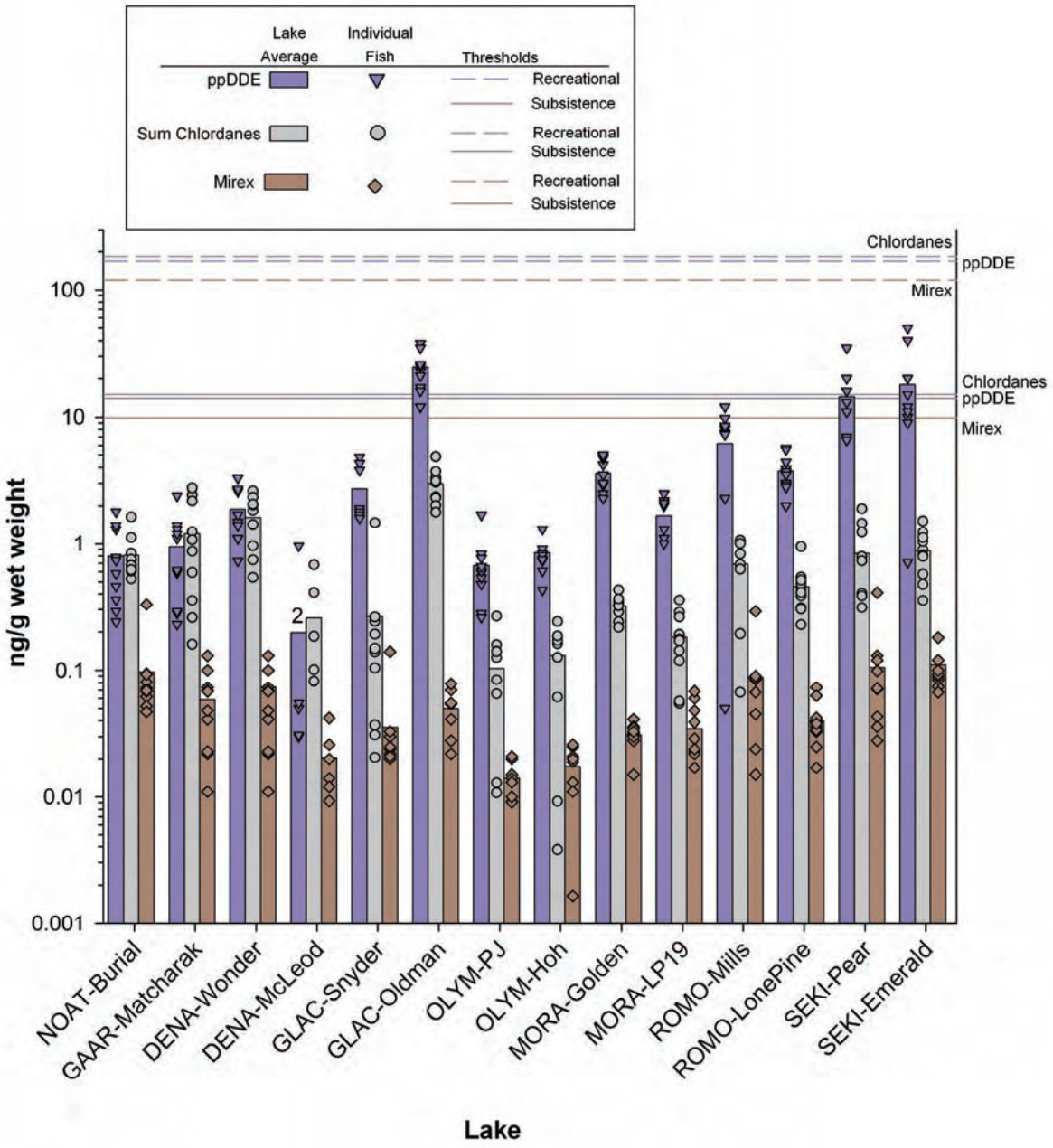


Figure 5-25. Concentrations of Historic-Use Pesticides (p,p′-DDE, chlordanes, mirex) in Individual Fish (symbols) and Lake Average Fish (bars) Compared to Contaminant Health Thresholds for Cancer for Fish Consumption for Recreational and Subsistence Fishers (USEPA, 2000). Data are plotted on a log scale; below detection limit values are reported as ½ the EDL. Concentrations of all compounds were below contaminant health thresholds for recreational fishers, but a lifetime consumption of fish from Pear and Emerald Lakes in SEKI and Oldman Lake in GLAC could increase cancer risk for subsistence fishers from p,p'-DDE. If no label is present at the top of a bar, the component was detected in at least 70% of the samples. "1" indicates the analyte was detected in 50–70% of the samples; "2" indicates the analyte was detected in less than 50% of the samples.

