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EXPLORATORY EVALUATION OF NUTRIENT ENRICHMENT AND FROG RESPONSE AT CONBOY LAKE NATIONAL WILDLIFE REFUGE

Environmental Contaminants On-Refuge Investigation
Project ID: 1N56

by

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EXECUTIVE SUMMARY

We sampled nutrient and water quality parameters and surveyed Oregon spotted frogs (*Rana pretiosa*) life stages (eggs, larvae and recently metamorphosed juveniles) to identify the potential for agriculture-related nutrient enrichment problems at Conboy Lake National Wildlife Refuge (Conboy Lake NWR), Klickitat County, Washington. Aside from selective conditions (exacerbated summer eutrophication during a drought year [2000]), primary nutrients levels (nitrogen and phosphorus) were generally low. Values for both nutrients and other water quality parameters were generally lower than those recognized as compromising the life stages of western North American ranid frogs. Moreover, available data, suggest no significant problem with eutrophication. Nonetheless, Conboy Lake NWR comprises part of a wetland system that exhibits a naturally eutrophic cycle that peaks in late summer. With climate change scenarios predicting increasingly droughty conditions for the region and a high potential existing for human development in the Glenwood Valley, at least periodic monitoring should be implemented to avoid jeopardizing this largest remaining population of Oregon spotted frogs through inadvertent nutrient enrichment.

KEYWORDS

Division of Environmental Contaminants (DEC) On-Refuge Investigation, 1N56, DEC identification number 200010010, Conboy Lake National Wildlife Refuge, Washington Congressional District 4, nutrients, water quality, Oregon spotted frog, *Rana pretiosa*.

INTRODUCTION

Located about 24 km (15 miles) southeast of Mount Adams in Washington State, Conboy Lake National Wildlife Refuge (hereafter Conboy Lake NWR) encompasses roughly 70% of a large (4,000 ha [10,000 acres]) seasonal marsh nestled within the mixed pine and fir forest landscape of the Glenwood Valley (FIGURE 1). As a consequence, Conboy Lake NWR seasonally supports numerous migratory waterfowl (ducks, geese, and swans); the only significant breeding population[1] of greater sandhill cranes (*Grus canadensis*) in Washington State; and a diverse assemblage of wetland-associated birds, including black terns (*Chlidonias niger*), yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), sora (*Porzana carolina*), Virginia rails (*Rallus limicola)* and American bitterns (*Botaurus lentiginosus*). The refuge also supports 12 amphibian and reptile species, including the Oregon spotted frog (*Rana pretiosa*), a candidate[2] species under the federal Endangered Species Act.

During early settlement of the Glenwood Valley, the historic Conboy Lake and its seasonal lakebed[3] were altered to increase pasture and hay production through channelization, diking, and water control structure construction. Today, agriculture remains a major land use in the valley, mostly as haying and livestock grazing, but limited crop farming also exists. Such land uses have the potential to increase nutrient loading in nearby wetlands (APHA *et al.* 1992, US EPA 1986, Rand and Petrocelli 1984). Nutrients like nitrogen and phosphorus are essential for plants, but at higher concentrations, these nutrients can encourage excessive plant growth, promote low dissolved oxygen levels, or directly impact organisms in the aquatic habitat (Correll 1998, Rouse *et al.* 1999).

Oregon spotted frogs have only been recognized to exist at Conboy Lake NWR since 1991, when Dr. Dennis Paulsen (then Director of the Slater Museum, University of Puget Sound) serendipitously encountered a few frogs in the ditch along Glenwood-BZ Road while collecting dragonflies. Moreover, the extent of their distribution throughout much of the refuge and adjoining private lands has been well known only since 1996, when two of us (JDE and MPH) initiated surveys of this system for Oregon spotted frogs. Information collected over the last nine years has revealed that the Oregon spotted frog population at Conboy Lake NWR is the largest remaining population of this species across its geographic range (JDE and MPH, unpubl. data). This range, which historically extended from southwestern British Columbia to northeastern California, includes fewer than six separate areas in Washington State where the species remains (McAllister and Leonard 1997, Hayes 1997; JDE, MPH, unpubl. data). Apparently extinct in California and the Willamette Valley of Oregon, the Oregon spotted frog has disappeared almost entirely from lowland marsh habitats across its geographic range, where a multiplicity of

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[1] Of 22 pairs of greater sandhill cranes recorded as nesting in Washington in 2004, the refuge provided habitat for 19 of those pairs (JDE, unpubl. data).

[2] Taxa for which the U.S. Fish and Wildlife Service has sufficient biological information to support a proposal to list as endangered or threatened.

[3] The historic Conboy Lake is thought to have included a perennial portion, which is located east of Glenwood-BZ highway; the rest of historic lake underwent an enormous seasonal flux in size. Today, all of Conboy Lake dries seasonally, and the only perennial areas are the ditch system and channelized streams that drain Glenwood Valley.

factors (e.g., habitat loss, hydrological alteration, exotic species) are thought to be responsible for its disappearance (McAllister and Leonard 1997, Hayes 1997).

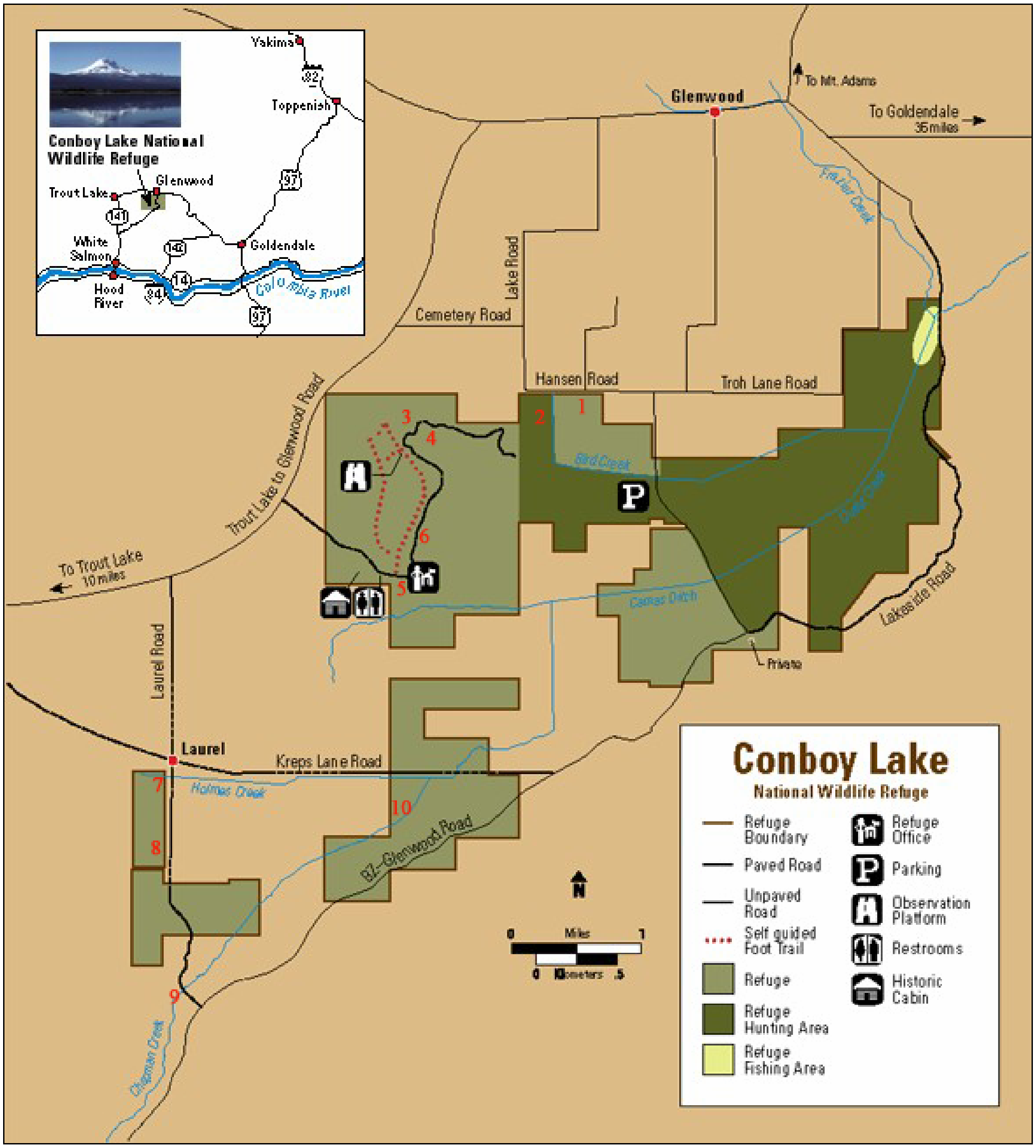

FIGURE 1. MAP OF CONBOY LAKE NWR Inset indicates the position of Conboy Lake NWR in south-central Washington. Red numbers indicate locations that were sampled in this study (see TABLE 1).

Almost always found in water (McAllister and Leonard 1997, Hayes 1997), the Oregon spotted frog is the most aquatic frog native to the Pacific Northwest (Owens 1999). Larvae (or tadpoles) are exclusively aquatic; adults depend on aquatic habitats for reproduction, foraging, and overwintering; and in the few circumstances in which they are encountered terrestrially, they typically occur within a leap of water (McAllister and

Leonard 1997, Hayes 1997). This high reliance on aquatic habitats makes Oregon spotted frogs vulnerable to exposure (via dermal absorption and/or ingestion) to chemicals entering their wetland habitats. Increased nutrients levels also have the potential to degrade their habitat. Consequently, concern existed that nutrient enrichment of wetlands had the potential to impact the Oregon spotted frog or its habitat at Conboy Lake NWR.

Recent laboratory investigations demonstrate that agriculture-linked nitrogen-based compounds may contribute to amphibian population declines. Ammonium nitrate (NH_3NO_3) and ammonium sulfate (NH_3SO_4), common components of many fertilizers (e.g., Matthews 1994, Akiyama *et al.* 2000), have the potential to kill frog developmental stages at concentrations of their component ions lower than typical fertilizer application levels (Marco *et al.* 1999). When nitrate (NO_3^{-2}) and nitrite (NO_2^-) ions were added to water, larvae of some amphibian species reduced feeding activity, swam less vigorously, showed disequilibrium and paralysis, suffered abnormalities and edemas, and eventually died (Marco *et al.* 1999). Observed effects increased with ion concentration and time. In acute and chronic exposures on five amphibian species (western toad [*Bufo boreas*], northern red-legged frog [*Rana aurora*], Oregon spotted frog, Pacific treefrog [*Hyla regilla*] and northwestern salamander [*Ambystoma gracile*]), northwestern salamander displayed the highest acute effect to nitrate and nitrite, whereas, in chronic exposures, the Oregon spotted frog was the most sensitive to both ions (Marco *et al.* 1999). All species showed 15-day LC50s[4] < 2 mg/L nitrite, with behavioral effects manifest at low (< 1 mg/L) concentrations. All species showed a high mortality at U.S. Environmental Protection Agency (EPA)-recommended limits of nitrite for warmwater fishes (i.e., 5 mg/L), and a significant larval mortality at the recommended limits of nitrite concentration in drinking water (1 mg/L). Moreover, the recommended levels of nitrate for warmwater fishes (90 mg/L) were highly toxic to Oregon spotted frog and northwestern salamander larvae. Based on these findings, Marco *et al.* (1999) suggested that nitrogen-based fertilizers may have contributed to the decline of the Oregon spotted frog in the lowland portions of its range. Further, exposure to sublethal nitrite and nitrate concentrations was shown to protract larval development by depressing growth rates in the related Cascades frog (*Rana cascadae*; Marco and Blaustein 1999) and northern red-legged frog (Schuytema and Nebeker 1999). This cumulative evidence indicated that examining anionic nitrogen forms potentially having agricultural origins at Conboy Lake NWR would be worthwhile.

RESEARCH OBJECTIVES

To evaluate whether nutrient inputs into wetlands supporting the Oregon spotted frog population at Conboy Lake NWR were sufficiently elevated to negatively impact the frogs, this study measured different forms of nitrogen and phosphorus at several Oregon spotted frog-occupied wetland sites. We quantified embryonic survivorship and evaluated the conditions of both larval and recently metamorphosed frogs to assess whether some of the effects reported in controlled laboratory exposures might be displayed in the wild. Results of the study will include the formulation of management strategies to improve seasonal water quality.

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[4] LC50 is the lethal concentration at which 50% are killed.

The scientific objectives of this investigation were to:

1. Determine the nutrient levels in refuge wetlands and waters supplying the wetlands.
2. Determine if differences in Oregon spotted frog survival or condition exist on potentially nutrient-influenced areas versus reference areas lacking such influence.
3. Measure conventional water quality parameters (dissolved oxygen [DO], pH, temperature, conductivity) in refuge wetlands supporting the Oregon spotted frog.

METHODS

A. SITE SELECTION

Study objectives focused site selection. We selected sites to ensure both the greatest and the least potential influence from agricultural areas on locations for Oregon spotted frog reproduction and rearing. Sites with the greatest potential agricultural influence were chosen to have the greatest likelihood of detecting a nutrient-related effect; sites with the least possible influence represented our best approximation to reference[5] sites. Oregon spotted frogs reproduce along shallow exposed stillwater margins (Licht 1969); at Conboy Lake NWR this resulted in Oregon spotted frog reproductive habitat necessarily being spatially separated from springs and streams contributing water to the refuge.

As water quality from inflows to the refuge and from Oregon spotted frog reproductive sites (hereafter, egg mass sites [EMS]) were both of interest, this spatial separation led us to select sites in both categories (TABLE 1). The wetland system of which Conboy Lake NWR is a part is extensive, so special effort was made to pair inflow and egg mass sites in a manner that each of the former contributed at least some water to the latter. Pairing was done to help identify differences that might result from agriculturally-influenced waters. Only Headquarters Spring (Site 5) and the Headquarters EMS (Site 6) were not thus paired. The Headquarters EMS was partly influenced by water from a spring north of Headquarters Spring. The Headquarters EMS was unlikely to be influenced by water from Headquarters Spring because the latter is located downstream in the flow sequence along Cold Springs Ditch, the channel into which both of these springs feed.

B. WATER QUALITY

***Sampling*:** Water was collected at each site during spring-summer 2000 for analysis of nutrient and other water quality parameters. Samples were collected on 28 March to coincide with Oregon spotted frog oviposition, on 26 April and 31 May to coincide with larval rearing, and on 20 July to follow metamorphosis.

Not all sites were sampled on every survey date, in part due to logistics and in part because water area shrank with the advancing season. Most sites were sampled during at least three of the four sampling periods; one site (the C&H EMS) was limited to two sample collections because the site had dried by the 31 May sampling date. At EMSs that did not dry completely through the survey period, the actual sample location shifted somewhat as water levels decreased and the water surface area shrank through the season.

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[5] We used the term "reference sites" to approximate "experimental controls." Complexity at Conboy Lake NWR as well as our inability to control key field conditions prevented our labeling these sites controls; the term reference sites better characterizes the complex, variable conditions we faced.

For the Headquarters EMS, the oviposition site was dry by July; the July sample for this site was collected in Cold Springs Ditch, which supplies water to the oviposition site during the Oregon spotted frog breeding interval in March.

TABLE 1. SAMPLING LOCATIONS AT CONBOY LAKE NWR Coordinates for latitude and longitude are based on the World Geodetic System 1984 (WGS84) grid. Site numbers correspond to the map in FIGURE 1.

Site Number and Name		Latitude	Longitude
1	Bird Creek inflow	45° 59' 10" N	121° 18' 48" W
2	C & H EMS	45° 58' 58" N	121° 19' 11" W
3	Willard Spring	45° 58' 56" N	121° 20' 42" W
4	Willard EMS	45° 58' 54" N	121° 20' 22" W
5	Headquarters Spring	45° 57" 54" N	121° 20' 29" W
6	Headquarters EMS	45° 58' 16" N	121° 20' 23" W
7	Holmes Creek inflow	45° 56' 30" N	121° 22' 48" W
8	Laurel West EMS	45° 56' 01" N	121° 22' 51" W
9	Chapman Creek inflow	45° 55' 04" N	121° 22' 37" W
10	Chapman Creek EMS	45° 56' 19" N	121° 20' 34" W

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, handheld sampler developed in 1948) that consisted of a 0.47-L glass container (milk bottle) 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 during each sample collection, a water-sediment mixture flows through a Teflon nozzle into the glass container, which displaces air that then escapes through a small exhaust vent. Sampling was also width-integrated by collection from at least three different spots at each wetland site (Edwards and Glysson 1999). Samples from each site were composited in a 6-L clear plastic jar and returned to refuge headquarters for subsequent processing. At the time of sample collection, we measured dissolved oxygen (DO), pH, temperature, and conductivity in situ either with handheld meters (YSI 550 DO meter; Orion 63 pH and conductivity meter) or with a Hydrolab Datasonde™ unit.

Composited samples returned to refuge headquarters were mixed in a 14-L churn splitter (Sylvester *et al.* 1990) for separation into suspended and dissolved fractions. Whole water samples were decanted from the churn splitter directly to 250-ml high-density brown polyethylene bottles. Prior to decanting into the polyethylene bottles, water for dissolved constituents was filtered through a 0.45-μm cellulose nitrate membrane within a peristaltic pump-enabled pancake filtration unit. Samples were stored on ice in the field and refrigerated at 4°C at the Oregon Fish and Wildlife Office (OFWO) until delivered to the analytical laboratory. Equipment cleaning procedures followed Shelton (1994) for the

USGS National Water Quality Assessment Program (NAWQA). The contract laboratory supplied clean polyethylene bottles.

Procedures for transport and shipment of samples followed quality assurance/quality control (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 by Oregon Analytical Laboratory (OAL [now North Creek Analytical], Beaverton, Oregon), the contract laboratory.

***Nutrient Analysis*:** Samples were submitted to OAL for determination of total and dissolved fractions of ammonia, total Kjeldahl nitrogen, and total phosphorus; and dissolved fractions of nitrate, nitrite, and orthophosphate. Kjeldahl nitrogen consists of ammonia and organic nitrogen determined together (APHA *et al.* 1992). The OAL was Oregon Department of Health and Washington Department of Ecology accredited.

Water samples were analyzed for nutrients according to EPA methods [numerical procedure label in brackets]: 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 [EPA 365.1/365.2]; total Kjeldahl nitrogen [EPA 351.2]. Detailed descriptions of these methods are available on the National Environmental Methods Index (2004) website.

