

# Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians

JAY BOWERMAN,<sup>1</sup> PIETER T. J. JOHNSON,<sup>2,4</sup> AND TRACY BOWERMAN<sup>3</sup>

<sup>1</sup>Surviver Nature Center, Box 3533, Sunriver, Oregon 97707 USA

<sup>2</sup>Ecology and Evolutionary Biology, University of Colorado, Ramaley N122, Campus Box 334, Boulder, Colorado 80309 USA

<sup>3</sup>Ecology Center, Watershed Sciences, Utah State University, Logan, Utah 84322 USA

**Abstract.** While many predators completely consume their prey, others feed only on blood or tissue without killing the prey, sometimes causing ecologically significant levels of injury. We investigated the importance of sublethal predator attacks in driving an emerging issue of conservation importance: missing-limb deformities in amphibians. We combined long-term field data and manipulative experiments to evaluate the role of sublethal predation in causing abnormalities in two regions of central Oregon, USA. Since 1988, western toads (*Bufo boreas*) in Lake Aspen have exhibited abnormalities dominated by partially missing limbs and digits at annual frequencies from <1% to 35%. On Broken Top volcano, we found comparable types and frequencies of abnormalities in Cascades frogs (*Rana cascadae*). Field sampling and observational data implicated two aquatic predators in these abnormality phenomena: introduced sticklebacks (*Gasterosteus aculeatus*) at Lake Aspen and corduliid dragonfly larvae (*Somatochlora albicincta*) at Broken Top. In experiments, these predators produced limb abnormalities identical to those observed in the respective regions. At Lake Aspen, in situ predator enclosures effectively eliminated abnormalities in toads, while comparisons among years with low and high stickleback abundance and between wetlands with and without sticklebacks reinforced the link between fish and amphibian abnormalities. Neither trematode parasite infection nor pesticide contamination could explain observed abnormalities. Our results suggest that predators are an important explanation for missing-limb abnormalities and highlight the ecological significance of sublethal predation in nature.

**Key words:** amphibian decline; amphibian deformities; amphibian malformations; *Bufo boreas*; community ecology; *Gasterosteus aculeatus*; generalized estimating equations; invasive predators; limb development; Oregon, USA; *Rana cascadae*; *Somatochlora albicincta*.

## INTRODUCTION

Predation is a central governing force in ecology and evolution, often with wide-ranging effects on prey survival, growth, reproduction, behavior, and phenotypic development. While ecological studies generally focus either on how predators directly affect prey survival or indirectly affect prey growth and behavior, predator attacks can also cause sublethal injuries (e.g., Kaliszewicz 2003). Sublethal predator attacks often occur when predators are smaller or similar in size to their prey, have a limited gape size, or have specialized mouthparts for extracting tissue or fluids (e.g., Schoener 1979, Pape-Lindstrom et al. 1997, Meyer and Byers 2005). Sublethal predation is well documented among herbivores and among blood-feeding insects (e.g., mosquitoes, deerflies, reduviid bugs). Some sublethal predators, however, can inflict severe and long-lasting injuries with costly consequences for prey fitness.

Examples include sea lampreys, vampire bats, cookie cutter sharks, and fangblennies. Mouritsen and Poulin (2003), for example, reported that fish can attack and injure up to 34% of marine cockles, resulting in between 9% and 21% loss of foot tissue per individual.

A better understanding of sublethal predation may provide insights into the etiology of emerging conservation issues. Since the 1990s, increasing reports of amphibians with skeletal abnormalities, including deformed, missing, and extra limbs, have caused concern (Souder 2000, Ankley et al. 2004). Proposed causes of amphibian limb abnormalities include chemical pollution, ultraviolet B radiation, predation, and parasite infection (see Ouellet 2000, Ankley et al. 2004; Johnson et al., *in press*). Morphological patterns may hold important clues to proximate causative factors. The trematode parasite *Ribeiroia ondatrae*, for example, can cause missing limbs, bony triangles, skin webbings, and duplicated structures (Sessions and Ruth 1990, Sessions et al. 1999, Johnson et al. 1999, 2001a), but is rarely associated with accounts involving predominantly missing limbs (Skelly et al. 2007, Reeves et al. 2008).

An important challenge in the continued study of amphibian abnormalities is to identify the proximate

Manuscript received 11 September 2008; revised 20 March 2009; accepted 16 April 2009. Corresponding Editor: S. P. Lawler.

<sup>4</sup> Corresponding author.  
E-mail: pieter.johnson@colorado.edu

causes of abnormalities in cases in which trematode infection can be ruled out (see Lannoo et al. 2003). Previous investigations have discounted predator-induced limb abnormalities on the grounds that (1) a predator would likely consume an entire frog rather than just a leg, (2) injuries ought to produce obvious scar tissue at the wound site, and (3) a frog is unlikely to survive the traumatic loss of an entire limb (Meteyer et al. 2000, Lannoo 2008). Although these assumptions may hold for large vertebrate predators and for amphibians injured after metamorphosis, they may not apply to larval amphibians injured by smaller predators such as aquatic insect larvae, leeches, small fishes, and crayfish, all of which may be more likely to attack an exposed portion of an amphibian such as a tail or a limb. Tail injury can be severe (up to 75% loss) and widespread (>30% prevalence) among larval amphibians (Feder 1983, Lawler et al. 1999, Blair and Wassersug 2000). Moreover, because larval amphibians have some capacity for regeneration, partial or complete regrowth following injury early in limb development might mask signs of trauma and create the appearance of a developmental malformation (e.g., Fry 1966). In Germany, investigators showed that leech attacks caused high prevalences of missing limbs or parts of limbs (ectromelia) in common toads (*Bufo bufo*) (Viertel and Veith 1992, Bohl et al. 1996, Bohl 1997). The role of predators in causing amphibian limb abnormalities has received little attention in North America, however.

We combined long-term monitoring data, field observations, and mechanistic experiments to evaluate the role of vertebrate and invertebrate predators in causing missing-limb abnormalities in native amphibians. We evaluated the hypothesis that sublethal predation by either stickleback fishes (*Gasterosteus aculeatus*) or dragonfly larvae (*Somatochlora albicincta*) was responsible for high frequencies (>20%) of limb abnormalities observed in metamorphosing western toads (*Bufo boreas*) and Cascades frogs (*Rana cascadae*) in central Oregon, USA. We further compared the modes of attack of each predator and the types of abnormalities induced to show that morphological clues can help identify the predator responsible. Our results help to account for missing-limb abnormalities in amphibians and have important implications for understanding the significance of sublethal predation in animal populations.

