National Protocol for Capturing, Banding, Radio-tagging, and Tissue Sampling Least Bitterns in Canada

prepared

for

Environment Canada - Canadian Wildlife Service

and

The National Least Bittern Recovery Team

by

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INTRODUCTION

In Canada, the Least Bittern (hereafter LEBI) breeds in freshwater marshes in the southern portions of Manitoba, Ontario, Quebec, and New Brunswick. The Canadian population is estimated to be fewer than 1500 pairs (COSEWIC 2009). It is a Threatened species in Canada under the federal *Species at Risk Act*, and has similar provincial designations in Manitoba, Ontario and Quebec.

Due to its inconspicuous, secretive nature, and its relatively inaccessible habitat, there are only limited data on the LEBI's current distribution, population size and status in Canada and across much of North America. Several important aspects of its breeding biology are also not well known (Gibbs et al. 2009). Management and recovery efforts are limited by a dearth of information, especially knowledge of broad and local-scale habitat use, site fidelity, wintering areas of the Canadian population, and within-year and between-year survivorship.

For the most part, collecting this information will require focused research projects that require the capture and marking of individuals. The primary purpose of this document is to provide an overview of the methods available to capture, mark (using bird bands and other auxiliary markers), and obtain tissue samples (blood/feathers) of LEBI.

In terms of the capture of adult birds, the LEBI poses particular challenges owing to the nature of its habitat and the secretive behaviour of the species. To ensure adequate sample sizes, any banding, radio telemetry, or marking project benefits greatly from having detailed *a priori* knowledge of the local distribution, behaviour, and status of the target species within the study area. As such, research studies that require the capture of adult birds should include a rigorous survey to locate birds, augmented by a nest searching component. The latter is essential for sampling nestlings. In the case of LEBI, surveys should probably be started well in advance of capture efforts.

There is a suite of potential capture methods for LEBI, but to date no one method has proven particularly efficient or successful, and several have apparently not been attempted. Capture success varies with time of year and with specific habitat and site variables (see Bogner and Baldassarre 2002a,b; Griffin et al. 2009). A description of potential trapping methods for both adult and young is outlined herein. Guidelines for handling and banding are also provided, followed by a synopsis of radio-telemetry and other marking methods, and tissue sampling. Options for auxiliary marker (transmitter) types and attachment methods are also described.

In order to get the most information from any mark-recapture project that employs transmitter devices, radio-tracking should commence immediately following transmitter deployment and continue until the transmitter battery dies, the transmitter is relocated (fallen off or bird found dead), or all individuals have moved from the study area. Ultimately, the intensity of transmitter monitoring depends on the specific objectives of the research study, accompanied by the necessary resources to support it.

All methods in this protocol are intended to follow the *Guidelines for the Use of Wild Birds in Research* (Fair et al. 2010). As with any research protocol, this is a living document intended to be modified as technologies improve and methods are tested and refined.

1. Least Bittern Surveys

LEBI are not uniformally distributed within a marsh; they have particular habitat needs and can often exhibit semi-colonial spatial patterns of occupancy (e.g., Meyer and Friis 2008). The primary purpose of surveys is to locate LEBI in the study area (especially areas where calling birds are regularly detected) and to identify areas that may support high densities of birds. Once such areas are identified, particular sites can be targeted for an efficient capture regime.

To maximize the usefulness of data, LEBI surveys should ideally be conducted in concert with standard methods provided by Jobin et al. (2010, 2011). However, these standard survey methods, which employ point counts and timed playbacks, are designed to collect data on abundance, distribution and habitat preferences of LEBI and are unlikely to be entirely sufficient for locating birds (and nests) in advance of capture efforts. Hence, targeted area-search efforts via canoe and on foot will also be necessary in order to maximize capture efficiencies.

Surveys should commence no later than 2 weeks prior to the intended start date for capture. Nest searching and looking for signs of breeding activity should be the focus of precapture surveys. Depending on the objectives of the research project, locating nests may not be necessary for capture, but will nevertheless ensure that nests are not disturbed during trapping exercises.

Nest Searching

Nest searching for any species becomes easier with practice and particularly if a search image can be developed. Researchers in the field that possess previous experience with Least Bitterns will be an asset. Prior to nest searching, all surveyors should read the species account in *The Birds of North America* (Gibbs et al. 2009). Further descriptions of LEBI nest characteristics and nesting behavior can be found in Weller (1961) and Meyer and Friis (2008).