Laboratory quality control samples for all nutrient analyses consisted of procedural blanks and duplicate samples. These were used to estimate within sample variance and measure analytical errors. Spike sample recovery and duplicate sample analysis were used to determine accuracy and precision, respectively.

The OAL conducted calibrations continually while performing analyses to show that the method and instrumentation were appropriate and effective for the analysis. Continual calibration meant that comparison with a laboratory control standard was done before a sample run and redone once every 20 samples. The laboratory control standard was a clean matrix spiked with analytes of interest.

Laboratory acceptance criteria for QA/QC were as follows: calibration with a correlation coefficient ≥ 0.995; initial and continuing calibration verification required a 90 to 110% recovery; laboratory control standard required a 75 to 125% recovery; laboratory matrix spike within 75 to 125%; and sample duplication ± 20%. Compliance with the laboratory control standard recovery limits was mandatory with failure resulting in corrective action and reruns, while matrix spike and duplicate results were occasionally qualified due to matrix effects and analyte concentration.

***Water Quality Monitoring*:** One Hydrolab unit was deployed at both Headquarters Spring and Bird Creek over the length of the field study (March-July) for continuous measurement of DO, pH, temperature, and specific conductance. Hydrolab multiprobes collected data at hourly intervals. Each multiprobe was maintained and calibrated on a 7- to 14-day schedule. Stored data were downloaded to a spreadsheet in July. A post-deployment equipment check was conducted in the OFWO laboratory as a quality assurance check for any differences between multiprobe units. During this check, multiprobes were placed in standing water at room temperature and recorded water quality information for 3 days. These recordings were downloaded, graphed, and visually assessed for variation between units. After the data were evaluated, each parameter was compared to Oregon Department of Environmental Quality (ODEQ) water quality standards as found in the Oregon Administrative Rules (ODEQ 2004).

Water quality data collected with multiprobe units went through two methods of data QA/QC following ODEQ protocols (ODEQ 1997). The first method compared the field calibration data to the multiprobe readings for the same date. 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 compared the multiprobes internally stored "setup" values to the "follow-up" values. The setup values are the data recorded by the multiprobe using the original calibration 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 comparison 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 measurement of pH, specific conductance, and DO was greater than 0.3, 10% µS/cm and 1.0 mg/L, respectively.

C. AMPHIBIAN SAMPLING

***Oregon Spotted Frog Egg Mass Monitoring*:** The five EMSs (Sites 2, 4, 6, 8, and 10 in TABLE 1, FIGURE 1) were used to monitor the pre-hatching life stages of Oregon spotted frogs. Prior to this exploratory survey, egg mass surveys had been conducted in each year between 1997 and 1999 on Conboy Lake NWR and to a lesser degree in the immediate vicinity (Engler and Friesz 1998). This study followed protocols developed for those surveys. As Oregon spotted frogs lay eggs in extremely shallow water (Licht 1969), the shallows (water depth < 0.4 m) of each site were systematically surveyed at least twice during the breeding season for Oregon spotted frog egg masses. Locations of each egg mass cluster (Oregon spotted frogs exhibit communal oviposition; Licht 1969) were georeferenced via GPS, and flagged and staged (based on Gosner [1960]) to avoid double-counting. For the last egg mass survey (the survey when embryos were closest to hatching), we estimated embryonic survival by counting the number of dead or non-developing embryos (evident from discoloration, deformity or fungal incursion). In most cases, the cause of mortality (e.g., freezing, desiccation) could be inferred based on examination of condition of the jelly, water levels, and the pattern of embryonic mortality within egg masses. We distinguished between mortality due to physical factors (e.g., freezing, desiccation) and mortality potentially caused by anomalous conditions during development (e.g., chemical or pesticide exposure).

We analyzed differences in both overall mortality and developmental mortality among EMSs using a Kruskal-Wallis non-parametric ANOVA (Siegel and Castellan 1988). For analyses where significant differences were found, we conducted *post hoc* independent contrasts using a Mann-Whitney U test on each pair of EMSs. We also correlated developmental mortality to nutrient levels for all forms of nitrogen and phosphorus analysis using a Spearman Rank correlation coefficient because of small sample sizes (Siegel and Castellan 1988).

Larval Oregon Spotted Frog Assessment: We also sampled the Oregon spotted frog larval cohort at each of the five EMSs during May 2000. At each site, we evaluated at least 50 larvae for three categories of conditions: body symmetry, non-symmetry deformities, and atypical behavior.

Body symmetry: Body symmetry was scored as abnormal if right and left sides differed in overall shape; larvae were scored normal if no obvious differences were visible.

Non-symmetry malformities: Six categories of non-symmetry malformities were scored:

1) abnormal gills – extra or misshapen gills
2) chewed tails – mechanical damage or tears of the tail
3) deformed tails – misshapen tail
4) deformed right side/eye absent – right side of head misshaped and eye absent
5) discolored right side – right side of body differently colored
6) normal – no obvious non-symmetry deformities

Atypical behavior: We also conducted a behavioral assessment of larvae based on a scoring of swimming behavior or response to disturbance into one of five categories:

1) no disturbance response – no reaction when water within 5 cm was disturbed
2) swam on side – larvae could not swim in typical upright position
3) awkward side-to-side motion – larvae swam with excessive lateral yaw
4) circular swimming – larvae could not swim in a straight line
5) normal – swimming and escape behavior normal

For each of the three categories we evaluated the number of individuals in the sample from each EMS. We used a Chi-square test to evaluate whether significant differences existed among categories (Zar 1999). For the analysis comparing non-symmetry deformity frequency across EMSs, we clustered larvae with chewed tails into the normal category because chewed tails indicate either a behavioral response to crowding or attempted predation, not a developmental anomaly. We performed the atypical behavior assessment analysis scoring failure to respond to disturbance as either normal or abnormal in separate analyses because individual larvae vary greatly in their response to disturbance and we could not be certain that failure to respond to disturbance was not within the typical range of larval behavior. We also correlated the frequency of larval asymmetry, non-symmetric deformities, and atypical behaviors to nutrient levels for all forms of nitrogen and phosphorus analysis using a Spearman Rank correlation coefficient because of small sample sizes (Siegel and Castellan 1988).

***Surveys of Recently Metamorphosed Juveniles*:** In this study, an attempt was also made to assess growth and condition of recently metamorphosed juvenile frogs at all five EMSs sampled. However, two of these sites (the Headquarters and Chapman Creek EMSs dried before the July sampling for these life stages) and we found no evidence of Oregon spotted frog recruitment at a third (the Willard EMS; see TABLE 1, FIGURE 1). Thus, recently metamorphosed juvenile data came exclusively from the remaining two EMSs, Laurel West and C&H (see TABLE 1, FIGURE 1).

Neither the Laurel West EMS nor the C&H EMS is isolated from potential sources of nutrient contamination, but the Laurel West EMS appears less influenced from nutrient inputs than C&H EMS, so comparison between sites was made in that context.

Surveys for recently metamorphosed Oregon spotted frogs were conducted during June and July. Each site was surveyed twice about three weeks apart. We found no evidence that metamorphosis had occurred on the late June survey dates, so all data presented here addresses the 18-19 July 2000 survey interval. Hand capture was the primary method used to obtain frogs, but this was supplemented with dip netting (APPENDIX I describes sampling methods). Basic habitat data and hydrological parameters were also recorded.

Bullfrogs (*Rana catesbeiana*) were present at both sites where recently metamorphosed Oregon spotted frog data were obtained, so we gathered parallel information on the metamorphosing bullfrog cohort[6] in part because recruitment of juvenile Oregon spotted frogs appeared limited in 2000.

Processing Recently Metamorphosed Juveniles**:** All amphibians captured during sampling were processed *in situ*, and processing generally followed the Amphibian Initiative Year 1 Work Plan guidelines (USFWS 2000). Frogs were examined for condition (appearance, vigorous or not), injuries (type and location on body), and presence/absence of any abnormalities; and their size (as snout-vent length [SVL] in millimeters [to the nearest 0.5 mm]), tail length (if present), and weight (to the nearest 0.5 g) were measured. We also recorded capture time, location (refuge unit and location within unit), and microhabitat (vegetation type and aquatic or terrestrial location) for each animal. Except for tail length and weight, these same data were estimated or recorded on individuals not captured. We estimated SVL to the nearest 5 mm on individuals not captured. Save abnormal individuals, all captured animals were released at their point of capture following processing. Abnormal individuals were retained, euthanized, and preserved following the Amphibian Initiative Year 1 Work Plan (USFWS 2000) guidelines.

Observations Outside the Sampling Period: We also used observations from the sample areas made outside the July 2000 sampling interval to assist in interpretation of findings made during the target period. We had collected data at all five EMSs during all of March; at the C&H EMS on 27-30 June, 17 July, and 5-7 September 2000; and at Laurel West EMS on 28 June and 6 September 2000.

RESULTS

A. WATER QUALITY

NUTRIENTS

Nitrogen: Only the two spring sites (Headquarters and Willard) had nitrate values at or above the detection levels (TABLE 2); the pattern was similar on all three dates sampled. The highest value, 0.3 mg/L at Headquarters Spring on 28 March, was still only slightly above the detection threshold. We did not detect nitrite in any sample. Ammonia was recorded in only the sample from the Headquarters Spring EMS on 28 March (TABLE 2); this value was at the detection limit.

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[6] A metamorphosing cohort simply means the group of bullfrogs metamorphosing that year. Unlike the Oregon spotted frog, which insofar as known, always metamorphoses in the year it developed from an egg, bullfrog larvae must overwinter at least once as larvae at Conboy Lake NWR. A metamorphosing cohort of bullfrogs may not represent a true annual cohort since a mix of individuals that overwintered either one or twice, or two or three times, may be represented, the variation largely depending on precisely when eggs were laid in each true annual cohort.

Total Kjeldahl nitrogen level varied between 0.2 and 1.7 mg/L among all sites except the Willard Spring EMS on 20 July, when 4.9 mg/L was recorded (TABLE 2). All sites except the Headquarters Spring and Holmes Creek inflows recorded detectable levels of dissolved Kjeldahl nitrogen between 0.2 and 1.3 mg/L at least once during sampling (TABLE 2).

Phosphorus: Only the two spring inflow sites had orthophosphate values at or above detection levels (0.01-0.03 mg/L) on all sampling dates (TABLE 2). It was detected on one sampling date (a different date depending on the site) from the Bird and Chapman Creek inflow sites and the Chapman and Willard Spring EMSs, and on two sampling dates from the Laurel West EMS (TABLE 2). Orthophosphate was not detected at each of the Headquarters and C&H EMSs and the Holmes Creek inflow site.

Total phosphorus concentrations were low (≤ 0.07 mg/L) on the early (28 March and 26 April) sampling dates, but generally showed an increase at sites where it was measured on the later dates (TABLE 2). The highest concentrations were recorded at two EMSs, Chapman Creek and Willard, on 31 May and 20 July, respectively.

OTHER PARAMETERS

Temperature: Water temperature varied among sites. The springs had relatively constant temperatures; spot sampling from 28 March to 20 July (n = 3) at Headquarters Spring revealed only a 0.2ºC range (7.5-7.7ºC; FIGURE 1, APPENDIX II). A nearly continuous record from the Hydrolab at Headquarters Spring was similar; temperatures between 7.3ºC and 7.7ºC were recorded between 28 March and 31 July (APPENDIX FIGURES 1A-D). Samples taken at Willard Spring from 26 April to 20 July (n = 3) had a 0.7ºC range (7.4-8.1ºC; FIGURE 1, APPENDIX II).

In contrast, the three inflowing creeks (Bird, Chapman, and Holmes) and the five EMSs (Chapman, C&H, Headquarters, Laurel West, and Willard) generally showed an increase in water temperature between 28 March and 20 July (FIGURE 2, APPENDIX II). This agreed with Hydrolab data from Bird Creek; March and April temperatures were mostly < 10ºC (APPENDIX FIGURE 1E), but July temperatures were often > 15ºC (APPENDIX FIGURE 1H).

The continuous record from both Hydrolabs also showed diel fluctuations in temperature (APPENDIX FIGURES 1A-D). However, fluctuations in Bird Creek were large (≥ 2ºC; up to 16ºC in July), but those in Headquarters Spring were small (0.1-0.3ºC) and barely beyond instrument resolution.

TABLE 2. NUTRIENT PARAMETERS MEASURED AT CONBOY LAKE NWR (locations georeferenced in TABLE 1). Parameters are ammonia (NH_3), nitrate (NO_3^{-2}), nitrite (NO_2^{-}), total dissolved Kjeldahl nitrogen (TKNd), total Kjeldahl nitrogen (TKN), phosphate phosphorus (P), and orthophosphate (PO_4^{-3}). Units are mg/L for all. Values for the Headquarters Spring inflow site on 28 March and 20 July, and the Chapman Creek EMS on 26 April and 31 May are each based on combined duplicate samples. An asterisk (*) means that the parameter was below the detection limit.

Parameter		**NH_3**	**NO_3^{-2}**	**NO_2^{-}**	**TKNd**	**TKN**	**P**	**PO_4^{-3}**
Detection Limit (mg/L)		0.05	0.1	0.1	0.2	0.2	0.01	0.01
Location	**Date**							
Headquarters Spring Inflow	28-Mar	*	0.3	*	*	*	0.03	0.03
	26-Apr	*	0.1	*	*	0.40	0.06	0.02
	20-Jul	*	0.1	*	*	*	0.04	0.01
Headquarters Spring EMS	28-Mar	0.05	*	*	0.60	1.30	0.05	*
	26-Apr	*	*	*	0.50	0.60	0.02	*
	20-Jul	*	*	*	*	0.20	0.02	*
Bird Creek Inflow	28-Mar	*	*	*	*	*	0.01	*
	26-Apr	*	*	*	*	*	0.02	*
	20-Jul	*	*	*	0.50	0.50	0.05	0.01
C&H EMS	28-Mar	*	*	*	0.70	1.20	0.04	*
	26-Apr	*	*	*	1.30	1.70	0.06	*
Willard Spring Inflow	26-Apr	*	0.1	*	*	*	0.02	0.02
	31-May	*	0.1	*	*	*	0.03	0.03
	20-Jul	*	0.1	*	*	*	0.04	0.01
Willard EMS	26-Apr	*	*	*	0.50	0.90	0.07	0.01
	31-May	*	*	*	1.00	1.40	0.11	*
	20-Jul	*	*	*	0.80	4.90	0.59	0.02
Chapman Creek Inflow	28-Mar	*	*	*	*	*	0.02	*
	26-Apr	*	*	*	0.50	0.50	0.02	0.01
	31-May	*	*	*	0.30	0.30	0.02	*
	20-Jul	*	*	*	*	0.30	0.05	*
Chapman Creek EMS	28-Mar	*	*	*	*	*	0.03	*
	26-Apr	*	*	*	0.35	0.35	0.02	0.01
	31-May	*	*	*	0.60	1.55	0.26	*
	20-Jul	*	*	*	0.30	1.00	0.08	*
Holmes Creek Inflow	28-Mar	*	*	*	*	*	0.02	*
	26-Apr	*	*	*	*	0.20	0.01	*
	20-Jul	*	*	*	*	*	0.04	*
Laurel West EMS	28-Mar	*	*	*	0.40	0.80	0.07	*
	26-Apr	*	*	*	0.50	0.60	0.04	0.01
	20-Jul	*	0.3	*	*	*	0.03	0.03

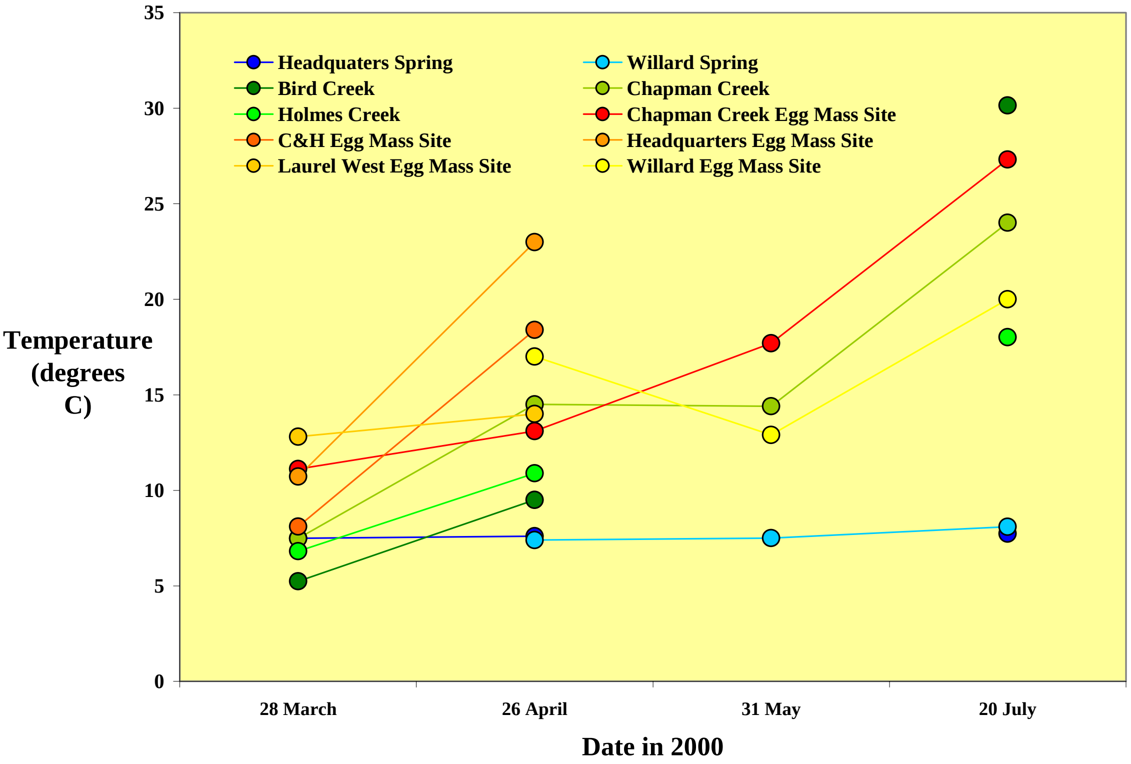

FIGURE 2. WATER TEMPERATURE DATA FROM CONBOY LAKE NWR, 2000 Time axis not precisely to scale.