## METHODS

### *Abnormalities in western toads*

*Study sites.*—Lake Aspen (“LA”; 43°53'26" N, 121°24'40" W) is an excavated impoundment of ~2 ha with a maximum depth of 2.5 m that has served as a major toad breeding site for 30 years (J. Bowerman, *personal observation*). Nonnative three-spined sticklebacks (*G. aculeatus*) were discovered in the Deschutes River in 1980 (Oregon Department of Fish and Wildlife, *unpublished data*) and quickly achieved high population

densities in LA that persist except during rare winterkill events. Rear Driving Range pond (“RDR”; <0.1 ha, <1.5 m depth) supports sticklebacks only during unusually mild winters, whereas Lake Penhollow (“LP”; 6 ha, 7 m depth), Paulina Junction (“PJ”; <0.5 ha, <1.5 m depth), and Fairway 11 (“FW”; <0.5 ha, <1.5 m depth) ponds are isolated from other water bodies and remain stickleback-free. These ponds are excavations within 15 km of LA that intermittently support toad reproduction. Lake Penhollow did not exist prior to 1999, PJ only intermittently produces toads, and FW last supported toads in 1997.

*Field observations.*—Since the discovery of abnormal toads at Lake Aspen in 1988, we have inspected a total of 13 443 metamorphic toads representing 11 seasons of major toad emergences at this site. To avoid bias that might result from greater mobility of normal vs. abnormal toads, all sampling was conducted during emergence events and within 10 m of the shoreline. Metamorphic toads were either hand-captured or netted from the water, held in buckets until inspection, then released within 10 m of capture location. Subsequent collections within the same year were conducted >100 m from prior collections to avoid resampling the same individuals. For comparison with Lake Aspen, we inspected a minimum of 200 emerging toads from each of the other four wetlands during opportunistic visits when these ponds supported toad reproduction, using the same inspection techniques (LP, RDR, and PJ in 2001 and FW in 1997). We visually inspected metamorphs by extending the hind limbs and comparing the shape, size, and position of all limb parts. We classified deformities into four categories of increasing severity of limb truncation: missing toes, missing foot, partial leg loss (between foot and femur), and whole leg loss (some or all visible portions of femur missing).

We also sampled LA for several factors with the potential to cause abnormalities: predators, trematodes, and pollutants. We conducted coarse-level monitoring of stickleback populations using Gee’s minnow traps (Cuba Specialty Manufacturing, Fillmore, New York, USA) at two locations within Lake Aspen. Traps were deployed for 24 h intermittently between May and August of each year. We classified sticklebacks as “abundant” (>100 individuals·trap<sup>-1</sup>·d<sup>-1</sup>) or “rare” (<10 individuals·trap<sup>-1</sup>·d<sup>-1</sup>). In a subset of years, including two years following severe winterkill events, we enumerated all captured fish to calculate a catch per unit effort (CPUE) and validate our coarse-level estimates. We necropsied a subset ( $n = 5$ ) of abnormal toads to quantify parasite infection with a focus on *Ribeiroia* abundance. We also tested water for 61 different pesticides and metabolite breakdown products (e.g., herbicides and organophosphates) as part of another study (Johnson et al. 2002).

*Predator exclosures.*—To evaluate whether isolating toad larvae from aquatic predators produced differences in tail and/or limb condition, we constructed two 1-m<sup>3</sup>

enclosures ( $1 \times 2 \times 0.5$  m) made of woven nylon mesh (5-mm openings) and suspended from floating log frames. This effort was not intended as a rigorous, replicated experiment, but rather as a qualitative comparison of larval toad development inside and outside the enclosures. Each enclosure received 500 field-caught tadpoles (stage 26 [Gosner 1960]) with intact caudal fins. Twice weekly we added aquatic plants (*Elodea*) in sufficient quantity to create a density similar to the elodea beds adjacent to the enclosures (after removing any sticklebacks in the plant material). Mesh openings were large enough to allow entrance of water-borne chemicals or free-swimming trematode cercariae. After two weeks (Gosner stage 30), we compared the tail condition of 400 toad larvae within the enclosures with 400 free-swimming larvae captured within 10 m of the enclosures, replacing all individuals after inspection. Garter snakes (*Thamnophis sirtalis*) invaded the enclosures during week 6 and consumed some of the tadpoles, necessitating that the experiment be terminated. We inspected the 120 remaining larvae (Gosner stages 38–39) from the enclosures and compared their tail and hind-limb condition with those of 400 free-swimming larvae collected adjacent to the enclosures. Snake predation was unlikely to have altered the nature of our results because limb development was too early to affect escape maneuvers and caudal fin reductions representing <5% of tail area (as seen within enclosures) typically have negligible effects on either maximum speed or escape time (Van Buskirk and McCollum 2000).

*Aquarium experiment.*—We investigated interactions between sticklebacks and toad larvae in glass aquaria ( $48 \times 25 \times 30$  cm) containing 30 L of well water and a small quantity of *Elodea*. Each tank received five *B. boreas* larvae (Gosner stages 35–38) without visible abnormalities. Predation treatments received four adult sticklebacks captured from Lake Aspen and acclimated in aquaria for 2–3 d prior to the experiment. Control aquaria were treated identically but received no sticklebacks. After 24 h, we removed and inspected all tadpoles. We used fish and tadpoles only once and rotated aquaria between experimental and control treatments. We conducted eight replicates of each treatment (16 trials in total) and observed experimental and control aquaria for a total of 4 h during experiments, noting fish and tadpole behavior. A subset of tadpoles that lost limbs during experiments was raised to metamorphosis.

#### *Abnormalities in Cascades frogs*

*Study sites.*—Broken Top Ponds 1 and 2 (BT1 and BT2) are small (<0.1 ha), shallow (<1 m), high-elevation wetlands (2200 m) located in Deschutes County, Oregon ( $44^{\circ}03'04''$  N,  $121^{\circ}41'40''$  W). Water is uniformly clear over a substrate of fine sediment and airfall pumice with <1% vegetation cover. Both ponds support breeding Cascades frogs (*Rana cascadae*) and

long-toed salamanders (*Ambystoma macrodactylum*), but lack fish or snails.

*Field observations.*—In September 2003, we found a high frequency (27.5%) of *R. cascadae* larvae with limb abnormalities consisting of missing or partially missing hind limbs. We sampled BT1 and BT2 twice in 2003 and twice in 2004, recording the prevalence and composition of abnormalities as described for examining toads. Using a D-frame dipnet (0.5-m opening  $\times$  0.5-cm mesh), we performed 2-m substrate sweeps at 11 evenly spaced points around each pond's perimeter and two additional sweeps 6 m offshore. Captured organisms were identified, counted, and released. Five abnormal *R. cascadae* were necropsied to assess parasite infection.