LEBI often nest preferentially along or near the edges of channels or discrete vegetation patches (Meyer and Friis 2008). Cattails (*Typha* spp.) are greatly preferred over other vegetation types. *Typha angustifolia* is preferred over *T. x glauca,* and to a lesser extent *T. latifolia*. Other vegetation is also used, including *Sparganium*, *Phragmites, Lythrum*, *Scirpus*, grasses, *Equisetum*, *Carex, Salix,* and *Cornus*.

Nest searching should primarily be conducted by small boat (canoe when possible) to reduce disturbance, although wading into the marsh vegetation will almost always be essential.

When nests are discovered, care must be taken not to disturb the surrounding area. Nest locations should be documented using a GPS and should be marked with a visible

stake (~2 m long) placed at least 5 m from the nest in a consistent direction. Details of each nest visit, nest location, habitat (e.g., dominant vegetation, plant species holding nest, height above water, distance to closest edge), contents, and activity should be recorded on every visit following provincial standards:

- Manitoba: Prairie Nest Records Scheme (see http://naturealberta.ca/alberta-natural-history/bird-projects/prairie-nest-records-scheme-pnrs)
- Ontario: Ontario Nest Record Scheme handbook (Peck et al. 2001); see http://www.birdsontario.org/onrs/onrsmain.html);
- Québec: base de données ÉPOQ in Québec (see (http://www.oiseauxqc.org/feuillet.jsp?ts=1299245883314)
- Maritimes: Maritimes Nest Records Scheme (see http://www.mba-aom.ca/english/mbbaguide.pdf and http://www.mba-aom.ca/english/form_nr_english.pdf)

Nest records should be submitted to the provincial databases or on-line to Project Nestwatch (http://www.birdscanada.org/volunteer/pnw/; Bird Studies Canada 2010) at the end of each field season. It is important to track the progress of each nest in order to correctly schedule trapping efforts of young. Ideally, nests will be checked once every 3-5 days after discovery.

Nest searching following transmitter deployment

Although initial nest searching should be conducted, nest finding will likely be more productive if radio-transmitters are deployed on adults that are captured away from the nest site. The radio signal from an adult bittern may even lead searchers directly to a nest.

2. Capture methods

Potential trapping methods are presented in Table 1, and are preferentially ordered based on levels of disturbance and likelihood of success. However, it is likely that several methods will need to be attempted and evaluated. One method may be preferred depending on site/habitat conditions, time of year, and the age/sex of the individuals being targeted.

Timing

If nest finding is an element of the study design (e.g., if nestlings are to be sampled), there is potential for increased success of locating nests after transmitter deployment, because birds can then be tracked back to the nest site. This is reason to begin trapping early. Catching individuals early in the season may also be more productive while males are territorial and females are actively looking for mates. Trapping too early in the season, however, may result in the trapping of migrants that will subsequently move out of the study area. Historical records and local knowledge from the study area should be

thoroughly examined to determine peak migration windows and the typical start of breeding activity. The timing of capture efforts will depend on the strategy employed and whether young or adults are targeted.

Special consideration for nestlings

Nestlings can be banded opportunistically, or systematically targeted. If targeted, nest searching and monitoring will need to be a strong component of the project. Once a nest has been found, nest checks should be done every 3-5 days in order to adequately assess the stage of the nest and the hatching/fledging time of young. Nest checks should be done as carefully as possible and from as far away as possible, especially after young have hatched to avoid premature fledging. If young prematurely fledge, they should be left alone and the area should be quickly evacuated. Nestlings can be captured following the method outlined in Table 2.

Young LEBI are semi-altricial and can leave the nest prematurely by day 5 or 6, but will usually linger nearby and return to the nest (Gibbs et al. 2009). Normal fledging time is between 12 and 16 days, but young are known to explore the nest area before this time. Banding should occur sometime between day 10 and day 16, because young will still strongly associate with the nest and leg size will be adequate for band application.

If nestlings are to receive radio transmitters (e.g., in order to study post-fledgling dispersal or survivorship), then the attachment method (Table 3) will need to be carefully considered to take into account the growth of the birds to adult body size. As such, harness designs are likely inappropriate for nestlings.

Mist net operations

If mist nets are used, the recommended size for LEBI is 60 mm mesh size (210 denier), which is available in 6, 10, and 12 m lengths.

