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Assessment of rates of deformity in wild frog populations using *in situ* cages: a case study of Leopard Frogs (*Rana pipiens*) in Ontario, Canada

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High rates of deformity in wild amphibian populations from north-eastern North America have been increasingly reported since 1995. In the St Lawrence River basin (Canada) elevated frequencies of limb and eye deformities in mudpuppies (*Necturus maculosus*) and leopard frogs (*Rana pipiens*) were recorded in the early 1990s. A caging study was conducted during 1998 to verify the rates recorded in leopard frogs and pursue the potential causes of deformities seen in juveniles and adults. Week-old leopard frog tadpoles were collected from a reference wetland and maintained through to metamorphosis in cages in previously identified high risk wetlands. Deformity frequencies were measured and compared with frequencies measured in wild populations of leopard frogs inhabiting the same wetlands. The results of caging studies and sampling of wild populations were also compared with corresponding data collected from a reference wetland. No deformities were observed in caged or wild reference animals. Very low deformity frequencies (up to 2.2%) were observed in frogs caged in high risk wetlands, but greater frequencies (3.4–10%) were observed in wild young-of-the-year frogs captured at the same sites. The types of deformities were similar among groups; they included fused, missing or extra digits and disproportionate hindlimb length or eye pupil size. In addition, mortality rates were elevated in two cages in high risk wetlands. In general, the caging procedure was effective in establishing the potential for production of deformities in the waters of a given wetland, but tended to underestimate the rates calculated for samples of wild populations. The ramifications of the first-year findings for similar assessments of amphibian deformity rates and establishment of cause–effect linkages are discussed.

Keywords: amphibian, wetlands, deformity, enclosure, leopard frog

Introduction

Incidences of deformity in wild frogs have been increasingly reported in North America since 1995 (Schmidt 1997). Documented cases of limb malformation include missing (ectromelia, ectrodactyly) (Ouellet *et al*. 1997) or extra (polymelia, polydactyly) (Johnson *et al*. 1999, Sessions *et al*. 1999) limbs or digits, and abnormal growth at joints (anteversions) (Ouellet *et al*. 1997). Documented eye malformation in wild frogs include lack of one eye (anophthalmy) (Northern Prairie Wildlife Research Centre [NPWRC] 1997, Johnson *et al*. 1999), or mismatched pupil or retina sizes (NPWRC 1997). Mouth deformities have been characterized as misshapen jaws (mandibular hypoplasia) (NPWRC 1997, Johnson *et al*. 1999). Where limb deformities are sufficient to hinder movement on land or in water, there is good reason to expect the individual concerned would be less fit to evade predators. High mortality rates have been associated with trematode-induced

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supernumerary hind limbs in wild frog populations (Sessions and Ruth 1990, Johnson *et al*. 1999). The lack of a functional eye might be expected to convey a similar predation risk. Hindrance of jaw function by a malformation may in turn reduce prey capture success by the affected individual. We expect that missing limbs or eyes, extra limbs or gaping jaws could be potentially life-threatening types of deformity and classify them as 'severe' malformations. Lesser deformities may also affect survival or reproduction, but the threshold below which an individual is able to compensate for loss of mobility, vision or jaw movement is unknown. Severe deformities could be associated with reductions in population recruitment if they were present at an as yet unquantified high frequency.

Even though observations of deformities in wild amphibians may be found in the published literature dating back a few centuries (Van Valen 1974), their prevalence at some sites in numbers sufficient to raise public alarm has been recent. Consequently, the knowledge base necessary to link causes and mechanisms of action with deformities, and subsequently deformities with population-level effects is weak. Since the identification of deformity 'hot' zones in 1995, several factors have conspired to impede progress in establishing a continuum of events that lead from causal agents to population- and community-level consequences. First, deformities in wild frogs and toads have been most obvious in juveniles or adults, even though evidence suggests that the deformities expressed were likely conceived at specific embryo-larval stages (Ankley *et al*. 1998, Harris *et al*. 2000). Unfortunately, wild tadpole populations are notoriously difficult to sample without detrimentally influencing recruitment success or destroying habitat (Wassersug 1997), and it has been difficult to confirm the laboratory findings of deformities in tadpoles with samples from wetlands of concern. Second, adult and larval frogs and toads often occupy structurally complex habitats which are difficult to sample in an unbiased fashion. That then brings into question how representative the deformity frequency in a captured sample of adults is for the true wild population, particularly given that the presence of a limb or eye deformity could influence capture rate. Third, larval development is generally highly plastic in amphibians (Burggren and Just 1992, Tejedo and Reques 1994) with many physiological feedback mechanisms ultimately determining recruitment in a manner which scientists have had little success in predicting from laboratory studies. That has made it more difficult to establish how laboratory-derived deformities may relate to the expression and frequency of a similar deformity in wild tadpoles.

