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Explanation for Missing Limbs in Deformed Amphibians

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ABSTRACT We present evidence that the most commonly found deformities in wild-caught amphibians, those featuring missing limbs and missing limb segments, may be the result of selective predation. Here we report that predatory dragonfly nymphs can severely injure and even fully amputate developing hind limbs of anuran tadpoles. Developmental responses of the injured/amputated tadpole limbs range from complete regeneration to no regeneration, with intermediate conditions represented by various idiosyncratic limb deformities, depending mainly on the developmental stage of the tadpole at the time of injury/amputation. These findings were reinforced by experimental amputations of anuran tadpole hind limbs that resulted in similar deformities. Our studies suggest that selective predation by dragonfly nymphs and other aquatic predators may play a significant role in the most common kinds of limb deformities found in natural populations of amphibians. *J. Exp. Zool. (Mol. Dev. Evol.) 312B:770– 779, 2009.*

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The occurrence of morphological abnormalities[1] in natural population of amphibians has been a major, and controversial, environmental issue for more than a decade (see Sessions, 2003; Lannoo, 2008 for recent reviews). Identifying the proximate cause(s) of these deformities is important because of the light it may shed on environmental degradation as well as amphibian disease and decline. Recent research has resolved several different categories of deformed amphibians, suggesting different causes or combinations of causes ranging from chemical pollution and UV-B radiation to parasites and predation (Meteyer et al., 2000; Ouellet, 2000; Johnson et al., 2001; Blaustein and Johnson, 2003; Sessions, 2003; Lannoo, 2008; Rohr et al., 2008). Although much research has focused on frogs with parasite-induced supernumerary hind limbs (Sessions and Ruth, '90; Johnson et al., '99; Sessions et al., '99; Stopper et al., 2002; Schotthoefer et al., 2003; Johnson et al., 2004), the vast majority of reports of deformed amphibians involve various species of frogs and toads with missing hind limbs, missing limb segments, or misshapen limbs, with no apparent involvement of parasites (Meteyer, 2000; Levey et al., 2003; Sessions, 2003; Skelly et al., 2007; Lannoo, 2008).

Since parasites do not seem to be involved in these most common deformities, several workers have pointed to chemical pollution without any direct evidence that chemical pollutants have a causal role (Levey et al., 2003; Taylor et al., 2005; Skelly et al., 2007; Lannoo, 2008). Other research suggests that missing limb deformities may be caused by predation (Martof, '56; Veith and Viertel, '93; Bohl, '97; Sessions, 2003; Eaton et al., 2004), but this possibility has not been well studied experimentally and is considered doubtful by some researchers (e.g. Meteyer et al., 2000; Skelly et al., 2007; Lannoo, 2008). Here we present results of a

[1] Some researchers refer to these abnormalities as ''malformations'' (e.g. Lannoo, 2008; Meteyer, 2000), implying an intrinsic developmental defect (Winter et al., '88; MedicineNet.com). However, since all of the suggested and known causes of morphological abnormalities in naturally occurring populations of amphibians involve extrinsic factors (e.g. parasites) disrupting the development of otherwise normal tissues, the more accurate term for these abnormalities is ''deformity'', and that is the term we have chosen to use here.

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study from both field observations and laboratory experiments, showing that deformities featuring missing limbs and missing limb segments, with an associated range of idiosyncratic limb deformities, represent normal regenerative responses in anuran tadpoles to injury from *selective predation* by invertebrate predators. By selective predation we refer to predators that are too small, or have mouthparts that are too small, to consume whole tadpoles and instead selectively snip or chew off small pieces without necessarily killing their prey. Selective predation of tadpoles by various predators has been reported by other workers (Licht, '74; Johnson et al., '75; Brodie et al., '78; Manteifel and Reshetnikov, 2002) but has not been explicitly linked with the occurrence of hind limb deformities. Our results show that the peculiar range of hind limb deformities produced by selective predation is the result of ontogenetic decline in regenerative ability, a well-known characteristic of limb development in anurans (Muneoka et al., '86). We suggest that many limb deformities in natural populations of amphibians probably stem from selective predation.

MATERIALS AND METHODS

This study involves both field observations and laboratory experiments focused primarily on European Common toads (*Bufo bufo*). Field observations were complemented by experimental predation simulations in the laboratory. In a separate experiment, North American wood frogs (*Rana sylvatica*) were used to investigate the regenerative response to surgical limb amputation at different stages of tadpole development.