Dissolved Oxygen (DO): Except for the Willard EMS, DO levels were high (about 8-12 mg/L) on the early (28 March and 26 April) sampling dates across all sites (FIGURE 3). The 20 July sampling showed still high DO (> 10 mg/L) in the springs, moderate levels (6.6-8.2 mg/L) in the creeks, and low levels (1.5-2.7 mg/L) at the only two EMSs measured (Chapman Creek and Willard). At the latter two sites, DO dropped progressively between 26 April and 20 July (FIGURE 2). These data agree with Hydrolab data from Bird Creek and Headquarters Spring that show DO consistently higher in Bird Creek through June and then dropping below the values seen in the spring in July (APPENDIX FIGURES 1E-H).

Bird Creek had greater diel fluctuations in DO than Headquarters Spring (APPENDIX FIGURES 1E-H). Daily fluctuations in Bird Creek were often in the range of 2 mg/L, occasionally reaching the 4-5 mg/L range (as on 7 May and 18-20 July). In contrast, DO fluctuations in Headquarters Spring were typically ≤ 1 mg/L, although occasional daily fluctuations ranged to 2 mg/L (as on 21 June and 11 July).

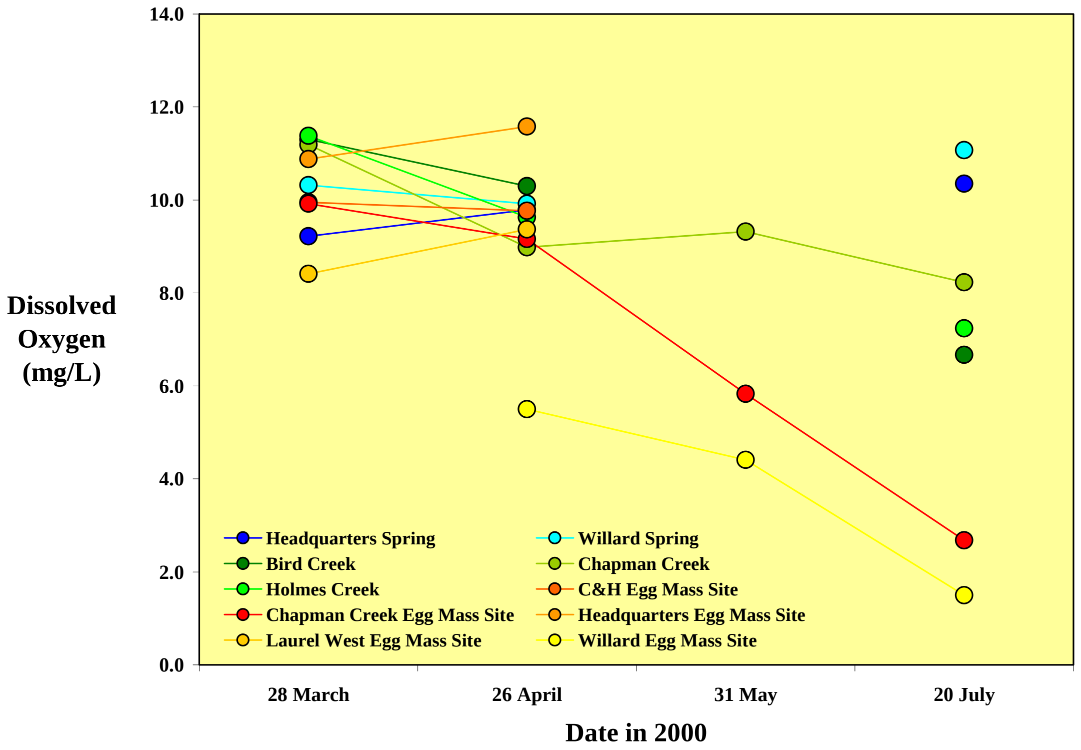

Figure 3. Dissolved Oxygen Data from Conboy Lake Nwr, 2000 Time axis not precisely to scale.

pH: Most sites had circumneutral pH, but spring sites were generally slightly more acidic than other sites, the exceptions being the Chapman Creek and Willard Emss (Figure 4). Hydrolab data agreed with spot measurements in that Headquarters Spring was consistently more acidic than Bird Creek (Appendix Figures 1i-l). Like temperature measurements, pH in Bird Creek exhibited a greater diel flux than pH in Headquarters Spring; diel variation in Bird Creek was 0.2-0.3, whereas that in Headquarters Spring was typically < 0.1.

Breaks in the continuous Hydrolab record for pH reflect unit maintenance periods (Appendix Figures 1i-l). Some reading drift is evident between maintenance intervals. Correcting for drift was not possible because too few independent pH readings were taken, but general differences in pH remained identifiable.

Specific Conductance: Except for the spring sites and Bird Creek, specific conductance showed a generally seasonal increase across sites (Figure 5). Specific conductance values for each of the spring sites and Bird Creek remained relatively similar through the study interval (Figure 4, Appendix Figures Im-p). Despite reading drift and power failure problems in the continuous record, specific conductance in Headquarters Spring was consistently in the 60-70 µS/cm range and uniformly higher than specific conductance in Bird Creek, which was in the 10-45 µS/cm range (Appendix Figures Im-p).

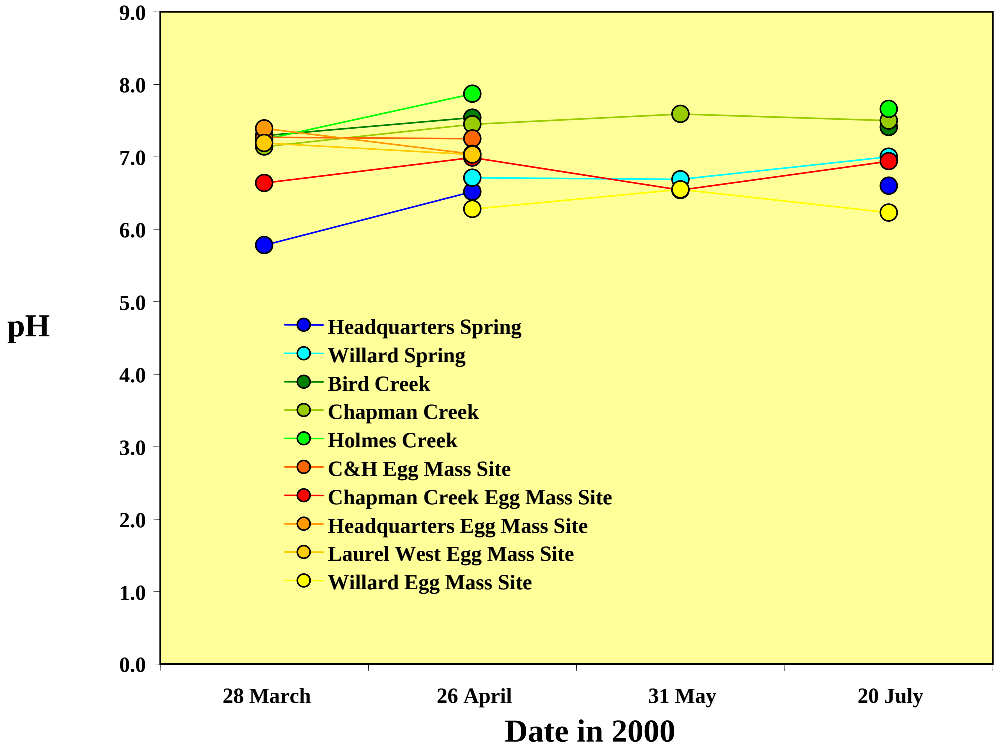

FIGURE 4. pH DATA FROM CONBOY LAKE NWR, 2000 Time axis not precisely to scale.

B. AMPHIBIAN DATA

EMBRYONIC MORTALITY

Among the five EMSs, we evaluated embryonic mortality in 724 Oregon spotted frog egg masses. Average overall mortality ranged from a low of 0.9% at the Chapman EMS to a high of 20.3% at the Laurel West EMS. Average developmental mortality ranged from a low of 0.5% at the Chapman EMS to a high of 5.8% at the Laurel West EMS. The percentage of all mortality attributable to developmental origin ranged from a low of 16% at the Headquarters EMS to a high of 56% at the Chapman EMS. We found significant differences in both overall mortality and developmental mortality among EMSs (Kruskal-Wallis non-parametric ANOVA: overall: $H = 94.6$, $P < 0.0001$; developmental: $H = 75.5$, $P < 0.0001$). Independent contrasts revealed significant differences among EMS pairs for both overall and developmental mortality (Mann-Whitney test: $P < 0.05$) except that we found no significant differences in overall mortality in the pairs among C&H, Headquarters, and Willard EMSs; and we also found no significant differences in developmental mortality between C&H and Willard EMSs. We also found no significant relationship between the levels of any of the nutrient forms of nitrogen or phosphorus and developmental embryonic mortality (Spearman Rank correlations: $\rho = 0.000\text{-}0.400$, $P > 0.4884$ for all nutrients forms except total phosphorus, which was $\rho = 0.800$, $P = 0.1659$; $n = 4$ for all).

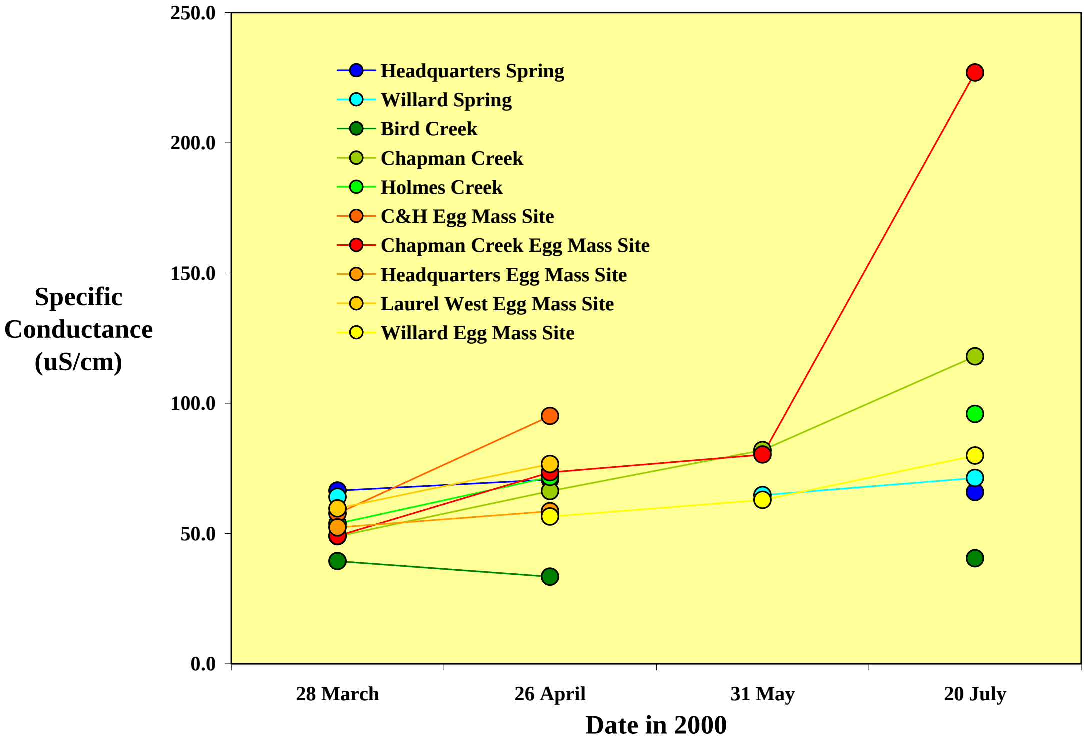

FIGURE 5. SPECIFIC CONDUCTANCE DATA FROM CONBOY LAKE NWR, 2000 Time axis not precisely to scale.

TABLE 3. EMBRYONIC MORTALITY ESTIMATES AT THE CONBOY LAKE NWR EGG MASS SITES (EMSS)

EMS	Egg Masses Evaluated (n = 724)	Overall % Mortality		Developmental % Mortality		Percentage Developmental Mortality of Overall Mortality
		Mean	SE	Mean	SE	
C&H	280	8.2	1.09	1.9	0.23	23
Chapman	74	0.9	0.40	0.5	0.01	56
Headquarters	226	6.4	1.23	1.0	0.16	16
Laurel West	32	20.4	3.41	5.8	1.70	28
Willard	112	6.1	1.38	2.1	0.37	34
Grand Means		7.1		1.7		24

LARVAL ASSESSMENT

Body Symmetry: One larva could be scored as abnormally symmetric among the 468 larvae sampled across the five EMSs (TABLE 4). The single abnormally symmetric larva was recorded from the Headquarters EMS. No significant difference was found in body symmetry among the five EMSs ($\chi^2 = 6.1$, df = 4, $P = 0.1914$). Too little variation existed in the data to run correlation coefficients between asymmetry frequency and the levels of the various nutrient forms of nitrogen and phosphorus.

TABLE 4. SCORING FOR LARVAL ASSESSMENT CATEGORIES AT CONBOY LAKE NWR EGG MASS SITES (EMSS)

Category		Egg Mass Site				
		C&H	**Chapman**	**Headquarters**	**Laurel West**	**Willard**
		n = 103	**n = 99**	**n = 100**	**n = 66**	**n = 100**
Symmetry	Normal	103	99	100	65	100
	Abnormal	0	0	0	1	0
Malformities	None (Normal)	102	98	88	50	85
	Abnormal Gills	1	0	0	0	0
	Chewed Tails	0	1	10	15	13
	Deformed Tails	0	0	2	0	1
	Deformed Right Side	0	0	0	1	0
	Discolored Right Side	0	0	0	0	1
Behavior	Normal	102	96	97	62	95
	No Disturbance Response	1	2	1	4	2
	Swam on Side	0	1	0	0	0
	Odd Side-to-side Motion	0	0	2	0	2
	Circular Swimming	0	0	0	0	1

Malformities: Six of the 468 larvae sampled across the five EMSs exhibited some kind of deformity (TABLE 4). Most EMSs had one or two larvae with deformities. Thirty-nine separate larvae had chewed tails; no larvae with deformities had a chewed tail. No significant difference was found in deformity frequency among the five EMSs ($\chi^2 = 2.2$, df = 4, $P = 0.6977$). We found no significant relationship between the percentage of larval deformity in each EMS and the levels of any form of nitrogen or phosphorus nutrients examined (Spearman Rank correlation: $\rho \leq 0.289$, $P \geq 0.5626$, n = 5).

Behavioral Assessment: Sixteen larvae were scored as displaying abnormal behavior; 10 displayed no response to disturbance, whereas six others displayed one of three kinds of odd swimming behavior (swimming on side, awkward side-to-side motion, or circular swimming; TABLE 4). Regardless of how we scored failure to respond to disturbance (see METHODS), we found no significant differences among the five EMSs (scoring failure to respond as abnormal: $\chi^2 = 4.1$, df = 4, $P = 0.3900$; scoring failure to respond as normal: $\chi^2 = 4.9$, df = 4, $P = 0.2881$). We also found no significant correlation between the frequency of atypical behaviors and the levels of any forms of nitrogen or phosphorus, whether we scored failure to respond to disturbance as normal or abnormal (Spearman Rank correlation: scoring failure to respond as abnormal: $\rho \leq \pm 0.344$, $P \geq 0.4913$ for all nutrients except PO_4^{-3}, for which $\rho = 0.740$, $P = 0.1386$; scoring failure to respond as normal: $\rho \leq \pm 0.344$, $P \geq 0.4913$ for all nutrients; n = 5 for all comparisons).

ASSESSMENT OF RECENTLY METAMORPHOSED FROGS

Sampling consisted of 6.33 person-hours of effort over 18-19 and 21 July 2000 (APPENDIX IV). Data for both the target Oregon spotted frog and the non-target American bullfrog data are included here. No other amphibians were observed at either study site during the 18-21 July sampling period, although we observed three other amphibian species at the study sites outside the target sampling period (APPENDIX V).

Oregon Spotted Frog: Oregon spotted frogs were found at both the C&H and Laurel West EMS (TABLE 5), but the number observed at each site differed markedly. Twenty-two observations of 21 Oregon spotted frogs were made at the C&H EMS over the interval 18-21 July, but only one observation of an Oregon spotted frog larva that was about to enter metamorphosis was made at the Laurel West EMS. No recently metamorphosed Oregon spotted frogs were observed at the Laurel West EMS either within or outside of the target 18-21 July interval.

During the 19 July sampling of the C&H EMS we found nine Oregon spotted frogs (TABLE 5), seven recently metamorphosed juveniles and two metamorphs. All nine of these individuals were young-of-the-year (i.e., individuals that developed from eggs laid in March 2000). During the 21 July sampling of the C&H EMS we found 13 Oregon spotted frogs (TABLE 3), 10 of them were recently metamorphosed juveniles and the remaining three individuals were metamorphs. Likewise, all 13 individuals were young-of-the-year. Only one individual (a metamorph) caught on the 21 July was a recapture of an individual caught on 19 July.

Two of the Oregon spotted frogs, both observed at the C&H EMS, exhibited physical peculiarities. One juvenile found on 19 July had a slight bone callus on the right femur, and one metamorph found on 21 July had a subdermal gas bubble on its back.

American Bullfrog: American bullfrogs also occurred at both study sites (TABLE 5). In contrast to the Oregon spotted frog, bullfrogs were seen more frequently at the Laurel West EMS than at the C&H EMS. We made 48 observations of bullfrogs at Laurel West, but only three bullfrog observations at the C&H EMS.

Within the 18-21 July interval, bullfrogs were observed at the C&H EMS only on 21 July, at which time one juvenile and two subadults were encountered. These individuals represented, respectively, frogs that metamorphosed in 2000 and in 1999.

During the 18-19 July sampling, we found 10 bullfrogs at the Laurel West EMS, five of which were juveniles and five of which were metamorphs; all represented the 2000 cohort of metamorphosing individuals. On 21 July we found 38 bullfrogs at the Laurel West EMS, 32 of which were juveniles and six of which were subadults or small adults.

No bullfrogs observed had malformations.