In-situ caging studies may be instrumental in ultimately establishing causal links between environmental or genetic factors and amphibian deformities. Although there is no published reference to their use for addressing this recently recognized problem for amphibians, cages have been used to investigate other aspects of amphibian ecology (Kupferberg *et al*. 1994, Tejedo and Reques 1994, Gascon 1995) and toxicology (Cooke 1981, Clark and Hall 1985, Freda and McDonald 1993, Materna *et al*. 1995, Harris *et al*. 1998, Rowe *et al*. 1998). In particular, several groups have used cages in ponds or mesocosms to test for effects of relevant UV-B exposure on amphibian early development at high altitudes (Blaustein *et al*. 1995, Ovaska *et al*. 1997, Corn 1998, Lizana and Pedraza 1998). Caging studies can incorporate some of the environmental factors that may mediate embryo-larval responses to anthropogenic stressors (e.g. temperature, dissolved oxygen, water depth), while ensuring that there will be sufficient sample size to test hypotheses. Cages also allow one to control some variables, such as predation, parasitism by trematodes, nutrition and exposure route,

and eliminate some uncertainty associated with sampling an open, mobile wild population. Ideally, caging studies would not be employed in isolation, but would instead form part of a larger investigation including field and mechanistic laboratory components. This paper discusses the feasibility of using caging studies as one quantitative methodology for the investigation of deformities in wild frog populations. It describes an application of cages in the assessment of reported high frequencies of deformities in leopard frog populations near Cornwall, Ontario, Canada. The data presented were collected during the first year of a 2-year study.

Case study

Reaches of the St Lawrence River immediately adjacent to Cornwall, Ontario and the Akwesasne Mohawk Territory (44°58'N, 74°38'W) have been designated an 'Area of Concern' (AOC) by Environment Canada because high concentrations of persistent contaminants were identified in biota (including the salamander, *Necturus maculosus*) (A. Gendron, pers. comm.), river water and sediments. Local historical or continuing contaminant inputs to the area include discharge of effluent to the river from a pulp and paper mill, leaching of polychlorinated biphenyls (PCBs) to the river from an automotive parts plant, and emissions of fluoride and polycyclic aromatic hydrocarbons (PAHs) to the atmosphere from an aluminium smelter.

In 1997, biologists with the Raisin Region Conservation Authority found elevated rates of deformity in juvenile and adult northern leopard frogs (*Rana pipiens*) and green frogs (*Rana clamitans*) in two of four wetlands surveyed around Cornwall. Frequencies ranged from 2.7 to 20.6% (B. Hickey, pers. comm.). Acknowledging that sample sizes were small ($n = 34$–58, with one to seven individuals deformed), frequencies were nonetheless sufficient to warrant further investigation.

In 1998, a 2-year assessment of deformity frequencies in leopard frogs began in several local wetlands, including those that had already been surveyed by the Conservation Authority. In the following text, sites that were considered to be at low risk of producing elevated levels of abnormalities in frogs are referred to as reference sites (abbreviated REF). This categorization was based on prevailing wind patterns, an upstream orientation from local contaminant sources (described above), and the relative isolation of the site from human activities (including agriculture). Sites that were considered to be at high risk of producing elevated levels of abnormalities in frogs are referred to as deformity stressor sites (abbreviated DEF). This categorization was based on downstream location with respect to contaminant sources, and the preliminary findings of the Conservation Authority.