Field observations

Specimens of toads were collected at three localities in Yorkshire, England during the summers of 2006–2008, during a survey to determine the occurrence of deformities in populations of free-living native British amphibians. A pilot study of one of the sites (a garden green pond in Havercroft Village, Wakefield), to find out whether deformities occurred in British amphibians and what kinds, revealed a substantial number of deformed, newly metamorphosed Common toads (*B. bufo*), the majority exhibiting abnormalities in the hind limbs, and a few with a missing eye. Subsequent collections were made at this and two other sites: Upton Colliery Eastern Ponds (Pontefract) and Campsall Country Park Clay Pond, a two-hectare fishing lake stocked with various game fish species in Doncaster. Specimens were collected over a three-week period using dip-nets. All animals were carefully examined and scored for deformities, and most were released. Selected specimens with deformities and injuries were collected, photographed, euthanized in MS222, and fixed in a 10% buffered formalin solution. The presence of potential predators, including both vertebrates and invertebrates, was noted at each site.

Experimental simulations

Selective predation

Tadpoles representing a range of developmental stages were collected from a permanent fish-free wetland in Hoyland Bank Pond, Barnsley, West Yorkshire, England. This site was selected because it contained a large population of Common toad (*B. bufo*) larvae and less than 5% of the sampled specimens displayed obvious injuries or deformities. Tadpoles were kept for observation in an outdoor tub with 100 L of aged water for 14 days and were fed fish food flakes daily to minimize any potential competitive injuring effects such as autopredation. Removal of feces and 10% water changes occurred daily before feeding. After initial observations and acclimation, tadpoles were sorted and grouped according to Gosner staging (Gosner, '60; McDiarmid and Altig, '99). Any tadpoles with injuries or other abnormalities were rejected along with tadpoles at stage 31 or earlier or stage 38 or later. Remaining tadpoles (stages 32–37) were subdivided into two sets according to developmental stage: Set number 1 (stages 32–34) and Set number 2 (stages 35–37). The two sets of tadpoles were kept at ambient room temperatures with a natural daylight/night cycle in acrylic tubs with 20 L aged water for 48 hr before experiments. Feeding and cleaning methods continued daily.

Dragonfly nymphs (*Sympetrum* sp.) were collected from a permanent fish-free wetland in the Upton Colliery Eastern Ponds, Upton, Pontefract, West Yorkshire. This site was selected because of the large population of *Sympetrum* dragonfly nymphs (1–3 per dip-net) and because deformed and newly injured tadpoles and newly metamorphic toadlets had been found at the site on an earlier visit. Dragonfly nymphs were grouped according to estimated developmental instar based on size and wing development. Each of a total of 37 individuals from the same stage were placed in individual containers with 5 L aged water kept at ambient room temperature with a natural

daylight/night cycle and three ''sprigs'' of the Common waterweed (*Elodea canadensis*) for partial habitat creation and oxygenation of water. Twenty of the nymphs were selected for the experimental groups and 17 reserved as controls and potential replacements of dead experimental nymphs. Nymphs were separated to prevent cannibalism and maintained by feeding one common frog tadpole (*R. temporaria*) every 48 hr.

The 20 experimental nymphs were starved for 72 hr before the introduction of toad tadpoles. Ten experimental nymphs were selected to feed on the younger *Bufo* tadpoles (Set 1, stages 32–34) and ten were selected to feed on the older *Bufo* tadpoles (Set 2, stages 35–37). Ten *Bufo* tadpoles were then added to each tank containing a single hungry nymph. Injured or dead tadpoles were replaced with same stage tadpoles every 24 hr for 11 days, allowing nymphs to gorge themselves on *Bufo* larvae. Dead tadpoles and remains from all tanks were described, photographed, and fixed in 10% buffered formalin. Injured tadpoles with visible trauma to the limb(s), abdomen, cranium, or tail (if severe enough that less than 50% of tail remained) were removed, euthanized in MS222, photographed, and fixed in 10% buffered formalin to record nonlethal injuries. The remaining live injured tadpoles from five out of ten tanks per set were removed, described, photographed, and placed in isolated tanks containing 500 mL aged water, fed and cleaned daily to allow tadpoles to continue to develop postinjury. Postinjury tadpoles were grouped into tanks by injury date and type of injury, and allowed to develop until tail absorption, at which point they were described, euthanized in MS222, photographed, and fixed in 10% buffered formalin.

