Survival and Body Condition of Captive-Reared Juvenile Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Following Translocation to the Wild

Author(s): Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Tony Mong, Jeff Beringer, Mark D. Wanner, Chawna D. Schuette, Jeff Ettling, and Joshua J. Millspaugh

Source: Copeia, 2012(1):150-159. 2012.
Published By: The American Society of Ichthyologists and Herpetologists
DOI: http://dx.doi.org/10.1643/CH-11-024
URL: http://www.bioone.org/doi/full/10.1643/CH-11-024

Copeia 2012, No. 1, 150–159

Survival and Body Condition of Captive-Reared Juvenile Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Following Translocation to the Wild

Catherine M. Bodinof[1], Jeffrey T. Briggler[2], Randall E. Junge[3], Tony Mong[4], Jeff Beringer[5], Mark D. Wanner[6], Chawna D. Schuette[6], Jeff Ettling[6], and Joshua J. Millspaugh[1]

We used radiotelemetry and recapture to monitor survival and body condition of 36 captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) released at two sites on the North Fork of the White River, Missouri, from May 2008 to August 2009. At the end of our study 16 salamanders were alive, 13 had died, and the fate of seven could not be determined. Captive-reared hellbenders released at a site with densely arranged boulders exhibited approximately 1.5-fold higher annual survival (0.7467; daily survival = 0.9992 ± 0.0004 95% CI) than hellbenders released at a site where boulders were patchily distributed (0.4816; daily survival = 0.9980 ± 0.0007 95% CI). When compared to log-transformed length–mass relationships developed for wild hellbenders from the same river in the 1970s, mean body condition of hellbenders at the patchy boulder site was about average at the end of the study (mean residual distance = **2**0.0273 ± 0.0234 SE, *n* = 7; range = **2**0.1375–0.0486), while mean body condition of hellbenders at the dense boulder site was above average (mean residual distance = 0.0423 ± 0.0402 SE; *n* = 8; range = **2**0.0374–0.1088). In addition to lower survivorship and body condition, a greater proportion of hellbenders at the patchy site accrued physical abnormalities (6 of 13 vs. 2 of 14), carried leech parasites (9 of 16 vs. 4 of 14), and carried the fungus *Batrachochytrium dendrobatidis* (3 of 11 vs. 1 of 13). A 'site only' model of survival was most supported, though additional supported models suggested increased mass at release may have increased daily survivorship. While more work is needed to determine the impact of translocation on long-term population dynamics of Ozark Hellbenders, our study demonstrated that about half of a translocated population of captive-reared hellbenders can survive while maintaining or increasing in body condition during their first year post-release, given release sites are well selected.

EFFECTIVE application of translocation to improve wild animal populations requires identifying reasons for translocation success or failure. With over 40% of amphibians currently in decline (Stuart et al., 2004), captive-rearing and repatriation are increasingly used to improve wild populations of frogs and salamanders (Gascon et al., 2007). However, the persistence of original drivers of decline (Rickard, 2006; Fellers et al., 2007), emigration of translocated animals away from release sites (Matthews, 2003), and inadequate habitat (White and Pyke, 2008) have plagued amphibian translocation programs. Survivorship of translocated populations can directly influence establishment of a self-sustaining population (Muths et al., 2001; White and Pyke, 2008). Pilot studies involving relatively small but closely monitored release cohorts can identify survivorship of translocated populations and help determine whether translocation may be an effective conservation strategy for a particular species.

Captive-rearing and translocation are being considered as potential strategies to augment wild populations of Hellbender salamanders (*Cryptobranchus alleganiensis*) throughout the species' range. Hellbenders are long-lived (Taber et al., 1975), fully aquatic, lotic amphibians that require cool water, abundant rock cover, and crayfish for prey (Nickerson and Mays, 1973a, 1973b; Williams et al., 1981). Two subspecies exist, including the Eastern Hellbender (*C. a. alleganiensis*) that ranges from New York south to Georgia and west into Missouri, and the Ozark Hellbender (*C. a. bishopi*) that is endemic to the Black and White river drainages in southern Missouri and northern Arkansas. Hellbenders have declined throughout their native range (Mayasich et al., 2003; Foster et al., 2009; Federal Register, 2010a), and evidence in Missouri alone indicates populations have decreased by 77% since the mid-1980s (Wheeler et al., 2003). Among the 17 states where hellbenders occur, they are listed as endangered in five, as a species of concern in four, and are legally protected in five others (Federal Register, 2010a). Due to a small endemic range, the Ozark Hellbender was recently proposed to be federally listed as endangered under the Endangered Species Act (Federal Register, 2010b). Reasons for hellbender declines are poorly understood but may include alterations to water quality via land use changes (Foster et al., 2009) or aquatic contaminants (Huang et al., 2010), illegal harvest (Nickerson and Briggler, 2007), introduced species (Gall and Mathis, 2010), or disease caused by infection of the chytrid fungus, *Batrachochytrium dendrobatidis* (hereafter *Bd*; Briggler et al., 2008; Bodinof et al., 2011). Captive-rearing and translocation may be one way to increase the number of juveniles surviving to adulthood as well as the number of young females (7–16 years), which have great reproductive potential (Peterson et al., 1988). However, the cost of captive-rearing hellbenders is considerable, and no studies have investigated survival of captive-reared hellbenders in the wild.

Our goal was to monitor survival and body condition of captive-reared Ozark Hellbenders. Specifically our objectives

[1] University of Missouri, 302 ABNR, Department of Fisheries and Wildlife Sciences, Columbia, Missouri 65211; E-mail: (CMB) bodinofc@missouri.edu; and (JJM) millspaughJ@missouri.edu. Send reprint requests to CMB.
[2] Missouri Department of Conservation, Jefferson City, Missouri 65102; E-mail: jeff.briggler@mdc.mo.gov.
[3] Saint Louis Zoological Park, Department of Animal Health, Saint Louis, Missouri 63110; E-mail: junge@stlzoo.org.
[4] Wyoming Game and Fish Department, Baggs, Wyoming 82321; E-mail: tony.mong@wgf.state.wy.us.
[5] Missouri Department of Conservation, Resource Science Center, Columbia, Missouri 65201; E-mail: jeff.beringer@mdc.mo.gov.
[6] Saint Louis Zoological Park, Department of Herpetology and Aquatics, Saint Louis, Missouri 63110; E-mail: (MDW) wanner@stlzoo.org; (CDS) schuette@stlzoo.org; and (JE) ettling@stlzoo.org.
Submitted: 25 February 2011. Accepted: 7 October 2011. Associate Editor: M. J. Lannoo.
 DOI: 10.1643/CH-11-024

were to estimate daily survival rates of captive-reared Ozark Hellbenders released at two study sites on the North Fork of the White River Missouri and identify factors correlated with survival, compare body condition of captive-reared hellbenders before and after release, and document factors associated with health of captive-reared hellbenders following release, including leech parasitism, accrual of physical injuries, and contraction of *Bd*.