Our objectives were (1) to verify the observed rates of deformity in wild frogs using larger samples of the originally surveyed populations, (2) to establish how widespread the apparently elevated rates were in the St Lawrence River 'AOC', and (3) to determine whether a water-borne vector could be responsible for deformities. Cages and field surveys were used in the pursuit of these three objectives.

Materials and methods

Cage design

Cages constructed to house tadpoles or larvae must be designed to accommodate the changing physiological needs of amphibians during development (table 1). In studies extending to metamorphic climax, tadpoles require access to the air–water interface during the transition from gill respiration to lung respiration (Cooke 1981, Burgrren and Just 1992). Based on two studies (Tejedo and Reques 1994,

Table 1. Comparison of cage designs used to hold amphibian early life stages in aquatic environments. NI = no information.

Dimensions[a] (cm)	Material	Mesh Size (μm)	Species	Density (number per litre)	Surface Access	Other Features	Reference
For use with embryos and hatchlings							
9.5 × 10 (c)	Plastic + fibreglass	505	*Rana sylvatica, Bufo americanus, Ambystoma maculatum*	40 to 145	No	Enclosed in 22-L perforated buckets	Clark and Hall 1985
NI × 38 (p)	Nylon mesh	1500	*R. sylvatica, B. americanus, A. maculatum*	~265 to 885	No		Freda and McDonald 1993
NI × 8.9 (c)	Polystyrene	NI	*B. boreas*	~402	No	UV covers (Saran wrap, polystyrene or mylar)	Corn 1998
For use with embryo-larvae (some up to metamorphosis)							
30 × 30 × 30 (b)	NI	NI	*R. temporaria*	1.5	Yes		Cooke 1981
18 × 30 (c)	White plastic	1000	*Hyla regilla*	0.4	No	Anchored	Kupferberg *et al.* 1994
50 × 37 × 28 (b)	Plastic	~1000	*B. calamita*	0.6	No	Predator removal necessary	Tejedo and Reques 1994
30 × 25 × 25 (b)	Nylon mesh	2000	Amazonian sp.[b]	0.5 to 1.1	NI		Gascon 1995
244 × 61 × 61 (b)	Wood + mesh	NI	*R. blairi, R. sphenocephala*	0.1	No	In lake littoral enclosures	Materna *et al.* 1995
20 × 15 (c)	Nytex nylon	500	*R. clamitans, R. pipiens*	3.0	Yes	Inner basket for embryos; anchored	Harris and Bogart 1997
42 × 20 × 20 (b)	Plastic + Lumite or Nytex mesh + foam	NI	*H. regilla*	0.7 to 1.8	Yes	UV covers (mylar)	Hansen 1998
27 × 27 × 14 (b)	White plastic + polystyrene foam	NI	*B. calamita, B. bufo*	14.7	Yes	UV covers (Llumar, PVC)	Lizana and Pedraza 1998
75 × 75 (c)	Wood + fibreglass	2000	*R. catesbeiana*	0.15	Yes		Rowe *et al.* 1998
60 × 35 (c)	Nytex nylon + wood	500 or 750	*R. pipiens*	0.9	Yes	Outer muskrat barrier	This study

[a]Length × width × height for boxes (b); height × diameter for cylinders (c) or pouches (p).
[b]*Osteocephalus taurinus, Epipedobates femoralis, Phyllomedusa tomopterna, H. geographica.*

Harris and Bogart 1997) a mesh size of 1000 μm or more may be insufficient to preclude some common predators on young tadpoles, such as dragonfly larvae. Also, we suggest that cylinders may be superior to boxes in optimizing tadpole swimming energetics, but could not find reference to relevant studies. In several studies where individuals were contained until metamorphosis, densities of up to 1.5 individuals per litre did not appear to compromise survival (Cooke 1981) or growth (Flores-Nava and Vera-Mu oz 1999).