Mist nets should be monitored continuously from an appropriate distance (close enough to ensure quick access to the net, but far away enough to minimize potential interference). If they cannot be monitored continuously, then they must be checked at least once every 30 minutes.

Mist nets should be taught, but not tight enough to cause injury if birds hit trammel lines. They should be tight enough to eliminate any droop to the net and so that a captured bird will not touch the water. This should be tested each time nets are set, with a bird bag weighing ~100 g. The bottom of the net should be between 50 and 100 cm from the water surface. Every effort must be made to keep birds from getting wet.

Nets should be placed as close to vegetation as possible. In some circumstances (e.g., netting in the proximity of known nest sites), any clearing of vegetation to accommodate the net should be restricted as much as possible, owing to disturbance and time. Generally, nets should be placed along the edges of openings and channels. Nets should be erected as quickly and efficiently as possible. Because of the time it takes to set up an array of nets, it may be useful to establish the net array several hours to a day in advance of capture efforts.

Netting should not be attempted if there is any threat of rain, under winds >15 km/h, or under extreme temperatures (>30°C or <5°C).

Upon capture in the net, the bird should be extracted as soon as possible. Birds can be extracted using the general feet-first or body grasp method as outlined in the North American Bander's Study Guide (North American Banding Council 2001). Upon extraction, birds are to be immediately placed in a large cloth carrying bag (50 x100 cm) and transported to a nearby processing station.

All personnel who are expected to be handling LEBI must undergo adequate training and have the necessary permits (see Section 9).

Caution when handling bitterns: Herons and bitterns have long, sharp bills and are very adept at making quick and accurate strikes – sometimes apparently targeting the eyes. A conscious effort must be made to keep them away from the face during handling. Safety glasses should be considered.

Table 1. Potential methods for capturing adult Least Bitterns, ordered from least to most invasive.

Method	Description	Pros	Cons
1) LURE NETTING 2-3 people PASSIVE (e.g.:Bogner and Baldassare 2002a,b)	**Methods and considerations:** In areas with high LEBI density (or known territories) that are identified during surveys, set-up a V- or U-shaped net array (or complete triangle/trapezoid) surrounding a discrete vegetation patch. Set-up a play-back loop of the LEBI 'coo' call close to the water's edge in the middle of the net array. The playback should consist of continuously looping series of 15s 'coo', 15s silence. As many of these stations can be set-up as resources and time permit. **Timing:** This method will be most useful early in the breeding season when males are setting up territories and when females are looking for mates. Later in the breeding season, it will likely only work on targeted individuals within a small area.	• Relatively low disturbance • Likely to yield good success • Can target particular individuals, though breeding status not necessarily confirmed	• Potential male bias • Captured individuals may not breed locally • Nest location and breeding status for individuals may not be known (potential for capture of unmated individuals)
2) FLUSH NETTING 2-3 people ACTIVE (e.g. Bogner and Baldassare 2002a,b)	**Methods and considerations:** In areas with high LEBI density, identified during surveys, set-up a V or U-shaped net array (or complete triangle/trapezoid) surrounding a discrete vegetation patch, or set a line of nets along the edge of a continuous vegetation patch. Once set, one person hides nearby and watches the net while the other two walk through the vegetation patch with the intent of flushing the bitterns into the awaiting net. This method will work best in discrete patches that are known to contain LEBI. The two 'flushing' personnel could also drag a line between them with tin cans (or other noisemakers) hanging every metre or two. **Timing:** This method can be employed at any time throughout the season but should be avoided during periods when nestlings could be active.	• Likely to yield good success • Can target particular individuals, though breeding status not necessarily confirmed	• Moderate disturbance (particularly to habitat) • Nest site not identified • Capture of unmated individuals • Labour intensive

