

**Humboldt Bay National Wildlife Refuge 2004 Northern Red-Legged Frog
Malformation Report**

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Abstract Surveys for abnormal post-metamorphic northern red-legged frogs (*Rana aurora*) were conducted 2002, 2003, and 2004 at the Humboldt Bay National Wildlife Refuge in northern California. In 2004, two primary sites were designated for bi-weekly surveys from June 11 until August 6. Pond 1 had 14 abnormal frogs detected out of 144 surveyed for an estimated 10% abnormal rate. Pond 5 had 22 abnormal frogs detected out of 238 for an estimated 9% abnormal rate. A total of 37 abnormal frogs were detected out of 407 surveyed for the entire refuge and accounted for an overall estimated 9% abnormal rate in 2004. This is above the considered background rate of 5% abnormal rates in anuran populations. In the 2003 surveys, 37 abnormal frogs out of 460 surveyed for the entire refuge accounted for an 8% abnormal rate. The 2002 surveys had one abnormal frog out of 165 surveyed for a rate of 0.6% of abnormal post-metamorphic northern red-legged frogs. Across years and across season, the potential for change in the prevalence of abnormal frog detections can change and should be considered when surveying for such animals. The presence of malformed *R. aurora* from both primary study ponds and the absence of the trematode *Ribeiroia* and of the first intermediate host, *Helisoma*, from Pond 5 makes a parasite hypothesis less likely. The cause of the malformations at Humboldt Bay NWR may therefore be due to either a chemical stressor or to ultraviolet β radiation exposure or other unknown effects.

Introduction

Amphibian malformations were brought to the public's attention in 1996 when middle school children found high numbers of deformed frogs in Minnesota. Since then, many additional reports have been made in North America in many species of amphibians. According to the North American Reporting Center for Amphibian Malformations (NARCAM, 2002), over 2,100 reports from 1,032 sites involving 82 species were made as of September 30, 2002, when project funding was discontinued. There were 52 species reported to have malformations from 46 states and 4 Canadian provinces with 944 verifiable cases.

With concerns over worldwide declines and range reductions in amphibians, a partnership to research the phenomenon in North America was undertaken by the U. S. Geological Survey (USGS), U. S. Fish and Wildlife Service (USFWS) and academic institutions. It is known as the Amphibian Research and Monitoring Initiative (ARMI) with the primary lead being the USGS for monitoring and directed research, and USFWS contributing investigation efforts of National Wildlife Refuges for malformed amphibians.

Amphibian malformations have been attributed to three primary causes: 1) parasites; 2) chemical exposure; and 3) increase in ultraviolet (UV) radiation (Blaustein and Johnson 2003). Parasites of the genus *Ribeiroia* have been demonstrated to cause deformities in amphibians that include multiple limbs (Sessions and Ruth 1990, Johnson et al. 1999). However, there is a large body of literature on amphibian parasites and not all parasites cause malformations (Gilliland and Muzzall 2002). Amphibian malformation has been found in association with agricultural practices in Quebec, Canada suggesting a link to herbicide or pesticide contamination (Ouellet et al. 1997). In a laboratory experiment with *Xenopus laevis* embryos exposed to water where a high incidence of wild frog malformations occurred, animals experienced a higher incidence of mortality and malformation compared to embryos exposed to water from sites with no associated malformations in wild frogs (Burkhart et al. 1998). After filtering of the water through activated carbon, limited experimental evidence suggested that malformations were not induced by infectious organism, imbalance of ion concentration or exposure to heavy metals. Ultra-violet radiation experiments have also demonstrated the effect of causing malformations (Ankley et al. 2002). Although all three of these hypotheses have been demonstrated in both laboratory and under field conditions, determining exact cause of deformities is quite often unattainable (Stocum 2000) due to insufficient data.

Study Area

The study area was located on the Humboldt Bay National Wildlife Refuge U. S. Fish and Wildlife Service located in Humboldt County, California. Two ponds, Pond 1 and Pond 5, are located in the southern portion of the refuge. These ponds were also surveyed in 2002 and 2003. Surveys were also conducted on a newly acquired Lamphere Dunes in April 2004, but no water bodies contained ranid larva and were therefore dropped from the summer surveys. Site 6 was an area adjacent to Pond 1 that was a riparian corridor along Salmon Creek. Site 6 was chosen opportunistically on July 16th, 2004 when the visit to Pond 1 revealed that it was completely dry and only one post-metamorphic northern red-legged frog (*Rana aurora*) was detected.

Table 1. Latitude and Longitude for the two ponds investigated for abnormal northern red-legged frogs (*Rana aurora*) at Humboldt Bay National Wildlife Refuge in 2004.

