

**Overwintering of the Oregon Spotted Frog (*Rana pretiosa*)  
at Conboy Lake National Wildlife Refuge, Klickitat County,  
Washington, 2000-2001**

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**FINAL REPORT**

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# **Overwintering of the Oregon Spotted Frog (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge, Klickitat County, Washington**

**Marc P. Hayes, Joseph D. Engler, Susan Van Leuven, Daniel C. Friesz, Timothy Quinn, and D. John Pierce**

**Abstract:** We studied overwintering behavior in the Oregon spotted frog (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge over the 22-week interval from 21 September 2000 to 17 February 2001. Ten adult (mass > 27 g) frogs (8 females, 2 males) from two sites 3 linear km apart were tracked on a weekly basis using radio-transmitters with an external belt attachment. During the study, we obtained 162 radio-locations, and most (n = 153) were based on non-visible, submerged frogs or transmitters. During successive surveys, we recorded straight-line radio-location changes of 0 to 160 m. We interpreted most location changes > 1 m, about 67% of the data, to be frog movements. Longer and more frequent location changes before the significant decline fall temperature are thought to represent directed movements to overwintering habitat. Frogs on which we placed transmitters in lentic habitats moved to lotic ones during the temperature decline interval that preceded freeze up, but not vice versa. During the period of ice cover, we saw significantly greater movements from sites where dissolved oxygen (DO) was lower. Frog use of less vegetated, shallower sites nearer to shorelines during this interval also agreed with the idea of DO limitation. Several frogs selected sites associated with beaver workings (i.e., beaver step dams, submerged pathways). These sites may be favorable for overwintering because they provide a better DO environment, refuge from predators, or both. Recovery of frog remains or transmitters from six frogs suggests that one slipped its transmitter, two were preyed upon, and three may have died from low oxygen stress. Regardless of cause, we estimate overwintering mortality to be high. This pattern agrees with available demographic data, which indicates that annual turnover is high and Oregon spotted frogs are relatively short-lived. Study will be needed to determine how much of the observed pattern of mortality and low oxygen stress conditions is a typically annual event or the consequence of extreme drought conditions, as observed in winter 2000-1.

## **Introduction**

The Oregon spotted frog (OSF; *Rana pretiosa*), a candidate species for listing under the Endangered Species Act, is currently known from 35-40 population segments across its historical range in the Pacific Northwest (Hayes 1997, Watson *et al.* 2000; K. McAllister, pers. comm.). The species, thought extinct in the California portion of its geographic range, is known to persist at three sites in British Columbia (Canada), at at least four sites

in Washington State, and at about 30 sites in Oregon (McAllister *et al.* 1993, Hayes 1997, McAllister and Leonard 1997, Watson *et al.* 2000; K. McAllister, pers. comm.). Roughly 30 years ago, research addressing a spotted frog population now assignable to this species compared it to the northern red-legged frog (*Rana aurora aurora*: Licht 1969, 1971, 1974, 1986a, 1986b) and to other spotted frog populations (Licht 1975) that are now assigned to a different species (Green *et al.* 1997). Further, recent and ongoing research to address this relatively warm-water specialized frog has focused on oviposition patterns (Hayes *et al.* 2000), its relationship to bullfrogs (M. Hayes and C. Pearl, *in prep*), its active-season ecology (Watson *et al.* 2000) and its status or surveys that address different geographic regions (McAllister *et al.* 1993, Hayes 1997, Hayes *et al.* 1997, Leonard 1997, McAllister and Leonard 1997). However, in a management context, knowledge of its overwintering sites remains a basic data gap. Hayes (1997) speculated that the frequent association of extant Oregon spotted frog populations with springs might arise from overwintering needs because use of springs may reduce the likelihood of exposure to freezing conditions. A pilot study conducted at Conboy Lake National Wildlife Refuge (CLNWR) by two of us (JDE, MPH) during the winter of 1998-9 implied that movement into deep channels with some flow seemed to agree with this hypothesis. However, ineffective waterproofing of epoxy around transmitters ultimately resulted in only one frog being followed through that winter season. For this reason, a more definitive determination of overwintering locations and behavior during overwintering was required to identify basic patterns.

These issues led us to study overwintering of the OSF using telemetry at Conboy Lake National Wildlife Refuge (CLNWR), one of the few sites from which this species is known in Washington State. This study is one portion of a Washington Department of Transportation overwintering study that was conducted at three of the four sites from which OSF were known in Washington State during the planning phase of this study<sup>1</sup>.

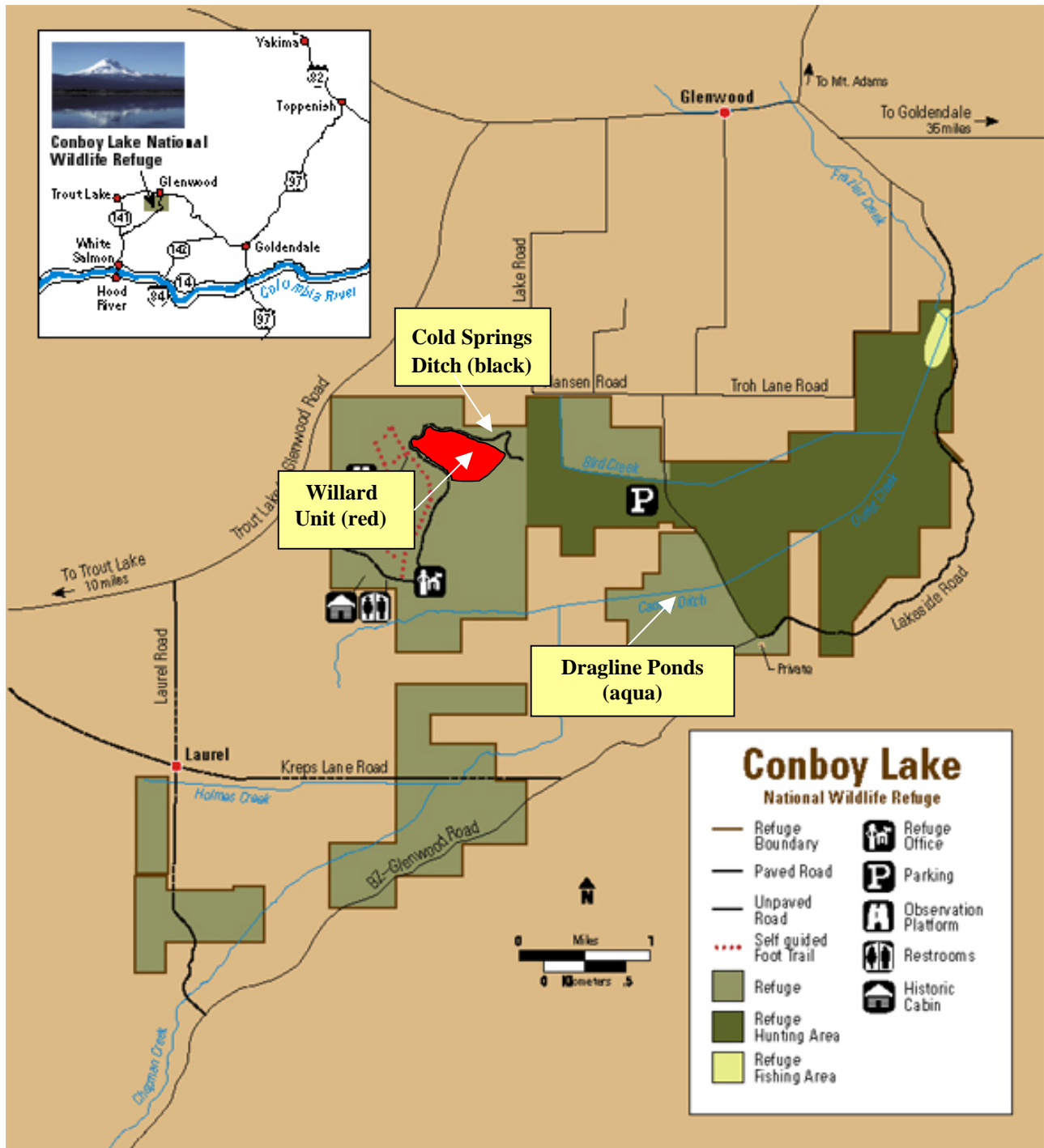
### Study Area and Sites

Study Area: The study area was CLNWR, a 2,354 ha [5,814-ac] NWR that is part of the Ridgefield NWR Complex and is located in the Glenwood Valley southwest of Mt. Adams in Klickitat County (Washington; Figure 1). The refuge encompasses roughly 67% of the 1,989 ha [4,914 ac] of marsh and other seasonal aquatic habitat that make up the historical Conboy Lake and Camas Prairie wetland system. The refuge is actually three separate parcels that interdigitate with private lands in a complex pattern (Figure 1). The remaining third of this seasonal wetland system (ca. 648 ha [1,600 ac]) is on private land. Hydrological change and use of a large segment of the lakebed for hay production

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<sup>1</sup> Oregon spotted frogs were discovered at locations satellite to the two known sites in Thurston County (Beaver and Dempsey Creeks) partly during the course of this study (K. McAllister, pers. comm.).





**Figure 1 – Area Map of Glenwood Valley Displaying Oregon Spotted Frog Overwintering Study Sites (Klickitat County, Washington)**

or grazing has altered much of the wetland complex from its historic condition. In particular, the two key sources of water to this system, four inflow streams and springs along the northwest periphery of this system have been channelized. As a consequence, permanent water is largely restricted to these channels. Although the historic hydrology of this wetland system is poorly known, permanent water is thought to have comprised springs, selected inflow streams that were not constrained by long lengths of continuous dikes, and at least one, late-season stillwater pool (i.e., Conboy Lake proper) that had a surface area of approximately 150 ha [ $\sim$ 370 ac] (H. Cole, pers. comm.)<sup>2</sup>.

The climate of CLNWR is cold during the winter, with temperatures regularly extending below freezing between mid-October and early March, and the winter precipitation that falls as snow typically accumulates 2-4 feet annually. Under extreme conditions, winter temperatures reach  $-20^{\circ}\text{C}$  [ $-4^{\circ}\text{F}$ ], but most wintertime temperatures are rarely colder than  $-10^{\circ}\text{C}$  [ $14^{\circ}\text{F}$ ] (Figure 2). Nonetheless, temperatures become cold enough for ice to cover significant areas of the permanent water channels. The spatial and temporal extent of ice cover depends primarily on an interaction between the length of time winter temperatures remain at subfreezing levels and how far below freezing winter temperatures extend.

The CLNWR was one of the only four or five<sup>3</sup> sites known to harbor the Oregon spotted frog at the initiation of this study (Watson *et al.* 2000; K. McAllister, pers. comm.).

Study Sites: We studied frogs at two sites, the Willard Unit and its vicinity and Dragline Ponds (Figure 1). The Willard Unit, located in the northwest corner of the refuge, has a permanent water pool because of a diked perimeter. This pool is deepest at its south end, where a tule (*Scirpus* sp.) marsh occurs. The north, northwest, and west margins of the Willard Unit border the Cold Springs Ditch (CSD; Figure 3). The CSD is the conduit for water from the springs and from Bird Creek, the largest inflow stream to this complex from the north. Water enters the Willard Unit from overflow points along the CSD dike as well as porous areas in the dike, some of which are the result of beaver activity.