Method	Description	Pros	Cons
3) NETTING AT NESTS 2 people PASSIVE	**Methods and considerations:** A net array similar to that in #1 and #2 is set-up around an active nest during late incubation or the early nestling stage (not during egg laying). Simply surround the nest area with a suitable array of nets and wait for an adult to be captured while it makes visits to and from the nest. To minimize disturbance to the nest, 'flushing' should not be employed, and nets should be set up at least 5 m from the nest. **Timing:** Can only occur during periods of high nest activity – late incubation and feeding of young.	• Likely high success • Can target specific individuals of known breeding status • Known nesting locations are useful for telemetry results for spatial studies of home range/territory • Allows measure of nest abandonment in relation to capture	• Relatively high disturbance, and potential for nest disturbance/abandonment (especially if conducted too early in the nesting phase)
4) HAND 2 to 3 people ACTIVE (Griffin et al. 2009)	**Methods and considerations:** This is an opportunistic method. LEBI often seek refuge in dense vegetation as opposed to taking flight. This behavior makes capture by hand or with a small fish-landing net possible. Chase the individual into dense vegetation and grab by hand or use a small fishing net. This method should not be used in the close proximity of known or suspected nests. **Timing:** Can be employed at any time.	• Opportunistic • Can target individuals, though breeding status not necessarily confirmed	• Likely labour intensive • Some increased risk of injuring individual birds is expected • Moderate success • Capture of individuals with unknown breeding status • Nest site probably not identified in relation to home range/territory studies
5) NIGHT-TIME DAZZLING (spot-lighting) 2 people ACTIVE	**Methods and considerations:** The theory behind this method is to 'stun, or dazzle' individuals with a bright spotlight, while birds are sitting on the nest at night. Birds are captured by hand or with a small landing net. Requires locating nest in advance and getting accurate GPS coordinates. Cease motorized boat travel about 100 m from the nest. Approach the nest silently and carefully under the cover of darkness. Attempt to get within 10 m without the aid of a light and when a light is used shine it directly at the nest and use peripheral light to navigate. Final	• Likely high success • Can target individuals • Known nesting locations	• High disturbance • Nest disturbance; potential for disturbance of nest contents (e.g., eggs/young may be damaged or spill out) • Moderate success

Method	Description	Pros	Cons
	approach can be made by foot. Extreme caution should be exercised if attempting to capture individuals on the nest as damage may occur to eggs or young. **Timing:** This method can only be employed during late incubation or early brooding stages.		
6) HAND CAPTURE AT NEST 2 people ACTIVE	**Methods and considerations:** Similar to #5, but occurs in daytime when adults are on or close to the nest. This method might be employed during nest searching or nest checks if individuals are 'sitting-tight' on the nest or leave the nest without taking flight and can be seen in the vegetation. Extreme caution should be exercised if attempting to capture individuals on the nest as damage may occur to eggs or young. Researchers may not wish to attempt to capture individuals on the nest. This should only be attempted during incubation or when young younger than 5 days old. **Timing:** This method can only be used during incubation or brooding stages.	• Opportunistic • Can target individuals of known breeding status • Known nest location useful for home range/territory use studies	• High disturbance/ stress • Moderate success • Nest disturbance; potential for disturbance of nest contents (e.g., eggs/young may be damaged or spill out)

Table 2. Potential methods for capturing nestling Least Bitterns, ordered from least to most invasive.

Method	Description	Pros	Cons
1) Nest 2 people ACTIVE	**Methods and considerations:** When nestlings are ~12 d old they can be captured at the nest. The nest should be approached quietly and slowly to avoid causing the young to prematurely fledge. A small fish landing-net or towel can be placed over the nest for capture. If young fledge, they can be captured manually in the vegetation if deemed not to be too disruptive. After banding, young should be placed back in the nest with a towel placed over them for up to 2 min to calm them down before departing the area. **Timing:** See special consideration for young. Technique can only be conducted when young are between 10 and 15 d old.	• Potential for high success • Can target individuals • Known nest location	• High disturbance/stress Nest disturbance (risk of premature fledging)
2) Post-fledging 2 people ACTIVE	**Methods and considerations:** Young remain in close proximity to the nest for several days after fledging. With sufficient search effort within a radius of about 10 m around the nest, they can be located in the vegetation and then captured by hand (Bogner and Baldassarre 2002b). **Timing:** Technique can likely only be conducted within about 5 d after fledging.	• Likely moderate success, but relatively moderate disturbance • Can target individuals • Known nest location	• Probably labour intensive

3. Banding

Adults: A separate processing station should be set-up nearby, either on land or in a stable boat (preferably not a canoe). Before the bird is removed from the carrying bag, weigh it using a 100 g Pesola scale. Be sure to subtract the weight of the empty bag. Weight will be useful information for transmitter attachment later.