In this study, a cage design previously used for frog embryos and young tadpoles (up to 2 weeks old) (Harris and Bogart 1997) was modified to accommodate development up to (but not including) metamorphic climax (figure 1). A cage cylinder (60 cm depth, 35 cm diameter) of either 500 or 750 μm white Nytex nylon (Tetko Inc., New York) was created by stitching together the ends of a 116 × 60 cm rectangle and adding 40 cm diameter circles as a bottom and lid (the latter attached by 12 mm Velcro® strips). Unlike fibreglass screening commonly used as mesh (table 1), Nytex contains enough structural

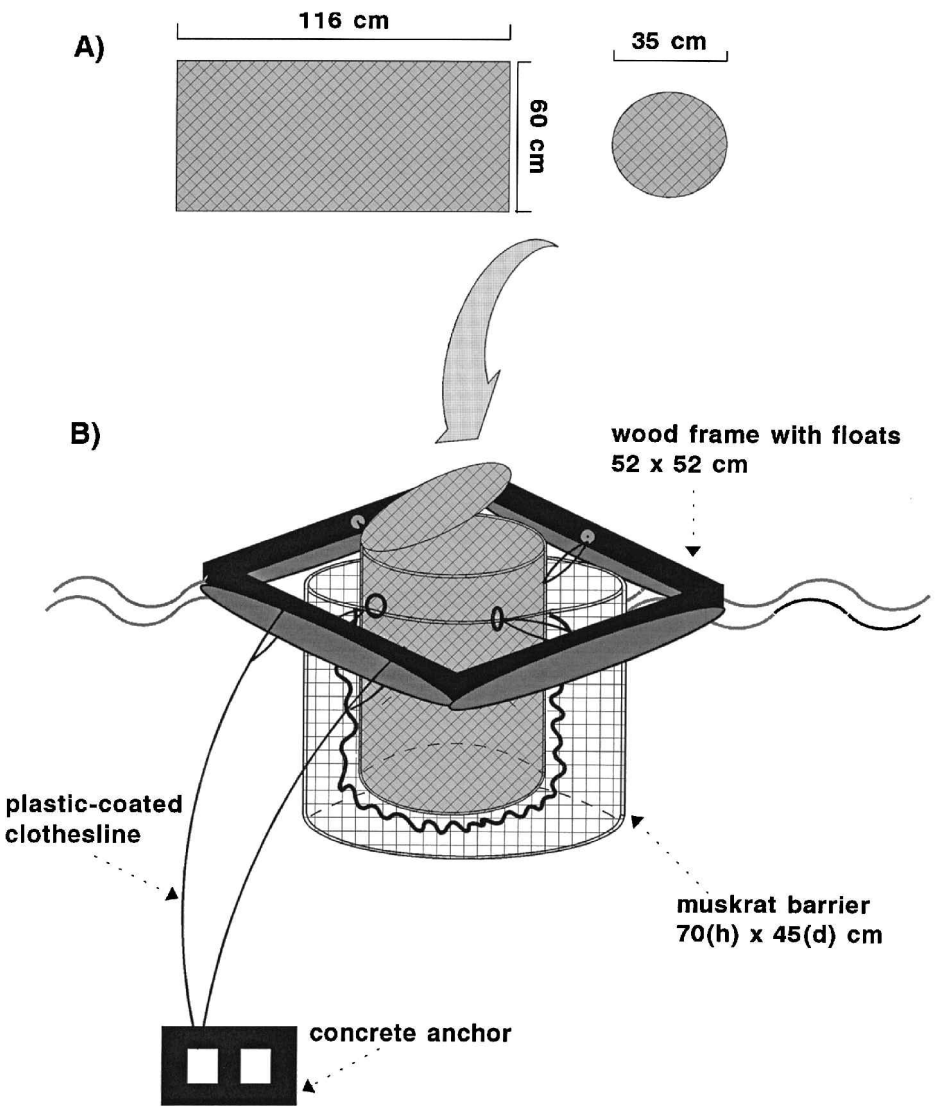

Figure 1. Cage dimensions and construction details. (A) Dimensions of the Nytex cylinder; (B) cage placement within the muskrat barrier (with cut-away to reveal cylinder inside), also showing flotation and anchor systems (not shown: muskrat barrier was attached to the wood frame with cable ties).