The Dragline Ponds consist of two “wildlife duck donuts” excavated in October 1993 that are relatively centrally located on CLNWR (Figure 1). Based on an aerial photograph for 23 September 1998 (Figure 4), the west Dragline Pond (herein Dragline 1) had a surface area of 0.20 ha [0.49 ac] and an elongated, C-shaped central island with a surface area of 0.11 ha [0.27 ac]. The easternmost Dragline Pond (herein Dragline 2) had a surface area

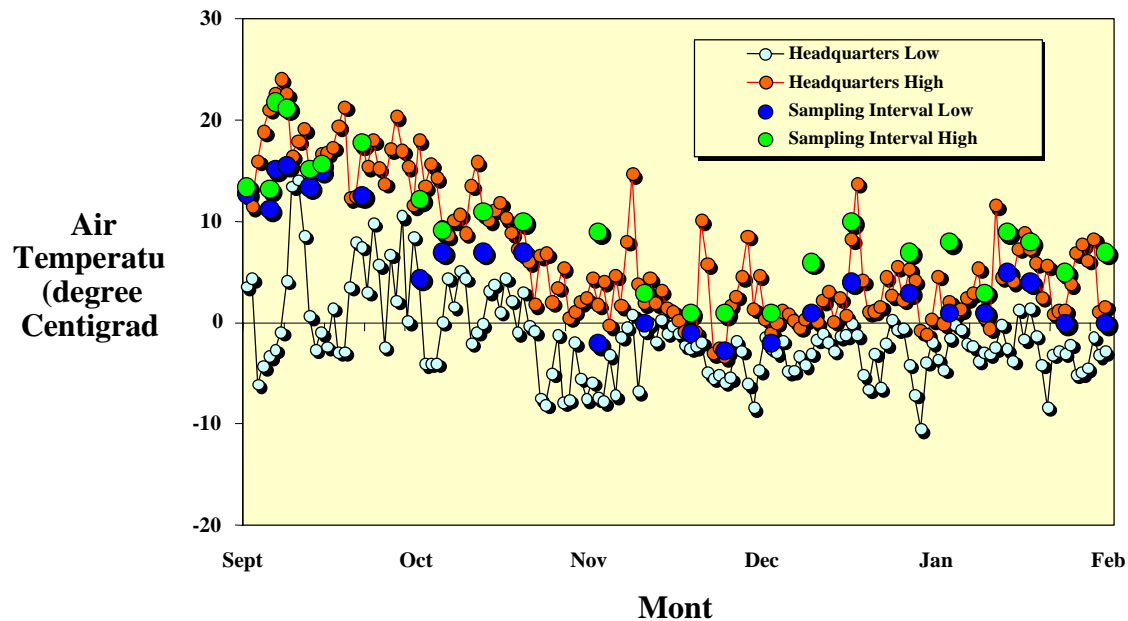
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<sup>2</sup> Data are a composite of information from several sources, but primarily notes of at least two individuals associated with the George McClellan-headed expedition through the Glenwood Valley in 1853, and an old pre-drainage district era map that the Kreps family, longtime Glenwood Valley residents, owns.

<sup>3</sup> Five sites if the two locations ca. 1.6 (1 mi) apart in the vicinity of Trout Lake are considered two sites.

of 0.40 h [0.98 ac] and an elongated, inverse L-shaped central island with a surface area of 0.13 ha [0.32 ac]. The seasonal extent of surface water in both varies significantly. These ponds merge with the Camas Prairie seasonal wetlands during high water in the lakebed and only become recognizable as distinct units at moderate water levels. In

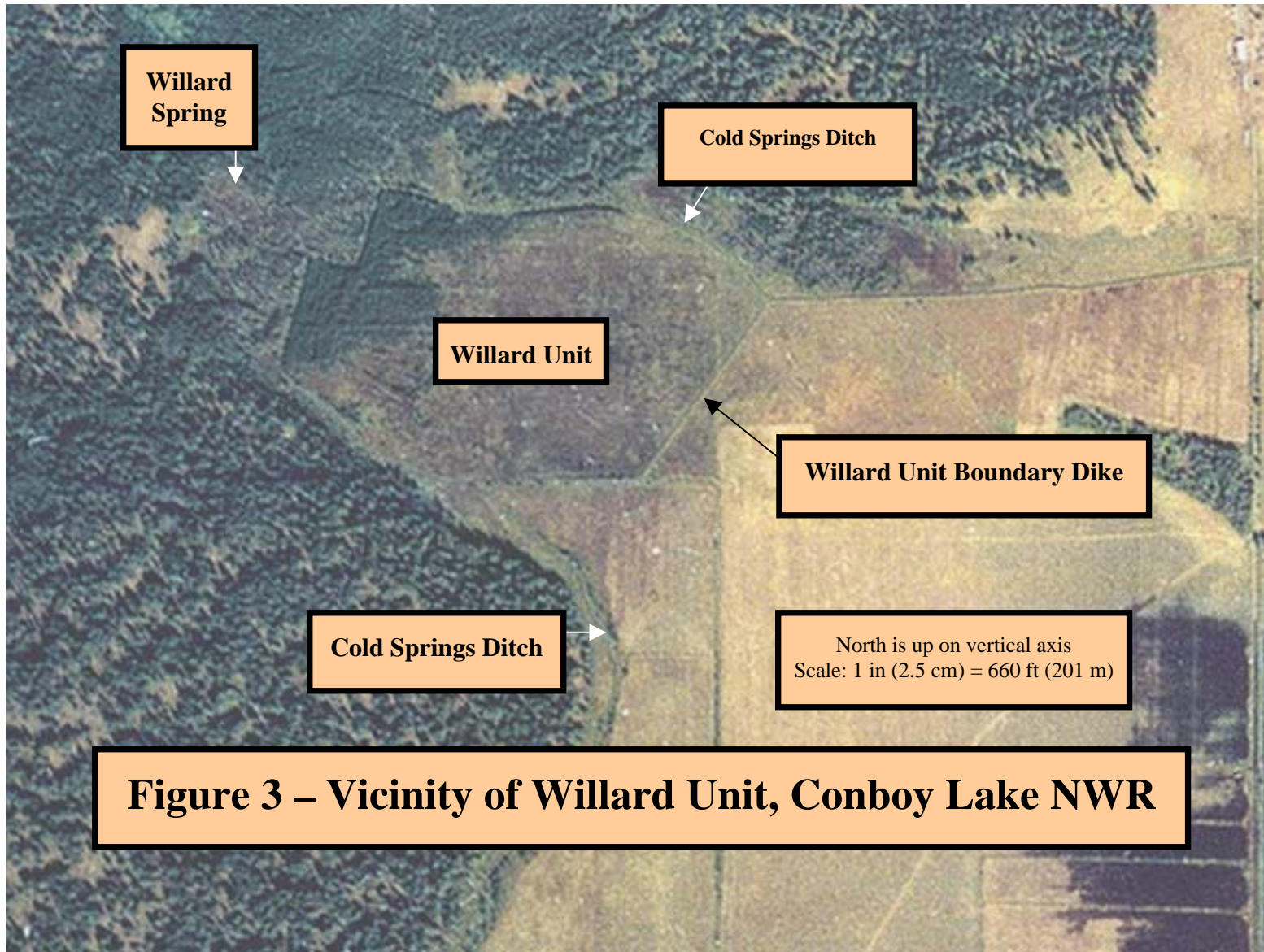
**Figure 2 - Daily Air Temperature Variation at Conboy Lake NWR during the Overwintering Study, 2000-2001**



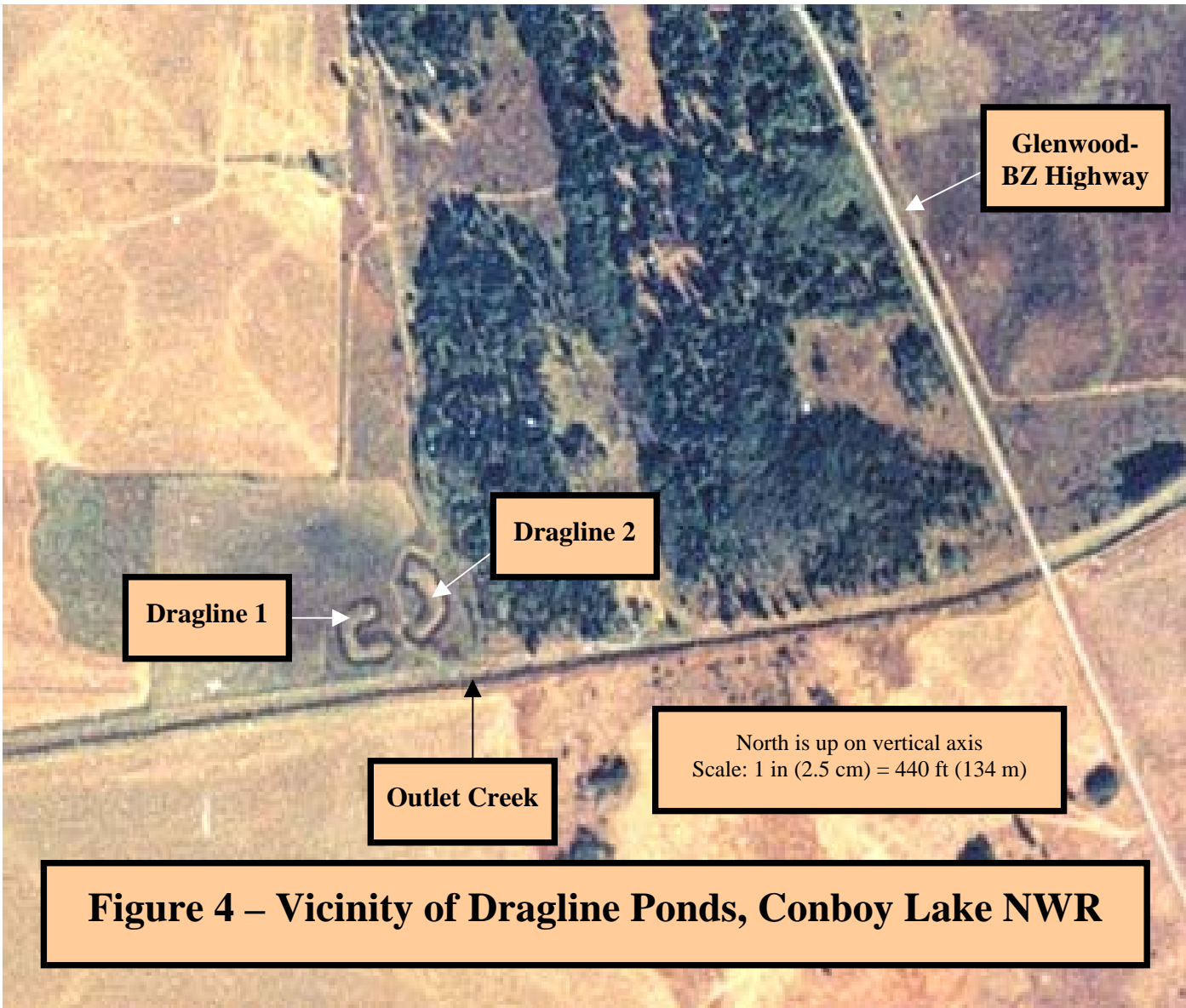
severe drought years, both can become dry for brief intervals in late summer-early fall, but the ponds retain some water during the dry season in most years. At typical early summer water levels, Dragline Ponds are connected to each other by two channels and to Outlet Creek (Figure 4), the main outflow channel for the wetland complex, through a culvert south of Dragline 2. Dragline Ponds are located 3.0 km [1.9 mi] southeast of the Willard Unit (Figure 1).