Table 5. Ranid Frogs Observed at the C&H and Laurel West EMSs during the 18-21 July 2000 Sampling Period for Metamorphosing or Recently Metamorphosed Oregon Spotted Frogs Time is 24-hr clock, midnight = 00:00; American bullfrog = *RACA*, Oregon spotted frog = *RAPR*; # = number of individuals; LS = life stage (L = larva, M = metamorph, J = juvenile, SA = subadult [subadults were juveniles one year class older than frogs indicated as juveniles; juveniles had metamorphosed in 2000]); SVL = snout-vent length in millimeters (mm) to the nearest 0.5 mm (SVL was estimated to the nearest 5 mm where the animal was not caught; estimates are indicated in **bold**); Mass = animal mass in grams (g) to the nearest 0.1 g; Notes provide key descriptive features.

Date	Time	Unit	Species	#	LS	SVL	Mass	Notes
18 July	23:30	Laurel	*RACA*	1	M	48.0	15.0	
18 July	23:45	Laurel	*RACA*	1	M	53.0	12.0	
19 July	00:00	Laurel	*RACA*	1	J	56.0	15.0	no tail scar
19 July	00:15	Laurel	*RACA*	1	J	53.0	9.0	tail scar small, but visible
19 July	00:30	Laurel	*RACA*	1	M	53.0	10.0	
19 July	00:45	Laurel	*RACA*	1	M	54.0	12.0	
19 July	01:00	Laurel	*RACA*	1	J	65.0	18.0	tail scar absent
19 July	01:10	Laurel	*RACA*	1	M	50.0	10.0	
19 July	01:20	Laurel	*RACA*	1	J	55.0	10.0	small tail scar visible
19 July	01:30	Laurel	*RACA*	1	J	56.0	12.0	tail scar not visible
19 July	12:25	C&H	*RAPR*	1	J	29.0	2.5	tail scar barely visible
19 July	12:28	C&H	*RAPR*	1	J	31.0	2.8	slight bone bridge on right femur
19 July	12:31	C&H	*RAPR*	1	J	30.0	2.1	large tail scar
19 July	12:34	C&H	*RAPR*	1	J	29.0	1.9	tail scar not visible
19 July	12:37	C&H	*RAPR*	1	M	27.0	3.9	right front leg not yet emerged
19 July	12:40	C&H	*RAPR*	1	J	29.0	2.1	tail scar not visible
19 July	12:43	C&H	*RAPR*	1	J	31.5	3.1	tail scar large
19 July	12:44	C&H	*RAPR*	1	J	32.0	3.4	
19 July	12:45	C&H	*RAPR*	1	M	28.0	4.5	missing end of tail, 40 mm hind legs
19 July	13:30	Laurel	*RAPR*	1	L	28.0	4.2	missing end of tail, hind legs 31 mm
21 July	14:00	Laurel	*RACA*	1	J	58.0	15.0	
21 July	14:08	Laurel	*RACA*	1	J	60.0	18.0	
21 July	14:17	Laurel	*RACA*	1	SA	102.0	74.0	
21 July	14:59	Laurel	*RACA*	1	SA	96.0	90.0	
21 July	15:06	Laurel	*RACA*	1	J	66.0	23.5	
21 July	15:26	Laurel	*RACA*	33	---	---		J-SA; SVL range = 50-115 for series
21 July	17:01	C&H	*RAPR*	1	J	**35.0**		Frog eaten by 500 mm SVL garter snake
21 July	17:00	C&H	*RACA*	1	SA	**80.0**		
21 July	17:10	C&H	*RAPR*	1	J	**35.0**		
21 July	17:15	C&H	*RACA*	1	SA	**80.0**		
21 July	17:20	C&H	*RACA*	1	J	**60.0**		
21 July	17:28	C&H	*RAPR*	1	J	38.0	4.9	no tail scar
21 July	17:30	C&H	*RAPR*	1	M	31.0	3.5	skin on back inflated with air bubble
21 July	17:36	C&H	*RAPR*	1	J	30.5	2.6	tail scar small
21 July	17:53	C&H	*RAPR*	1	M	**33.0**		
21 July	17:53	C&H	*RAPR*	1	J	33.0	2.4	tail scar gone (not visible)
21 July	18:05	C&H	*RAPR*	1	J	38.0	3.2	small tail scar visible
21 July	18:14	C&H	*RAPR*	1	J	**33.0**		swimming underwater
21 July	18:14	C&H	*RAPR*	1	J	39.0	4.7	small tail scar
21 July	18:15	C&H	*RAPR*	1	M	34.0	3.7	recap from past week marked RR4
21 July	18:18	C&H	*RAPR*	1	J	35.0	3.3	small tail scar
21 July	18:24	C&H	*RAPR*	1	J	34.0	3.0	very small tail scar

DISCUSSION

A. WATER QUALITY

NUTRIENTS

Our analysis of the different forms of nitrogen and phosphorus at Conboy Lake NWR in 2000 found little evidence of problematic eutrophication. Only the two spring sites (Headquarters, Willard) consistently had measurable levels of macrobiotically available nutrients[7] (i.e., nitrate and orthophosphate). Presence of these particular nutrients in only the springs implies a groundwater origin, as these forms tend to be either rapidly consumed biotically (Spalding and Exner 1993) or become unavailable in the more oxidizing environment characteristic of surface waters (Dillon and Kirchner 1975, Wetzel 2001). Moreover, hydrologically rich igneous-dominated lithologies of the Washington Cascade Range frequently result in spring waters that carry varying amounts of such nutrients (e.g., Mariner *et al.* 2003).

A seasonal eutrophication signature is evident from the general increase in total Kjeldahl nitrogen and total phosphorus in all locations monitored except Headquarters Spring; the latter showed an apparent decrease in these nutrients. Moreover, concurrent measurement of ammonia showing it to be largely below detectable limits indicates that total nitrogen values (as Kjeldahl nitrogen) were almost entirely due to the organic nitrogen fraction (APHA *et al.* 1992). Collectively, these data are consistent with a seasonal accumulation of organic biomass in this largely seasonal wetland (Brinson *et al.* 1981); data from the spring may indicate seasonal nutrient depletion from that source. Notwithstanding a seasonal eutrophic signature, values for total Kjeldahl nitrogen, total phosphorus, and ammonia were relatively low (see Wetzel 2001), especially in contrast to other lowland landscapes where agricultural influences play a role in the Pacific Northwest (Bonn *et al.* 1995, Rinella and Janet 1998, de Solla *et al.* 2002).

In the summaries that follow, we comment briefly on specifics of the different forms of nitrogen and phosphorus, and address the potential risk that each may pose to Oregon spotted frogs.

Nitrogen: In waters and wastewaters, the important forms of nitrogen (nitrate [NO_3^{-2}], nitrite [NO_2^-], organic nitrogen [N], and ammonia [NH_3]) are chemically interconvertible components of the nitrogen cycle (APHA *et al.* 1992). Oxidation-reduction of aqueous forms of nitrogen is closely tied to biological activity, and both the paths followed and the reaction end products strongly depend on the biota.

***Nitrite*:** Nitrite, a naturally occurring anion in freshwaters, is rapidly oxidized to nitrate in oxygenated waters, although water may be nitrite-bearing in reducing environments (Hen 1985 in Stednick 1991). Nitrite levels in oxygenated waters are typically < 0.0005 mg/L (APHA *et al.* 1992) and below the detection limit resolvable in this study (see TABLE 2). Perhaps not surprisingly, nitrite was not detected at aquatic sites sampled at Conboy Lake NWR. Oregon spotted frog larvae begin to show the negative effects of nitrite exposure at concentrations somewhere between 0.4 and 0.8 mg/L (TABLE 6). Since the instrumental

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[7] Macrobiotically available nutrients are those nutrient forms directly useable by large plants and animals; several nutrient forms (e.g., elemental nitrogen) are useable directly only by the microbiota (bacteria and selected microorganisms).

detection limit for nitrite in this study was 0.1 mg/L, we infer that if nitrite was present, the concentrations were about one-fourth of that required to manifest negative effects. We emphasize, however, that the threshold level of nitrite needed to induce a negative effect, even if closer to 0.8 mg/L, is still relatively low. As a consequence, monitoring nitrite may be among the best ways to detect threshold negative effects on Oregon spotted frogs.

***Nitrate*:** Nitrate generally occurs in trace quantities in surface water but may attain high levels in selected groundwater (APHA *et al.* 1992; Spalding and Exner 1993). In groundwater, nitrate is often the only common form of nitrogen. An essential nutrient for many photosynthetic autotrophs, it has been identified as growth-limiting in some cases (Wetzel 2001).

Nitrate, with one exception, was only detected in springs at Conboy Lake NWR and was recorded at concentrations between 0.1 and 0.3 mg/L (TABLE 2). This value range is over an order of magnitude lower than nitrate levels shown to cause increased mortality in Oregon spotted frog larvae, depressed growth rates in northern red-legged frog (*Rana aurora*) embryos, and depressed survivorship in the northern leopard frog (*Rana pipiens*) (TABLE 6). At Conboy Lake NWR, Oregon spotted frogs do not use springs for reproduction (unpubl. data). Uptake (by vegetation) or chemical conversion of nitrate in water originating in springs probably occurs before that water reaches oviposition sites. So, if nitrate was present at oviposition sites, it was below the 0.1 mg/L detection limit and would not pose a threat to Oregon spotted frogs. Nonetheless, the relatively low threshold at which nitrate can cause negative effects in Oregon spotted frogs also makes this form of nitrogen a good candidate to use in monitoring.

Ammonia: Ammonia is present naturally in surface waters and wastewaters as a result of bacterial decomposition of nitrogen-containing organic material and organism excretion, although the contribution of the latter is considerably less important (e.g., fishes, aquatic invertebrates; Wetzel 2001). In groundwater, ammonia is generally rare as it adsorbs to clays and soil particles and thus is not readily leached (APHA *et al.* 1992). Concentrations vary from < 0.01 mg/L in some surface waters and groundwaters to > 30 mg/L in selected wastewaters (APHA *et al.* 1992).

In water, ammonia exists largely either as un-ionized ammonia or ammonium ion (NH_4^+) in a temperature and pH-labile equilibrium (US EPA 1999). This equilibrium is significant because un-ionized ammonia is much more toxic than ammonium ion.

No data exist on effects of un-ionized ammonia on the Oregon spotted frog, but research on other ranid frogs indicates that sublethal effects (decreased growth and increased deformities) occur at concentrations in the 0.6-1.5 mg/L range (TABLE 6). The potential for ammonia to negatively affect aquatic organisms, based largely on the sensitivity of the early life stages of fishes, is often evaluated using chronic criteria, typically defined as four- or 30-day mean concentrations (US EPA 1999, ODEQ 2004)[8]. The temperature and pH-labile equilibrium for ammonia in water requires knowledge of both temperature

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[8] Chronic criteria are regarded as protective of aquatic life if threshold values are not exceeded more than once every three years (US EPA 1999, ODEQ 2004).

TABLE 6. EXPERIMENTAL ANALYSES INVESTIGATING EXPOSURE TO FORMS OF NITROGEN IN DIFFERENT RANID FROG SPECIES

Species[1]	Form[2]	Form(s) of Interest	Life Stage	Dose	Condition	Response	Reference
Rana aurora	NH_4SO_4	NH_4^+	larvae	82.7 mg/L	10-day exposure	no effect	Nebeker & Schuytema (2000)
				134.0 mg/L		↓ growth rates	
	NH_4NO_3	NH_4^+, NO_3^-	embryos	6.4 mg/L	16-day exposure	no effect	Schuytema & Nebeker (1999)
				13.2 mg/L		↓ growth rates	
	$NaNO_3$	NO_3^-		0.6 mg/L		no effect	
				29.1 mg/L		↓ growth rates	
	$NaNO_2$	NO_2^-	larvae	0.9 mg/L	15-day exposure	no effect	Marco *et al.* (1999)
				1.8 mg/L		↓ feeding	
						↓ swimming	
						disequilibrium	
						abnormalities[3]	
						paralysis	
						↑ mortality	
Rana cascadae	$NaNO_3$	NO_3^-	larvae	3.5 mg/L	14-day exposure	↓ development	Marco & Blaustein (1999)
						↓ tail loss rate	
						↑ exposure	
Rana clamitans	NH_3	NH_3	embryos	≥ 0.6 mg/L	114-day exposure	↓ survival	Jofre & Karasov (1999)
						↑ deformities[4]	
						↓ growth rates	

[1] *Rana aurora* (northern red-legged frog), *Rana cascadae* (Cascades frog), and *Rana clamitans* (green frog)

[2] Ammonia (NH_3), ammonium sulfate (NH_4SO_4), ammonium nitrate (NH_4NO_3), sodium nitrate ($NaNO_3$), and sodium nitrite ($NaNO_2$)

[3] Mainly edemas and bent tails

[4] Body curled up or down, asymmetric body, curled spine, short tail, abnormal tail fins, and deformed tail

TABLE 6. EXPERIMENTAL ANALYSES INVESTIGATING EXPOSURE TO FORMS OF NITROGEN IN DIFFERENT RANID FROG SPECIES (continued)

Species[1]	Form[2]	Form(s) of Interest	Life Stage	Dose	Condition	Response	Reference
Rana pipiens	NH_4NO_3	NH_4^+, NO_3^-	larvae	10.0 mg/L	chronic test	↓ survival	Hecnar (1995)
	NH_3	NH_3	embryos	≥ 1.5 mg/L	114-day exposure	↓ survival ↑ deformities[3] ↓ growth rates	Jofre & Karasov (1999)
Rana pretiosa	KNO_3	NO_3^-	larvae	2.5 mg/L	15-day exposure	no effect	Marco *et al.* (1999)
				12.5 mg/L		↓ feeding ↓ swimming disequilibrium abnormalities[4] paralysis ↑ mortality	
	$NaNO_2$	NO_2^-		0.4 mg/L		no effect	
				0.8 mg/L		↓ feeding ↓ swimming disequilibrium abnormalities[4] paralysis ↑ mortality	

[1] *Rana aurora* (northern red-legged frog), *Rana cascadae* (Cascades frog), and *Rana clamitans* (green frog)

[2] Ammonia (NH_3), potassium nitrate (KNO_3), and sodium nitrite ($NaNO_2$)

[3] Body curled up or down, asymmetric body, curled spine, short tail, abnormal tail fins, and deformed tail

[4] Mainly edemas and bent tails

and pH to determine chronic criteria under different conditions. Based on temperature (2-30°C) and pH (5.8-7.9) conditions across all sites sampled at Conboy Lake NWR in 2000 (TABLE 2, APPENDIX III), chronic criteria would vary between 1.02 and 7.00 mg/L. Based upon these data, ammonia being detected only once at Conboy Lake NWR at the limit of detection (0.05 mg/L; see TABLE 2) likely does not represent a threat to Oregon spotted frogs.

Kjeldahl Nitrogen (TKN): Except for the 4.9 mg/L TKN outlier value recorded at the Willard EMS on 20 July 2000, dissolved TKN and total TKN levels, respectively, did not exceed 1.3 and 1.7 mg/L (TABLE 2). These values extensively overlap those reported for streams (≤ 0.2-1.1 mg/L) monitored under the Oregon Plan for Salmon and Watersheds Program (ODEQ 2000). The 20 July Willard Spring outlier is probably a valid reading as both that value and total phosphorus (for the same date and location) were outliers among values for these nutrients at Conboy Lake NWR (FIGURE 6). Parallelism of outlier values strongly suggests that an episodic event (of unknown origin) occurred sometime after 31 May that resulted in elevated levels of both nutrients on 20 July.

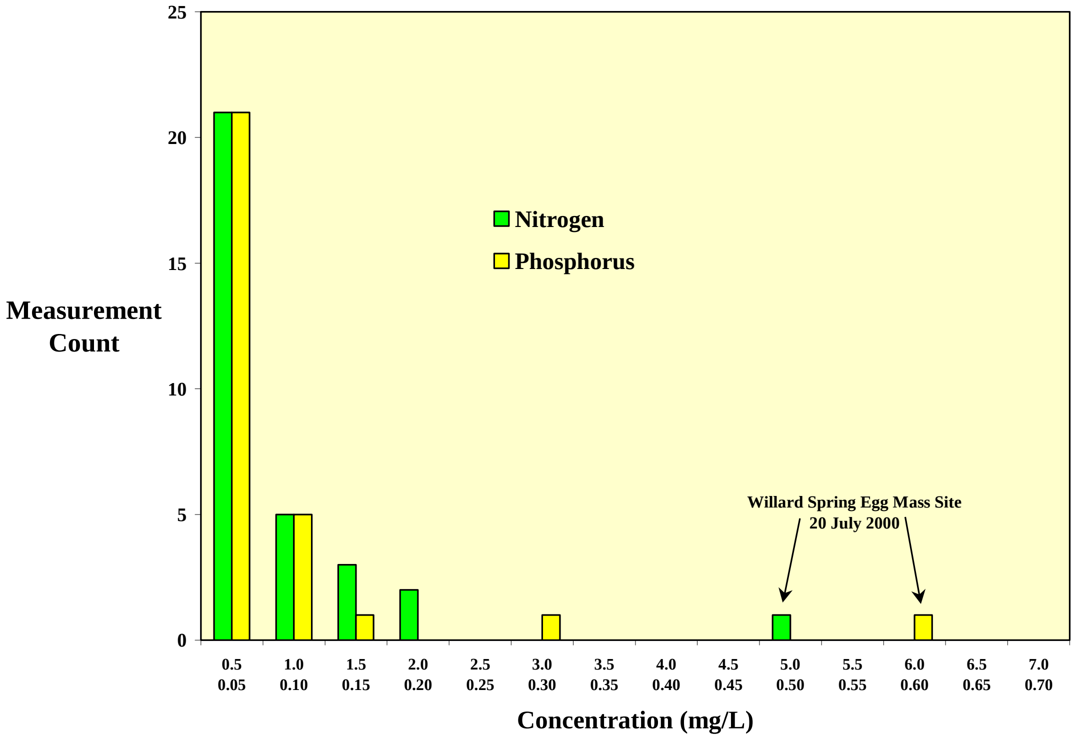

FIGURE 6. FREQUENCY DISTRIBUTION OF ALL NITROGEN (AS TOTAL TKN) AND PHOSPHORUS (AS TOTAL PHOSPHORUS) VALUES RECORDED AT CONBOY LAKE NWR, 2000 Upper and lower horizontal axis labels indicate concentration for nitrogen and phosphorus, respectively.

Higher total TKN levels detected at EMSs (as distinguished from the spring or stream inflow sites) are consistent with their shallow and ephemeral marsh character that differs hydrologically and physically from streams and lakes; this habitat difference results in a characteristic seasonally elevated TKN signature in shallow marshes (see Wetzel 2001).