*Predator experiment.*—We identified two predators with the potential to cause limb loss in *R. cascadae*: larval long-toed salamanders (*Ambystoma macrodactylum*) and larval dragonflies (*Somatochlora albicincta*). Naiads of *S. albicincta* consume aquatic insects, freshwater shrimp, small fishes, and tadpoles (Corbet 1999). Long-toed salamanders are known to attack other amphibians and sometimes cause limb damage (see Wildy et al. 2000, Johnson et al. 2006). We compared the effects of salamander larvae and dragonfly naiads on *R. cascadae* limb development within covered storage containers ( $65 \times 35 \times 15$  cm), each of which received 5 cm of pond water, 1 cm of mud (strained through a 0.5-cm filter to remove any potential predators), and 10 *R. cascadae* larvae (Gosner stages 35–40; 20.9 mm mean snout-vent length [SVL]). Containers were assigned randomly to one of three treatments with 21 replicates per treatment: dragonfly larvae ( $n = 5$ , 21.3 mm mean length), salamander larvae ( $n = 5$ , 32.7 mm mean SVL), or no predators. All larvae were captured and examined immediately prior to each trial. We maintained containers in sheltered locations near the ponds and rotated them among treatments and positions between trials. Containers were rinsed thoroughly between trials and neither predators nor larval *R. cascadae* were used more than once. After 48 h, we removed and inspected all *R. cascadae* larvae for abnormalities. A subset of abnormal *R. cascadae* larvae was raised through metamorphosis while remaining predators and tadpoles were released.

*Analysis.*—To compare the frequency of abnormalities among toad larvae from inside and outside the enclosures, we used a contingency table analysis that evaluated whether the status of each animal (normal vs. abnormal) was independent of its source (free-living vs. protected). For experiments involving sticklebacks in aquaria and salamander or dragonfly predators in outdoor chambers, we used generalized estimating equations (GEE). Generalized estimating equations are an extension of the generalized linear model that allow for correlations among observations from the same subject (e.g., individuals within the same container) and use an iterative approach to estimate the correlations among observations (Liang and Zeger 1986, Horton and

Lipsitz 1999). Generalized estimating equations are most often used for longitudinal or clustered data when the outcome variable is discrete or binary. Because multiple tadpoles were maintained in containers with predators, the likelihood of one tadpole experiencing an injury may be influenced by the fate of co-occurring tadpoles (i.e., if predators become satiated after feeding on one tadpole). We therefore used GEEs to explicitly allow for correlations among individual tadpoles within a single container and evaluated the effect of predator treatment on the likelihood of limb abnormality (abnormal vs. normal) or death (dead vs. alive) during the experimental trials. We used the procedure “geepack” (Halekoh et al. 2001) in the statistical package R (R Core Development Team, Vienna, Austria). We assumed an unstructured covariance matrix for our data and evaluated the significance of each treatment using ANOVA following the methods outlined in Halekoh et al. (2001).

## RESULTS

### *Abnormalities in western toads*

*Field patterns.*—Between 1988 and 2008, the prevalence of limb deformities among toad metamorphs at Lake Aspen ranged from <1% to 34.2% ( $12.9 \pm 3.9$  [mean  $\pm$  SE];  $n = 13,443$ ; Fig. 1A). We typically observed tail damage in larval *B. boreas* by Gosner stage 30 whereas limb abnormalities became evident in later-stage larvae and metamorphs. Tail damage consisted of small tears or nicks to the margin and typically affected <5% of total fin area. In most years, sticklebacks were abundant in LA ( $>100$  sticklebacks $\cdot$ trap $^{-1}\cdot$ d $^{-1}$ ). In 1993 and 2008, however, following severe winterkill events, we caught an average of 1.7 and 8.5 sticklebacks $\cdot$ trap $^{-1}\cdot$ d $^{-1}$ , respectively, compared to 176 sticklebacks $\cdot$ trap $^{-1}\cdot$ d $^{-1}$  in 1998 and 104 sticklebacks $\cdot$ trap $^{-1}\cdot$ d $^{-1}$  in 2003. Correspondingly, abnormality frequencies in 1993 and 2008 were 0.05% ( $n = 2000$ ) and 1.2% ( $n = 1016$ ), respectively, which contrasted sharply with the average abnormality frequency of 15.6% in years with typical stickleback abundances (Fig. 1A). Comparative data from surrounding water bodies in Deschutes County supported the link between limb abnormalities in toads and the occurrence of sticklebacks. In RDR, which supported an average of 90 sticklebacks $\cdot$ trap $^{-1}\cdot$ d $^{-1}$  in 2001, 12.3% of examined toads exhibited limb abnormalities, whereas <1% of toads were abnormal among wetlands without sticklebacks (Fig. 1B).

Toad abnormalities from LA and RDR ranged from missing digits to partially and completely missing limbs (Fig. 2). Missing digits and feet were most common, (65.1% of all abnormalities), followed by partially (22.2%) and completely missing limbs (7.9%; Table 1). Bilateral deformities were uncommon and were rarely symmetrical when they occurred. Among more than 1000 abnormal toads observed, we saw only two instances of polymely and no individuals with bony triangles, skin webbings, or other abnormalities charac-

teristic of trematode-related malformations (see Johnson et al. 1999, 2001a). Necropsies of five abnormal toads from LA detected *Ribeiroia* in only one individual. This observation, combined with the absence of more typical parasite-related malformations (e.g., bony triangles and skin webbings) suggests that trematode infection did not contribute significantly to the observed pattern of abnormalities (see detailed discussion in Johnson et al. 2001a). No pesticides or pesticide metabolites were detected in the water analyses (Johnson et al. 2002).

*Predator exclusions.*—Predator exclusions in LA eliminated limb and tail abnormalities in nearly all toad larvae. Among unprotected larvae from LA, 12.3% of early-stage individuals and 89% of late-stage larvae exhibited ragged caudal fin margins, whereas 0.8% of early-stage and 1.7% of late-stage toads from exclusions exhibited any tail damage (Pearson  $\chi^2 = 331.3$ ,  $df = 1$ , two-tailed  $P < 0.0001$ ). Similarly, while 4.5% of free-living larvae exhibited limb abnormalities, all larvae inside the exclusions had normal limbs (Pearson  $\chi^2 = 5.59$ ,  $df = 1$ , two-tailed  $P < 0.02$ ; Fig. 3).

*Aquarium experiment.*—Among 40 tadpoles exposed to sticklebacks in aquaria, 53% exhibited tail damage and 7.5% suffered hind-limb abnormalities compared to 2.5% of individuals with tail damage and 0% with limb abnormalities among tadpoles maintained only with conspecifics. Tail and limb damage among larvae in the tanks with sticklebacks was consistent with field observations and yielded post-metamorphic deformities indistinguishable from those seen in wild-caught individuals. Results of the GEE analysis revealed a strong effect of sticklebacks on the risk of limb and tail damage in larval toads (limbs, estimate of coefficient =  $42.82 \pm 0.61$ , Wald statistic = 4890.7,  $P < 0.0001$ ; tails, estimate of coefficient =  $3.34 \pm 1.11$ , Wald statistic = 9.15,  $P < 0.005$ ).