MATERIALS AND METHODS

Study site description.—The North Fork of the White River (hereafter NFWR) is a seventh order, primarily spring-fed, stream flowing south into Arkansas from south-central Missouri. The NFWR watershed is dominated by forested woodland and crop and grassland. The river is a popular canoeing and fishing destination, especially from late May through August. Substrate throughout the river is characterized by long stretches of pebble and gravel beds interspersed with bedrock, and beds of dolomite and limestone slabs that historically provided (Nickerson and Mays, 1973a, 1973b; Peterson et al., 1983), and still appear to provide, suitable habitat for Ozark Hellbenders. Crayfish are the predominant prey of hellbenders (Peterson et al., 1989) and three species, including *Orconectes neglectus*, *O. longidigitus*, and *O. punctimanus* are common throughout the NFWR. Nickerson and Mays (1973b) reported densities of approximately one hellbender/8–10 m^2 of streambed in a riffle with numerous suitably sized rocks. However, populations throughout the river declined by approximately 70% between the early 1980s and late 1990s (Wheeler et al., 2003). Predators of hellbenders may include the Common Raccoon (*Procyon lotor*), North American River Otter (*Lontra canadensis*), and American Mink (*Mustela vision*), all of which are common along the NFWR. The disease causing fungus *Bd* has occurred in NFWR hellbender populations since at least 1969 (Briggler et al., 2008; Bodinof et al., 2011). However, while *Bd* infection causes high rates of mortality in some amphibians (Berger et al., 1998), its lethality for hellbenders remains poorly understood.

We selected two release sites (hereafter upper and lower), each approximately 1 km long, that contained at least one relatively large and continuous patch of boulder substrate that we defined as 'core habitat.' Study sites were separated by approximately 17 km of river. We choose not to reveal the discrete location of sites due to the status of Ozark Hellbenders and threats of illegal collecting. Fewer than ten wild hellbenders were detected in core habitat of either site during prior surveys. Therefore, the density of wild conspecifics was assumed to be extremely low compared to historical accounts from the same river (Nickerson and Mays, 1973b).

Wetted width throughout the upper site ranged from 15–50 m, and mean monthly water temperature, estimated from random samples collected three to five times per week, ranged from 5.03uC in January 2009 to 23.55uC in August 2009. Upper site core habitat (extent 5 3,300 m^2) consisted of patchily arranged cobble and boulder clusters that overlapped bedrock slabs with deep crevices, along a 350 m reach. Crayfish density in core habitat, estimated from random sampling of runs and riffles (,1 m deep), using a 1 m kick-seine technique (Mather and Stein, 1993) in August 2008 was 12.16 6 2.4 SE crayfish/m^2.

In general, the lower site was wider, deeper, and reached less extreme temperatures than the upper site. Wetted width ranged from 48–88 m, and mean monthly water temperatures estimated from random sampling within the lower site ranged from 8.61uC in January 2009 to 20.96uC in August 2008. Lower site core habitat (extent 5 7,700 m^2) lacked bedrock with crevices, but included densely arranged cobble and boulder over a relatively continuous extent throughout a 320 m reach. The lower site occurred within a portion of the NFWR designated as a Blue Ribbon Trout Zone (daily limit is 1 trout - 18 inches) that is periodically stocked with non-native Rainbow (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta*). Random sampling (described above) indicated that lower site crayfish density in August 2008 was 12.61 6 1.52 SE crayfish/m^2.

Study animals.—We monitored 36 juvenile captive-reared Ozark Hellbenders that were released in an effort to augment remnant wild populations. Hellbenders were hatched from eggs collected by Unger (2003) from the NFWR in 2002 and were reared in captivity from 2003 to 2008 at the Saint Louis Zoo's Ron Goellner Center for Hellbender Conservation. In captivity, hellbenders were maintained in groups of six to ten individuals per 75-Liter aquarium from 2003 through 2006, and in groups of two to four individuals per 150-Liter aquarium from 2006 through 2008. We lined aquaria with pebble, cobble, and boulder substrate and continuously circulated chilled, oxygenated water to mimic wild conditions. Initially we tong fed hellbenders a diet of crayfish, krill, lake smelt (*Osmerus* sp.), and night crawlers. We successfully weaned hellbenders from tong feeding approximately six months prior to release, after which they demonstrated successful foraging in captivity. Hellbenders received regular health exams by zoo veterinarians prior to release. Polymerase chain reaction (PCR) assay confirmed absence of *Bd* from the entire release cohort from swabs collected at weekly intervals during the three weeks immediately prior to release.

Prior to release, each hellbender was implanted with an AVID passive integrated transponder used for individual identification, and a Sirtrack Limited model RVI 118 (*n* 5 9) or RVI 218 (*n* 5 18) radio transmitters, permitting the model weighed #5% of hellbender mass. Model RVI 118 measured approximately 30 mm 3 13 mm 3 8 mm, weighed 5–6 g, and had an estimated battery life of 7.5 months. Model RVI 218 measured approximately 35 mm 3 15 mm 3 15 mm, weighed 9–10 g, and had an estimated battery life of 15 months. We implanted hellbenders in three cohorts between May and September 2008 to allow smaller animals to reach sufficient weights for transmitter attachment (140 g for RVI 118 and 180 g for RVI 218). Mean mass of hellbenders at surgery was 202 6 9 g SE (*n* 5 36; range 5 142–334 g), mean total length was 319 6 4 mm SE (*n* 5 36; range 5 285–368 mm), and gender of hellbenders was unknown. At surgery, we anesthetized each hellbender in a 250 mg/L solution of tricaine methanesulfonate (MS222) buffered with sodium bicarbonate (baking soda). Coelomic implantation of transmitters followed methods described in Bodinof (2010). We closed the body wall and muscle layers with three sutures and closed the skin with three (3/0 polydioxane or nylon) sutures. After surgery, we injected each hellbender with 10 mg/kg of enrofloxacin (antibiotic) and monitored them until voluntary swimming occurred (within 30 minutes). We released hellbenders 14 to 28 days following transmitter implant unless we observed dehiscing of the sutures, in which case we repaired sutures and held animals for an additional 14 to 28 days prior to release. Following the staggered fashion of surgeries, we released 18 randomly selected hellbenders at each site over four discrete release events, occurring on 19 May (*n* 5 10), 29 May (*n* 5 7), 11 August (*n* 5 9), and 3 October (*n* 5 10). On the day of release we transported hellbenders to the field by vehicle and released them by hand at pre-selected rocks spaced $5m from the nearest known hellbender. In addition to initial implant surgeries, seven hellbenders were removed from the wild and experienced a second surgery to replace a transmitter that failed prematurely or was scheduled to expire $four months prior to the end of our study (Bodinof, 2010). Recaptures for transmitter replacement occurred 82, 220, and 155 days post release, respectively, for three upper site hellbenders; and at 154, 155, 162, and 299 days post release, respectively, for four lower site hellbenders. Hellbenders that received a second surgery were recaptured by hand, quarantined, and transported to the Saint Louis Zoo. We removed expired transmitters before implanting a new unit following methods identical to initial implantation surgeries. We re-released hellbenders on 12 May 2009, resulting in an approximate eight-week absence from our study, with the exception of one hellbender recaptured in August 2008 (82 days post release) that was absent for nine months to ensure healing and avoid a winter re-release.

Monitoring survival.—We monitored translocated hellbenders by wading or canoeing (if water depth $ 1.5 m) and using homing procedures (White and Garrott, 1990) with a three element Yagi antenna and a handheld (Advanced Telemetry Systems, Isanti, MN) receiver. To monitor survival, we located hellbenders approximately every 3264 hours from release through 14 November 2008 and from 26 March through 21 August 2009. During winter (15 November 2008–25 March 2009), we located hellbenders approximately once each week. Throughout the study, we attempted to visually confirm status (alive or dead) of each hellbender at least once per week by snorkeling or with an Aqua-Vu SV 100 (Outdoor Insights, Inc., Crosslake, MN) camera.

