DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION 1

ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

Assessment of Impacts to Aquatic Organisms from Pesticide Use on the Willamette Valley National Wildlife Refuge Complex

by

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List of Acronyms

ANOVA	Analysis of Variance
BEST	Biomonitoring of Environmental Status and Trends
BLM	Bureau of Reclamation
BEECS	Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences
7DADM	7 day average of the daily maximums
2,4-D	(2,4-dichlorophenoxy)acetic acid
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl)ethane
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane
DO	dissolved oxygen
E_2	17β-estradiol
EPA	Environmental Protection Agency
GSI	gonadosomatic index
HPLC	high-pressure liquid chromatography
IPM	integrated pest management
11- KT	11- ketotestosterone
NAWQA	National Water Quality Assessment
NH_3	ammonia
NH_4^+	ammonium ion
NO_2^-	nitrite
NO_3^-	nitrate
NWR	National Wildlife Refuge
OC	organochlorine
ODEQ	Oregon Department of Environmental Quality
OFWO	Oregon Fish and Wildlife Office
P	probability
PCB	polychlorinated biphenyl
PPR	posterior lobe of plastron

List of Acronyms (continued)

PUP	Pesticide Use Proposal
QA	quality assurance
QC	quality control
SRP	soluble reactive phosphorus
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
USGS	U.S. Geological Survey
WRD	Water Resources Division

Keywords
Pesticides, herbicides, nutrients, water quality, William L. Finley National Wildlife Refuge, Willamette Valley National Wildlife Refuge Complex, DEC ID 199710001, FFS 1N33, Oregon Congressional District 4, common carp, *Cyprinus carpio*, western pond turtle, *Clemmys marmorata marmorata*, estrogen testosterone, hormone.

ABSTRACT

The primary habitat management objective for the three refuges of the Willamette Valley NWR Complex is to provide high quality forage for wintering Canada geese. To accomplish this, much of the land within the refuge is managed for grass production involving applications of herbicides, fungicides, and fertilizers. Other agrochemicals such as insecticides are applied to agricultural land outside the refuge. Some agrochemicals used in the area have the potential to enter aquatic habitats of the refuge and could impact species such as amphibians, turtles, or the federally-listed Oregon chub. The objective of this investigation was to sample both biotic and abiotic matrices and use a weight-of-evidence approach to determine if agrochemicals used on or around the refuge pose a risk to aquatic species.

This investigation included seven components: 1) evaluation of pesticide use practices; 2) collection of continual water quality measurements; 3) water sampling; 4) *in-situ* bioassay; 5) analysis of blood plasma from carp and turtles for endocrine-disrupting compound exposure; 6) fish health assessment; and 7) fish tissue analysis for organochlorine pesticides. The components of the study were used as individual lines of evidence to evaluate risk of agrochemicals to aquatic organisms on the refuge. Multiple lines of evidence create more confidence in making decisions suitable for refuge management because the approach considers all the information gathered from the investigation. By framing the assessment with this weight-of-evidence approach, risk to the receptors of concern and organizational levels is more clearly evident and the information can be used in carrying out the Fish and Wildlife Service mission to protect and conserve natural resources.

The pesticide monitoring component of this study indicated that there could be effects to aquatic communities from exposure to atrazine and chlorpyrifos. The greatest potential impact is at Brown Creek which reflects a source of pesticides outside the refuge while Muddy Creek pesticide concentrations indicate sources from both on- and off-refuge.

Nutrient monitoring indicated that several forms of nitrogen and phosphorus exceeded aquatic life criteria in three creeks sampled with the greatest potential for impact at Gray Creek (Cattail Pond), Brown and Muddy Creeks. Sources of nutrients appear to be from both on- and off-refuge.

The hormone values measured in biotic samples from refuge sites were within normal ranges except for western pond turtles at Finley National Wildlife Refuge, where higher testosterone values were observed in females and the female hormone ratio was atypically low compared to reference turtles. This indicates exposure to some type of antiestrogenic compound that blocks the conversion of testosterone to estrogen. All other parameters measured (health, histopathology, and analytical concentrations) were similar to reference values or considered within normal ranges. Only the two non-refuge sites showed both elevated contaminants in tissue and abnormal hormone results.

The weight-of-evidence risk assessment indicates that the receptors of greatest concern at the refuge are amphibians and aquatic life. Nutrient concentrations could affect aquatic life at nearly all locations sampled on the refuge based on extent of exposure, although the greatest potential impact from both pesticides and nutrients is at Brown Creek reflecting a source outside the refuge.

The results of this study indicate that there are numerous sources of pesticides and fertilizers in and around the refuge, and some of these agrochemicals are entering waterways important to the refuge. The pesticides of most serious concern originate outside the refuge, and refuge personnel have limited ability to manage pesticide application occurring off-refuge lands. However, we recommend several management actions be taken on the refuge to help minimize the pesticides and nutrients entering waterways and to further assess the health of western pond turtle populations.

GENERAL INTRODUCTION

The Willamette Valley National Wildlife Refuge Complex consists of three National Wildlife Refuges (NWRs), William L. Finley (Finley), Ankeny, and Baskett Slough (Figure 1). A variety of wetland, forest, and prairie habitat types occur on these three refuges of the lower Willamette Valley that support numerous species of large and small mammals, migratory birds, anadromous fish, and several threatened or endangered species, including the bald eagle (*Haliaeetus leucocephalus)*, Oregon chub (*Oregonichthys crameri*), Fender's blue butterfly (*Icaracia icarioides fenderi*), Kincaid's lupine (*Lupinus sulphureus kincaidii*), Willamette daisy (*Erigeron decumbens decumbens*) Bradshaw's lomatium (*Lomatium bradshawii*), and Nelson's checker mallow (*Sidalcea nelsoniana*).

A primary habitat management objective for the three Willamette Valley refuges is to provide high quality, palatable forage for wintering Canada geese (*Branta canadensis*). To accomplish this, the majority of land on the refuges is cultivated farmland managed for grass production (annual ryegrass, perennial ryegrass, fescue, timothy, birdsfoot trefoil, and a grass and clover mix) with a minor amount in corn and oat production. The Willamette Valley's is the major producer of cool season grasses in the United States (Oregon Agricultural Statistics Service 2004). Refuge plots are cooperatively farmed by local grass seed farmers with the farmers conducting all aspects of the agricultural operations. Standards for grass seed certification developed by the turf industry require very strict weed control, which used to be conducted primarily with fall field burnings. Public concerns about air quality drastically reduced this practice (Jenkins et al. 1994), which led to increased use of fungicides and herbicides to control or eliminate any undesirable plant species or fungus (Jenkins et al. 1994). Managing for grass production also typically involves repeated applications of chemical fertilizers. The refuges also receive drainage from surrounding croplands, dairies, and timberlands where agricultural chemicals (insecticides, herbicides, fungicides, and fertilizers) are widely used. Chemicals used in the area have the potential to enter aquatic communities on the refuge and affect species of special concern such as northern red-legged frog (*Rana aurora aurora*) and western pond turtle (*Clemmys marmorata marmorata*), as well as listed species like Oregon chub. A proposed delivered water system which would bring additional irrigation water to both surrounding agricultural lands and refuge lands is also of concern to the refuge complex. Additional irrigation water could allow farmers to diversify their crops resulting in changes in pesticide use. Herbicides and fungicides are currently the primary pesticides used in the area and on the refuge, although insecticides are used in some areas on row crops outside refuge lands. Crop diversification may result in increased use of insecticides in the area.

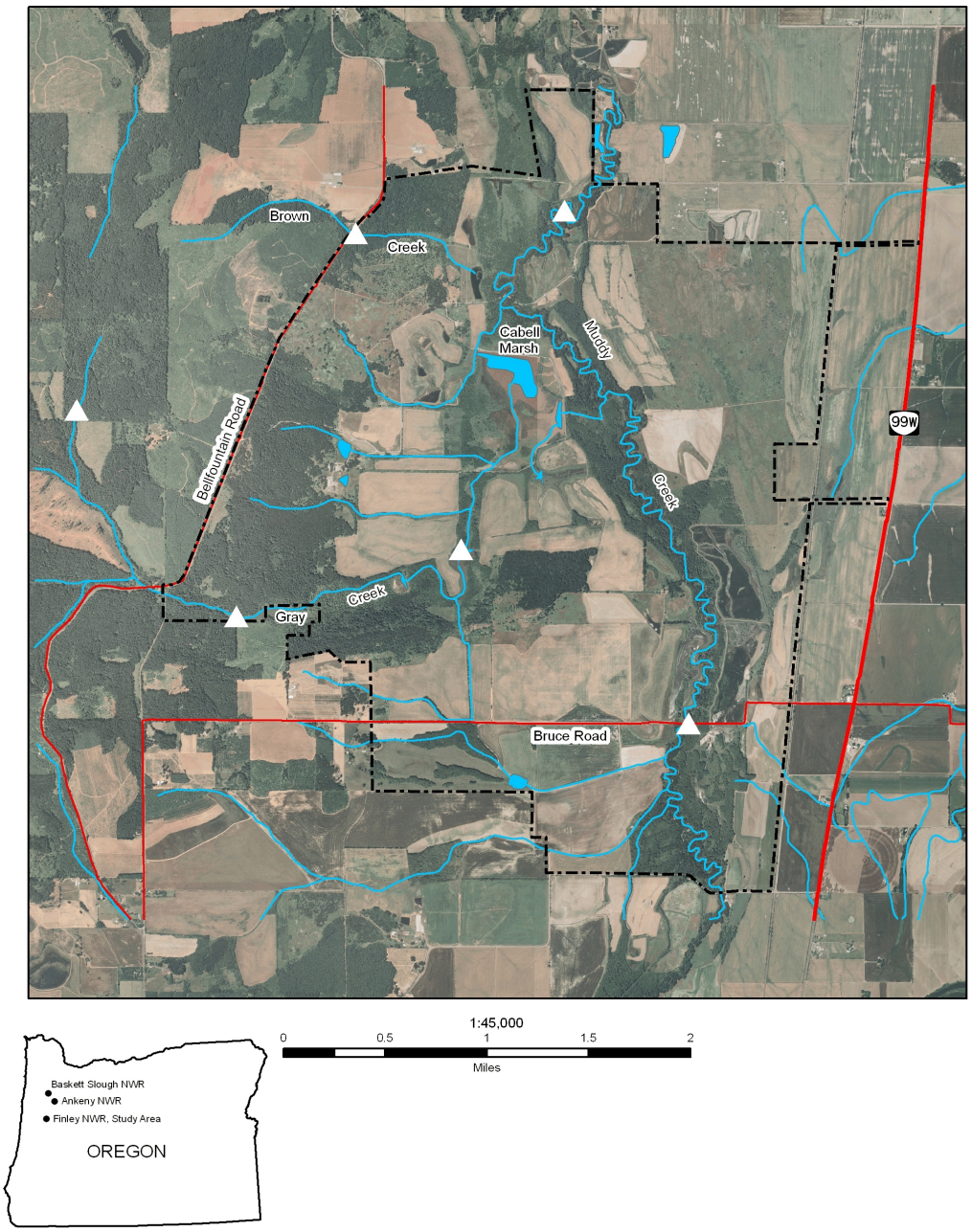


Figure 1. Agricultural land uses associated with Finley NWR. Black dashed line indicates refuge boundary; red lines indicate roads; and white triangles indicate sample site locations.

A variety of herbicides and fungicides are approved for use on the refuge lands (Table 1). The U.S. Geological Survey's (USGS) National Water Quality Assessment Program (NAWQA) has documented a variety of pesticides in surface waters associated with similar agricultural uses in the Willamette Basin. These pesticides include metalochlor, carbaryl, carbofuran, diazinon, diuron, atrazine, and 2,4-dichlorophenoxyacetic acid (2,4-D) (Anderson et al. 1997). Two of these pesticides detected in surface water (2,4-D and diuron) are included in Table 1 as approved for use on the refuge

The fungicides and herbicides used on the refuge or nearby may be acutely toxic to many aquatic organisms if the chemicals enter the aquatic system in sufficient concentrations. In the spring of 1993, a large fish kill of approximately 8,000 sticklebacks (*Gasterosteus aculeatus*) was reported on the Baskett Slough NWR by refuge staff. The episodic nature of the fish kill was suggestive of pesticide poisoning, but the true cause of the kill remains unknown.

A number of fungicides and herbicides used on or around the refuge currently or in the past are suspected or documented endocrine disruptors (Colborn et al. 1993, Keith 1997). These chemicals, such as 2,4-D and many triazine herbicides, can act as exogenous estrogens (reproductive hormones originating outside the organism) or antiestrogens in the endocrine system. Studies on alligators, turtles, fish and other species have revealed permanent and irreversible reproductive impacts from exposure to exogenous estrogens and endocrine-disrupting compounds (Colborn et al. 1993, Guillette et al. 1994, National Wildlife Federation 1994, Kime 1998, Sumpter and Johnson 2005). Development of the endocrine system and the organs that respond to endocrine signals can be disturbed by indirect exposure to exogenous estrogens and endocrine-disrupting compounds during prenatal or early postnatal life, or by direct exposure of the offspring after birth or hatching (Colborn et al. 1993). Persistent endocrine-disrupting chemicals can also be released from body fat and mobilized, such as during egg laying, and expose across generations (Colborn et al. 1993, Kime 1998).

The impact of endocrine-disrupting chemicals on western pond turtles and fish on the refuge is of particular concern. Recently, female common carp (*Cyprinus carpio*) from several streams in the Willamette Valley revealed low estrogen/testosterone ratios, indicating exposure to endocrine-disrupting compounds (Goodbred et al. 1997). Exposure to estrogen-disrupting compounds could impact reproduction of fish species such as the Oregon chub, whose populations have declined in the Willamette Valley even though suitable habitat exists (U.S. Fish and Wildlife Service 1998, Scheerer et al. 2003). Western pond turtle populations in the Willamette Valley have declined precipitously over the past several decades (Holland 1991), and studies on winter and summer distribution and movements of western pond turtles at Finley NWR found that this population is heavily adult biased, indicating low recruitment (Loegering 1998).

Table 1. Common and trade names[1] and select properties of chemicals approved for use on the Finley National Wildlife Refuge.

Trade Name	Common Names	General Uses[2]	Average hydrolysis half-life[3] (days)	Acute Toxicity Range[4]
2,4-D	2,4-D	control of broadleaf weeds and brush in cereal crops, pasture, forest management, turf and grass seed crops	39	practically nontoxic to moderately toxic
MCP Amine 4	MCPA	control of annual and perennial weeds in grasslands, grass seed crops, and forestry applications	not reported	practically nontoxic to moderately toxic
Karmex	diuron	control of broadleaf and grass weeds and mosses in grasslands/pasture and fruits	1,285	slightly to moderately toxic
Rodeo	glyphosate	control of annual and perennial weeds and woody species around aquatic sites and non-crop areas	not reported	practically nontoxic to moderately toxic
Roundup	glyphosate	control of broadleaf weeds and woody plants in cereal and grains, pastures, and grass seed crops	not reported	practically nontoxic to moderately toxic
Crossbow	triclopyr and 2,4-D	broadleaf and woody plant weed control in pastures and grasslands	not reported	moderately toxic[5]
Tilt	propiconazole	fungicide for use in cereals, corn, and grass seed crops	442	moderately toxic
Sencor	metribuzin	control of broadleaf and grass weeds in alfalfa, cereals, grasses, and turf	4,760	practically nontoxic to slightly toxic
Nortron	ethofumesate	control of broadleaf and grass weeds in grass seed crops and other crops	2,900	slightly to moderately toxic
Banvel	dicamba	control of broadleaf weeds and wood brush species in grain crops, pasture, non-cropland, turf and grass	30	practically nontoxic to slightly toxic

Table 1. Continued.

[1] Based on the Farm Chemicals Handbook (Meister 1995).
[2] General uses gathered from The Extension Toxicology Network (1998), Pesticide Action Network (2005), Farm Chemicals Handbook (Meister 1995), and/or chemical-specific labels (Crop Data Management Systems, Inc. 2007).
[3] Time required for half the pesticide to degrade from reaction with water, as reported by Pesticide Action Network (2005).
[4] Toxicity range reported for fish by the Pesticide Action Network (2005) as classified according to the following toxicity categories (LC50 in µg/L): very highly toxic <100; highly toxic 100-1,000; moderately toxic 1,000-100,000; slightly toxic 10,000-100,000; and practically nontoxic >100,000.
[5] Toxicity for triclopyr only.

Finley NWR was selected for this investigation because it is the largest refuge within the Willamette Valley NWR Complex and has the most cultivated land. In addition, it has the endangered Oregon chub and two species of concern, red-legged frog and western pond turtle. Data gathering for this investigation was conducted over a 2-year period. The first year involved collection of background information characterizing pesticide applications on the refuge and in areas adjacent to the refuge. The second year of the study involved all the field sampling, including collection of water samples for detection of fungicides/herbicides and nutrients, water quality measurements, *in-situ* bioassays, and blood collection from carp and turtles for sex steroid hormone analysis.

The purpose of this investigation was to determine if pesticides occur or enter and move through refuge aquatic habitats and to assess the potential impacts of pesticides on fish or other trust resources on the refuge. Specifically, the scientific objectives were as follows:

1. Determine pesticide use practices (type of pesticides, application rates, and period of use) for refuge lands and surrounding areas;

2. Determine if agrochemicals used on or off refuge lands are present in refuge aquatic habitats;

3. Evaluate seasonal trends in water quality;

4. Evaluate acute toxicity of water during pesticide application using an *in-situ* bioassay;

5. Investigate endocrine-disrupting compounds in common carp and western pond turtles by comparing differences in hormone levels between agricultural and non-agricultural sites;

6. Assess the health of common carp between agricultural and non-agricultural sites; and

7. Determine organochlorine pesticide concentrations in carp from historic use of “legacy” pesticides.

This study applied the following procedures to address our objectives: collection of pesticide-use information; collection and analysis of water samples for agrochemicals (pesticides and nutrients); measurement of water quality parameters; assessment of pesticide impacts through *in-situ* bioassays; analysis of blood plasma sex steroid hormone levels in common carp and western pond turtles; assessment of fish health; and analysis of fish tissue for historic organochlorine pesticide residues. The techniques used in this study allow for multiple species assessment of the potential acute or chronic and reproductive impacts of pesticides used on and around the refuge. The various procedures used in this study provide measurement endpoints that are used to evaluate the level of risk to species on the refuge and assist the refuge in formulating management and conservation strategies to limit pesticide exposure to fish and wildlife dependent on these lands.

Table 2 outlines the foundation for evaluating the stressors to aquatic life on the refuge through a weight of evidence approach. Each step of the evaluation is designed to link sources and pathways to measurements of exposure and effects to increase the confidence used in decision making for managing the refuge. Measurement endpoints are each considered a line of evidence which may vary in confidence and can be used to either support or negate other lines of evidence.

Table 2. Stressors, pathways, and measurement endpoints used to assess exposure of aquatic receptors to agrochemicals used on or near the Finley National Wildlife Refuge.

Stressor/ Source	Pathway	Sample Medium/Receptor	Measurement Endpoint
Fertilizers	Surface water runoff	Surface water	Nutrient analysis Fathead minnow bioassay (survival) Fish health assessment
Pesticides Endocrine-disrupting chemicals	Surface water runoff	Surface water Carp Turtle plasma	Pesticide analysis (water) Fathead minnow bioassay Plasma hormones Histopathology (carp) Fish health assessment
Lipophilic insecticides Endocrine-disrupting chemicals	Water and food chain transfer	Whole body carp	Tissue analysis for organochlorine pesticides and PCBs

Information regarding current pesticide practices associated with the refuge and concentrations of pesticides and nutrients in water provides pertinent data for evaluating the contaminant pathway or availability of the pesticides and nutrients to aquatic organisms. Measurements of water quality parameters provide information on the current state of the aquatic habitats, which can strongly influence the response of aquatic organisms to pesticides. *In-situ* fish bioassays were intended to serve as an indicator of fish exposure to toxic concentrations of agrochemicals. Measurement of blood plasma hormone levels in carp and turtles serve as an indicator of

pesticides possibly acting as endocrine disruptors and altering reproduction and other important functions in animals. Fish health assessments were conducted to understand the overall condition of fish. Analysis of legacy pesticide concentrations in carp tissue identifies persistent pesticides that may potentially impact aquatic populations.

This report is organized into two chapters, and further divided into sections within each chapter according to the measurement endpoints identified in Table 2. Each section introduces the endpoint and presents the methods, results, and discussion for evaluating that endpoint. Chapter 1, entitled *Sources and Pathways*, includes sections on information gathering and monitoring of pesticides, nutrients, and water quality. Chapter 2, *Exposure and Effects*, includes sections on the *in-situ* bioassays, endocrine biomarkers, fish health assessment, and fish tissue chemistry. The report is concluded with a summary and management recommendations.

Some of the data collected for this report (Chapter 1) were compiled and analyzed by refuge staff or contracted out to independent biologists and the USGS. Separate reports have been prepared by each of these sources and are summarized within the applicable section in Chapter 1. Each independent report is provided in full as an appendix.

CHAPTER 1

SOURCES AND PATHWAYS

Introduction

To evaluate the possible effects of pesticides to aquatic organisms inhabiting the refuge, it is necessary to identify the types of pesticides being used on and around the refuge and determine if those chemicals are being found in aqueous systems. In addition, it is important to ascertain the ambient water quality conditions which may influence a chemical reaction or an organism's response to a pesticide. The first three study objectives, repeated below from the *General Introduction*, were designed to answer these questions.

1. Determine pesticide use practices (type of pesticides, application rates, and period of use) for refuge lands and surrounding areas;

2. Determine if agrochemicals used on or off refuge lands are present in refuge waterways; and

3. Evaluate seasonal trends in water quality.

Sections of the study designed to address these objectives are entitled Information Gathering, Pesticide Monitoring, Nutrient Monitoring, and Water Quality Monitoring.