integrity to maintain its shape and provide an open, consistent volume of water to swimming tadpoles. Cage cylinders were attached by four plastic rings and medium gauge fishing line to an untreated pine $52 \times 52 \times 5$ cm^3 frame outfitted with foam floats (PoolNoodle®). Approximately 7–10 cm of the cylinder extended above the water surface, to ensure tadpoles had access to the air–water interface. The floating cage was anchored in a wetland by looping plastic-coated clothesline wire through an eight pound concrete cinder block and attaching the two ends to the pine frame with stainless steel clips (figure 1). The length of the anchor line was equal to the water depth plus one and one half times the cage depth (90 cm), which allowed investigators to lift the cage almost fully out of the water for surveillance and cleaning.

One month into the 1998 caging study, the cage cylinders showed signs of interference, most likely by muskrats (*Ondatra zibethica*, an aquatic rodent). To protect the Nytex from muskrat teeth and claws, a second cage was constructed around the first (figure 1). The barrier was composed of green plastic garden fencing, which was sturdy and bite-proof yet did not restrict water movement significantly (mesh size 3×3 cm^2). The 70 cm wide plastic fencing was attached to the inside of the pine frame with plastic cable ties and a barrier bottom was then attached to the sides, also with cable ties.

Surveillance and maintenance of caged leopard frog tadpoles

On 13 May 1998, wild leopard frog tadpoles were collected by dip-netting from ephemeral pools at a wetland, REF2, just upstream of Cornwall. REF2 had been surveyed the previous year and the resident leopard frog population had shown no incidence of deformities (B. Hickey, pers. comm.). The tadpoles were estimated to be approximately 1 week old at the time of collection and were of a mixed genetic stock. Tadpoles were transported in an ice-filled cooler to wetlands where cages had been installed 7 days previously. Since the air temperature was high at the time of transport (~30°C), the ice only brought the water temperature down to slightly below room temperature (~19°C), which was not expected to affect rates of deformity (Cooke 1981). Fifty tadpoles were randomly selected and placed in each of four cages at one reference site (REF1) and two potentially impacted sites (DEF1, DEF2). Initial densities were, therefore, 0.9 tadpoles per litre in each cage. Transport of tadpoles to all cage sites was completed in ~5 h.

REF1 was located 31 km northwest of the St Lawrence River at Cornwall. It was a slow-moving creek with extensive associated marshlands created by beaver (*Castor canadensis*) dams upstream. Cages were placed a few km from the headwaters, with no substantial agricultural activity upstream. DEF1 was a natural marsh and wildlife sanctuary that had been modified by dyking to create more wildlife habitat. It received water diverted from the St Lawrence River and was 18 km downstream of Cornwall. DEF2 was another slow-moving creek with a four-lane highway and agricultural activity upstream, and a marina less than one km downstream. The cages were placed less than one km from the mouth of the creek, where it emptied into the St Lawrence River. The creek also received drainage from several stormwater runways within Cornwall and was 0.3 km downstream from the city limits.

Cages were monitored from a canoe. Tadpoles were fed boiled lettuce *ad-libitum* every second day for 2.5 weeks. After 2.5 weeks, tadpoles were fed 0.4 g boiled lettuce per tadpole per day, and the ration was increased upon demand until it peaked just before metamorphic climax at 1.0 g per tadpole per day. Every second day, tadpoles were counted by lifting the cage two-thirds out of the water and faecal material plus uneaten lettuce were removed with a kitchen strainer. Periodically, all tadpoles were removed and held temporarily in a shallow container of water while the cages were thoroughly cleaned and the mesh defouled. Water temperature was recorded upon each visit using a maximum/minimum thermometer (Fisher Scientific, Mississauga, ON) that was attached to one pine frame at each site.