Monitoring body condition.—To monitor body condition, we recaptured 19 hellbenders (upper site: *n* 5 7; lower site: *n* 5 12) in fall 2008 (29 September–6 December), 13 hellbenders (upper site: *n* 5 5; lower site: *n* 5 8) in spring 2009 (6 March–1 June), and 15 hellbenders (upper site: *n* 5 7; lower site: *n* 5 8) at the end of our study (14 July–29 August). We recaptured hellbenders by hand, lifting cover rocks only when necessary, and making an effort to minimize habitat disturbance. To prevent disease transmission, we sterilized equipment and replaced gloves before handling each hellbender. Upon recapture, we identified gender of hellbenders based on the presence of testes or egg follicles via a portable ultra-sound, or when hellbenders exhibited a swollen cloaca (male) or appeared gravid (female). We measured total length and snout-to-vent length to the nearest mm, and weighed each animal to the nearest gram using an Ohaus (Ohaus Corporation, Pine Brook, NJ) digital balance.

Monitoring health.—To monitor factors associated with health of translocated hellbenders, we swabbed hellbenders at each recapture to detect presence of *Bd* via PCR assay following methods of Briggler et al. (2008), counted the number of external parasites (leeches), and documented physical abnormalities on recaptured and opportunistically re-sighted individuals. We recorded the number and location of leeches, along with tail abnormalities (notches or tears), abnormalities of the digits (missing, supernumerary, fused, or reduced), abrasions or scars (including scrapes, bite marks, or scratches), and open sores (necrotic sores or open flesh wounds) on diagrams representing the dorsal and ventral surface of each animal.

Survival analysis.—We completed one preliminary analysis to determine if there were differences in survival rates by gender (males and females), dispersal type (abrupt-long-distance, non-disperser, and slow-and-steady disperser; Bodinof, 2010), or site (upper or lower). Given the low number of hellbenders in this analysis, we wanted to increase the viability of a single analysis by including the least number of group variables. We compared a gender model, a dispersal type model, and a site model to a constant model using the known-fate model with a logit link function within Program MARK (White and Burnham, 1999) and Akaike's Information Criterion for small sample sizes (AIC_c; Burnham and Anderson, 2002). We left-censored (fate was unknown and not included in the estimation) individuals until the day they were released, and we right-censored data (fate is defined as alive on the last observation and included in estimation only to that point) if the transmitter failed, a hellbender was removed temporarily for transmitter replacement, or if an individual was not relocated on the last day of the study. Mortalities were coded on the day of discovery unless more than one day had passed since the last observation, in which case the hellbender was coded as a mortality on the day immediately following the last day the animal was found alive (e.g., hellbender alive on 1 December but found dead on 3 December, was coded as a mortality on 2 December). Results of this preliminary analysis demonstrated support for the site model only. The constant only model was supported over gender or dispersal type models, indicating we could not differentiate survival rates by gender or dispersal type. Consequently, we pooled data across gender and dispersal type and used site as a grouping variable in the next stage of analysis.

After pooling data by gender and dispersal type, we developed eight models to assess the relative importance of factors in explaining hellbender survival rates. We used site and time covariates that considered month (m), sampling season (19 May–14 November, 15 November–25 March, 26 March–21 August), and days post initial release (time). Our saturated model included an interaction term between *t* and site (time 3 site). We fit reduced models for site, the biologically relevant time scale (month and season) and interaction terms between site, month, and season (site 3 month; season 3 site). Also, we modeled survival based on the interaction between initial mass (mass) and the number of days held after surgery before initial release (dtr) and site (mass 3 site and dtr 3 site). We used the known-fate model with a logit link function within Program MARK (White and Burnham, 1999) to estimate survival rates of hellbenders. We used Akaike's Information Criterion for small sample sizes (AIC_c; Burnham and Anderson, 2002) to assess the relative support among candidate models. We report the logit-transformed 95% confidence intervals for survival estimates.

Body condition analysis.—To evaluate body condition, we plotted log-transformed length–mass associations for hellbenders in our study against regression lines representing

Table 1. Model Selection Results for Survival Analysis of Captive-Reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Released at Two Sites on the North Fork of the White River, Missouri, USA, 2008–2009.

Model	k[a]	AIC_c[b]	$DAIC_c$	w_i[c]	Deviance[d]
S(site)	2	198.651	0.000	0.605	194.650
S(site 3 dtr[e])	4	201.207	2.556	0.168	193.203
S(site 3 mass[f])	4	201.621	2.970	0.137	193.617
S(season)	4	202.689	4.035	0.080	194.681
S(site 3 season)	8	207.287	8.636	0.008	191.272
S(month)	12	210.313	11.663	0.002	186.281
S(site 3 month)	24	221.598	22.947	0.000	173.471
S(site 3 time[g])	920	2122.401	1923.750	0.000	83.803

[a] Number of parameters
[b] AIC_c 5 Akaike Information Criterion for small samples
[c] Akaike weight
[d] Difference in 22log(Likelihood) of the current model and 22log(Likelihood) of the saturated model
[e] Days from surgery to release
[f] Mass at time of release
[g] Time (days) since release

log-transformed length–mass relationships for hellbenders in the NFWR that were developed in the late 1970s, when populations appeared healthy (Peterson et al., 1983). We measured the difference between observed and predicted log-transformed body mass (residual distance) of hellbenders in our study based on total length for all 36 hellbenders prior to release and for all individuals recaptured at the end of the study. We calculated residual distance for individual hellbenders using gender specific (when gender was confirmed during the study) or pooled-gender regression equations (Peterson et al., 1983); and report mean residual distance as a population level measure of body condition after pooling across known and unknown genders.

RESULTS

At the end of our study, 16 hellbenders (6 F, 5 M, 5 U) were alive, 13 (5 F, 4 M, 4 U) had died, and status of seven hellbenders (2 F, 1 M, 4 U) was undetermined. Five mortalities occurred within 30 days post release, including three hellbenders whose sutures had dehisced post-release and two cases where only the transmitter was recovered within 1 m of the stream bank or on land. Of the other eight mortalities, two occurred in captivity following transmitter replacement surgery; one case was symptomatic of chytridiomycosis which was later confirmed from histological examination of epidermis from a digit; one case occurred where a hellbender was buried alive by bed load during a high water event; and six mortalities were due to unknown causes with only the transmitter recovered, though in one of these cases the radio signal was tracked to a Common Snapping Turtle (*Chelydra serpentina*).

Hellbender use of bedrock crevices and immovable rocks resulted in unequal catchability. Of the 36 individuals released, 25 were captured alive at least once, eight were never recaptured, and three were only recaptured post-mortem.