Refuge	State	Site Name	Sample Season	Potential Contaminants	Latitude	Longitude
Humboldt Bay NWR	CA	POND 1	2	Cattle use / EDCs ¹	40 40 37	124 12 30
Humboldt Bay NWR	CA	POND 5	2	Cattle use / EDCs ¹	40 40 39	124 12 17
Humboldt Bay NWR	CA	SITE 6	1	Cattle use / EDCs ¹	40 40 37	124 12 26

¹EDC's = endocrine disruptor chemicals

Methods

Surveys were focused on ranid frogs and therefore on northern red-legged frogs (*Rana aurora*) for the Humboldt Bay National Wildlife Refuge. Generally, the same protocol was followed between 2003 and 2004, with several of the same field biologists involved.

Collections of aquatic snails were made at both Pond 1 and Pond 5 by dip-netting with aquarium nets in water about 30 cm deep. Gross examination was made in the field to collect representatives from different family level taxons. Identification of aquatic gastropods to family and genus level taxonomy follows Burch's North American Freshwater Snails (1989).

Biologists and/or technicians conducted diurnal visual encounter surveys (VES) on the margins of the ponds. Metamorphosed *Rana aurora* were captured with aquarium dip-nets or by hand. Biologists worked individually or in pairs to capture metamorphs, place them in a bucket, and then examine the animals after 20 individuals were collected. Data recorded included species, snout-vent-length (SVL), tail-length, Gosner stage, and whether or not the animal exhibited a physical abnormality (Gosner 1960, Meteyer 2000). Metamorphs were examined for abnormalities while in a zip-lock bag or held in hand. Normal animals were given a mark by clipping the number 4 digit on the left rear foot so that on subsequent visits to the pond, recaptures could be identified. Digit number 4 is the fourth phalange counting from proximal to the body and moving distally. Toe-clips were retained and preserved in 70% ethanol as genetic voucher material.

If animals were suspected to be diseased, they were retained as vouchers for submission to the National Wildlife Health Care Center in Madison, Wisconsin for diagnostic examination to determine type of pathogen. If animals were not submitted alive, they were euthanized by overdose in MS-222 (Tricaine methanesulfonate) and then placed in 70% or 95% ethanol.

Animals identified as malformed in the field were assigned an abnormal frog ID following the U.S. Fish and Wildlife Service Standard Operating Procedures Abnormal Amphibian Surveys (Malphibian SOPs.doc April 2004) and had the type of abnormality noted (Meteyer 2000). Digital photos were taken when a camera was available.

Abnormal specimens were euthanized by overdose in MS-222 and then placed in 70% or 95% ethanol. Voucher specimens were submitted to Dr. Michael Lannoo at the Muncie Center for Medical Education, Ball State University, Muncie, Indiana. Animal vouchers of *R. aurora* (n = 37) were submitted on October 4, 2004. A total of 18 Pacific treefrogs (*Hyla* (= *Pseudacris*) *regilla*) were collected on July 30 and submitted alive to Dr. Dan Sutherland at the Department of Biology and River Studies, University of Wisconsin- La Crosse for parasitology examinations, with 9 collected at Pond 1 and 5 collected at Pond 5. Ten *R. aurora* were collected (five from Pond 1 and five from Pond 5) on August 13, 2004 and submitted alive for parasite examination by Dr. Sutherland.

The population for Ponds 1 and 5 were estimated using the Schumacher-Eschmeyer model based on a Schnabel mark recapture estimator (Krebs 1998), as shown by the equation:

$$N = \frac{\sum C_t M_t^2}{\sum R_t M_t}$$

Where N = Estimate of population size, C_t = number of individuals caught in survey period t , R_t = number of individuals recaptured in survey period t , and M_t = number of total marked individuals at large at survey period t .

Sampling methods followed U.S. Fish & Wildlife Service - Standard Operating Procedures – Abnormal Amphibian Surveys – “Malphibian SOPs.doc revision April 2004”.

Results

Ponds 1 and Ponds 5 demonstrated a difference in the species assemblage of Gastropoda. Pond 1 had only one genera present, *Fossaria*, where Pond 5 had two genera present, *Fossaria* and *Helisoma* (see Appendix B for classification division and keys from Burch 1989 that lead to identification). The first intermediate hosts for the trematode parasite *Ribeiroia* are snails in the genus *Helisoma* (formerly genus *Planorbis*).