Compared to other stillwater and wetland marsh systems where TKN has been measured, values recorded at Conboy Lake NWR are not extraordinary (see Wetzel 2001).

TKN represents combined ammonia and organic nitrogen, so low or absent ammonia (see previous section) indicates that organic nitrogen dominated TKN values at Conboy Lake NWR. Organic nitrogen exists in numerous forms and no attempt has been made to evaluate the risk of ecological relevant forms of organic nitrogen to Oregon spotted frogs or related anurans. However, given that TKN values at Conboy Lake NWR contrast favorably with those recorded in other marsh wetland systems, the risk to Oregon spotted frogs is probably low. Still, better understanding of seasonal and spatial variation in organic nitrogen at Conboy Lake NWR would be valuable, especially to understand the extent of spatial and seasonal restriction of outlier values.

Phosphorus: Phosphorus occurs in soils, plants, and microorganisms in varied organic and inorganic forms and is always present in animal metabolic waste. Phosphorus occurs in natural and waste-waters almost exclusively as phosphates, which are classified as orthophosphates, condensed phosphates, and organically bound phosphates (APHA *et al.* 1992). Orthophosphate, typically far less concentrated in natural waters than nitrate (Hem 1985 in Stednick 1991), is the basic form of phosphorus directly available to organisms for uptake and use. Orthophosphates applied to agricultural or cultivated residential land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Reactive phosphorus (available for organic metabolism and inorganic reactions) occurs in both dissolved and suspended forms (APHA *et al.* 1992). Total phosphorus represents the immediately available form (i.e., orthophosphate) plus the phosphorus that may become available through release from sediment, organic material, and ion exchange (Garman *et al.* 1986).

Surface waters generally receive most of their phosphorus in surface flows rather than in groundwater, as phosphates are typically bound up in soils and sediments (Correll 1998). Exceptions occur in watersheds of volcanic origin or waterlogged, anoxic soils. Phosphorus, essential to the growth of organisms, can often be the nutrient that limits aquatic primary production (Wetzel 2001). Phosphorus tends to be retained efficiently in these aquatic systems, leading to higher primary production, especially during summer and fall. High primary production leads to high rates of decomposition and depletion of dissolved oxygen. Such a seasonal eutrophic cycle can result in major shifts in species composition at all trophic levels (Correll 1998).

As previously noted, measurable orthophosphate parallels nitrate in having most of the highest values recorded from the two springs (TABLE 2), which suggests a groundwater origin for both nutrients. This agrees with the finding that many volcanically influenced areas that have naturally elevated levels of phosphorus originate from groundwater sources (Dillon and Kirchner 1975)[9]. In contrast to nitrate, mostly EMSs (three of five) had orthophosphate levels at or just above detection levels, whereas only one of three stream inflow sites (Chapman Creek) recorded orthophosphate; the value recorded was

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[9] In streams in Oregon monitored under the Oregon Plan for the Salmon and Watersheds Program (ODEQ 2000), orthophosphate levels ranged from ≤ 0.005 to ~ 0.17 mg/L, with most values falling below 0.025 mg/L. However, how comparable these streams are to the Conboy Lake wetland system is questionable since their hydrological dynamics differ substantially.

just at the detection limit on one date (TABLE 2). Like organic nitrogen, orthophosphate remains unstudied for its effects on the Oregon spotted frog, and data are also lacking for its effects on other anurans. However, an effect on developing Oregon spotted frog eggs is unlikely since elevated values are associated with springs, where Oregon spotted frogs do not lay eggs. Further, non-spring orthophosphate values were at or only slightly above detection limits, which would require an effect to be manifest on Oregon spotted frogs at low levels, an unlikely condition. Given that orthophosphate effects on Oregon spotted frogs are unstudied and that orthophosphate concentrations are labile due to the dynamic interactions and conversions between the sediments and water column (Correll 1998), orthophosphate is probably a poor nutrient form to use in trend monitoring for Oregon spotted frogs at this time.

Except for Headquarters Spring on 26 April with a total phosphorus value of 0.06 mg/L, all springs and stream inflow streams had total phosphorus values ≤ 0.05 mg/L. Stream inflow values agree with those for Oregon streams (ODEQ 2000), which had total phosphorus concentrations ranging from ≤ 0.01 to ~0.45 mg/L, with most values falling below 0.05 mg/L. Phosphorus values also agree with groundwater concentrations (as the springs) being typically low since phosphorus adheres to soil particles (Garman *et al.* 1986).

In contrast, seven of 15 total phosphorus values at four of five EMSs were > 0.05 mg/L, with maximum values reaching 0.26 mg/L and 0.59 mg/L on 31 May and 20 July, respectively, at Chapman Creek and Willard EMS. Wetzel (2001) reported phosphorus in most nonpolluted natural waters ranging from 0.01 to 0.05 mg/L, but recognized that natural variation is high, noting that the groundwater influence in volcanic landscapes contributes to the high end of this spectrum. Collectively, these data require caution in interpretation of phosphorus patterns at Conboy Lake NWR because elevated phosphorus at EMSs may simply reflect a natural eutrophic signature resulting in the seasonal release of bound organic phosphorus that had its ultimate origin from inorganically enriched groundwater. We can neither exclude an agricultural influence as an alternative source of phosphorus nor can we understand how much may be contributed from that source. Total phosphorus is probably a more reliable nutrient than orthophosphate for trend monitoring (Corell 1998), but we need to better understand the pathways contributing phosphorus. In particular, we need to understand the spatial and temporal contributions of phosphorus from groundwater and agricultural compartments before we can evaluate whether trend monitoring using total phosphorus will be useful at Conboy Lake NWR.

OTHER PARAMETERS

Variation in other water quality parameters (temperature, pH, DO, specific conductance) largely reflected differences among the flowing-water habitats (springs and streams) and stillwater habitats examined (ephemeral marsh EMSs). Spot data for these parameters (FIGURES 2-5) provide the broader view of patterns at Conboy Lake NWR (since they address all 10 sites sampled) albeit with few points. These data generally agree well with the large continuous datasets from Bird Creek and Headquarters Spring (APPENDIX FIGURES 1A-1P).

Variation in water quality at Conboy Lake NWR seems generally consistent with what is known about variation for these habitat types. Springs being the least variable of the three types for all four water quality parameters (see FIGURES 2-5 and APPENDIX FIGURES 1A-1P) is consistent with their water (groundwater) having been largely sheltered from solar, thermal, and other influences (Erman and Erman 1992). For example, stream water chemistry also often varies more than groundwater because of solute dilution during periods of heavy rain or snowmelt (Wetzel 2001). Moreover, higher specific conductance of springs versus the stream and ephemeral marsh habitats we examined is consistent with groundwater usually containing more dissolved minerals than surface water since it remains in contact with rocks and soils for longer periods (Leopold 1997).

In contrast to springs, greater diel and season variation in water quality at both stream and EMSs is consistent with the exposure of water in these habitats to physical and biotic diel and seasonal changes. Notably, the seasonal increase in air temperature is reflected in a general seasonal increase in water temperatures (APPENDIX FIGURES 1A-1D; see also FIGURE 2) that indirectly influences the seasonal decline in DO (APPENDIX FIGURES 1E-1H; see also FIGURE 3). Seasonal decrease in stream flow[10], which declines to a small volume (personal observations)[11], likely exacerbates the seasonal increase in water temperature. The seasonal decline in DO reflects the respiratory demands of consumer and producer organisms exceeding the DO production capacity of producers in seasonally shrinking aquatic habitats, conditions that can occur in small, low gradient streams and ephemeral marsh habitats like those at Conboy Lake NWR (Mitch and Gosselink 2000). The slight seasonal alkaline shift in pH (APPENDIX FIGURES 1I-1K) may also result from these changes[12]. By contrast, springs showed a slight elevation in DO after spring thaw and DO remained almost constant thereafter; this condition likely reflects an increase in producers to capacity at the springhead during late March-early April, and subsequent seasonal demand for DO by consumers (and producers) never exceeding that capacity. Episodic events may explain short-term changes in this general seasonal template. For example, the 63.5 mm (2.5 inches) of rain that fell between June 6 and 12 may have caused the slight drop in pH in Bird Creek around June 12. The event resulting in the sudden drop in DO in Bird Creek on 7 May is unknown.

The relatively large diel variation in temperature in streams (and presumably EMSs) reflects diel variation in insolation (APPENDIX FIGURES 1A-1D), which in turn reflects diel variation in the balance between DO production and consumption (APPENDIX FIGURES 1E-1H). During the day, DO increases as production exceeds consumption; at night, when production ceases, DO declines as consumption exceeds production (Wetzel 2001). The change in DO is linked to a change in pH because producers (plants) consume carbon dioxide (CO_2), the photosynthetic substrate, during the day in excess of its production (by consumers and producers [animals and plants] through respiration). Reduced levels of CO_2 result in a slight alkaline shift in pH because carbonic acid is removed in an equilibrium with dissolved CO_2 (Wetzel 2001). At night, consumer and producer respiration accumulates carbon dioxide, resulting in an acid shift in pH because carbonic acid increases as the CO_2 produced is dissolved. Seasonal changes in the amplitude of

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[10] In 2000 (a moderately droughty year), the flow decline had occurred by July (personal observation).

[11] In severe drought years, Chapman and Holmes Creeks actually cease to flow in summer.

[12] Drift and error associated with resetting the apparatus following power failures limit identifying the magnitude and reality of the shift in pH (see APPENDIX III).

variation in temperature, DO, and pH were also generally correlated; for example, higher variation in temperature in July was reflected in a higher range in DO and pH.

Data on water quality parameters at EMSs comes exclusively from spot measurements as continuous recording dataloggers (Hydrolabs) could not be deployed at any EMS because we could not precisely predict when the sites would dry (the sensitive probes of Hydrolabs must be submerged in water or they will be damaged). Variation in EMSs generally paralleled that for the creek sites except that seasonal changes recorded were more extreme (FIGURES 2-5). In particular, temperature and specific conductance rose faster seasonally, and DO and pH generally dropped to lower levels at EMSs than at stream sites.

Collectively, water quality data agree with those expected for relatively small streams (Chapman, Holmes – 2nd order[13]; Bird – 4th order) in a low-gradient landscape, springs in volcano lithologies, and seasonal marshes (the EMSs) (see Mitch and Gosselink 2000, Wetzel 2001). We have obtained a perspective on water quality variation that is spatially truncated (study sites are only a small part of a larger wetland system), seasonally truncated (study sites were examined only from March to July), and truncated on a inter-year scale (our study was done in one moderately droughty year). Recognizing this truncation is critical because spatial truncation may have excluded habitats with water quality patterns different from those we sampled; seasonal truncation excluded time intervals when flows were lower and conditions drier than during our sample period; and inter-year truncation excluded years with both wetter and drier conditions (personal observations on the latter two). We emphasize that the seasonal and inter-year truncation omit especially important components of our understanding because late summer low-flow conditions and more extreme drought years are expected to manifest more extreme water quality (and nutrient) conditions. This is critical as we expect that the naturally eutrophic low-flow signature of this system to manifest extreme values for selected water quality parameters and nutrients; any agricultural enrichment would potentially simply amplify that signature. For example, the work of three of us (JDE, MPH, and CJR) has revealed high water temperatures in this system under typical late summer conditions. Specifically, surface water temperature in Camas Ditch (or Outlet Creek), the drain that collects waters from Bird, and Chapman and Holmes Creeks, develops water temperatures consistently over 20°C, with local pockets over 25°C in wet years; drought years result in an upward shift of water temperature in the 3-6°C range (unpubl. data). This perspective poses two important difficulties in understanding the dynamics of this system:

1) The existing Conboy Lake wetland system is hydrologically altered and agricultural enterprises affect that system to varying degrees (location-dependent), distinguishing how agriculture has altered the seasonal signature, if at all, will be difficult.
2) Determining that a water quality (or nutrient) "problem" exists will have little meaning outside of the context of better understanding this wetland system. For example, July temperatures we recorded in Bird Creek exceeded the designated use

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[13] Stream order is a mode of indicating relative stream size according to the pattern of tributaries that was developed by Strahler (1952), where the smallest headwater streams are 1st order, two 1st order streams make a 2nd order stream, two 2nd order streams make a 3rd order stream and so forth.

temperature standard for Bird Creek at 17.5 to 18.0°C depending on whether revised standards (not yet EPA approved) or existing standards (EPA approved) are used. Depending on glacial snowmelt pattern on Mt. Adams (which feeds Bird Creek at its origin)[14], standards may be exceeded through August and possibly September. We should note here that Bird Creek represents the colder and most permanent of the streams contributing water to the Conboy Lake system, so the other streams contributing water to Conboy Lake wetlands are unlikely to fare better (see our previous discussion of Camas Ditch). This standards problem is not unique to temperature, and we anticipate that the seasonal signature of this system will consistently locally violate standards for DO and pH, (WDOE 2004) and likely some of the nutrients (APPENDIX VI).

Instrumentation errors (data logger drift and instrument reset error following power failures) prevented us from confidently identifying whether some of the observed patterns in water quality variation were real. In particular:

1) We could not reject the hypothesis that specific conductance in Headquarters Spring was constant. Headquarters Spring readings may have been somewhat suspect since the datalogger often read higher than the standards during maintenance checks. One should also recognize that some of this problem may arise simply from the difficulty of measuring low conductivity freshwater systems.
2) We could not be certain that the slight seasonal alkaline shift in pH in Headquarters Spring was real.

B. AMPHIBIAN DATA

Embryonic stages: Low levels of deformity-related (0.5-5.8%) embryonic mortality in Oregon spotted frogs at Conboy Lake NWR are similar to background developmental mortality levels that have been identified in Oregon spotted frogs and other ranid frogs elsewhere (Licht 1969, McAllister and Leonard 1997; MPH, unpubl. data). Moreover, we found no correlations between levels of any nutrients across EMSs and levels of deformity-related embryonic mortality, and we were unable to detect either nutrient (nitrate or nitrite) for which a risk to Oregon spotted frog life stages is known at any EMS site in March, when embryos are present.

Larval stages: Asymmetries, deformities and atypical behaviors in Oregon spotted frog larvae were infrequent at Conboy Lake NWR. Further, the lack of significant correlation between levels of any nutrients across EMSs and frequencies of deformities or atypical behaviors make it unlikely that these anomalies are attributable to nutrient enrichment.

Metamorphic and post-metamorphic stages: The only peculiarities found were the subdermal gas bubble seen in a metamorphosing Oregon spotted frog and the femoral bone bridge found in a juvenile Oregon spotted frog, both from the C&H study site. The subdermal gas bubble represents is a low-frequency phenomenon that typically arises from a localized infection of the lymph hearts that can be induced by a variety of

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[14] If late summer conditions are warm, greater glacial melt on Mt. Adams may actually cool Bird Creek more than if late summer conditions are cooler (personal observations).

typically non-lethal bacterial pathogens, and rarely results in mortality, at least in the wild (Zimmermann 1986). Moreover, this phenomenon is typically not symptomatic of contaminant-induced conditions. The only peculiarity that might be construed a malformation was the bone bridge found in the femur of one juvenile. As this condition is characteristic of calluses associated with bone break trauma (Meteyer *et al.* 2000), our sampling revealed no malformities that could be considered problematic in either Oregon spotted frogs or American bullfrogs. We consider the latter as a lack of biological response that can been associated with any habitat impacts at Conboy Lake NWR. However, this finding has only weak support because of the small sample size.

Lack of malformations observed in bullfrogs at Conboy Lake NWR is consistent with available data that indicate that bullfrogs are more tolerant to environmental insults than other amphibians (Kruse and Francis 1977; Bury and Whelan 1984). Thus, bullfrogs may be a less suitable species in which to look for malformations. A related factor to consider is the fact that at Conboy Lake NWR, attempt is made to manage water levels to negatively impact bullfrogs, which reduces the number of individuals which survive to metamorphosis (JDE, unpubl. data) and may limit the ability to obtain the appropriate sample size for such monitoring.

CONCLUSIONS

Primary nutrients levels (nitrogen and phosphorus) were generally lower than those recognized as compromising the life stages of western North American ranid frogs, and generally lower than standards recognized as being a risk to human health by Canadian and U.S. regulatory agencies. Moreover, available data, albeit few, suggest no significant problem with eutrophication. However, a relatively small sample size must temper these conclusions.

Seasonal increases in total Kjeldahl nitrogen and total phosphorus suggest that the Conboy Lake marsh system is seasonally eutrophic. Whether this pattern reflects the historic (pre-development) condition, and whether agriculture (livestock grazing) actually contributes to this seasonal eutrophic pattern, and to what degree if it does, is currently unclear. Special effort will be needed to precisely identify the sources of eutrophication in this system, and determine how much, if any, of the current seasonal variation does not reflect a historic pattern. Since the Oregon spotted frog is a warmwater-adapted species (Hayes 1997), we believe it is likely that this seasonal pattern is characteristic of sites where this species occurs. This will require a re-evaluation of how water quality standards are applied (as previously discussed) in evaluating the quality of sites where this species occurs, especially those standards based on cool-water adapted salmonid fishes.

Given these conclusions, we recognize certain limitations of this study. First, the complexity of the Conboy Lake system prevented clear delineation between sites with agricultural influence versus sites lacking such an influence. The five EMSs we examined were all influenced by water with some agricultural influence; Willard and Headquarters EMS likely had the least agricultural influence while Chapman, C&H, and Laurel West EMSs had water that was more directly influenced by livestock. Second, between-year variation can make inference from a single drought year risky. At minimum, two years of data would provide a much stronger basis for inferring patterns.

Thirdly, the recommended minimum sample of 50 metamorphs per USFWS (2000) guidelines was not met. Reduced sample sizes may reduce the likelihood of observing malformed frogs. Finally, localized structural habitat limitation for native amphibians and domination of some of the existing habitat by introduced (exotic) aquatic predators (e.g., bullfrogs, brown bullheads) at Conboy Lake NWR has the potential to be confounded with contamination in that each could result in depressed frog numbers.

MANAGEMENT RECOMMENDATIONS

We recommend periodic monitoring to detect augmentation of the natural seasonal eutrophic signature. Absent analytical measurement for nutrients in water, wetlands should be observed for signs of increased eutrophication through visual detection of increasing or changing vegetative growth.