During processing, a sock with a hole cut in it for the bill can be placed over the head and neck to keep the bird calm and personnel safe. If necessary, a rubber band can be placed over the bill to avoid snapping. First a standard USFWS/CWS aluminum bird band (size 4) is placed on the leg, ideally above the tarsal joint if there is sufficient room. Apart from body weight, detailed measurements should not be taken, in order to reduce processing time, and because they are not useful for ageing or sexing (Pyle 2008; Gibbs et al. 2009).

Four digital pictures should be quickly taken of each individual for data integrity and for cross-checking age/sex field determinations: 1) full back (including tail, body, neck and head); 2) extended left wing; 3) extended right wing; and 4) full underside (including tail, body, neck and head). To clearly associate pictures with each individual, the date and band number should be written on a piece of card stock and made clearly visible in each photo. The age and sex can then be re-confirmed later. Once the bird is banded and the photos are taken, transmitter attachment can begin (see transmitter attachment below).

Nestlings: Young should be processed as close to the nest as possible. Young should all be captured at once and placed in separate cloth bags. Young should be banded one at a time and placed back in their bag until all of them are ready to be placed back in the nest at the same time. Young that are being fitted with radio-transmitters should be processed first (ideally by additional team members), so that they can be returned to the nest at the same time as their siblings.

If the young appear very active and restless after being returned to the nest, place a towel or bird bag over top of the nest in order to settle them down. If necessary, light pressure can be applied to the group through the bag. When young appear settled in the nest, the cover can be slowly removed and the area can be immediately evacuated.

3.1 Colour marking

There are several different kinds of colour markers that could be used for LEBI research work, including coloured leg bands, coloured leg flags, and coloured wing tags. Because of their size and prominence (see Figures 1 and 2), the last two markers may interfere with LEBI behaviour, and we don't advocate their use at this time. However, we do acknowledge that they would be easier to resight and "read" than standard color bands, especially if alpha-numeric coding is used.

Standard colour banding LEBI, while unlikely to interfere with behaviour, is unlikely to be of use in achieving project objectives. It should only be pursued if the project will run a minimum of 2 years and intensive effort is made to resight individuals. It is extremely difficult to see LEBI, let alone read colour-band combinations in dense marsh. It may be useful, however, to use a simple colour-banding scheme merely to distinguish different capture sites, broad geographic areas, ages, and/or years.

Celluloid colour bands come with a special applicator that is used for proper application. Colour bands should be applied after the standard USFWS/CWS band is applied and can be applied to either leg. Depending on the colour banding scheme, it is probably best to place the colour bands on the leg opposite the standard band to avoid confusion when trying to read combinations or colours. Colour bands should be sealed with acetone or superglue and held for 10 to 15 seconds to ensure bonding.

Specifications: Celluloid (size 4FB); colours available = Dark Green*, Light Blue**,** Orange***,** Mauve, Dark Pink***,** Light Pink. Note that some celluloid bands are known to change colour over time (Lindsey et al. 1995). Only those colours marked with * should be used to reduce potential confusion in later years.

Figure 1: A coloured flag affixed to the leg of a young night-heron.

Figure 2: A coloured wing tag on a Great Egret.

4. Radio Telemetry[1]

4.1 Telemetry equipment

Telemetry technology is continually improving and is very competitive. Therefore, the following information, while current, is likely to be outdated within the next year. The methods of telemetry tracking also evolve with technology, but do not change at near the same rate. A complete list of suppliers and manufacturers of remote marking technologies for wildlife is available at www.biotelem.org. Most systems from most manufacturers will do the required job, but the old adage that you get what you pay for applies.

There are two main types of telemetry systems: “beeper” and “coded.”

- Beeper tags emit a pulse that is identified by a receiver. Each tag is typically tracked on a unique frequency, although tracking of up to three individuals on a single frequency is possible, resulting in a high variation in tag-life. Tracking of numerous individuals therefore requires systematic or automated cycling through the active frequencies. The use of this technology can therefore be quite time consuming and cumbersome, especially if many individuals are being tracked simultaneously, but they are economical (~$1000 to $2000/receiver). Beeper tags are typically a little heavier than coded tags, with weights typically >2 g.