Information Gathering

Introduction

To identify potential risk from agricultural chemicals to aquatic organisms on the refuge, a background search and review of pesticide use was conducted. This information was compiled by Brunkal (1997) and a complete report entitled, *Potential Environmental Contaminants on William L. Finley National Wildlife Refuge, Corvallis, Oregon* is included as Appendix A. The information in this report provided direction for the sampling design, most notably in selecting dates for water analysis and *in-situ* bioassay placement. The report is summarized briefly below.

Methods

Information was gathered during the spring of 1997 from 1) refuge records and refuge personnel, 2) county extension agents, 3) interviews with refuge cooperative farmers, and 4) interviews with private landowners. Pertinent information gathered for this survey included the type, amount, and application method for agricultural chemicals. The Willamette Valley NWR Complex maintains an Integrated Pest Management (IPM) Plan which contains Pesticide Use Proposals (PUP) for chemical pesticides proposed for use on refuge lands. Information obtained from

PUPs was also used for this study component, as were resource publications from Oregon State University Extension Service.

Results and Discussion

In the area of the Willamette Basin where the refuge is located, grass seed crops dominate and make up the majority of cultivated refuge land (Brunkal 1997, Appendix A). The use of particular herbicides, fungicides, or insecticides varies depending on the presence of pest species. It is difficult to predict what species may occur during any particular growing season, therefore, information presented here is considered typical of a particular crop in the Willamette Valley. Dates are somewhat variable depending on life history of the particular crop or pest being managed and other environmental influences. Table 3 was compiled based on information in Appendix A, and summarizes the approximate timing and chemicals being applied on or near the refuge.

Pesticide Monitoring

Introduction

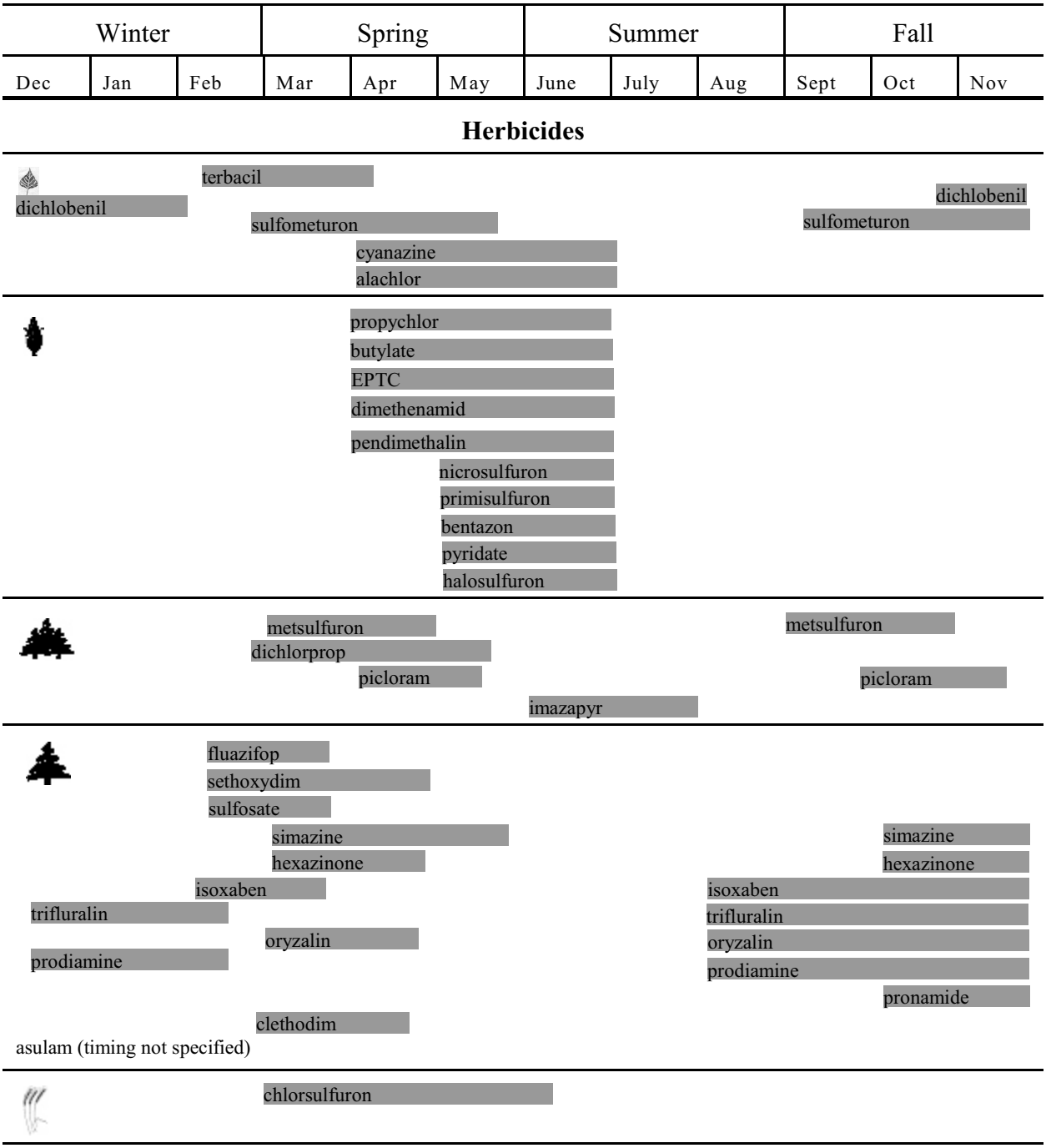
As of 1999, Oregon's chief industry was agriculture (Uhrich and Wentz 1999) and the State leads the world in production of high quality grass seed and Christmas trees (Oregon Agricultural Statistics Service 2003). Within the Willamette Basin, most of the agricultural land is located in the Willamette Valley (Bonn et al. 1995), as are the refuges. Farming operations, including the use of agricultural chemicals, have occurred on the refuge since it was established in 1964. It is also likely that chemicals were used on this area prior to 1964 (Chris Seal, Biologist, William L. Finley NWR, Corvallis, Oregon, pers. comm., 2003).

The primary purpose of monitoring pesticides at Finley NWR was to assess the risk of aqueous exposures to fish and wildlife resources on the refuge. The pesticide use survey indicated that there were numerous agricultural chemicals applied at various times of the year. This information provided the basis for our selection of sample collection times.

Anderson et al. (1997) found that in general, concentrations of most pesticides exhibit seasonal patterns which are lowest in the summer during low-flow conditions and highest in either the spring or the fall, coincident with higher stream flows and initial fall runoff periods. Based upon their conclusion, this study was designed to determine if there are seasonal fluctuations in pesticide concentrations at Finley NWR.

Table 3. Approximate application timing of pesticides used on various crops grown within and near the Finley National Wildlife Refuge. Crop symbols defined at bottom of table.

Winter			Spring			Summer			Fall		
Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov
Herbicides											
glyphosate											glyphosate
2,4 D											
			dicamba						dicamba		
			clopyralid						clopyralid		
			oxyfluorfen								oxyfluorfen
diuron									diuron		
triclopyr											
						bromoxynil					bromoxynil
			MCPA						MCPA		
oryzalin trifluralin paraquat			atrazine			paraquat			atrazine oryzalin trifluralin paraquat		
											metalochlor metribuzin
		ethofumesate							ethofumesate		
			tribenuron methyl fenoxaprop						tribenuron methyl		


Winter
Spring
Summer
Fall
Dec
Jan
Feb
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Herbicides
terbacil
dichlobenil
dichlobenil
sulfometuron
sulfometuron
cyanazine
alachlor
propychlor
butylate
EPTC
dimethenamid
pendimethalin
nicrosulfuron
primisulfuron
bentazon
pyridate
halosulfuron
metsulfuron
dichlorprop
picloram
metsulfuron
picloram
imazapyr
fluazifop
sethoxydim
sulfosate
simazine
hexazinone
isoxaben
trifluralin
oryzalin
prodiamine
clethodim
simazine
hexazinone
isoxaben
trifluralin
oryzalin
prodiamine
pronamide
asulam (timing not specified)
chlorsulfuron

Winter			Spring			Summer			Fall		
Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov
Insecticides											
			malathion methyl parathion								
				ethoprop chlorpyrifos fonofos							
					Lorsban 4E						
									metaldehyde		
Fungicides											
				propiconazole							
				Baytan							
					Benlate 50 WP chlorothalonil						
Fertilizers											
	nitrogen							NPK nitrogen		NPK nitrogen	

Grass seed

Christmas trees

Forestry

Corn

Hybrid cottonwood

Oats

Methods

Site Selection. Selected sampling sites included five refuge stream segments directly influenced by agricultural runoff water and one reference site not influenced by agricultural runoff. The sites were located on three streams that run through the refuge, Muddy, Gray, and Brown Creeks (Table 4, Figures 1 and 2). Photographs of each sampling site are included in Appendix B. A local reference site representing a stream typical of the lower elevation landscapes of the Willamette Basin was not located because most streams in the area are influenced to some extent by agriculture. Instead, we selected a reference site on the upper segment of Gray Creek which differs in its physical characteristics from typical Willamette Valley streams and other study sites in that it originates in a steep forested area, has a streambed of primarily cobbles, and is much narrower in channel width. However, this segment is surrounded by timber with no adjacent agricultural land and inputs of agricultural chemicals are limited. Gray Creek enters the refuge at its southwestern boundary and the reference site was located approximately 1 mile upstream of the refuge boundary on land managed by the U.S. Bureau of Land Management.

The segment of Gray Creek at Beaver Pond was located approximately 0.5 miles downstream of the refuge boundary (Figure 2), where a beaver dam and low-lying terrain create an area of slow, diffuse stream flow with substantial aquatic vegetation. Refuge staff refer to this site as upper Gray Creek Swamp and it should not be confused with the Beaver Pond on Gray Creek that is immediately upstream of Cattail Pond. This site was chosen to characterize the water quality of an off-refuge source as it flows through a marsh habitat. Another segment on Gray Creek (Gray Creek at Cattail Pond) was located near the center of the refuge and was chosen as a sample site to characterize outflow from the pond, which could contain agrochemicals used on the refuge (Figures 1 and 2). Some restoration and dike maintenance activities conducted by refuge personnel had been initiated at Gray Creek at Cattail Pond (primarily near the outflow) prior to our first sampling event, but activities did not continue until after study completion.

Muddy Creek is the largest stream on the refuge and serves as the ultimate surface-water drain for the area (Figure 2). The creek flows through an agricultural area before entering the refuge at its southern boundary, and the site on Muddy Creek at Bruce Road was selected to characterize agriculturally-impacted inflow to the refuge (Figure 2). Muddy Creek exits the refuge at the northern boundary, and the Muddy Creek at North Bridge site was chosen to represent the integrated surface-water outflow from the refuge.

Brown Creek enters the refuge at the northwestern boundary after flowing near a Christmas tree farm and forested area (Figure 2). The sample site on Brown Creek at Bellfountain Road was selected to characterize refuge inflow impacted by Christmas tree farming practices.

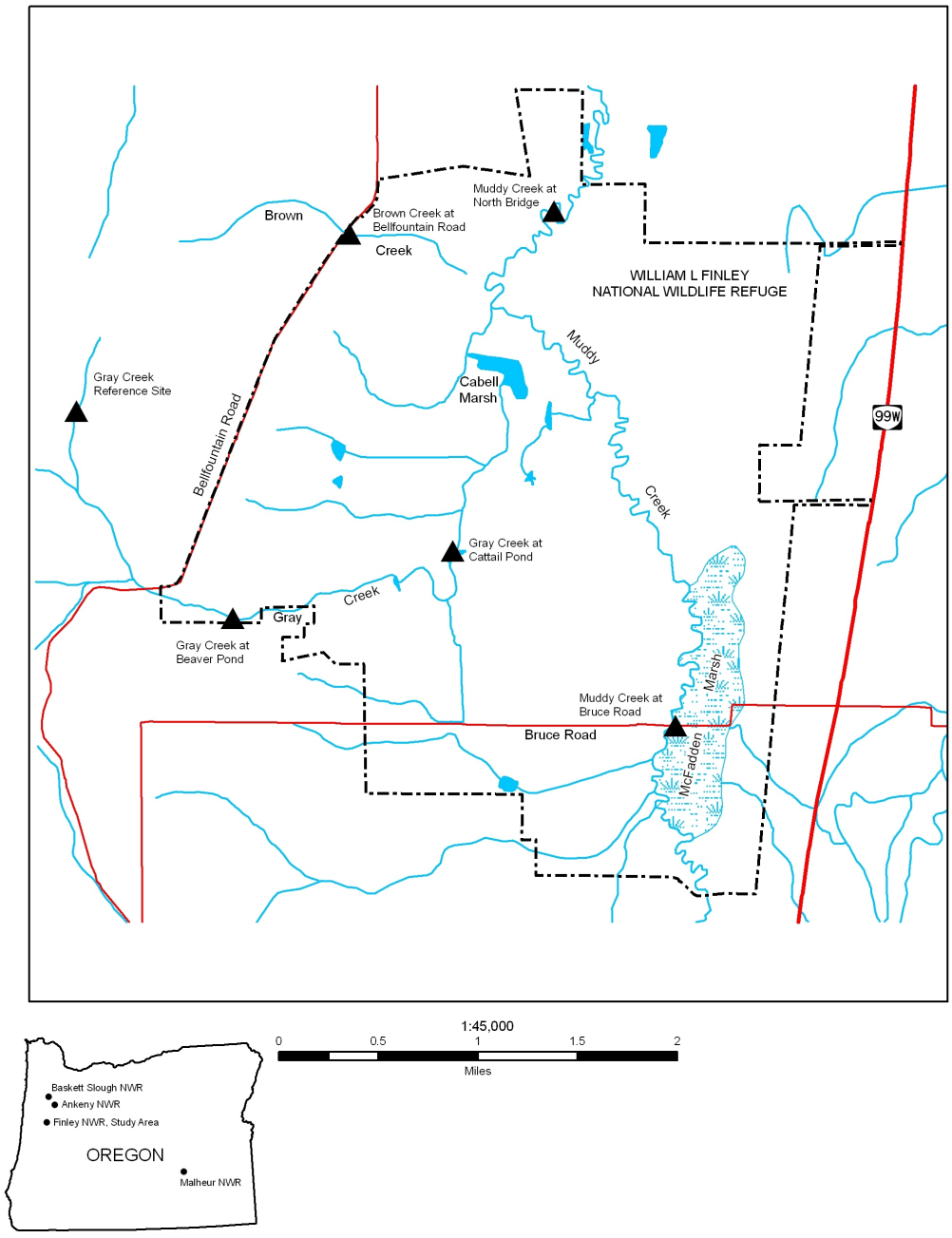


Figure 2. Sample collection sites at the Finley National Wildlife Refuge.

Table 4. Stream segments selected for monitoring at Finley National Wildlife Refuge, 1998.

Site Name	Latitude	Longitude
Gray Creek Reference Site	44° 24' 44"	123° 21' 36"
Gray Creek at Beaver Pond[1]	44° 23' 50"	123° 20' 43"
Gray Creek at Cattail Pond	44° 24' 08"	123° 19' 25"
Brown Creek at Bellfountain Road	44° 25' 30"	123° 20' 01"
Muddy Creek at Bruce Road	44° 23' 22"	123° 18' 07"
Muddy Creek at North Bridge	44° 25' 31"	123° 18' 47"

[1] This site is identified by the Finley NWR as Gray Creek Swamp and should not be confused with Beaver Pond on Gray Creek immediately upstream of Cattail Pond. Samples for this study were collected from Gray Creek Swamp in an area backed up by a beaver dam.

Water Sample Collection, Preparation, and Handling. We collected water samples for pesticide analysis six times during the winter, spring, summer, and fall of 1998 and these sampling occurrences are termed monitoring events. The objective of this sampling was to monitor water concentrations to detect seasonal changes. The six monitoring events occurred on February 10, March 4, March 24, April 22, August 18, and November 9. All six sites (Table 4) were sampled during each event except in August, when two sites (Muddy Creek at Bruce Road and Gray Creek at Cattail Pond) were excluded due to funding limitations. Two additional collection events occurred at four sites in the spring to evaluate pesticide concentrations entering refuge waters in association with storm runoff events. These samples were collected on March 21 and April 24 during or immediately following rain events (rain event samples). On March 21, all sites were sampled except the Muddy Creek at Bruce Road and Gray Creek at Beaver Pond sites. On April 24, all sites except Muddy Creek at North Bridge and Gray Creek at Beaver Pond were sampled. The March 24 monitoring event occurred within days of the March 21 rain event sample and rain continued in this intervening 3 days.

Two unplanned pesticide samples were collected to investigate if pesticide concentrations were detectable in the water following a die-off of native sticklebacks. The fish were observed dead in minnow traps on September 28 during an in-stream fish assessment in the vicinity of the Gray Creek at Beaver Pond site (Scheerer et al. 1999). The two samples were collected on October 1, approximately 100 m upstream and downstream of the trap placement, at sites identified as Gray Creek below Beaver Pond and Gray Creek above Beaver Pond (Appendix C, Table 7).

Water samples were collected in chemically-cleaned, 1-L amber glass bottles with Teflon-lined caps. Water samples were collected according to USGS methods (Shelton 1994). Bottles were submerged, either by hand or in a weighted bottle sampler attached to a hand line (rope). Sample collection was depth integrated by lowering and raising the bottles from the surface to the bottom at a constant rate, and width integrated by repeating this process at three locations across the

width of the channel (Edwards and Glysson 1999). Bottles were lowered in the weighted sampler from a bridge or by hand in smaller wadable streams (collection occurred upstream of the wading point).

Water samples were stored in a cooler with ice during transport to the Finley NWR headquarters for filtering within 2 hours of collection. Samples for pesticide analysis were filtered to remove suspended particulate matter with glass-fiber filters (0.7 µm pore size) set in an aluminum pancake filter following USGS methodology (Sandstrom 1995). Filtered samples were transported on ice to the Oregon Fish and Wildlife Office (OFWO) laboratory in Portland, Oregon, and stored at 4 °C overnight prior to processing at USGS, Water Science Center laboratory in Portland, Oregon. Equipment cleaning occurred at the USGS laboratory following procedures described by Shelton (1994) for the USGS NAWQA program.

Analyte Extraction, Resolution, and Determination. Standard techniques involving liquid to liquid extraction for evaluating pesticides in water samples are often limited due to insufficient detection limits, poor recoveries of some pesticides, or incomplete analyte lists that exclude important degradation products. For this study, we used the same methodology developed by the USGS for their NAWQA program to gain improved detection limits for pesticides and to obtain results comparable to NAWQA studies conducted elsewhere in the Willamette Basin. The method incorporates use of solid-phase cartridges to extract analytes from water samples in the field, which greatly improves detection and increases recovery of pesticide analytes over standard liquid-liquid extraction techniques (Zaugg et al. 1995). This method has been extensively used by the NAWQA program, and sampling and extraction protocols are well developed and field tested (Sandstrom 1995, Zaugg et al.1995, Lindley et al. 1996, Werner et al. 1996). The information gained using solid phase extraction compared to standard methods is critical to accurately assess potential impacts of endocrine-disrupting compounds, as their effects can occur at very low concentrations.

Analyte extraction occurred at the USGS Water Science Center laboratory and consisted of transferring compounds from the filtered water sample onto solid phase C-18 cartridges containing octadecyl-bonded porous silica (Zaugg et al. 1995) and Carbopak-B graphitized carbon-based cartridges (Werner et al. 1996) under controlled flow conditions. The solid-phase extraction occurred within 48 hours of sample collection, and the cartridges were then stored at -20 °C until overnight shipment to USGS National Water Quality Laboratory in Lakewood, Colorado. At the Lakewood Laboratory, adsorbed analytes were eluted from the C-18 solid phase cartridges using hexane-isopropanol at a 3:1 ratio. Analytes were resolved and determined by capillary-column gas chromatography/mass spectrometry with selected ion-monitoring of three characteristic ions (Zaugg et al. 1995, Lindley et al. 1996). Reported method detection limits range from 0.001 to 0.018 µg/L. Analytes were eluted from the Carbopak-B cartridges using methylene chloride and methanol to remove the adsorbed base and neutral compounds in the first fraction of the elution, and methylene chloride and methanol with trifluoroacetic acid to remove the acidic compounds in the second fraction. Analytes eluted from the Carbopak-B

cartridges were resolved by high pressure liquid chromatography (HPLC) and identified by photodiode-array detection (Werner et al. 1996). Reported method detection limits range from 0.006 to 0.032 µg/L. A complete list of the pesticides and degradation products analyzed and analyte-specific method detection limits are included in Appendix C, Table 6.

Quality Control (QC). The field QC sampling plan followed NAWQA program recommendations as described by Shelton (1994) and included duplicate samples, field equipment blanks, field-matrix spikes, and surrogate samples. Duplicate samples were collected to assess sampling and processing variability and analytical precision; one duplicate sample was collected during each monitoring event (Appendix C, Table 3). Four field blanks were collected to quantify contamination from sample handling and processing and consisted of organic-free blank water processed in the same manner as field-collected samples (Appendix C, Table 3). Field matrix spikes were used to evaluate the efficiency of analyte extraction as well as analytical recovery and precision. A matrix spike solution was added to a sample after filtration and before extraction; one sample was spiked for each monitoring event (Appendix C, Table 2). In addition, a surrogate solution consisting of a series of organic compounds in known concentrations was added to all samples after extraction at the USGS Water Science Center laboratory to assess analytical recovery and precision (Appendix C, Table 4). The surrogate compounds added to the samples would not be expected in the samples, but are chemically similarly to the select targeted analytes (Rinella and Janet 1998).