### *Abnormalities in Cascades frogs*

*Field observations.*—At Broken Top Ponds 1 and 2, we observed a high prevalence (5–21%) of hind-limb abnormalities among pre- and post-metamorphic Cascades frogs (Fig. 4A). The types of abnormalities were similar to those observed in LA toads but often more severe, with limb abnormalities in Cascades frogs often affecting >50% of a limb (e.g., missing tibiafibula and portion of femur). We also observed several cases of micromelia (limb reduced in size but otherwise complete). Tail abnormalities occurred in <20% of late-stage tadpoles from BT1 and BT2.

Dipnet sweeps of substrate captured an average of 3.7 dragonfly larvae and 0.8 salamander larvae per sweep. On several occasions, we observed dragonfly larvae clasp the hind limb of individual *R. cascadae* tadpoles. Although the much heavier tadpoles would periodically thrash violently, none managed to dislodge the dragonfly. Necropsies performed on five deformed metamorphs from BT1 found no *Ribeiroia* parasites and the absence of snails from BT1 or BT2 further ruled out

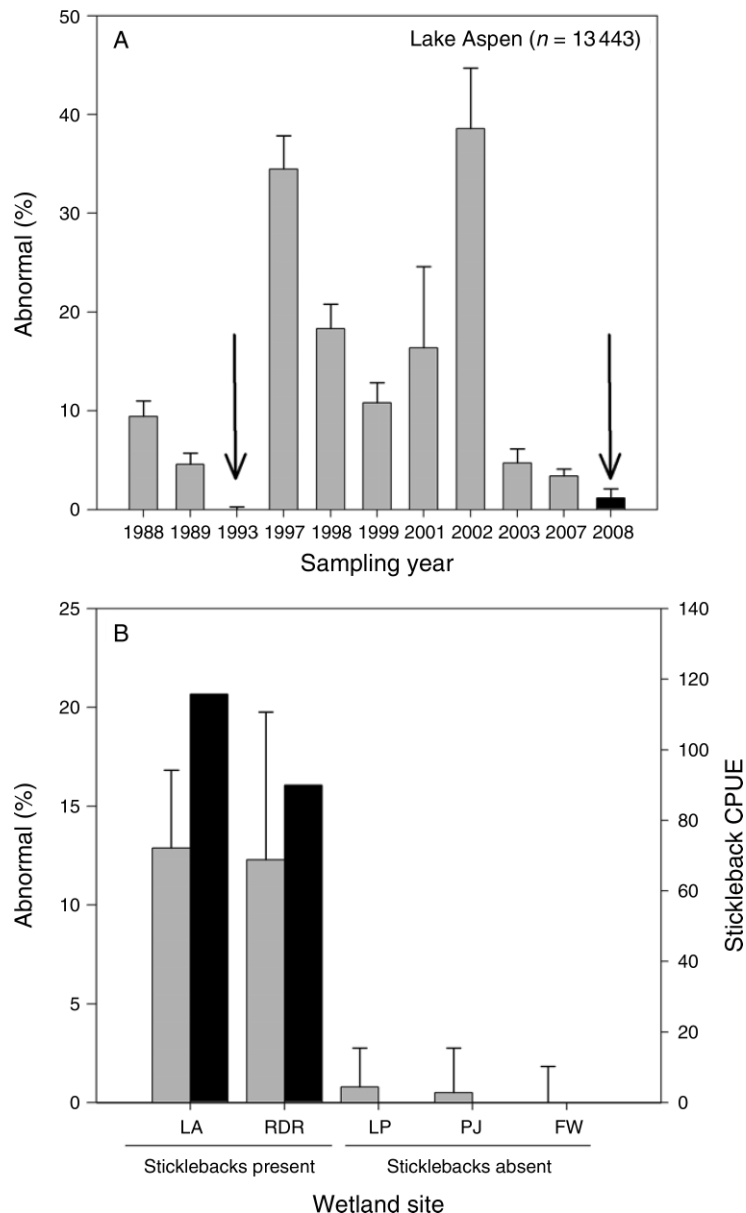


FIG. 1. Pattern of limb abnormalities in western toads (*Bufo boreas*) in central Oregon, USA. (A) Frequency of limb abnormalities in metamorphosing toads from Lake Aspen (1988–2008). Only years with successful toad emergence are displayed. Bars in black with vertical arrows above them indicate years in which the abundance of three-spined sticklebacks (*Gasterosteus aculeatus*) was low ( $<10$  individuals-trap $^{-1}$ ·d $^{-1}$ ) due to winterkill events. In all other years, stickleback abundance was estimated at or above 100 individuals-trap $^{-1}$ ·d $^{-1}$ . (B) Comparison of limb abnormality frequency in wetlands as a function of stickleback abundance (CPUE is catch per unit effort). The wetland sites Lake Aspen (LA) and Rear Driving Range (RDR) pond supported high densities of sticklebacks whereas Lake Penhollow (LP), Paulina Junction (PJ), and Fairway 11 (FW) lacked sticklebacks in the year of sampling. Error bars in both panels represent the 95% confidence interval for a proportion.

trematode infection as a cause of these abnormalities. No water quality analyses were performed at these wetlands.

**Container experiments.**—Of the 210 tadpoles maintained with dragonfly larvae, 37 (17.6%) suffered complete or partial amputation of one or both hind limbs (Fig. 4B). Four tadpoles lost both hind limbs. In contrast, two tadpoles in containers with salamander

larvae and one from the control exhibited limb abnormalities. The GEE analysis indicated a significant effect only for dragonfly larvae, with no difference between the salamander and control treatments (dragonfly treatment, coefficient estimate =  $2.25 \pm 0.73$ , Wald statistic = 9.43,  $P < 0.005$ ; salamander treatment, coefficient estimate =  $0.75 \pm 0.87$ , Wald statistic = 0.74,  $P = 0.3$ ). Of the 37 abnormal hind limbs produced

