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The Biology of Crawfish Frogs (*Lithobates areolatus*) Prevents the Full Use of Telemetry and Drift Fence Techniques

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One assumption, implicit or explicit, often made by researchers when designing field studies is that techniques that work well in one species will work without qualification in other species, often closely related, in which they have never been employed. This is not always true. During our ongoing work to understand the natural history and life history of Crawfish Frogs (*Lithobates areolatus*) on reclaimed surface coal mines in Indiana, we employed two techniques—internal transmitters for a radiotelemetry-based movement study and drift fences with pitfall traps for a mark-recapture demographic study—that are recommended and proven tools for amphibian field research (Dodd and Scott 1994; Gibbons and Bennett 1974; Gibbons and Semlitsch 1981; Madison 1997; Richards et al. 1994), though not without qualification (Bull 2000; Dodd 1991; Goldberg et al. 2002; Weick et al. 2005). In fact, both drift fence and radiotelemetry studies have been used successfully with closely related species, Gopher Frogs (*L. capito*; Palis 1998; Roznik and Johnson 2009) and Dusky Gopher Frogs (*L. sevosus*; Richter and Seigel 2002; Richter et al. 2001). We report here two previously undescribed difficulties with the implementation of these techniques in Crawfish Frogs.

We have discovered that surgically weakened abdominal musculature following the implantation of internal transmitters can lead to visceral herniation through the incision site in calling male Crawfish Frogs. Secondly, we have observed that upon exiting wetlands and encountering a drift fence, postbreeding Crawfish Frogs do not usually move laterally along the drift fences to then fall into buckets. Instead—perhaps because they must travel in a specific direction, often over great distances, to find a burrow (J. Heemeyer, unpubl. telemetry data)—they often remain against the fence until early in the morning when they then turn around to re-enter the wetland or surrounding vegetation. This appears to be the first time that these two issues have been reported in amphibian field studies.

Transmitter implantation can result in visceral herniation.—The advantages of radiotelemetry are well known, with the one limitation most cited being transmitter longevity, which is related to transmitter size (Richards et al. 1994; Van Nuland and Claus 1981). Smaller transmitters, often necessary for use in smaller animals such as amphibians, have smaller batteries, which have a shorter life, either making for short-term studies or adding to the number of transmitter replacements. External harnesses have been used successfully on Gopher Frogs (Richter et al. 2001; Roznik and Johnson 2009). However, because Crawfish Frogs at our study site must negotiate densely vegetated upland prairie habitat and use small burrows (made by crayfish), we felt external harnesses might interfere with mobility and decided to implant transmitters intraperitoneally.

Intraperitoneal transmitter implantation is often used in amphibians and may be the most effective way of tracking these animals for long periods (Goldberg et al. 2002; Johnson 2006; Madison 1997; Richards et al. 1994; Weick et al. 2005). However, problems can arise both during and after surgery (Goldberg et al. 2002; Weick et al. 2005). Complications among anuran species arise from response to anesthesia (Goldberg et al. 2002; Weick et al. 2005), infection (Werner 1991), tearing of sutures, lesions around sutures, and transmitter expulsion (Weick et al. 2005). Goldberg et al. (2002) note that many anuran telemetry studies fail to discuss deleterious effects of implantation surgeries.

In the present study, Crawfish Frogs (72–188 g) were captured during the spring of 2009 either on their way into breeding wetlands (drift fences) or in breeding wetlands (minnow traps). Because Crawfish Frogs will often quit calling at the least sign of disturbance (Parris and Redmer 2005), and because intraperitoneal transmitters had been considered safe in calling frogs (Goldberg et al. 2002; Lamoureux and Madison 1999), we decided to implant a subset of males (8 out of 59 Crawfish Frog males encountered entering the pond) in order to identify wetland calling sites and where animals find refuge when they are not calling. We implanted animals with Holohil Systems Ltd. (Carp, Ontario) PD-2 transmitters with internal helical antennae (average weight of 3.8 g). Implantation surgeries were initially practiced (by JLH) on Southern Leopard Frogs (*L. sphenocephalus*) under the supervision of a researcher with over 20 years experience with animal surgeries (MJL). Richards et al. (1994) recommend the use of transmitters weighing no more than 10% of body weight; Goldberg et al. (2002) recommends no more than 5%. Our transmitters accounted for 2–5% of total body weight.

