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

EFFECTS OF NUTRIENT ENRICHMENT ON WETLANDS AT CONBOY LAKE NATIONAL WILDLIFE REFUGE

Environmental Contaminants On-Refuge Investigation

Project ID: 1N56

Interim Report

by

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INTRODUCTION

Located at the base of Mount Adams in Washington, Conboy Lake National Wildlife Refuge (NWR) is a large seasonal marsh nestled within dense pine and fir forests. The refuge supports large numbers of swans, geese, ducks, and sandhill cranes (*Grus canadensis*) during spring and fall migrations, as well as a wide variety of wetland-associated bird species, including black terns (*Chlidonias niger*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), sora (*Porzana carolina*) and Virginia rails (*Rallus limicola)* and American bitterns (*Botaurus lentiginosus*). Of the 17 pairs of greater sandhill cranes nesting in Washington, the refuge provides habitat for 14 of those pairs. In addition, the refuge supports twelve species of reptiles and amphibians including the Oregon spotted frog (*Rana pretiosa*) which is a candidate species under the Endangered Species Act (taxa for which the Fish and Wildlife Service has sufficient biological information to support a proposal to list as endangered or threatened).

During early settlement of the area, the natural lake was altered to increase native pasture and hay production. A series of channels were created to improve lake bed conditions for farming. Agricultural activities including livestock grazing, haying, and crop farming continue to be major activities occurring in the valley. It is suspected these land use activities are resulting in increased levels of nutrients in the canal system and wetlands on the refuge. Consequently, there is concern that nutrient enrichment of the wetlands may have the potential to impact the Oregon spotted frog.

Although Oregon spotted frogs have been known to exist at the refuge for many years, their widespread distribution throughout much of the refuge and adjoining private lands has only been known since 1995. The current population at Conboy Lake NWR is one of the two largest known populations of Oregon spotted frogs presently remaining within its range.

Oregon spotted frogs are highly aquatic, spending most of their lives in water (McAllister and Leonard 1997). This species is the most aquatic native frog in the Pacific Northwest (Owens 1999). While larval stages of the spotted frog are restricted to the aquatic environment, adults remain dependent on aquatic habitats for reproduction, hibernation, and foraging. With this close, almost constant contact with the aquatic environment, Oregon spotted frogs can be exposed to chemicals in the water through dermal absorption and ingestion.

Recent data suggest that nitrogen-based compounds related to agriculture may be contributing to the decline of some amphibian populations. Nitrate and nitrite compounds have been shown to reduce feeding activity, reduce swim vigor, alter equilibrium, cause paralysis and even death in frog and toad species (Marco 1997). In a series of acute and chronic tests conducted with four species of frogs and toads, Marco (1997) found the Oregon spotted frog was the most sensitive to nitrite and nitrate, with effects observed at nitrite concentrations of <1 mg/L. Marco (1997) suggests that nitrogen-based fertilizers may have contributed to the spotted frog's decline in the lowland areas of distribution. In addition, exposure to sublethal concentrations of nitrite in water has been shown to induce behavioral and morphological changes in a closely- related species to the spotted frog, the Cascades frog (*Rana cascadae*) [Marco and Blaustein 1999]. The

researchers found that tadpoles exposed to nitrite transformed more slowly than controls and development was retarded such that they emerged at an earlier developmental stage. The Cascades frog and spotted frog occur together at certain locations and have been known to hybridize (Leonard et al. 1993).

This study examined nutrient levels at various wetland sites at Conboy Lake NWR used by Oregon spotted frogs to determine if nutrient concentrations are such that they could impact the population. Frog populations were surveyed to assess whether effects seen in controlled exposures to nutrients were being displayed in the wild. Results of the study will be used to assist the refuge in formulating management strategies to improve seasonal water quality entering the refuge.

The scientific objectives of this investigation are to:

1. Determine the nutrient levels in refuge wetlands and waters supplying the wetlands.

2. Determine if there are differences in Oregon spotted frog growth and/or survival on the refuge as compared to a reference area.

3. Determine conventional (dissolved oxygen, pH, temperature, conductivity) water quality conditions in refuge wetlands supporting the Oregon spotted frog.