If analytical measurements are utilized in monitoring for eutrophication, the focus should be on target nutrients that have the most direct impact on amphibians, including nitrate, nitrite, and ammonia. Other constituents could be measured if funding is available. A sampling design should be well thought out to ensure a suitable framework for statistical analysis.

Nutrient inputs from livestock and other off-refuge sources should be minimized by encouraging livestock users to protect channels from direct livestock use by pumping water to an off-channel watering location. Where this cannot be effectively done, sections of the channel could be fenced to allow vegetative growth to help filter excess nutrients.

Agricultural activities have been identified as a potential source of nutrient input to the refuge, yet there may be other contributors. For example, numerous goose droppings were observed near many of these sites during sample collection. We recommend more definitive identification of nutrient inputs, perhaps via isotopic signature.

Adaptive feedback should be implemented with management actions to test whether activities are working in the manner expected. If management activities are not working as desired, the approach should be adjusted. Feedback loops to monitor design effectiveness would benefit by being conducted with community involvement, such as landowners and local stakeholders.

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APPENDIX I

Sampling Methods

Frogs were captured by hand or with net sampling or if not captured, recorded via visual encounter survey, whereas net sampling was the primary method used to capture larvae or tadpoles. Each method is briefly described below.

Hand Capture: To use this method effectively on the metamorphic or post-metamorphic stages of frogs, a stealth approach was employed while simultaneously moving directly down the line of sight of target animals in order to minimize detection from one's lateral movements. Frogs that remained stationary long enough for a surveyor to approach sufficiently close to make a capture attempt were grabbed rapidly and retained for processing.

Net Sampling: Net sampling (with a kicknet) was used to assist in the capture of premetamorphic amphibian life stages, and to capture metamorphic and post-metamorphic frogs under circumstances where hand capture had a lower probability of effecting a capture. Across the study sites, most sampling efforts were directed at the specific aquatic habitats with the highest likelihood of having amphibians, such as aquatic and emergent vegetation patches, which are known refuge areas. Net sampling of these areas was conducted in a systematic pattern. First, net sweeps approximately 1 m in length would be made both 1) *around* the selected habitat, quickly and repeated sufficiently to cover the entire water column, and 2) *through* the selected habitat, again with sufficient repetitions to thoroughly sample the selected habitat. All suitable habitat identified within sites was sampled in this method.

Visual Encounter Observations: Observations resulting from direct visual encounters with animals were a third method used to gather data. This method was used primarily to record data on individual amphibians that were not captured, especially in situations in which capturing those individuals would have been time prohibitive.

APPENDIX II

SPOT DATA FOR WATER QUALITY PARAMETERS MEASURED AT CONBOY LAKE NWR, 2000

APPENDIX TABLE I. SPOT DATA FOR TEMPERATURE, DISSOLVED OXYGEN, pH, AND SPECIFIC CONDUCTANCE MEASURED AT CONBOY LAKE NWR, 2000 (Georeferencing for sites in TABLE 1).

Parameter		**Temperature**	**Dissolved Oxygen**	**pH**	**Specific Conductance**
Units		degrees C	mg/L	standard units	µS/cm
Location	**Date**				
Headquarters Spring inflow	28-Mar	7.49	~9.22	5.78	66.4
	26-Apr	7.60	9.79	6.52	70.7
	20-Jul	7.74	10.35	6.60	65.9
Headquarters Spring EMS	28-Mar	10.73	10.88	7.39	52.3
	26-Apr	23.00	11.58	7.04	58.5
	20-Jul	16.00	5.50	6.58	70.3
Bird Creek inflow	28-Mar	5.24	11.30	7.29	39.4
	26-Apr	9.50	10.30	7.54	33.4
	20-Jul	30.15	6.67	7.41	40.5
C&H EMS	28-Mar	8.11	9.95	7.27	57.7
	26-Apr	18.40	9.77	7.25	95.1
Willard Spring inflow	26-Apr	7.40	10.32	6.71	64.0
	31-May	7.50	9.92	6.69	64.7
	20-Jul	8.10	11.07	7.00	71.3
Willard EMS	26-Apr	17.00	5.50	6.28	56.5
	31-May	12.90	4.41	6.55	62.9
	20-Jul	20.00	1.50	6.23	79.9
Chapman Creek inflow	28-Mar	7.48	11.19	7.14	48.9
	26-Apr	14.50	8.98	7.45	66.3
	31-May	14.40	9.32	7.59	82.0
	20-Jul	24.01	8.23	7.50	118.0
Chapman Creek EMS	28-Mar	11.12	9.92	6.64	49.1
	26-Apr	13.10	9.16	6.99	73.5
	31-May	17.70	5.83	6.54	80.3
	20-Jul	27.32	2.68	6.94	227.0
Holmes Creek inflow	28-Mar	6.82	11.38	7.24	53.8
	26-Apr	10.90	9.63	7.87	71.7

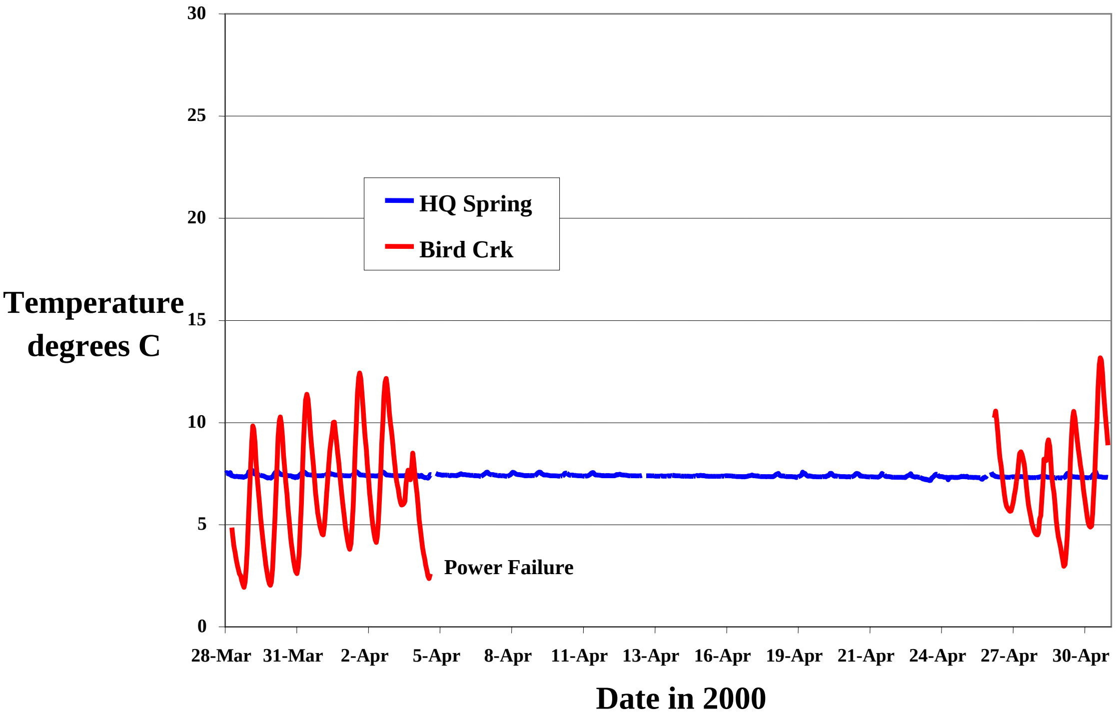
APPENDIX III

CONTINUOUS DATA FOR WATER QUALITY PARAMETERS MEASURED AT CONBOY LAKE NWR, 2000

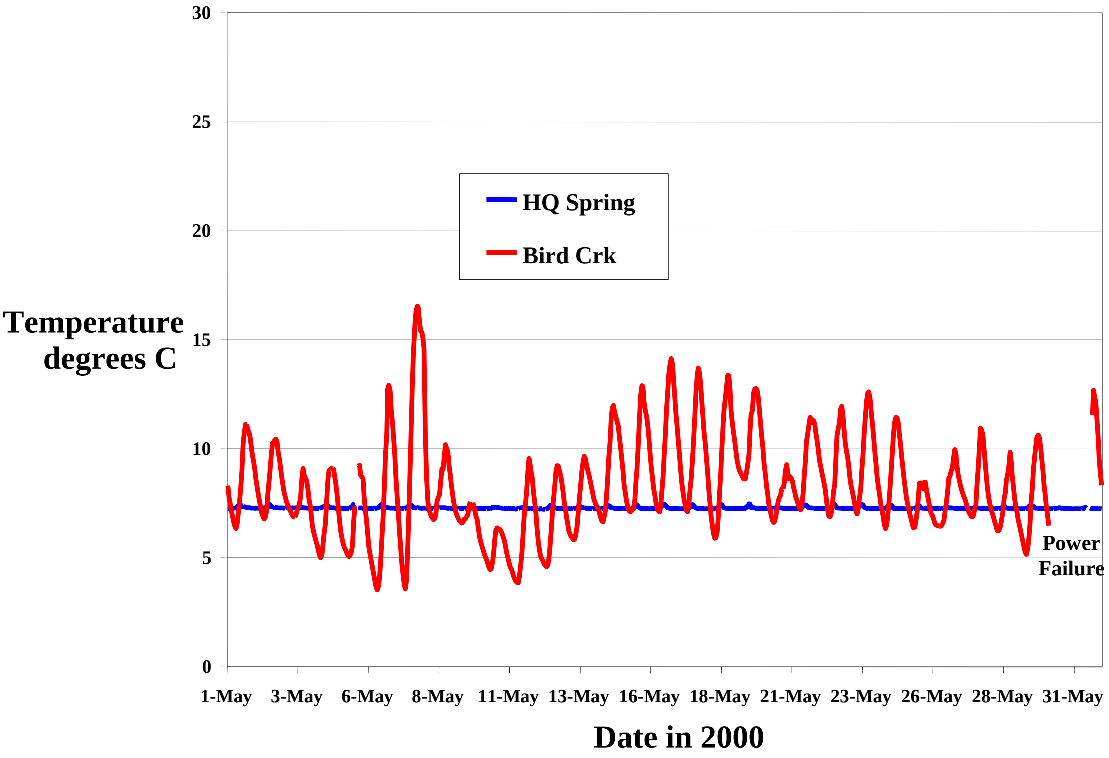
This APPENDIX provides graphics illustrating the four water quality parameters obtained from the Hydrolab Datasonde units deployed at each of Headquarters Spring and Bird Creek over the length of the field study. Data are in figures as follows:

1) Temperature: APPENDIX FIGURES IA-ID
2) Dissolved Oxygen: APPENDIX FIGURES IE-IH
3) pH: APPENDIX FIGURES II-IL
4) Specific Conductance: APPENDIX FIGURES IM-IP

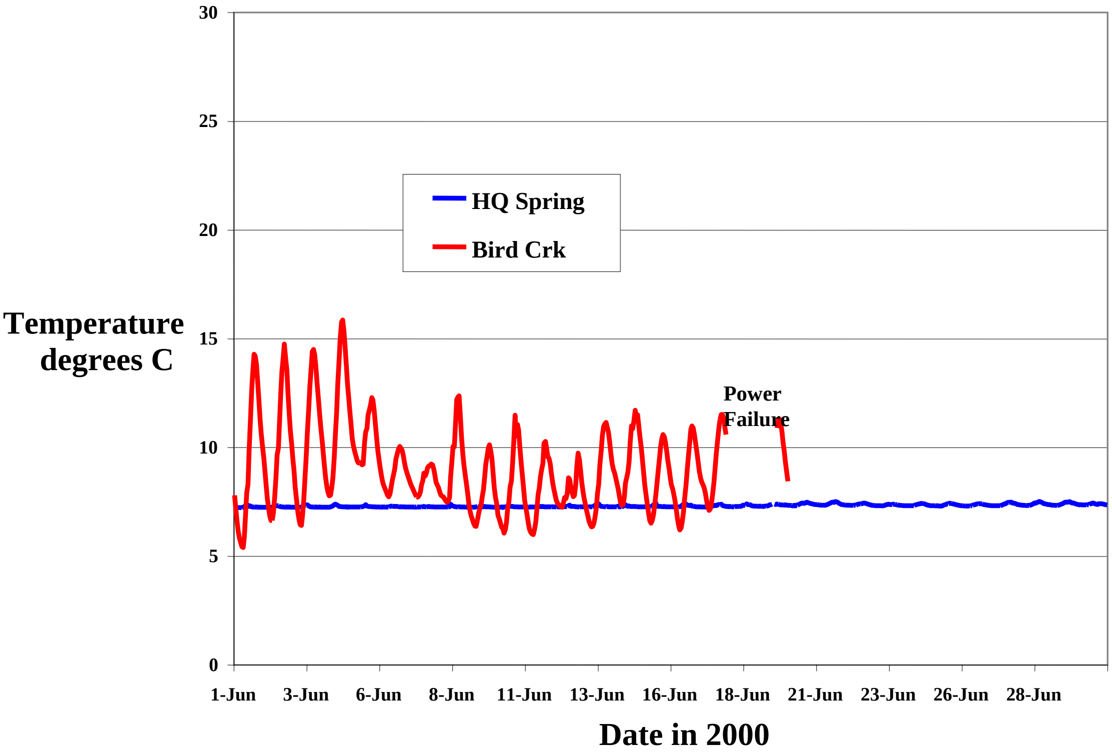
Interruptions in the data strings due to power failure or unit servicing are indicated as "Power Failure." Occasionally, capricious readings signaled the "Initiation of Power Failure"; those points are so indicated.



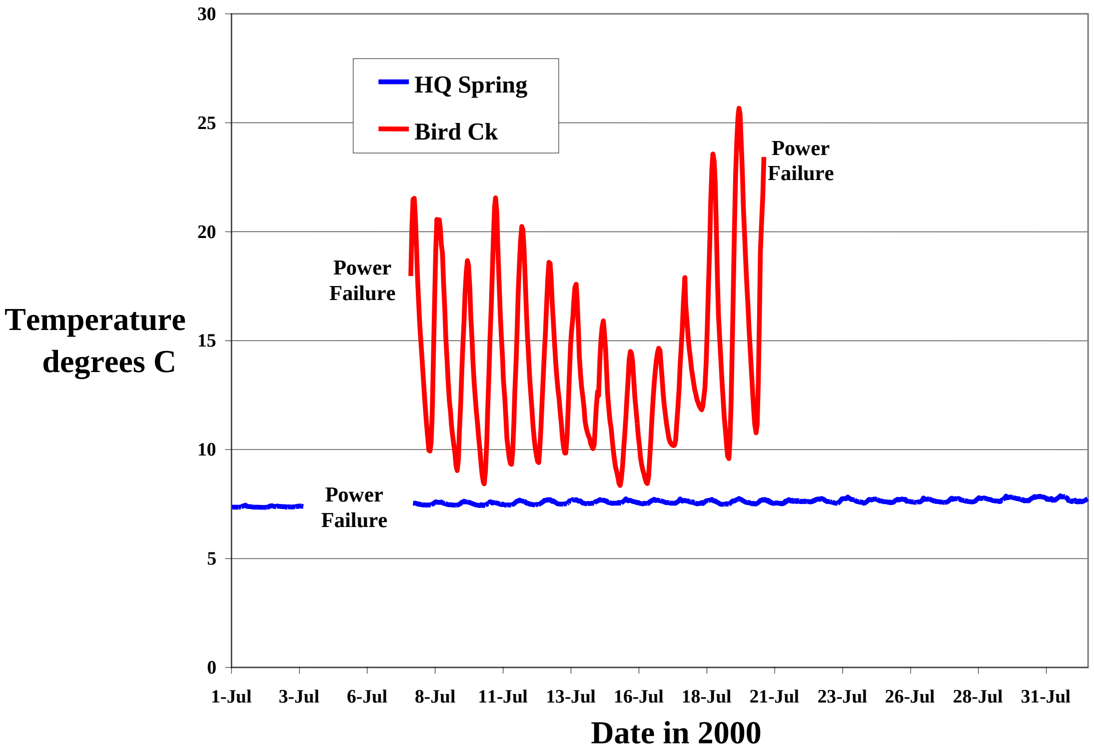
APPENDIX FIGURE 1A – WATER TEMPERATURE, 28 MARCH TO 30 APRIL 2000



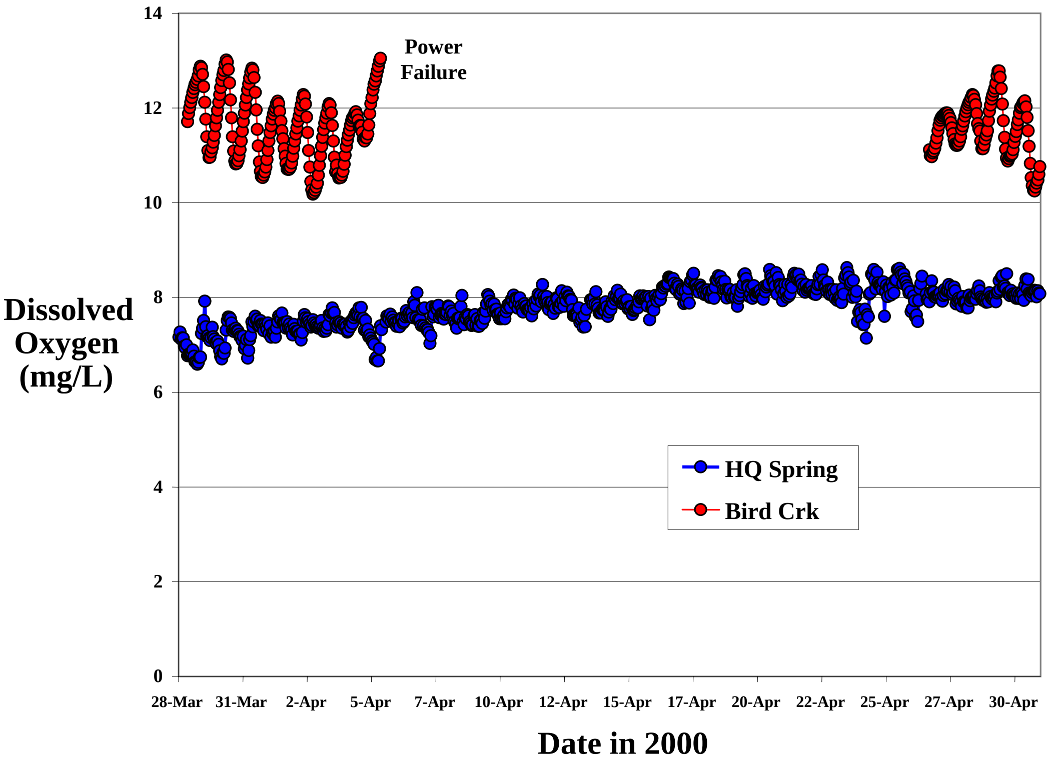
APPENDIX FIGURE 1B – WATER TEMPERATURE, MAY 2000