Tadpoles were removed from the cages once a forelimb emerged from the branchial chamber (developmental stage 42, Gosner 1960). Once this began (on 25 June) cages were visited once each day to ensure that no individuals drowned. Tadpoles at metamorphic climax were transferred to a laboratory in Cornwall, where they were held in Rubbermaid® containers ($17 \times 30 \times 8.5$ cm^3) with many small holes poked in the lids. Each container was placed on a slant of approximately 20 degrees, and sufficient site water was added to submerge the individual only in the lower one-third of the pan. The remainder of the pan bottom was covered with a moist unbleached paper towel. Young-of-the-year were held until their tails were fully resorbed. Each individual was checked for deformities before being released near the caging site. Deformed individuals and a small sample of normal individuals were kept and sacrificed for histological examination (data not shown).

Field surveys of leopard frog young-of-the-year

From 6 to 23 July, the three caging sites and two additional wetlands (DEF3, REF2) in the 'AOC' were surveyed by canoe and on foot. DEF3 was a deep flooded gravel quarry stocked with rainbow trout (*Oncorhynchus mykiss*) and with limited associated areas of marsh. It was located at the northwest corner of Cornwall (1.5 km outside city limits), and had no connection with the St Lawrence River or obvious influences from roads, agriculture or industry. REF2 was a collection of small ephemeral pools and one permanent shallow pond from which tadpoles were collected for cages. It also had no surface water connection with the St Lawrence River and was upstream of Cornwall proper, but within the city limits.

Wild leopard frogs were captured by netting and any gross deformities were recorded. No estimates of catch-per-unit-effort were made, because large variability in capture efficiency among collectors and in habitat complexity among sites weakened inter-site comparisons. However, the right, fore, outermost digit of each captured individual was clipped to identify recaptures. Each site was surveyed two to four times during the two-and-a-half week period.

Statistical analyses

The frequency of deformities in caged and wild young-of-the-year leopard frogs from potentially impacted (DEF) sites was compared to a background rate of 1% using the binomial goodness-of-fit test modified for a Poisson distribution (Zar 1984). The 1% frequency was based on published reports of background rates of deformity in wild amphibian populations (Dubois 1979, Borkin and Pikulik 1986, Meyer-Rochow and Asashima 1988, Fort *et al*. 1999). Of particular significance was the deformity frequency of 0.5–1% reported for wild leopard frog populations in reference areas of Minnesota (Fort *et al*. 1999). Because predatory scars could be misdiagnosed as deformities, cages suffering tadpole losses from muskrats were dropped from the analysis.

Results

Two cages at REF1 and two at DEF1 had to be dropped from statistical analyses because of losses due to muskrat interference. There was high survival of tadpoles through to metamorphic climax in the two remaining cages at REF1 (96%, table 2). Survivorship was also high in most cages at other sites. Only one cage each from DEF1 and DEF2 had notable mortality, reducing survivorship to 64% (DEF1, C-3) or 82% (DEF2, M-3).

A small number of deformities were observed in caged individuals upon

Table 2. Survivorship and deformity frequency for caged leopard frog young-of-the-year.

		Survivorship		Deformity frequency		
Site	Cage	*N*	% of Initial	*N*	% of Transformed	Deformity characterization
DEF1	C-1[a]	22	44	0	0.0	
	C-2	47	94	1	2.1	Pupil of one eye smaller than the other
	C-3	32	64	0	0.0	
	C-4[a]	13	26	0	0.0	
	all	114	57	1	0.9	
	all[b]	79	79	1	1.3	
DEF2	M-1	48	96	2	4.2	(1) Small black growth under one eye that also caused the mouth to be misshapen (2) Only 4 of 5 digits on one hindlimb
	M-2	49	98	0	0.0	
	M-3	41	82	2	4.9	(1) Only 4 of 5 digits on one hindlimb with 2 of the 4 fused (2) 2 of 5 digits fused on hindlimb
	M-4	48	96	0	0.0	
	all	186	93	4	2.2	
REF1	H-1	47	94	0	0.0	
	H-2	49	98	0	0.0	
	H-3[a]	1	2	0	0.0	
	H-4[a]	11	22	0	0.0	
	all	108	54	0	0.0	
	all[b]	96	96	0	0.0	

[a]Cage was interfered with, probably by muskrats, and all or some of the individuals inside were lost.
[b]All, excluding cages that were influenced by muskrats.

completion of metamorphosis (table 2). While no reference metamorphs had gross abnormalities ($n = 108$), one individual at DEF1 and four from DEF2 had minor limb, eye or face abnormalities. The affected metamorph from DEF1 had disproportionate pupil sizes. One affected metamorph at DEF2 had a black growth on its face and a misshapen jaw, while the other three had missing or fused digits (table 2). None of the caged groups showed deformity frequencies that were significantly different from 1% ($p>0.05$). All of the deformed individuals transformed during the second half (13–23 July) of the transformation period (which started July 2 and finished July 23).