Surgical amputations

The second experiment was designed to compare the results of experimentally amputated limbs in tadpoles at different stages of limb development with those from the wild. We collected wood frog (*R. sylvatica*) eggs within one day of being laid in a small pond located near West Davenport, Delaware, New York. The eggs were kept in aged, filtered tap water and allowed to hatch and develop to limb bud stages. Tadpoles were anesthetized in 0.1% neutral-buffered MS222. We used iridectomy scissors and watchmaker's forceps to amputate one hind limb from each of 69 tadpoles at the prospective knee joint. Limb amputations were performed at three different stages of limb development: Gosner stage 28, Gosner stage 34, and Gosner stage 37. Tadpoles were then allowed to continue development to near metamorphosis (stages 42+, complete hind limb development and emergence of forelimbs; Field stage 6 and beyond; Gosner, '60) and examined for regenerative response. All specimens were euthanized in 0.1% neutral-buffered MS222, fixed in 10% buffered formalin, cleared, and stained (Sessions and Ruth, '90).

RESULTS

Field observations

The pilot study revealed a wide range of missing, partial, and misshapen hind limb deformities among late stage tadpoles and newly metamorphosed toads (''peri-metamorphs''). A total of 35 peri-metamorphic toads were found with deformities, the majority exhibiting abnormalities in the hind limbs and three with normal hind limbs but single missing eyes. The limb deformities included complete absence of limbs, presence of cartilaginous spikes (tapered cartilage growths at the tip of a truncated limb bone), reduced hind limbs, and one individual with epidermal webbing binding a reduced hind limb that prohibited full use. A newly metamorphosed Common frog (*R. temporia*) with an abnormal hind limb, and an adult Smooth newt (*Triturus vulgaris*) with a partial hind limb with missing foot were also observed. All the animals were alive at the time of surveys except one toad metamorph with a completely missing hind limb. Population counts of normal individuals were not generated in the pilot study.

Systematic collections at the above and the two other sites yielded deformed peri-metamorphic toads, including some fresh injuries and freshly amputated hind limbs, at all three ponds (Fig. 1). The proportion of deformed toads ranged from 16 out of 1,214 toads (1.3%) at Havercroft, 22 out of 1,879 (1.2%) at Campsall Clay, and 4 out of 41 toads (9.8%) at Upton Colliery. Except for the last site, these rates of deformities fall well within the suggested baseline ($\leq$5%) for deformities in natural populations of amphibians (Lannoo, 2008).

Potential predators found at the sites included three-spined stickleback fish (*Gasterosteus aculeatus*), newts (*T. helviticus* and *T. vulgaris*), and several species of aquatic insect predators including diving beetles (*Dytiscus* sp.), water scorpions (*Nepa cinerea*), and predatory odonata nymphs (including *Sympetrum* sp.). Odonata nymphs were

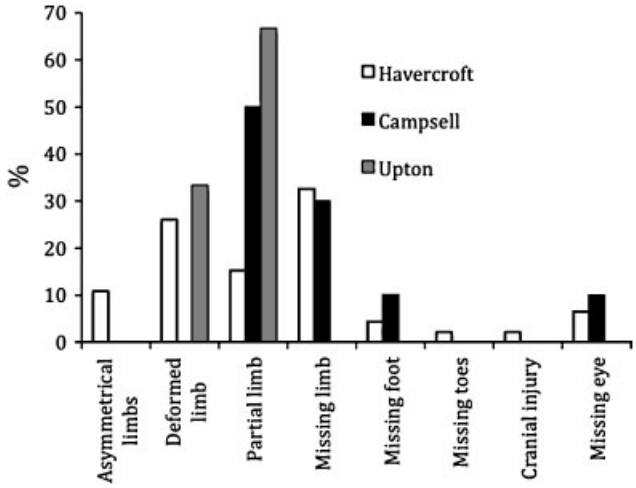

Fig. 1. Graph showing major kinds of deformities in wild-caught European toads from three sites (presented as percent of total deformities in each case). One specimen could be scored for more than one kind of deformity; specimens with missing foot or toes had otherwise intact limbs.