Results

The pesticide data collected in water at Finley NWR during our study were compiled by McCarthy (2001) in the report entitled *Pesticides and Nutrients in Surface Water on the William L. Finley National Wildlife Refuge, Oregon, 1998*. The complete report is included as Appendix C. A summary of pesticide detections is provided in Table 5, and detailed results of the pesticide concentrations are presented in Table 7 of Appendix C. Of the 83 pesticides investigated during this study, 17 were detected at least once in collected water samples. Atrazine and its degradation product, deethylatrazine, were the most frequently detected compounds, found in 76 and 67% of samples, respectively (Table 5). Atrazine was detected at the reference site during a rain event sampling on March 21. At other sites, atrazine or deethylatrazine were detected throughout the year during both rain and monitoring sampling events. The only other compounds that were detected in more than 10 samples (>25% detections) over the course of the study were diuron, metolachlor, and simazine. The number of pesticides detected, the frequency of detection, and the concentrations measured were lowest at the reference site and generally increased from Gray Creek to Brown Creek and highest in Muddy Creek. Comparisons of pesticides in Muddy, Brown, and Gray Creeks (Figure 3 of Appendix C) show that for many of the most frequently detected pesticides, the highest concentrations occurred in Muddy Creek.

Table 5. Summary of maximum detected values of pesticides in water samples collected at Finley National Wildlife Refuge compared to Canadian and U.S. aquatic life chronic criteria. Only detectable pesticides are reported. Refer to Appendix C for complete data on pesticide concentrations. Chemicals listed in italics are endocrine-disrupting compounds based on Keith (1997). Underlined listings are above criteria at maximum concentration.

Chemical	no. of detects/ %	Max. conc. (µg/L)	Criteria (µg/L)	Reference	Site of max. detection
Herbicides					
2,4-D	4/10	1.3	4[a]	CCME (2002)	Muddy C. @ North Bridge
Alachlor	2/5	0.005			Muddy C. @ North Bridge
Atrazine	32/76	3.0	1.8	CCME (2004)	Brown C. @ Bellfountain Rd.
Bromacil	1/2	0.090	5.0	CCME (2004)	Muddy C. @ North Bridge
Deethylatrazine	28/67	0.078			Brown C. @ Bellfountain Rd.
Dicamba	1/2	0.03[b]	10	CCME (2004)	Muddy Creek @ Bruce Road
Diuron	16/38	0.39			Muddy C. @ North Bridge
EPTC	4/10	0.004			Muddy C. @ North Bridge
MCPA	1/2	0.17	2.6	CCME (2004)	Muddy C. @ North Bridge
Metolachlor	15/36	0.056	7.8		Gray C. @ Cattail Pond
Metribuzin	6/14	0.014	1.0	CCME (2004)	Muddy C. @ North Bridge
Pronamide	6/14	0.077			Muddy C. @ Bruce Road
Simazine	11/26	0.019	10	CCME (2004)	Brown C. @ Bellfountain R.
Tebuthiuron	5/12	0.022	1.6	CCME (2004)	Muddy C. @ North Bridge
Triclopyr	1/2	0.03[b]			Muddy C @ Bruce Road
Insecticides					
Chlorpyrifos	2/5	0.012	0.0035 0.041[c]	CCME (2004) EPA (2006)	Brown C. @ Bellfountain Rd.
Ethoprop	1/2	0.003			Gray C. @ Cattail Pond

[a] Criteria for phenoxy herbicides.
[b] Estimated concentration.
[c] Criteria continuous concentration (CCC), an estimate of the highest concentration of a material in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect.

Figure 3 (Figure 2 from Appendix C) illustrates the rain pattern experienced during the 1998 sampling period. One rain event sampling occurred on March 21 followed shortly thereafter by a monitoring event sample on March 24. A second rain event sample was collected on April 24, just after monitoring event samples were collected on April 22. The rainfall in March was dramatically greater than in April. Although the collection that occurred on March 21 is termed a rain event sampling, samples collected on the March 24 (termed monitoring) were also influenced by the rain event as the rain and the runoff continued over several days. Concentrations were slightly higher on March 21 or 24 than the other sampling dates for several of the pesticides reported in Table 7 of Appendix C. The pesticides that show increased concentrations during either of the March sampling events are: 2,4-D at Muddy Creek at North Bridge; atrazine at Gray Creek at Cattail Pond, Brown Creek, Muddy Creek at North Bridge and Bruce Road; diuron at Muddy Creek at Bruce Road; pronamide at Muddy Creek at Bruce Road and North Bridge; simazine at Brown Creek and Muddy Creek at Bruce Road. During the April rain event, an increase relative to the April monitoring event was noted in the simazine concentration at Brown Creek and the atrazine concentration at Muddy Creek at Bruce Road, although concentrations of the herbicides at these sites were still higher in March. Metolachlor was detected at all sites, but at the reference area it was only detected in November, which was prior to the onset of significant fall rains. Metolachlor was also detected at Gray Creek above and below Beaver Pond during the added October sampling related to the Oregon chub die-off, outside of any major rain event.

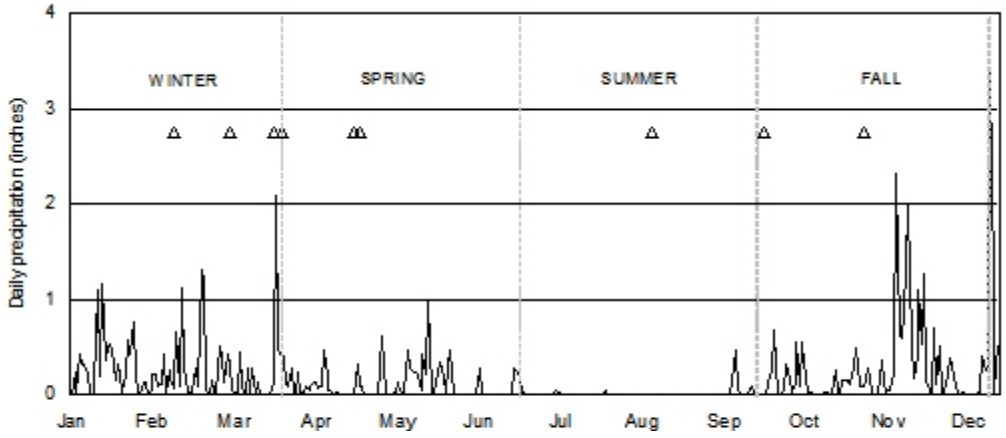


Figure 3. Daily precipitation for 1998 measured near Finley National Wildlife Refuge at Oregon Climate Service station 351826 (data from Oregon Climate Service). Triangular symbols indicate sampling dates.

QC data indicate that pesticide results were within acceptable ranges. Spike results were within the range of 60 to 140%. A few compounds exhibited slightly lower than acceptable recovery, which is typical for the particular compounds, and these are noted in Appendix C. Although QC data were acceptable, spike ranges were higher than observed in field samples, and so recovery data may not necessarily be indicative of recovery efficiencies for analytes present at low concentrations. However, other QC data, such as duplicate samples, showed good agreement and acceptable relative differences for compounds that were detected in both samples. In addition, pesticide surrogate recoveries were acceptable, ranging from 82 to 129%. Pesticide data generated during this study were comparable to data from other studies involving similar analytes and sampling methodology. QC and quality assurance (QA) data are reported and further discussed in Appendix C (Tables 2 to 4).

Discussion

Of the pesticides detected, all but three (bromacil, deethylatrazine, and tebuthiuron) have documented use on or near Finley NWR (Table 1 and Appendix A). Because deethylatrazine is a breakdown product of atrazine, use on the refuge would not be documented. Bromacil and tebuthiuron are herbicides used on non-cropland, industrial sites, and in rights-of-way (Weed Science Society of America 1994, Anderson et al. 1997, Rinella and Janet 1998). This information suggests that the Information Gathering stage of this study was highly successful in identifying nearly all pesticides with potential to enter aquatic systems on the refuge. Appendix A documented nearly all of the pesticides detected during this study as being used on or near the refuge for crop cultivation, facilities maintenance, or forestry activities. Results of the pesticide use survey also indicate there is a chance of multiple chemicals being used at the same time, but pesticide analysis indicated that detections of multiple chemicals within the same sample occurred on few occasions. Although the available data cannot support a thorough explanation of pesticide occurrence and distribution in refuge streams, the observed patterns can be attributed to factors such as 1) timing, frequency, location, and mode of application of each pesticide, 2) physical and chemical properties of individual compounds such as environmental partitioning characteristics, solubility in water, and degradation potential, and 3) precipitation patterns.

Concentrations of many of the most frequently detected pesticides were similar at both Muddy Creek sites (Figure 3 in Appendix C), which indicates that sources upstream of the refuge contribute much of the pesticide load to the stream. In contrast, concentrations of 2,4-D in water collected on March 24, at the end of the heaviest (inches/day) spring rain (Figure 3), showed an increase in concentration in Muddy Creek from Bruce Road to North Bridge, indicating that the major source of the chemical came from the refuge. The herbicide 2,4-D is approved for use on the refuge (Table 1).

Some qualitative patterns are apparent in the pesticide analytical results (Figure 3 and Table 7 in Appendix C). The three compounds most frequently detected (atrazine, deethylatrazine, and diuron) were found in samples during all four seasons, while other chemicals were detected only in spring (2,4-D, chlorpyrifos, dicamba, EPTC, MCPA, tebuthiuron, and triclopyr). All

detections of 2,4-D, alachlor, dicamba, ethoprop, metribuzin, and triclopyr were in samples collected during rainy weather patterns. The maximum detected concentration of all compounds, except bromacil, chlorpyrifos, and EPTC, occurred in samples collected during rainy periods. In contrast, three of the four EPTC detections occurred during the April 22 sample collection (monitoring) which occurred during dry weather.

Similar to Willamette Valley NAWQA studies that targeted the same 83 analytes (Anderson et al. 1997, Rinella and Janet 1998), we found the most frequently detected compounds were atrazine, deethylatrazine, diuron, metolachlor, and simazine. However, considerably fewer pesticides were detected at the Finley NWR sites compared to the NAWQA sites (Table 6 in Appendix C). Compared to the 17 pesticides detected in our study, Anderson et al. (1997) detected 36 and Rinella and Janet (1998) detected 49. Furthermore, the maximum concentrations detected in the NAWQA studies also were considerably higher than the maximum concentrations measured on the refuge for most compounds (Table 6 in Appendix C). The NAWQA studies encompass more of the Willamette Valley, and the differences in our study compared to the NAWQA results reflect the limited diversity of crops and the managed use of pesticides on and near the refuge.

In this study, atrazine was the most frequently detected compound followed by its metabolite, deethylatrazine. Atrazine was even detected at the reference site during the two sampling events in March (Table 7 in Appendix C). Because atrazine is not known to be applied upstream of the reference site, its presence is likely due to atmospheric transport and subsequent deposition by precipitation (Thurman and Cromwell 2000). Anderson et al. (1997) found atrazine and metolachlor detected throughout the year among seasonal time periods, implying that there is a steady supply of both compounds entering streams in the basin. Based on our information gathering, atrazine is applied nearly year-round from February to July and October to November (Table 3).

Diuron, metolochlor, and simazine were detected with relatively similar frequencies on the Finley NWR. Diuron and metolochlor are used on grass seed, while simazine is used on Christmas trees; diuron is also used on corn and oats. Anderson et al. (1997) estimated 2,4-D, MCPA, and EPTC to be among the four most used compounds in the Willamette Basin, yet detections of these chemicals were only occasional in their study and ours. Their estimated use of atrazine, metolachlor, and simazine in the Willamette Basin was moderately low, yet these pesticides were detected with relatively high frequencies in both the NAWQA and our studies.

Chemical concentrations measured in water collected on the Finely NWR were more than one to two orders of magnitude below concentrations considered protective of aquatic life for most pesticides for which criteria are available. However, atrazine concentrations in two samples from Brown Creek (3.0 and 2.2 µg/L) collected around the heaviest spring rain in March exceeded Canadian Environmental Quality Guideline of 1.8 µg/L for protection of aquatic life (Canadian Council of Ministers of the Environment 2004). Samples collected on March 24 from both sites on Muddy Creek (1.2 µg/L) approached the Canadian guideline. All atrazine concentrations

from samples collected in this study were well below EPA's (2003) draft atrazine acute criterion of 1,500 µg/L. The maximum concentration of 2,4-D measured in Muddy Creek (1.3 µg/L) approached the Canadian Environmental Quality Guideline which is 4 µg/L for phenoxy herbicides (Canadian Council of Ministers of the Environment 2004); currently there are no criteria for 2,4-D in the U.S. (Environmental Protection Agency 2006). In addition, the highest concentration of chlorpyrifos (0.012 µg/L), which was measured in Brown Creek, exceeded the Canadian guideline of 0.0035 µg/L (Canadian Council of Ministers of the Environment 2004) and approached the national chronic criterion of 0.041 µg/L for protection of freshwater aquatic life (Environmental Protection Agency 2006).

The Canadian Water Quality Guidelines for the Protection of Aquatic Life are designated to protect all plants and animals that live in Canadian lakes, rivers, and oceans (Canadian Council of Ministers of the Environment 2004). As long as conditions are within the levels established by the guidelines, negative effects to the environment are not expected. The guidelines are based on toxicity data for the most sensitive species of plants and animals found in Canadian waters and act as science-based benchmarks for the protection of 100% of the aquatic life species in Canada, 100% of the time. EPA Water Quality Criteria for the Protection of Aquatic Life are designated to accurately reflect the latest scientific knowledge (Environmental Protection Agency 2002). The criteria present guidance on the environmental effect of pollutants which can be useful to derive regulatory requirements based on considerations of water quality impacts. Canadian guidelines for those chemicals noted above are considerably more conservative than U.S. guidelines. The exceedances of Canadian guidelines for atrazine and chlorpyrifos in water samples collected on the Finley NWR indicate that there may be some negative effects to the environment. Negative effects would be expected to be subtle given these exceedances are relatively small.

In a study of herbicide movement in runoff from grass seed fields in a drainage basin of the Willamette Valley, Jenkins et al. (1994) monitored three herbicides commonly used during winter months (diuron, metribuzin, and oxyfluorfen). Monitoring sites were selected to represent drainage waters originating at the edge of grass seed fields and extended downstream to larger drainages. The three herbicides selected were identified in our Information Gathering (Table 3). Based on the LC50 values for these three herbicides to aquatic organisms and upon EPA's standard evaluation procedures for ecological risk assessment, Jenkins et al. (1994) postulate that there are no indications of adverse effects to aquatic species from application of these chemicals to grass fields.

The October sampling in Gray Creek to investigate conditions shortly after the September 28 fish kill revealed only metolachlor at a concentration above the detection limit, both upstream (0.004 µg/L) and downstream (0.007 µg/L) of Beaver Pond. During the November sampling, approximately 1 month later, a metolachlor concentration of 0.007 µg/L was detected in Gray Creek at Beaver Pond and further downstream in Cattail Pond (0.056 µg/L). The Pesticide Action Network Pesticide Database (2003) indicates that mortality in a variety of fish species is observed at metolachlor concentrations several orders of magnitude greater than results from

October or November sample events. The Canadian guideline for freshwater aquatic life, 7.8 µg/L, is also much greater than the concentrations observed in Gray Creek. The fish kill at Beaver Pond occurred several days prior to the October sampling, and in-stream concentrations may not reflect what fish were exposed to in the water at time of death. It is possible that residual pesticide concentrations could remain in the water several days after the fish kill at the time of sampling, but many pesticides could have caused mortality and degraded quickly to concentrations below detection limits. Therefore, the role of pesticides in the stickelback mortality remains unknown. However, very low dissolved oxygen levels (2.8 mg/L) were recorded soon after the dieoff and were likely related to the event. Although no dead fish were noticed outside of the minnow traps, some observed fish appeared to be gulping air at the surface about 500 m upstream from the traps (Paul Scheerer, Biologist, Oregon Department of Fish and Wildlife, Corvallis Oregon, pers. comm. 1998). Low dissolved oxygen levels also were apparent in October of 1999 (VanDatta 2000).

The only detectable concentration of chlorpyrifos in our study was 0.012 µg/L (detection limit = 0.004 µg/L) at Brown Creek on April 22, 1998. The pesticide use survey (Appendix A) indicates chlorpyrifos is only used on corn. Literature suggests that the concentration detected in our study does not seem to pose a threat to aquatic life on the refuge. Adverse effects have been documented on survival, reproduction, metabolism and species diversity to a variety of fishes, terrestrial and aquatic invertebrates, freshwater flora, and waterfowl over various lengths of exposure at reported concentrations from 0.08 to 1.0 µg/L, above the level of chlorpyrifos detected in this study (Odenkirchen and Eisler 1988). Chlorpyrifos concentration at the refuge also was well below the median effective concentration (EC50) associated with physical malformations, such as spinal abnormality and edema in metamorphic African clawed frog (*Xenopus laevis*) reported at 0.24 mg/L, or significant decreases in cholinesterase activity at 0.01 mg/L (Richards and Kendall 2002).

Atrazine, one of the most used pesticides in North America, is a relatively mobile and persistent herbicide which does not bioaccumulate appreciably (Solomon et al. 1996). Although environmental concentrations of atrazine vary widely in aquatic systems, Solomon et al. (1996) concluded that a concentration of 20 µg/L is rarely exceeded in permanent waters and does not pose a significant risk to the aquatic environment because the value is at the no-observable-effect-level for ecosystem response. These authors do stipulate that aquatic organisms in small reservoirs (such as ponds) in an area with intensive atrazine use may be at greater risk of exposure. Eisler (1989) reports typical concentrations of atrazine range from 0.1 to 30.3 µg/L in lakes and streams, but runoff waters directly adjacent to treated fields can exceed 740 µg/L. Howe et al. (1998) also cite studies where atrazine levels of 500 to1,000 µg/L have been detected in waters adjacent to treated fields. Levels of atrazine sampled at Finley NWR appear to be relatively low, especially in comparison to aquatic systems directly adjacent to application. The maximum detected atrazine concentrations found in this study were from Brown Creek during the heaviest spring rain, 3.0 µg/L on March 21 and 2.2 µg/L on March 24. Other than the Muddy Creek concentration of 1.2 µg/L on March 24, all other samples had concentrations of atrazine below 1 µg/L.

Although the levels of atrazine observed in this study are below concentrations reported to cause adverse effects in a large variety of aquatic organisms (Pesticide Action Network 2003), they exceed levels reported by Hayes et al. (2002a, 2002b, 2003) which may interfere with metamorphosis and sex differentiation by producing gonadal abnormalities (retarded development and testicular oogeneis) in leopard frogs (*Rana pipiens*) with similar effects in African clawed frogs. In their research, endocrine-disrupting mechanisms resulted in up to 20% of the animals having multiple gonads or being hermaphrodites (multiple testes and ovaries). Field collections from sites associated with atrazine water-borne concentrations above 0.2 µg/L also produced males with testicular oocytes similar to those induced in the laboratory at concentrations as low as 0.1 µg/L. This hermaphroditism was not evident in the absence of exposure to atrazine. Conversely, Carr et al. (2003) concluded that environmentally-relevant concentrations of atrazine (<10 µg/L) do not influence metamorphosis or sex ratios in the African clawed frog. Work by Tavera-Mendoza et al. (2002) showed reproductive system effects (decreases in testicular volume, number of spermatogonial cell nests and nursing cells) in *X. laevis* at exposures of 21 µg/L atrazine. Internal reproductive system effects may go unnoticed unless specifically part of an experiment, yet in the wild they may impair reproductive function and ultimately the reproductive success of exposed amphibian populations. Water concentrations of atrazine found in three sites at Finley NWR (Brown and Muddy Creeks) were elevated above the lowest concentration reported in the Hayes et al. (2002a, 2003) research to elicit endocrine disruption-like responses in *Xenopus* and leopard frogs. More work needs to be done to determine if the atrazine in refuge waters is having a detrimental effect on amphibian populations.

Results from field and laboratory experiments conducted by Keisecker (2002) suggest that the occurrence of trematode-mediated limb deformities in wood frogs (*Rana sylvatica*) depends on the presence of agricultural chemicals, including atrazine. In their field experiments, limb deformities were more common at sites adjacent to agricultural runoff. Amphibian limb deformities have been noted for centuries, however, the incidence of cases being reported has increased in relatively recent history. Increases in these developmental abnormalities could be due to changes in the ability of the host to resist infection or prevalence of the parasite. A recent (2001) survey conducted on the Finley NWR as part of the U.S. Fish and Wildlife Service's Amphibian Initiative indicated the incidence of external developmental abnormalities was not high (>3%) in northern red-legged frogs (Materna and Seal 2001). Of three ponds sampled, one was in the Gray Creek drainage and two adjacent ponds were within the Muddy Creek drainage. Muddy Creek had a concentration of atrazine of 1.2 µg/L while Gray Creek had atrazine levels below 1 µg/L. The ponds in the Muddy Creek drainage were directly adjacent to agricultural fields with little buffer in between. Although the presence of external abnormalities were not elevated, the 2001 survey did not include examination of frogs for internal abnormalities or endocrine impacts. Atrazine levels detected on the refuge may be impacting the frog populations at the cellular and organ system level, which could lead to population effects.

Analytical results show that the concentration of any single pesticide at Finley NWR is relatively low and likely not to cause any direct effect on aquatic organisms, with the exception of subtle

endocrine effects. However, the effects of any combination of these compounds on the ecosystem is essentially unknown. If pesticide use increases, then the threat may also increase.

Nutrient Monitoring

Introduction

Nitrogen and phosphorus are essential nutrients for aquatic plants, yet high concentrations of these nutrients can cause excessive growth that reduces dissolved oxygen and decreases the available habitat in aquatic systems and can impact aquatic organisms. Rinella and Janet (1998) report that about 63,000 tons of nitrogen and 20,000 tons of phosphorus fertilizer were applied in the Willamette Basin in 1991. USGS (Wentz et al. 1998) notes that in general, nitrate concentrations in streams increased as the percentage of drainage area in agriculture increased in the Willamette Basin. The quantity of nutrients applied for agricultural purposes in the basin raises the question as to whether nutrients are entering refuge waters to the degree that they alter the system and affect aquatic organisms.