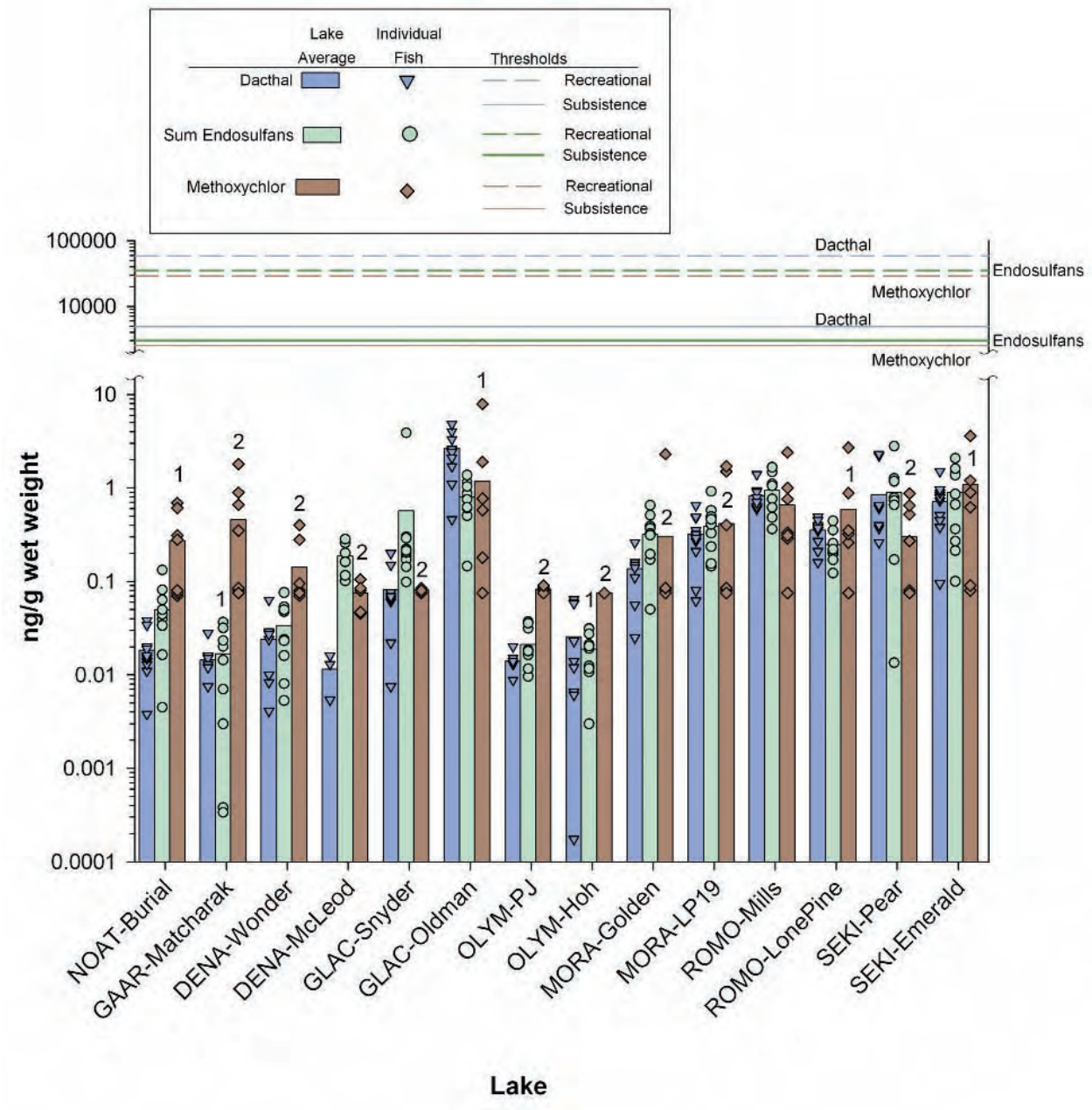


Figure 5-26. Concentrations of Current-Use (dacthal, endosulfans) and Historic-Use (methoxychlor) Pesticides in Individual Fish (symbols) and Lake Average Fish (bars) Compared to Contaminant Health Thresholds for Chronic Disease for Fish Consumption for Recreational and Subsistence Fishers (USEPA, 2000). Data are plotted on a log scale; below detection limit values are reported as ½ the EDL. Concentrations of all compounds were below contaminant health thresholds at all lakes. If no label is present at the top of a bar, the component was detected in at least 70% of the samples. “1” indicates the analyte was detected in 50–70% of the samples; “2” indicates the analyte was detected in less than 50% of the samples.

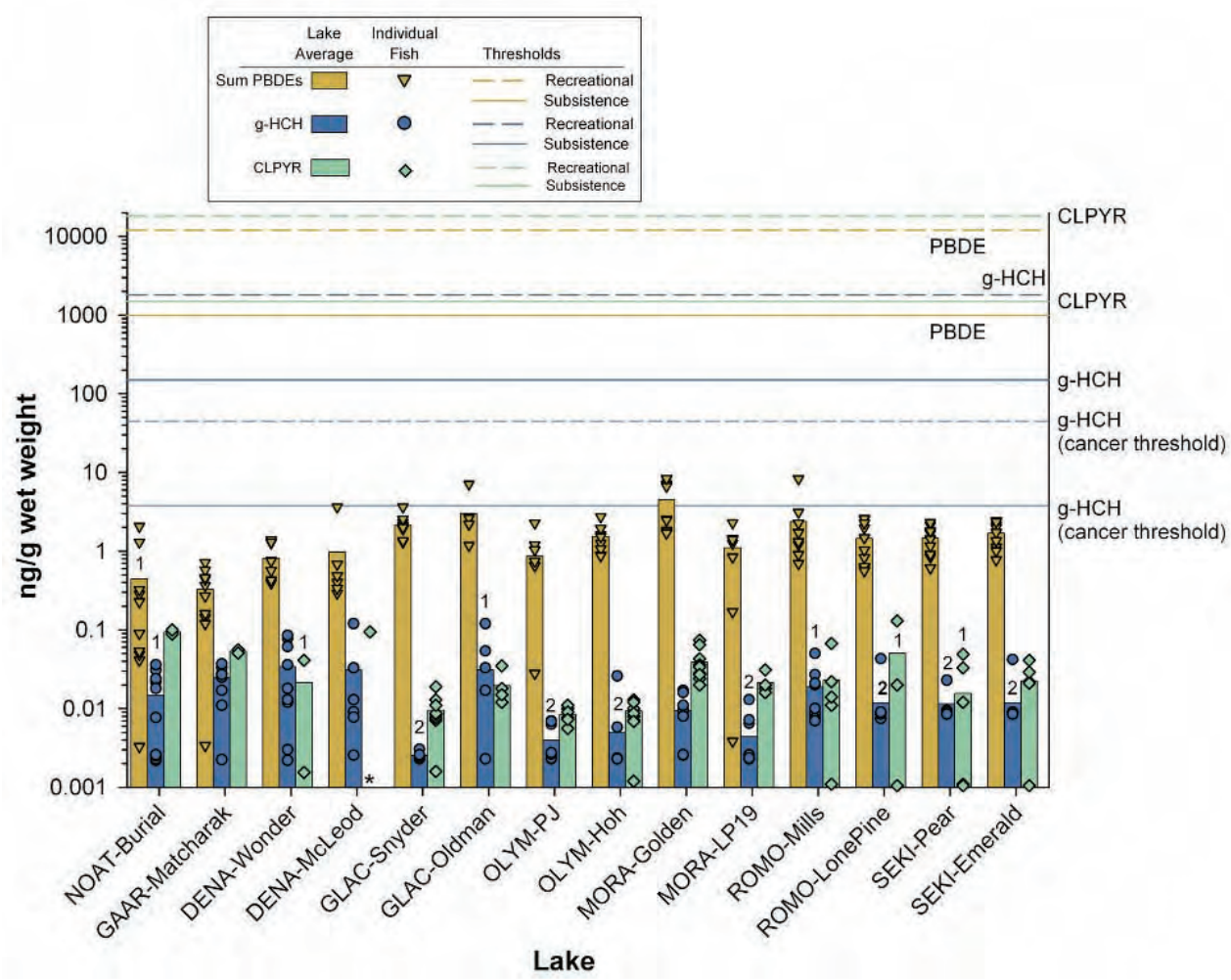


Figure 5-27. Concentrations of Current-Use Contaminants PBDEs, g-HCH, and Chlorpyrifos (CLPYR) in Individual Fish (symbols) and Lake Average Fish (bars) Compared to Contaminant Health Thresholds for Chronic Disease (and Cancer Thresholds for g-HCH) for Fish Consumption for Recreational and Subsistence Fishers (USEPA, 2000). Data are plotted on a log scale; below detection limit values are reported as ½ the EDL. Concentrations of all compounds were below contaminant health thresholds at all lakes. If no label is present at the top of a bar, the component was detected in at least 70% of the samples. "1" indicates the analyte was detected in 50–70% of the samples; "2" indicates the analyte was detected in less than 50% of the samples. * indicates results were available from only one sample from the site, and no average is presented.

The numbers of fish in each lake that exceeded these thresholds and the recreational fishing threshold for dieldrin are listed in Table 5-14. Although some individual fish (5) exceeded recreational fishing thresholds for dieldrin, the average fish contaminant concentration per lake did not exceed recreational fishing thresholds in any of the 14 lakes, but did exceed the subsistence fishing thresholds for dieldrin or p,p′-DDE in 9 of the 14 lakes. No other contaminant concentrations measured in fish from the core WACAP parks exceeded human contaminant health thresholds, and concentrations of the other target contaminants detected in fish were one to seven orders of magnitude below the adjusted recreational human contaminant health thresholds. PBDEs and the current-use pesticides dacthal, endosulfan, chlorpyrifos, and methoxychlor were at least three orders of magnitude below all contaminant health thresholds.

Table 5-14. Number of Fish Exceeding Human Cancer Thresholds

Population		Number of Fish out of 10 Sampled that Exceeded Threshold		
		Subsistence Fishing		Recreational Fishing
Contaminant		p,p′-DDE	Dieldrin	Dieldrin
Park	Lake			
NOAT	Burial	0	7	0
GAAR	Matcharak	0	3	0
DENA	Wonder	0	7	0
DENA	McLeod*	0	2	0
GLAC	Snyder	0	0	0
GLAC	Oldman	9	9	1
OLYM	PJ	0	0	0
OLYM	Hoh	0	0	0
MORA	Golden	0	5	0
MORA	LP19	0	4	0
ROMO	Mills	0	8	2
ROMO	LonePine	0	10	1
SEKI	Pear	3	10	1
SEKI	Emerald	4	9	0
	Cancer Threshold (ng/g wet weight)	14	0.31	3.7

* Number out of 6 total from lake

The EPA's calculated contaminant health thresholds offer a uniform approach to evaluating human health risks from consumption of contaminated fish, but *individual* risk is probably higher or lower. Potential interactions or synergistic effects from the multiple contaminants present in fish could yield higher risks than those reported here. However, because most of the total risk is attributed to the contaminant dieldrin, cancer risks from additive interactions are not significantly different from exceedances of the dieldrin threshold. Also, the likelihood of these risks being realized is small, because (1) the lakes are remote, with small fish populations (which limit the people present and the frequency of fishing), (2) the risk scenario assumes lifetime consumption (which is probably rare), and (3) the acceptable risk values (1:100,000) add a safety factor.