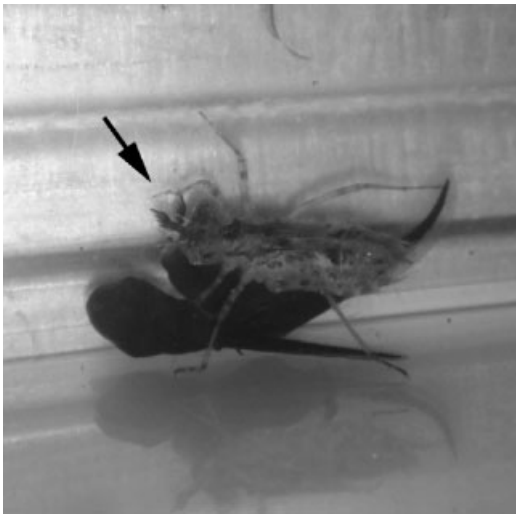

Fig. 2. Dragonfly nymph attacking one of two toad tadpoles, shortly after selectively removing the hind limbs (visible in the nymph's mandibles, arrow).

abundant at all three sites, as they have been historically (Sunter, '97, 2000).

Experimental manipulations

Selective predation

Dragonfly predation appeared to be a major source of injury and mortality via selective predation in toad larvae. Preliminary observations (data not shown) involving various potential predators of Common toads, including stickleback fishes, Palmate newts, Smooth newts, and various invertebrates came out negative. Invertebrates included several insect species, including larval and adult Great diving beetles (*Dytiscus marginalis*), Water scorpions (*N. cinerea*), and nymphs of several species of damsel flies, and all generated negative results. Although *Dytiscus* larvae and adults maimed and killed *B. bufo* tadpoles, these attacks did not generate deformities in surviving tadpoles. The only toad predators found to induce deformities via selective predation were three species of dragonfly nymphs, *Aeshna mixta, Libellula depressa*, and especially *Sympetrum* sp. (probably either *S. striolatum* or *S. sanguineum*).

In our experimental observations, we examined a total of 427 *Bufo* tadpoles that had been predated by *Sympetrum* dragonfly nymphs in experimental enclosures. Selective predation was observed in 14 out of 20 tanks beginning in the first hour after introduction of prey tadpoles. Nymphs were observed (and videoed) capturing tadpoles and chewing on selected body parts before releasing the tadpoles (Fig. 2; http://blip.tv/file/1418583). Recapture of injured tadpoles was occasionally observed, though it appeared nymphs were attracted more to movement in noninjured tadpoles than returning to less active, previously injured prey. Our observations suggest that *Sympetrum* use visual over tactile hunting techniques, at least when prey are abundant. Occasionally, tadpoles were able to escape after being captured (''predation attempt''), but this was rarely recorded relative to successful capture followed by selective predation (e.g. of a limb) and release. Most nymphs continued to feed for several days, some for the entire duration of the experiment. Only 2 of the 20 nymphs did not feed at all.

Full consumption of an entire *Bufo* tadpole was never observed in the toad/dragonfly interactions. Instead, nymphs performed selective predation, removing body parts (often limbs) and inducing a range of both lethal and nonlethal injuries (Fig. 3). Nonlethal injuries included facial/cranial damage such as missing eyes, but the most common injuries were various degrees of damage to tails and hind limbs including partial and sometimes full amputation of both hind limbs (Figs. 3 and 4). Damage to developing limbs occurred frequently in both younger (Fig. 3A) and older (Fig. 3B) *Bufo* tadpoles, but with different developmental consequences (Fig. 3). Lethal damage most often included major injuries to the cranium and abdomen and/or the loss of greater than 75% of the tail (Fig. 3).