Samples of northern red-legged frogs (*Rana aurora*) from the Humboldt Bay NWR collected in 2004 had several forms of abnormalities, and slightly different prevalence's of abnormalities between sites surveyed. The prevalence of Pond 1's abnormalities was at 9%, with 14 out of 144 *R. aurora* exhibiting some degree of abnormality. The prevalence of Pond 5's abnormalities was at 10%, with 22 out of 238 *R. aurora* exhibiting some degree of abnormality (Figure 1). Site 6 had one out of 25 animals surveyed as abnormal, for a rate of 4%. The estimated refuge wide abnormality rate across all sites was 10%, with 37 out of 407 post-metamorphic *R. aurora* exhibiting an abnormality.

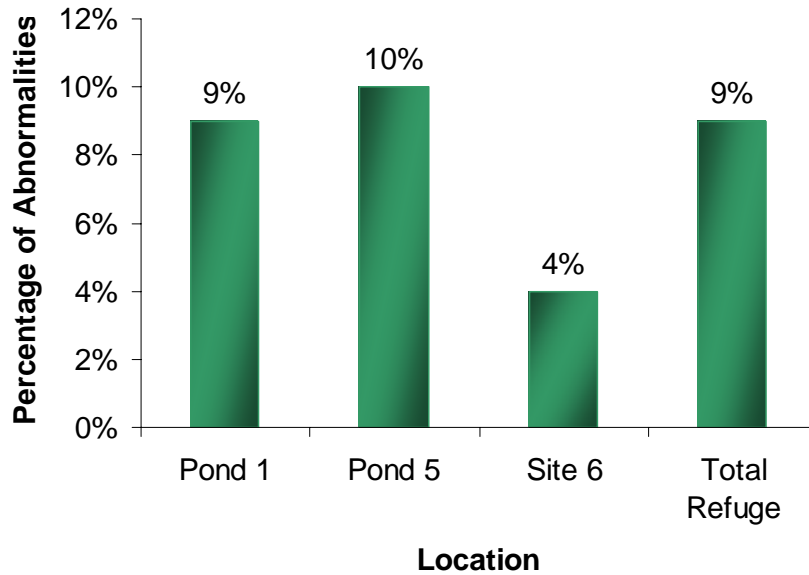


Figure 1. Abnormalities rates in northern red-legged frogs (*Rana aurora*) by site and for all sites combined observed during surveys conducted within Humboldt Bay National Wildlife Refuge, CA, in 2004.

The types of abnormalities seen in the *R. aurora* were dispersed across the reported malformation types (Figure 2). Hindlimb abnormalities (n = 26) made up the majority of the 37 *R. aurora* vouchered in 2004, and accounted for 72% of the abnormalities detected. Ten vouchered *R. aurora* exhibited reduced lower jaws, or micrognathia of the mandible, with five vouchers from Pond 1 and five vouchers from Pond 5.