METHODS

Data Collection and Analysis

Site Selection and Water Sampling

Water samples for nutrient analyses were collected from five sites (Table 1) on the Conboy Lake NWR during the spring and summer of 2001. Feeder creeks and canals entering the refuge from agricultural areas were targeted as sample sites, as well as locations where frogs had actually oviposited (referred to as eggmass sites). Water samples were collected in March, April, and May to coincide with Oregon spotted frog oviposition and in July to coincide with metamorphosis.

For the four sampling periods described (March, April, May, July), all sites were not sampled each time. Most sites were sampled at least three of the four sampling periods with only one site sampled during two sample events because it did not retain water for the duration of the investigation. At the eggmass sites, exact sample site collection had to be adjusted as the season progressed due to water evaporation. In the case of the Headquarters eggmass site, the actual site where the eggmasses were laid and tadpoles grew was dry in July, therefore the sample was collected in an adjoining ditch which supplied water to the site.

Water samples were analyzed for nutrients according to the following U.S. Environmental

Protection Agency (EPA) methods: ammonia as N, EPA 350.1; nitrate as N, EPA 300.0; nitrite as N, EPA 300.0; orthophosphate as P, EPA 365.1; total phosphorus 365.1/365.2; total Kjeldahl nitrogen, EPA 351.2. Nutrient analysis included total and dissolved fractions of ammonia, total Kjeldahl nitrogen, dissolved fractions of nitrate, nitrite, and orthophosphate, and total fraction of phosphorus. Water quality parameters of dissolved oxygen (DO), pH, temperature, and conductivity were measured at all sampling sites when water was collected for nutrient analysis. These water quality measures were taken with either handheld meters (YSI 550 dissolved oxygen meter; Orion 63 pH and conductivity meter) or with a Hydrolab Datasonde unit. In addition, two Hydrolab Datasonde units were deployed over the entire length of the field study (March-July) for continuous measurements of pH, DO, temperature, and conductivity.

Water samples for nutrient analysis were collected by an equal-width increment method with sampling equipment fashioned by the U.S. Geological Survey (USGS), Ocala Laboratory. Samples were composited with a churn splitting apparatus also fashioned by the USGS, Supply Center. After splitting a sample for analysis of total concentrations (dissolved and suspended), the dissolved fraction was collected by filtering through a cellulose nitrate membrane within a pancake filtration unit enabled by a peristaltic pump. Water samples were decanted into clean, 250 ml brown polyethylene bottles with Teflon-lined caps. The samples were stored on ice in the field and refrigerated at 4EC at the Oregon Fish and Wildlife Office (OFWO) until pick-up or delivery to the analytical laboratory. Samples were submitted to Oregon Analytical Laboratory (OAL), Beaverton, Oregon, for determination of nutrient concentrations.

Sample Shipment, Handling, and Quality Assurance/Quality Control (QA/QC)

Procedures for transport and shipment of samples followed QA/QC guidelines specified in Rope and Breckenridge (1993) and the OFWO Standard Operating Procedures (SOP). Samples were tracked with chain of custody forms supplied OAL.

Laboratory quality control samples for all nutrient analyses consisted of procedural blanks and duplicate samples to estimate within sample variance, and measure analytical errors. Accuracy and precision were determined by spike sample recovery and duplicate sample analysis.

Data Analysis

Mean values will be tested for significant differences among sites on the refuge. Analysis of variance (ANOVA) will be used to test for mean differences and appropriate mean separation procedures will be used to distinguish sites with differences. Nutrient concentrations will be compared to published values to determine if levels are sufficient to cause effects to aquatic biota and compared to other monitoring data from other waterbodies within the region.

Oregon Spotted Frog Surveys

For the past several years, the refuge has conducted surveys of Oregon spotted frog. Egg mass surveys were conducted in 1997, 1998, and 1999 and this study continued to follow protocols established for the previous work. Data collected includes: GPS coordinates; site description; primary vegetation cover; presence, number, gender and size of adult frogs; condition of egg mass; and Gosner (1960) stage of egg mass development. From these surveys, embryonic

survival was estimated based upon available water, egg developmental stage and subsequent site visits. In this study, growth in recently metamorphosed juveniles was an additional measurement that had not been included in previous frog survey work.