Finally, salmonid consumption is associated with nutritional and health benefits, such as increased consumption of omega-3 fatty acids and reduced consumption of unhealthy fats. For some people, these benefits probably outweigh contaminant risk. Health risks from contaminants in fish can be reduced by removing the skin before cooking and by draining fats during cooking.

5.4.4 Potential Ecological Effects of SOC and Metal Contaminant Loads on Aquatic Systems in the Parks

Information generated from sampling allows hypotheses to be generated about the health and reproductive fitness of individual fish. We were also interested in making inferences regarding potential effects of contaminants on the fish populations and other components of the biotic communities of the lakes. We wanted to know if the presence of contaminants at levels with potential biological effects on the fish would affect the demographic structure of the fish populations. Furthermore, we conducted analyses to allow inferences about potential effects to bird and mammal populations that are dependent on these specific lakes for at least some part of their life cycle.

We obtained faunal lists for each of the respective lakes studied. These lists focused on birds and mammals known to use the lakes (see Table 5-13). For some lakes, the identity of species representing other taxa was available. For assessment of ecological risk of the vertebrate fauna of the parks to contaminant exposure, the use of population viability analyses would be desirable. However, quantitative data is lacking for such an assessment. There is no information on the abundance, age structure, birth or death rates, or predation rates. It is thus unfeasible for us to conduct any quantitatively-based risk assessment. Instead, we used a qualitative analysis (loop analysis) that is useful for allowing inference about systems such as these that are only partially specified.

We constructed generalized trophic webs for communities of the lakes sampled. Li and Li (1996) discuss how organisms can be classified into functional groups (guilds) and we followed their recommendations. Briefly, we assigned the biota into trophic guilds as follows: (1) mammals that eat fish, (2) birds that eat fish, (3) birds that eat invertebrates, (4) fish that eat invertebrates, (5) fish that eat fish, (6) predacious invertebrates, (7) herbivorous and detritivorous invertebrates, and (8) plant material and other detritus. Two different loop analyses were run. One was for lakes in parks in the conterminous United States that contain mainly one species of fish. Such systems are characterized by straight chain relationships between guilds. The second was for systems such as those in Alaska, and perhaps for Emerald Lake in Sequoia, where more than one fish species was present (Figure 5-28). Of course, piscivorous fish are also prey during early life stages. In addition, fish of two fish systems can shift between guilds 4 and 5 over time, as feeding ecology and abundance of various prey species change through time.

Considerable variation is likely, regarding the potential impact to the fish populations, because of the use of lakes by birds and mammals. That is, depending on whether predators were resident or transitory, potential impact to the fish population (and to the predators) could vary according to the relative predatory pressure on the population. In the case of resident keystone predators (birds and mammals), a top-down effect on fish would probably occur. At these lakes, birds and perhaps mammals use *central place foraging.* That is, these top fish predators would be resident over a substantial period of time and feed almost solely at this site, foraging for young as well as themselves. For such systems, predator guilds would be considered omnivorous. Although warm-blooded vertebrate species forage, such as the ouzel (*Cinclus mexicanus*), a bird

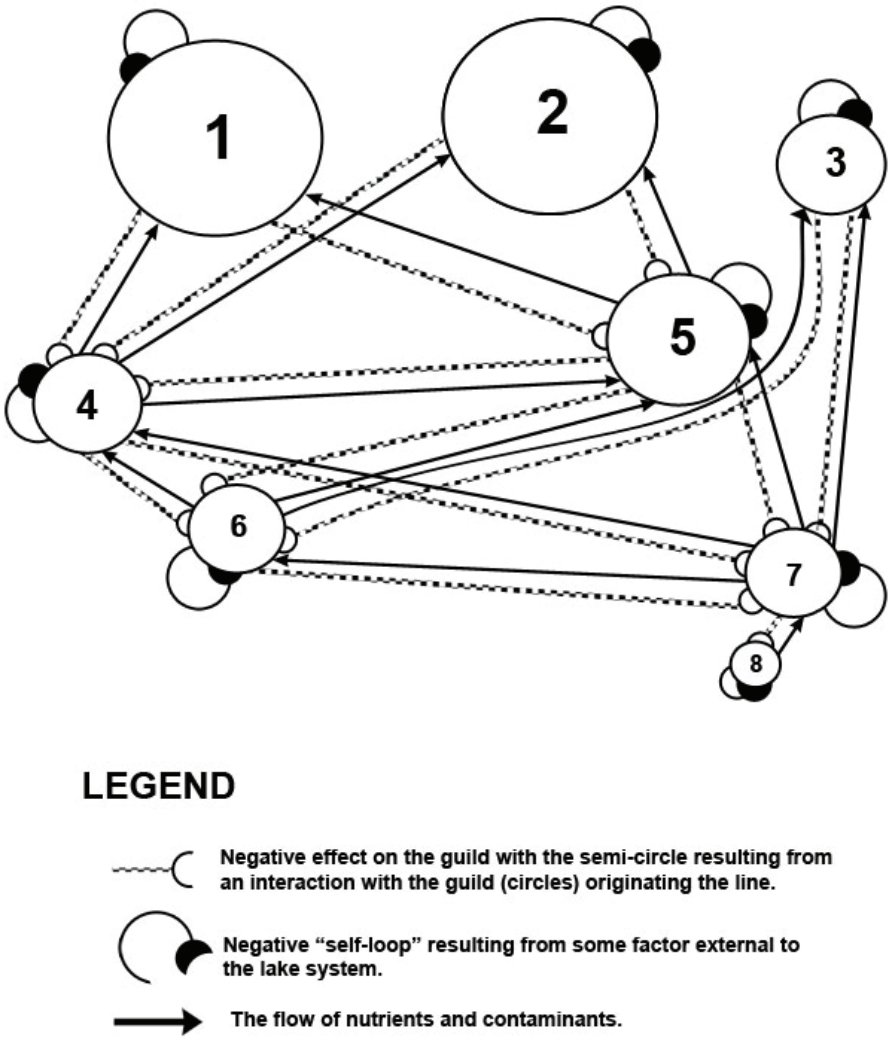


Figure 5-28. Trophic Model for Lakes with Two-Fish Guilds Representing Alaska Systems. Circle with numbers are guilds and 1= piscivorous mammal; 2 = piscivorous bird; 3 = insectivorous bird; 4 = insectivorous fish; 5 = piscivorous fish; 6 = invertebrate predator; 7 = invertebrate; and 8 = detritus. One-fish models would be similar, except that they would contain only one of the fish guilds. The self loops represent self-regulation, such as in the form of logistic growth, which accounts for intraspecific competition. The relative size of the circles indicates relative approximate position in the food web with larger circles indicating higher trophic level.

representative of a trophic guild that forages on aquatic invertebrates, we do not believe that the competition between members of this guild and fish would result in any population-level impact on the fish. This contention is based on their small biomass and the fact that the foraging habitat for this guild is relatively sparse in these lakes. Hence, there would be no top-down effect by this guild on the fishes, but there could be bioaccumulation and amplification of contaminants up the food chain from invertebrates into species such as ouzels. In the case of transitory top-order predators (perhaps only as a brief stopping place during migration), pressure on the fish would not be likely to affect the fish population. However, there could still be effects of toxicants via

trophic transfer on these top-order predators, adding to concentrations accumulated elsewhere. Given that concentrations of certain endocrine disrupting contaminants found in fish in the WACAP lakes exceed tolerance thresholds for several species of predaceous birds and mammals (Figures 5-18 to 5-22), we also ran two other variations of the model. For non-omnivory systems, there would be no top-down control by the predators on the fish; for systems with omnivory, there would be population-level effects. In either case, the contaminants could have an effect on the warm blooded vertebrates foraging in the lakes.