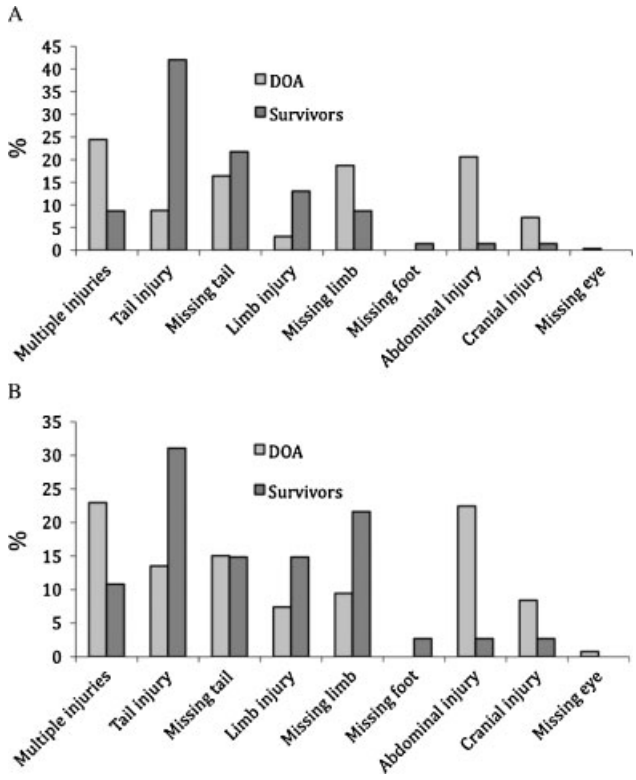

Fig. 3. Abnormalities (percent of total observed) resulting from selective predation by captive dragonfly nymphs on toad tadpoles at two different stages of tadpole limb development; (**A**): attack at Gosner stage 32–34; (**B**): attack at Gosner stage 35–37. Lethal injuries (DOA) are compared with injuries in survivors in each case. Deformities scored as exclusive categories and counted only once per frog; single specimens could be scored for more than one deformity.

Many of the tadpoles survived the dragonfly-induced injuries showing partial to complete regeneration of their tails and hind limbs. Regenerative response ranged from complete regeneration to partial regeneration to healing with no regeneration. By metamorphosis, this variation in regenerative response manifested itself as various kinds of limb deformities including missing limbs and limb segments, resembling field sampled deformed *Bufo* (Fig. 4).

Our observations also showed that the extent of deformity varies with the developmental stage at which the injury occurred. Selective predation of hind limbs in older tadpoles (stages 35–37; 16/75 tadpoles = 21.3%) was more than three times as likely to result in permanent limblessness than amputation in younger tadpoles (stage 32–34; 4/64 tadpoles = 6.3%) (Fig. 5). Even extreme injury in a late-stage tadpole, such as complete removal of a hind limb including portions of the body wall and pelvic girdle, was not always fatal and could be followed by complete healing (but no regeneration) within a few days (Fig. 6).

Surgical amputations

Experimental surgical amputations confirmed the results of the dragonfly study that the extent of limb deformity increases with developmental stage of the tadpole, even if the initial injury was otherwise identical (Figs. 7 and 8). Later stage tadpoles showed an incidence of reduced or

Fig. 4. Deformed hind limbs in wild-caught *B. bufo* tadpoles (top row) compared with hind limb deformities in tadpoles (bottom row) induced by selective predation by captive dragonfly nymphs. Note protruding bone in the tadpoles second from left end in each row.

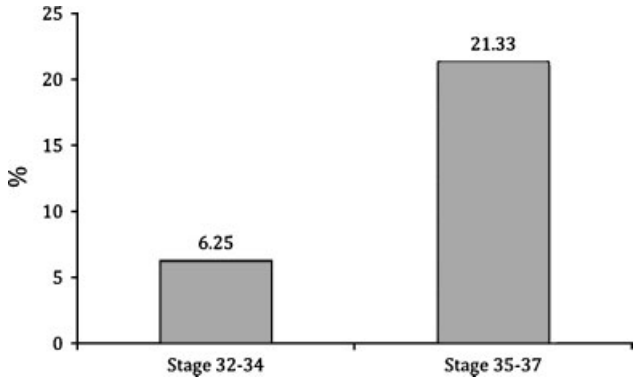

Fig. 5. Percent of toad tadpoles with missing limbs (i.e. entire hind limb absent) in surviving tadpoles that were selectively predated by dragonfly nymphs at two different stages (Gosner, 1960). Differences are statistically significant (χ^2; $P<0.01$).

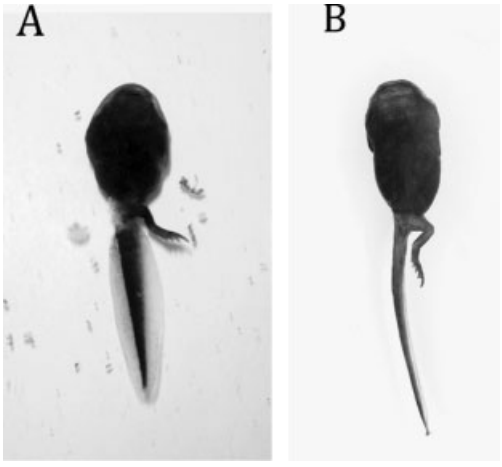

Fig. 6. Selective predation by dragonfly nymph on a toad (*B. bufo*) tadpole resulting in amputation of the right hind limb; (**A**): immediately after attack; (**B**): same tadpole 10 days after attack. Right hind limb area has completely healed, resulting in a permanent limb loss.