Survival.—The site model was most supported (Table 1); however, there was some model uncertainty and we considered models ,3 AIC units of the top model as supported. Daily survival was higher at the lower site (0.9992 6 0.0004 CI) compared to the upper site (0.9980 6 0.0007 CI, Fig. 1). There was no support for season or month; therefore, we assumed this constant daily survival rate over time (Fig. 1). The annual survival rate of hellbenders at the lower site (0.7467) was about 1.5-fold higher than at the upper site (0.4816; Fig. 1). The second most supported model contained an interaction between site and the number of days from time of surgery to release (dtr 3 site), and indicated that survival decreased as individuals were held longer following surgery (Fig. 2). However, survival estimates according to the 'dtr 3 site' model were highly variable, as noted by the confidence intervals (Fig. 2). The only other model within three AIC units of the top model indicated that as weight increased, daily probability of survival increased (Fig. 3).

Body condition.—Residuals based on both pre-release and end of study data fell within the 95% confidence limits of length–mass relationships developed by Peterson et al. (1983), indicating that throughout the study, body condition of captive-reared hellbenders was consistent with body condition of hellbenders historically encountered in the same river when populations appeared healthy. Residuals generated from pre-release data typically fell just below regression lines for both upper (mean residual distance 5 20.0210 6 0.0143 SE; *n* 5 18; range 5 20.1209–0.0997) and lower populations (mean residual distance 5 20.0716 6 0.0094 SE; *n* 5 18; range 5 20.1429–0.0038; Fig. 4). While hellbenders from the upper site tended to maintain average body condition throughout the study (end of study mean residual distance 5 20.0273 6 0.0234 SE; *n* 5 7; range 5 20.1375–0.0486), most hellbenders at the lower site had achieved above-average body condition by the end of the study (end of study mean residual distance 5 0.0423 6 0.0402 SE; *n* 5 8; range 5 20.0374–0.1088; Fig. 4). At the end of the study, body mass of individuals from the upper site was more variable but averaged about 5% less than predicted based on total length (mean 5 94.07 6 4.90% SE; *n* 5 7; range 5 72.87–111.84%), while body mass of lower site individuals averaged about 10% higher than predicted, regardless of gender (mean 5 110.65 6 3.59% SE; *n* 5 8; range 5 92.63–128.47%).

Health.—Three of 11 upper site hellbenders and one of 13 lower site hellbenders that were recaptured at least once and swabbed to detect presence of *Bd* via PCR assay tested

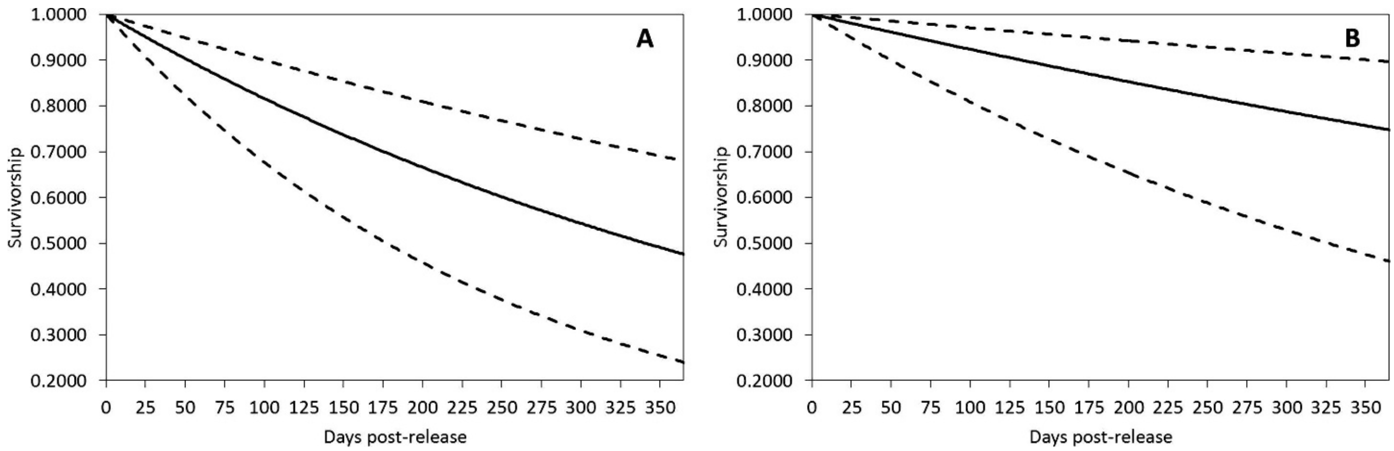

Fig. 1. Survival rate over one year for captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (A) and lower (B) sites on the North Fork of the White River, Missouri, USA, 2008–2009. The solid line represents the survival estimate and the dashed lines represent the 95% confidence intervals.

positive for the fungus. A male hellbender contracted the fungus and died within 70 days post release. Three hellbenders tested positive but survived to the end of the study. An upper site female was blue-gray in color, shedding heavily, and tested positive at 73 days post release; however, swabs collected at 337 and 372 days post release were negative for *Bd*. A lower site female was negative at 157 days post release but positive at 454 days post release, and one upper site hellbender (unknown gender) was first recaptured and tested positive on day 446 post release.

Thirteen of 30 translocated hellbenders that were recaptured, opportunistically re-sighted, or recovered after death were observed carrying the leech *Placobdella cryptobranchii,* which is thought to be an endemic external parasite of Ozark Hellbenders (Moser et al., 2008; Huang et al., 2010). Twice as many animals carried the leech at the upper site (9 of 16) as at the lower site (4 of 14). The mean parasite load was 5.3 6 1.8 SE leeches per individual (range 5 1–20).

Three times as many hellbenders accrued physical abnormalities at the upper (patchy-boulder) site (6 of 13) as at the lower (dense boulder) site (2 of 14). Following translocation, almost half of the hellbenders at the upper site accrued scars or abrasions (*n* 5 6) or open sores or flesh wounds (*n* 5 3). In comparison, hellbenders at the lower site accrued only tail notches (*n* 5 1) or open sores (*n* 5 1). Three of the six hellbenders that accrued scars or abrasions at the upper site simultaneously tested positive for *Bd*. None of the salamanders observed with open sores, which typically occurred on the ventral surface of the feet, simultaneously tested positive for *Bd.*

DISCUSSION

Our study demonstrated that approximately half of a translocated population of captive-reared hellbenders could survive and maintain or improve in body condition during their first year post-release. In contrast, despite releasing more than 1000 individuals, translocated populations of Boreal Toads (*Bufo boreas;* Muths et al., 2001), Blanchard's Cricket Frogs (*Acris crepitans blanchardi*; Rickard, 2006), and Mountain Yellow-Legged Frogs (*Rana muscosa*; Fellers et al., 2007) were non-evident or had declined precipitously at most release sites within one year following release. In addition to the potential persistence of factors driving original declines (Dodd and Seigel, 1991), translocated animals face the added stress of translocation (Tiexiera et al., 2007), lack of prior knowledge of release sites (Stamps and Swaisgood, 2007), and captive-reared animals in particular may be limited by a naivety to stressors in a wild environment (i.e., predators, food availability). While some studies report