A) *Surveys for Oregon spotted frog egg masses*
Surveys were conducted at three treatment sites adjacent to stream inflow sites, as well as three control sites not exposed to agricultural water influences. Surveys occured in March and April to correspond with the peak timing of oviposition. Each site was surveyed three times to count egg masses and assess egg hatching and tadpole survival. Data collected included locational information; number; hydrological parameters; condition and stage of egg mass development; and survival to hatching.

B) *Surveys of recently metamorphosed juveniles*
Surveys were conducted during June and July to assess the population of recently metamorphosed juvenile spotted frogs at the treatment and control sites. Each site was surveyed twice, approximately three weeks apart to evaluate changes in the population. The population of juvenile spotted frogs captured and examined correlated with the number of egg masses located for the given site. Frogs were examined for malformations and overall health. Snout-vent lengths and weights were used to compare growth parameters between the treatment and control sites. Basic habitat characteristics and hydrological parameters were examined to evaluate each site's suitability for juvenile rearing.

RESULTS

Water Sampling
Results of nutrient analysis are presented in Table 1.

Nitrogen
Nitrite (NO_2^-)
There was no nitrite detected (0.1 mg/L reporting limit (RL)) in any of the samples collected.

Nitrate (NO_3)
Only two sites showed the presence of nitrate over above reporting levels. Nitrate levels at Headquarters Spring ranged between 0.1and 0.3 mg/L on all 3 of the dates sampled (March, April, July) and concentrations at Willard Spring were 0.1 mg/L, the RL, on all 3 dates sampled (April, May, July).

Ammonia (NH_3)
The only sample that contained ammonia was collected from the Headquarters eggmass site on March 28, 2000, and the concentration was at the RL of 0.05 mg/L.

Kjeldahl Nitrogen
Total Kjeldahl nitrogen levels were measured between 0.2 and 4.9 mg/L (RL 0.2 mg/L), while

dissolved Kjeldahl nitrogen levels were between 0.2 and 1.3 mg/L.

Phosphorus

Total phosphate ranged from 0.01 to 0.59 mg/L (0.01 mg/L RL) at sample sites on Conboy Lake NWR. Phosphorus was detected at every site with the highest values from Willard egg mass (May and July) and Chapman egg mass (May) sites.

Orthophoshate was detected at levels $\leq$ 0.03 mg/L (0.01 mg/L RL). Orthophosphate was not detected at the Headquarters egg mass, C&H egg mass, or the Holmes Creek Inflow sites. It was detected from only one of the sampling periods at Laurel West egg mass, Chapman egg mass, Chapman inflow, and Bird Creek Inflow sites. Headquarters Spring and Willard Spring had detectable orthophosphate during all sampling periods.

Quality Control/Quality Assurance

Blanks collected in April and May had dissolved total Kjeldahl nitrogen (TKN) detected at 0.2 mg/L (RL). No other nutrients were detected in any of the blanks. Duplicates were very close (0.1 mg/L) for all compounds, with the exception of one sampling of dissolved TKN which spanned a 0.3 mg/L difference.

Table 1. Concentrations of ammonia at various sites throughout the Conboy Lake NWR. NH_3=ammonia, NO_2^-=nitrate, NO_3^-=nitrite, TKN=total Kjeldahl nitrogen, P=phosphate phosphorus, PO_4^{3-}=orthophosphate. For reporting purposes, duplicates were averaged.