Knowing the potential negative effects of anesthesia on anurans (Goldberg et al. 2002), we started with a concentration of 200 mg/L MS-222 (ethyl 3-aminobenzoate methanesulfonic acid salt;

Sigma-Aldrich, St. Louis, MO) dissolved in a buffer solution (500 ml phosphate buffered saline [PBS], pH 7.2, giving the anesthetic solution a pH of 6.8) at room temperature. We observed the animal for 20–30 min and if it was still responsive we added 200 mg/L MS-222. This continued every half hour until the animal became fully anesthetized, indicated by loss of righting reflex and lack of pain response to toe pinching (Johnson 2006). After several surgeries, we were able determine that a concentration of 600 mg/L was optimal. At this concentration, animals usually took 20–30 min to become anesthetized and remained anesthetized through the duration of the surgery (~30 min). Transmitters were placed intraperitoneally by making a left side, off-midline abdominal incision through the skin and a parallel incision through the rectus abdominus (Johnson 2006). After transmitter insertion, the rectus abdominus was closed with five or six continuous (Weick et al. 2005) sutures (Vicryl™ [polyglactin 910] 5-0 RB1, #36; Ethicon, Somerville, NJ), and the skin was closed with five or six continuous sutures (Vicryl™) and glued (Vetbond™ [n-butyl cyanoacrylate] adhesive). Postoperatively, animals were placed in deionized water and observed until they awoke. They were allowed to recover overnight in a cold, dark environment (a cooler placed in a refrigerator) to minimize stress, and then released on the inside (opposite side) of the drift fence, or near the minnow trap, where they were captured.

Postoperatively, we periodically examined animals in the field. At various times following surgeries (5–27 days), we observed asymmetrical ventral swellings in five animals. Swellings were caused by a hard mass of tissue (Figs. 1A, B), not by fluid or air accumulation. We collected these animals, anesthetized them, did exploratory surgery, and found visceral herniations through the rectus abdominus at the point of surgical incision. The liver had herniated in all animals; the left lung, stomach, and/or intestine herniated in a subset of animals. To repair these herniations, the viscera were carefully re-inserted into the peritoneal cavity, the incised edges of the rectus abdominus were trimmed (ensuring blood flow and enabling healing in case the wound was dehisced), the muscle and skin were individually sutured closed as before, and Vetbond™ was applied to the skin. The animals were again kept overnight before they were released at their respective capture locations.

All five animals exhibiting herniations were males entering or trapped in breeding wetlands. The three other males implanted at the same time did not show signs of herniation. Herniations were not, and have not been at any time, seen in females (four females were implanted at the time and eight more females have been subsequently implanted). We (JLH and MJL) observed one of the implanted males calling (distended vocal sacs) and in amplexus prior to discovering its hernia. The breeding call of male Crawfish Frogs has enormous energy, generating sound capable of carrying over a kilometer under favorable acoustic conditions (Busby and Brecheisen 1997; Minton 2001). We suspect the abdominal pressure necessary to generate these calls (Wells 2007) caused the viscera to herniate through the healing incision and the sutures in the muscular wall.

Four of the five reconstructive surgeries were successful. A herniation in one animal was initially misdiagnosed as internal swelling underneath the muscle layer (necropsy showed liver herniation); this animal died the next day. Of the four surviving

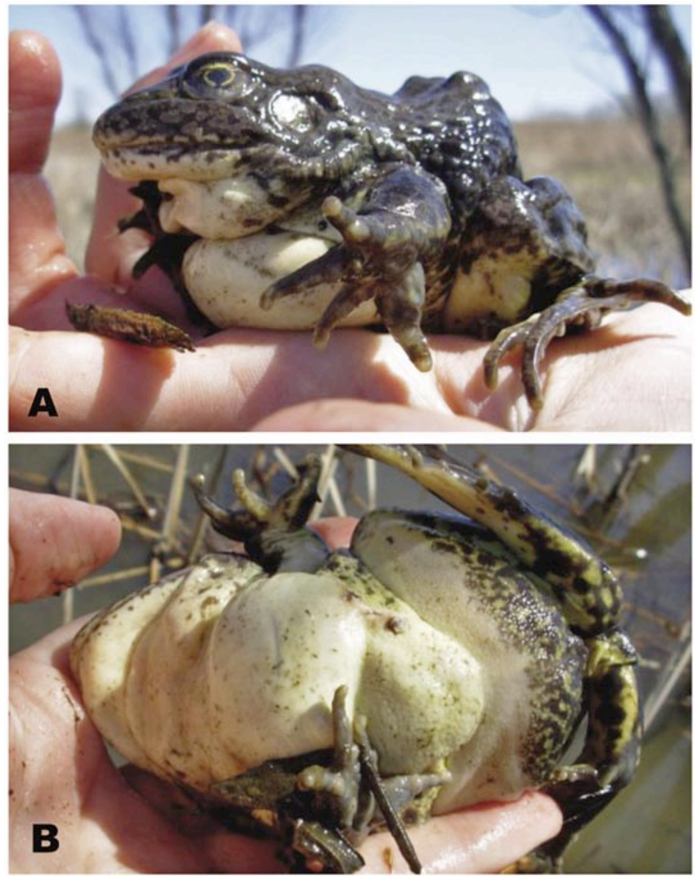

Fig. 1. A) Left lateral oblique view of a male Crawfish Frog (93 mm SVL) showing external appearance of visceral herniation through the rectus abdominus. B) Ventral view of same animal. The more anterior bulge is due to liver herniation and the posterior bulge is due to intestinal herniation.