The aqueous forms of nitrogen of interest, in order of decreasing oxidation state, are nitrate (NO_3^-), nitrite (NO_2^-), ammonia (NH_3), and organic nitrogen (N). These forms of nitrogen, along with nitrogen gas, are biochemically interconvertible and are components of the nitrogen cycle (American Public Health Association et al. 1992). Organic nitrogen is defined functionally as organically-bound nitrogen in the trinegative oxidation state. Analytically, organic nitrogen and ammonia can be determined together and are referred to as "Kjeldahl nitrogen," a term that reflects the technique used in their determination (American Public Health Association et al. 1992).

The fraction of phosphorus generally thought to be immediately available to biota is soluble reactive phosphorus (SRP), usually representing the orthophosphate portion of total phosphate phosphorus in the sample. Orthophosphate is the primary form of phosphorus directly available to organisms for uptake and use. Total phosphorus represents the immediately available form plus the phosphorus that may become available through release from sediment, organic material, and ion exchange (Garman et al. 1986). Total dissolved phosphorus represents the SRP plus the dissolved organic phosphorus, and often total dissolved phosphorus approximates SRP. Phosphate can be elevated in waters receiving discharges from treated municipal wastewater due to detergents, animal wastes, and fertilizers. Orthophosphate inputs from sewage and fertilizer releases to surface waters are the most easily used form of phosphorus for algal growth (American Public Health Association et al. 1992). Phosphorus correlates well with the eutrophication in water bodies, as it is often the limiting nutrient in freshwater systems (Garman et al. 1986).

Methods

Sites selected for nutrient monitoring were the same six sites described above under Pesticide Monitoring. These monitoring sites were sampled less frequently for nutrients than for pesticides. Nutrient sampling was conducted only during the monitoring event sampling on February 10 or 11, March 4, March 24, April 22, and November 9. Nutrient samples were not collected during the two rain events nor during the summer monitoring event on August 18.

Water samples were collected with a depth-integrated sampler to account for changes in suspended materials that occur with depth. We used a DH48 (depth-integrated, hand-held sampler developed in 1948) which consisted of a glass bottle (0.47 L) mounted in a metal device attached to a wading rod (Hauer and Lamberti 1996). As the sampler is lowered and raised in the water column, a water-sediment mixture flows into a Teflon nozzle and then into the glass bottle; displaced air escapes through a small exhaust vent. Samples were also width integrated by collecting water along three spots across the width of the stream (Edwards and Glysson 1999). In larger streams, the DH48 sampling device was deployed from a bridge while in smaller streams the device was lowered just upstream of the wading point.

Water samples were composited in a clear plastic jar (6 L) and returned to refuge headquarters where they were poured into a 14-L churn splitter (Sylvester et al. 1990) for mixing and separation into suspended and dissolved fractions. Whole water samples were decanted from the churn splitter directly into 125-ml, high density polyethylene, brown bottles. Water for dissolved constituents was filtered through 0.45 µm cellulose filters prior to being decanted into the polyethylene bottles. Nutrient samples were transported on ice in a cooler to the OFWO and the USGS Water Science Center laboratory and stored at 4 °C until express shipment to the USGS National Water Quality Laboratory in Lakewood, Colorado, for analysis. Equipment cleaning procedures followed those described by Shelton (1994) for the USGS NAWQA Program.

Nutrient samples were analyzed according to methods described by Fishman (1993). Whole water samples were analyzed for total phosphorus and ammonia plus organic nitrogen (total Kjeldahl nitrogen or TKN). Filtered samples were analyzed for ammonia nitrogen, nitrate nitrogen, ammonia plus organic nitrogen (dissolved Kjeldahl nitrogen or DKN), dissolved nitrite plus nitrate nitrogen, dissolved phosphorus, and orthophosphorus. Total nitrogen concentrations were calculated as the sum of the TKN and the filtered nitrite plus nitrate concentrations, as described by Bonn et al. (1995) and Rinella and Janet (1998). Dissolved nitrate concentrations were calculated by subtracting the concentration of nitrite from the concentration of nitrate plus nitrite.

Quality Control (QC). The QC sample plan for nutrients included collection of duplicate samples and field equipment blanks. Duplicate samples, collected on March 24 and November 11, were used to evaluate sampling and processing variability and analytical precision. Field blanks quantified any contamination from sample handling and processing and consisted of

inorganic blank water processed in the same manner as field-collected samples. Field blanks were collected on February 11, April 4, and April 24.

Results

The nutrient information collected during this study was compiled by McCarthy (2001), and the complete report entitled *Pesticides and Nutrients in Surface Water on the William L. Finley National Wildlife Refuge, Oregon, 1998* is included as Appendix C. Measured concentrations of nitrogen and phosphorus compounds in surface water are reported in Table 8 of Appendix C.

Nutrient concentrations were fairly low in all samples. Maximum concentrations reported for the various constituents are summarized below in Table 6. The maximum concentration of ammonia was detected in Gray Creek at Cattail Pond and the maximum concentrations of phosphorus were detected at the Muddy Creek at North Bridge site. Dissolved nitrite plus nitrate nitrogen concentrations were highest at Brown Creek, with concentrations exceeding 1 mg/L in all but the November sample. Ammonia concentrations and organic nitrogen were relatively low in all three sites on Gray Creek with the exception of the November sampling when levels rose at all three sampling locations. Muddy Creek had the highest concentrations of ammonia and organic nitrogen. As observed with the pesticide results, phosphorus species showed a trend of lowest concentrations in Gray Creek, higher in Brown Creek, and highest in Muddy Creek.

The results from the QC samples were within acceptable ranges for most nutrients (Table 5 of Appendix C). The relative differences in duplicate samples from the March 24 event ranged from 0 to 11% for all nutrients analyzed, and ranged from 0 to 21% for the November 9 event. The TKN duplicate in the November event exhibited the highest percent difference. All nutrient concentrations in field blank samples were below detection limits except for orthophosphorus during the February and March events and dissolved nitrite plus nitrate nitrogen in the February event. The orthophosphorus results were near the method reporting limit; therefore, results for dissolved orthophosphorus that are near the reporting limit could be biased high and should be interpreted cautiously. The dissolved nitrite plus nitrate nitrogen result of 0.10 mg/L in the field blank is twice the method reporting limit, and results for this sampling event in February could be biased high.

Discussion

Nitrogen and phosphorus compounds were distributed similarly to pesticides, which indicates their occurrence in streams is associated partially with agricultural practices. As with several of the pesticides, similar concentrations at both Muddy Creek sites suggest that nitrogen and phosphorus levels may be largely attributed to sources upstream of the refuge (Appendix C). Median and maximum values of nitrogen and phosphorus were all lower than corresponding values reported in studies elsewhere in the Willamette Valley (Rinella and Janet 1998) (Table 9 in Appendix C).

Table 6. Maximum nutrient concentrations, as nitrogen (N) or phosphorus (P), in water samples collected from Finley National Wildlife Refuge investigation as compared to aquatic life criteria. Refer to Appendix C for complete data on nutrient concentrations.

Nutrient	Max. Conc. mg/L	Criteria mg/L	Reference	Site and date of max. detection
Dissolved ammonia N	0.27	3.97[a]	EPA (1999)	Muddy @ Bruce Road March 22
Dissolved nitrite N	0.035	0.06	CCME (2002)	Muddy @ North Bridge April 24
Dissolved Kjeldahl N	0.66			Gray @ Cattail Pond November 11
Total Kjeldahl N	0.72	0.21	EPA (2001)	Gray @ Cattail Pond November 11
Dissolved nitrite + nitrate	1.3	0.15	EPA (2001)	Brown @ Bruce Road March 24
Total N (calculated)	1.66[b]	0.36	EPA (2001)	Brown @ Bellfountain February 2
Total P	0.13	0.04[c]	EPA (2001)	Muddy @ Bruce Road March 24 Muddy @ North Bridge March 24, April 22
Dissolved P	0.061			Muddy @ North Bridge March 4
Dissolved ortho P	0.069			Muddy @ North Bridge March 4

[a] Ammonia criteria are temperature and pH dependent. Criteria noted are for the maximum temperature and pH recorded for all sites except Gray Creek at Cattail Pond which had a much higher temperature.

[b] Calculations for total nitrogen followed methods outlined in Bonn et al. (1995) and Rinella and Janet (1998).

[C] Criteria recommendation for rivers and streams in the Willamette Valley. The criteria recommendations are empirically derived to represent surface waters that are minimally impacted by human activities and to be protective of aquatic life. EPA expects the value presented generally represents a nutrient level that protects against the adverse effects of cultural overenrichment based on available information, but expects States and Tribes to refine criteria for specific waterbody types and specific beneficial uses to be protected.

Results of nutrient samples collected over a 2.5-year period for the NAWQA study revealed seasonal patterns with variability apparently related to surface and subsurface-water runoff from winter and spring rains (Rinella and Janet 1998). Bonn et al. (1995) note similar seasonal variations in their analysis of historical water quality data for the Willamette Basin. No seasonal pattern in nutrient concentrations was apparent in our results (Table 8 in Appendix C). In this

study, although nutrient samples were not included in either rain event sampling, they were collected on the March 24 sampling which is considered to be influenced by the rain event of March 21. Maximum concentrations of total phosphorus, nitrite nitrogen and nitrite plus nitrate nitrogen were recorded at various sites on March 24.

EPA (2001) provides technical guidance to assist States and Tribes in developing regionally-based numeric nutrient criteria for stream and river systems. Table 6 indicates that nutrients detected in some of the waterways on the Finley NWR exceed recommended levels for protection of aquatic life based upon EPA guidance for rivers and streams in the Willamette Valley (Environmental Protection Agency 2001). On several sampling events, nitrite plus nitrate nitrogen concentrations exceeded the concentration of 0.15 mg/L recommended by EPA (2001). These events include: 1) Brown Creek at Bellfountain Road on all sample dates; 2) Muddy Creek at Bruce Road on the three sample dates in March and April; 3) Muddy Creek at North Bridge on all but the November sampling; and 4) Gray Creek at Cattail Pond in November. Calculated concentrations of total nitrogen (TN) exceed EPA's recommended value of 0.36 mg/L for rivers and streams at similar locations as the nitrite plus nitrate nitrogen exceedances, including: 1) all dates for Brown Creek at Bellfountain Road; 2) all but the November sampling for both sites on Muddy Creek; and 3) the November sampling at Gray Creek at Cattail Pond. At the Gray Creek reference site, nitrite plus nitrate nitrogen and total nitrogen concentrations were below the EPA guidance during all sampling events.

TN concentrations in this study ranged from <0.15 mg/L at the reference site to 1.66 mg/L at Brown Creek. In Rinella and Janet's (1998) report, TN concentrations ranged from 0.25 to 24 mg/L with a median of 1.5 mg/L. The highest concentration of TN noted in our study was very close to the median calculated for the NAWQA study and is within Dodds and Welch's (2000) suggested criteria range of 0.3 to 3 mg/L TN.

In the Willamette Basin, nitrate concentrations are quite variable and are frequently influenced by anthropogenic sources such as agricultural and urban runoff, sewage treatment outfalls, and other discharges. Rinella and Janet (1998) reported that Willamette River Basin nutrient concentrations were associated with land use categories such as agriculture, urban, and forest, and report nitrate nitrogen concentrations from 289 water samples at 51 locations ranged from 0.054 to 22 mg/L (median = 1.1 mg/L). The upper 10% of the nitrate concentrations exceeded 5.9 mg/L and were associated with sites receiving primarily agricultural runoff. In our study, nitrate concentrations in Brown Creek were by far the highest with values close to the NAWQA median (Rinella and Janet 1998). Nitrate concentrations in our reference samples (<0.10 mg/L) were below the median value reported in the NAWQA study for a forested reference site in the Oregon Coast Range (0.15 mg/L) and well below the median value for a forested site in the Cascade Range (0.35 mg/L).

Nitrite and nitrate nitrogen concentrations detected in creeks from Finley NWR are lower than those shown by Marco et al. (1999) to cause mortality in several species of amphibians native to the Willamette Valley, including Oregon spotted frog (*Rana pretiosa*), northern red-legged frog,

western toad (*Bufo boreas*), Pacific tree frog (*Hyla regilla*), and northwestern salamander (*Ambystorma gracile*). In addition, nitrate levels detected in Finley water samples were below those shown to affect survivorship (10 mg/L) in leopard frog and chorus frog (*Pseudacris triseriata*) tadpoles chronically exposed to ammonium nitrate fertilizer (Hecnar 1995), effects which were attributed to nitrate and not ammonia. Although results indicate amphibian survivorship is not likely to be directly affected by nitrate or nitrite concentrations on the refuge, subtle sublethal effects may occur. In Hecnar's study, less vigorous swimming and feeding were noted at the lowest treatment of 2.5 mg/L nitrate which is close to the highest concentration of nitrate detected at the refuge (approximately 1.3 mg/L). Marco et al. (1999) also report that *B. boreas* and *H. regilla* larvae experienced very low effects (activity level, behavior, and abnormalities) at concentrations of nitrate which bracket the highest concentrations observed at Brown Creek and both sites on Muddy Creek.

In aqueous solution ammonia primarily exists in two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+), and their equilibrium is highly dependent on temperature and pH (Environmental Protection Agency 1999). Un-ionized ammonia is much more toxic than ammonium ion. From February to April 1998, the maximum temperature recorded for all sites except Gray Creek at Cattail Pond was 16 °C; Gray Creek at Cattail Pond had temperatures up to 23 °C (Appendix D). Values for pH ranged from 6.0 to 7.5 among all sites for the same period. For the temperature and pH range of all sites, with the exception of Gray Creek at Cattail Pond, the corresponding chronic ammonia criteria is 3.97 mg/L (Environmental Protection Agency 1999). The maximum concentration detected in water samples from these sites was 0.27 mg/L collected in April from Muddy Creek at Bruce Road. The corresponding ammonia criteria for maximum measurements recorded for Gray Creek at Cattail Pond is 2.37 mg/L, while the maximum ammonia concentration detected in water samples from that site was 0.19 mg/L collected in November. Instantaneous measurements of temperature and pH collected in August indicate that the ammonia criteria should have remained the same during the summer.

Total phosphorus concentrations on four of five sampling dates in Brown Creek and all sampling dates at both sites on Muddy Creek exceed the criteria recommended by EPA (2001) for the Willamette Valley. Dodds and Welch (2000) suggest criteria within the range of 0.02 to 0.4 mg/L for total phosphorus, depending on the stream conditions. Total phosphorus concentrations at the reference site of Gray Creek were below or just slightly above the 0.02 mg/L concentration which Dodd and Welch (2000) suggest for pristine systems. Total phosphorus concentrations from all other sites did not exceed 0.13 mg/L. The range of total phosphorus found in the Willamette Basin for a variety of land use types, as reported by Rinella and Janet (1998), is 0.01 to 7.0 mg/L. In Gales Creek (100% forested), the median total phosphorus for 23 samples was 0.02 mg/L while in the Pudding River, with 58% agricultural cover type and an upstream discharge of a sewage outflow, the median total phosphorus for 31 samples was 0.11 mg/L.

Concentrations of SRP (orthophosphorus) from all sites on Gray Creek are consistent with the those reported in the NAWQA study for forested Gales Creek, with a median of 0.02 mg/L. Muddy Creek concentrations are closer to the median SRP values of 0.07 mg/L reported for the

more agricultural Pudding River (Rinella and Janet 1998). Brown Creek concentrations fall between medians for forested and agricultural land uses.

Water Quality Monitoring

Introduction

Physical and chemical properties of water have traditionally served as the primary means for monitoring and evaluating water quality. Parameters such as pH, dissolved oxygen (DO), conductivity, and temperature are commonly measured because they are sensitive to various sources of pollution, as well as important to aquatic ecosystems (MacDonald et al. 1991).

Our monitoring of basic water quality parameters was done to determine if aquatic organisms were exposed to undesirable temperatures, oxygen levels, or pH levels during the study period, provide an assessment of how variable these parameters were over time, and allow an evaluation of how these parameters may influence pesticide concentrations or fate in the water column. The water quality information collected at Finley NWR during our study was compiled by Mochan (2000), and the complete report entitled *A Summary of Several Water Quality Parameters for Six Sites in the Finley National Wildlife Refuge, Oregon* is included as Appendix D.

Methods

Water quality data were collected with Hydrolab® Datasonde3® multiparameter water quality instruments (multiprobes) deployed at each of the six sites where pesticide and nutrient samples were collected (see *Pesticide Monitoring* section above for the site selection process). Multiprobes collected data on temperature, DO, pH, and specific conductance at hourly intervals for the 4-month period from February to early May in 1998. Each unit was secured in place using steel cable and fence posts. A multiprobe was used to collect single water quality data points during summer and fall pesticide and nutrient water sample collections.

Each multiprobe was maintained and calibrated on a 7- to 14-day interval. Stored data were downloaded into a spreadsheet in May. At the end of the study period, a post-deployment equipment check was conducted as a QA check for any differences among multiprobes by placing all units in standing water at room temperature in the OFWO laboratory. The units recorded water quality information for 3 days, and recordings were downloaded, graphed, and visually assessed for variation among units. Suspect data were compared to an average of the readings taken by the majority of the multiprobes. After the data were evaluated, each parameter was compared to Oregon Department of Environmental Quality (ODEQ) State water quality standards as found in the Oregon Administrative Rules (Oregon Department of Environmental Quality 2004).

For water quality data collected with multiprobes, two methods of data QA/QC were completed following ODEQ protocols (Oregon Department of Environmental Quality 1997). The first method compares the field calibrations data to the multiprobe readings for the same date (Appendix D). Multiprobe readings were considered questionable if the data were not within the following audit standards: ± 1.5 degrees in temperature, ± 0.3 pH units, ± 10% µS/cm specific conductance, and ± 1.0 mg/L DO. The second method compares the multiprobe's internally stored "setup" values to the "follow-up" values. The setup values are the data recorded by the multiprobe using the original calibrations and variables in effect at the time the logging run was set up. The follow-up values are the data recorded using the calibrations and variables that followed any subsequent calibration or variable changes made by the operator during the logging period. This allows comparisons of the beginning of the first audit to the beginning of the next audit. Data drift was considered to occur if the difference between each parameter of pH, specific conductance, and DO was greater than 0.3, 10% µS/cm and 1.0 mg/L, respectively (Appendix D).

Results

The post-deployment QA check conducted in the OFWO laboratory revealed consistency in temperature and specific conductance readings among the six multiprobe units deployed during the study at Finley NWR (Appendix D). The pH readings were more variable among the multiprobes, and the unit placed at Gray Creek at Cattail Pond did not pass the setup and follow-up checks, which indicated drift in pH values. Therefore, all pH readings from the Gray Creek at Cattail Pond unit were considered questionable. In addition, the DO readings from the Brown Creek at Bellfountain Road multiprobe were very different from other units in the laboratory QA check and were more than 4.0 mg/L from the average reading, so all DO readings recorded from the Brown Creek unit were considered questionable and are not discussed in this report.

Multiprobe temperature readings recorded at all sites were quite similar (Figures 5 to 7 in Appendix D). However, towards the end of the study period, air temperatures were increasing and the temperatures in Gray Creek at Cattail Pond were higher than the other sites. Gray Creek at Cattail Pond also experienced the highest diurnal temperature fluctuations, while the BLM reference site experienced the lowest.

The pH and DO readings from the multiprobes were variable for all sites (Figures 11 to 16 in Appendix D). As observed with temperature data, Gray Creek at Cattail Pond had the largest diel fluctuation of pH and DO and the BLM reference site had the smallest. The solubility of oxygen in water is dependent on the temperature of water, so similar fluctuations in these two parameters are expected.

All measurements of specific conductance fell within the range of 30 to 90 µS/cm (Appendix D, Figures 8 to 10). Gray Creek at Beaver Pond had the lowest conductance and Gray Creek at Cattail Pond had the highest. On two occasions, abrupt increases in specific conductance were observed. These increases occurred in February at Brown Creek, where conductance increased

approximately 45 µS/cm (Figure 8 in Appendix D), and in April at Muddy Creek at North Bridge, where specific conductance rose about 20 µS/cm (Figure 10 in Appendix D).

Discussion

Results of water quality monitoring indicate that temperature, specific conductance, pH and DO are quite variable but fall within normal conditions for most sites. Data collected in winter and spring that passed QA/QC audits met ODEQ water quality standards for all parameters at all sites except Gray Creek at Cattail Pond, which had the highest 7-day average maximum temperature and violated Oregon temperature standards. The most remarkable differences among sites was between Gray Creek at Cattail Pond and the reference site. The difference in patterns between these two sites can be primarily attributed to habitat and hydrodynamic differences. The Gray Creek at Cattail Pond is a slow-moving system open to solar radiation, and would be expected to contain more algae and macrophytes and have greater temperature, pH and DO fluctuations. In a well-shaded system with faster water flow, as is the setting for the reference site, fluctuations in these parameters are not as dramatic.

Only Gray Creek at Cattail Pond violated the Oregon temperature standard. However, the State standard is applied as the 7-day average of the daily maximums (7 DADM), which typically occurs in July or August and sampling for this study was terminated on May 5. Although there are no data from the summer months, water temperatures at other sites may have also been in violation of the State temperature standard. State water quality temperature standards in the Willamette Basin are intended to protect native cold water salmonids and fall into designated use categories of salmonid spawning (13 °C 7 DADM) and core cold water habitat (16 °C 7 DADM) (Oregon Department of Environmental Quality 2004).

Some sites monitored for this study provide habitat for the endangered Oregon chub. Although several of the chub sites may have been in violation of the State standard for temperature during the summer months, these warmer temperatures are actually preferred by Oregon chub. Oregon chub are found in slack water off-channel habitats such as beaver ponds, oxbows, backwater sloughs, low gradient tributaries, and flooded marshes (Fish and Wildlife Service 1998), typically less than 6 feet deep with summer temperatures exceeding 16 °C, and spawning occurs when water temperatures are between 16 and 28 °C (Fish and Wildlife Service 2003). Therefore, no risk to Oregon chub is expected based on the violation of the Oregon temperature standard.