[1] *Note: The authors have no affiliation to the products or companies, and make recommendations based on previous experience, word of mouth, and information provided by the manufacturers.*

- Coded tags are superior to beeper tags in that up to 200 individuals can be simultaneously monitored on a single frequency. They also allow for simultaneous data recording with geographic positioning system integration and time synchronization capabilities between one or multiple receivers. The tags themselves are comparably priced, but the receiver units are approximately five times the price (~$5000 to $10,000/receiver). These tags also allow for continuous monitoring of individuals (presence/absence and basic location or flight direction information) from stationary locations within a very broad area (Mills et al. 2011). Weights of coded tags can be as low as 0.5 g.

4.2 Transmitter specifications

Beeper technology is suitable for most potential research projects on LEBI, though it does require intensive manual localization work.

The tags (with harness) should weigh ≤ 3-5% of body mass of the bird, following Caccamise and Hedin (1985) and Fair et al. (2010). The range of recorded mass for LEBI is quite substantial, ranging from 45.9 to 95 g (Gibbs et al. 2009). Given current transmitter weights, a realistic target weight for transmitter and harness is ~2.5 to 3 g. Therefore, any bird weighing less than 75 g should not be affixed with a transmitter, unless tag or harness weight can be reduced.

- Recommended Tag: Model # Pip Ag386 beeper transmitter from Lotek Wireless. Weight = 2.4 g. Tag life = 3.9 months at 20 millisecond (ms) pulse width, with 50 pulses per minute (ppm); up to 9.4 months with a 12ms/30ppm application. Two individuals can easily be tracked on the same frequency with some variation in tag life.

- Recommended Receiver: Model # Biotracker receiver available from Lotek Wireless.

4.3 Transmitter preparation and calibration

All transmitters should be turned on and tested with all receivers, antennae, and cables prior to deployment. Before deployment, tags should be activated and displaced at a series of distance intervals from an activated receiver to observe reduction in signal strength at certain distances and to document the maximum range of the transmitters in a variety of conditions and habitats.

4.4 Transmitter attachment

No bird should be held for longer than 15 minutes from time to capture to release, including all measurements, banding, and transmitter attachment. After capture, birds should immediately be placed in a cloth carrying bag ~50 x 150 cm, and carried to a stable location nearby for processing (not in a canoe).

Potential methods for transmitter attachment are outlined in Table 3, and are the same for adults and young, but see below. Methods are ordered preferentially based on previous success, level of invasiveness, and simplicity in the field. Of the four methods of transmitter attachment described in Table 3, two (the leg band and neck collar methods) are not recommended without further investigation.

In some cases, harnesses can be made to fit prior to capture. Every effort should be made to minimize attachment time. All methods will likely require two people; one to hold the bird, the other to attach the transmitter.

Special consideration for young

The number of transmitters to be deployed at each nest should be determined before the visit to the nest (no more than 2 transmitters per nest). Transmitters should be placed on the largest and healthiest-looking young. Least Bitterns have asynchronous hatching so there will be a large range of ages in the nest. Young that are being fitted with radio-transmitters should be processed before those just being banded. Ideally, additional team members can affix the transmitters, while other young are being banded. All young should be returned to the nest at the same time.

If the young appear very active and restless after being returned to the nest, place a towel or bird bag over top of them in the nest in order to settle them down into the nest. If necessary, light pressure can be applied to the group through the bag. When young appear settled in the nest, the area can be quickly evacuated.

4.5 Release procedure for adults

Individuals should be released as close to the capture site as possible without risk of immediate recapture. They should be released directly into dense vegetation, not over open water.

5. Bird Handling Times

In all cases, bird handling times for banding/transmitter attachment and for any additional tissue sampling must be minimized. All birds will be processed and released within ~20 minutes of capture. Times of capture/release will be recorded in the field, and a stop-watch with a beeper set for 15 min will be used.

6. Radio tracking

There are four main components to a radio-telemetry system: transmitter, receiver, cable and antennae. All units need to be in proper working order and handled appropriately to ensure accurate data collection.

The frequency of tracking tagged individuals depends on project objectives and available resources. The guidelines below should be considered as the minimum effort needed to collect sufficient behavioural and spatial data. Some important terms are as follows:

Localization or triangulation is the process of identifying the exact location of an individual either by direct observation or an accurate estimate.

Signal strength is the strength of the signal emitted from each transmitter as recorded by the receiver. It is recorded as an index from ~20 to 255 which is linearly related to the $\log_{10}$ of the received signal in dB.