## Methods

**Transmitters:** We attached 10 1.8-g BD-2G radio-transmitters (Holohil Systems Limited, 112 John Cavanagh Road, Carp, Ontario, Canada K0A 1L0) to 10 adult OSF (snout-vent length (SVL) > 70 mm, mass (M) > 27 g) obtained from the two aforementioned areas at CLNWR during the interval 17-19 September 2000. The BD-2G transmitters are guaranteed to have a life of 16 weeks. Since information from the pilot study conducted







during the winter of 1998-9 suggested that Oregon spotted frogs sometimes moved into lotic sites during entry into overwintering, we intentionally placed transmitters on three adult frogs within the Willard Unit and on two adult frogs in the Dragline Ponds (both lentic habitats) to aid in identification of a potentially similar pre-overwintering move. These sites were both adjacent to lotic habitat, CSD and Outlet Creek, respectively, that were known to have been used for overwintering (J. Engler, M. Hayes, *unpubl. data*).

The five remaining frogs were obtained from points in Cold Springs Ditch adjacent to the Willard Unit. To avoid problems with potential frequency drift, radio-transmitters had been built with a dominant frequency that differed by at least 20 kHz from other transmitters. In this text, we use the kHz transmitter frequency of each frog to identify it. Transmitters each had a 10-cm [4 in] trailing whip antenna and were modified with an anterior sleeve through which attachment belts were strung. Our belt attachments were a modification of the methods of Bull (2000) and Bull and Hayes (2001) using a 6-mm wide silk ribbon. Belt ends were secured by hand sewing a multiple stitch using a heavy-duty composite 70% cotton-30% polyester thread. We adjusted belts snugly enough that they could not easily slip over the back legs, but were loose enough that they could rotate around the waist. We released transmittered frogs at their capture points and used white PVC poles to mark each release location. We obtained Universal Transverse Mercator (UTM) data (northing and easting) for release locations with Garmin 12CX or Rockwell HNV-560-B GPS units. Both could resolve data to a minimum error of  $\pm 3.0$ -3.5 m.

Tracking: We tracked frogs using a TR-4 telemetry receiver (Telonics, Inc., 932 East Impala Avenue, Mesa, Arizona 85204-6699) tuned to 164 MHz. Except for one week in mid-November, when tracking did not occur, it typically took place at least once a week from 21 September 2000 to 17 February 2001. We tracked frogs with greater frequency during the first two weeks in an attempt to verify their condition (especially to check for abrasions or injuries related to their attached belts) and in the last two weeks to improve the likelihood of detecting a pre-breeding move. Wherever possible, we tried to obtain a visual detection to verify tracked signals as representing a frog, but opportunity to detect frogs visually was infrequent. In general, we avoided disturbing the location thought to represent a submerged frog except where we suspected a dropped transmitter requiring recovery or to remove transmitters at the end of the study. We resolved transmitter signal locations to  $\pm 0.25$  m if ice was absent, but were usually unable to resolve locations more accurately than  $\pm 0.50$  m if ice was present. We marked the signal location of the first move after a frog's release using a second white PVC pole that was offset 0.25-1.0 m to avoid disturbing the transmittered frog. We obtained the declination-corrected azimuth from the previous location to the new location and the distance between these locations for each new signal location. In each week from the 3<sup>rd</sup> onward, we moved this second

pole to mark each new location. Occasionally, where a debris matrix interfered with pole placement, the new location was flagged. We obtained northing and easting UTM values for most transmitter signal locations. We terminated the study on 17 February 2001, and were able to remove transmitters from two of four frogs thought to be alive on that date; we lost the signal from the remaining two frogs before attempting transmitter removal.

Frog and Habitat Data: We obtained selected habitat data at each signal location. These data included scoring or measuring:

- 1) the transmitter or transmittered frog as:
  - a) as *visible* or not.
  - b) as *submerged* or not.
- 2) whether habitat at the point of the signal was *aquatic* or *terrestrial*.
- 3) circumference of right thigh of frog (measured at the visually estimated point of maximum thickness). The thigh circumference-mass ratio was used as a condition index.
- 4) cloud cover (to nearest 5%).
- 5) wind speed (estimated on Beaufort scale [[www.crh.noaa.gov/lot/webpage/beaufort/](http://www.crh.noaa.gov/lot/webpage/beaufort/)])
- 6) wind direction (to the nearest half joint direction)
- 7) precipitation as:
  - a) type (*fog, hail, rain, sleet, snow*); and
  - b) amount (*light, moderate, heavy*).
- 8) water temperature (to the nearest 0.1C).
- 9) air temperature (to the nearest 0.1C) at:
  - a) the time the transmitter or frog location was obtained.
  - b) the beginning and the end of the sample date.
- 10) water depth (to the nearest 0.01 m).
- 11) distance from shore (to the nearest 0.1 m).
- 12) dissolved oxygen (to the nearest 0.1 mg/L).
- 13) water velocity (to the nearest 0.1 m/sec)

We measured air temperatures with a hand-held, digital Taylor thermometer. We took water temperatures close to the depth at which transmittered frogs were suspected with the thermometer on the dissolved oxygen meter or the water velocity meter. Depths were measured with the handle of a kicknet incremented in centimeters. We measured depths to the relatively solid substrate and distances with a tape or hypsometer. Dissolved oxygen (DO) was measured with a YSI 95<sup>TM</sup> oxygen meter beginning 2 October 2000. During periods with ice cover, we used an ice auger to obtain temperatures and dissolved oxygen measurements beneath the ice. We made special effort to avoid disturbing frogs when obtaining depth or dissolved oxygen measurements using a slight offset from what we thought was the frog location. We measured flow with a Flowwatch<sup>TM</sup> flow meter (JDC Electronics, S.A., Rue de Uttins 40, CH-1400 Yverdon, Switzerland) for the first 8 weeks, but discontinued measurements because flow under most conditions was lower than the threshold limit of measurement of the instrument (0.1 m/sec). We discontinued

attempts to measuring flow after 8 November 2000 because we were rarely able to record data over the threshold for the meter.

Air and water temperatures were also obtained from data loggers at two nearby stations. We obtained air temperatures using a HOBO Pro Temp/Relative Humidity™ logger at a station near CLNWR headquarters located ca. 1.6 km [1 mi] south of the Willard Unit (Figure 1). Water temperatures were recorded with a Stowaway XTI™ logger sealed in a waterproof housing at a station in Bird Creek just south of Hansen Road. The location of this station represents measurement of water temperature for the major inflow of water as it enters the refuge, and this location is ca. 1.6 km [1 mi] east of the Willard Unit.

Vegetation and habitat structure was assessed in two different ways. The first consisted of determining the percentage (to the nearest 5%) for each of the following seven habitat classes within a 0.5-m and a 5-m radius of the signal point:

- 1) low emergents ( $\leq 1$  m in height).
- 2) tall emergents ( $> 1$  m in height).
- 3) submerged vegetation (not in contact with the water surface).
- 4) floating vegetation (at the water surface).
- 5) upland (any land positive habitat at the time of the survey).
- 6) open water/ice (a water column lacking macrovegetation).
- 7) other (any habitat category not included in 1-6).

The second was an assessment along a biaxial grid described in Appendix I. We did not implement the biaxial system until week 8 (the week beginning 5 November 2000) as the WDOT study team decision to use these was not made until mid-week 7. The habitat class system (first method) was used throughout the study, and habitat data were obtained using both systems from week 8 through the end of the study.

Analyses: We used standard statistical procedures (Zar 1996), relying predominantly on non-parametric statistics. Non-parametric test results were corrected for ties wherever appropriate. Comparative non-parametric tests are based on differences in medians, but we frequently provided the mean ( $\bar{x}$ ), standard deviation ( $s$ ), standard error of the mean ( $se$ ), and range to clarify descriptions.

We analyzed effort both as time and surveyor-adjusted time (i.e., person-minutes (pers-min)) because number of surveyors varied among surveys. We used Mann-Whitney U comparisons to examine differences in effort between the initial two and remaining 20 weeks of the study, favorable and inclement weather conditions, and conditions with one versus two surveyors. We used a Kruskal-Wallis test to compare effort among individual surveyors with significant survey time.

Air and water temperatures, dissolved oxygen (DO), distances from shore, and movement distances all had distributions that approximated a negative logarithm, so non-parametric statistics were used for all analyses involving these variables. We performed a Spearman rank correlation to characterize relationship strength between air and water temperature.



Physical habitat changes lent themselves to partitioning three intervals (*pre-ice* = before substantial ice was present, *ice* = when substantial ice was present, and *post-ice* = the ice-melt interval) for selected analyses. Our water depth variable had a normal distribution, so we used a standard single-factor ANOVA was used to analyze water depth across the three time intervals.

For movement analyses, we excluded the terminal location change, if it was potentially attributable to a predator. Furthermore, because in terms of number of days, the intervals between surveys were somewhat irregular, we analyzed movement data collected within each week standardized on a per day basis as well as based on the raw data. Moreover, because of the aforementioned resolution issues with telemetry locations, we disregarded all values < 1.0 m as being movements. For analyses, we truncated these values to zero. For dissolved oxygen analyses, we excluded data points where surveyors indicated they had difficulty obtaining reliable readings because of ice- (e.g., slushy ice) or vegetation-related interference.

We analyzed data with Statview™ 5.0 (SAS Institute, Inc.) and did most manipulations using Microsoft Excel™ 2000. We discuss significance based on a rejection criterion placed at  $\alpha = 0.05$ , but we believe it equally useful to examine probabilities as a guide of what to expect when more data become available.

## Results

**Effort:** Beside the roughly 2.5 days spent capturing and fitting 10 frogs for telemetry, one or two surveyors tracked frogs on 25 different days over a total of ca. 89 hours or ca. 167 person-hours. Tracking effort averaged 4.0 hr ( $s = 1.4$  hr,  $se = 0.3$  hr, range = 1.1-6.2 hr) per day overall, but 4.7 hr ( $s = 0.7$  hr,  $se = 0.2$  hr, range = 3.6-6.2 hr) over the 18 days for which all transmittered frogs available were tracked. For the latter, surveys that involved one surveyor ( $n = 6$ ) did not differ significantly from surveys that involved two surveyors ( $n = 12$ ) in per day tracking effort (one surveyor:  $\bar{0} = 4.7$  hr,  $s = 0.7$  hr,  $se = 0.3$  hr, range: 3.9-5.9 hr; two surveyors:  $\bar{0} = 4.6$  hr,  $s = 0.8$  hr,  $se = 0.2$  hr, range: 3.6-6.2 hr; Mann-Whitney U:  $U = 35$ ,  $U' = 37$ ,  $z = -0.094$ ,  $p = 0.9254$ ).