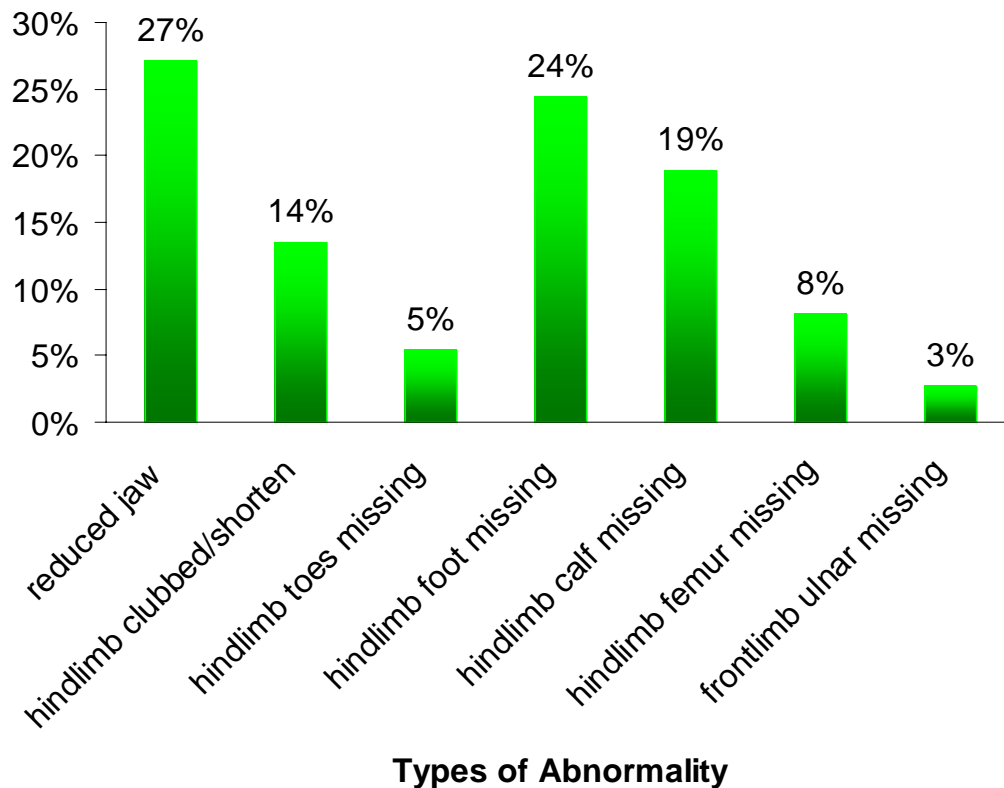


Figure 2. Types of abnormalities found in northern red-legged frogs (*Rana aurora*) at Humboldt Bay National Wildlife Refuge in 2004 field surveys. Sample size of n = 37 pooled from all sites on refuge.

The population of *R. aurora* was estimated to be 829 for Pond 1 and 829 for Pond 5 (Appendix C). Average snout-vent length (SVL) for each survey ranged from 26.3 mm to 31.4 mm and the percentage of abnormal post-metamorphic *R. aurora* ranged from 3.8 % to 13.3 % (Figure 3).

The examinations of parasites in *R. aurora* and in the Pacific treefrogs (*Hyla regilla*) results are reported in Table 2, and it is notable that the malformation-causing trematode, *Ribeiroia ondatrae*, was not detected in any of the ten *R. aurora* or the 18 *H. regilla* examined. Specimens of each species did have some level of infection from three types of trematodes (genera: *Fibricola*, *Megalodiscus*, and *Echinostoma*) and the *R. aurora* had a light infection of the lung nematode, *Rhabdias*. The percentage of abnormalities detected at the 2004 detection sites can be seen in Appendix D.

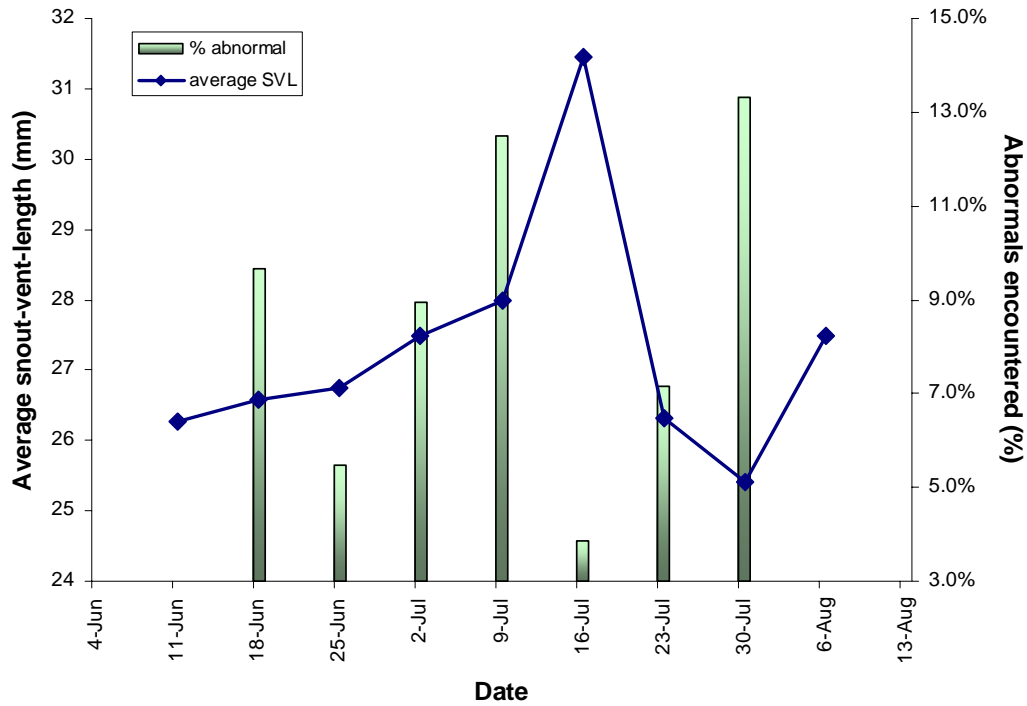


Figure 3. Weekly snout-vent-length (SVL) and percentages of abnormalities detected during the nine surveys for northern red-legged frogs (*Rana aurora*) at Humboldt Bay National Wildlife Refuge in 2004.

Table 2. Helminth parasites from northern red-legged frogs (*Rana aurora*) and Pacific treefrogs (*Hyla regilla*) from Humboldt Bay National Wildlife Refuge, CA, in 2004. Numbers below parasite genera represent average number of helminths per infected host.