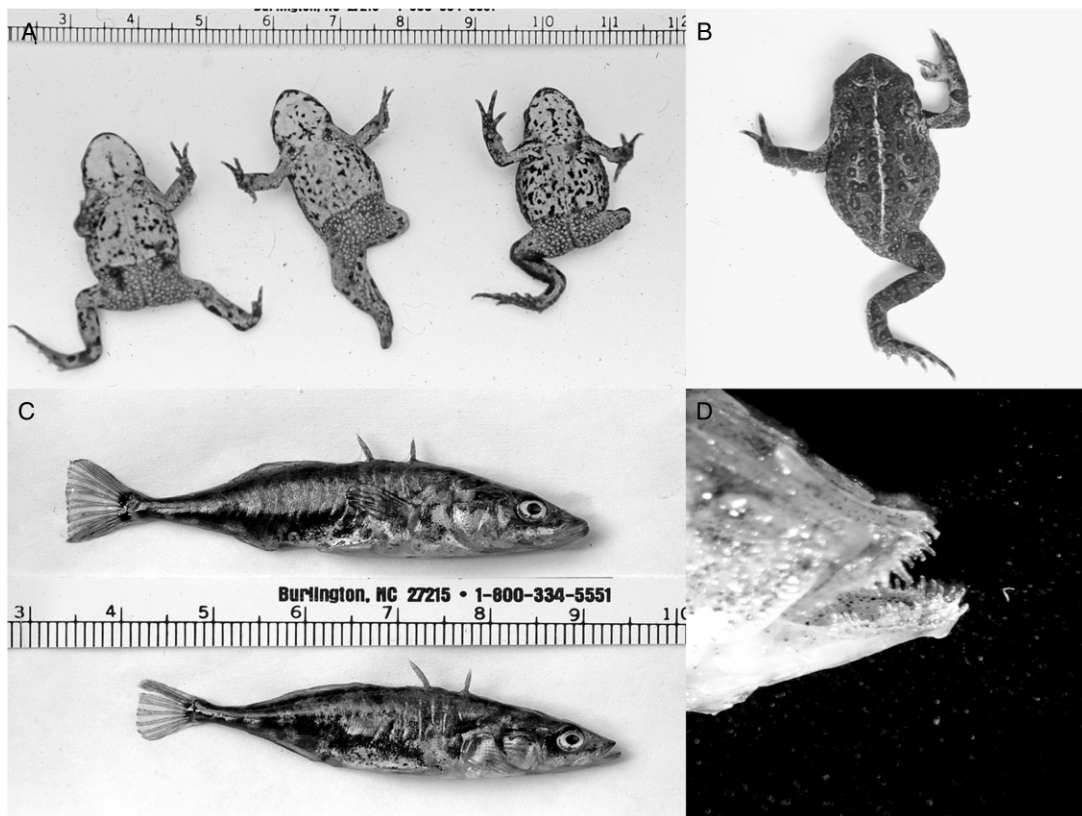


FIG. 2. Representative limb abnormalities and predators from Lake Aspen, Oregon: (A) metamorphic western toads (*Bufo boreas*) with typical missing-limb abnormalities (scale in cm); (B) toad with a completely missing hind limb; (C) sticklebacks (*Gasterosteus aculeatus*) from Lake Aspen; (D) jaws and teeth of a stickleback with overlying tissue removed.

in the dragonfly treatment, 22 involved amputations of an entire hind limb, 13 amputations removed more than 50% of a hind limb, and two resulted in loss of a hind foot. Tadpoles with amputations healed quickly leaving no sign of scarring by metamorphosis.

DISCUSSION

After more than a decade of focused attention on amphibian deformities, some causes of malformations, such as the trematode parasite *Ribeiroia* (Johnson et al. 1999, 2002, 2006, Sessions et al. 1999, Kiesecker 2002,

Stopper et al. 2002) have been well-documented. However, despite numerous reports of abnormalities involving predominantly missing or truncated limbs in the absence of *Ribeiroia* infection, few studies have explored the contributing role of sublethal predation. Although some researchers have suggested that predators are unlikely to explain limb abnormalities, citing the improbability of a predator removing only the limb of a frog and the absence of obvious scar tissue in abnormal animals (e.g., Meteyer et al. 2000, Lannoo 2008), we suggest that the hind limbs of larval anurans may be

TABLE 1. Distribution of limb abnormalities found in a subset of metamorphosing western toads (*Bufo boreas*) from Lake Aspen and in metamorphosing Cascades frogs (*Rana cascadae*) from Broken Top ponds in central Oregon, USA.

Deformity type	Lake Aspen ( <i>Bufo boreas</i> )		Broken Top ( <i>Rana cascadae</i> )	
	No. deformities	Percentage of all deformities	No. deformities	Percentage of all deformities
Missing digit(s)	182	53.1	1	1.7
Missing foot	50	14.6	5	8.6
Partially missing limb	79	23.0	17	29.3
Missing limb†	28	8.2	31	53.4
Micromelia	2	0.6	3	5.2
Forelimb	2	0.6	1	1.7
Total	343	100	58	100

† Defined here as more than two-thirds of limb missing.

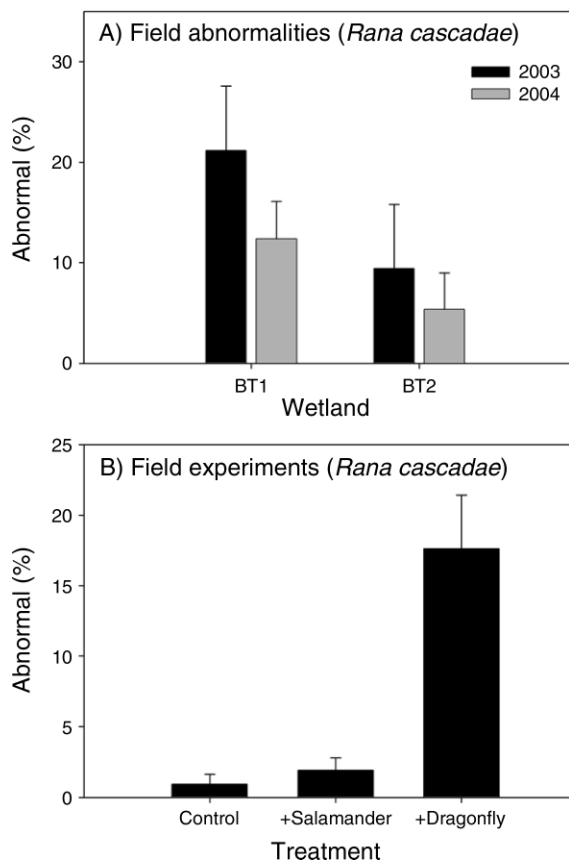


FIG. 3. Results of field and experimental studies from wetlands of Broken Top volcano, Oregon. (A) Percentage of limb abnormalities in larval and metamorphic Cascades frogs (*Rana cascadae*) inspected from Broken Top (BT) ponds 1 and 2 in both 2003 and 2004. Error bars represent the 95% confidence interval for a percentage. (B) Results of experimental trials examining the effect of predator identity on limb development in Cascades frog larvae. Illustrated is the percentage of amphibian larvae with missing-limb abnormalities as a function of predator treatment, including no predator (control), exposure to larval long-toed salamanders (*Ambystoma macrodactylum*), and exposure to larval dragonflies (*Somatochlora albicincta*). Predation trials lasted 48 h. Error bars represent +SE.

particularly vulnerable to predator attack. Because the hind limbs of larval anurans lack hard skeletal elements and become increasingly exposed over time, they may be especially susceptible to predators that are gape-limited or too small to consume an entire larva. Moreover, the partial regenerative ability of larval anurans may obscure obvious signs of trauma such as scarring.