Standardized effort analysis showed that the daily tracking effort averaged 6.7 person-hr ( $s = 3.1$  pers-hr,  $se = 0.6$  pers-hr, range = 1.1-12.3 pers-hr). For the 18 days for which all transmittered frogs available were tracked, daily effort averaged 7.8 pers-hr ( $s = 2.6$  pers-hr,  $se = 0.6$  pers-hr, range = 3.9-12.3 pers-hr), and two surveyors expended significantly greater effort than one surveyor (one:  $n = 6$ ,  $\bar{0} = 4.7$  pers-hr,  $s = 0.7$  pers-hr,  $se = 0.3$  pers-hr, range: 3.9-5.9 pers-hr; two:  $n = 12$ ,  $\bar{0} = 9.3$  pers-hr,  $s = 1.6$  pers-hr,  $se = 0.5$  pers-hr, range: 7.2-12.3 pers-hr; Mann-Whitney U:  $U = 0$ ,  $U' = 72$ ,  $z = -3.372$ ,  $p = 0.0007$ ).

Tracking episodes (time per signal tracked) averaged 37 min ( $s = 19$  min,  $se = 2$  min), but varied over an order of magnitude (10-119 min). The 12 episodes during the first two weeks averaged 18 minutes longer than the 152 episodes in the last 20 weeks, but the difference was not significant (Table 1). The seven episodes during which frogs were captured averaged 7 minutes longer than the 155 episodes during which frogs were not captured, but the difference was not significant (Table 1). The 128 tracking episodes that occurred during favorable weather did not differ significantly in length from 36 episodes that occurred during inclement weather (Table 1). The only significant difference found among effort variables related to the number of surveyors; 54 single-surveyor episodes required significantly longer (7 minutes on average) than the 110 two-surveyor episodes (Table 1).

**Table 1 – Variation in survey effort (per target frog tracked) during telemetry of Oregon spotted frogs (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.**

Partitioned Variable	Categories	Survey Effort (min)						Mann Whitney U Test Results	
		n	0	s	se	range		z	p <sup>1</sup>
						min	max		
Time	First 2 Weeks	12	52	40	1	10	119	-0.782	0.4344
	Last 20 Weeks	152	36	17	11	10	105		
Capture	Captured	7	43	35	13	10	119	-0.074	0.9408
	Not Captured	157	36	18	1	10	115		
Weather	Favorable	128	37	20	2	10	119	-0.181	0.8562
	Inclement <sup>2</sup>	36	35	17	3	10	115		
Surveyors	One	54	42	18	2	12	119	-3.329	<b>0.0009</b>
	Two	110	35	20	2	10	115		
Partitioned Variable	Categories	Standardized Survey Effort (pers-min)						Mann Whitney U Test Results	
		n	0	s	se	range		z	p <sup>1</sup>
						min	max		
Time	First 2 Weeks	12	88	71	21	20	230	-0.693	0.4883
	Last 20 Weeks	152	58	32	3	12	210		
Capture	Captured	7	58	33	13	30	119	-0.074	0.9408
	Not Captured	157	60	37	3	12	230		
Weather	Favorable	128	60	37	3	12	210	-0.657	0.5112
	Inclement <sup>2</sup>	36	60	36	6	17	230		
Surveyors	One	54	42	18	2	12	119	-5.561	<b>&lt; 0.0001</b>
	Two	110	69	40	4	20	230		

<sup>1</sup> Significant results based on an  $\alpha = 0.05$  rejection criterion are emboldened.

<sup>2</sup> Inclement weather was all categories of precipitation.

Analysis of standardized effort indicated that tracking episodes averaged 60 pers-min ( $s = 37$  pers-min,  $se = 3$  pers-min), but also varied greatly (12-230 pers-min). Variables category comparisons displayed the same patterns as the raw data. However, although we also found a significant difference between episodes with one versus two surveyors, the difference was opposite in direction to that of the raw data. Two-surveyor episodes averaged 27 person-minutes longer than those with one surveyor.

Ten different individuals conducted these surveys, but only five tracked frogs on four or more days (Appendix II). Comparison of these five surveyors revealed no differences in the effort required to track frogs (Kruskal-Wallis:  $H = 1.980$ ,  $df = 4$  and  $p = 0.7376$ ).

Frog Data At Release: The 10 frogs to which transmitters were attached were adults of reproductive size averaging 41.5 g M ( $s = 11.1$  g,  $se = 3.5$  g, range: 27.7-59.7 g; Table 2). The eight females averaged 44.6 g M ( $s = 10.1$  g,  $se = 3.6$  g, range: 31.2-59.7 g), whereas the two males averaged 29.0 g M (range: 27.7-30.2 g). Maximum circumference of the right thigh of transmittered frogs at release averaged 44.3 mm ( $s = 4.4$  mm,  $se = 1.4$  mm, range: 39.0-50.0 g; Table 2). Eight females averaged 45.4 mm ( $s = 4.3$  mm,  $se = 1.5$  mm, range: 39.0-50.0 g), whereas the two males averaged 40.0 mm (range: 39.0-41.0).

Climate Phenology: Over the 150-day study interval (21 September 2000 to 17 February 2001), air temperatures ranged from a high of 24.0°C (recorded on 27 September 2000) to a low of -10.5°C (recorded on 16 January 2000; Figure 2). Daily low air temperatures reached a freezing level in every month of the study interval, but occurred almost daily after 1 November. Daytime temperatures regularly reached highs  $> 15^{\circ}\text{C}$  through mid-October, declined sharply to mid-November, and except for four days, remained  $< 10^{\circ}\text{C}$  through the about 90 days to the termination of the study (Figure 2). Except for a more contracted range of variation, the pattern of water temperatures in Bird Creek, the major inflow to this system, largely paralleled air temperatures (Figure 5). High and low air

**Table 2 – Basic Data at Release on Oregon Spotted Frogs (*Rana pretiosa*) Tracked at Conboy Lake National Wildlife Refuge during Winter 2000-2001**

Date	Time	Location	PIT Tag	Radio Transmitter Frequencies		Mass (g)	Thigh Circumference (mm)	Sex
				Specs <sup>1</sup>	Field <sup>2</sup>			
9/19	13:40	Dragline	41035E1157	0.290	0.289-0.290	54.7	50.0	F
9/19	13:54	Dragline	501C6D594E	0.641	0.639-0.642	41.2	50.0	F
9/19	14:20	Willard Unit	501D232C4A	0.372	0.371	32.2	42.0	F
9/19	14:25	Willard CSD <sup>4</sup>	501D553403	0.598	0.598-0.600	59.7	48.0	F
9/19	14:30	Willard CSD	501D1E043B	0.431	0.430-0.432	30.2	41.0	M
9/19	14:37	Willard CSD	501C79361F	0.513	0.510-0.513	42.2	41.0	F
9/19	14:44	Willard CSD	501D1A7433	0.315	0.313-0.318	31.2	39.0	F
9/19	14:48	Willard CSD	501D18516E	0.684	0.683-0.688	27.7	39.0	M
9/19	15:10	Willard Unit	501D505158	0.300	0.301	50.7	48.0	F
9/19	15:10	Willard Unit	501D4A751D	0.340	0.339-0.342	45.2	45.0	F

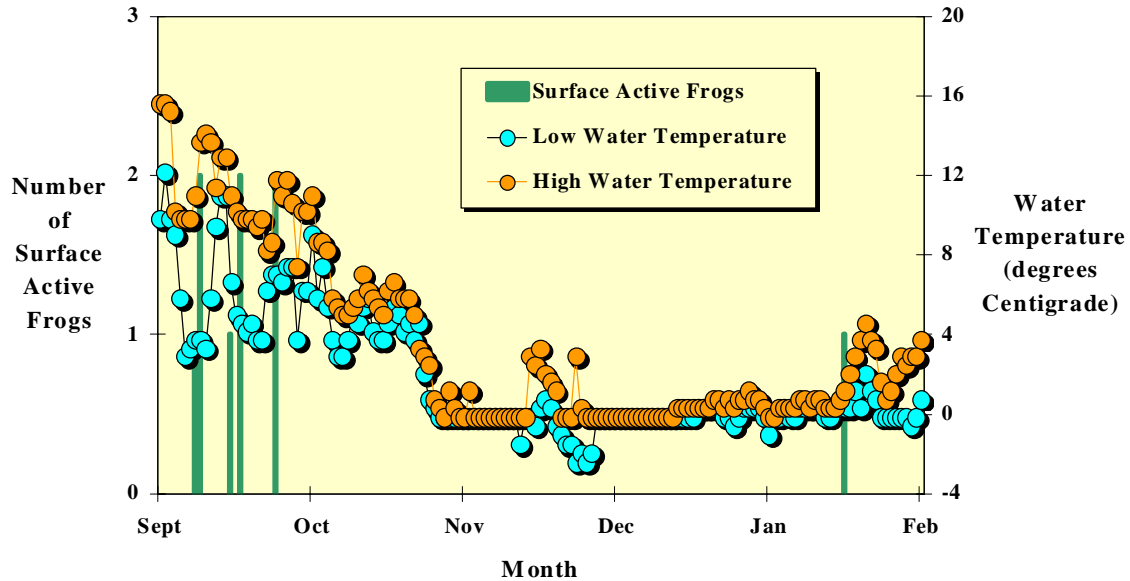
<sup>1</sup> Specs = Manufacturer (Holohil Systems Limited)-specified transmitter frequency.

<sup>2</sup> Field = Field-determined transmitter frequency.

<sup>3</sup> WT = Water temperature.

<sup>4</sup> CSD = Cold Springs Ditch.

**Figure 5 - Daily Water Temperature Variation in Bird Creek during the Overwintering Study Interval, 2000-1**



temperatures were strongly correlated with high and low water temperatures, respectively (Spearman rank correlation:  $\rho_{\text{lows}} = 0.635$ ,  $p < 0.0001$ ;  $\rho_{\text{highs}} = 0.811$ ,  $p < 0.0001$ ). Water temperatures reached freezing or supercooled levels in Bird Creek on 10 November 2000 (Figure 5), however, ice was present on some water surfaces as early as 21 October 2000. Ice was limited in extent and thickness until later November. During late November, ice became thick enough to support the weight of investigators conducting telemetry in many places. The ice layer remained coherent into early February 2001, but then began to melt from the slow warming of inflow waters (see Figure 5) and daytime temperatures (Figure 2). Ice was present in several areas at the termination of this study on 17 February 2000.

Analysis of temperatures and relative humidity revealed significant differences among pre-ice ( $n = 48$ ), ice ( $n = 85$ ), and post-ice ( $n = 17$ ) periods (Table 3). Post-hoc analysis showed that significant differences existed in temperature and relative humidity for all interval combinations except for the ice versus post-ice period comparison for low air temperature and low relative humidity (Table 4). Generally, the least differences were between the ice and post-ice intervals.

**Table 3 – Variation in temperature and relative humidity during pre-ice, ice, post-ice periods at Conboy Lake National Wildlife Refuge, 2000-2001**

Variable	Category	Interval	Descriptive Statistics					Kruskal-Wallis Test Result	
			0	s	se	range		H	p <sup>1</sup>
						min	max		
Air Temperature	Daily Low	Pre	2.2	4.8	0.7	-6.1	14.1	41.0	< <b>0.0001</b>
		Ice	-3.2	2.6	0.3	-10.5	3.1		
		Post	-2.9	2.3	0.6	-8.3	1.4		
	Daily High	Pre	15.0	4.1	0.6	7.3	24.0	94.3	< <b>0.0001</b>
		Ice	2.8	3.4	0.4	-3.4	14.7		
		Post	4.8	2.9	0.7	0.9	8.9		
Relative Humidity	Daily Low	Pre	51.8	21.7	3.1	16.9	102.2	43.7	< <b>0.0001</b>
		Ice	78.1	17.2	1.9	28.4	102.2		
		Post	68.5	13.0	3.1	52.9	98.6		
	Daily High	Pre	87.9	10.0	1.4	50.3	99.3	35.6	< <b>0.0001</b>
		Ice	96.1	7.7	0.8	61.7	103.7		
		Post	97.3	4.8	1.2	87.7	103.7		
Water Temperature	Daily Low	Pre	5.5	1.9	0.3	3.0	11.0	116.6	< <b>0.0001</b>
		Ice	-0.1	0.7	0.1	-2.0	3.0		
		Post	0.3	0.7	0.2	-1.0	2.0		
	Daily High	Pre	8.8	2.8	0.4	5.0	14.0	118.3	< <b>0.0001</b>
		Ice	0.5	0.9	0.1	0.0	5.0		
		Post	2.6	1.2	0.3	1.0	5.0		

Note: The sample size was 155 for all variable categories. Sample sizes for intervals were Pre = 48, Ice = 85, and Post = 17.