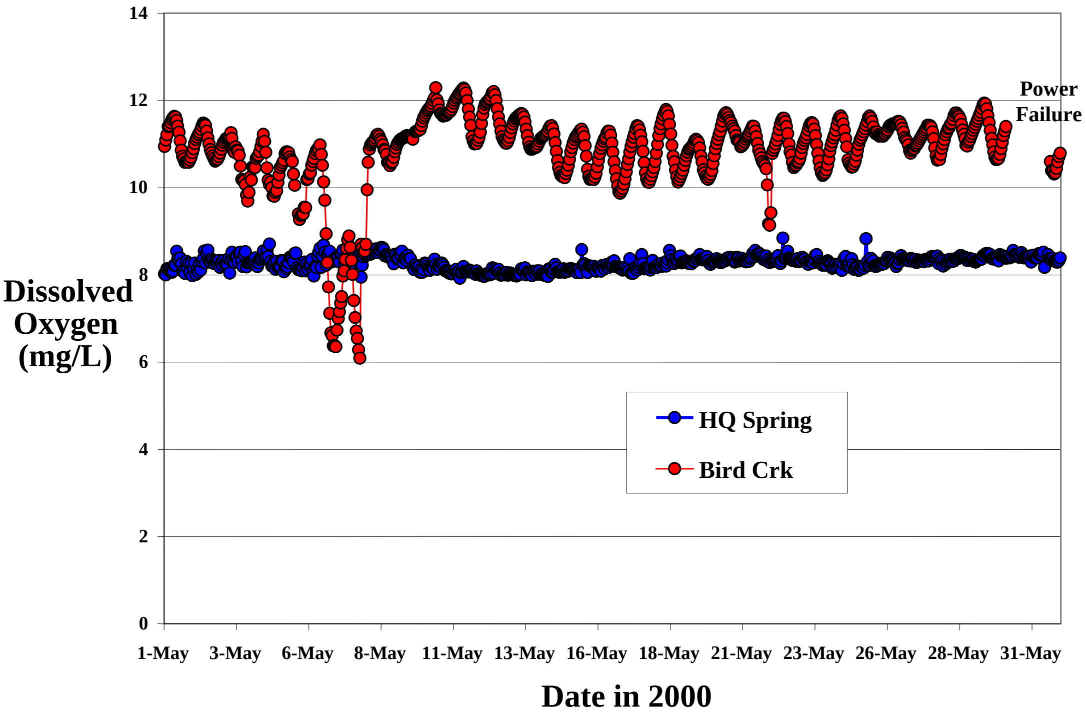
APPENDIX FIGURE 1C – WATER TEMPERATURE, JUNE 2000



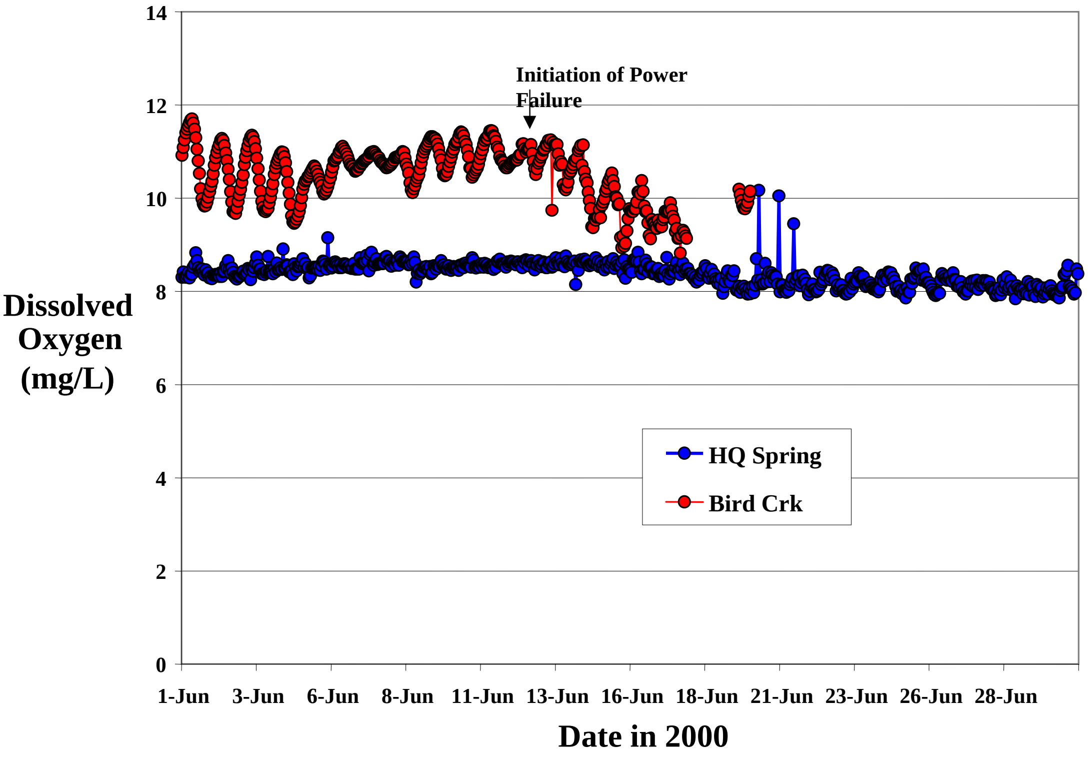
APPENDIX FIGURE 1D – WATER TEMPERATURE, JULY 2000



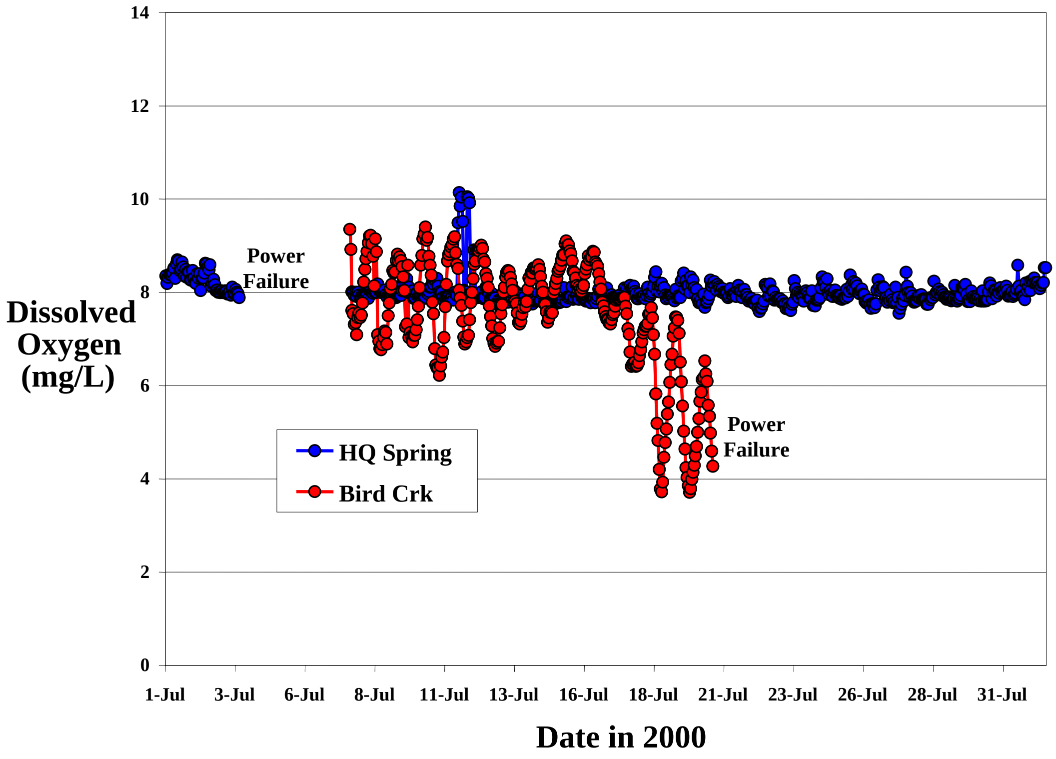
APPENDIX FIGURE 1E – DISSOLVED OXYGEN, 28 MARCH TO 30 APRIL 2000



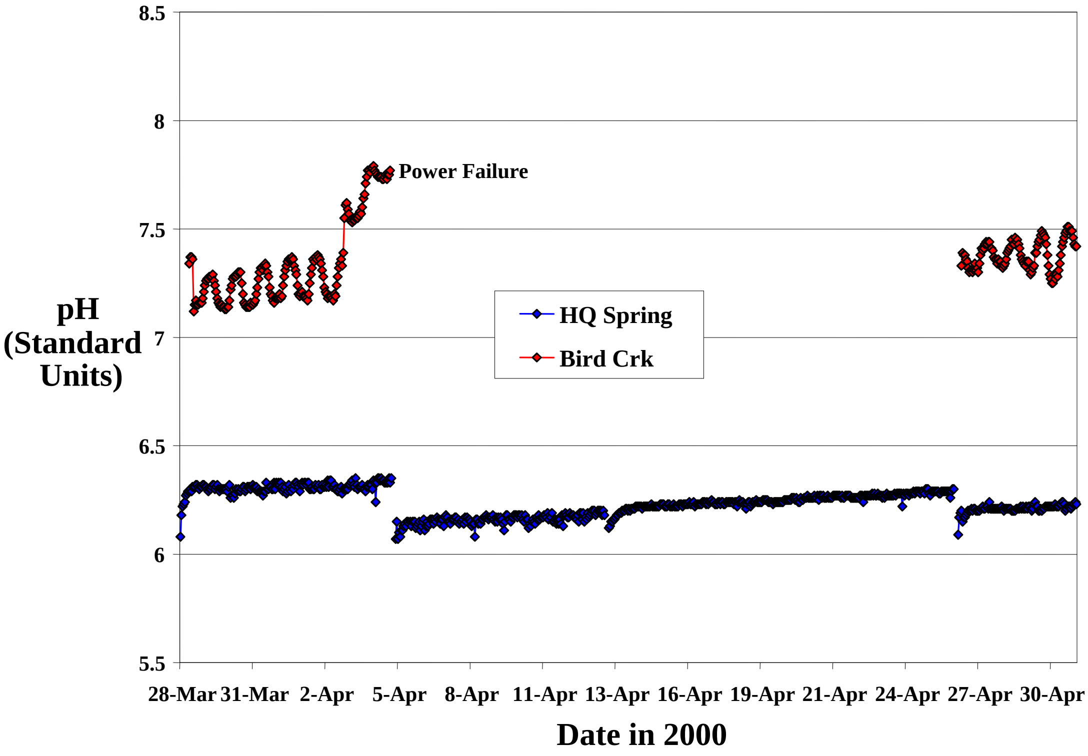
APPENDIX FIGURE 1F – DISSOLVED OXYGEN, MAY 2000



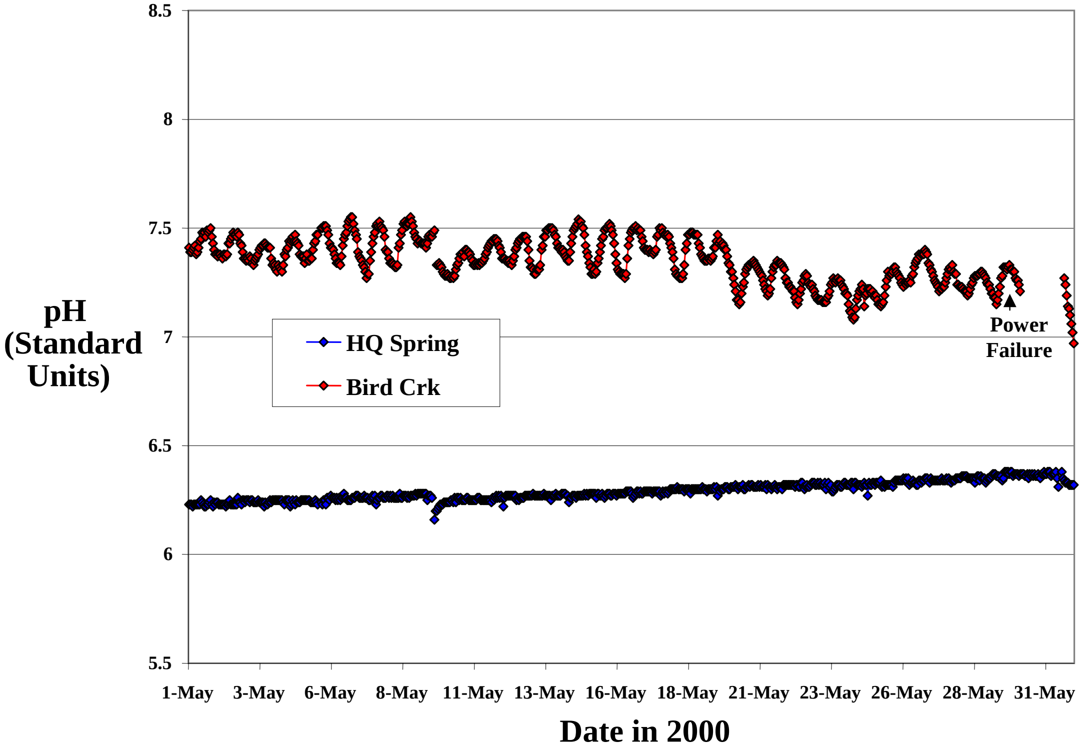
APPENDIX FIGURE 1G – DISSOLVED OXYGEN, JUNE 2000



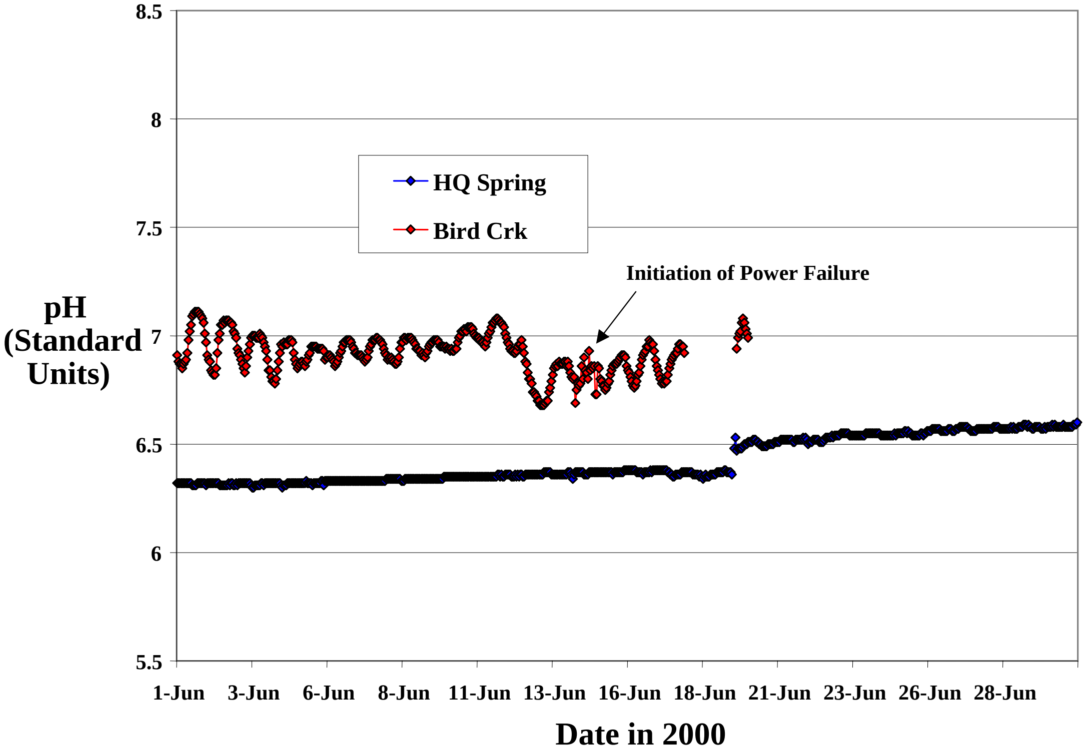
APPENDIX FIGURE 1H – DISSOLVED OXYGEN, JULY 2000



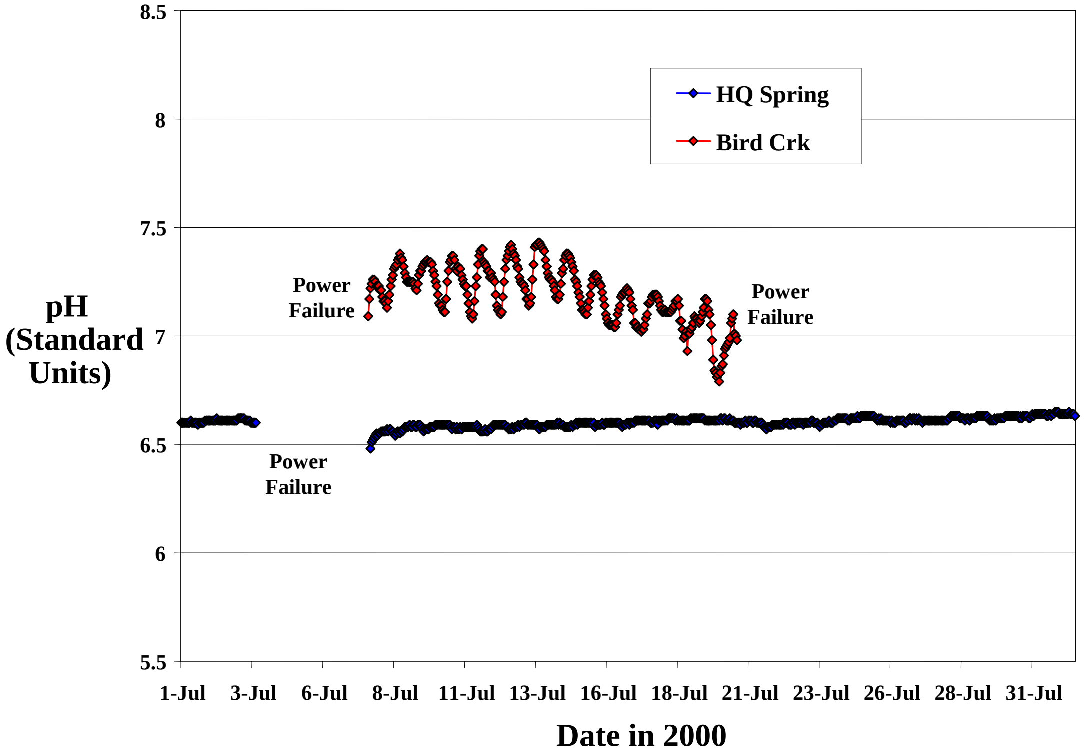
APPENDIX FIGURE 1I – pH, 28 MARCH TO 30 APRIL 2000