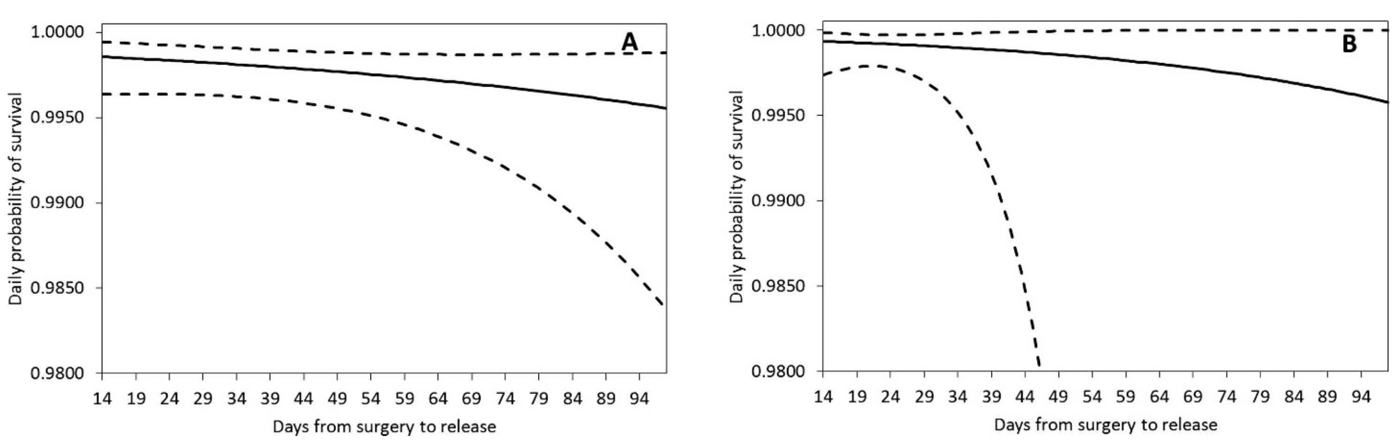

Fig. 2. Daily survival (survival from one day to the next) of captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (A) and lower (B) sites on the North Fork of the White River, Missouri, USA, 2008–2009, based on days from the time of surgery to release. The solid line represents the predicted survival estimate and the dashed lines represent the 95% confidence intervals.

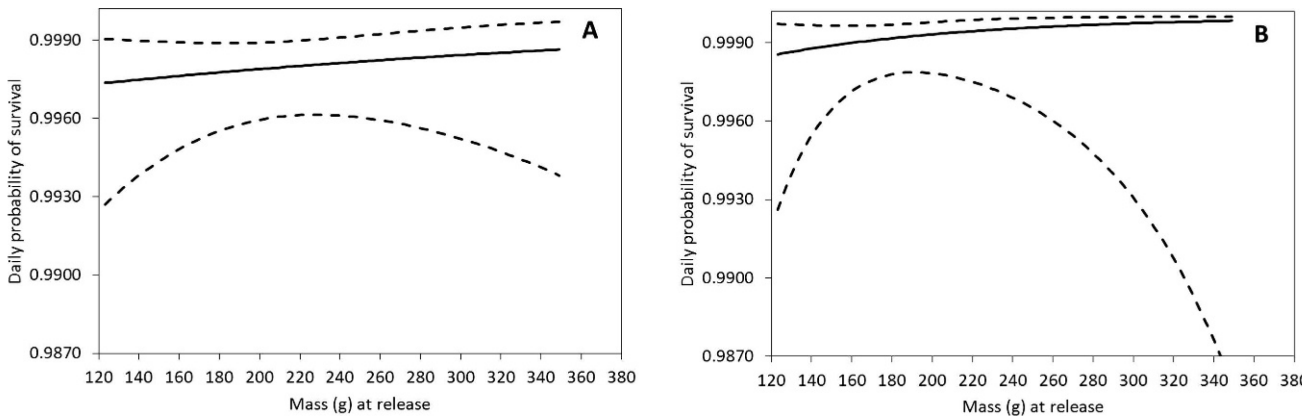

Fig. 3. Daily survival (survival from one day to the next) of captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (A) and lower (B) sites on the North Fork of the White River, Missouri, USA, 2008–2009, based on mass (g) at time of release. The solid line represents the predicted survival estimate and the dashed lines represent the 95% confidence intervals.

reduced survival of translocated amphibians immediately following release (Cooke and Oldham, 1995; Tocher and Pledger, 2005), we detected no influence of time since release on hellbender survival. Therefore, acute impacts to survival resulting from captive-rearing or the stress of translocation may have been minimal or absent for hellbenders in our study. More work is needed to determine the impact translocation has on long-term population