5.4.4.1 Assumptions

We assumed that avian and mammalian predators at a lake function as though they are resident, foraging for themselves and for their progeny during the time that the aquatic system is free from ice. Any negative impacts on the fish predators are assumed to result from a reduced prey base as well as directly from contaminants. This predation pressure would have an impact on the biotic system of the lakes. We acknowledge that this is a significant assumption because we have no measurements on the impact of predators on the fish or the impact of contaminants on piscivorous biota. We are aware that mammals and birds, such as kingfishers and ouzels (the duck-like birds), and birds such as ospreys and eagles, probably forage beyond the confines of a sampled lake, but for this exercise, we assumed that the other foraging sites would have similar or greater (see Tables 5-1 and 5-2) concentrations of contaminants. Of course, birds are migratory and can be exposed to different contaminants when they are not resident at WACAP lakes, and we do not address the interactive effects of contaminant loading and unloading over the seasons for these species. Therefore, we used negative self loops in the model (Figure 5-28). In addition, birds and mammals export contaminants from the lake system.

We also did not consider the following in the analysis. Allochthonous matter, such as leaves and needles, which provides energy input into a lake system, is assumed to be accessed by higher trophic levels through the detritus; primary production in the lake is also subject to other inputs to the system. Thus negative self loops are also used for that guild. We also assume that allochthonous fish prey (e.g., terrestrial insects) are probably unimportant over the course of a year, compared with prey produced within the lake itself. We also ignored in the model the fact that constituents of aquatic organisms that are not removed from the lake will ultimately find their way into the detritus. Biomagnification of persistent organic pollutants through the food web, including aquatic ones, has been well documented (Kelly et al., 2007).

5.4.4.2 Loop Analysis

We used loop analysis (constructing signed diagraphs; see Figure 5-28) to analyze the trophic network of the lake systems. It resulted primarily in inferences about the flow of nutrients from which hypotheses about numbers of individuals could be made. Flow of contaminants through the system would be similar to that of nutrients. Hence, hypotheses can be generated about effects of contaminants on the various trophic guilds.

Loop analysis has been elaborated upon and validated in a series of recent publications (Hulot et al., 2000; Dambacher et al., 2002, 2003a, 2003b, 2005; Arkoosh et al., 2004; Zavaleta and Rossignol, 2004; Ramsey and Veltman, 2005). The computer programs are available in Dambacher et al. (2002), updated 2006, and at http://www.ent.orst.edu/loop/. We initiated the press (negative demographic effect of the contaminants) at the level of the fish in the system (Figure 5-28, Table 5-15a). For the two-fish system (Figure 5-28, Table 5-15b), separate presses were performed on each fish guild, but we interpret the results together as an intact system. The

Table 5-15a. Effects of a Negative Press Perturbation[1] on Fish in a One-Fish Guild Ecosystem.

Guild[2]	**No Omnivory**	
	Δ[3] in Life Expectancy [4]Press 4	**Δ in Abundance Press 4**
1	No effect	No effect
2	No effect	No effect
3	Increase	No effect
4	Increase	No effect
Guild[2]	**Omnivory**	
	Δ[3] in Life Expectancy [4]Press 4	**Δ in Abundance Press 4**
1	Increase	Decrease
2	Increase	Decrease
3	Increase	Decrease
4	Increase	Ambiguous

Table 5-15b. Effects of a Negative Press Perturbation[1] on Fish in a Two-Fish Guild Ecosystem.

Guild[2]	**No Omnivory**	
	Δ[3] in Life Expectancy [4]Press 4	**Δ in Abundance Press 4**
1	No effect	No effect
2	No effect	No effect
3	Increase	Decrease
4	Increase	Decrease
5	Increase	Ambiguous
Guild[2]	**Omnivory**	
	Δ[3] in Life Expectancy [4]Press 4	**Δ in Abundance Press 4**
1	Increase	Decrease
2	Increase	Decrease
3	Increase	Decrease
4	Increase	Ambiguous
	Increase	Ambiguous

[1]Press perturbation = decreased birth to fish resulting from contaminant induced fish death.
[2]See Figure 5-28 for key to guilds.
[3]Δ = change.
[4]The guild receiving a negative press perturbation.

modeling and interpretation of the results were performed by Drs. Phil Rossignol and Hiram Li and we acknowledge their contributions (Department of Fisheries and Wildlife and Oregon Cooperative Fish and Wildlife Research Unit, USGS, respectively, Oregon State University).

5.4.4.3 Loop Analysis Sensitivity

Two assessments were made to determine the sensitivity of the loop analysis model, one for stability and the other for predictability. The most complex community run by us in the loop analyses was used to test sensitivity.

5.4.4.3.1 Sensitivity of Stability

Sensitivity of stability is evaluated with simulations of the community matrix over a range of randomly selected interaction values, as explained on the Loop Analysis site: http://www.ent.orst.edu/loop/

The stability test is based on the signed digraph, in which the relationships between species are (+1, -1, 0). The qualitative stability offers no insight into the stable structure of quantitative domain of the system. For example, a signed digraph has an overall negative feedback with two negative loops (-2) and one positive loop (+1). However, if we have the measure of interaction strengths between species, the values of two negative loops could be -0.2 and -0.3, and the value of a positive loop could be 0.6. In this case, the strength of overall feedback would be 0.1 and then we would obtain a positive overall feedback.

In order to know the probability that the system is also stable in a quantitatively specified matrix, 5,000 quantitative matrices are constructed based on the unchanged sign structure of the system. Non-zero elements of each matrix are quantitatively specified with a pseudorandom number generator that assigns interaction strength but not a sign from a uniform distribution between 0.01 and 1.0. The stability of each quantitatively specified matrix is then examined in terms of Hurwitz criteria I and II.

- Hurwitz criterion I: Characteristic polynomial coefficients are all of the same sign.
- Hurwitz criterion II: Hurwitz determinants are all positive

The results of 5,000 simulations indicate that the system is very stable, particularly considering its fairly high connectivity:

- Pass Hurwitz criteria I and II: 4,305
- Pass Hurwitz criterion I, but not II: 50
- Pass Hurwitz criterion II, but not I: 0
- Not pass Hurwitz criteria I and II: 645

5.4.4.3.2. Sensitivity of Predictability

Sensitivity of predictions can be estimated from the weight of the predictions. These weights represent the likelihood that the prediction will be in the direction generated by the adjoint matrix. These weights correspond to simulation results, by the method discussed in Dambacher et al. (2003b). This matrix represents the probability, based on average proportion of correct sign, of a correct sign in a prediction (adjoint matrix) from simulations with uniformly distributed interaction strengths and interdependent trophic relationships:

0.88	0.75	0.66	0.82	0.55	0.57	0.63	0.63
0.75	0.88	0.66	0.82	0.55	0.57	0.63	0.63
0.69	0.69	0.85	0.50	0.73	0.64	0.77	0.77
0.50	0.50	0.50	0.95	0.95	0.50	0.50	0.50
0.80	0.80	0.71	0.75	0.95	0.57	0.66	0.66
0.70	0.70	0.83	0.77	0.55	0.90	0.57	0.57
0.50	0.50	0.50	0.83	0.83	0.87	0.85	0.85
0.50	0.50	0.50	0.83	0.83	0.87	0.85	0.83

5.4.4.4 Results

We assume that the primary impact of a toxin would be a negative press perturbation (i.e., a deleterious effect on fish). The mechanism would be reduced reproduction. Impact is assessed as changes in abundance and life expectancy (inverse of turnover). During our analysis, both systems tested appear to be stable, indicating that inferences can be drawn on the result of the press perturbation.