Site	date	NH_3	NO_2^-	NO_3^-	TKN dissolv	TKN total	P	PO_4^{3-}	temp EC	DO mg/L	pH	spec cond Fs/cm
	Report Limit	0.05 mg/L	0.1 mg/L	0.1 mg/L	0.2 mg/L	0.2 mg/L	0.01 mg/L	0.01 mg/L				
Headquarters Spr. inflow	**3/28**[1]	ND	0.3	ND	ND	ND	0.02	0.025	7.49	~9.2[2]	5.78	66.4
	4/26	ND	0.1	ND	ND	0.4	0.06	0.02	7.6	9.79	6.52	70.7
	7/20	ND	0.1	ND	ND	ND	0.035	0.01	7.74	10.35	6.6	65.9
Headquarters eggmass	3/28	0.05	ND	ND	0.6	1.3	0.05	ND	10.73	10.88	7.39	52.3
	4/26	ND	ND	ND	0.5	0.6	0.02	ND	23	11.58	7.04	58.5
ditch	7/20	ND	ND	ND	ND	0.2	0.02	ND	16	5.5	6.58	70.3

Site	date	NH_3	NO_2^-	NO_3^-	TKN dissolv	TKN total	P	PO_4^{3-}	temp EC	DO mg/L	pH	spec cond Fs/cm
Bird Creek inflow	3/28	ND	ND	ND	ND	ND	0.01	ND	5.24	11.3	7.29	39.4
	4/26	ND	ND	ND	ND	ND	0.02	ND	9.5	10.3	7.54	33.4
	7/20	ND	ND	ND	0.5	0.5	0.05	0.01	30.15	6.67	7.41	40.5
C&H eggmass	3/28	ND	ND	ND	0.7	1.2	0.04	ND	8.11	9.95	7.27	57.7
	4/26	ND	ND	ND	1.3	1.7	0.06	ND	18.4	9.77	7.25	95.1
Willard Spring inflow	4/26	ND	0.1	ND	ND	ND	0.02	0.02	7.4	10.32	6.71	64
	5/31	ND	0.1	ND	ND	ND	0.03	0.03	7.5	9.92	6.69	64.7
	7/20	ND	0.1	ND	ND	ND	0.04	0.01	8.1	11.07	7	71.3
Willard eggmass	4/26	ND	ND	ND	0.5	0.9	0.07	0.01	17	5.5	6.28	56.5
	5/31	ND	ND	ND	1	1.4	0.11	ND	12.9	4.41	6.55	62.9
	7/20	ND	ND	ND	0.8	4.9	0.59	0.02	20	1.5	6.23	79.9
Chapman Ck. inflow	3/28	ND	ND	ND	ND	ND	0.02	ND	7.48	11.19	7.14	48.9
	4/26	ND	ND	ND	0.5	ND	0.02	0.01	14.5	8.98	7.45	66.3
	5/31	ND	ND	ND	0.3	0.2	0.02	ND	14.4	9.32	7.59	82
	7/20	ND	ND	ND	ND	0.3	0.05	ND	24.01	8.23	7.5	118
Chapman Ck. eggmass	3/28	ND	ND	ND	ND	ND	0.03	ND	11.12	9.92	6.64	49.1
	4/26	ND	ND	ND	0.35	ND	0.02	0.01?	13.1	9.16	6.99	73.5
	5/31	ND	ND	ND	0.6	1.55	0.26	ND	17.7	5.83	6.54	80.3
	7/20	ND	ND	ND	0.3	1	0.08	ND	27.32	2.68	6.94	227
Holmes Ck. inflow	3/28	ND	ND	ND	ND	ND	0.02	ND	6.82	11.38	7.24	53.8
	4/26	ND	ND	ND	ND	0.2	0.01	ND	10.9	9.63	7.87	71.7
	7/20	ND	ND	ND	ND	ND	0.04	ND	18.02	7.24	7.66	95.9
Laurel West eggmass	3/28	ND	ND	ND	0.4	0.8	0.07	ND	12.81	8.41	7.19	59.6
	4/26	ND	ND	ND	0.5	0.6	0.04	0.01	14	9.37	7.03	76.7

Site	date	NH_3	NO_2^-	NO_3^-	TKN dissolv	TKN total	P	PO_4^{3-}	temp EC	DO mg/L	pH	spec cond Fs/cm
	7/20	ND	ND	ND	0.5	1	0.12	ND	21.64	3.11	6.63	125

[1] Bold indicates duplicate samples collected. Results are an average of both values.

[2] Estimate based on temperature and percent saturation.

Frog Surveys

Frog surveys were completed by September 2000 through a contract with Marc Hayes, currently with the Washington Department of Ecology, but the final report has not been received.

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