<sup>1</sup> Probability (p); significant results using a rejection criterion of  $\alpha = 0.05$  are emboldened.

During tracking sessions, which were entirely diurnal, the low air temperatures recorded reached freezing levels on seven survey dates (Figure 2). However, subfreezing levels were recorded on only four of these dates, all of which occurred during November and December. Air temperatures obtained during several tracking sessions during the winter were higher than the temperatures recorded by the headquarters data logger because the latter was positioned in the shade and sampling interval temperatures were obtained in sun-exposed positions. During surveys, cloud cover was varied from a low averaging 58% (s = 43%, se = 9%, range: 0-100%) to a high averaging 75% (s = 40%, se = 8%, range: 0-100%). Range of variation in cloud cover during each survey averaged 16% (s = 30%, se = 6%, range: 0-100%). Beaufort wind values during surveys reflected the general pattern of light wind, with low values averaging 0.2 (s = 0.5, se = 0.1, range: 0-2) and high values averaging 1.5 (s = 1.6, se = 0.3, range: 0-6). Range of variation in wind during each survey averaged 1.3 (s = 1.6, se = 0.3, range: 0-6).

Table 4 – Post hoc analysis addressing pairwise interval comparisons of relative humidity and temperature at Conboy Lake National Wildlife Refuge, 2000-2001

Variable	Category	Interval Comparison	Mann-Whitney U Test Result	
			z	p <sup>1</sup>
Air Temperature	Daily Low	Pre-ice versus Ice	-6.185	< <b>0.0001</b>
		Ice versus Post-ice	-0.027	0.9785
		Pre-ice versus Post-ice	-4.009	< <b>0.0001</b>
	Daily High	Pre-ice versus Ice	-9.242	< <b>0.0001</b>
		Ice versus Post-ice	-2.519	<b>0.0118</b>
		Pre-ice versus Post-ice	-5.994	< <b>0.0001</b>
Relative Humidity	Daily Low	Pre-ice versus Ice	-5.622	< <b>0.0001</b>
		Ice versus Post-ice	-0.189	0.8503
		Pre-ice versus Post-ice	-4.159	< <b>0.0001</b>
	Daily High	Pre-ice versus Ice	-6.356	< <b>0.0001</b>
		Ice versus Post-ice	-2.622	<b>0.0087</b>
		Pre-ice versus Post-ice	-2.881	<b>0.0040</b>
Water Temperature	Daily Low	Pre-ice versus Ice	-10.303	< <b>0.0001</b>
		Ice versus Post-ice	-2.623	<b>0.0087</b>
		Pre-ice versus Post-ice	-6.155	< <b>0.0001</b>
	Daily High	Pre-ice versus Ice	-9.983	< <b>0.0001</b>
		Ice versus Post-ice	-6.151	< <b>0.0001</b>
		Pre-ice versus Post-ice	-6.071	< <b>0.0001</b>

<sup>1</sup> Probability (p); significant results using a rejection criterion of  $\alpha = 0.017$  are emboldened. The standard rejection criterion ( $\alpha = 0.05$ ) was conservatively adjusted for the number of tests in each variable category ( $n = 3$ ) using Sidak's multiplicative inequality:  $\alpha' = 1 - (1 - \alpha)^{1/n}$ .

**Frog Behavior:** All 162 radio-locations obtained except one were from aquatic habitats. The sole terrestrial telemetry location obtained was from frog 340, which was found dead on 14 January 2001 in partly eaten condition next to the water entry point of a beaver run.

Of 162 radio-locations, we obtained visual observations only infrequently ( $n = 16$ ). Only a few frogs ( $n = 9$ ; about 5.6% of all locations) were observed surface active during the study interval, and all except one of these were seen when daytime water temperatures were  $\geq 8^\circ\text{C}$  (Figure 5). Remaining visual observations ( $n = 7$ ) were all of submerged frogs<sup>4</sup>. Observations of submerged frogs were made on or before 8 November 2000, or on or after 10 February 2001, when little or no ice could obscure the view into the water.

<sup>4</sup> Visual observations of submerged frogs refers to the position of the frogs *in situ*, it excludes observations of frogs that were removed from a concealed position for examination.

Table 5 – Variation in interval-specific movements for Oregon spotted frogs (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.

Interval	Straight-Line Distance Data (m)					Kruskal-Wallis Test Result	
	0	s	se	Range		H	p <sup>1</sup>
				min	max		
Pre-ice (n = 54)	21.5	31.0	4.2	0.0	106.5	29.070	<b>&lt; 0.0001</b>
Ice (n = 87)	8.7	26.7	2.9	0.0	159.8		
Post-ice (n = 15)	0.7	0.6	0.2	0.0	1.5		

Post-hoc Comparison	Mann-Whitney U Test Result	
	z	p <sup>1</sup>
Pre-ice versus Ice	-4.799	<b>&lt; 0.0001</b>
Ice versus Post-Ice	-0.943	0.3455
Pre-Ice versus Post-Ice	-4.077	<b>&lt; 0.0001</b>

Interval	Standardized Straight Line Distances (m/dy)					Kruskal-Wallis Test Result	
	0	s	se	range		H	p <sup>1</sup>
				min	max		
Pre-ice (n = 54)	3.2	4.7	0.6	0.0	16.9	30.971	<b>&lt; 0.0001</b>
Ice (n = 87)	1.0	3.1	0.3	0.0	20.0		
Post-ice (n = 15)	0.1	0.1	0.0 <sup>2</sup>	0.0	0.4		

Post-hoc Comparison	Mann-Whitney U Test Result	
	z	p <sup>1</sup>
Pre-ice versus Ice	-5.103	<b>&lt; 0.0001</b>
Ice versus Post-Ice	-0.719	0.4722
Pre-Ice versus Post-Ice	-3.908	<b>&lt; 0.0001</b>

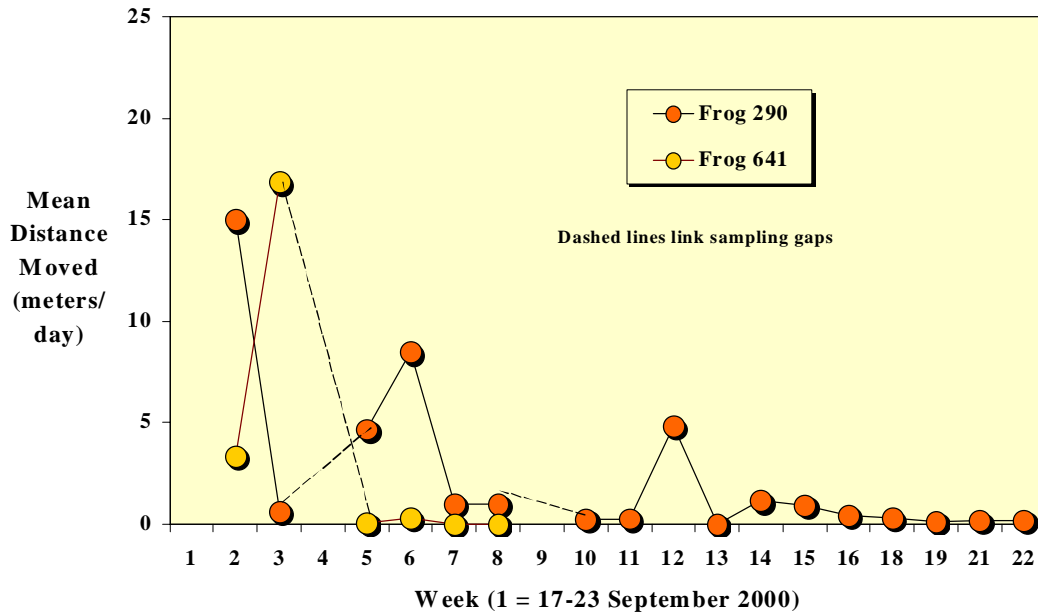
<sup>1</sup> Significant results are emboldened; significant results for overall tests (i.e., Kruskal-Wallis analyses) are based on an  $\alpha = 0.05$  rejection criterion; significant results for post-hoc comparisons are based on a rejection criterion of  $\alpha = 0.017$ , which represents a criterion conservatively adjusted for the number of comparisons ( $n = 3$ ) based on Sidak's multiplicative inequality:  $\alpha' = 1 - (1 - \alpha)^{1/n}$ .

<sup>2</sup> Standard error was  $< 0.1$  m/dy.

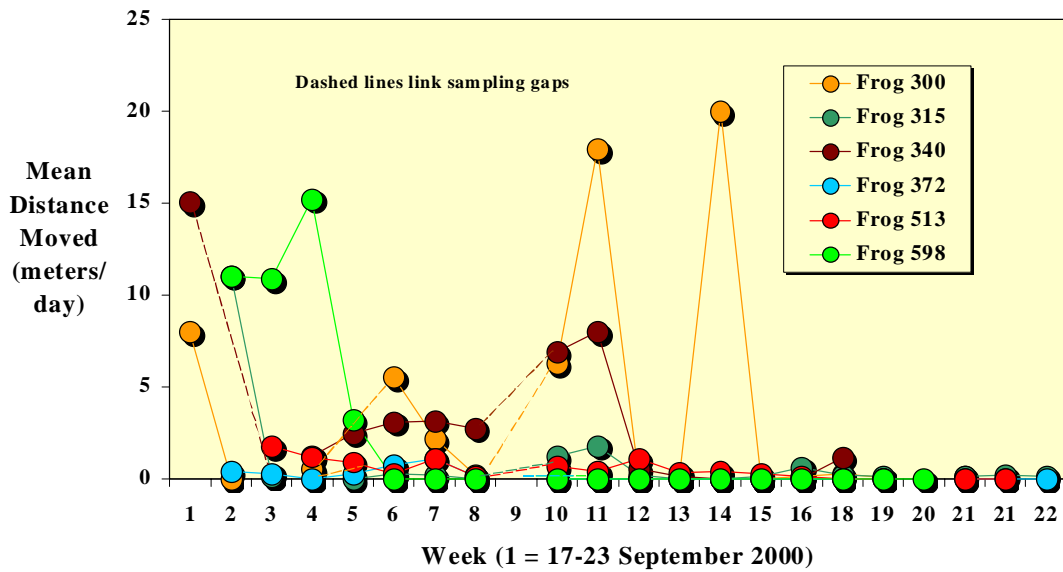
Distances frogs moved were variable. The straight-line distances between radio-locations taken at survey intervals of 1-13 days varied from 0 to 160 m [0 to 525 ft] (0 = 12.4 m,  $s = 27.8$  m,  $se = 2.2$  m). Standardized straight-line distances (mean distances moved per day) varied from 0 to 20 m/dy [0 to 66 ft/dy] (0 = 1.7 m/dy,  $s = 3.8$  m/dy,  $se = 0.3$  m/dy).