Gain is a simply measure of receiver and antennae sensitivity typically on a scale from ~ 10 to 95. Higher gain will increase the range of the receiver but also increase the amount of potential interference and reduce accuracy. Lower gains will reduce the range of the receiver but will increase accuracy. Optimal gain will vary depending on local topography, habitat and environment conditions. Ideally a range of gains will need to be used during every localization attempt.

Table 3. Methods for transmitter attachment.

1. BACK PACK (legs or wings; see Rappole and Tipton 1991; Bogner and Baldassare 2002a,b; Mong and Sandercock 2007; Griffin et al. 2009)	
Harness Preparation	Using elasticized cord, harnesses can be premade to fit most individuals. Minor adjustments may need to occur in the field. Following Naef-Daenzer (2007), a leg-loop harness span of ~70 mm should be appropriate. Loops are fixed in a figure-8 shape affixed in one or two locations on the transmitter using Super Glue (see Rappole and Tipton 1991; Naef-Daenzer 2007). If LEBI are found to break the elasticized cord, a non-elastic more permanent material (Herculite or Teflon) may need to be used, but will require some sewing or application in the field.
Description	Place a loop around one leg and pull up and away from the body dorsally to ensure the loop goes around the tibia, underneath the crural feathers and well into the 'pit' between the tibia and the body of the birds. With tension, extend the transmitter and other loop around the back/rump of the bird and stretch the loop to extend over the foot of the other leg. Keeping the first loop high up on the leg, and transmitter in place on the lower back is essential. When the second loop is around the leg, the transmitter can be pulled dorsally to ensure that both loops are pulled high up and nestled between the thigh and body of the bird. A similar method can be used around the wings although this may cause flight difficulty and will require experimentation as to loop size.
Pros and Cons	**PROS** • Fast • Pre-fabrication • Temporary attachment, allowing transmitter to fall off (depends on material) • Non-invasive **CONS** • Transmitter may become dislodged via preening activity or through active removal by the bird
2. BACK SURGICAL IMPLANT (see Pietz et al. 1995; Bogner and Baldassare 2002a,b)	
Harness Preparation	Super Glue a small piece of stainless steel wire to the underside of the transmitter with a few centimetres remaining at the end opposite the antennae. Once the glue is fixed, the wire can be bent into an anchor shape for insertion (see Pietz et al. 1995).
Description	On the back of the bird, between the wings, pinch the skin and make a small incision with a hypodermic needle (large enough to fit the wire inside). Take the anchor-wire and thread it into the hole so that each anchor 'spoke' is on either side of the hole. Ensure the transmitter is oriented in an anterior-posterior position. Place one drop of super glue on the underside of the transmitter and press against the bird's body. Another drop of glue should be applied to seal the incision. Alternatively, a suture can be used to close the incision.
Pros and Cons	**PROS** • Fast • Pre-fabrication • High success

	CONS • Invasive • Semi-permanent
3. LEG BAND (see Griffin et al. 2009)	
Harness Preparation	None
Description	Place an additional unmarked metal or colour band on the leg opposite the standard USFWS/CWS band. After band application, super-glue the transmitter to the band with the antennae pointed dorsally.
Pros and Cons	**PROS** • Little preparation • Fast • Non-invasive • Pre-fabrication **CONS** • Awkward antennae and transmitter position can cause signal disturbance and may be annoying to the bird • Semi-permanent • Additional band and uneven distribution of weight
4. NECK COLLAR (see Brininger 1996, Bogner and Baldassare 2002a,b)	
Harness Preparation	Super-glue a transmitter to the centre of a piece of Herculite or Teflon approximately 1 cm wide and long enough to create a bib wrapping around the LEBI neck (~5 to 10 cm).
Description	Place the bib around the neck of the bird with the transmitter tucked under the chest feathers with antennae pointing to the posterior end of the bird. The two ends of the material can then be sewn or glued together. Ensure that the bib can move freely around the neck, but is snug enough to reduce potential interference.
Pros and Cons	**PROS** • Fast • Non-invasive **CONS** • Awkward antennae and transmitter position is apt to cause signal disturbance and may be annoying to the bird • Previous problems have occurred with the bib getting caught in the bill and in vegetation (Heidi Kennedy pers. comm.). • Semi-permanent • May interfere with feeding