A greater number of deformities were observed in wild young-of-the-year from the caging sites and other wetlands in the area in comparison to the frequencies in caged individuals (table 3). At the two caging sites, DEF1 and DEF2, 6 and 10% of individuals showed gross abnormalities, respectively. Sampled populations from two other wetlands (DEF3 and REF2) showed slightly lower deformity rates of 3.4 to 4.1%. As in the caged individuals, wild young from REF1 showed no abnormalities. Unfortunately, habitat restrictions at REF1 (i.e. flooded marshlands) limited collections to those from a canoe and the subsequent sample size was low ($n = 16$). Aside from REF1, all sites surveyed showed deformity frequencies that were significantly greater than 1% (table 3).

Most of the deformities observed in wild individuals were not severe (as per the classification described in the Introduction) and were similar to those observed in caged metamorphs. At DEF1, one metamorph had a misshapen jaw, one had a growth on a limb, and the other two had missing or stunted digits. Most of the deformed individuals at DEF2 and DEF3 had either missing or extra digits. One metamorph at DEF3 had disproportionate pupil sizes. The three types of abnormality seen in individuals from REF2 were not typical of those seen in wild or caged frogs at other sites. One had a large lump under the skin of the abdomen. The other two showed limb abnormalities, one missing the lower half of a hindlimb, and the other showing disproportionate development of the two hindlimbs. There were no cases of completely missing limbs (ectromely), extra limbs (polymely) or missing eyes (anophthalmy).

Discussion

Abnormalities were observed in several leopard frog populations in the St Lawrence River 'AOC', whereas none were observed in a remote reference population. Cages placed in wetlands were successful in maintaining tadpoles through to metamorphic climax with no cage-associated mortality, and metamorphs from cages expressed deformities in a similar relative fashion among sites to that observed in samples of wild young-of-the-year. That is, in both caged and wild samples, the frequency of deformities increased in the order REF1 < DEF1 < DEF2. In addition, the types of deformities expressed in wild and caged individuals were similar. However, the proportion of individuals deformed at any given site, other than REF1, was consistently greater in the wild sample compared to the caged sample.

The discrepancy in deformity frequencies between caged and wild young may be indicative of the presence of many mediating factors, and it is difficult to confirm which rate of abnormality is most accurate for each leopard frog population. Assuming that the frequency in the wild sample was the best reflection

Table 3. Deformity frequency in wild leopard frog young-of-the-year (y-o-y) captured between 6 July and 23 July, 1998.

	Total captured			Deformities[a]		
Site	All	Y-o-Y	Recaptures[b]	*N*	%	Deformity characterization
DEF1	157	67	5	4	6.0**	(1) Small black growth behind a forearm (2) Misshapen lower jaw, resulting in a gaping mouth (3) Only 4 of 5 digits on one hindlimb (4) Stunted digit on one forelimb
DEF2	30	20	2	2	10.0*	(1) 6 digits on one hindlimb, with 3 fused (2) Only 4 of 5 digits on one hindlimb
DEF3	121	117	1	4	3.4*	(1) Fused digits (2) Only 4 of 5 digits on one hindlimb (3) Only 4 of 5 digits on one forelimb (4) Pupil of one eye smaller than the other
REF1	16	16	0	0	0.0	
REF2	76	74	1	3	4.1*	(1) Large persistent lump under the skin of the abdomen (2) One hindlimb severed above the knee (possibly mechanical damage) (3) One hindlimb much smaller than the other (both in length and girth)

[a]Deformities listed only for y-o-y; stars indicate deformity frequencies greater than 1% (** — $p < 0.01$, * — $p < 0.05$). Another y-o-y kept for histology, but not included in these calculations had a badly swollen hindlimb that could have been due to a break or other mechanical injury or a deformity. If the latter, the frequency for DEF1 would change to 7.5%.