Natural levels of pH in streams and rivers typically vary between 6.0 and 8.5 (Stumm and Morgan 1996). ODEQ has set the pH standard for cool-water aquatic life in the Willamette Basin between 6.5 and 8.5 (Oregon Department of Environmental Quality 2004) and nationally the standard is 6.5 to 9.0 (Environmental Protection Agency 2006). Hydrolab readings were variable for all sites, ranging from pH 6.0 to 7.5. Daily pH fluctuations occur naturally and pH values are often greater in the afternoon when photosynthetic activity is higher (Stumm and Morgan 1996). EPA (1986) states that a pH range of 5 to 9 is not directly lethal to fish; however,

the toxicity of several common pollutants is markedly affected by pH changes within this range. For example, ammonia concentration is strongly influenced by pH and the national criteria is dependent on both pH and temperature.

The ODEQ DO standard for cool-water aquatic resources is >6.5 mg/L (Oregon Department of Environmental Quality 2004). Disregarding the Brown Creek DO readings, which were considered questionable due to QA problems, all sites were above 6.5 mg/L DO. Notwithstanding, it should be noted that DO concentrations vary seasonally and reach their lowest when temperatures are warmest during the summer months. Our water quality monitoring did not extend to the warmest time of the year, so it is unknown whether oxygen levels fell below the State standard during that time. As with temperature and pH, DO showed the largest daily fluctuation in Gray Creek at Cattail Pond and the lowest at the BLM reference site.

In a study prompted by concerns regarding water quality at Finley NWR, VanDatta (2000) collected water quality data for temperature, pH, and DO on a bimonthly basis from October 1999 through May 2000 at three locations in Gray Creek, all located upstream of our Beaver Pond site. Results of VanDatta's study are attached as Appendix E. The DO readings were commonly >5 mg/L, however levels dropped to 2.3 mg/L in October 1999 and <4 mg/L in May 2000. Iron precipitate was observed in October 1999 prior to the fall rains, and again during the spring at the margins of beaver ponds. The iron precipitate and low dissolved oxygen conditions indicate a strong groundwater flux into the system. From 1995 to 1999, dead stickelback were occasionally found in minnow traps used to obtain population estimates of Oregon chub. The dead fish are unique to the Gray Creek location and are likely associated with low DO in the creek and inability of the fish to avoid the low DO concentrations while in the minnow traps.

Specific DO requirements for Oregon chub are unknown, but Scheerer and Apke (1998) report that Oregon chub have been observed at sites with DO concentrations ranging from 3.0 to 9.9 mg/L. The slackwater environments which Oregon chub inhabit typically experience low DO concentrations (Gordon et al. 1992). However, observed concentrations of DO below 3 mg/L could be detrimental to the species.

Specific conductance is a measure of the electrical conductance of water and an estimate of total dissolved ions. Lack of Statewide or national standards for specific conductance indicates the relative insensitivity of aquatic biota to this water quality parameter. Conductivity of streams emanating from forested areas in the Pacific Northwest almost always falls at the low end of the range (MacDonald et al. 1991). In our study, abrupt increases in specific conductance measurements were observed on two occasions (February and April). Specific conductance can change quickly in response to an influx of stormwater or groundwater containing different dissolved ions than the receiving water and we suspect these increases are associated with rain events increasing suspended sediment in the stream due to erosion and runoff, as the visual clarity of Brown Creek was noted to decrease quickly after rain events.

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CHAPTER 2

EXPOSURE AND EFFECTS

Introduction

This chapter focuses on measures of exposure and effects of pesticides to aquatic organisms at Finley NWR. An array of tools are available to use as indicators of exposure to environmental chemicals. In some cases, the tool used to indicate exposure may also provide information as to biological response at the organismal or population levels.

Specifically, the objectives covered by this chapter include the last four objectives stated in the *General Introduction* and reiterated below.

- Evaluate acute toxicity of water during pesticide application using an *in-situ* bioassay;
- Investigate endocrine-disrupting compound exposure in common carp and western pond turtles to compare differences in hormone levels between agricultural and non-agricultural sites;
- Assess the health of common carp between agricultural and non-agricultural sites; and
- Determine organochlorine pesticide concentrations in carp from historic use of "legacy" pesticides.

Components of the study included in this chapter are entitled Aquatic Bioassays, Endocrine Biomarkers and Fish Tissue Analysis, and Fish Health Assessment.

Aquatic Bioassays

Introduction

Bioassays are used extensively to determine the effects of potentially toxic materials on aquatic organisms during short-term exposure (Rand and Petrocelli 1984). These tests can provide a rapid, inexpensive, and reproducible estimate of toxic effects. Detailed, standardized methods have been developed for conducting these experiments in the laboratory where there is rigid control of many variables. In the field where conditions cannot easily be controlled, methods have to be conformed to fit more complex situations.

The most straightforward bioassay endpoint to measure is mortality; however, growth and reproduction provide useful measures of sublethal effects. These tests also have their limitations, such as lack of predictability of potential chronic toxicity or cumulative effects. Oftentimes bioassays are the initial step, or one of a combination of steps, in an evaluation of environmental

conditions. In this investigation, bioassays were intended as one of several methods to provide information for assessing conditions of aqueous systems on the refuge.

Methods

Two types of bioassays were conducted during pesticide application periods, *in-situ* and static renewal. Fathead minnows (*Pimephales promelas*) were used as the test organism in both types of bioassays. Fathead minnows are frequently recommended for use in freshwater toxicity tests due to their widespread distribution in freshwater environments (Wydoski and Whitney 2003) and ease of handling in the laboratory (American Society for Testing and Materials 1980). In addition, fathead minnow growth and survival tests have been found to be a reliable and relatively precise toxicity test method (Environmental Protection Agency 1989, Anderson and Norberg-King 1991).

Larval fathead minnows were obtained via overnight delivery from Aquatic Research Organisms in Hampton, New Hampshire. Upon arrival, minnows were transferred to an aquarium held in a temperature-controlled waterbath and for the first 24 hours the waterbath remained within 2 °C of shipping temperature. Following the initial 24-hour period, the waterbath temperature was decreased by 2 °C per day to acclimate fish to field temperatures. Larvae were 5 days old upon arrival at the OFWO and less than 10 days old upon initiation of the bioassay.

In-situ bioassays were based upon methods described by Nebeker et al. (1984) and Bennett (1994). Test chambers consisted of polyvinyl chloride compression couplers equipped with open screw-on endcaps. Endcaps were covered with Nitex mesh to contain the minnows while allowing water flow through the chamber. For transport to test sites, test organisms were placed into each chamber within 1-quart Ziploc plastic bags containing culture water. Each plastic bag contained three test chambers which were placed in coolers with ice to maintain temperature during transport. At the field site, chambers were transferred underwater from the plastic bag to plastic screen cages fitted with a layer of Styrofoam on the top screen for flotation. To evaluate minnow mortality during transport to the field sites, a duplicate set of three chambers served as travel controls and were inspected for mortality at the end of the deployment route.

The *in-situ* bioassay was conducted at the same six sites described in the Pesticide Monitoring section in Chapter 1. Three test chambers were placed at each site and observed at 24-hour intervals. Ideally, chambers would have been retrieved after 96 hours of exposure but high mortality (live minnow count in controls <80%) resulted in early retrieval. Multiprobe units were deployed at each bioassay site during the same time period to collect water quality measurements as described in the Water Quality Monitoring section of Chapter 1. Deployment of the *in-situ* bioassays during March and April occurred within 24 hours of pesticide and nutrient monitoring sample events.

Static renewal bioassays primarily followed techniques described in American Society for Testing and Materials (1980) and EPA (1985). The 96-hour static test was conducted at Finley

NWR headquarters. Site water was collected according to procedures described in Pesticide Monitoring (Chapter 1) and poured into1-L glass beakers with two duplicate beakers per site. Beakers were held within a fiberglass waterbath attached to a flow-through cooling system to maintain temperatures similar to sample streams. Each beaker held 10 test organisms. One-half of the site water was replaced every 24 hours. For the duration of the test, beakers were aerated and organisms were not fed. Organisms were observed for behavior and mortality every 24 hours. Temperature, DO and pH were measured at initiation, 48 hours, and termination of the test. Ammonia levels were also monitored periodically during testing.

Results and Discussion

On both attempts at *in-situ* bioassays, high mortalities ($\geq$50%) were observed in control cages deployed in the reference stream within the initial 24-hour exposure period, thus invalidating the tests. Mortality may have been a result of high streamflows during winter and spring deployments; the young fish not yet strong enough to maintain swimming within the fast current.

The results of the static renewal bioassay were also unacceptable due to high mortality of test organisms during the acclimation process, as well as in controls throughout the test. American Society for Testing and Materials (1980) standard practices for conducting toxicity tests specify that organisms must not be used for a test if more than 3% die during the 48 hours immediately preceding the test. Mortality during acclimation was most likely the result of ammonia buildup in aquaria. Thus, results of the bioassay are not included as a line of evidence in evaluating conditions on the refuge.

Endocrine Biomarkers and Fish Tissue Analysis

Introduction

The endocrine system is made up of all the hormone-secreting glands of the body and acts in concert with the nervous system to maintain homeostasis (stability) in organisms. The endocrine system serves as a communication system where the hormones serve as blood-borne messengers which control and integrate many functions including reproduction (Vander et al. 1980). Endocrine glands secrete hormones into the bloodstream and these chemical messengers travel to other parts of the body where they attach to receptor molecules to affect transduction, or the cells ability to receive signals (Dempsey and Costello 1998). Each hormone plays a different role in an animal's biology, including growth regulation, sexual maturation and reproduction. The major vertebrate organs and glands involved in hormone secretion include the testes, ovaries, placenta, pancreas, kidney, adrenal, parathyroid, thyroid, pituitary, and the hypothalamus. Disruption of the endocrine system as a result of exposure to exogenous estrogens and other endocrine-disrupting compounds has been documented in various species (Purdom et al. 1994, Crisp et al. 1998, Dempsey and Costello 1998, Sumpter and Johnson 2005), and measuring endocrine parameters can be a valuable tool to assess health of fish and wildlife.

The underlying mechanisms of the endocrine system, disruption of the system, and the biomarkers used to assess disruption are complex. In the simplest model of hormone disruption, environmental chemicals can mimic an endogenous hormone by binding to its receptor and eliciting a spectrum of biological effects, or they could bind to a hormone receptor as an inactive compound and block the response to the natural hormone (Dempsey and Costello 1998). Many synthetic and natural chemicals foreign to living systems (xenobiotic chemicals) are so similar in structure to natural hormones that they can bind to or block the hormone receptor and activate or block transduction. Xenobiotic chemicals that activate transduction are agonists and bind to cellular receptors to mimic the cellular response characteristic of the natural hormone. Chemicals that block transduction and prevent natural expression of the cellular response are antagonists (Dempsey and Costello 1998). Endocrine disruption can occur when xenobiotic chemicals bind or block the cellular receptors of natural hormones and cause abnormal cell activity, impair normal cell activity, amplify the impact of hormones, change the natural hormone message, or disrupt hormone synthesis and cause an improper balance or quantity of circulating hormone (National Wildlife Federation 1994).

The effects of endocrine disruptors vary by species and the life-stage at exposure and could result in morphological, functional, or behavioral abnormalities (Dempsey and Costello 1998). The reproductive disorders reported in wildlife include reduced fertility, reduced hatchability, reduced viability of offspring, poor growth, wasting and lower rates of activity in neonates, impaired hormone activity, and modified adult sexual behavior (Guillette 1994).

Studies of endocrine disruption in fish and wildlife have primarily focused on plasma concentrations and receptor function of androgens and estrogens, which are sex steroid hormones synthesized by the gonads and required for normal reproductive activity. Estrogens (including progesterone) are hormones responsible for the primary and secondary sexual characteristics in females. They are primarily produced and secreted from the ovaries, and in smaller amounts by the testes, upon receipt of hormonal messages from the anterior pituitary gland in response to messages from the hypothalamus (McDonald et al. 2000). Androgen hormones (e.g., testosterone) are produced by the testes and stimulate the development, growth, and secretory activity of the male reproductive duct and glandular system as well as male secondary sexual characteristics. These sex steroid hormones are present in the plasma of all vertebrates, although the ratios of estrogens to androgens vary dramatically between males and females. Females typically have ratios significantly greater than one, whereas males have a ratio smaller than one (Goodbred et al. 1997). The actual ratio will vary between species or within a species depending upon factors such as reproductive stage, age, season, time of day, level of stress, or nutritional level (McDonald et al. 2000). However, the difference between males and females should remain dramatically different unless unique biological factors or biological abnormalities exist.

Recent toxicological studies have measured responses of the endocrine system to chemical stressors as an indicator of exposure in fish and wildlife. Many environmental contaminants elicit estrogenic activity in animals, and studies have shown that chemical exposure and altered sex steroid levels in plasma have been associated with abnormal reproductive development

(Purdom et al. 1994, Crisp et al. 1998, Sumpter and Johnson 2005). Suspected endocrine disruptors include some chemicals of the following groups: chlorinated compounds, polychlorinated biphenyls (PCBs), dioxins, natural and synthetic hormones, and heavy metals (Colborn et al. 1993, Keith 1997). Some commonly-used herbicides also fall within this group, including 2,4-D, atrazine, metribuzin, and trifluralin (Keith 1997). In their compilation of environmental chemicals reported to have reproductive and endocrine-disrupting effects, Dempsey and Costello (1998) also identify malathion. These chemical pesticides are used on or near the refuge. Aquatic resources using Finley NWR could be exposed to endocrine dirsuptors based on past or current use of agricultural chemicals on or near the refuge.

Monitoring sex steroid concentrations has been the primary method used to study the endocrine-disrupting effects of various environmental chemicals (McDonald et al. 2000). Monitoring sex steroids as a biomarker provides a convenient method for assessing subtle physiological alterations induced by xenobiotic chemicals. In fish, the major androgens include testosterone, 11-ketotestosterone, and androstenedione. Levels of 11-ketotestosterone typically correlate with testosterone and are more important for spermatogenesis in male fish (Goodbred et al. 1997). The predominant estrogens are 17β-estradiol (E_2) and estrone. While sex steroids in immature fish probably influence gonadal differentiation, these same hormones play an important role in gametogenesis, ovulation, and spermiation in mature fish (Barry et al. 1990, Patino and Thomas 1990a, Patino and Thomas 1990b, Redding and Patino 1993). These steroids collectively control the development of the gonads and gametes, secondary sexual characteristics, and reproductive behavior such as pheromonal attraction, spawning, and parenting (Fostier et al. 1983, Liley and Stacey 1983).

Studies demonstrate that exposure to a variety of contaminants, including agricultural pesticides, can lead to alterations in plasma sex steroid concentrations in fish species (Singh and Singh 1987, Goodbred et al. 1997). Goodbred et al. (1997) found a negative correlation between hormone ratios and dissolved pesticides for both male and female carp collected in streams across the U.S. Contaminants can alter sex steroid levels by interfering at multiple sites along the hypothalamo-pituitary-gonadal axis. Measurement of sex steroid hormones combined with gonadal histopathology are the most reliable techniques available for measuring reproductive function as well as effects of contaminants (McDonald et al. 2000).

In this study, we selected common carp and western pond turtle to evaluate endocrine parameters as part of a weight-of-evidence approach to better assess water quality at Finley NWR. With the apparent increase in herbicide use on or near the refuge, endocrine biomarkers were an important tool in the risk assessment for these two aquatic organisms. We also analyzed carp tissue for organochlorine pesticides ("legacy pesticides") and PCBs to determine if these compounds were present at levels that could influence the endocrine system.

Carp were used in this study because they are widely distributed and available, relatively easy to collect, and have been used by others in studies on endocrine disruption (Folmar et al. 1996, Goodbred et al. 1997, Gimeno et al. 1998, Schmitt et al. 2002). In addition, considerable

information exists on the biology of common carp (Penak 1987), including the annual cycles of sex steroid profiles in males (Barry et al. 1990, Chang and Chen 1990). Although carp are not a desired species at the refuge, they can serve as indicators of the health of other fish species, and they are in the same family (Cyprinidae) as the endangered Oregon chub, so they serve as a suitable surrogate for this species. Oregon chub are of particular concern at the refuge because their populations have declined in the Willamette Valley even though suitable habitat exists (U.S. Fish and Wildlife Service 1998, Scheerer et al. 2003).

We selected the western pond turtle as another indicator or test species because alteration of sex hormone concentrations associated with contaminants has been shown in reptiles (Guillette et al. 1994), and specifically in turtles (Palmer and Palmer 1995, Willingham et al. 2000). In addition, samples could be obtained sublethally with minimal risk to captured adults. Although knowledge of reproductive ecology of western pond turtles is growing, data are limited primarily to nesting females and oviposition (Todd 1999). Most females do not appear to develop eggs until they are over 120 mm in length and at least 8 to 10 years of age (Holland 1994). Clutch size varies from one to 13 eggs (average for the Willamette Basin drainage is about seven) with oviposition occurring in June and July. The majority of females probably oviposit in alternate years (Holland 1994). In males, age and size at first reproduction is not well known, but may follow a similar pattern to females. In the Willamette Valley, western pond turtle populations were formerly quite common but have declined by 96 to 98% since the 1900s (Holland 1991, Csuti et al. 1997), and studies at Finley NWR found that the population there is heavily adult biased, indicating low recruitment (Loegering 1998). By including the western pond turtles in our study, we hoped to learn more about possible reasons for population declines.

In addition to population declines, juvenile western pond turtle survivorship has become an important issue for conservation of the species. Given the low fecundity of the species, current survivorship is too low to maintain population levels (Jennings et al. 1992). High juvenile mortality, low nest success, and human-induced environmental factors have severely reduced recruitment (Jennings et al. 1992).

The objective of this study component was to investigate endocrine-disrupting compound exposure in common carp and western pond turtles by comparing differences in reproductive hormone levels, and contaminant concentrations in carp, between agricultural and non-agricultural sites on Finley NWR lands. In addition, carp from two known contaminated sites in the Willamette Basin were also collected and analyzed for endocrine biomarkers and contaminant residues for comparison to carp collected from the refuge.

Methods

Animal Capture and Sample Collection. Common carp and western pond turtles were captured for blood collection to determine concentrations and ratios of reproductive hormones. The minimum target number was 20 individuals (10 males and 10 females) of each species collected from a field site where pesticides were applied on Finley NWR and from a reference site

receiving no pesticide inputs. Mature adults were targeted in order to ensure that animals were of breeding age. Carp at breeding reach an average length of about 300 mm (at 2 years of age) for males and 430 mm (at 3 years of age) for females (Penak 1987). Turtles were considered juveniles if carapace length was less than about 110 mm (Holland 1991). Although these sizes were targeted, a few carp of each sex collected at Finley NWR, and a few females at Malheur NWR, were slightly under these measurements. For turtles, it was not possible to meet the target number of females at either the Finley NWR or the reference site. However, the number of males captured exceeded the target number at both sites.

Common Carp. Carp were collected in 1998 between September 30 and October 7 at Cabell Marsh at Finley NWR (Figure 2), and on September 26 at Double-O Pond at Malheur NWR. Fish were collected during a postspawn period (Penak 1987) when gonadal recrudescence occurs (Down et al. 1990). Carp from Malheur NWR were collected by pulsed direct current electroschocking, while seining was used to collect carp from Cabell Marsh because the shallow depth of the marsh prevented access with electroshocking-equipped boats or rafts. The Malheur NWR site was considered a reference site because the water in the pond is spring fed and the pond is distant from industrial, urban, or agricultural areas; the dominant land use near the pond is ranching. Therefore, few sources other than atmospheric deposition would introduce organochlorine contaminants into the pond. Carp samples collected in 1998 were processed according to protocols established by the Biomonitoring of Environmental Status and Trends (BEST) program of the USGS, Biological Resources Division (Schmitt et al. 1999) as described under the *Fish Health Assessment* section below.

In addition to carp collected at the two NWRs, we obtained previously unreported data from the USGS (Steve Goodbred, NAWQA liaison, USGS, Sacramento, California, pers. comm., 1999) which included plasma steroid concentrations and other parameters for carp samples collected in 1995 by the USGS's Water Resources Division (WRD) in Portland, Oregon. These samples were collected for the NAWQA program in Oregon on July 17, 1995, from the Mill Race Pond near Springfield (a waterbody receiving surface water diverted from the Middle Fork Willamette River), and on July 18, 1995, from the Willamette River at St. Johns Bridge in Portland (within the Portland Harbor Superfund site). These carp samples were collected and processed in a similar manner to the fish collected in 1998 for our study; specific collection methods for the 1995 fish are reported in Goodbred et al. (1997). We did not make statistical comparisons between the 1995 and 1998 data due to confounding factors between the studies such as differences in collection year, season (fish obtained in 1995 may have been collected during spawning rather than during recrudescence), and sample size. Rather, we used these fish to make a gross comparison or evaluate the "upper bound" of potential values because the 1995 fish came from areas with known contamination.

Whole blood samples were collected from the caudal vein of carp using a 5-cc syringe and 20-gauge needle, transferred to a heparanized Vacutainer®, and chilled on wet ice. Blood samples were then centrifuged in the field for 10 minutes. The plasma was removed from the sample, placed in a clean storage vial, and frozen on dry ice. Samples were shipped overnight on dry ice

to the University of Florida's Biotechnologies for the Ecological, Evolutionary, and Conservation Sciences (BEECS) Program in Gainsville for hormone analysis.

Methods of aging and specifics of gonadal stage determination are described in the *Fish Health Assessment* section of this chapter.

Western Pond Turtles. Western pond turtles were captured at Finley NWR in various wetland habitats including Cabell Marsh, Muddy Creek, Greenberry Oxbow, and Beaver Pond. Turtles were captured from late July to early September 1998. Blood samples were taken from 18 individuals at Finley NWR, including 11 males, six females, and one juvenile of undetermined sex.