Class:	Trematoda				Nematoda
Genera:	Ribeiroia	Fibricola	Echinostoma	Megalodiscus	Rhabdias
Red-legged frogs	0	3.4	52.4	7.2	1.5
Pacific treefrogs Pond 1	0	52.3	199.6	2.7	0
Pacific treefrogs Pond 5	0	27.5	57.3	0	0

Discussion

The overall malformation rate in northern red-legged frogs (*Rana aurora*) across all sites at the Humboldt Bay National Wildlife Refuge was 9.0% (37 out of 407). This is above the expected background rate of 2-3% (Stocum 2000). An abnormality rate of 5% or higher is suggested as a level that warrants continued National Wildlife Refuge sampling (Eaton-Poole et al. 2003). Site-specific abnormality rates were 9% (14/144) for Pond 1 and 10% (22/238) for Pond 5. Many of the malformations seen were probably caused by factors other than parasites, such as failed predation attempts or possible contamination impacts (Rohr et al. 2003). Notable abnormalities included ten individuals with lower mandibles reduced (micrognathia) (Figure 2), an abnormality that has not been associated with parasitic infections (Johnston et al. 1999).

Pond 1 was the only site surveyed for all three years of study on the Humboldt Bay NWR. In 2002, 71 *R. aurora* post-metamorphic frogs were surveyed on June 13, 2002 without detection of abnormal individuals. In 2003, Pond 1 was surveyed four times from June 13 until July 25 of 2003, and had 23 abnormal frogs out of 181 total frogs for a 13% prevalence (Appendix E). That rate dropped the following year to 10% (14 abnormal frogs out of 144 total frogs) at Pond 1. Pond 5 was surveyed in 2003 and 2004, with abnormal prevalence of 6% and 9%, respectively.

Parasites identified by Dr. Dan Sutherland fall into two categories, intestinal & kidney helminths, and an assemblage of rectal protozoans. Of the three helminth parasites identified to the class Trematoda (*Echinostoma*, *Fibricola* and *Megalodiscus*), none of these are associated with malformations in amphibians. There were no *Ribeiroia* found in any of the 18 Pacific treefrogs (*Hyla regilla*) or the 10 *R. aurora* submitted to Dr. Sutherland (Table 2). Regarding the protozoan assemblage of four species (*Opalina*, *Nyctotherus*, *Tritrichomonas* and a *Hexamita*-like flagellate) found in the rectum of the *H. regilla* and *R. aurora*, there has been no link of these protozoans causing malformations in amphibians and they may act as commensals (Dr. Sutherland, pers comm.).

The presence of the primary intermediate host of *Ribeiroia*, the planorbid snail *Helisoma* spp. at Pond 5, would allow the malformation-causing trematode to exist on this refuge. Surveys should be conducted for the first intermediate molluscan host to better elucidate the potential presence of the *Ribeiroia* and its association with *Helisoma*. The absence of the *Ribeiroia* in the 10 *R. aurora* and 18 *H. regilla* may be due to the small sample size, and not necessarily to an absence of the parasite in the population. To have a 95% confidence level of detection of a parasite or pathogen in a population at a 10% prevalence, 29 specimens would need to be sampled (DiGiacomo and Koepsell, 1986). The timing of the sample collection for voucher specimens in relation to when the *Ribeiroia* parasite may be present in a metamorphosing population of anurans could be an important consideration in the detection of the parasite. According to recent work by Schotthoefer et al. (2003) with *Rana pipiens* tadpoles infected with *Ribeiroia ondatrae*, the timing of the infection in relation to the developmental stage of the tadpoles can vastly change the number of infected individuals in the population. It may be easier to

survey for the snail host *Helisoma* and inspect for the cercariae of *Ribeiroia* to locate the infective stage of the trematode without impacting the anuran population.

These monitoring efforts detect certain external types of deformities, but exclude internal, non-skeletal, and physiological deformities not detectable with an external examination. Several studies have drawn conclusions about internal malformations from exposure to chemicals, both structurally and physiologically. In a laboratory study with African clawed frogs (*Xenopus laevis*) and in field studies of northern leopard frogs (*R. pipiens*) exposed to the common herbicide atrazine, animals experienced malformations of the larynx and the production of hermaphrodites with impaired sexual development (Hayes et al. 2002). Sexual development was also impaired by complete feminization or intersex condition in both northern leopard frogs (*R. pipiens*) and wood frogs (*R. sylvatica*) exposed to estrogenic and antiestrogenic compounds (MacKenzie et al. 2003). There are additional examples (Tavera-Mendoza et al. 2002) of these forms of internal deformities that are caused by endocrine-disrupting chemicals (EDCs). Limb malformations and altered sex hormone production was seen in bullfrogs (*R. catesbeiana*) from a survey in central and southern New Hampshire (Sower et al. 2000). These researchers concluded that exposure to EDCs in early life history of this amphibian manifested in both limb deformities and an alternation of brain gonadotropin-releasing hormone and androgen levels. These internal deformities would go unnoticed by field biologists conducting external examinations, yet could have an extreme impact on the population structure of these frogs. It may be important to find other biomarkers such as altered hormone levels to assess the impacts of chemicals on amphibians in the National Wildlife Refuge system.