animals, the transmitter in one was removed during surgery. This frog was returned to its wetland and was captured three days later at the drift fence as it was leaving; it was behaving normally. The three remaining animals were released postoperatively at the point of capture; one died two and a half weeks later from unknown causes. At the time of this writing (~150 days post surgery), the two remaining herniated frogs with transmitters had been tracked since their release and are behaving similarly to implanted animals that did not suffer visceral herniations. One of the two animals was examined 12 days (Fig. 2A) and 40 days (Fig. 2B) after hernia reconstruction and showed no signs of recurrent herniation. Its cutaneous incision had completely healed, although portions of suture material and Vetbond™ remained adherent. These have since dissolved or been worn away.

Drift fences inhibit postbreeding migrations.—Drift fences in combination with pitfall traps constitute a useful tool for amphibian biologists interested in the timing and demographics of breeding populations, as well as measures of reproductive success (Dodd and Scott 1994; Gibbons and Bennett 1974; Richter and Seigel 2002; Semlitsch et al. 1995). One assumption with this technique is that upon encountering a drift fence, an animal will turn left or right to move laterally along the fencing until they tumble into a pitfall trap to be captured and processed by the researcher. Crawfish Frogs entering breeding wetlands seemed to do this, but Crawfish Frogs

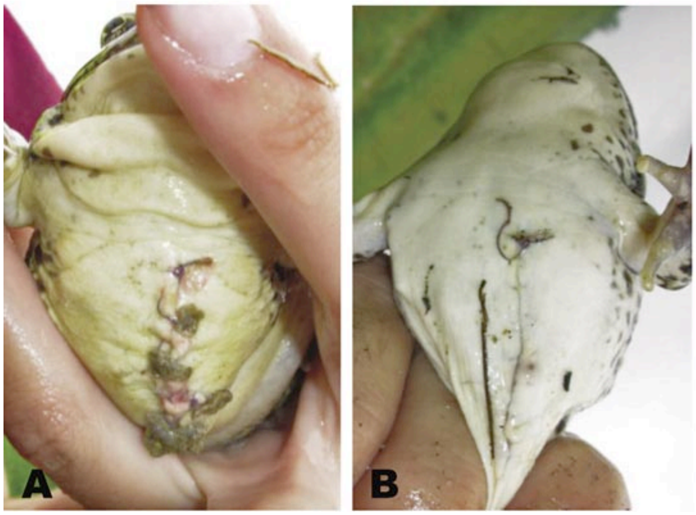

FIG. 2. Ventral view of a male Crawfish Frog (85 mm SVL) showing healing following hernia repair surgery on 16 April 2009. A) The scar on 28 April, 12 days following surgery. B) The scar on 26 May, 40 days after surgery.

exiting wetlands often did not. Difficulties with capturing animals in pitfall traps had not been specifically reported in studies that involved the closely related Gopher Frogs (Palis 1998) and Dark Gopher Frogs (Richter et al. 2001; Richter and Seigel 2002).

On the night of 12–13 April (11°C with 2.0 cm of rainfall), several postbreeding frogs were observed on the inside of the silt drift fence after having left their breeding wetland. Despite this potential emigration activity, on the morning of 13 April, no Crawfish Frogs were found in pitfall traps at this wetland. We suspected escapes, but had had no evidence that frogs trespassed entering this wetland and so doubted Crawfish Frogs could negotiate fences upon leaving. After noting emigration behavior, but an absence of frogs in pitfall traps, we began returning to our study ponds during night-time rains. We found that when emigrating animals encountered the silt drift fence they often tried to work their way through the fence to continue their direction, sometimes laboring until, if at the aluminum hardware cloth portion of our fences, they abraded their snouts (Fig. 3). The hardware cloth—installed in drainage areas to prevent washing out of the silt fences—comprises two sections at one pond (2.86 m, 1.14% of the whole fence) and four sections at the second pond (4.87 m, 1.87% of the whole fence). The silt fence itself, made from woven polypropylene composite, did not injure frog snouts. After realizing this, we began to relocate emigrating Crawfish Frogs across fences. One night alone (19–20 April) we transferred a total of 40 (23 at one wetland, 17 at a second) postbreeding Crawfish Frogs to the outside of fences (a total of 97 animals entered our two drift-fenced breeding wetlands). Overall, 35% of emigrating Crawfish Frogs were relocated in this way, the other 65% were caught in pitfall traps. The use of drift fences may lengthen the duration of Crawfish Frogs in breeding wetlands, and therefore bias results.