Turtles also were captured in August and September 1998 at a reference site in the South Umpqua River drainage (Umpqua National Forest, Douglas County, Oregon) at Dumont Creek, Three C Rock, Jackson Creek, and at Carmine Lake, a 2-ha lake 0.5 km west of the Black Rock Fork of the South Umpqua River. The South Umpqua River locations were collectively considered a reference site because they were in the upper drainage of a forested area and received no agricultural inputs. At the reference site, 26 individuals were sampled, including 16 males, eight females, and two juveniles of undetermined sex.

Turtles were captured by hand or by trapping. Funnel traps were placed along creek and marsh banks in perpendicular fashion with the entrance directed toward deeper water. Traps were baited with dead salmon and steelhead smolts, sardines, or both. Bait was positioned at the rear of the trap. Traps were checked daily and bait replaced as necessary. Hand capture involved snorkeling in pools where turtles had been observed basking. Upon capture, turtles were transported to a suitable location for blood sampling and data recording. Following the blood draw, turtles were aged, sexed, weighed, and the maximum length of carapace measured. The sex of turtles was determined by a number of characteristics including shape of plastron, cloacal opening, tail base enlargement, carapace height, and chin coloration (Todd 1999). Age of mature turtles was determined by counting concentric ridges on scutes (Germano and Bury 1998). Turtles older than 16 years could not be reliably aged and data were recorded as "16+" for these individuals. Individual turtles were marked by filing notches into the marginal scutes of the carapace using the numbering system described by Holland (1991). All turtles were released at the point of capture as soon as possible after processing. Specific measurements and collection information are reported in Appendix F (Brunkal 1998).

Blood was collected from the jugular vein of turtles using 1-cc syringes with 25-gauge needles. Sample blood was transferred from the syringe to a heparinized Vacutainer® tube or a serum tube and immediately placed on ice. Samples were then centrifuged for 10 to 15 minutes to separate cells from plasma, and the plasma removed and placed in a clean storage vial and frozen on dry ice. Samples were transferred to an ultracold freezer (-80 °C) for storage until analysis. Frozen samples were shipped overnight on dry ice to the BEECS laboratory for hormone analysis.

Analysis of Sex Steroid Hormones from Common Carp and Western Pond Turtle. Analysis of sex steroid hormones in plasma was conducted by radioimmunoassay (RIA) at the BEECS laboratory. Carp plasma was analyzed for 17β-estradiol and 11-ketotestosterone as described by Goodbred et al. (1997), and turtle plasma was analyzed for 17β-estradiol and testosterone following methods of Gross et al. (1995). Carp or turtle plasma was extracted twice with diethyl ether and analyzed in duplicate, and results were corrected for extraction efficiencies. Standard curves were generated based on various dilutions of individual radio-inert sex steroids. Cross-reactivities of individual sex steroids and antiserum were characterized by Dr. Tim Gross, BEECS laboratory.

In addition to plasma steroids, the phosphoprotein vitellogenin was analyzed in carp collected in 1995 by Goodbred et al. (1997). Vitellogenin is produced by the liver as a precursor of egg yolk in egg-bearing vertebrates. Synthesis of vitellogenin in male carp can be an indication of endocrine disruption (Purdom et al. 1994, Folmar et al. 1996). Vitellogenin was assayed by capture enzyme-linked immunosorbent assay, following the methods as described in Goodbred et al. (1997). The vitellogenin data reported here were provided by Dr. Steve Goodbred (NAWQA liaison, USGS, Sacramento, California, pers.comm., 1999) and have not been previously reported. Vitellogenin was not analyzed in carp collected from the Finley or Malheur NWRs.

Analysis of Chemicals in Carp Tissue. Carp analyzed for contaminant residues included samples collected from both refuge locations and the samples collected by USGS in 1995. The latter samples were stored frozen at the USGS, WRD in Portland, Oregon, until analysis (samples provided by Ian Waite, Biologist, USGS, WRD, Portland, Oregon). Carp from the refuge were separated by sex and each composite sample consisted of 10 to 12 fish, resulting in two samples per refuge. Carp from the non-refuge sites were separated by sex and each composite sample consisted of five fish, resulting in two samples per site.

Carp samples were processed according to protocols established by the BEST program of the USGS's Biological Resources Division (Schmitt et al. 1999) as described in the methods of the the *Fish Health Assessment* section below. Procedures for transport and shipment of samples to contract laboratories followed QA/QC guidelines documented in Rope and Breckenridge (1993) and OFWO Standard Operating Procedures. Samples were shipped on dry ice by overnight mail to contract laboratories performing chemical analyses.

Whole-body carp tissue was analyzed for OC pesticides and total PCBs at Patuxent Analytical Control Facility (PACF) in Patuxent, Maryland. Analytical methods for the preparation, extraction, and clean up of tissue samples followed the methods of Cromartie el al. (1975). Briefly, a tissue sample was extracted under a solvent using Soxhlet apparatus and glass extraction thimbles. Silica gel chromatography was used to separate pesticides from PCBs. Methods of Cromartie et al. (1975) were modified to include four fractions instead of three to enable the separation of dieldrin and endrin from the rest of the pesticides. The pesticides in each fraction were quantified with a gas-liquid chromatograph (GLC) with a 30 m Megabore column coated with a 1.0 micron film of 7% cyanopropyl and 7% phenyl polysiloxane. The GLC

was equipped with a nickel 63 electron capture detector. Residues in 10% of the samples were confirmed by gas chromatography/mass spectrometry (GC/MS). The nominal lower limit of detection was 0.01 µg/g for pesticides and 0.05 µg/g for PCBs.

Fish tissue samples were analyzed for trace elements at PACF. Samples were lyophilized to a constant mass. An aliquot of freeze-dried material (about 1 g) was digested in 10 ml ultrapure nitric acid and 4 ml of 30% hydrogen peroxide using an OI Model 7301 Microwave Digestion System. A scandium internal standard was added after digestion. Quantitation of trace elements other than arsenic, selenium, and mercury was performed using a Perkin Elmer Plasma II sequential inductively coupled plasma emission spectrometer. Arsenic and selenium were quantified by stabilized temperature platform graphite furnace atomic absorption spectroscopy using a Perkin Elmer Zeeman 3030 atomic absorption spectrophotometer. Mercury was determined by cold vapor atomic absorption spectroscopy as described by Hatch and Ott (1968) modified for use with a Perkin Elmer atomic absorption spectrophotometer 3100 equipped with a Perkin Elmer Flow Injection Analysis System 200. The detection limits for trace elements were primarily below 1 µg/g.

Percent moisture and percent lipid were determined on all tissue samples. Percent lipid was determined by placing a portion of the extracted lipid solution on a pre-weighed aluminum pan, evaporating the solvent, and reweighing the pan.

Statistical Analysis. Results of physical characteristics (length and width) and sex steroid concentrations for turtles and carp were compared using two-way analysis of variance (ANOVA) with sex and site as classification categories. Data were only compared statistically if collected during the same season and year for a species. Data from juvenile turtles were excluded from statistical tests. Individual parameters were evaluated for normality by examining probability plots, and tested for equal variances among groups using the F-test. Homogeneity of variances in a set of samples is an important precondition for statistical tests such as ANOVA (Sokal and Rohlf 1981). Results of these tests indicated that variances in sex steroid concentrations were not equal, and $\log_{10}$ transformation of 17β-estradiol and 11-ketotestosterone (or testosterone for turtles) values slightly improved homoscedasticity (homogeneity of variances). Significant tests were performed using transformed sex steroid concentrations and non-transformed physical characteristics at the 95% confidence level ($\alpha = 0.05$) using SYSTAT® 9 software (SPSS 1999). Linear relationships were tested by regressing hormone levels against mass, length, and age; age against gonadal stage; and gonadosomatic index (GSI) against length, mass, age, and gonadal stage.

Gonadal stages were similar between sites, therefore all gonadal stages were grouped for purposes of statistical analysis. For a description of the gonadal stages, see the Histopathology Results of the *Fish Health Assessment* section below.

Hormone ratios of 17β-estradiol/11-ketotestoserone (or testosterone for turtles) were ranked and compared between sexes within a site using the Kruskal-Wallis test at $\alpha = 0.05$ (Sokal and Rohlf

1981). This nonparametric method was used because ratios are distribution free (i.e., the ratios of normally distributed random variables are not distributed normally but have a Cauchy distribution [Stefan Baratto, Mathematics Instructor, Clackamas Community College, Oregon City, Oregon, pers. comm., 2002]). Although ranked for statistical purposes, ratio data were reported as arithmetic means and standard errors in tables.

Contaminant concentrations in carp were compared to data from previous tissue monitoring programs in the northwest, and to concentrations estimated to protect fish-eating wildlife. Insufficient data were available to compare concentrations between sites or to identify relations between hormone values.

Results

Carp from Finley and Malheur NWRs. Gonads of female fish were classified according to six stages of sexual development. All female carp from both sites were classified in gonadal stage 3, late development. In stage 3 the majority of developing follicles are late vitellogenic, which is characterized by increasing oocyte diameter and chorion thickness and yolk globules distributed throughout the cytoplasm. Stage 3 is typical of fish approaching spawning, although stages 1 to 3 are all representative of sexually mature female fish.

Male gonads were classified according to four stages of sexual maturation. All males collected from Finley and Malheur NWRs were either at stage 2 or 3. Stage 2 is classified as mid-spermatogenesis where the germinal epithelium is moderately thick and some proliferation and maturation of sperm can be observed. Stage 3 is classified as late spermatogenesis where the germinal epithelium is thick and spermatozoa predominate; roughly equal proportions of spermatocytes, spermatids, and spermatozoa are present. Stages 1 to 3 are characteristic of sexually mature fish with the least activity occurring in stage 1 and the most activity occurring immediately prior to and during spawning season of stage 3. Immature, undeveloped testes are classified as stage 0 and spent testes are stage 4.

All fish exceeded the target lengths considered mature except for four males and seven females from Finley NWR and one female from Malhuer NWR (Table 1). The female from Malheur NWR and the males and one female from Finley NWR were slightly below the target values of 300 mm for males and 430 mm for females (Penak 1987). Five of the females from Finley NWR were about 100 mm below the target length. Although carp ages at the two sites indicated differences, gonadal stage was similar between the two sites (Table 1).

Table 1. Physical characteristics and concentrations of plasma hormones 17β-estradiol (E_2) and 11-ketotestosterone (11-KT) from common carp collected in 1998 from William L. Finley National Wildlife Refuge (NWR) and a reference pond at Malheur NWR.

	William L. Finley NWR		Malheur NWR reference site	
	Male	Female	Male	Female
Sample size	12	11	10	10
Physical characteristics[a]				
length (mm)[b]	326 ± 9.7 A[c] (282-401)	407 ± 25 B (316-556)	412 ± 13 BC (376-516)	475 ± 11 C (412-528)
mass (g)	460 ± 49 A (285-891)	932 ± 179 BC (385-2,130)	829 ± 69 AB (598-1,410)	1,308 ± 91 C (906-1,852)
age (yrs)	1.8 ± 0.1 (1-2)	2.2 ± 0.1 (2-3)	3.8 ± 0.4 (2-6)	4.0 ± 0.3 (3-5)
gonadal stage	2.6 ± 0.2 (2-3)	3.0 ± 0.0 (3-3)	2.8 ± 0.1 (2-3)	3.0 ± 0.0 (3-3)
Hormone concentrations[d]				
plasma E_2 (pg/ml)	532 A 377-752 (109-851)	1,510 B 1,050-2,173 (791-3,750)	566 A 367-873 (104-853)	1,191 B 861-1,644 (536-2,539)
plasma 11-KT (pg/ml)	1,183 A 752-1,862 (244-2,601)	385 B 267-555 (169-824)	993 A 564-1,750 (149-2,409)	416 B 274-634 (138-951)
E_2/11-KT[e]	0.49 ± 0.1 A (0.21-0.95)	5.9 ± 1.6 B (1.0-16)	0.63 ±0.1 A (0.28-1.4)	3.4 ± 0.6 B (1.3-6.6)

[a] Physical characteristics are reported as the arithmetic mean ± standard error and range.
[b] Length above 300 mm for males and 430 mm for females is considered mature (Panek 1987).
[c] Results of pairwise comparisons are displayed using capital letters. Two means within a row are significantly different ($P < 0.05$) if they do not share at least one capital letter.
[d] Hormone concentrations are reported as the geometric mean, 95% confidence interval, and range unless otherwise noted. Reported means and 95% confidence limits are antilogarithms of transformed ($\log_{10}$) data (Sokal and Rohlf 1981).
[e] The E_2/11-KT ratio results are reported as the arithmetic mean ± standard error and range. Ratio data were ranked and means were compared only within sites using a Kruskal-Wallis test (Sokal and Rohlf 1981).

Regression analysis conducted to test for linear relationships among physical characteristics (length and mass) in carp to histopathological and physiological parameters indicated correlations only between GSI and length ($P < 0.001$, $r = 0.89$) and GSI and mass ($P < 0.001$, $r = 0.86$) for females collected from Finley NWR. The GSI relates the mass of the gonads expressed as a percentage of body mass. Appendix G lists the correlation coefficients and probabilities for each test.

Length of carp was different ($P < 0.001$) between males and females, and was different ($P < 0.001$) between carp from the Finley and Malheur NWRs. Likewise, mass of carp was different ($P < 0.001$) between males and females, and between sites ($P = 0.002$). Female carp were longer and heavier than males, and fish at the Malheur NWR reference site were longer and heavier than at Finley NWR. Interactive effects of sex*site were not significant for either the length ($P = 0.56$) or mass ($P = 0.98$) variable. Within sites, pairwise comparisons of length indicated differences between sexes at Finley NWR ($P = 0.004$) but not at Malheur NWR ($P = 0.06$) (Table 1). Between sites, differences in length were significant between males ($P = 0.003$), between females ($P = 0.03$), and between males at Finley NWR and females at Malheur NWR ($P < 0.001$) (Table 1). Pairwise comparisons of mass within sites indicated differences between sexes at Finley NWR ($P = 0.02$) and at Malheur NWR ($P = 0.03$), and differences between males at Finley NWR and females at Malheur NWR ($P < 0.001$) (Table 1).

Mean 17β-estradiol concentrations in females were higher ($P < 0.001$) than males, but 17β-estradiol was not different between the Finley and Malheur NWRs sites ($P = 0.60$). Interactive effects of sex*site were not significant ($P = 0.37$). Within sites, pairwise comparisons indicated 17β-estradiol concentrations were higher in females compared to males at Finley NWR ($P < 0.001$) and at Malheur NWR ($P = 0.02$) (Table 1).

Similar to 17β-estradiol, mean concentrations of 11-ketotestosterone were different between sexes with males exhibiting higher concentrations ($P < 0.001$), but 11-ketotestosterone was not different between the Finley and Malheur NWRs sites ($P = 0.82$). There were no significant interactive effects of site*sex ($P = 0.54$). Pairwise comparisons indicated 11-ketotestosterone was higher in males compared to females at Finley NWR ($P = 0.001$) and at Malheur NWR ($P = 0.03$) (Table 1).

Mean 17β-estradiol/11-ketotestosterone ratios were well above 1 for females and well below 1 for males at both sites (Table 1). Only one individual male carp at Malheur NWR exhibited a ratio exceeding 1. Within sites, hormone ratios were higher in female carp than male carp at Finley NWR ($P<0.001$) and at the reference site ($P < 0.001$) based on the Kruskal-Wallis test (Table 1). Hormone ratios between sites were not statistically compared.

Very few OC pesticides were detected in carp samples from the refuge sites. Concentrations were all below detection limits in fish from Malheur NWR reference site, and only DDE was detected at the detection limit in the samples from Finley (Table 2). Likewise, no PCBs were detected in fish from the two refuge sites (Table 2).

Table 2. Contaminant concentrations (µg/g wet weight) in whole body, single composite carp samples collected from two national wildlife refuges (NWRs) in 1998 and from two locations in the Willamette River in 1995. The Malheur NWR site was considered a reference site.

	Malheur NWR Double-O Pond		Finley NWR Cabell Marsh		Middle Fork Will R. Mill Race Pond		Will R. St. Johns Bridge	
	Female	Male	Female	Male	Female	Male	Female	Male
Sample Information								
lipid (%)	1.8	1.7	3.6	2.3	1.3	2.2	5.7	7.2
moisture (%)	77	78	73	75	77	77	71	73
no. fish/composite	11	12	10	10	5	5	5	5
mean length (mm) [a]	475	412	407	326	393	358	476	445
mean mass (g) [a]	1,308	829	932	460	884	631	1,648	1,218
composite mass (g)	10,255	5,520	13,081	8,291	4,419	3,157	8,240	6,092
Organic contaminants								
PCB-1242	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
PCB-1248	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.09	0.36
PCB-1254	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.10	0.42
PCB-1260	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.13	0.26
total Aroclor PCBs	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.32	1.03
p,p′-DDT	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
p,p′-DDE	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.03	0.11
p,p′-DDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.03
o,p′-DDT	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
o,p′-DDE	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
o,p′-DDD	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
alpha-BHC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
beta-BHC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
gamma-BHC	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
alpha-chlordane	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
gamma-chlordane	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
oxychlordane	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
dieldrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
endrin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
HCB	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
heptachlor epoxide	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
mirex	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
toxaphene	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
cis-nonachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
trans-nonachlor	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2. Continued.

	Malheur NWR Double-O Pond		Finley NWR Cabell Marsh		Middle Fork Will R. Mill Race Pond		Will R. St. Johns Bridge	
	Female	Male	Female	Male	Female	Male	Female	Male
Trace Elements								
aluminum	21.8	53.2	59.8	39.1	146	300	210	153
arsenic	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33
boron	11.3	6.68	30.2	29.1	2.27	2.72	<1.33	<1.3
barium	1.13	1.65	9.98	12.3	4.76	5.5	6.53	3.74
beryllium	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
cadmium	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07	0.07	0.117
chromium	0.81	0.94	0.79	0.70	0.82	0.85	0.95	0.94
copper	3.1	2.7	2.7	2.2	3.3	4.0	3.5	4.4
lead	2.3	<0.33	3.3	2.9	1.9	1.5	<0.33	2.7
iron	76.6	105	101	85.3	161	365	441	362
manganese	4.15	4.88	16.9	29.4	25.9	32.9	23.3	17.8
magnesium	1173	1433	1326	1470	1513	1638	1615	1289
mercury	<0.13	<0.13	<0.13	<0.13	<0.13	<0.13	0.24	0.18
molybdenum	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
nickel	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33
selenium	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33	<0.33
strontium	30	41	63	85	62	67	54	32
vanadium	0.40	0.39	<0.33	<0.33	0.38	0.83	0.78	0.89
zinc	241	171	241	189	284	224	224	241

[a] Mean based on the number of fish within a composite sample.

A total of 19 trace elements were analyzed in fish tissue (Table 2). Trace elements detected in all carp from the refuges included aluminum, boron, barium, chromium, copper, lead, iron, manganese, cadmium, magnesium, strontium, vanadium (detected at Finley NWR only), and zinc. Arsenic, beryllium, mercury, molybdenum, nickel, and selenium were below detection limits in fish from the refuges. Concentrations were relatively similar between samples from Finley NWR and the reference site with the exceptions of boron, barium, manganese, and strontium, which were higher at the Finley NWR site.

Western Pond Turtles. In western pond turtles, mean carapace length and total mass were not different between sexes (P=0.11 and 0.30, respectively) but were different between sites (P = 0.003 for length and P < 0.001 for mass). Turtles from the Finely NWR site had larger measurements compared to the Umpqua reference site (Table 3). Interactive effects of sex*site were not significant for length (P = 0.48) or mass (P = 0.53). Pairwise comparisons indicated that males at Finely NWR were longer (P = 0.01) than males at Umpqua, but females at Finley NWR were not different (P = 0.78) than females at Umpqua (Table 3). Likewise, males at Finley

NWR were heavier (P = 0.003) compared to males at Umpqua, but no differences (P=0.33) were found between females at Finley NWR and Umpqua (Table 3). Average age of turtles was similar between sites, although turtles could not be reliably aged above 16 years (Table 3).

Table 3. Physical characteristics and concentrations of plasma hormones 17β-estradiol (E_2) and testosterone (T) from western pond turtles collected in 1998 from William L. Finley National Wildlife Refuge (NWR) and the South Umpqua River reference site.

	William L. Finley NWR		South Umpqua River reference	
	Male	Female	Male	Female
Sample size	11	6	16	8
Physical characteristics[a]				
carapace length (mm)	181 ± 5.3 A (154-218)	168 ± 7.8 AB (133-188)	160 ± 3.6 B (129-182)	154 ± 6.1 B (131-181)
mass (g)	908 ± 90 A (590-1,710)	789 ± 92 AB (370-1,000)	595 ± 39 B (305-820)	565 ± 47 B (385-790)
age (years)[c]	13[d] (7-16+)	14[d] (9-16+)	14 (9-16+)	15 (11-16+)
Hormone concentrations[e]				
plasma E_2 (pg/ml)	646 A 564-740 (475-865)	553 A 417-733 (473-951)	585 A 499-685 (252-827)	668 A 418-1,069 (364-1,334)
plasma T (pg/ml)	1,222 A 998-1,496 (816-2,219)	676 AB 449-1,019 (324-915)	944 A 622-1,429 (221-2,952)	294 B 199-435 (211-910)
E_2/T[f]	0.58 ± 0.1 A (0.22-1.1)	0.92 ± 0.2 A (0.54-1.7)	0.76 ± 0.1 A (0.19-1.7)	2.6 ± 0.6 B (1.5-5.3)

[a] Physical characteristics are reported as the arithmetic mean ± standard error and range.
[b] Results of pairwise comparisons are displayed using capital letters. Two means within a row are significantly different (P < 0.05) if they do not share at least one capital letter.
[c] Age was determined by counting concentric ridges on scutes and was reported as 16+ for turtles older than 16 years.
[d] Sample size of four individuals for age determination at this site.
[e] Hormone concentrations are reported as the geometric mean, 95% confidence interval, and range unless otherwise noted. Reported means and 95% confidence limits are antilogarithms of transformed ($\log_{10}$) data (Sokal and Rohlf 1981).
[f] The E_2/T ratio results are reported as the arithmetic mean ± standard error and range. Ratio data were ranked and means were compared only within sites using a Kruskal-Wallis test (Sokal and Rohlf 1981).