For the one-fish system, we can infer from the predicted results that, with little predation pressure from higher trophic levels, the abundance of fish would decrease. The results about abundance are ambiguous for single-fish systems where there is top-down control on the fish by predators, assuming that there would be fewer insectivorous birds, and those remaining would have a reduced life span. The populations of fish in both types of systems would have an older age structure, because of a decreased birth rate. Piscivorous mammals and birds are expected to live longer, but maintain fewer numbers (5-15a) in cases where they forage substantially on the contaminated fish. Invertebrate-eating birds would be expected to have a decreased abundance and an increase in the proportion of older individuals in the population.

The loop analysis infers that a press on fish in the two-fish system would lead to a response in piscivorous and invertebrate-eating birds and mammals similar to that of the one-fish system. There would probably be an increased life expectancy and reduced abundance of invertebrate-eating birds and those birds and mammals eating large numbers of contaminated fish. The birth rate would be expected to decrease in fish, with or without top-down control by predators, and the abundance of fish would probably decrease in predatory fish in systems without top-down control. Effects of a press on piscivorous fish in general and on invertebrate-eating fish in lakes with top-down control by avian or mammalian predators are ambiguous (Table 5-15b), because these fish, being more or less in the middle of the food chain, would be responsive to the contaminants directly, but also indirectly through the action of the contaminants on their predators.

For the analyses for scenarios that were complex and that included considerable interaction between various guilds (not just in a single straight chain food web), there was considerable ambiguity in the results. This is a logical result and stems from the fact that predators affect forage and the forage can be toxic to the predators. Therefore the system does not behave according to a simple predator-prey relationship. Paradoxical results were often found in these situations; for example, the presence of a predator could actually result in an increase in abundance of its prey because the contaminant in the prey would reduce the abundance of predators. We therefore thought it would be best to be conservative and not interpret these results (ambiguous cases in the tables). Results related to the age structures of the populations must also

be interpreted with caution. The sign diagraph analyses show effects on birth rate. Therefore, in those cases where a decrease in birth rate was suggested, the interpretation would be that the age structure favored older individuals. So, there could be the same number, or even fewer, older individuals in that population, but proportionately there would be many fewer younger individuals than there were before the press. A population with proportionately older individuals does not *de facto* infer a population that is healthier than one with younger individuals.

For the cases in which the model results are ambiguous, one could rely on other information upon which to make an inference about contaminant effects. For example, in systems where the top predators use central place foraging, the toxicity tolerance information available (see Figures 5-18 to 5-22) would suggest the hypothesis that the abundance of those species would be negatively impacted.

5.4.4.5 Conclusion

Birds eating only invertebrates could be subjected to bioaccumulation and magnification of contaminants, and thus experience toxic effects. The ultimate risk to birds and mammals preying on fish, and especially species of fish that eat other fish, would be both the negative effects of a decreased prey base and the potential negative effects of bioaccumulation and magnification of contaminants.

The model runs allow the following inferences: Fish populations in lake communities with only one species of fish represented, hence with an absence of the omnivory loop, would be expected to experience a decrease in abundance and a decrease in life expectancy. These decreases could happen because of the potential negative effects of bioaccumulation and magnification of contaminants. Effects on abundance of fish in systems with more than one species present are ambiguous, but there would be fewer births and hence an older-aged population. Mammals and birds preying on these fish would experience double impacts (altered prey base and contaminants), leading to fewer births and a reduced abundance in systems without the omnivory loop. Birds eating only invertebrates could be subjected to bioaccumulation and magnification of contaminants and hence experience toxic effects.

We believe that piscivorous predators (birds, mammals, and fish) that forage on fish could be affected by contaminants. This is likely only if the predators forage on fish in the national parks with concentrations of contaminants that exceed tolerance levels for the birds and mammals. On a lake-by-lake and park-by-park basis, judgment regarding applicability of our conclusions would rest on knowledge unavailable to us regarding the period of time the birds and mammals were actually using those respective lakes.

5.5 Nitrogen Deposition Effects and Relationships

5.5.1 Ecological Effects of Enhanced Nitrogen Deposition in the Western United States

Nitrogen inputs to the United States from anthropogenic sources doubled between 1961 and 1997, mainly from inorganic N fertilizer use and emissions of nitrogen oxides from fossil fuels (Howarth et al., 2002; Burns, 2003). Chronic enhanced nitrogen deposition and excess available nitrogen can lead to a myriad of adverse ecological effects in forests in the western United States, such as eutrophication of water bodies, nitrate-induced toxic effects on aquatic biota,

changes in plant community composition and aquatic communities through removal of N limitations on biotic activity, disruptions in nutrient cycling, decreased soil capacity for N retention, and increased emissions from soil of nitrogenous greenhouse gases (Fenn et al., 1998; Fenn et al., 2004). Current upward trends in population growth and energy use throughout the western United States suggest a need for continued monitoring of atmospheric deposition N and its ecological effects.

5.5.2 Evidence of Enhanced Nitrogen Deposition in Some Parks from Lichen N

The previous chapter explains that we found indication of enhanced N deposition to terrestrial ecosystems based on WACAP lichen N data at SEKI, GLAC, BIBE, and BAND. Lichens from these parks exceeded thresholds for background sites in the western United States; N in lichens from other parks was within expected ranges for clean sites. Elevated lichen nitrogen concentrations are associated with adverse changes to lichen community composition (Geiser and Neitlich, 2007; Jovan and McCune, 2005) and are an indicator that other N-sensitive ecosystem components could be affected.

Another measurement of nitrogen availability obtainable at most of the WACAP parks are ambient concentrations of fine particulate (<2.5 µm) ammonium nitrate and ammonium sulfate sampled by IMPROVE. The IMPROVE network was established by federal land management agencies to meet federal land manager responsibilities under the Clean Air Act to monitor visibility in Class I areas. Three days a week, synchronized nationally, 24-hour samples of particulate matter <2.5 µm diameter are collected onto a filter from a height of about 3 m (these fine particulates are associated with declines in visibility and adverse human health effects). The chemistry of the particulates is determined following national protocols at the University of California, Davis. Particulates composed of ammonium sulfate and ammonium nitrate are reported in $\mu g\ m^{-3}$. These data have value as indicators of nitrogen availability at the site, from agricultural as well as urban-industrial sources. IMPROVE data and trends analyses can be obtained from the IMPROVE website at http://www.coha.dri.edu/index.html.

Mean ambient atmospheric ammonium nitrate and ammonium sulfate concentrations ($\mu g/m^3$) reported from WACAP IMPROVE sites, 1999-2004, are displayed in Figure 5-29. SEKI has higher concentrations of both pollutants than all other parks except BIBE, which has the highest ammonium sulfate concentrations. Among the core parks, GLAC, ROMO, MORA, and OLYM are not different from each other and DENA is lowest (Tukey-Kramer multiple means comparisons, $\alpha = 0.05$). Only nitrate and sulfate concentrations are measured, as these anions are assumed to be balanced by ammonium. IMPROVE data can be further explored by examining trends over seasons and years and by comparing peaks during worst days instead of averages. The point of including the data here is to provide further evidence of enhanced nitrogen deposition at SEKI and BIBE. There is no IMPROVE monitor at KATM; data from the Tuxedni National Wildlife Refuge monitors were used in surrogate.

5.5.3 Correlations between Agricultural Chemicals and Measures of Agricultural Intensity, Atmospheric Pollutants that Contain Nitrogen, and Human Population

Correlations among mean annual ammonium nitrate concentrations in airborne fine particulates sampled by park IMPROVE monitors from 1998-2004, the agricultural intensity index, nitrogen concentrations in WACAP lichens and SOC concentrations in WACAP vegetation were

calculated (Table 5-16). Agricultural intensity calculations are described by Hageman et al. (2006). See also Chapter 3 for more information about agricultural intensity, IMPROVE, and population density calculations.