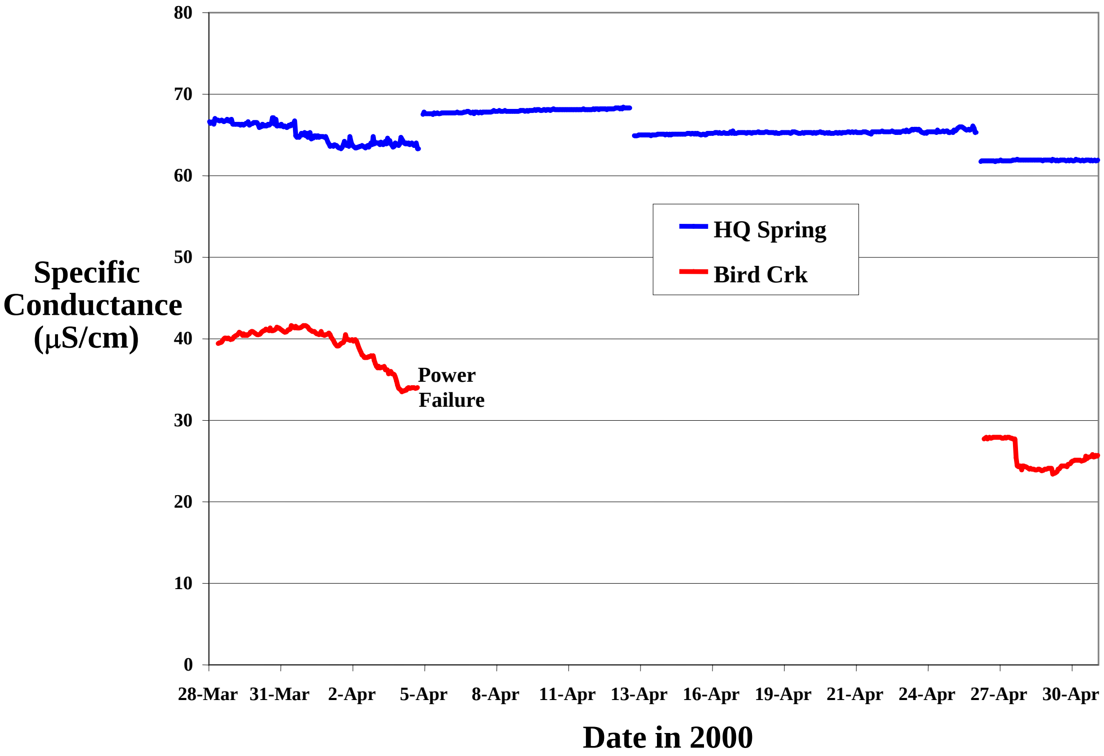
APPENDIX FIGURE 1J – pH, MAY 2000



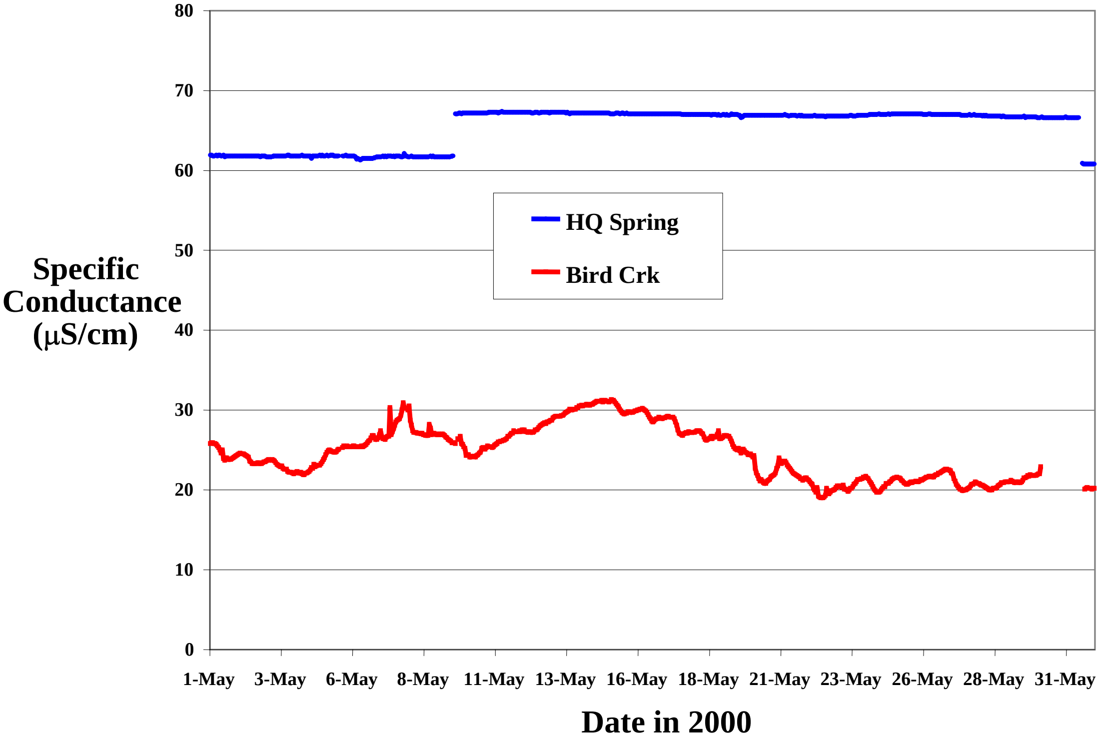
APPENDIX FIGURE 1K – pH, JUNE 2000



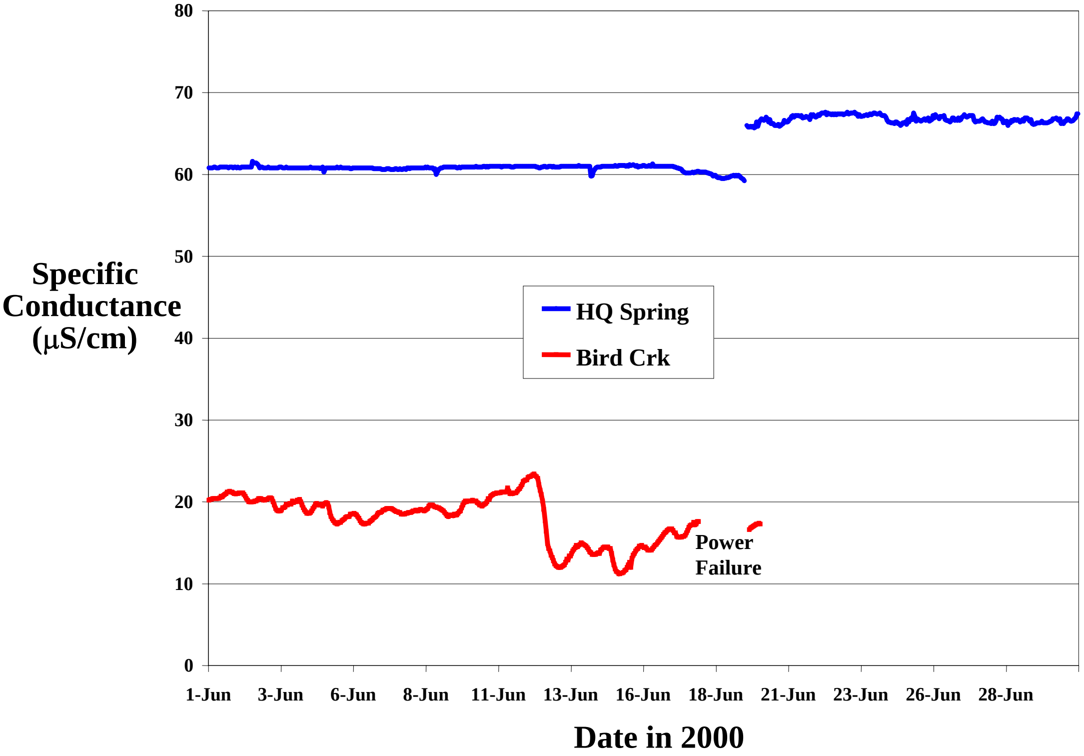
APPENDIX FIGURE 1L – pH, JULY 2000



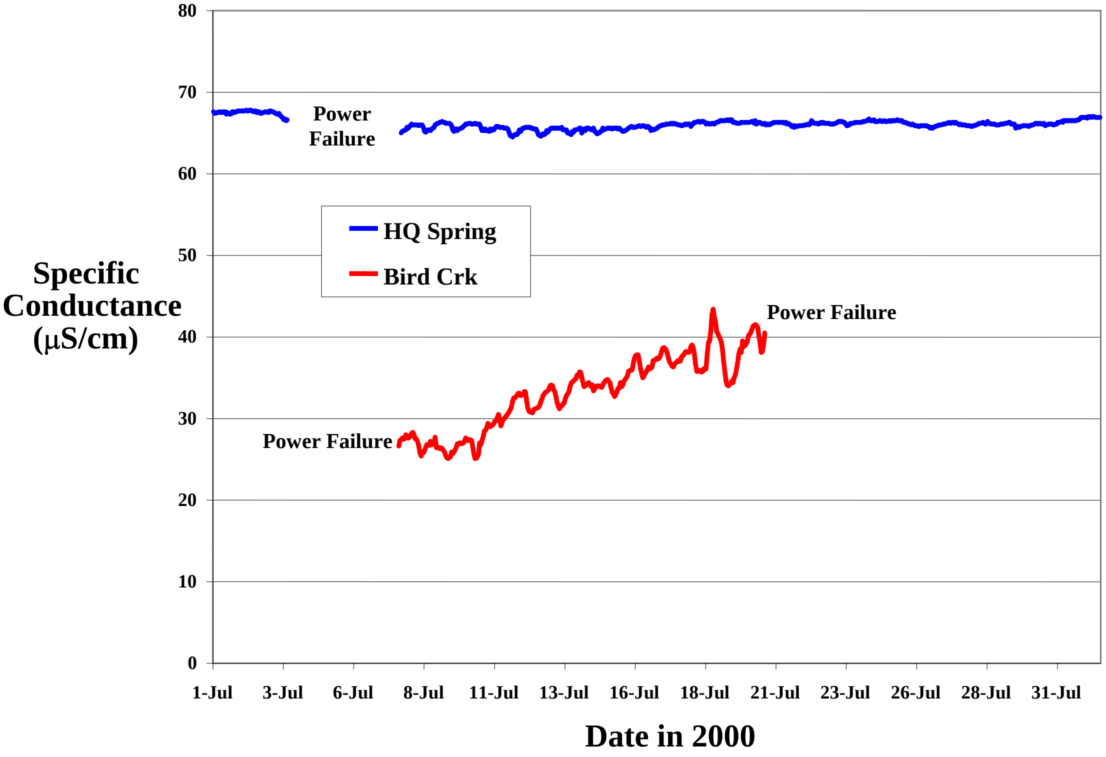
APPENDIX FIGURE 1M – SPECIFIC CONDUCTANCE, 28 MARCH TO 30 APRIL 2000



APPENDIX FIGURE 1N– SPECIFIC CONDUCTANCE, MAY 2000



APPENDIX FIGURE 1O– SPECIFIC CONDUCTANCE, JUNE 2000



APPENDIX FIGURE 1P– SPECIFIC CONDUCTANCE, JULY 2000

APPENDIX IV

SAMPLING EFFORT FOR POST-LARVAL RANID FROGS AT CONBOY LAKE NWR, 2000

APPENDIX TABLE II. SAMPLING EFFORT FOR METAMORPHOSING AND POST-METAMORPHIC RANID FROGS AT CONBOY LAKE NWR, 2000

Date	Start Time	End Time	Total Hours	Unit
18-July	23:30	00:00	0.50	Laurel West
19-July	00:00	01:30	1.50	Laurel West
19-July	12:25	12:45	0.33	C&H
19-July	13:20	13:40	0:33	Laurel West
21-July	13:50	15:30	1.67	Laurel West
21-July	17:00	19:00	2:00	C&H

APPENDIX V

Other Amphibians Recorded at Conboy Lake NWR

Besides the Oregon spotted frog and the American bullfrog we sampled in this study, six additional amphibian species have been recorded at Conboy Lake NWR. Three of these species (long-toed salamander [*Ambystoma macrodactylum*], the Pacific treefrog [*Hyla regilla*], and the rough-skinned newt [*Taricha granulosa*]) were recorded at sites used in this study in 2000. Brief description for each follows:

Long-toed Salamander (*Ambystoma macrodactylum*): The long-toed salamander has been observed at Conboy Lake NWR primarily in association with Cold Springs Ditch and its associated spring system. In 2000, it was observed at the Headquarters and Willard Springs sites; it was also recorded at the Headquarters EMS in March 2000. It was not observed at the remaining seven sites used in this study during 2000.

Pacific Treefrog (*Hyla regilla*): Pacific treefrogs were found at many sites on Conboy Lake NWR, and may be the most widespread amphibian on the refuge. Pacific treefrogs were most common at sites that either lacked fish entirely or possessed only fish species not typically predatory on amphibian life stages. Pacific treefrogs were observed at all sites sampled in this study, but not during the 18-21 July 2000 sampling for metamorphosing and recently metamorphosed ranid frogs.

Roughskin Newt (*Taricha granulosa*): Similar to the long-toed salamander, the rough-skinned newt has been observed in Cold Springs Ditch and its associated spring system, but it has also been recorded from Bird Creek and Camas Ditch (see FIGURE 1). In 2000, it was observed at the Bird Creek inflow site, and the Headquarters and Willard Springs sites. It was not observed at the remaining seven sites used in this study during 2000, including any of the five EMSs used in this study.

The remaining three amphibian species (Northwestern Salamander [*Ambystoma gracile*], Western Toad [*Bufo boreas*], and Northern Red-legged Frog [*Rana aurora*]) recorded at Conboy Lake NWR are all much less frequently encountered. Western toad has been found near the C&H EMS and in Camas Ditch. The northwestern salamander and northern red-legged frog have both only been recorded from near the Laurel West EMS.

APPENDIX VI

COMPARISON OF THE CONCENTRATIONS FOR FORMS OF NITROGEN AND PHOSPHORUS DETECTED AT CONBOY LAKE NWR WITH WATER QUALITY CRITERIA AND GUIDELINES FROM PACIFIC NORTHWEST REGULATORY AGENCIES

U.S. Environmental Protection Agency criteria for nutrients are empirically derived to represent surface waters with minimal human impact and be protective of aquatic life (US EPA 2000). Based on available data, EPA considers the values presented to represent nutrient levels that would generally protect against adverse cultural overenrichment, but expects States and Tribes to refine criteria for specific waterbody types, and that specific beneficial uses would be protected. Canadian Water Quality Guidelines for the Protection of Aquatic Life are designated to protect all plants and animals that live in Canadian waters (CCME 2004). If conditions remain within guidelines, negative environmental effects are not expected. 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 all aquatic species in Canada. We reference criteria for Canadian waters only where Federal or State criteria are lacking.

APPENDIX TABLE III. MAXIMUM CONCENTRATIONS OF FORMS OF NITROGEN (N) AND PHOSPHORUS (P) FROM WATER COLLECTED AT CONBOY LAKE NWR IN 2000 COMPARED TO ALLOWABLE WATER QUALITY CRITERIA AND GUIDELINES FOR PACIFIC NORTHWEST REGULATORY AGENCIES NCA indicates no criterion available.

Nutrient	Detection Limit	Maximum Concentration	Criterion	Reference	Conboy Lake NWR site	Date of Maximum Detection
	mg/L	mg/L				
Dissolved ammonia	0.05	0.05	1.73[a]	US EPA 1999[b]	HQ EMS	28 March
Dissolved nitrite	0.01	no data	0.06	CCME 2004	-	-
Dissolved nitrate	0.01	0.03	NCA	-	HQ Spring	28 March
Dissolved TKN	0.2	1.3	NCA	-	C&H EMS	26 April
Total TKN	0.2	4.9	0.05	US EPA 2000	Willard EMS	20 July
Total nitrogen	calculated	4.9	0.06	US EPA 2000	Willard EMS	20 July
Total phosphorus	0.01	0.59	0.03	US EPA 2000	Willard EMS	20 July
Dissolved orthophosphate	0.01	0.03	NCA	-	HQ Spring Willard Spring	28 March 31 May

[a] Ammonia criteria are temperature and pH dependent. Lowest criteria for water quality measured at sites on Conboy Lake NWR.

[b] Ammonia criteria are national criteria developed by EPA and enacted in Washington and Oregon.

[c] Criteria recommendation for rivers and streams in the Eastern Cascade Slopes and Foothills Level III Ecoregion.

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

APPENDIX VII

LIST OF ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
CCME	Canadian Council of Ministers of the Environment
CJR	Christopher J. Rombough
DEC	Division of Environmental Contaminants
DO	dissolved oxygen
EMS	egg mass sites
EPA	Environmental Protection Agency
JDE	Joseph D. Engler
L	liter
LC50	lethal concentration
MPH	Marc P. Hayes
µS	microsiemen
Mg	milligrams
N	nitrogen
NAWQA	National Water Quality Assessment Program
NH_3	ammonia
NH_4^+	ammonium
NO_3^{-2}	nitrate
NO_2^-	nitrite
NWR	National Wildlife Refuge
OAL	Oregon Analytical Laboratory
ODEQ	Oregon Department of Environmental Quality
OFWO	Oregon Fish and Wildlife Office
N	nitrogen
P	phosphate phosphorus
PO_4^{-3}	orthophosphate
QA/QC	quality assurance/quality control
SVL	snout-vent length
TKN	total Kjeldahl nitrogen
TKNd	total dissolved Kjeldahl nitrogen
USFWS	U.S. Fish and Wildlife Service
WDOE	Washington Department of Ecology