Hopkins et al. (2000) found the presence of axial malformations in the tadpoles of bullfrogs (*R. catesbeiana*) was higher from sites polluted with ash-coal waste than from reference sites. This type of malformation in an earlier life-stage of the tadpoles may prevent successful metamorphosis and therefore go undetected in surveys of recent metamorphic individuals. However, the focus of these investigations has been to target recent metamorphs to better elucidate the prevalence of limb-deformities in amphibians.

Synergism between the trematode parasites, *Ribeiroia* and *Telorchis*, and exposure to the herbicide atrazine and the insecticide malathion increased malformations in wood frogs (*R. sylvatica*) (Kiesecker 2002). In a complex experiment that subjected tadpoles to different treatments of pesticide exposure, as well as challenge and protection from trematode cercariae, Kiesecker was able to demonstrate an increase in parasite infected individuals subjected to pesticides. Pesticide exposed tadpoles also had lower eosinophil counts that indicated a possible immunocompetency mechanism to allow for establishment of the trematode parasites. This form of experiment could be considered for refuges that have been identified with high levels of parasitism by malformation-causing trematodes. By using field enclosures that exclude trematode cercariae, one could determine if malformations in amphibians are manifesting only in animals exposed to trematodes or also in those that are protected from the parasites. If individuals from the parasite excluding field enclosures develop malformations, then you may be able to suspect that there is either a direct chemical pathway or possibly UV radiation impact on the amphibians.

There continues to be an over-emphasis in the current literature on the role that the trematode parasite *Ribeiroia* plays in the causation of abnormal amphibians reported across the United States (Ankley et al. 2004, Johnson et al. 2004). In a study from Minnesota that used several locations where malformed frogs were known to exist, it was noted that the highest infestations of *Ribeiroia* occurred at “hotspots”, but conversely there were other “hotspots” of malformed frogs with a complete absence of the *Ribeiroia* parasite (Lannoo et al. 2003). There is also an increasing breadth of knowledge on the impacts of chemical contaminants affecting wildlife at a sub-lethal level (Colborn and Thayer 2000, Jobling and Tyler 2003) and the large amounts of commercially applied herbicides that have the potential to disrupt the endocrine system of wildlife (Short and Colborn 1999). The impact of chemical contamination at sub-lethal levels across several life stages of amphibians could be contributing to malformations (Bogi et al. 2003, Kadokami et al. 2004). A possible source of environmental contaminants that could have an influence would be the waste from cattle treated with hormone therapy (Orlando et al. 2004). Water quality test should be considered to better determine the causative agents of the abnormalities where they are found in the absence of *Ribeiroia*.

Recommendations

Continued effort at the Humboldt Bay National Wildlife Refuge and other locations may be warranted based on multiple criteria. First, it is important to establish some long-term study sites that have demonstrated some level of malformations and to develop an associated population estimate for detecting trends between malformation and population levels (Burkhart et al. 2000). Second, techniques for surveying amphibians with malformations could be refined to better estimate the number of malformations being produced at a site. The use of pitfall arrays around pond systems may be a better approach of detection in that deformed amphibians emerging from the pond may be the first animals that predation occurs upon within the population. Deformed animals that are captured during a passive sampling effort such as pitfalls may help increase the level of malformation detection. Third, we recommend additional disease monitoring to detect the occurrences of emerging diseases (Green 2002). Research at the USGS National Wildlife Health Center determined that the most common cause for the reported amphibian die-offs were from infection with an iridovirus, followed by infection with the pathogenic amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). Monitoring of designated populations for both increase in malformation rates and occurrence of epizootic outbreaks would further our understanding of amphibian population fluctuations due to these two phenomena. A fourth reason to continue monitoring at designated refuges is to evaluate the importance of amphibian population health to that of human population health. Cohen (2001) contends that there are three aspects to the importance of amphibians to humans: importance of frogs to human society; medical implications of frog studies; and the comparison of frog and human disease factors. His last point is the most practical contribution that a monitoring program on National Wildlife Refuges system for determining landscape epidemiology of frog malformations can make.