deformed hind limbs (including abnormal tissue growths) that are more than 10 times higher than that of younger tadpoles (Figs. 7 and 8). Interestingly, nearly all (85% for stage 28; 100% for stages 34 and 37) amputated tadpole limbs, whether fully regenerated or not, developed abnormal pigmentation, seen as distorted or missing bands of pigment (Figs. 7 and 9). Experimental amputations resulted in similar-looking deformities to wild-caught anurans (Fig. 10).

DISCUSSION

The problem of deformed amphibians has been a major environmental issue for more than a decade. The first study that linked specific types of

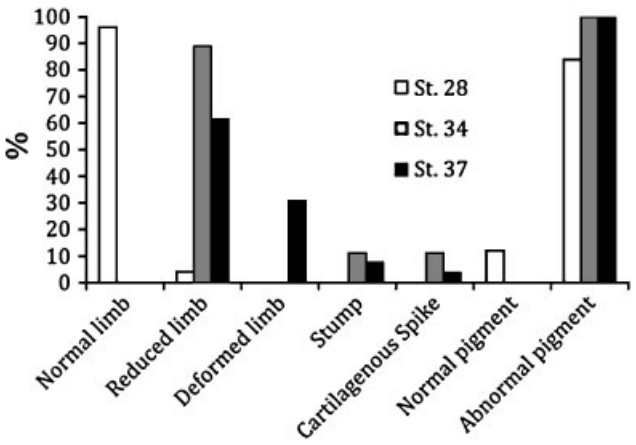

Fig. 7. Hind limb abnormalities resulting from experimental amputations at the indicated Gosner stages of limb development (expressed as percent of total deformities; single specimens could be scored for more than one deformity).

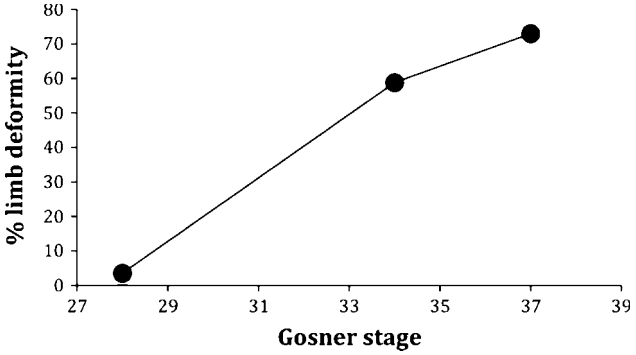

Fig. 8. Relationship between limb abnormalities and developmental stage at which amputation was performed in *R. sylvatica* tadpoles.

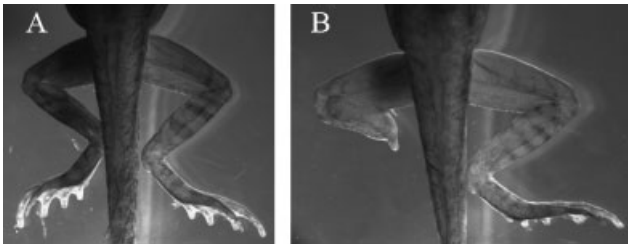

Fig. 9. Regenerative response to experimental amputations at different stages of limb development in wood frogs (*R. sylvatica*); (**A**): amputation of left hind limb at Gosner stage 28 showing completely regenerated limb; (**B**): amputation of left hind limb at Gosner stage 33 showing incomplete regeneration with cartilagenous spike at the end of the stump.

deformities in natural populations of amphibians with a definitive cause was Sessions and Ruth ('90) who found that deformities involving supernumerary limbs were caused by trematode infection.