[b]Number of individuals recaptured (adults + y-o-y).

of the frequency in the wild population, the most obvious explanation for reduced frequencies in caged samples is that the factor(s) responsible for malformations at each site was not predominantly water-borne. Several of the contaminants characteristic of waterways in the 'AOC' (e.g. metals, chlorinated hydrocarbons) would likely be more concentrated in sediment than in water, and wild tadpoles are in frequent contact with sediment. However, there is also the possibility that the cage environment mediated the expression of deformities by reducing tadpole encounters with other contributing stressors. For example, Cooke (1981) found that exposing tadpoles to an environmental stress (e.g. cold) increased the incidence of deformities by 5% in *Rana temporaria*. Two stressors absent within the cages in this study were predation and some forms of parasitism. Parasitic trematodes may directly induce hindlimb deformities in amphibians (Johnson *et al*. 1999, Sessions *et al*. 1999). Other variables were modified inside cages, including nutrition and UV-B exposure. The fact that the same types of deformities were expressed, with a similar pattern among sites, in caged and wild metamorphs suggests that there was some element in the water responsible for at least a portion of the abnormalities observed in leopard frog populations.

If the frequency of deformities in the wild sample was not representative of the leopard frog population as a whole, there are also reasons why rates in that sample might be artificially elevated. The number of recaptures identified during repeat

surveys at caging sites was low (less than 10% of those captured), suggesting that only a small fraction of the total leopard frog population at each site was assessed. The subset captured might have shown an inflated prevalence of deformities if the presence of such abnormal morphology hindered those individuals' ability to escape a capture net. Given that the limb and eye deformities observed were not severe, this explanation seems unlikely. Although one individual with a missing eye (anophthalmy) and one with an extra limb protruding from the throat (polymely) were previously described from DEF1 (B. Hickey, pers. comm.), there were no deformities found in 1998 that could be considered to have a substantial effect on predator (or net) evasion or prey capture abilities of the individual.

Another possibility is that the collection period was too restrictive, such that the sample was taken from a temporally distinct metamorphic group. During the cage study, individuals expressing deformities were slightly delayed developmentally, transforming in the second half of the full temporal range. This observation supports Cooke's (1981) finding that a susceptibility to malformation is indicative of generally poor fitness in tadpoles. The initiation of metamorphic events in the wild were not precisely recorded; hence, the possibility that only individuals which transformed late and were predisposed to malformation were captured cannot be dismissed. Deformity frequencies observed in adult (≥2 years old) wild leopard frogs (unpublished data) do not clarify the situation, as some rates were far less than rates in young-of-the-year at the same site while some remained approximately the same.

To summarize, an elevated (e.g. >1%) rate of deformity was found in 1998 wild frogs, but not to the extreme that had been previously recorded in smaller sample sets (20.6% at DEF1, $n = 34$, B. Hickey, pers. comm.). Also, the high deformity rates were characteristic of several ($n = 4$) leopard frog populations in the area of Cornwall, including populations where they had not been observed the previous year. Caged individuals expressed similar types of deformities as wild individuals in this study, and further work will be conducted to elucidate the true frequency of expression in those populations. Contaminant sampling of water and sediment, increased cage replication and concerted collection efforts during further, planned field surveys will all assist in delineating the potential environmental factors producing deformities in these wild frogs. We conclude that caging studies show promise as a method of establishing the relative importance of the factors potentially contributing to deformity expression in wild frogs. In regions where the reported frequency and severity of abnormalities is far greater (e.g. Minnesota, Quebec), we expect that caging studies can play a vital role in establishing links between effects observed in the field and mechanisms of action established in the laboratory, by allowing investigators to manipulate which potential deformity inducers are present in a tadpole's environment. Caging studies in more impacted environments may also help clarify the relationship between absolute frequencies of abnormality in wild and caged samples.

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