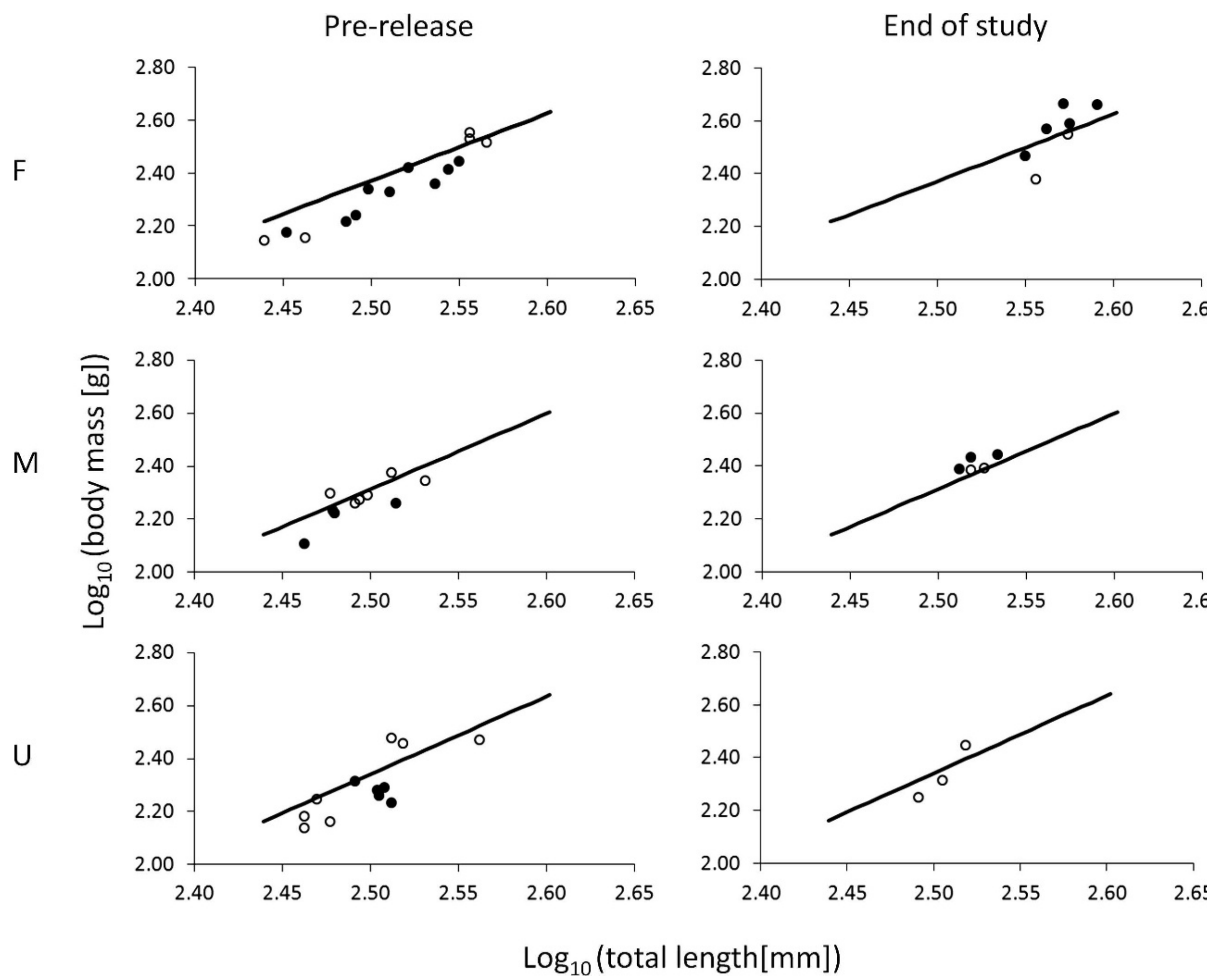

Fig. 4. Comparison between length–mass associations for captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis* bishopi) translocated to the upper (white circles) and lower (dark circles) sites on the North Fork of the White River, Missouri, USA, 2008–2009, and historic length–mass associations (regression lines) developed separately for male (M), female (F), and pooled gender (U) wild Ozark Hellbenders from the same river in the late 1970s. Regression lines represent log-transformed length–mass relationships developed by Peterson et al. (1983). Residuals around the line in 'pre-release' plots represent all 36 captive-reared hellbenders released, and in the 'end of study' plots represent the seven upper and eight lower site hellbenders recaptured at the end of the study.

dynamics of Ozark Hellbenders, but translocation led to a short-term increase in the number of sexually mature individuals in a population.

The survival rate of captive-reared Ozark Hellbenders released at the lower (dense boulder) site was similar to survival estimates of similar age wild conspecifics. Mark–recapture data collected from NFWR hellbender populations in 1977 and 1978, when hellbender abundance appeared robust, indicated annual survivorship of post-metamorphic male and lumped sexed-and-unsexed individuals was approximately 0.81 and fairly constant across age classes (Peterson et al., 1983). Although we found no support for an effect of time since release on captive-reared hellbender survivorship, our study occurred over short temporal scale relative to the 20+ year life span of hellbenders. Data collected by Taber et al. (1975) indicated that survivorship of Eastern Hellbenders in the Niangua River, Missouri, followed a Deevey (1947) Type III curve where probability of survival gradually increased with age. We hypothesize that survivorship of captive-reared hellbenders may also increase over time.

Though we released all captive-reared hellbenders within the same stream, release site was associated with a nearly a 1.5-fold difference in annual survivorship. Resource selection analyses generated independently for both captive-reared populations and the site differences we noted in arrangement of boulders throughout core habitats suggest that habitat quality may have affected survival. Resource selection functions indicated that utility of a resource at both sites was maximized when coarse substrate (cobble or boulder) was present and adjacent to (touching) a substrate particle large enough to provide cover (i.e., with at least one axis $ 15 cm; Bodinof, 2010). While both populations exhibited similar resource selection patterns, the extent of habitat selected by hellbenders was greater at the lower site. For example, cobble or larger particles were about three times more likely to be encountered at randomly selection locations within the lower site (591 of 1761, or 33% of locations) than within the upper site (171 of 1387, or 12% of locations). Additionally, in areas where cobble or boulders occurred, mean distance to the nearest cobble or boulder at the lower site (0.35 m 6 0.11 m SE; *n* 5 319) was approximately half that of the upper site (0.59 m 6 0.26 m SE; *n* 5 100; Bodinof, 2010). Therefore, availability of high quality habitat may have been a limiting factor for upper site hellbenders. Although previous surveys of both release sites indicated relatively few wild hellbenders were present, bedrock ledges and extremely large boulders embedded along the bank were more prevalent within upper site core habitat and may have limited detectability, and led to underestimates of resident density. We speculate that the density of conspecifics, density or abundance of predators, prevalence of sub-lethal stressors, or a synergy of these factors may explain the site differences we noted in survivorship of captive-reared hellbenders. Regardless of the ultimate causes for mortality in our study, our observations demonstrate that survival can differ among discrete habitat patches within the same stream. We encourage the use of pilot translocations to identify site differences that may not be evident otherwise, especially prior to releasing large numbers of individuals, which has been associated with translocation success (Germano and Bishop, 2009).

In addition to survival rates, the site differences we observed in body condition, injuries, and leech parasitism indicated that habitat within the upper site presented a harsher environment for captive-reared hellbenders. However, it is unclear whether the difference between upper and lower sites should be described as ‘poor versus good’ or ‘good versus better.’ For example, despite the slightly higher body condition exhibited in lower site hellbenders at the end of the study, comparisons between our data and length–mass relationships produced for apparently healthy historical populations indicated that upper site hellbenders exhibited average body condition, while lower site animals exhibited slightly higher than average body condition. We are unable to determine the proximate cause for reduced body condition in upper site hellbenders. Several herpetofauna translocations report body mass loss or reduced weight gain correlated with an abnormally high rate of movement post-release (Reinert and Rupert, 1999; Matthews, 2003; King et al., 2004). In contrast, we see no evidence to suggest that body condition was related to hellbender movement. For example, upper site hellbenders moved less often and covered shorter distances on a daily basis than lower site hellbenders (Bodinof, 2010). Additionally, among upper site hellbenders surviving at the end of our study, two individuals exhibiting lower than predicted body condition had dispersed ,20 m from the point of release, while a hellbender exhibiting higher than predicted body condition had dispersed over 545 m. The increased prevalence of leeches, injuries, and *Bd* that we observed in upper site hellbender may have attributed to reduced body condition. Sub-lethal injury has been linked to reduced growth, survival (Semlitsch and Reichling, 1989), and reproductive effort in other amphibians (Bernardo and Agosta, 2005). The frequency of leech parasitism and parasite loads we observed in upper site hellbenders were similar to frequencies (67–100%) and parasite loads (4.5 6 3.5 SD) observed in wild NFWR hellbenders from 2002–2005 (Moser et al., 2008). Therefore, while parasitism appeared more common at the upper site, we see no evidence to suggest captive-reared Ozark Hellbenders were parasitized at higher rates than wild individuals. Because stress is likely to increase with parasitism (Esch et al., 1975), injury, or disease, and heightened stress can inhibit feeding in amphibians (Carr, 2002), the reduced survival and body condition of captive-reared hellbenders at the upper site may be explained by the differences in sub-lethal stressors.