If there is continued research that brings in multiple disciplinary approaches, it could serve as a model for epidemics that may occur in other populations. Most of the research on amphibian malformations calls for an integration of the sciences of developmental biology, disease ecology, aquatic toxicology, hydrogeochemical morphology, and field biology. Agencies, academia, private and non-profit organizations should embrace this form of collaborative science.

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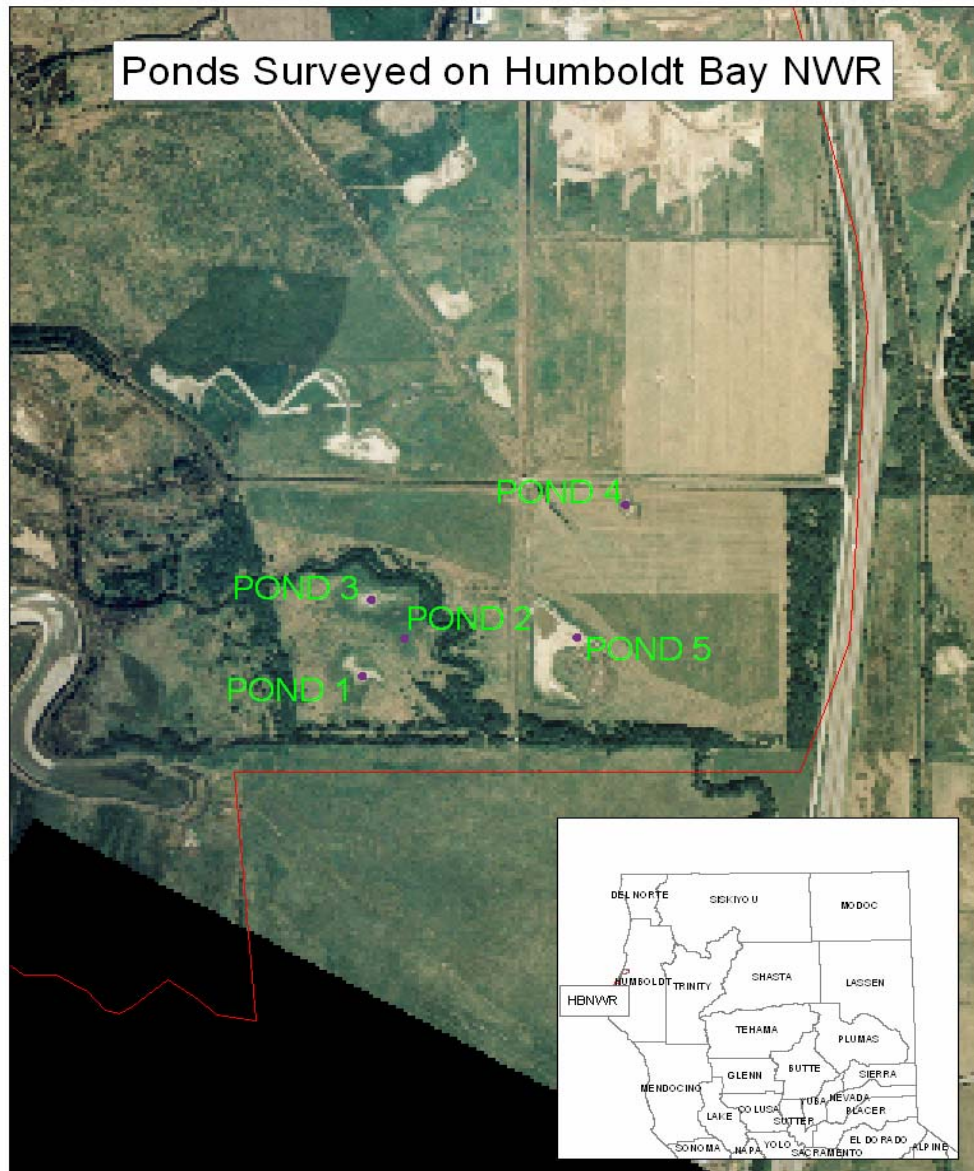
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Appendices

Appendix A. Enlargement of the 5 Ponds on Humboldt Bay National Wildlife Refuge that were investigated for abnormal northern red-legged frogs (*Rana aurora*) in 2003. Pond 1 was also surveyed in 2002. Ponds 1 and 5 were resurveyed in 2004.



Appendix B. Identification of aquatic Gastropoda from Humboldt Bay NWR

Without operculum, and mantle cavity a lung

Shell with a spine, even if reduced or depressed

Shell with raised spire

Aperture on right side of shell

Shell without microsculpture, whorls often shouldered length generally < 15mm.