Results from long-term field observations and experiments indicate that sublethal predation by vertebrate and invertebrate predators can contribute to missing-limb abnormalities in amphibians. Three lines of evidence point to sublethal predation by sticklebacks as the principle cause of the limb deformities in studied toad populations. First, toad deformities were most common at sites with abundant sticklebacks but rare at

nearby wetlands lacking sticklebacks. Moreover, following severe winterkill events that reduced stickleback abundances within Lake Aspen, toad deformities declined to low levels (<5%) (Fig. 1). Second, preliminary in situ predator exclosures effectively eliminated tail damage and limb deformities among developing toads, suggesting that neither water quality nor trematode infection was involved (observations that were supported by pesticide analyses and parasite examinations). We acknowledge, however, that invasion by garter snakes into the exclosures make such results preliminary and in need of additional confirmation. Finally, sticklebacks attacked tadpoles in aquaria and produced damage to tails and hind limbs consistent with, and indistinguishable from, abnormalities observed in the field.

In two high-elevation wetlands on Broken Top volcano, metamorphosing Cascades frogs exhibited high frequencies (5–25%) of missing limbs, partially missing limbs, and shrunken limbs (micromelia). In this case, direct observations and predation experiments suggested that the causative agent was a corduliid dragonfly larva (*Somatochlora albicincta*). While larval dragonflies such as *Aeshna* and *Anax* spp. are widely recognized as important lethal predators of larval amphibians (e.g., Smith 1983, Wilbur and Fauth 1990), this is the first report of which we are aware linking odonate predation to mass limb abnormalities in amphibians. Unlike the penetrating labial palps of *Aeshna* and *Anax* spp., corduliid and libellulid dragonfly larvae have palps better suited for clasping and cutting (Fig. 4D), possibly contributing to their disproportionate effects on developing limbs. Further, larval *Rana cascadae* are large relative to *S. albicincta*, which may explain why injury was a more common outcome than death in experiments. Neither trematode parasites nor other predators could account for observed abnormalities.

Among abnormal toads and Cascades frogs, we rarely observed obvious signs of trauma such as scarring following metamorphosis. Anurans have varying degrees of regenerative ability that decline as larval development proceeds (e.g., Kurabuchi and Inoue 1982), which may help to explain the lack of visible trauma. Fry (1966) found that if leopard frog (*R. pipiens*) limbs were amputated prior to differentiation of the digits, injured limbs regenerated into fully patterned albeit smaller limbs (micromelia); when amputation occurred later in development, however, limbs healed into stumps lacking joints, toes, or other limb-like features. We observed all of these conditions in Cascades frogs (Fig. 4). The absence of rigid, calcified skeletal elements in developing tadpole limbs, combined with their limited regenerative capacity, may help explain why observers seldom see evidence of injury, such as protruding bone or scarring. Nonetheless, while sticklebacks and dragonfly larvae caused generally similar deformities in anurans, deformities caused by sticklebacks primarily affected the toes and feet of toads,

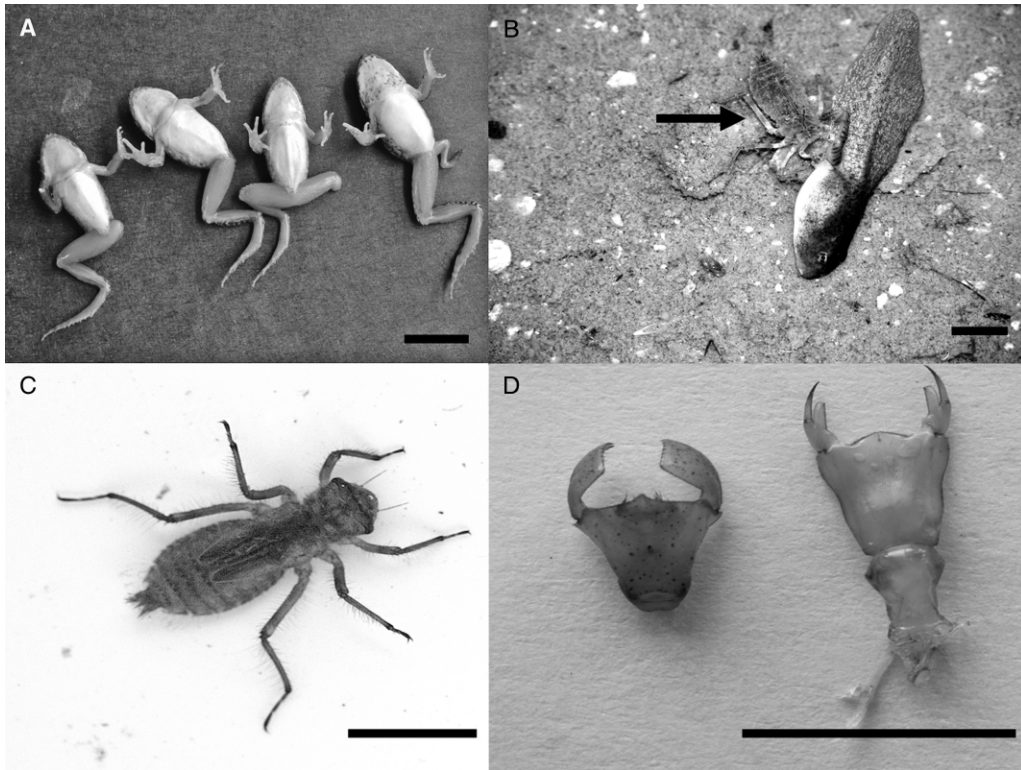


FIG. 4. Representative abnormalities and predators from wetlands of Broken Top volcano, Oregon: (A) typical limb abnormalities in metamorphosing Cascades frogs (*Rana cascadae*), illustrating partially missing limbs, completely missing limbs, and shrunken limbs (micromelia); (B) *Rana cascadae* larva with proximal portion of left leg clasped by labial palps of a larval dragonfly (black arrow); (C) larval dragonfly (*Somatochlora albicincta*); (D) comparison of the labial palps of *S. albicincta* (left) and an aeshnid dragonfly (right), contrasting the clasping apparatus of the corduliid with the piercing apparatus of the aeshnid. Scale bars indicate 1 cm.

whereas dragonfly attacks more often removed all or most of the hind limb and only rarely caused the loss of toes or feet (Table 1). This difference likely reflects differences in the anatomy and attack behavior between the respective predators: sticklebacks attack rapidly and tear away small pieces of tissue whereas corduliid dragonfly larvae capture and hold prey with their labial palps before chewing through prey tissue with their mandibles (see Figs. 2D and 4D).