In addition to considering the influence of site, the mass at which to release captive-reared hellbenders may be an important consideration for future translocations. The ‘dtr 3 site’ model indicated that holding hellbenders longer post-surgery might negatively influence survival; however, this model may have been confounded by other factors. Because we required a minimum weight for transmitter implant the largest hellbenders tended to be released in the first cohort at each site, and because we observed dehiscing sutures in some animals from the first release cohort, hellbenders in later cohorts were held longer (30–98 days) than the first cohorts (13–24 days). As a result, the ‘site 3 dtr’ model likely reflected higher survival of larger animals, similar to the ‘site 3 mass’ model, rather than higher survivorship of animals held for fewer days post-surgery. Among translocated herpetofauna, pre-release mass was not a strong predictor of survival for Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*; King et al., 2004) or Egyptian Tortoises (*Testudo klienmanni*; Attum et al., 2010); however, higher survival was noted in larger captive-reared juvenile Redbelly Turtles (*Pseudemys rubriventris*;

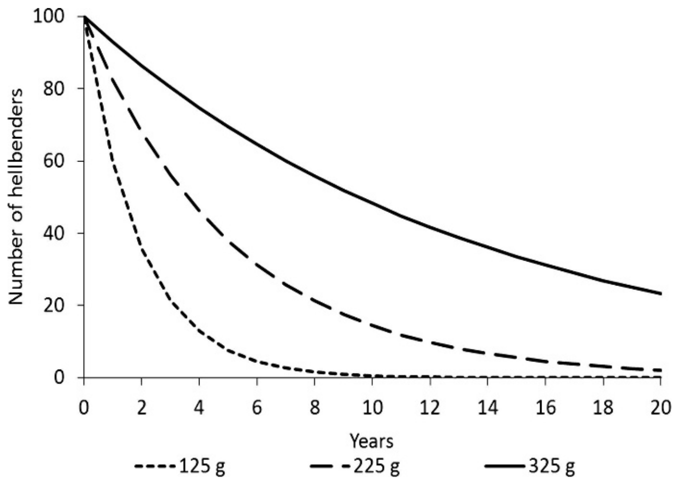

Fig. 5. The number of captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) predicted to be surviving in years post-release, based on estimates of daily survival for hellbenders released at 125 g (short dash), 225 g (long dash), and 325 g (solid line). Daily survival estimates are based on the 'site 3 mass' model of daily survival developed for captive-reared Ozark Hellbenders released at the lower site on the North Fork of the White River, Missouri, 2008–2009.

Haskell et al., 1996), Oregon Spotted Frogs (*Rana pretiosa*; Chelgren et al., 2008), and in sexually mature, rather than immature, Gopher Tortoises (*Gopherus polyphemus*; Tuberville et al., 2008). Size can be an important determinant of survival because smaller herpetofauna can be more susceptible to predation (Sauer and Slade, 1987) and disease such as chytridiomycosis (Garner et al., 2009). Our 'site 3 mass' model predicted that daily survival rates for hellbenders released at 325 g may be as much as 0.0012 greater than for animals released at 125 g. Such a slight difference in daily survival rates (e.g., 0.9986 vs. 0.9998) can make a considerable difference in annual survival (e.g., 0.5996 vs. 0.9296, respectively) and longer term persistence of a population (Fig. 5). While rearing hellbenders to a greater mass prior to release may improve survival, it would be more expensive primarily due to enclosure space required of larger individuals. Therefore, captive-rearing programs may need to carefully weigh the pros and cons of releasing various sized hellbenders.

Though our survival analysis assumed that implant of transmitters, radio-tracking, and recaptures of hellbenders did not influence survival, it is likely that these assumptions were not entirely met. For example, dehiscing sutures caused mortality in at least three animals, two other individuals died after transmitter replacement surgery, and one additional hellbender died within seven days of recapture and having blood drawn. Dehiscing sutures were observed in a review of transmitter attachment methods for adult hellbenders (Blais, 1989) and a telemetry study of newts (Jehle and Arntzen, 2000), suggesting urodeles may be particularly susceptible to problems with implantation of transmitters. For at least two hellbenders, dehiscing sutures occurred prior to release, but holding hellbenders $30 days post-surgery allowed veterinarians to monitor healing and repair wounds as needed, thereby reducing needless mortality. We also noted no dehiscing sutures in hellbenders implanted with the smallest size transmitter (RVI 118), despite the fact that animals receiving the implant tended to be the smallest in the study. However, the short battery life of the small unit required a second (replacement) surgery and involved removing animals from the wild for several weeks before re-release which likely caused considerable stress. We recommend holding implanted animals at least 30 days post-surgery, selecting the smallest transmitter units possible for the study, and that only qualified veterinarians perform surgeries.

ACKNOWLEDGMENTS

We thank the University of Missouri, the Missouri Department of Conservation, and the Saint Louis Zoo for logistic and financial support of this research. We especially acknowledge K. Goellner, the late R. Goellner, and N. McClure for making this research possible. We thank B. Sandercock for advice on our survival analysis and thank J. Ackerson, J. Calfee, T. Crabill, R. J. Distefano, L. Goodrick, J. Howard, B. Ousterhout, A. J. Pratt, S. Prutzman, and M. Sobotka for assistance with field research. Intensive handling involved in captive-rearing and surgical procedures were performed in entirety by veterinarians, staff, and interns of the Saint Louis Zoo, according to AZA accredited institutional protocol. Monitoring of translocated animals took place under scientific collection permits issued by the Missouri Department of Conservation (#13830 and 14180).

LITERATURE CITED

Attum, O., W. E. Farag, S. M. B. E. Din, and B. Kingsbury. 2010. Retention rate of hard-released translocated Egyptian tortoises *Testudo kleinmanni*. Endangered Species Research 12:11–15.

Berger, L., S. Rick, and P. D. Daszak. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proceedings of the National Academy of Sciences of the United States of America 95:9031–9036.

Bernardo, J., and S. J. Agosta. 2005. Evolutionary implications of hierarchical impacts of nonlethal injury on reproduction, including maternal effects. Biological Journal of the Linnean Society 86:309–331.

Blais, D. P. 1989. Movements, home range and other aspects of the biology of the hellbender (*Cryptobranchus alleganiensis alleganiensis*): a radiotelemetric study. Unpubl. Master's thesis. State University of New York at Binghamton, Binghamton, New York.

Bodinof, C. M. 2010. Translocation and conservation of hellbenders (*Cryptobranchus alleganiensis*) in Missouri. Unpubl Master's thesis. University of Missouri, Columbia, Missouri.

Bodinof, C. M., J. T. Briggler, M. C. Duncan, J. Beringer, and J. J. Millspaugh. 2011. Historic occurrence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in hellbender (*Cryptobranchus alleganiensis*) populations from Missouri. Diseases of Aquatic Organisms 96:1–7.

Briggler, J. T., K. A. Larson, and K. J. Irwin. 2008. Presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on hellbenders (*Cryptobranchus alleganiensis*) in the Ozark highlands. Herpetological Review 39:443–444.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science, New York.

Carr, J. A. 2002. Stress, neuropeptides, and feeding behavior: a comparative perspective. Integrative and Comparative Biology 42:582–590.

Chelgren, N. D., C. A. Pearl, M. J. Adams, and J. Bowerman. 2008. Demography and movement in a relocated population of Oregon spotted frogs (*Rana pretiosa*): influence of season and gender. Copeia 2008:742–751.

Cooke, A. S., and R. S. Oldham. 1995. Establishment of populations of the common frog, *Rana temporaria*, and common toad, *Bufo bufo*, in a newly created reserve following translocation. Herpetological Journal 5:173–180.

Deevey, E. S., Jr. 1947. Life tables for natural populations of animals. The Quarterly Review of Biology 22:283–314.

Dodd, C. K., Jr., and R. A. Seigel. 1991. Relocation, repatriation and translocation of amphibians and reptiles: Are they conservation strategies that work? Herpetologica 47: 336–350.

Esch, G. W., J. W. Gibbons, and J. E. Bourque. 1975. An analysis of the relationship between stress and parasitism. American Midland Naturalist 93:339–353.

Federal Register. 2010a. Inclusion of the hellbender, including the eastern hellbender and the Ozark hellbender in Appendix III of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). 75(173):54579–54585. *In:* V.75, No. 173.