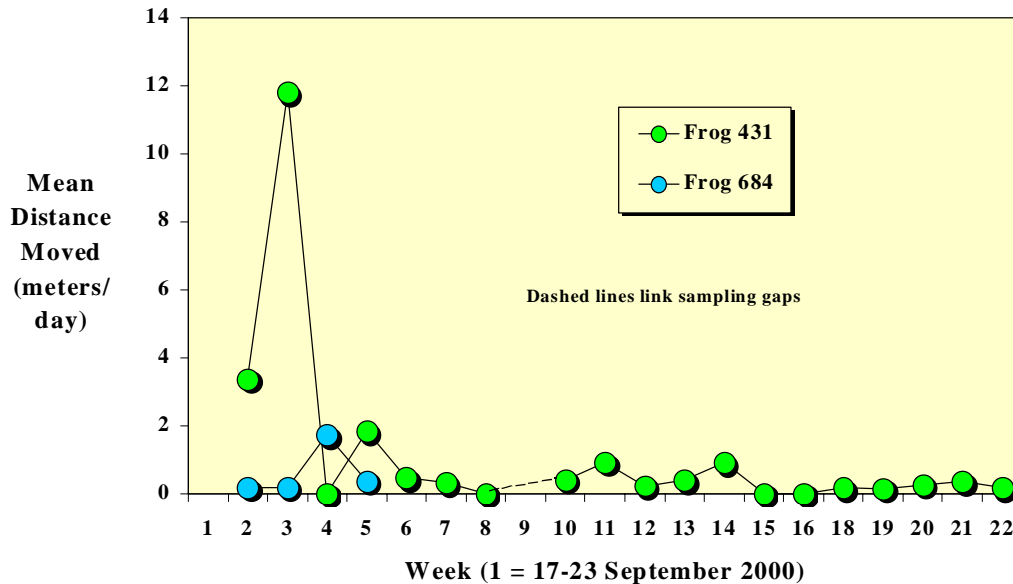
**Figure 6 - Movement Distances of Female Oregon Spotted Frogs at the Dragline Ponds, 2000-2001**



**Figure 7 - Movement Distances of Female Oregon Spotted Frogs in the Willard Unit Vicinity, 2000-2001**



**Figure 8 - Movement Data for Male Oregon Spotted Frogs in the Willard Unit Vicinity, 2000-2001**



Significant differences were found in movement distances among pre-ice, ice, and post-ice intervals (Table 5). Post-hoc comparison revealed significantly greater movements during the pre-ice interval than either of ice or post-ice intervals; movements during the ice and post-ice intervals did not differ significantly (Table 5).

Movement of individual frogs was also variable, but two intervals with substantial moves were evident (Figures 6, 7 and 8). The first, which occurred in the first four weeks of the study (19 September-21 October 2000), involved large mean daily movement distances (i.e.,  $\geq 5$  m/dy) in 7 of the 10 radio-transmitted frogs (Figures 6, 7, and 8). The second interval was during weeks 10-14 (19 November-23 December 2000), and involved mean daily mean movement distances of a similar magnitude that was observed during at least one week in each of three frogs (Figures 6 and 7). The latter moves were made during the interval of ice cover.

Of four female frogs fitted with transmitters in lentic habitats (290 and 641 in Dragline 2; 300 and 340 in the Willard Unit), three moved into lotic habitats during the interval 1-21 November 2000, prior to the formation of significant ice. Movement history of the fifth frog (641) is incomplete. Female 641 had moved to west side of Dragline 1 by 2 October 2000; subsequently, we recorded only a few radio-location changes greater than those of

resolution error until transmitter 641 was recovered without a frog on 8 November 2000. All six frogs (4 females, 2 males) that were fitted with transmitters in lotic habitats (Colds Springs Ditch and Outlet Creek) continued to be recorded in those habitats for the

remainder of the study, until found dead, or we located their transmitter.

We found a significant difference among pre-ice, ice, and post-ice intervals in DO at locations where we tracked frogs (Table 6). Post-hoc analysis revealed that while the pre-ice versus ice, and ice versus post-ice intervals differed significantly in DO, the pre-versus post-ice intervals did not. Some difference existed among units, but a general reduction in DO during the interval of heavy ice cover was apparent (Figure 9).

**Table 6 – Variation in dissolved oxygen at locations with Oregon spotted frogs (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.**

Interval	Dissolved Oxygen (mg/L)					Kruskal-Wallis Test Result	
	0	s	se	range		H	p <sup>1</sup>
				min	max		
Pre-ice (n = 42)	4.4	3.8	0.3	0.0	15.7	37.121	<b>&lt; 0.0001</b>
Ice (n = 81)	1.6	2.5	0.3	0.0	12.6		
Post-ice (n = 15)	5.5	5.2	1.3	0.0	15.7		

Post-hoc Comparison	Mann-Whitney U Test Result	
	z	p <sup>1</sup>
Pre-ice versus Ice	-5.395	<b>&lt; 0.0001</b>
Ice versus Post-Ice	-3.991	<b>&lt; 0.0001</b>
Pre-Ice versus Post-Ice	-0.308	0.7580

<sup>1</sup> Significant results are emboldened; significant results for overall test (i.e., Kruskal-Wallis analysis) is based on an  $\alpha = 0.05$  rejection criterion; significant results for post-hoc comparisons are based on a rejection criterion of  $\alpha = 0.017$ , which represents a criterion conservatively adjusted for the number of comparisons ( $n = 3$ ) based on Sidak's multiplicative inequality:  $\alpha' = 1 - (1 - \alpha)^{1/n}$ .

We also identified differences in DO between areas during selected periods (Table 7). In particular, DO was significantly higher (over 5.5 mg/L higher) in Outlet Creek than in the CSD around Willard from 1 November 2000 onwards. During the short segment of the pre-ice interval for which we have data (2 October-8 November 2000), DO in the Willard Unit averaged about 2 mg/L higher than in the CSD around Willard, but the difference

was not significant. We found no difference in DO between the combined Dragline Pond data and Outlet Creek through the interval 2 October-15 December 2000, but data for this comparison were few. Data were lacking to make comparisons between areas at other times.

**Table 7 – Comparison in dissolved oxygen between areas for selected time intervals across the Oregon spotted frog overwintering interval, 2000-2001.**

Comparison		Dissolved Oxygen (mg/L)						Mann-Whitney U Test Result	
		n	0	s	se	range		H	p <sup>1</sup>
						min	max		
<b>1</b>	Dragline Ponds <sup>2</sup>	7	6.6	2.3	0.8	3.5	9.5	-0.244	0.8075
	Outlet Creek	5	5.5	3.0	1.3	0.6	8.3		
<b>2</b>	CSD <sup>3</sup> (near Willard)	34	3.3	3.6	0.6	0.4	15.4	-1.602	0.1093
	Willard Unit	8	5.2	3.9	1.4	0.7	12.3		
<b>3</b>	CSD <sup>3</sup> (near Willard)	81	1.5	2.5	0.3	0.0	15.7	-3.542	<b>0.0004</b>
	Outlet Creek	12	7.2	4.6	1.3	0.3	12.6		

Comparison Time Intervals: (1) 2 October-15 December 2000, (2) 2 October-8 November 2000, and (3) 1 November 2000-2 February 2001.

<sup>1</sup> Significant results are emboldened.

<sup>2</sup> Combines data from both Dragline Ponds.

<sup>3</sup> Cold Springs Ditch.

For the time interval after 2 October 2000 (i.e., the entire data period for which DO was measured), DO levels were significantly correlated with movement distances, whether the raw (Spearman Rank:  $n = 137$ ,  $\rho = 0.309$ ,  $p = 0.0003$ ) or day-standardized movement data were used (Spearman Rank:  $n = 137$ ,  $\rho = 0.309$ ,  $p = 0.0003$ ). However, this relationship worsened if the data were restricted to the ice interval (Spearman Rank: raw data:  $n = 81$ ,  $\rho = 0.219$ ,  $p = 0.0506$ ; day-standardized data:  $n = 81$ ,  $\rho = 0.215$ ,  $p = 0.0549$ ). Moreover, DO levels from the immediately previous site at which a radio-location was obtained were also significantly correlated with movement distances, whether the data were raw (Spearman Rank:  $n = 137$ ,  $\rho = 0.283$ ,  $p = 0.0014$ ) or day-standardized (Spearman Rank:  $n = 137$ ,  $\rho = 0.296$ ,  $p = 0.0009$ ). For the two frogs for which we took DO measurements at the immediately previously location on the same date as DO was taken at the new location, DO averaged 1.41 mg/L higher at the new location (range: 1.34-1.48).

Water depths at which we obtained radio-locations did not differ among the pre-ice, ice, and post-ice intervals (Table 7), and the power of this test was limited ( $\beta = 0.162$ ). Water

**Table 8 – Variation in water depth at locations with Oregon spotted frogs (*Rana pretiosa*) among time intervals at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.**

Interval	Water Depth (m)					ANOVA Test Result	
	0	s	se	range		F	p <sup>1</sup>
				min	max		
Pre-ice (n = 54)	0.59	0.27	0.04	0.06	1.11	0.707	0.4950
Ice (n = 80)	0.55	0.20	0.02	0.10	1.00		
Post-ice (n = 15)	0.61	0.07	0.07	0.21	0.95		

<sup>1</sup> Significant results are emboldened.

**Table 9 – Variation in water depth at locations with Oregon spotted frogs (*Rana pretiosa*) among areas at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.**

Area Category	Water Depth (m)					Kruskal-Wallis Test Result	
	0	s	se	range		F	p <sup>1</sup>
				min	max		
Boundary Ditch <sup>2</sup> (n = 1)	0.35	-	-	0.35	0.35	0.707	0.4950
CSD <sup>3</sup> near Willard (n = 117)	0.62	0.21	0.02	0.06	1.11		
Dragline (n = 9)	0.49	0.30	0.01	0.21	1.02		
Outlet Creek (n = 10)	0.34	0.20	0.06	0.10	0.68		
Willard Unit (n = 12)	0.29	0.07	0.02	0.20	0.40		

Area Category	Water Depth (m)					Mann-Whitney Test Result	
	0	s	se	range		z	p <sup>1</sup>
				min	max		
CSD <sup>3</sup> near Willard (n = 82) <sup>4</sup>	0.59	0.19	0.02	0.25	1.00	-3.407	<b>0.0007</b>
Outlet Creek (n = 10) <sup>4</sup>	0.34	0.20	0.06	0.10	0.68		

<sup>1</sup> Significant results are emboldened.

<sup>2</sup> Boundary ditch on southeast edge of Willard Unit.

<sup>3</sup> Cold Springs Ditch.

<sup>4</sup> Time interval of comparison is 8 November 2000-17 February 2001.

Table 10 – Variation in distance from shore at locations with Oregon spotted frogs (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge across the winter season, 2000-2001.