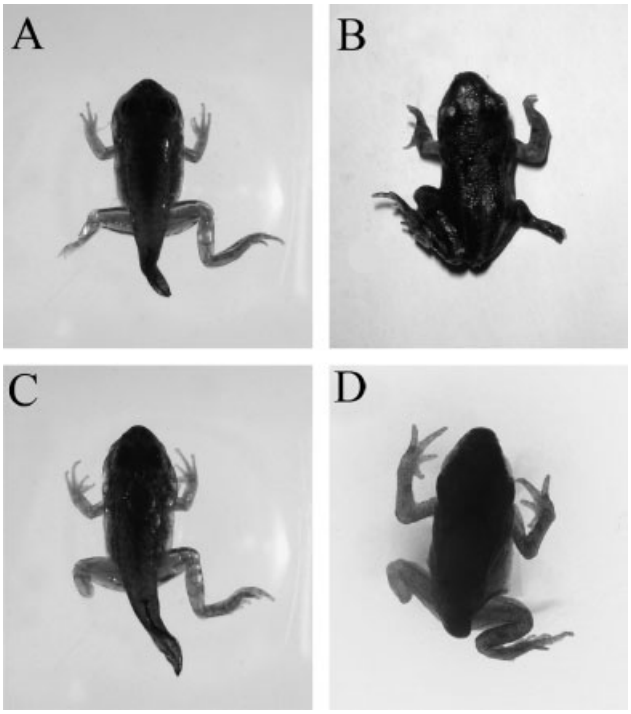

Fig. 10. Experimental amputations (*R. sylvatica* Gosner stage 37, (**A**, **C**) compared with wild-caught deformed frogs (**B**: *R. temporaria*; **D**: *B. bufo*).

A subsequent study identified the trematode as a species of *Ribeiroia* (Sessions et al., '99), later identified as *R. ondatrae* (Johnson et al., 2001). The vast majority of reported deformed amphibians from natural populations of amphibians, however, are deformed anurans, including several species of frogs and toads that feature missing limbs and missing limb segments, with an associated suite of idiosyncratic deformities with no evidence of trematode involvement, and they have turned out to be much more difficult to explain (Ouellet et al., '97; Helgen et al., '98; Meteyer, 2000; Levey et al., 2003; Sessions, 2003; Hoppe, 2005; Skelly et al., 2007; Lannoo, 2008). Although Sessions and Ruth ('90) attributed some limbless deformed amphibians to parasites or predation, the cause of these types of deformities has remained elusive and somewhat controversial (reviewed in Lannoo, 2008). In this study we present evidence, from field observations and experimental studies, that selective predation may be a primary cause of this major category of deformities in natural populations of amphibians. By selective predation we mean predation by those predators that are too small, or have mouth parts that are too small, to consume whole tadpoles and instead snip off or chew small pieces (partial consumption), often releasing their prey who may survive to metamorphosis.

Our experimental studies showed that dragonfly predation appeared to be a major source of traumatic injury to tails and hind limbs of toad larvae, often resulting in the loss of one or sometimes both hind limbs. Without exception, the dragonfly nymphs practiced selective predation in which they ate no more than a portion of the tadpole. Subsequent regenerative response in surviving tadpoles produced abnormal morphologies in the peri-metamorphic toads that resembled those of wild-caught toad metamorphs. Given the ubiquity of dragonfly nymphs in ponds inhabited by amphibians, such selective predation is likely to be a major source of these kinds of deformities in frogs and toads; what is peculiar is not that these kinds of deformities occur, but that there are not more of them.

Some workers have rejected predation as a significant cause for these kinds of deformities on what we consider to be spurious grounds (Gardiner and Hoppe, '99; Meteyer et al., 2000; Lannoo et al., 2003; Levey et al., 2003; Skelly et al., 2007; Lannoo, 2008). Lannoo (2008) for example has argued against ''predation attempt'' as a feasible cause of limb deformities in frogs because most predators would swallow or kill tadpoles at early stages of limb development, and would unlikely be able to precisely remove hindlimbs without causing lethal damage to the rest of the tadpole. However, we found that dragonfly nymphs often release badly injured tadpoles, which can survive at least to metamorphosis. Our results show definitively that injuries by certain kinds of predators can readily account for missing hind limbs in deformed frogs. Simultaneously, selective predation explains the rarity of missing forelimbs, since anuran forelimbs develop within the protection of the gill chamber (McDiarmid and Altig, '99).