Federal Register. 2010b. Endangered and threatened wildlife and plants; proposed rule to list the Ozark hellbenders salamander as endangered, p. 54561–54579. *In:* V. 75, No. 173.

Fellers, G. M., D. F. Bradford, D. Pratt, and L. L. Wood. 2007. Demise of repatriated populations of mountain yellow-legged frogs (*Rana muscosa*) in the Sierra Nevada of California. Herpetological Conservation and Biology 2:5–21.

Foster, R. L., A. M. McMillan, and K. J. Roblee. 2009. Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York state. Journal of Herpetology 43:579–588.

Gall, B. G., and A. Mathis. 2010. Innate predator recognition and the problem of introduced trout. Ethology 116:47–58.

Garner, T. W. J., S. Walker, J. Bosch, S. Leech, J. M. Rowcliffe, A. A. Cunningham, and M. C. Fisher. 2009. Life history tradeoffs influence mortality associated with the amphibian pathogen *Batrachochytrium dendrobatidis*. Oikos 118:783–791.

Gascon, C., J. P. Collins, R. D. Moore, D. R. Church, J. E. McKay, and J. R. Mendelson, III. 2007. Amphibian Conservation Action Plan. IUCN/SSC Amphibian Specialist Group, Gland, Switzerland.

Germano, J. M., and P. J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology 23:7–15.

Haskell, A., T. E. Graham, C. R. Griffin, and J. B. Hestbeck. 1996. Size related survival of head started redbelly turtles (*Pseudemys rubriventris*) in Massachusetts. Journal of Herpetology 5:121–126.

Huang, C. C., Y. Xu, J. T. Briggler, M. McKee, P. Nam, and Y. Huang. 2010. Heavy metals, hematology, plasma chemistry, and parasites in adult hellbenders (*Cryptobranchus alleganiensis*). Environmental Toxicology and Chemistry 29:1132–1137.

Jehle, R., and J. W. Arntzen. 2000. Post-breeding migration of newts (*Triturus cristatus* and *T. marmoratus*) with contrasting ecological requirements. Journal of Zoology 251:297–306.

King, R., C. Berg, and B. Hay. 2004. A repatriation study of the eastern massasauga (*Sistrurus catenatus catenatus*) in Wisconsin. Herpetologica 60:429–437.

Mather, M. E., and R. A. Stein. 1993. Direct and indirect effects of fish predation on the replacement of a native crayfish by an invading congener. Canadian Journal of Fisheries and Aquatic Sciences 50:1279–1288.

Matthews, K. R. 2003. Response of mountain yellow-legged frogs, *Rana muscosa*, to short distance translocation. Journal of Herpetology 37:621–626.

Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern hellbender status assessment report. U.S. Fish and Wildlife Service, Ft. Snelling, Minnesota.

Moser, W. E., D. J. Richardson, B. A. Wheeler, K. J. Irwin, B. A. Daniels, S. E. Trauth, and D. L. Klemm. 2008. *Placobdella cryptobranchii* (Rhynchobdellida: Glossiphoniidae) on *Cryptobranchus alleganiensis bishopi* (Ozark hellbender) in Arkansas and Missouri. Comparative Parasitology 75:98–101.

Muths, E., T. L. Johnson, and P. S. Corn. 2001. Experimental repatriation of boreal toad (*Bufo boreas*) eggs, metamorphs, and adults in Rocky Mountain National Park. Southwestern Naturalist 46:106–113.

Nickerson, M. A., and J. T. Briggler. 2007. Harvesting as a factor in population decline of a long-lived salamander; the Ozark hellbender, *Cryptobranchus alleganiensis bishopi* Grobman. Applied Herpetology 4:207–216.

Nickerson, M. A., and C. E. Mays. 1973a. The Hellbenders: North American ''Giant Salamanders''. Milwaukee Public Museum Press, Milwaukee, Wisconsin.

Nickerson, M. A., and C. E. Mays. 1973b. A study of the Ozark hellbender *Cryptobranchus alleganiensis bishopi*. Ecology 54:1163–1165.

Peterson, C. L., D. E. Metter, B. T. Miller, R. F. Wilkinson, and M. S. Topping. 1988. Demography of the hellbender, *Cryptobranchus alleganiensis*, in the Ozarks. The American Midland Naturalist 119:291–303.

Peterson, C. L., J. W. Reed, and R. F. Wilkinson. 1989. Seasonal food habits of *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae). The Southwestern Naturalist 34: 348–441.

Peterson, C. L., R. F. Wilkinson, M. S. Topping, and D. E. Metter. 1983. Age and growth of the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*). Copeia 1983:225–231.

Reinert, H. K., and R. R. Rupert, Jr. 1999. Impacts of translocation on behavior and survival of timber rattlesnakes, *Crotalus horridus*. Journal of Herpetology 33:45–61.

Rickard, A. 2006. Assessment of translocation of blanchard's cricket frogs (*Acris crepitans blanchardi*) in southeast Michigan. Endangered Species Update 23:3–12.

Sauer, J. R., and N. A. Slade. 1987. Size-based demography of vertebrates. Annual Review of Ecology and Systematics 18:71–90.

Semlitsch, R. D., and S. B. Reichling. 1989. Density-dependent injury in larval salamanders. Oecologia 81:100–103.

Stamps, J. A., and R. R. Swaisgood. 2007. Someplace like home: experience, habitat selection and conservation biology. Applied Animal Behaviour Science 102:392–409.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Taber, C. A., R. F. Wilkinson, Jr., and M. S. Topping. 1975. Age and growth of hellbenders in the Niangua River, Missouri. Copeia 1975:633–639.

Tiexiera, C. P., C. S. de Azevado, M. Mendl, C. F. Cipreste, and R. J. Young. 2007. Revisiting translocation and reintroduction programmes: the importance of considering stress. Animal Behaviour 73:1–13.

Tocher, M. D., and S. Pledger. 2005. The inter-island translocation of the New Zealand frog *Liopelma hamiltoni*. Applied Herpetology 2:401–413.

Tuberville, T. D., T. M. Norton, B. D. Todd, and J. S. Spratt. 2008. Long-term apparent survival of translocated gopher tortoises: a comparison of newly released and previously established animals. Biological Conservation 141:2690–2697.

Unger, S. D. 2003. Sperm production and larval development in hellbenders *(Cryptobranchus alleganiensis alleganiensis* and *C. a. bishopi)*: a comparison of declining and stable populations. Unpubl. Master's thesis. Southwest Missouri State University, Springfield, Missouri.

Wheeler, B. A., E. Prosen, A. Mathis, and R. F. Wilkinson. 2003. Population declines of a long-lived salamander: a 20+-year study of hellbenders, *Cryptobranchus alleganien- sis*. Biological Conservation 109:151–156.

White, A. W., and G. H. Pyke. 2008. Frogs on the hop: translocations of green and golden bell frogs *Litoria aurea* in greater Sydney. Australian Zoologist 34:249–261.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:120–138.

White, G. C., and R. A. Garrott. 1990. Analysis of Wildlife Radio-tracking Data. Academic Press, San Diego, California.

Williams, D. R., J. R. Gates, C. H. Hocutt, and G. J. Taylor. 1981. The hellbender: a nongame species in need of management. Wildlife Society Bulletin 9:94–100.