Interval	Distance from shore (m)					Kruskal-Wallis Test Result	
	0	s	se	range		H	p <sup>1</sup>
				min	max		
Pre-ice (n = 43)	1.8	4.0	0.6	0.0	23.8	20.334	<b>&lt; 0.0001</b>
Ice (n = 83)	0.5	0.6	0.1	0.0	4.2		
Post-ice (n = 15)	0.3	0.4	0.1	0.0	1.2		

Post-hoc Comparison	Mann-Whitney U Test Result	
	z	p <sup>1</sup>
Pre-ice versus Ice	-4.063	<b>&lt; 0.0001</b>
Ice versus Post-Ice	-1.254	0.2099
Pre-Ice versus Post-Ice	-3.356	<b>0.0008</b>

<sup>1</sup> Significant results are emboldened; significant results for overall test (i.e., Kruskal-Wallis analysis) is based on an  $\alpha = 0.05$  rejection criterion; significant results for post-hoc comparisons are based on a rejection criterion of  $\alpha = 0.017$ , which represents a criterion conservatively adjusted for the number of comparisons ( $n = 3$ ) based on Sidak's multiplicative inequality:  $\alpha' = 1 - (1 - \alpha)^{1/n}$ .

depths differed significantly among areas (Table 8), but too few data were available to identify the basis of differences between most areas because of temporal differences in data collection. The only comparison that could be made with some confidences was between the CSD near Willard and Outlet Creek over the interval 11 November 2000-17 February 2001. During this interval, radio-locations in the CSD near Willard were at sites with roughly twice the depth of radio-locations in Outlet Creek (Table 8).

Distances to the nearest shoreline among pre-ice, ice interval, and post-ice intervals were significantly different (Table 9). Post-hoc analysis revealed that significant differences existed between the pre-ice and ice intervals, and the pre- and post-ice intervals, but not the ice and post-ice intervals (Table 9).

We found significant variation the near-space habitat structure close to radio-locations (Table 10). In particular, within a 1-m diameter circle around the radio-location point, we found that floating vegetation and open water/ice was significantly more frequent and uplands were significantly less frequent than within area defined by 5-m diameter circle outside of the 1-m diameter circle. The low emergent category was more frequent outside the 1-m diameter circle and the other category (almost exclusively logs) was less

frequent inside the 1-m diameter circle, but comparisons for both of these were not significant.

**Table 11 – Variation in habitat structure of the near-space around Oregon spotted frog (*Rana pretiosa*) locations through the overwintering interval at Conboy Lake NWR, 2000-2001**

Structural Variable	Category	Percentage (%) of Area in Structural Category					Wilcoxon Signed Rank Test Result	
		0	s	se	range		z	p <sup>1</sup>
					min	max		
Low Emergents	1 m circle <sup>2</sup>	11.5	23.3	1.9	0.0	100.0	-1.979	0.0478
	5 m circle <sup>2</sup>	15.6	28.2	2.3	0.0	98.8		
Tall Emergents	1 m circle	9.8	23.9	1.9	0.0	100.0	-1.037	0.2997
	5 m circle	7.7	14.7	1.2	0.0	83.8		
Submerged Vegetation	1 m circle	1.5	8.6	0.7	0.0	100.0	-0.221	0.8252
	5 m circle	1.1	4.1	0.3	0.0	26.9		
Floating Vegetation	1 m circle	26.9	33.1	2.7	0.0	100.0	-5.932	< <b>0.0001</b>
	5 m circle	8.3	13.3	1.1	0.0	76.2		
Uplands	1 m circle	21.0	23.8	1.9	0.0	100.0	-9.634	< <b>0.0001</b>
	5 m circle	60.2	28.1	2.3	0.0	95.2		
Open Water/Ice	1 m circle	34.5	31.2	2.5	0.0	100.0	-4.230	< <b>0.0001</b>
	5 m circle	22.6	29.9	2.4	0.0	100.0		
Other	1 m circle	2.7	10.8	0.9	0.0	70.0	-1.544	0.1227
	5 m circle	0.7	2.3	0.2	0.0	16.0		

Note: The sample size was 155 for all comparisons.

<sup>1</sup> Probability (p); significant results (emboldened) use a rejection criterion of  $\alpha = 0.007$ , which represents a criterion conservatively adjusted for the number of comparisons ( $n = 7$ ) based on Sidak's multiplicative inequality:  $\alpha' = 1 - (1 - \alpha)^{1/n}$ .

<sup>2</sup> Dimension refers to the diameter of the circle centered on the frog location.

Examining the habitat complexity (interspersion index: see Appendix I) of a 1-m<sup>2</sup> space around radio-locations, we found a steady decrease in the range and variation of habitat complexity from week 7 (when these data were first collected) to week 11 (Figure 9). Simplification was associated with a combination of the progressive development of ice cover, vegetation dieback, and location in more open-water habitat. After week 11, the interspersion index remained relatively low with limited variation through the balance of the study.

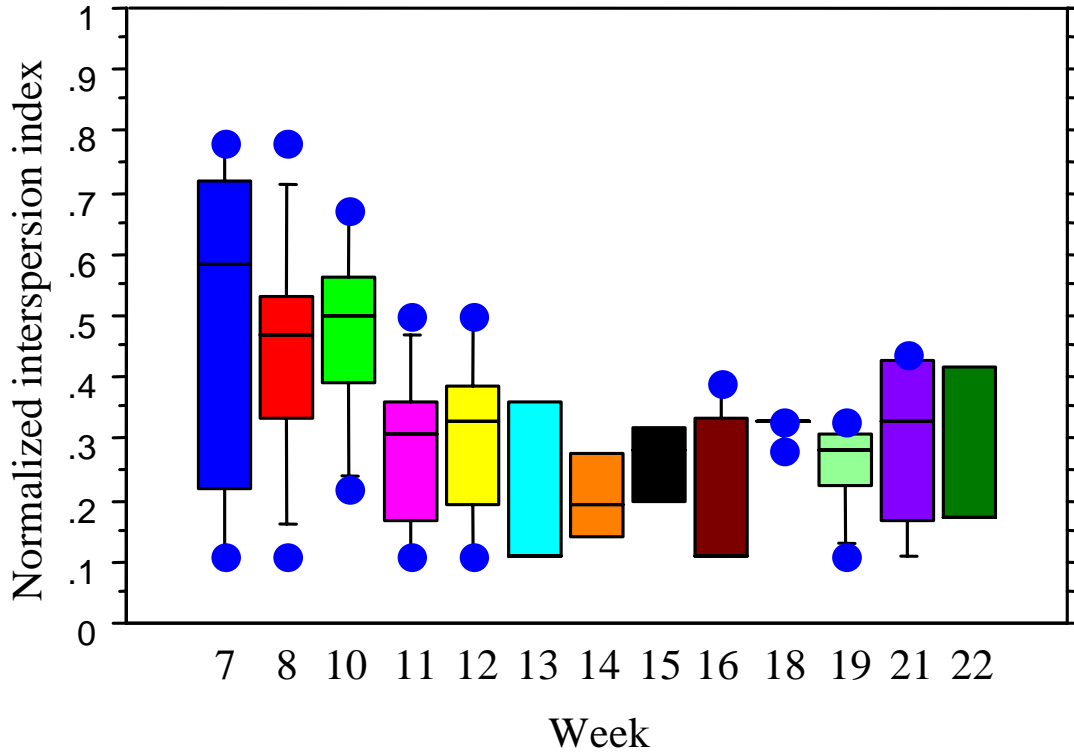
Fate of Frogs: Of 10 frogs that were radio-transmitted, the transmitter from one female (641) was left at Dragline 1 sometime before 8 November 2000 and had no evidence of having been handled, and the transmitter left by a second female (684) at the CSD had some incisions and depressions on it. Remains of a partly eaten female (340) were found on 14 January 2001 in the CSD, and a well-decomposed male (598) was also found in the CSD on 31 January 2001. The radio-location history for the latter frog had been unchanged for 13 weeks (no position movement since week 6). Two other frogs (male 513 and female 315, both from the CSD near Willard) had shown no position changes during the last 10 weeks. The signal for frog 513 was lost during the terminal week of the study; frog 315 was never recovered. We removed transmitters from three live frogs at the end of the study, female 290 at Dragline and females 372 and 431 at the CSD near Willard. The signal on female 300 was lost during the terminal week of the study and the frog was never recovered.

## Discussion and Conclusions

Variation in Effort: Tracking effort among surveyors was variable. The only pattern we analyzed that was significant was that two surveyors could conduct a telemetry survey in significantly less absolute time, but the cost of the standardized effort (person-hours) was still greater. The implication of this finding is that it remains less costly to conduct such surveys with one surveyor despite the increase in absolute time required for one surveyor to complete a survey. We anticipate that this difference was a function of two surveyors facilitating the partitioning of recording and tracking survey tasks on relatively few frogs, and that a difference in standardized cost between one and two surveyors may be reduced as number of frogs tracked increases. Not all costs are linked to survey time. Safety, an important consideration, more than balances the added cost of using two surveyors.



**Figure 9 – Variation in interspersion (habitat complexity) index from week 7 (29 October-4 November 2000) to week 22 (11-17 February 2001). No interspersion data were collected during weeks 9 and 17.**



We suspect that some of the lack of significant variation between other categories may be concealed by relatively small sample sizes (e.g., number of frogs captured, frogs tracked in the first two weeks) and a relatively high variance in among- and within-individual effort (Appendix I). In particular, the indication that greater effort may be required early in a telemetry study is likely real, and we anticipate that it may reflect a surveyor learning curve related to increasing familiarity with study areas and frog behavior. Although more data will be needed, these indications can provide guidance for conducting such studies. On the basis of available information, we suggest the following:

- 1) Allocating sufficient time for surveyors to gain enough pre-study familiarity with areas prior to tracking. This should reduce the need for general orientation during surveys, thereby decreasing overall time spent tracking during the initial tracking sessions.
- 2) Not switching surveyors in the course of the study since every new surveyor will likely add time for general orientation.

- 3) Using two surveyors to allow partitioning of data gathering and recording tasks. We believe the latter would especially increase efficiency where the number of radio-transmitted animals is  $\geq 10$ .

Frog Behavior: A basic aspect of OSF overwintering behavior appears to be the use of aquatic overwintering sites. Both terrestrial and aquatic overwintering sites have been described for ranid frogs (Berger 1982; Bohnsack 1951, 1952; Bradford 1983; Breckenridge 1944; Cunjak 1986; Friet 1993; Emery *et al.* 1972; Rand 1950; Ritson and Hayes 2000; Savage 1951; Stinner *et al.* 1994; Willis *et al.* 1956). Some investigators have suggested that selection of overwintering site type may be species-specific, but use of different overwintering habitat types has been observed in some species (e.g., *Rana pipiens* [aquatic: Breckenridge 1944, Cunjak 1986, Emery *et al.* 1972; terrestrial: Rand 1950]; *Rana catesbeiana* [aquatic: Stinner *et al.* 1994, Willis *et al.* 1956; terrestrial: Bohnsack 1952]). In species where use of different overwintering habitat types has been recorded, the aquatic site use encountered at higher latitudes is thought to afford greater protection from winter extremes (Pinder *et al.* 1992). However, data for the OSF to date has not revealed terrestrial overwintering site use. As CLNWR is among OSF sites that have the more extreme winter conditions, terrestrial overwintering sites might not be expected. Yet, some data from the more benign climate of Pacific slope drainage sites where OSFs are extant (western Washington and British Columbia) also imply that overwintering sites are aquatic (R. Haycock, K. McAllister, pers. comm.). Thus, for the OSF, use of aquatic sites may be unrelated to severe winter conditions. As we have observed that OSFs dry rapidly during handling, requiring frequent immersion (e.g., during radio-transmitter belt attachment), we suspect that OSFs have a high water turnover physiology that may constrain them to using aquatic overwintering sites.