FIG. 3. Head-on view of Crawfish Frog (103 mm SVL) whose snout was abraded during attempts to negotiate a direct route through drift fencing.

Recommendations.—The advantages of telemetry and drift fence trapping techniques will typically outweigh disadvantages if certain precautions are heeded (e.g., Gibbons and Bennett 1974). Several measures can be taken to adapt the telemetry and drift fence modifications described here to Crawfish Frogs, which in turn could also be useful for research on other species. In our opinion, implantation of transmitters remains a viable method for Crawfish Frogs, however, we suggest only implanting postbreeding animals to avoid the possibility of visceral herniation. We also recommend using interrupted sutures (Weick et al. 2005), which may provide stronger closure, to close the peritoneal incision. We have wondered whether the notoriously unstable, acidic, nutrient poor, and droughty soils of mine spoils (Brothers 1990) stressed Crawfish Frogs to the point of hindering healing and producing these herniations. At the present time we have no data to support this idea.

Drift fence studies for Crawfish Frogs must be accompanied by all-night visual encounter surveys for emigrating adults during or following rainfall. Animals at, or approaching, fences should be captured and relocated to the opposite side. To prevent abrasion, we recommend using softer perforated fencing in areas where water accumulates, or attaching strips of cloth or Gorilla Tape© (Gorilla Glue Inc., Cincinnati, Ohio) across the bottom of the hardware cloth. A height of two inches would provide ample protection for Crawfish Frogs, and still allow water flow through the fence.

When utilizing field techniques, even well-established techniques, we recommend not assuming that techniques that have worked well on other species will work well on novel species. We suggest incorporating either a period of preliminary analysis or an intense initial observational component to prevent unintended animal injury.

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AMPHIBIAN DISEASES

This section offers a timely outlet for streamlined presentation of research exploring the geographic distribution, host range, and impact of emerging amphibian pathogens, especially the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) and ranaviruses. *Bd* is an emerging pathogen linked to mass mortality and declines of amphibians worldwide, yet Bd has also been detected in amphibians without disease. Ranaviruses also cause mass mortality, but have not yet been linked to large-scale declines. We know relatively little about their global distribution, host range, or impacts on host populations. To improve our understanding of the scope of this issue, we encourage submission of studies that illuminate the geographic distribution, host ranges, and impact of these pathogens on amphibian populations, including research on individual species or groups of species, wild or captive animals, native or non-native species, live animals or museum specimens, environmental samples, and, provided there is sufficient sampling[1], reports of non-detections.

We ask authors to: 1) restrict the Introduction of their paper to a **maximum** of two paragraphs to highlight the context of their study; 2) briefly include both field and laboratory Methods; 3) present Results in a Table, although a map might also be useful, and limited text; and 4) have a short discussion of a **maximum** of three paragraphs to touch upon key findings. Please include the following information in submissions as appropriate: coordinates and description of sampling areas (or please note if locations are extremely sensitive to reveal, and provide general area instead); species name(s) and life history stages examined, as well as other species present; whether samples were collected randomly or just from dead or moribund animals; date of specimen collection; evidence of unusual mortality; numbers of positive and negative samples; disposition of voucher specimens; name of collaborative laboratory or researcher conducting histological sections or PCR analyses; and names of cooperative land owners or land management agencies. We encourage researchers to conduct post-mortem examinations when possible to identify the cause of death when reporting mortalities. We aim to expedite the review and publication process! Please e-mail submissions directly to Associate Editor, Dr. Dede Olson: dedeolson@fs.fed.us.

[1]If a sample of 30 individuals of a particular life history stage of a particular species yields no positive results, and the diagnostic test is highly sensitive, one can conclude that the prevalence of infection is less than 10% with 95% confidence. With a sample of 10 an infection in one of four individuals could go undetected. We encourage researchers to collect sufficient samples that negative results are meaningful.

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Batrachochytrium dendrobatidis in Adult *Notophthalmus viridescens* in North-Central Alabama, USA

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Batrachochytrium dendrobatidis (Bd) has devastated many amphibian populations, especially in tropical areas (Berger et al. 1998; Daszak et al. 1999). However, the geographic distribution of this emerging pathogen is not well known (see http://www.spatia-