We suggest that sublethal predation may be an important explanation of missing-limb abnormalities in other amphibian populations. Working in the United Kingdom and the Netherlands, Wisniewski (1958) and Van Gelder and Strijbosch (1995), respectively, each documented large numbers of common toads (*Bufo bufo*) with missing-limb abnormalities. The specific causes of the abnormalities were not isolated, but both groups suspected predators might be important contributors. In Panama, Duellman and Trueb (1994) repeatedly observed crabs attack frogs (*Colostethus inguinalis*), often resulting in missing limbs. Gray et al. (2002) similarly suggested the involvement of crabs in explaining high levels (12%) of digit abnormalities in the green poison dart frog (*Dendrobates auratus*), also in Panama.

In an unpublished study, C. Corkran and colleagues noted missing-limb abnormalities in >40% of metamorphosing toads from Frog Lake, Oregon. Based on the composition of the abnormalities and field observations, the researchers suggested that the introduced speckled dace (*Rhinichthys osculus*) was responsible for the abnormalities (C. Corkran, *personal communication*).

Studies of leech (*Erpobdella octoculata*) attacks on common toads (*Bufo bufo*) in Germany provide some of the best evidence of the importance of sublethal predation in causing amphibian abnormalities (Viertel and Veith 1992, Veith and Viertel 1993, Bohl et al. 1996, Bohl 1997). High frequencies (>20%) of partially and completely missing limbs in metamorphic toads were not associated with genetic anomalies or contaminants; however, experimental enclosures permeable to water but excluding predators eliminated the abnormalities (Bohl 1997). Further, experimental exposure to leeches in the laboratory induced the same suite of abnormalities in toads (Viertel and Veith 1992). An experimental reduction of leech density within a wetland sharply reduced abnormality levels in the toad population, offering ecosystem-level evidence for the causal role of leeches (Bohl et al. 1996).

A pressing question surrounding the occurrence of predation-related injuries is whether they pose a threat to affected individuals and populations. Meyer and Byers (2005), for example, reported the sublethal predation injuries to the siphons of marine bivalves. Shortened siphons forced bivalves to remain at shallow depths in the sediment, thereby making them more vulnerable to lethal attacks by excavating predators. Similarly, severe tail injury in tadpoles can significantly increase their vulnerability to turtle and crayfish predators (Feder 1983, Figiel and Semlitsch 1991). We suspect that observed limb abnormalities substantially reduce survivorship among affected amphibians (see Johnson et al. 2001*b*). Unlike tail injuries, which disappear at metamorphosis, limb abnormalities are most likely to reduce the survival of post-metamorphic frogs.

In summary, our results indicate that attacks by vertebrate and invertebrate predators can cause high frequencies of missing-limb abnormalities similar to those observed in nature. Considering that amphibians with missing limbs and digits are among the most commonly reported anuran abnormalities in the United States (e.g., Guderyahn 2006), we suggest that the role of predators deserves further investigation. Although there are other causes of missing limbs in amphibians besides sublethal predation, the potential involvement of predators, which can often be examined through relatively inexpensive techniques (e.g., enclosure studies), should not be discounted a priori. Sublethal predation and the resulting injuries in amphibians may be important not only as ecological or evolutionary influences, but may also have significant implications for conservation and contemporary concerns over widespread amphibian population losses (Stuart et al. 2004). While predators are a ubiquitous component of most aquatic ecosystems, not all amphibian populations exhibit high frequencies of limb abnormalities. Thus, determining which predators cause sublethal injury and under what conditions they are most likely to do so are important priorities in the continued study of amphibian abnormalities. We emphasize the need for more work to understand (1) under what circumstances sublethal predation causes higher-than-baseline levels of abnormalities and (2) the consequences of sublethal predator attacks for amphibian ecology and conservation.

#### ACKNOWLEDGMENTS

We thank Margaret Beard, Michael Bell, Michael Blouin, Chuck Kimmel, and Stan Sessions for comments and suggestions. Thoughtful critique and suggestions by two anonymous reviewers improved the manuscript.

#### LITERATURE CITED

- Ankley, G. T., S. J. Degitz, S. A. Diamond, and J. E. Tietge. 2004. Assessment of environmental stressors potentially responsible for malformations in North American anuran amphibians. *Ecotoxicology and Environmental Safety* 58:7–16.
- Blair, J., and R. J. Wassersug. 2000. Variation in the pattern of predator-induced damage to tadpole tails. *Copeia* 2000:390–401.
- Bohl, E. 1997. Limb deformities of amphibian larvae in Aufseß (Upper Franconia): attempt to determine causes. *Münich Contributions to Wastewater Fishery and River Biology* 50: 160–189.
- Bohl, E., E. Bohl, J. Heise, R. Fischer, and M. Popp. 1996. Untersuchungen zu Mißbildungen an Amphibienlarven im Natureich des Beispielsbetriebs in Aufseß (Oberfranken). Report to the Bayerisches Landesamt für Wasserwirtschaft, Institut für Wasserforschung, München, Germany.
- Corbet, P. S. 1999. Dragonflies: behavior and ecology of Odonata. Cornell University Press, Ithaca, New York, USA.
- Duellman, W. E., and L. Trueb. 1994. Biology of amphibians. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Feder, M. E. 1983. The relation of air breathing and locomotion to predation on tadpoles, *Rana berlandieri*, by turtles. *Physiological Zoology* 56:522–531.
- Figiel, C. R., and R. D. Semlitsch. 1991. Effects of nonlethal injury and habitat complexity on predation in tadpole populations. *Canadian Journal of Zoology* 69:830–834.
- Fry, A. E. 1966. Hind limb regeneration in *Rana pipiens* larvae. *Copeia* 1966:530–534.
- Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Gray, H. M., M. Ouellet, D. M. Green, and A. S. Rand. 2002. Traumatic injuries in two Neotropical frogs, *Dendrobates auratus* and *Physalaemus pustulosus*. *Journal of Herpetology* 36:117–121.
- Guderyahn, L. 2006. Nationwide assessment of morphological abnormalities observed in amphibians collected from United States National Wildlife Refuges. CBF0-C0601. U.S. Fish and Wildlife Service, Annapolis, Maryland, USA.
- Halekoh, U., S. Højsgaard, and J. Yan. 2001. The R Package geeppack for generalized estimating equations. *Journal of Statistical Software* 15:1–11.
- Horton, N. J., and S. R. Lipsitz. 1999. Review of software to fit generalized estimating equation regression models. *American Statistician* 53:160–169.
- Johnson, P. T. J., K. B. Lunde, R. W. Haight, J. Bowerman, and A. R. Blaustein. 2001*a*. *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). *Canadian Journal of Zoology* 79:370–379.
- Johnson, P. T. J., K. B. Lunde, E. G. Ritchie, and A. E. Launer. 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* 284:802–804.
- Johnson, P. T. J., K. B. Lunde, E. G. Ritchie, J. K. Reaser, and A. E. Launer. 2001*b*. Morphological abnormality patterns in a California amphibian community. *Herpetologica* 57:336–352.
- Johnson, P. T. J., K. B. Lunde, E. M. Thurman, E. G. Ritchie, S. N. Wray, D. R. Sutherland, J. M. Kapfer, T. J. Frest, J. Bowerman, and A. R. Blaustein. 2002. Parasite (*Ribeiroia ondatrae*) infection linked to amphibian malformations in the western United States. *Ecological Monographs* 72:151–168.
- Johnson, P. T. J., E. R. Preu, D. R. Sutherland, J. Romansic, B. Han, and A. R. Blaustein. 2006. Adding infection to injury: synergistic effects of predation and parasitism on salamander limb malformations. *Ecology* 87:2227–2235.
- Johnson, P. T. J., M. K. Reeves, S. K. Krest, and A. E. Pinkney. *In press*. A decade of deformities: advances in our understanding of amphibian malformations and their implications. *In* D. W. Sparling, G. Linder, C. A. Bishop, and S. K. Krest, editors. *Ecotoxicology of amphibians and reptiles*. Second edition. Society of Environmental Toxicology and Chemistry, Pensacola, Florida, USA.
- Kaliszewicz, A. 2003. Sublethal predation on *Stylaria lacustris*: a study of regenerative capabilities. *Hydrobiologia* 501:83–92.