Concentrations of 17β-estradiol in western pond turtle plasma were not different between sexes ($P = 0.93$) or between sites ($P = 0.69$). In contrast, differences in testosterone concentrations were significant between males and females ($P < 0.001$) and between turtles from the Finley NWR site compared to the Umpqua reference site ($P = 0.007$) with turtles from Finley NWR having higher values. Interactive effects of sex*site were not significant for 17β-estradiol ($P = 0.22$) or testosterone ($P = 0.14$). Pairwise comparisons indicated that testosterone concentrations were higher ($P < 0.001$) in male turtles at Umpqua compared to female turtles at Umpqua, and concentrations in male turtles at Finley NWR were higher ($P < 0.001$) than female turtles at Umpqua (Table 3). Other pairwise comparisons were not significant ($P > 0.05$), although probability values ($P = 0.07$) were suggestive of higher testosterone in females at Finley NWR compared to females at Umpqua (Table 3).

Mean 17β-estradiol/11-ketotestosterone ratios were below one for male turtles at both sites and well above one for female turtles at Umpqua (Table 3). One male turtle from Finley NWR (17%) had a ratio slightly above one, and six (38 %) male turtles from Umpqua had ratios slightly exceeding one. The mean ratio was below one for females at Finley NWR, and two individual females (33%) at the site were well above one. In contrast, no female turtles collected at Umpqua had a ratio below one (Table 3). Within sites, hormone ratios were not different ($P = 0.23$) between male and female turtles from Finley NWR, but were different ($P < 0.001$) between males and females at Umpqua based on the Kruskal-Wallis test (Table 3). Hormone ratios between sites were not statistically compared.

Western pond turtle physical characteristic regressed against $\log_{10}$ transformed hormone values and gonadal measures revealed significant correlation only in males collected at the Umpqua site; estrogen was correlated to mass ($P = 0.029$, $r = 0.55$) and carapace length ($P = 0.02$, $r = 0.58$). Appendix G lists the correlation coefficients and probabilities for each test.

Carp from Non-refuge Sites. Carp collected from the Willamette River at St. Johns Bridge and from the Mill Race Pond site exhibited significant site and gender differences in mass and length (Table 4). Female carp were heavier ($P = 0.01$) and longer ($P = 0.02$) compared to male carp, and fish from the St. Johns Bridge were heavier ($P < 0.001$) and longer ($P < 0.001$) than fish from the Mill Race Pond site. Interactive effects of sex*site were not significant for either the length ($P = 0.89$) or width ($P = 0.48$) variable. Between sites, pairwise comparisons indicated that differences in length were significant between males ($P = 0.001$), between females ($P = 0.002$), and between males at Mill Race Pond and females at St. Johns Bridge ($P < 0.001$) (Table 4). For mass, pairwise comparisons indicated a difference for males ($P = 0.02$) and for females ($P = 0.002$) between sites (Table 4). Females at St. Johns Bridge were also heavier ($P < 0.001$) than males at Mill Race Pond (Table 4).

Age data were not collected on fish from the St. Johns Bridge and Mill Race Pond sites. All male fish collected from the two sites exceeded the size associated with mature adults (300 mm), and four of the five females exceeded the target length of 430 mm indicating maturity.

Table 4. Physical characteristics, plasma hormone concentrations (17β-estradiol [E_2], 11-ketotestosterone [11-KT]), and the egg-yolk protein vitellogenin in plasma from common carp collected from the Willamette River at St. Johns Bridge and the Middle Fork Willamette River at Mill Race Pond.

	Willamette River at St. Johns Bridge		Mill Race Pond	
	Male	Female	Male	Female
Sample size	5	5	5	5
Physical characteristics[a]				
length (mm)[b]	445 ± 9.8 AB[c] (416-477)	476 ± 16 A (428-509)	358 ± 8.4 C (336-380)	393 ± 16 BC (345-442)
mass (g)	1,218 ± 60 AB (999-1,360)	1,648 ± 199 A (1,052-2,209)	631 ± 34 C (547-714)	884 ± 121 BC (550-1,213)
age (years)	NC[d]	NC	NC	NC
gonadal stage	NC	NC	2.4 ± 0.4 (1-3)	2.8 ± 0.2 (2-3)
Hormone concentrations[e]				
plasma E_2 (pg/ml)	506 A 265-964 (215-771)	392 A 228-673 (249-687)	224 A 144-349 (121-299)	412 A 213-783 (259-846)
plasma 11-KT (pg/ml)	1,469 A-I 589-3,664 (695-4,310)	759 AB-I 505-1,140 (487-1,124)	344 B-I 145-817 (149-775)	608 AB-I 791-469 (459-744)
E_2/11-KT[f]	0.44 ± 0.1 A (0.10-0.68)	0.64 ± 0.2 A (0.29-1.4)	0.79 ± 0.3 A (0.30-1.8)	0.77 ± 0.2 A (0.39-1.4)
vitellogenin[f] (mg/ml)	0.0 ± 0.0 (0.0-0.0)	39.9 ± 13 (2.66-66.7)	0.0 ± 0.0 (0.0-0.0)	27.3 ± 4.5 (11.9-39.6)

[a] Physical concentrations are reported as the arithmetic mean ± standard error and range.
[b] Length above 300 mm for males and 430 mm for females is considered mature (Panek 1987).
[c] Results of pairwise comparisons are displayed using capital letters. Two means within a row are significantly different ($P<0.05$) if they do not share at least one capital letter. An "I" indicates interaction of sex*site was significant and therefore confounded interpretation of main effects.
[d] NC=data not collected.
[e] Hormone concentrations are reported as the geometric mean, 95% confidence interval, and range unless otherwise noted. Reported means and 95% confidence limits are antilogarithms of transformed (log_{10}) data (Sokal and Rohlf 1981).
[f] The E_2/11-KT ratio and vitellogenin results are reported as the arithmetic mean ± standard error and range. Ratio data were ranked and means were compared only within sites using a Kruskal-Wallis test (Sokal and Rohlf 1981).

In contrast, only one female from the Mill Race Pond site was above the length indicating maturity. However, the gonadal stage of this fish indicated it was sexually mature. The gonadal stages of male fish from Mill Race Pond were primarily stage 3, with one male at stage 1 and another at stage 2 (Table 4). All female fish from Mill Race Pond were at gonadal stage 3 except for one individual that was at stage 2. Females at stage 3 are typical of fish approaching spawning, although stages 1 to 3 are all representative of sexually mature female fish. Gonadal stage information was not collected for carp from the St. Johns Bridge site.

Regression analysis conducted to test for linear relationships among variables of hormones (log_{10} transformed) and physical characteristics for carp from the Willamette River-associated sites yielded no correlations. Appendix G lists the correlation coefficients and probabilities for each test.

Levels of 17β-estradiol and 11-ketotestosterone were not different (P = 0.41 and 0.85, respectively) between sexes at the St. Johns Bridge and Mill Race Pond sites (Table 4). Concentrations of 17β-estradiol were not significant (P = 0.08) in carp (sexes combined) from the St. Johns Bridge compared to Mill Race Pond, but concentrations were different (P = 0.003) between these two sites for 11-ketotestosterone. However, interaction of sex*site was suggestive of significance for 17β-estradiol (P = 0.05) and was significant for 11-ketotestosterone (P = 0.02) which could have influenced results. Pairwise comparisons of 11-ketotestosterone indicated males at St. Johns Bridge had higher (P = 0.004) concentrations than males at Mill Race Pond (Table 4). Other comparisons were not significant.

Mean 17β-estradiol/11-ketotestosterone ratios were all below one for both male and female carp at the Mill Race Pond and St. Johns Bridge sites (Table 4). Females exhibited lower 17β-estradiol and/or higher 11-ketotestosterone at both sites than would be expected for normal, sexually mature adults. Within sites, hormone ratios were not different between males and females at the St. Johns Bridge site (P = 0.47) and at the Mill Race Pond site (P = 0.92) based on the Kruskal-Wallis test (Table 4). Hormone ratios between sites were not statistically compared.

Vitellogenin was not detected in male fish from either of the Willamette River-associated sites. All female fish from these sites exhibited concentrations of vitellogenin.

In contrast to carp sampled from the refuge sites, concentrations of some organochlorine compounds in samples from one of the non-refuge sites were well above detection (Table 2). DDE and DDD were both detected in male and female fish from the St. Johns Bridge site. At this site, DDE was three to ten times higher in carp compared to the detection limit or to concentrations at the other sites (Table 2). Differences were also apparent between gender; male carp exhibited DDE concentrations more than three times greater than in females at the St. Johns Bridge site. Aroclor PCBs also were elevated in male and female carp from the St. Johns Bridge site (Table 2). Aroclor 1254 was the most elevated, ranging up to 0.42 µg/g in male carp. Similar to the DDE results, all Aroclor PCBs were at least double the concentration in the male

composite sample compared to the female composite sample (Table 2). No organochlorine compounds were detected in fish from the Mill Race Pond site (Table 2).

Trace elements detected in carp from the non-refuge sites were similar to those found in refuge carp, with the addition of mercury found in carp from the St. Johns Bridge site ranging up to 0.24 µg/g (Table 2). Concentrations of most trace elements were similar to the refuge sites with the exceptions of aluminum, iron, mercury, and vanadium, which were much higher in samples from the St. Johns Bridge location (Table 2).

Discussion

Common Carp. Based on a limited evaluation of sex steroid hormones in blood plasma, carp at Finley NWR showed no evidence of endocrine disruption. The differences in hormone levels observed between sexes were expected and considered normal, and no differences were observed in hormone values from the Finley NWR site when compared to reference carp. Carp hormone ratios were considered normal (males primarily below one and females above one) at both sites.

In contrast to the results in carp from the refuge sites, carp collected at the non-refuge sites (Mill Race Pond in Springfield and Willamette River at St. Johns Bridge) showed altered hormone levels. No differences in 17β-estradiol levels were observed between male and female carp, whereas healthy females would be expected to have significantly higher 17β-estradiol levels than males. Females at the St. Johns Bridge and Mill Race Pond sites appear to have lower 17β-estradiol levels when compared to values for the reference female fish at Malheur NWR, whereas males from the non-refuge sites have 17β-estradiol levels more similar to the male fish from the refuge-reference site. Similarly, the 11-ketotestosterone levels were not different between sexes in contrast to what is considered normal for adult fish, although interactive effects between sex and site complicated this interpretation. The data indicate that males at the Mill Race Pond site have unexpectedly low 11-ketotestosterone levels, and female fish at both non-refuge sites appeared to have higher 11-ketotestosterone compared to reference fish at Malheur NWR. Moreover, female carp from both the Mill Race Pond and St. Johns Bridge sites exhibited mean hormone ratios well below one and hormone ratios within sites were not different between sexes; these conditions would be abnormal for healthy adult females. The evidence of endocrine disruption at St. Johns Bridge and Mill Race is indicative of exposure to antiestrogenic (low 17β-estradiol/11-ketotestosterone ratios in females) or antiandrogen (low 11-ketotestosterone at Mill Race) compounds rather than xenoestrogens (no vitellogenin detection in male carp at non-refuge sites) as vitellogenin induction in male fish would have indicated exposure to xenoestrogens. A confounding factor in this analysis is that non-refuge carp were captured early in the reproductive cycle and possibly just after spawning, so hormone values could have been influenced differently than the refuge fish which were captured during a time of gonadal recrudescence. Additional fish from the non-refuge sites would need to be collected before reliable comparisons to refuge fish can be made.

Hormone levels in fish not exposed to any endocrine-disrupting compounds can exhibit considerable natural variation, even within the same period of the reproductive cycle, and exacting a cause related to chemically-altered hormone levels requires intensive effort and examination of multiple parameters. Folmar (1993) found significant variability in hormone values based on species, age or size, and time of year the samples were collected in reviewing effects of organic and inorganic chemical contaminants on serum chemistry and hematology of teleost fish. In the laboratory, large variation was noted in serum measurements of feral fish sampled in the field compared to those fish collected from the same location but maintained in the laboratory and fed a standard ration of food (Folmar 1993). In addition to the natural variability of hormone levels in fish, other factors unrelated to chemical exposure such as stress could alter the levels of reproductive hormones in plasma (McMaster et al. 1994, Barton and Iwama 1991).

In our study, we controlled for variation in hormone levels occurring naturally, and variation introduced during handling, by comparing a potentially chemically-exposed population of carp at Finley NWR to carp at a reference site that were collected during the same season and handled under the same procedure. Other sources of variation introduced into our study were differences between sites in size and age of carp, water temperature, and capture techniques (electroshocking versus seining). These factors could influence hormone levels and disguise variations due to chemical exposure. However, size and age differences were not considered to affect hormone levels at the Malheur and Finley NWRs sites because gonadal stages were similar among sites and there were no correlations between hormone levels and size measurements for these carp. Water temperature was the variable with the greatest uncertainty between the sites. Although temperatures were not measured at the time of fish collection, subsequent monitoring conducted in August 2004 in Double-O Pond indicated the water temperature was 17.8 °C (Rick Roy, Supervisory Wildlife Biologist, Malheur NWR, Princeton, Oregon, pers. comm., 2004), which is within the range (13 to 19 °C) of water temperatures at four of the Finley NWR sample sites in August 1998. However, water temperatures at the reference site are likely to be colder all year because the pond is spring-fed, and reproductive cycles and hormone values of carp could have been influenced by temperature compared to carp at the Finley NWR site.

As described in the introduction to this section, hormone levels can be altered by contaminant concentrations. Contaminant concentrations in Finley NWR carp were primarily below detection or similar to values from the reference carp at Malheur NWR. The value of DDE at the detection limit in carp from Finley NWR could result from historical use of DDT at agricultural areas near the Finley NWR, but the relatively low value suggests there are no longer distinct sources of organochlorine contaminants in the area. Carp at the refuge sites had much lower lipid values than the non-refuge carp, and the refuge carp may not have bioaccumulated organochlorines to the extent of the carp at the more contaminated, non-refuge site which were older and had higher lipid values. Most trace elements were at similar concentrations in fish between the refuge sites except for boron and barium which were much higher in carp from Finley NWR. Boron levels at Finley NWR are also higher than levels found in fish from other areas (Eisler 1990). However,

toxicity data for boron and barium based on tissue concentrations are too limited to determine if effects would be expected at the observed concentrations.

The highest concentrations of organochlorine compounds were found in carp from the St. Johns Bridge site in the lower Willamette River. Concentrations of DDE and PCBs were well above detection limits in carp from this site, but no other organochlorine compounds were detected in these fish. Carp at the St. Johns Bridge site also had the highest lipid values and were larger and older than carp from the refuge sites, and older fish typically accumulate greater amounts of bioaccumulative contaminants. Male carp from the site contained higher contaminant concentrations than females, which was likely due to their much higher lipid content. It is unknown why male carp exhibited higher lipid values than females, although female carp could have eliminated some contaminants while using lipid reserves during reproduction and shedding of eggs.

Similar to results from previous studies on fish in the lower Willamette and Columbia Rivers (Schmitt et al. 1990, U.S. Environmental Protection Agency 1992, Tetra Tech 1994, U.S. Fish and Wildlife Service 2004), concentrations of DDE and PCBs in carp were the most elevated contaminants. This lower section of the Willamette River is part of the Portland Harbor Superfund Site, and contaminants such as organochlorine compounds previously have been documented in sediment and biota. Whole body, composite carp sampled from the lower Willamette River between RM 3 to 6 (St. Johns Bridge is at RM 6) in 1990 contained 0.23 µg/g total PCBs (U.S. Fish and Wildlife Service 2004), which is similar to values found in our 1995 study of carp. However, concentrations of DDE in our 1995 study of carp were lower than concentrations of 0.27 µg/g in carp collected in 1990 (U.S. Fish and Wildlife Service 2004) and lower than concentrations (0.33 µg/g) estimated in whole body, composite carp sampled near Portland from 1987 to 1989 by the Environmental Protection Agency (1992). Total PCB concentrations (reported as Aroclor 1254) in male carp from St. Johns Bridge were seven times higher than the value of 0.06 µg/g estimated by the U.S. Fish and Wildlife Service (2004) to be protective of bald eagles in the lower Columbia River, and were nearly four times higher than the New York State Department of Environmental Conservation (NYSDEC) value of 0.11 µg/g considered protective of piscivorous fish and wildlife (Newell et al. 1987). Concentrations of DDE in male carp were nearly three times higher than the value of 0.04 µg/g estimated for the protection of Columbia River bald eagles consuming fish (U.S. Fish and Wildlife Service 2004) but were about half the NYSDEC value of 0.11 µg/g. No other carp samples from other refuge or non-refuge sites outside of the St. Johns Bridge site exceeded the estimated protective values for fish and wildlife for organochlorine compounds.

Some trace elements were higher in carp from the St. Johns Bridge site compared to the other sites, which likely represents the more industrialized section surrounding the St. Johns area. Mercury in the 1995 carp samples was much greater than found in carp sampled in 1990 in the Willamette River near Portland, where concentrations were below detection limits (U.S. Fish and Wildlife Service 2004). Mercury concentrations in male and female carp (0.18 and 0.24 µg/g, respectively) from the St. Johns Bridge site exceeded concentrations in food items of 0.1 µg/g

considered protective of fish-eating birds (Eisler 1987). Mercury, a potential endocrine disruptor (Colborn et al. 1993, Keith 1997), could influence hormone levels in the St. Johns Bridge fish. However, the concentration at which mercury would influence hormone concentrations is unknown and would require further study.

Although too few data points were available to relate contaminant concentrations to hormone levels, our study results indicated that evidence of endocrine disruption was only found in carp associated with known contaminated areas. In contrast, fish from the refuge sites did not exhibit abnormal hormone levels and had contaminants in tissue at concentrations at or below detection. In addition, the hormone ratios in refuge carp did not reflect differences between the sites, and histopathology (see next section in this chapter) indicated no abnormal tissue changes that were suggestive of endocrine disruption. Although size and age of fish can affect hormone levels (Folmar 1993) and were different between refuge sites, these factors were not thought to confound the results because they were not correlated to hormone values. Additional evaluation of non-refuge fish during a period of recrudescence would be needed to determine if the lowered 17β-estradiol and possibly higher 11-ketotestosterone in female fish are associated with contaminants observed in sediment at these sites.

Western Pond Turtles. Evidence of possible endocrine disruption was observed in turtles at Finley NWR compared to a reference site. Levels of 17β-estradiol were not different between males and females, and female turtles from the Finley site had significantly higher testosterone levels than female turtles from the reference site. The higher testosterone values in females also were reflected in the atypically low hormone ratio for the female turtles at Finley NWR compared to the reference turtles. In some species of turtles, altered or decreased levels or circulating sex steroid hormones have been correlated with reproductive impairment (Colborn and Clement 1992, Mayer et al. 1992) and can result in incomplete or improper gonadal development (Hileman 1994). In addition, hormone application directly to turtle eggs has been shown to influence the sex in what otherwise are temperature-dependent, sex-determined reptiles (Gutzke and Bull 1986, Bull et al. 1988, Crews et al. 1990). This latter effect is primarily a result of inhibiting the enzyme aromatase. Aromatase converts androgens such as testosterone to estrogens (testosterone is the precursor for both 11-ketotestosterone and 17β-estradiol), and is the primary steroidogenic enzyme in the sex-determining pathway. Aromatase activity exhibits temperature dependence in reptiles such as the red-eared slider (*Trachemys scripta elegans*), and inhibiting aromatase can cease production of estrogens and affect sex determination in this species (Willingham and Crews 2000). Turtle data from our study did not indicate that estradiol levels were increased in males or females from either study site. It is unknown what effects would be exhibited in western pond turtles from altered hormone levels because the specific responses of higher testosterone in females has not been reported.

Altered hormone levels in reptiles also has been associated with a number of contaminants. Willingham et al. (2000) found xenobiotic treatment during embryonic development in red-eared slider turtles significantly influenced plasma testosterone levels. Testosterone levels were significantly lower in chlordane-treated females compared to control females while exposure to

PCBs (as Arochlor 1242) decreased concentrations of testosterone in both males and females. The authors postulate that Aroclor 1242 may increase aromatase activity because of observed increases in 17β-estradiol concentrations in males, thereby decreasing the concentration of testosterone. Pesticide compounds applied to turtle eggshells during development also have been implicated in shifting the sex ratio outcomes (Willingham and Crews 2000). For example, embryonic exposure of red-eared slider turtles to the pesticides p,p'-DDE and chlordane has been shown to alter expected sex outcomes (Willingham and Crews 2000) and hatchling steroid physiology and growth rates (Willingham 2001). Little work has been conducted on contaminant effects in western pond turtles in the Northwest, but Henny et al. (2003) analyzed eggs of western pond turtles from western Oregon for 20 organochlorine pesticides or metabolites, 42 congener-specific PCBs, and 16 trace elements and metals. Concentrations of organochlorine pesticides and PCBs were generally low and similar to those found in eggs of snapping turtles (*Chelydra serpentina*) from a remote reference site in Ontario, Canada (Bishop et al. 1998). Henny et al. (2003) did not find any relationship between contaminant concentrations and nest success or failure.