Concentrations of the CUPs, chlordanes, dacthal, and endosulfans in lichens and in conifer needles, DDTs in conifer needles, and PAHs in lichens correlated well (Spearman's Rho 0.62 to 0.85) with both agricultural intensity and concentrations of ammonium nitrate in fine particulates <2.5 µm diameter sampled by park IMPROVE monitors. Trifluralin in conifer needles was strongly correlated with IMPROVE ammonium nitrate.

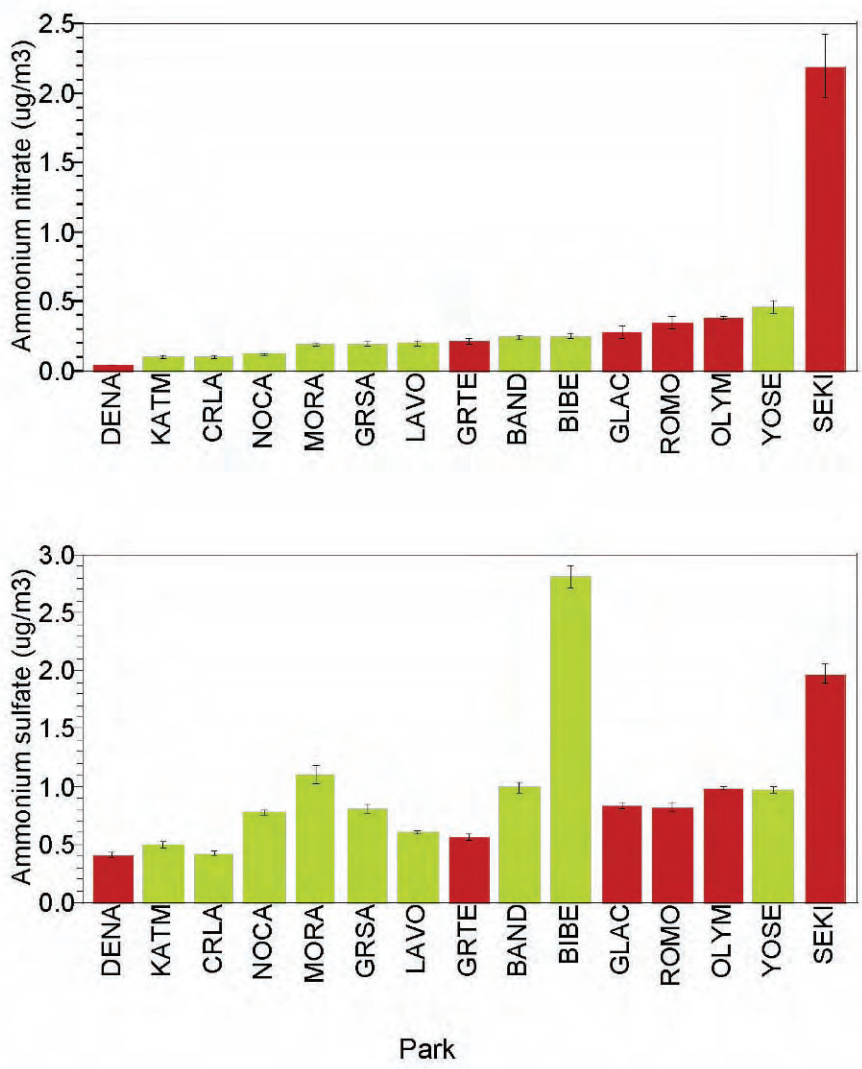


Figure 5-29. Mean Annual Concentrations (µg/m^3) of Ammonium Nitrate and Ammonium Sulfate in Ambient Fine Particulates (< 0.25 µm) Measured by IMPROVE at WACAP Parks, 1998-2004. Parks are sorted by increasing ammonium nitrate concentration. Red bars indicate core parks; green bars indicate secondary parks. Error bars indicate one standard error around the mean. SEKI has higher concentrations of both pollutants than all other parks except BIBE which has highest ammonium sulfate concentrations. Among the core parks GLAC, ROMO, MORA, and OLYM are not different from each other and DENA is lowest (Tukey-Kramer multiple means comparisons, α = 0.05).

Table 5-16. Rank Correlations among SOC Concentrations in Vegetation (pink), Agricultural Intensity, Mean 1998-2004 Ammonium Nitrate Concentrations in Fine Particulates Measured by IMPROVE (green), and Population Density (gray) for the 20 WACAP Parks. Bold-faced variables were highly significant in both lichen and conifer vegetation.

Conifer Variable	By Conifer Variable	Spearman Rho	Prob >|Rho|	Lichen Variable	By Lichen Variable	Spearman Rho	Prob >|Rho|
Agintensity	**Dacthal**	**0.873**	**<.0001**	**Agintensity**	**Dacthal**	**0.849**	**<.0001**
Agintensity	**Endosulfans**	**0.777**	**<.0001**	**Agintensity**	**Amm_NO_3 µg/m^3**	**0.787**	**<.0001**
Agintensity	**Amm_NO_3 µg/m^3**	**0.758**	**0.0002**	Agintensity	DDTs	0.779	0.0047
Agintensity	PAHs	0.745	0.0003	**Agintensity**	**Endosulfans**	**0.744**	**0.0002**
Agintensity	**Chlordanes**	**0.646**	**0.0038**	**Agintensity**	**Chlordanes**	**0.566**	**0.0116**
Amm_NO_3 µg/m^3	PAHs	0.655	0.0023	Amm_NO_3 µg/m^3	Triflualin	0.762	0.0280
Amm_NO_3 µg/m^3	**Endosulfans**	**0.644**	**0.0029**	Amm_NO_3 µg/m^3	DDTs	0.724	0.0117
Amm_NO_3 µg/m^3	**Dacthal**	**0.623**	**0.0058**	**Amm_NO_3 µg/m^3**	**Dacthal**	**0.700**	**0.0006**
				Amm_NO_3 µg/m^3	**Endosulfans**	**0.630**	**0.0029**
Pop. 150-km radius	**Pop. 75-km radius**	**0.900**	**<.0001**	Pop. 75-km radius	Dieldren	1.000	0
Pop. 75-km radius	**Amm_NO_3 µg/m^3**	**0.721**	**0.0005**	**Pop. 150-km radius**	**Pop. 75-km radius**	**0.914**	**<.0001**
Pop. 75-km radius	Endosulfans	0.698	0.0009	**Pop. 75-km radius**	**Amm_NO_3 µg/m^3**	**0.760**	**0.0001**
Pop. 75-km radius	PAHs	0.671	0.0016	**Pop. 150-km radius**	**Agintensity**	**0.677**	**0.0010**
Pop. 75-km radius	PCBs	0.664	0.0051				
Pop. 75-km radius	**Agintensity**	**0.629**	**0.0039**				
DDTs	**Chlorpyrifos**	**0.943**	**0.0048**	Chlordanes	Dieldrin	1.000	0
HCB	**a-HCH**	**0.874**	**<.0001**	Dacthal	Dieldrin	1.000	0
Endosulfans	**Chlordanes**	**0.864**	**<.0001**	DDTs	Dieldrin	1.000	0
a-HCH	Chlordanes	0.862	<.0001	PCBs	Dieldrin	1.000	0
PAHs	Chlordanes	0.847	<.0001	**Endosulfans**	**Dacthal**	**0.922**	**<.0001**
Endosulfans	**Dacthal**	**0.843**	**<.0001**	**Dacthal**	**Chlordanes**	**0.846**	**<.0001**
PAHs	A-HCH	0.828	<.0001	Dacthal	DDTs	0.818	0.0021
Endosulfans	**DDTs**	**0.821**	**0.0234**	PCBs	Chlordanes	0.798	<.0001
PCBs	DDTs	0.821	0.0234	a-HCH	g-HCH	0.795	<.0001
PAHs	Endosulfans	0.818	<.0001	**Endosulfans**	**Chlordanes**	**0.784**	**<.0001**
Endosulfans	A-HCH	0.807	<.0001	**HCB**	**a-HCH**	**0.773**	**<.0001**
HCB	Chlordanes	0.790	<.0001	**Endosulfans**	**DDTs**	**0.764**	**0.0062**
DDTs	**Chlordanes**	**0.786**	**0.0362**	PCBs	g-HCH	0.721	0.0007
PAHs	Dacthal	0.771	0.0002	PCBs	a-HCH	0.674	0.0016
PCBs	**Endosulfans**	**0.753**	**0.0008**	PCBs	Dacthal	0.663	0.002
Dacthal	**Chlordanes**	**0.738**	**0.0007**	PAHs	a-HCH	0.653	0.0018
PAHs	**HCH**	**0.735**	**0.0003**	PAHs	g-HCH	0.653	0.0025
Chlordanes	Chlorpyrifos	0.700	0.0037	**PCBs**	**Endosulfans**	**0.644**	**0.0029**
PCBs	Chlorpyrifos	0.685	0.0139	**PAHs**	**HCB**	**0.638**	**0.0025**
Endosulfans	HCB	0.672	0.0016				