Selective predation of tadpoles by aquatic arthropods or other predators has been reported in several studies (Johnson et al., '75; Brodie et al., '78; Manteifel and Reshetnikov, 2002) and may reflect optimal foraging strategies employed by certain predators that cannot fully consume their prey (Cook and Cockrell, '78; Sih, '80; Peckarsky, '82). Formanowicz ('84) observed this behavior in the aquatic predaceous diving beetle (*D. verticalis*) feeding on tadpoles. The advantage of selective predation is that different parts of the tadpoles contain varying degrees of food quality. Also, some portions of the tadpole may be relatively easily

removed, minimizing the cost/benefit ratio by increasing the quality of extracted food relative to handling time of the prey (Formanowicz, '84). Selective predation on toad tadpoles may also reflect the fact that mature skin glands contain bufotoxins, which may explain a preference by odonate nymphs for developing limb buds that are covered by immature skin. We have also observed stickleback fishes selectively predating the tails and hind limbs of *Bufo* and *Rana* tadpoles (J. Bowerman, personal communication; Sessions, 2003).

Selective predation of tadpoles, including the removal of their hind limbs, was reported by Licht ('74,) but was not explicitly linked with the occurrence of hind limb deformities. Several other studies have suggested that limb deformities in amphibians could be caused by predation by various predators. Martof ('56) attributed hind limb deformities in field-collected metamorphic Green frogs (*R. clamitans*) to predator ''attacks'' by aquatic arthropods, fish, and other animals. Veith and Viertel ('93) reported the induction of limb deformities in *Bufo* tadpoles by predatory leeches (*Erpobdella octoculata*). Bohl ('97) also reported possible predation-induced deformities in wild populations of amphibians found in Upper Franconia. Gray et al. (2002) found missing limbs and digits associated with predation in two species of neotropical frogs (*Dendrobates auratus* and *Physalaemus pustulosus*). Recently, Eaton et al. (2004) hypothesized that hind limb deformities found in their studies of western Canadian wood frogs (*R. sylvatica*) were results of predation injuries. Sessions ('97) reported hind limb deformities resulting from cannibalistic interactions in Bullfrog tadpoles at high population densities.

The regenerative response of developing anuran tadpoles to predation-induced hind limb injury probably reflects several factors including developmental stage of the tadpole, extent of traumatic injury, proximal–distal position of the injury along the length of the limb, and even the mode and mechanics of predation by the predator (in this case, dragonfly nymphs). Because of ontogenetic regenerative decline typical of anuran hind limb development (Muneoka et al., '86), injuries to the hind limbs lead to a vast range of possible morphologies between nonregenerated and completely regenerated hind limbs. The resulting array of deformities appears to encompass the range of limb deformities most commonly found in natural populations of anurans (i.e. those that are associated with reduced or missing hind limbs). This variability and diversity in regenerative response probably explains some of the confusing morphologies described in limbless deformed frogs (e.g. Meteyer et al., 2000; Lannoo, 2008).

As with supernumerary limbs (Sessions et al., '99), the deformities often provide morphological clues that give some indication of the cause, even in frogs with missing or reduced limbs. Morphologically irregular cartilaginous spikes and lumps on the ends of limb stumps represent the maximum regenerative response capable of a late stage tadpole and points to the most likely cause (beyond reasonable doubt) as a traumatic amputation. Furthermore, repeated trauma at the ends of limbs can induce tissue destruction and various kinds of cellular accumulations that resemble blastemas (Sessions and Bryant, '88). Our experimental limb bud amputations in wood frogs generated irregular patterns of pigmentation and tissue morphologies in the partially regenerated limbs, resembling the response of tadpoles to limb injury from stickleback attacks (J. Bowerman, personal communication; Sessions, 2003).

Our results provide further assistance in understanding the specific etiologies of deformities in amphibians, especially the most common reported deformities: missing limbs and limb segments. It is our opinion that most hind limb deformities in wild-caught anurans are the result of natural regenerative responses to traumatic injuries from selective predation and, in the case of extra limbs, parasitic infection. The logical conclusion from our study is that variation in the incidence of such deformities involves changes in population densities of predators and even of the tadpoles themselves in the context of extremely complex ecosystems. If predation is occurring at preternatural rates, it could represent changes in amphibian behavior or ontological defenses against predators. Certain types of environmental deterioration, such as increased salinity in freshwater habitats or pesticide contamination, have been shown to increase tadpole susceptibility to predation (Cook, '71; Squires et al., 2008). Organic pollution resulting in eutrophication could facilitate plant growth and increase food and shelter for tadpoles and other organisms, providing resources to support growth of both predator and prey populations in wetlands. The potential involvement of chemical pollution and/or UV-B radiation adds to the ecological complexities of amphibian deformities. However, our studies suggest that selective predation, together with parasite infection, may be sufficient to account for the vast

majority of deformities in natural populations of amphibians.

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