Although the turtle hormone data indicate abnormal hormone ratios for female turtles at Finley NWR, a number of factors may confound the results. First, the reference turtles did not exhibit sexual differences in estrogen levels, so these turtles may not be suitable for comparing normal estrogen levels. Second, sex determination in turtles was primarily based on secondary sexual characteristics and not by examining internal organs, and the secondary sex characteristics could be influenced by abnormal hormone levels and lead to misidentification of turtle sex. Secondary sex characteristics visible in the western pond turtles are based upon differences in the plastron, carapace, tail, coloration, and morphological differences in the rostrum (Holland 1994, Todd 1999). The characteristics that distinguish the sexes generally become more obvious with increasing size (Holland 1994). A small percentage (as high as 1 to 2% occasionally) of animals in any given population may display characteristics that are intermediate between the sexes and, in most cases, are females (Holland 1994). Also, an experiment conducted on snapping turtles indicates that environmental contaminants may affect sexually dimorphic morphology without affecting circulating testosterone or estrogen levels in adults (de Solla et al. 1998). Researchers found concentrations of organochlorine contaminants higher in the blood plasma of snapping turtles from contaminated sites compared to reference sites, and found the ratio of the precloacal length to the posterior lobe of the plastron (PPR), a sexually dimorphic characteristic, was significantly reduced at three contaminated sites versus two reference sites. A significantly higher proportion of PPRs from males from a contaminated site overlapped with females from a reference site. Therefore, identifying sexes based upon this secondary sex characteristic could give erroneous results. Because exposure to exogenous chemicals may alter these secondary sex characteristics, we cannot be certain, without sacrificing turtles to examine internal organs or conducting genetic analysis, as to the sex of the turtles used in our study.

Although significant differences in circulating hormone levels were observed in turtles, definitive conclusions cannot be made without additional investigation into possible causes. Turtle reproductive organs were not examined histopathologically, therefore we do not know if there

were any signs of abnormality associated with the different hormone levels. Consequently, it remains unknown whether or not the elevated testosterone in female turtles from Finley NWR has any effect on breeding success or reproduction. Additional turtle samples are needed to determine if the sex ratios observed in turtles are greater than would be explained by normal variation.

Fish Health Assessment

Introduction

Lewis (1998) defines health as the condition or overall state of being of an organism when it functions normally with no evidence of disease or persistent distress. If exposed to a stress of certain magnitude, fish may respond physiologically (Wedemeyer and McLeay 1981). Physiological responses to stressors (including chemicals) at the organ or organism level can be detected through observations of gross external abnormalities and condition indices and often represent an advanced condition (Blazer et al. 2002). Methods of varying complexity and specificity are available for assessing the health of an organism and are increasingly being used to assess environmental conditions. An example is the USGS's BEST Program where fish health is an important component in their nationwide assessment of large river basins. In the BEST report for the Columbia River Basin, numerous studies are cited which correlate a high prevalence of external anomalies (fin erosion, tumors, and skeletal deformities) with exposure to anthropogenic stressors (Hinck et al. 2004). Findings in fish from the Columbia River Basin BEST Program indicate that fish throughout the basin were exposed to a variety of contaminants. Waite and Carpenter (2000) used a simplified approach of investigating fish health by including external examination for abnormalities in their study of fish assemblages for the USGS NAWQA work. The authors found a high percentage of external abnormalities which correlated with high water temperature, low dissolved oxygen concentration, and low physical habitat diversity. Increased concentrations of nutrients and pesticides were often interrelated with physical habitat degradation, increased water temperature, and increased algal production.

The objectives of the fish health assessment aspect of this study were to: 1) evaluate the presence of gross abnormalities with visual observation in a necropsy-based examination; and 2) obtain general health conditions through measurements of length, mass, and organ mass.

Methods

Health assessments were conducted on fish collected at Finley and Malheur NWRs, but not on the carp collected in 1995 at the non-refuge sites. Fish were collected as described under the Endocrine Biomarkers section above, and were held in a live well for up to 2 hours while awaiting processing. Fish health assessments were conducted according to the procedure developed by the USGS for the BEST Program, which were based on methods developed by Goode (1989) as modified by Schmitt et al. (1999). Fish were visually examined internally and

externally for anomalies (e.g., lesions, parasites, tumors) before specific organs were excised and weighed. Information and tissues collected for the assessment included: 1) length and mass measurements to determine condition factor and organo-somatic indices; 2) blood plasma to determine sex steroid concentrations; 3) pieces of gill, spleen, liver, gonads, and kidney for histopathological examination; 4) scales near the lateral line to determine age of the fish; and 5) whole body composite samples for chemical analysis. For whole body chemical analysis, the remaining organs that had been removed for examination and histopathological sampling were placed back into the abdominal cavity of individual fish before the carcass was wrapped in aluminum foil (dull side contacting fish) and placed in double plastic bags. Results of the chemical analysis were reported in the previous section.

Histopathology. The entire spleen and a small section of gill, liver (five pieces), gonads (five pieces), and head and hind kidney were collected in the field placed in 10% neutral buffered formalin and shipped to the USGS's National Fish Health Research Laboratory in Leetown, West Virginia. Pieces of tissue were processed for routine histopathological slide preparations. Slices of the tissue pieces were placed in cassettes, dehydrated in 100% alcohol, and infiltrated with paraffin. Blocks were cut from the paraffin using a rotary microtome and 5 µm sections were placed on a glass slide and stained with hematoxylin and eosin. A more detailed description of this method is provided in Blazer et al. (2002).

Microscopic examination of these tissue for lesions provides evidence of chemical exposure and relates to overall organism health. Each tissue was examined for abnormalities, including inflammation, parasites, preneoplastic foci in the liver, and tubular necrosis. Each abnormality was rated as 1 (present) or 0 (absent). Gonads were staged following the BEST protocols (Schmitt and Dethloff 2000) which describe six developmental stages for females and four maturational stages for males. The stages are based on the size and developmental status of the oocytes (ovaries) and spermatozoa (testes).

Macrophages are important for the immune system as a first line of defense for the organism and as an antigen processing cell, and can be impacted by many contaminants. Macrophage aggregate analysis was conducted on spleen, hemopoetic kidney, and liver tissue. More detailed information on the method is described in Blazer et al. (2002).

Age. Fish collected in 1998 were aged by counting annuli on scales (DeVries and Frie 1996). Scales were collected from carp beneath the anterior portion of the dorsal fin, above the lateral line on the left side of the fish unless scales were missing or damaged, in which case the right side was used. Several scales were placed into a small envelope and allowed to air dry. Scales were shipped to the U.S. Fish and Wildlife Service, New Mexico Fishery Resource Office, Albuquerque, New Mexico, for aging. Age was not determined for fish collected by USGS in 1995.

At the Fishery Resource Office, scales were soaked in a 70% ethanol solution to clean debris accumulated during collection and processing, to increase transparency, and to soften scales for

flattening. Scales were read on a Microfiche reader Model 835. All scales in the sample were examined and the best scale was used by individuals determining age (agers). Regenerated scales were not aged because they do not contain circuli present in the original scales. Two agers were used on all samples except in the case of discrepancy, in which case a third ager examined the scale. One male fish sample from the Malheur NWR required right side scale reading because left side sample scales were all regenerated.

Results

Histopathology. Carp collected at Malheur NWR were about twice as old and significantly larger than those collected at Finley NWR. This seriously complicates data comparisons between sites for a number of morphological and histological measurements. For instance, larger GSIs for each sex at Malheur NWR may simply be due to age rather than any other factors. Also, since macrophage aggregates are known to be age-dependent they were not measured due to the age differences.

In general, there were no histological findings indicating health issues at either site. No neoplastic lesions were observed. Based on liver observations, more of the carp at Finley NWR had altered foci and inflammatory lesions, despite being younger. Altered foci are areas of hepatocytes that stain differently from surrounding ones. In some species of fish there have been suggestions that some of these altered foci are preneoplastic (a growth of tissue, possibly a tumor), however, this has not been evaluated in carp. Many of the inflammatory lesions in fish from Finley NWR consisted of pigmented macrophages (cells important for immune response) around vessels, and the interrenal (kidney) tissue at this site appeared hyperplastic (excessive development of normal tissue due to increased rate of cell division) and vacuolated (one or more cavities in the tissues containing air or liquid).

External and Internal Examination. External abnormalities were found in a relatively small percentage of the common carp examined (Table 5). The most prevalent condition observed was fraying of the fins, which results from numerous causes. At both sites, males had a higher incidence of fatty livers than females. Body abnormalities were slightly higher in the carp from Malheur NWR than from Finley NWR which may be due to the fact that carp collected at Malheur NWR were considerably older and significantly larger than those collected at Finley NWR.

Discussion

Necropsy-based assessments are conducted with minimal equipment and expense to provide a rapid indication of the health of the population being studied. These tests are not intended to be diagnostic but rather to alert investigators to possible problems that should be investigated with more specific tests. Our objectives were to use internal and external fish examination techniques to estimate stress, and, in concert with the other biomarkers in this study, to assess environmental conditions.

Table 5. Percentage of abnormal conditions[1] observed in common carp collected from Finley National Wildlife Refuge (NWR) and the reference site at Malheur NWR.

	Malheur NWR		Finley NWR	
	Males (n = 10)	Females (n = 10)	Males (n = 12)	Females (n = 11)
Body	20% lesions	40% hemorrhage	8% hemorrhage	18% lesions
Eyes	10% opaque spot	0	0	0
Opercles	10% hemorrhage	10% tumor	0	9% wound
Gills	10% pale 10% frayed	10% pale	0	9% eroded
Fins[2]	50% frayed 10% spotted	50% frayed 10% lesion 10% injury	50% frayed 42% hemorrhage 8% nodules	45% frayed 9% erosion 9% bumps 18% hemorrhage
Liver	90% fatty	40% fatty 10% discolored	83% fatty	9% fatty 9% discolored
Spleen	0	0	0	0
Kidney	30% mottled	20% mottled	8% mottled	18% mottled 18% swollen

[1] Classification of conditions adapted from USGS's Biomonitoring of Status and Trends (BEST) classifications (Blazer et al. 2002, Blazer 2000).

[2] Multiple fin abnormalities may have been observed on the same fish, such that 60% abnormalities for male fish from Malheur NWR does not necessarily equate to 60% of the fish.

Interpretations of the GSI measurements rely on a thorough understanding of the natural variability among fish of the same age, sex, and species, as well as the environmental influences, behavioral patterns, and reproductive strategies that may complicate or confound data. Larger gonadosomatic indices for each sex at Malheur NWR may simply be due to age because various fish species undergo gradual reproductive senescence (Patnaik et al. 1994). Both testes and ovaries can undergo changes with age that may be relatively subtle and not obviously apparent in histological examination but which account for the differences observed in the GSI. Another confounding factor is that several environmental factors might influence the rate of the aging process in fish, including nutrition, temperature, photoperiod, radiation, and chemical agents (Patnaik et al. 1994).

Visible lesions generally include fin erosion, skin ulcers, eye disorders, visible tumors, and skeletal deformities. Blazer (2000) notes that laboratory studies have demonstrated gross lesions can be induced by contaminant exposure and field studies have shown that fish in severely-polluted areas have a higher frequency of gross lesions than similar, less-polluted habitats. Most often the development of these grossly-visible lesions is the result of interacting conditions including susceptible hosts, stressors, and infectious disease organisms. Fish from Malheur NWR, the reference site, had a greater percentage of noted abnormalities than those from Finley NWR. Again, this may be due to the age difference. Fish from Finley NWR appear to have a relatively small portion of abnormal conditions noted and appear to be in relatively good health.

SUMMARY AND CONCLUSIONS

The Willamette Valley NWR Complex supports numerous fish and wildlife species and provides foraging habitat for wintering Canada geese. Much of the land within the refuge is managed for grass seed production involving applications of herbicides, fungicides, and fertilizers. Other agrochemicals such as insecticides are applied to agricultural land outside the refuge. Some agrochemicals used in the area have the potential to enter aquatic areas of the refuge and could impact species such as amphibians, turtles, or the federally-listed Oregon chub. The objective of this investigation was to sample both biotic and abiotic matrices and use a weight-of-evidence approach to determine if agrochemicals used on or around the refuge pose a risk to aquatic species.

The following specific components of the investigation were conducted to meet the objective: 1) evaluating pesticide use practices on and around the refuge; 2) collecting continual water quality measurements over the pesticide application season; 3) sampling water for pesticides and nutrients; 4) evaluating water using an *in-situ* bioassay; 5) collecting blood plasma from carp and turtles to examine exposure to endocrine-disrupting compounds; 6) conducting a health assessment on common carp from agricultural and non-agricultural sites; and 7) analyzing carp tissue for the presence of organochlorine pesticides.

The components of the study were used as individual lines of evidence to evaluate risk of agrochemicals to aquatic organisms on the refuge. Because ecological outcomes are extremely difficult to predict with a high degree of confidence (even with abundant data collected over multiple years), one or two lines of evidence is usually insufficient to reach conclusions regarding risk to a population or community (Fairbrother 2003). Complex environments make predictions less reliable as well. Multiple lines of evidence create more confidence in making decisions suitable for refuge management because the approach considers all the information gathered from the investigation. A weight-of-evidence approach is important in evaluating the risk to individuals, populations, communities, and to better assess the ecosystem. In the regulatory arena of protecting aquatic life from chemicals in the water, risk is based on the

population level. When addressing threatened or endangered species, concern usually lies with an individual. By framing the assessment with this weight-of-evidence approach, risk to the receptors of concern and organizational levels is more clearly evident. This information then can be used by refuge managers in carrying out their mission to protect and conserve natural resources using refuge land.

We employed a weight-of-evidence approach similar to the methods applied by Ankley and Giesy (1998) in assessing risk of endocrine-disrupting compounds to animal classes. Under this approach, we summarized our study components and represented them as: 1) the observed differences from the reference site in abiotic samples regarding concentrations of pesticides, nutrients, and water quality measurements and the degree to which these parameters exceeded levels reported to be harmful to aquatic organisms (Table 6); 2) the observed differences in biotic samples as measured by biomarker responses, health assessments, and tissue concentrations at Finley NWR compared to reference site (Table 7); and 3) an overall assessment of the potential for aquatic species to be adversely impacted at Finley NWR (Table 8). Each of these provides a different line of evidence within the risk assessment, each with different assumptions, degrees of confidence and predictive capabilities (Fairbrother 2003). The *in-situ* bioassay was excluded from this approach because the results did not pass QC criteria.

The pesticide monitoring component of this study (Table 6) indicated that there could be effects to aquatic communities related to exposures of atrazine and chlorpyrifos in Brown Creek and at both sites on Muddy Creek (Table 7 in Appendix C). This is related to levels of the pesticide that exceeded Canadian guidelines (Table 5, Chapter 1) and levels shown to produce reproductive effects in amphibians (see Chapter 1, Pesticide Monitoring, Discussion). The greatest potential impact is at Brown Creek which reflects a source of pesticides outside the refuge. Muddy Creek pesticide concentrations indicate that the sources of pesticides are from both on- and off-refuge or that pesticides are moving through the refuge.

The nutrient monitoring (Table 6) indicated that several forms of nitrogen and phosphorus exceeded aquatic life criteria in all three creeks sampled (see also Table 8 in Appendix C). The potential for the greatest impact associated with elevated nutrients would be at Gray (Cattail Pond), Brown and Muddy Creeks. Based on the location of the sites sampled, sources of nutrients appear to be from both on- and off-refuge.

Water quality temperature standards were exceeded in Gray Creek at Cattail Pond and likely would be exceeded in other sites. However, the temperature standards are based on protection of cold water salmonids and species more common to the refuge (e.g., Oregon chub) are more tolerant of warmer water. Measurements of pH for several sites were below the Oregon standard, however with the variation noted during the post QA/QC check, low pH cannot reliably be associated with potential effects to individuals or populations.

Table 6. Line-of-evidence summary table comparing observed measurements at Finley National Wildlife Refuge to the reference site and to water quality standards or levels from the literature shown to affect aquatic organisms.

Site	Pesticides	Nutrients	Water Quality
Gray Creek, Reference Site	−	−	−
Gray Creek, Beaver Pond	−	+	−
Gray Creek, Cattail Pond	−	+ +	+
Brown Creek, Bellfountain Rd	+ +	+ +	−
Muddy Creek, Bruce Road	+	+ +	−
Muddy Creek, North Bridge	−	+ +	−

− = reference value, or value did not exceed guidelines or literature effect levels
+ = exceeded guidelines or literature effect levels
++ = exceeded guidelines or literature effect levels by at least 2 times for at least one chemical or parameter

Table 7. Line-of-evidence table comparing results of carp and turtle assessments from Finley and Malheur National Wildlife Refuges (NWRs) and two non-refuge sites. The non-refuge sites have a known contaminant source and are only added for comparison purposes (serving as a "positive" control for analysis). A "+" indicates response different from reference or value considered normal, and a "−" indicates response not different from reference or value considered normal.

Site	Hormones		Health	Histopathology	Analytical
	Carp	Turtle	Carp	Carp	Carp
Malheur NWR	−	−	−	−	−
Finley NWR	−	+	−	−	−
Willamette River/St. Johns Br.	+	NA[a]	NA	NA	+
Mill Race Pond	+	NA	NA	NA	+

[a] NA=not assessed.

Table 8. Assessment of study components used in this investigation as to their potential effects to aquatic organisms at Finley National Wildlife Refuge (NWR). A "+" indicates response different from reference or value considered normal, and a "–" indicates response not different from reference or value considered normal.

Study Component	Potential Effects to Aquatic Life[a]	Receptor of Concern[b]	Extent of Exposure[c]	Source of Exposure[d]
Pesticide Monitoring	+	aquatic life anurans	50%	on- and off-refuge
Nutrient Monitoring	+	aquatic life	83%	on- and off-refuge
Water Quality Monitoring	+	salmonids	17%	on-refuge
Endocrine Biomarkers	+	turtles	NA[e]	unknown
Fish Health Assessment	–	–	NA	NA
Fish Tissue Analysis	–	–	NA	NA

[a] Exceedance of aquatic life criteria, literature effect levels, or, for endocrine biomarkers, values considered normal.
[b] Receptor most likely to be affected from stressor monitored in study component.
[c] Percentage of sites listed in Table 6 with a positive (+) outcomes.
[d] Stressor considered to originate from on- or off-refuge based on location where detected and, for pesticides and nutrients, how they were used on the refuge.
[e] NA-not applicable.

The hormone values measured in biotic samples from refuge sites were within normal ranges except for turtles at Finley NWR (Table 7), where higher testosterone values were observed in females and the female hormone ratio was atypically low compared to reference turtles. All other parameters measured (health, histopathology, and analytical concentrations) were similar to reference values or considered within normal ranges. Only the two non-refuge sites showed both elevated contaminants in tissue and abnormal hormone results.

Criteria used in Table 8 address whether or not there is potential for aquatic species to be adversely impacted and the species or group most likely to be affected. These assessment categories are primarily based upon comparison of results from this investigation (excluding the bioassay) with guidelines for protection of aquatic life. The criteria are directed to effects for receptors of concern and the extent of exposure as well as the source (on- or off-refuge). There are a number of sites that have impaired water quality based on exceedances of pesticide, nutrient, or water quality guidelines or levels known to affect aquatic organisms. The receptors of greatest concern are amphibians and aquatic life (Table 8). Nutrient concentrations could

affect aquatic life at nearly all locations sampled on the refuge based on extent of exposure (Table 8), although the greatest potential impact from both pesticides and nutrients is at Brown Creek, which reflects a source outside the refuge. Whether or not impacts to aquatic life would occur on the refuge is dependent upon the presence of chemicals or degraded water quality when receptors are present. The degree to which receptors may be affected by these chemicals is not known from this investigation and further work at the organismal and community level would be needed to determine a response.

Management Recommendations

The results of this study indicate that there are numerous sources of pesticides and fertilizers in and around the refuge, and some of these agrochemicals are entering waterways important to the refuge. The pesticides of most serious concern originate outside the refuge, and refuge personnel have limited ability to manage pesticide application occurring off-refuge lands. However, there are actions the refuge could take to better limit some pesticides used on the refuge from entering waterways, and the exceedance of water quality criteria for nutrients on the refuge should be addressed through better management of fertilizer application. We recommend the following management actions be taken on the refuge to help minimize the pesticides and nutrients entering waterways and to further assess the health of western pond turtle populations:

1) Refuge personnel should work with cooperative farmers to encourage them to use the most current IPM methodologies in applying fertilizers and to respect buffer areas near riparian corridors or other waterways to minimize runoff to streams. In the absence of resources to use these methods or technologies, cooperative farmers could be encouraged to lessen the amount of fertilizers and see if their crop is still viable. Alternatively, the use of organic fertilizers could be explored as an option.

2) Develop guidelines to maintain adequate buffers around lands managed by the refuge that support agriculture, or that were formerly used for these purposes. Vegetative buffers or riparian corridors should be present between agricultural land receiving agrochemical applications and refuge waterways.

3) The refuge should develop an IPM plan to try to lessen the amount of pesticides entering their waters. This plan could be developed in collaboration with cooperative farmers and provided to farmers directly adjacent to the refuge. Part of an IPM plan could incorporate use of bioswales, holding ponds for retaining sediment, or increased buffer zones in areas that are particularly prone to agrochemical runoff.

4) Refuge personnel should meet with Brown Creek watershed landowners to talk about herbicide runoff impacts to refuge water quality and cooperative measures to reduce such runoff.

5) Continue monitoring western pond turtle populations as part of a larger population and habitat assessment within the Muddy Creek watershed. Declines observed in this species along with low juvenile recruitment and endocrine biomarker results from this study indicate that the population is stressed. Monitoring of egg laying, egg viability, nest success, juvenile survival and recruitment, population numbers, male/female ratios, and other natural history parameters is important to determine the health of the refuge's turtle population and assess water and habitat quality on the refuge.

6) Once data are gathered and evaluated to inform the status of the western pond turtle populations in Willamette Valley, further study is needed to assess endocrine disruption in turtles at the Finley NWR and its role involving turtle population declines. This study should be conducted in coordination with the Service's Environmental Contaminants Program. Given the findings of turtle sex steroid levels between sites, any future investigation should be designed to characterize the hormone levels at both the reference and refuge sites and evaluate if the abnormal hormone results are repeatable. Development of a species-specific vitellogenin assay for pond turtles would also be helpful to determine if males are experiencing hormone and possibly reproductive problems. The utilization of other non-lethal biomarkers should be explored such as sperm quality, female fecundity, and gonad size (potentially measured via ultrasound). Contaminant burdens would also need to be determined to fully assess if endocrine disruption is occurring in western pond turtles. Ultimately this information would need to be evaluated in relation to results of population studies recommended in number 5 to determine if western pond turtles are experiencing individual or population level effects.

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