Human population size within a 75-km radius of WACAP parks correlated most strongly with endosulfan, PAH, and PCB concentrations in conifers (Spearman's Rho 0.66 to 0.70) and with dieldrin concentrations in lichens (Spearman's Rho 1.00). Other radii tested (25, 150, 300) did not predict SOC concentrations as well. Human population size, agricultural intensity, and ammonium nitrate concentrations in ambient fine particulate were all strongly correlated with each other. In the west, the most productive agricultural areas and largest urban areas are often located in the same geographical and climatic zones.

Concentrations of endosulfans were strongly correlated with chlordanes, dacthal, DDTs, and PCBs in vegetation samples, regardless of vegetation type. Chlordanes were strongly correlated with dacthal and DDTs, and HCB was strongly correlated with a-HCH and PAHs in both vegetation types. Many other SOCs were strongly correlated with other SOCs in one vegetation type but not the other. Although correlations do not imply direct or causal relationships between variables, they can serve as indicators of each other. For example, high IMPROVE Amm_NO3 is a fairly good predictor of high dacthal, endosulfan, trifluralin, DDT, and PAH concentrations in conifer needles and/or lichens relative to cleanest sites.

5.6 The Influence of Environmental Factors on Fish Hg_{tot}

Observational and experimental studies show that total mercury (Hg_{tot}) in fish is strongly influenced by watershed and food web characteristics (Wiener et al., 2006), and that the interplay among these variables is complex and varies in unpredictable ways. Thus we are not able to predict Hg_{tot} in fish from an unknown lake, even when we have basic information such as basin characteristics, area of wetlands, TOC in the lake water, and the general structure of the food web. The best we can currently do is to suggest that the top predatory fish in any system are likely to have the highest $Hg_{tot.}$ There appears to be no strong relationship between Hg atmospheric deposition and Hg concentration in fish at the site level. When aggregated at a large scale (i.e., the state level), it has been demonstrated, with important caveats, that wet atmospheric deposition of mercury can account for about two-thirds of the methyl mercury (MeHg) in largemouth bass (*Micropterus salmoides*) (Hammerschmidt et al., 2006). The WACAP data clearly demonstrate this inability to anticipate Hg_{tot} in fish.

Figure 5-30 is a plot of the average fish Hg_{tot} concentration for each WACAP lake against lake water total phosphorus (P_{tot}). Two of the four lakes with the highest Hg_{tot} concentrations in target fish are Arctic lakes. The lake with the highest average Hg_{tot} is Burial Lake; this average value exceeds the USEPA criterion for human consumption. The other WACAP Arctic lake, Matcharak, is the fourth highest of all. These results are surprising for two reasons. First, the sedimentary records show that Hg_{tot} flux to the Alaska lakes in the last 50 years was only about one-fourth of the Hg_{tot} flux observed in WACAP lake sediments in the lower 48 states (see Section 4.3.5). Secondly, it is also true that the Arctic lake food webs tend to have fewer levels and are simpler than those in the lower 48 states; by conventional interpretation, this would lead to less biomagnification of Hg in fish. LP19 (MORA) and Hoh Lake (OLYM) are second and third highest, respectively, with respect to fish Hg_{tot}.

All WACAP lakes, except Burial Lake, are considered to be oligotrophic (water column $P_{tot} < 5$ µg/L); Burial Lake approaches being mesotrophic with water column $P_{tot} = 9$ µg/L (Wetzel, 1983). Since Burial Lake has at least double the concentrations of P_{tot} observed in any other WACAP lake, this could be an important factor contributing to the higher total Hg found in fish. However, several studies have indicated that increased algal primary production can reduce the uptake of MeHg in fresh waters as a result of dilution in greater planktonic biomass (Pickhardt et al., 2002). Only the methyl form of mercury can enter the food web and bioaccumulate. Lake trout (the target fish in both Arctic lakes) have been shown to rely heavily on snails derived from the benthic food web (Hershey et al., 1999). This was confirmed at the time of WACAP sampling in that the gross anatomical evaluation showed stomach contents of fish from the

Arctic lakes comprised mostly snails. Our evidence and the literature suggest that the Arctic food web in which the lake trout reside is quite short and simple: periphyton, snails, and lake trout.

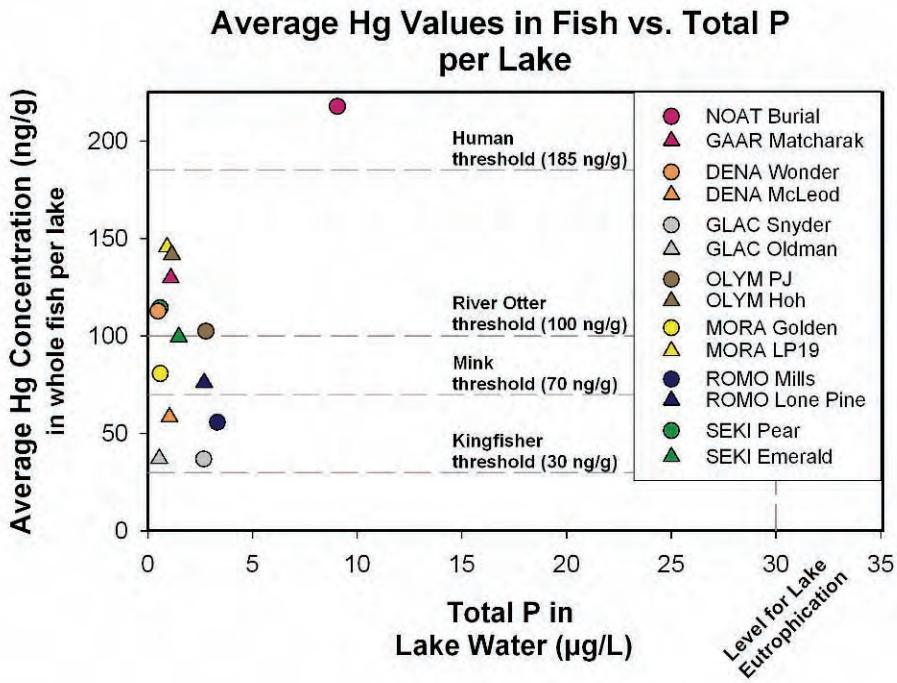


Figure 5-30. Average Total Mercury Values for Whole Fish Plotted Against Total Phosphorus (TP) in the Lake Water for All Lakes in the Core Parks. Notably, Burial Lake was mesotrophic with respect to TP and had the highest TP while also having the highest Hg_{tot}. All other lakes were oligotrophic. The human contaminant health threshold is 300 ng/g wet weight (USEPA, 2001), and is based on methyl-Hg in the fillet for a general population of adults with 70 kg body weight and 0.0175 kg fish intake per day. 95-100% of Hg in fish is methyl-Hg (Bloom, 1992), and 300 ng/g in the fillet is equivalent to 185 ng/g ww whole body methyl-Hg (Peterson et al., 2007). Contaminant health thresholds in piscivorous animals are based on 100% fish in the diet for whole body Hg_{tot} as determined by Lazorchak et al., (2003).