Phylum: Mollusca

Class: Gastropoda

Subclass: Pulmonata

Order: Lymnophila

Family: LYMNAEIDAE

Genera: *Fossaria*

Whorls increasing in size gradually, aperture width about $\frac{1}{2}$ or < of shell width

Shell without lamellae or teeth

Maximum frontal height (including aperture) more than $\frac{1}{2}$ shell diameter, aperture height usually greater than whorl height, specimens typically < 6mm in diameter, about 20 species widely distributed

Phylum: Mollusca

Class: Gastropoda

Subclass: Pulmonata

Order: Lymnophila

Family: PLANORBIDAE

Genera: *Helisoma*

Appendix C. Schnabel method population estimates for Ponds 1 & 5 from the mark-recapture surveys of northern red-legged frogs (*Rana aurora*) on Humboldt Bay NWR, CA, 2004.

Schnabel Method Population Estimates for Pond 1						
Date	Total Captures (C)	Recaptures (R)	New	# of frogs marked at large (M)	CM ²	RM
18Jun04	93	0	93	0	0	0
2Jul04	56	5	51	93	484344	465
16Jul04	1	1	0	144	20736	144
					505080	609
					Est. N =	829

Schnabel Method Population Estimates for Pond 5						
Date	Total Captures (C)	Recaptures (R)	New	# of frogs marked at large (M)	CM ²	RM
25Jun04	55	0	55	0	0	0
9Jul04	64	2	62	55	193600	110
23Jul04	42	1	41	117	574938	117
30Jul04	60	10	50	158	1497840	1580
6Aug04	20	9	11	208	865280	1872
					313658	3679
					Est. N =	851

Appendix D. Locations of sampling sites at Humboldt Bay NWR and the number of abnormal northern red-legged frogs (*Rana aurora*) vouchered compared to the number collected over the survey season in 2004. Table format follows request of the National Amphibian Malformation Surveys coordinator.

Refuge	Sample Date	Site Name	Common Name	Genus Species	Collected	Abnormal	%Abnormal	Malformed
Humboldt Bay NWR	18JUN04	Pond 1	northern red-legged frog	Rana aurora	144	14	10%	NA*
	2JUL04							
	16JUL04							
Humboldt Bay NWR	11JUN04	Pond 5	northern red-legged frog	Rana aurora	238	22	9%	NA
	25JUN04							
	9JUL04							
	23JUL04							
	30JUL04							
6AUG04								
Humboldt Bay NWR	16JUL04	Site 6	northern red-legged frog	Rana aurora	25	1	4%	NA
TOTALS					407	37	9%	NA

NA* confirmed diagnosis as to what the cause of the abnormality in the frogs has not been provided at the time of this report.

Appendix E. Yearly surveys of Pond 1 and Pond 5 at Humboldt Bay NWR and the number of abnormal northern red-legged frogs (*Rana aurora*) vouchered compared to the number collected over the survey seasons from 2002 to 2004. Table format follows request of the National Amphibian Malformation Surveys coordinator.

Refuge	Sample Date	Site Name	Common Name	Genus Species	Collected	Abnormal	%Abnormal	Malformed
Humboldt Bay NWR	13JUN02	Pond D1 ¹	northern red-legged frog	Rana aurora	71	0	0	NA ²
Humboldt Bay NWR	13JUN03	Pond 1	northern red-legged frog	Rana aurora	181	23	13%	NA
	27JUN03							
Humboldt Bay NWR	11JUL03	Pond 1	northern red-legged frog	Rana aurora	144	14	10%	NA
	25JUL03							
Humboldt Bay NWR	18JUN04	Pond 1	northern red-legged frog	Rana aurora	176	10	6%	NA
	2JUL04							
Humboldt Bay NWR	16JUL04	Pond 5	northern red-legged frog	Rana aurora	238	22	9%	NA
	20JUN03							
Humboldt Bay NWR	3JUL03	Pond 5	northern red-legged frog	Rana aurora	176	10	6%	NA
	18JUL03							
Humboldt Bay NWR	11JUN04	Pond 5	northern red-legged frog	Rana aurora	238	22	9%	NA
	25JUN04							
Humboldt Bay NWR	9JUL04	Pond 5	northern red-legged frog	Rana aurora	238	22	9%	NA
	23JUL04							
	30JUL04							
	6AUG04							

¹Pond D1, surveyed in 2002, is the same pond as Pond 1 for the 2003 and 2004 surveys.

²NA confirmed diagnosis as to what the cause of the abnormality in the frogs has not been provided at the time of this report.