The pattern of OSF surface activity in aquatic habitat around the overwintering interval appears to be water temperature related. We never observed surface-active OSFs when water temperatures were  $< 5^{\circ}\text{C}$ . Related northern red-legged frogs moved from terrestrial active-season habitat to aquatic habitat when fall soil temperatures reached  $8^{\circ}\text{C}$  (Ritson and Hayes 2000). As water temperatures were most tightly correlated with movements in OSF and soil temperatures were most strongly correlated with activity in northern red-legged frogs, a threshold temperature or a decline in temperature through a threshold region in the active-season environment that influences these ranid frogs the most may represent the cue to impending winter conditions. Lower variance for temperatures we found in the OSF aquatic environment, and the lower variance of temperatures Ritson and Hayes (2000) encountered in the terrestrial environment of northern red-legged frogs (in both cases relative to the higher variance of air temperatures) may make these the most reliable cuing variables. Other variables that might serve as cues (air temperature,

relative humidity) were highly unpredictable. Further, we expect that such a cue would precede winter conditions by a long enough interval to avoid placing frogs at risk. A temperature cue in the focal water environment at a temperature  $\geq 5^{\circ}\text{C}$  would occur early enough to allow OSF sufficient time to move to refuges before winter conditions set in.

We interpreted an increased magnitude of movements as resulting from two factors: 1) a search for suitable overwintering sites initiated by cues related to oncoming winter conditions (previous discussion); and 2) search for suitable overwintering sites that results from dissolved oxygen depletion. We believe that the first of these occurred when the larger magnitude movements around the time when the presumed threshold condition (perhaps water temperature) is reached. In contrast, the second can be identified from large-scale movements after ice-up that were disconnected temperature conditions. Low water conditions in Cold Springs Ditch<sup>5</sup> as a result of drought likely exacerbated oxygen depletion. Oregon spotted frog tolerance to low DO levels needs study, but DO levels in the largely ice-capped Cold Springs Ditch being extremely low (i.e.,  $< 2$  mg/L) for as much two months may have resulted in anoxia/hypoxia induced mortality. Male 598 may have been an anoxia casualty. Two additional frogs that showed on movements for long periods and were not recovered may represent similar casualties.

Interpretation of a pattern related to overwintering movements during the ice interval is ambiguous, but several lines of evidences imply that DO may be a factor. A significant positive relationship between DO at radio-locations and previous movement distances might be expected if frogs were tracking DO. What is puzzling, however, is that this relationship remains essentially the same if one compares movements to DO from the immediately previous location, for which one might expect an inverse relationship. In addition, the relationship is unimproved by examining exclusively the ice-bound interval, when one might expect this effect to be greater. The difficulty with these comparisons may be due to an approximately weekly tracking resolution, when in fact, volatility of DO variation may require a daily resolution. Other evidence also points to a dissolved oxygen-related pattern. This includes shallow water locations during the ice interval (greater DO has been demonstrated along shallow margins during ice-capping episodes [Barica and Mathias 1979]), and use of microhabitats with a greater proportion of open water (greater potential for DO exchange). The latter would not be expected if predation was the major factor on overwintering location selection since sites with open water are known to be more accessible to mink, a major wintertime predator on OSF (J. Engler, M. Hayes, *unpubl. data*). Our limited attempt to take simultaneous DO measurements at

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<sup>5</sup> The few gauge data from the Bird Creek inflow to the refuge were insufficient for proper interpretation of stage variation, but our own observations indicated that water levels in the CSD were lower than we had observed them in the five years since we began systematic work at CLNWR.

existing and previous frog locations also agree with a DO mediated hypothesis and imply that more resolved sampling would significantly improve understanding of this pattern.

If DO is an influence, we would expect it to be particularly pronounced effect during drought conditions. During higher water years, greater flows (and as a consequence less ice development) may prevent DO levels in channels at CLNWR from dropping to the low levels observed in the CSD during the winter of 2000-1. If this is true, special attention should be given to the management consequences of this condition, since water withdrawals that decrease flows into CLNWR will likely aggravate the problem. Since adults responsible for the next year's reproduction overwinter in these habitats, water mismanagement has the potential to reduce the adult OSF population levels rapidly. The likelihood of this occurring needs study. Given that historic patterns of management of water resources at CLNWR have been problematic, understanding the dimension of this problem is critical.

Although not well detailed in the habitat data, our observational data suggest that beaver structures of various kinds, but particularly dams, wood debris accumulations from old step dams, and undercut beaver runways may represent particularly important winter refuge habitats for OSF. The structure of most of such habitats may provide a favorable flow environment, that increases the likelihood of sufficient DO while simultaneously providing the structure that provides better refuge from predators. The quality of beaver structures as overwintering habitat needs comparison to alternative overwintering sites.

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## Appendix I

### Habitat Interspersion Data Collection Method

This appendix describes the habitat interspersion data collection method, which describes the method developed largely by Ken Risenhoover and Bill Leonard, two members of the OSF overwintering study team. We used a 1 m<sup>2</sup> micro-plot upon which microhabitat data were scored on two axes having 10 10-cm<sup>2</sup> cells with a center offset (Appendix Figure 1).

				X					
				X					
				X					
				X					
				X					
X	X	X	X	X	X	X	X	X	X
				X					
				X					
				X					
				X					

**Appendix Figure Ia – Diagram of a 1 m<sup>2</sup> micro-plot grid showing the two axes of 10-cm<sup>2</sup> cells scored (Xs).**

We scored vegetative data into one of 13 vegetation or structural forms, which included:

- 1) open water
- 2) emergent (upright) reed canarygrass (*Phalaris arundinacea*)
- 3) overhanging (horizontal, but not floating) reed canarygrass
- 4) emergent native vegetation
- 5) overhanging native vegetation
- 6) herbaceous overhanging
- 7) floating native vegetation
- 8) floating vegetation mat
- 9) upland
- 10) wood debris (e.g., logs, bark or other wood debris)
- 11) floating reed canarygrass
- 12) snow/ice covered – vegetation unknown
- 13) snow covered – substrate uncertain

We scored the emergent vegetation categories (2 and 4) as that vegetation the stems of which were  $\geq 45^\circ$ . The overhanging vegetation categories (3 and 5) had stems at  $< 45^\circ$ , but were not floating. Floating vegetation categories (7, 8, and 11) was vegetation that

lacked a significant emergent or overhanging component (e.g., pondweed [*Potamogeton* sp.]). Within each 10 cm<sup>2</sup> cell, we occasionally found variation involving more than one category; for those cases, we scored the dominant form within the cell.

To estimate habitat interspersions, we counted how many times a vegetation or structural form changed along each of the two axes in the microplot, and summed these together for a total sum of all changes along both axes. The cell total for both axes, 20, was divided into this sum of changes to obtain a raw interspersions index value. We divided this raw index value by 0.9 to obtain a normalized value that varied between zero and 1. In order for the index to reach a maximum value of 1, normalization of raw values was necessary because the maximum possible number of changes was 18, resulting in a maximum value the index could take on of 0.9. The equations are:

$$C_h + C_v = C \qquad C/20 = I_r \qquad I_r/0.9 = I$$

where  $C_h$  = total number of changes along the horizontal microplot axis  
 $C_v$  = total number of changes along the vertical microplot axis  
 $C$  = total sum of all changes along both horizontal and vertical microplot axes  
 $I_r$  = raw interspersions index value  
 $I$  = normalized interspersions index value

We also summarized vegetation and structural forms in microplots using the percentage of each form type. This percentage was obtained by taking the number of cells with each form type and dividing them by 19, which represents the total different cells scored in the 1 m<sup>2</sup> plot.

## Appendix II

**Variation in surveyor effort for tracking radio-transmitted Oregon spotted frogs (*Rana pretiosa*) at Conboy Lake National Wildlife Refuge during winter 2000-1.**

Surveyor	Survey Days	Frequencies Tracked	Effort per Frequency Tracked (person-minutes) <sup>1</sup>		
			0	s	se
Eric D. Anderson	1	8	68	39	14
Harold E. Cole	1	1	56 <sup>2</sup>	-	-
Daniel J. Dugger	2	19	69	40	9
Joseph D. Engler	7	53	61	42	6
Daniel C. Friesz	3	26	60	39	8
Marc P. Hayes	1	10	72	48	15
Don I. Ross	2	8	88	70	25
Sarra M. Russum	6	47	58	30	4
Susan Van Leuven	10	74	60	24	3
Teunis G. Wyers	7	28	72	56	11

<sup>1</sup> Effort was calculated in person-minutes because two surveyors conducted most surveys. Thus, effort per frequency tracked represents the effort for indicated surveyors that was done with or without assistance from a second person.

<sup>2</sup> Effort was for one transmitter frequency, not an entire survey day.

### Appendix III

#### Vegetation Structure and Taxon Codes

Code	Common Plant Species Name or Structure	Scientific Name or Explanation
ACHI	Yarrow	<i>Achillea millefolium</i>
CARX	Sedge	<i>Carex</i> spp.
CIRS	Thistle	<i>Cirsium</i> spp.
ELEO	Spike-rush	<i>Eleocharis</i> spp.
EQST	Horsetail	<i>Equisetum</i> spp.
FLGR	Floating grasses	Poaceae family
JUNC	Rush	<i>Juncus</i> spp.
LEMN	Duckweed	<i>Lemna</i> spp.
PELI	Beaver slash	fresh peeled limbs or old wood
PHAR	Reed canarygrass	<i>Phalaris arundinacea</i>
PICO	Lodgepole pine	<i>Pinus contorta</i>
POTA	Pondweed	<i>Potamogeton</i> spp.
ROSA	Rose	<i>Rosa</i> spp.
SALX	Willow	<i>Salix</i> spp.
SCIR	Bulrush	<i>Scirpus</i> spp.
SOLI	Goldenrod	<i>Solidago canadensis</i>
SPAR	Bur-reed	<i>Sparganium</i> spp.
SPIR	Hardhack	<i>Spiraea douglasii</i>
TYPH	Cattails	<i>Typha</i> spp.
UNAL	Unknown algae	
UNGR	Unidentified grasses	Poaceae
UNMI	Mint	Lamiaceae
WOOD	Woody debris	Logs, branches or other debris

Note: Scientific and common names follow Guard (1995) for the aquatic taxa and Pojar and McKinnon (1994) for other plant taxa.

<sup>1</sup> *Carex vesicaria* var. *major* (inflated sedge) was among the sedges identified.

<sup>2</sup> *Eleocharis palustris* (creeping spike-rush) was among the spike-rushes identified.

<sup>3</sup> *Juncus effusus* (common rush) was among the rushes identified.

<sup>4</sup> *Lemna minor* (common duckweed) was among the duckweeds identified.

<sup>5</sup> *Potamogeton natans* (floating-leaf pondweed) was among the pondweed identified.

<sup>6</sup> *Scirpus tabernaemontani* (*S. validus*) (soft-stem bulrush) was among the bulrush identified.

<sup>7</sup> *Sparganium emersum* (simple-stem bur-reed) was among the bur-reed identified.

<sup>8</sup> *Typha angustifolia* (narrow-leaf cattail) was among the cattails identified.