How might the high Hg_{tot} in Arctic fish be explained? One clue might be that the Arctic lakes had the highest DOC of all WACAP lakes (Burial = 3.3 mg/L; Matcharak = 4.7 mg/L). The range of DOC in the lakes in the lower 48 states was 0.65 – 2.25 mg/L (mean 1.3 mg/L). This suggests that there is a greater connection to sources of DOC, such as wetlands, sediment production littoral zones, and melting permafrost zones, than in other lakes. These locations are known and likely sites of mercury methylation (St. Louis et al., 1996) and DOC has been shown to be an important pathway for conveying methyl mercury from sites of methylation (i.e., anoxic, organic rich areas) to lakes. The high P content of the Burial Lake would stimulate periphytic algal growth on the lake sediment surface in the photic zone (depth ≤ ~ 10 m), providing the snail "grazers" with an abundant food source. It is probable that the combination of elevated P and DOC with the importance of the snail/ periphyton food web combined to provide a very efficient mechanism to transport MeHg into fish by using a short but efficient pathway. In other systems, lacking the snail/periphton linkage in an extensive photic zone, mercury reaching the sediment surface via "plankton rain" is typically lost to the accumulating sediment sink and little is conveyed back into the water column.

Another important function of DOC relating to MeHg in lakes and the bioaccumulation in fish is that DOC can attenuate light penetration and thereby decrease photodegradation of MeHg. This dynamic has been shown to be significant in an Arctic lake (Hammerschmidt et al., 2006) and would be likely to occur in Burial and Matcharak Lakes because of the fairly high DOC found there.

We determined that LP19 had the highest Hg flux (current vs. pre-industrial) ratio in lake sediments and that Hoh Lake had the lowest flux ratio of all WACAP lakes (Section 4.3.5), yet we observe that the mean Hg_{tot} in fish for these two lakes is almost identical. Clearly, there are factors other than mercury flux to the lakes, as indicated from the sediment record, responsible for Hg_{tot} concentrations in fish.

One of the best current tools available to scientists to examine the food web structure of an aquatic system and, therefore, to develop a quantitative understanding of the bioaccumulation of Hg_{tot}, is the application of stable isotope techniques (Kidd et al., 1995). Application of this technique to an exploratory observational research project such as WACAP was considered, but because we had no idea of the structure of the fish contaminant data, and of Hg concentrations in particular, it was not deemed to be cost effective. Application of this tool would be useful in deciphering causes behind the high concentrations of Hg_{tot} found in fish from selected WACAP lakes.

5.7 Summary

In this chapter we present an assessment of bioaccumulation in vegetation, fish, and moose; biological effects in fish; potential adverse ecological effects to piscivorous wildlife; and human health risks from atmospheric sources of anthropogenic semi-volatile organic compounds (SOCs), metals, and fixed nitrogen in national parks, preserves, monuments, and wildernesses of the western United States. The principal findings are itemized in the following subsections:

5.7.1 Bioaccumulation

- SOC concentrations were orders of magnitude higher in biota (fish, vegetation) and sediments compared to snow and air. Vegetation tended to accumulate more PAHs, CUPs, and HCHs, and fish tended to accumulate more PCBs and less volatile chlordanes, DDTs, and dieldrin.
- SOC burdens in conifer needles approximately tripled between first and second years.
- Western US coniferous forests have the capacity to accumulate annually in 2nd year needles amounts of pesticides that are comparable on a per ha basis to a significant fraction of regional pesticide application rates, providing an ecosystem service with un-examined ecological consequences. Forest productivity (annual needle biomass production), conifer species, proximity to sources, and application rates at the sources all appear to be important factors in needle concentrations.
- Fish lipid and age were the most reliable predictors of SOC concentrations.
- Whole-body mercury was age-dependent in all fish up to approximately 15 years of age. In lake trout older than 15, mercury was not age-dependent.

- Mean ammonium nitrate concentration in ambient fine particulates < 2.5 µm diameter, sampled at park IMPROVE monitors, was a fairly good predictor (Spearman's Rho correlation coefficient > 0.62) of dacthal, endosulfan, chlordane, trifluralin, DDTs, and PAH concentrations in vegetation.

5.7.2 Adverse Biological Effects Observed in Fish

- Most fish appeared normal during field necropsies. Lake trout from the Arctic had parasite infestations of varying severity but there was no evidence that this was related to contaminant concentrations.
- Kidney and spleen macrophage aggregates, a biomarker of tissue damage, varied considerably but between-site differences were not related to contaminant concentrations.
- Spleen macrophage aggregates were highly correlated with tissue mercury concentrations and age in brook, rainbow and cutthroat trout.
- Fish with both male and female characteristics (ova-testis) were found in ROMO and GLAC lakes. The incidence of intersex has significantly increased since the pre-organic pollutant era (pre-1930s).
- Elevated concentrations of vitellogenin, a female protein involved in egg production, were found in male fish from MORA, ROMO, and GLAC. At ROMO, vitellogenin appeared to be related to the concentration of several organochlorines. Small sample sizes limit the inferences that can be made and suggest that further sampling and analysis of SOC concentrations and vitellogenin might be warranted.

5.7.3 Potential Adverse Ecological Effects

- Mercury concentrations in fish exceeded contaminant health thresholds for some piscivorous mammals and birds in most parks (see Section 5.4.1 for caveats). Concentrations of the sum of the forms of DDT, DDD, and DDE in some fish in GLAC and SEKI exceeded contaminant health thresholds for piscivorous birds.
- Modeling of contaminant and nutrient transport through hypothetical food webs also suggests that contaminants may be adversely affecting life expectancy and abundance in piscivorous wildlife.
- IMPROVE fine particulate monitoring data and lichen nitrogen and sulfur concentrations in WACAP parks indicate that anthropogenic deposition of atmosphere nitrogen and sulfur-containing fertilizing and acidic compounds are enhanced in SEKI, GLAC, and BIBE. Elevated nutrient deposition is associated with adverse effects to sensitive species, community dynamics, and ecosystem processes.

5.7.4 Health Risks to Humans

- Over half (77 of 136) of the individual fish, from 11 of the 14 WACAP lakes, carried concentrations exceeding subsistence fishing contaminant health thresholds for dieldrin and/or p,p′-DDE; thresholds were calculated using USEPA guidelines. Consuming fish exceeding a contaminant health threshold implies an increased risk (by 1 in 100,000) of developing cancer during a lifetime of frequent fish consumption.

- Concentrations of chlorpyrifos, dacthal, endosulfans, methoxychlor, mirex, HCB, a-HCH, g-HCH, chlordanes, heptachlor epoxide, and PBDEs, the other pesticides, and industrial compounds detected in >50% of fish, were 1-7 orders of magnitude lower than contaminant health thresholds for subsistence fishing.
- Risks from recreational fishing consumption were lower than risks from subsistence fishing, but concentrations of dieldrin in five individual fish from SEKI, ROMO, and GLAC exceeded contaminant health thresholds for recreational fishing.
- The average mercury concentration in fish from Burial Lake (NOAT) and in some individual fish from PJ and Hoh lakes (OLYM), LP19 (MORA), and Pear Lake (SEKI) exceeded the USEPA contaminant health thresholds for humans.
- SOC and metal concentrations in the three moose samples, all from DENA, were low and not of concern with regard to human health effects. The DENA moose were nutritionally deficient in copper, which might be of interest to DENA wildlife biologists.