- Kiesecker, J. M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences (USA)* 99:9900–9904.
- Kurabuchi, S., and S. Inoue. 1982. Limb regenerative capacity of four species of Japanese frogs of the families Hylidae and Ranidae. *Journal of Morphology* 173:129–135.
- Lannoo, M. J. 2008. *Malformed frogs: the collapse of aquatic ecosystems*. University of California Press, Berkeley, California, USA.
- Lannoo, M. J., D. R. Sutherland, P. Jones, D. Rosenberry, R. W. Klaver, D. M. Hoppe, P. T. J. Johnson, K. B. Lunde, C. Facemire, and J. M. Kapfer. 2003. Multiple causes for the malformed frog phenomenon. Page 1443 in G. Linder, E. Little, S. Krest, and D. Sparling, editors. *Multiple stressor effects in relation to declining amphibian populations*. ASTM International, West Conshohocken, Pennsylvania, USA.
- Lawler, S. P., D. Dritz, T. Strange, and M. Holyoak. 1999. Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. *Conservation Biology* 13:613–622.
- Liang, K. Y., and S. Zeger. 1986. Longitudinal data analysis using generalized linear models. *Biometrika* 73:13–22.
- Meteyer, C. U., I. K. Loeffler, J. F. Fallon, K. A. Converse, D. E. Green, J. C. Helgen, S. Kersten, R. Levey, L. Eaton-Poole, and J. G. Burkart. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. *Teratology* 62:151–171.
- Meyer, J. J., and J. E. Byers. 2005. As good as dead? Sublethal predation facilitates lethal predation on an intertidal clam. *Ecology Letters* 8:160–166.
- Mouritsen, K. N., and R. Poulin. 2003. The risk of being at the top: foot-cropping in the New Zealand cockle *Austrovenus stutchburyi*. *Journal of the Marine Biological Association of the United Kingdom* 83:497–498.
- Ouellet, M. 2000. Amphibian deformities: current state of knowledge. Pages 617–661 in D. W. Sparling, G. Linder, and C. A. Bishop, editors. *Ecotoxicology of amphibians and reptiles*. Society of Environmental Toxicology and Chemistry, Pensacola, Florida, USA.
- Pape-Lindstrom, P. A., R. J. Feller, S. E. Stancyk, and S. A. Woodin. 1997. Sublethal predation: field measurements of arm tissue loss from the ophiuroid *Microphiopholis gracillima* and immunochemical identification of its predators in North Inlet, South Carolina, USA. *Marine Ecology Progress Series* 156:131–140.
- Reeves, M. K., C. L. Dolph, H. Zimmer, R. S. Tjeerdema, and K. A. Trust. 2008. Road proximity increases risk of skeletal abnormalities in wood frogs from National Wildlife Refuges in Alaska. *Environmental Health Perspectives* 116:1009–1015.
- Schoener, T. W. 1979. Inferring the properties of predation and other injury-producing agents from injury frequency. *Ecology* 60:1110–1115.
- Sessions, S. K., R. A. Franssen, and V. L. Horner. 1999. Morphological clues from multilegged frogs: Are retinoids to blame? *Science* 284:800–802.
- Sessions, S. K., and S. B. Ruth. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. *Journal of Experimental Zoology* 254:38–47.
- Skelly, D. K., S. R. Bolden, L. K. Freidenburg, N. A. Freidenfelds, and R. Levey. 2007. *Ribeiroia* infection is not responsible for Vermont amphibian deformities. *EcoHealth* 4:156–163.
- Smith, D. C. 1983. Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology* 64:501–510.
- Souder, W. 2000. *A plague of frogs*. Hyperion, New York, New York, USA.
- Stopper, G. F., L. Hecker, R. A. Franssen, and S. K. Sessions. 2002. How trematodes cause limb deformities in amphibians. *Journal of Experimental Zoology* 294:252–263.
- Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigue, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
- Van Buskirk, J., and S. A. McCollum. 2000. Influence of tail shape on tadpole swimming performance. *Journal of Experimental Biology* 203:2149–2158.
- Van Gelder, J. J., and H. Strijbosch. 1995. Adult common toads (*Bufo bufo*) with mutilated legs. *Alytes* 13:105–108.
- Veith, M., and B. Viertel. 1993. Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*): Analyse möglicher Ursachen. *Salamandra* 29:184–199.
- Viertel, B., and M. Veith. 1992. Predation by leeches and regeneration, a factor in larval development of *Bufo bufo* (L.). Pages 479–484 in Z. Korsos and I. Kiss, editors. *Proceedings of the Sixth Ordinary General Meeting of the Societas Europaea Herpetologica*, Budapest, 1991. Hungarian Natural History Museum, Budapest, Hungary.
- Wilbur, H. M., and J. E. Fauth. 1990. Experimental aquatic food webs: interactions between two predators and two prey. *American Naturalist* 135:176–204.
- Wildy, E. L., D. P. Chivers, J. M. Kiesecker, and A. R. Blaustein. 2000. The effects of food level and conspecific density on biting and cannibalism in larval long-toed salamanders, *Ambystoma macrodactylum*. *Oecologia* 128:202–209.
- Wisniewski, W. L. 1958. Characterization of the parasitofauna of a eutrophic lake. *Acta Parasitologica Polonica* 6:1–64.