6.1 Transmitter localization/triangulation

- From a stationary position point the antenna in a direction where the signal is thought to be originating, using a relatively high gain (80-95). Wait for approximately two pulse cycles and examine the signal strength.
- Move the antennae slightly (10-20^{o}) in the direction where the signal is thought to be strongest. Wait for two more cycles and reexamine signal strength.
- Repeat steps one and two until the direction where the signal strength is thought to be highest is ascertained.
- Once a direction is decided upon, move in that direction until the signal strength maxes out (255) at a particular gain. Lower the gain, and repeat above steps. This method should bring you very close to the transmitter.
- Due to local habitat conditions, it may not be possible to track signals from a specific direction. Once you have gotten as close as possible to an individual from a particular direction, mark the waypoint, signal strength, and the gain and direction of the signal. Move to an area where the origin of the signal may be accessible and repeat steps 1 through 3. Ideally, you would approach the signal from at least three of the main directions at ~45-60° apart, but this may not be necessary or possible if one has to negotiate 'navigable' waterways.
- Individuals should be localized to the nearest metre (bird is actually observed) whenever possible, or to the nearest 5-10 m based on triangulation methods when observation of the bird is not possible (see Lebevre and Poulin 2003; Montgomery et al. 2010).
- Antennae should always be held horizontally and straight out from the body of the surveyor. Elevation is very beneficial. Even standing in a stationary boat (not a canoe) will make signal strengths more clear. A step ladder may prove to be a beneficial tool for standing in the marsh to get above the vegetation.

Note on interference: Depending on the frequency of the transmitters, interference will occur from boat motors, personal electronic devices, airplanes, fish-finders, etc. All electronic and motorized devices should be turned off during tracking to minimize interference and to maximize signal strength.

6.2 Initial tracking surveys

Birds should be intensely tracked (twice daily localizations) during the first 5 days following deployment. This will help to ensure that a) individuals are not responding negatively to the transmitter harness, b) transmitters have not been lost, and c) territories or preferred locations are identified which will make subsequent tracking efforts more efficient. For these reasons, tagging may need to be staggered or trapping and tracking teams will need to work simultaneously.

6.3 Follow-up tracking surveys

After the initial surveys have been completed, the frequency of localizations can be reduced, depending on the specific project design, available resources, and the number and distribution of tagged individuals. Localizing all individuals two or three times a week should be a realistic target. Follow-up surveys can be conducted in one of two ways depending on desired accuracy of data.

Tracking from stationary sample points **:** Stationary sampling points are useful for determining whether individuals are present in a broad area and to locate specific areas to target for more detailed searching. This method provides presence/absence information with an accuracy of about 500 m. The following steps will need to be repeated for each frequency used, if beeper tags are deployed. Stations should be set up at 500 m intervals surrounding and within the survey area if possible. At a relatively high gain, point the antennae in each of the cardinal directions for 15 to 30 seconds. If more than one frequency is in use target area, frequencies will need to be switched and the process repeated as necessary.

Manual tracking searches: Manual searches can give locations with an accuracy of 1-10 m. Exact locations for individuals should be obtained as frequently as possible, but at least two to three times per week. General locations for individuals should be determined prior to manual searches from stationary points. Once the general locations for each individual are known, follow the instructions for localization above.

Supplementary tracking methods: In the event that many individuals have gone missing from target survey areas, a broader region may need to be surveyed in order to determine whether transmitters have failed or individuals have moved. Depending on the extent of potential habitat to be surveyed, additional sites may be able to be scanned by an intensive boat survey of adjacent wetlands, by foot through mainland access points, or by air using a light aircraft.

If aircraft are used, its engines and instruments will cause interference at certain frequencies. Optimum gains that provide good signal strength while minimizing interference should be determined prior to take-off, with engines and instruments running. Affix the antennae to the wheel-housing or wing of the plane pointing forward. Flight lines should occur 1-2 km apart to maximize potential hits from active transmitters. If multiple frequencies are being used, they will have to be systematically cycled through or more than one pass over an area will have to be made by the aircraft.

6.4 Tracking fledglings

Once young have fledged, frequency of localizations may need to be increased in order to keep track of dispersal rates and distance travelled. Use the same methodologies as above, but localizations should occur at least two to three times a week until the transmitter fails, individuals disperse away from the survey area, or migrate.

6.5 Retrieving lost radio tags

Depending on the attachment techniques, tags will undoubtedly fail or fall off. Unusual or subdued signal sounds (beeps), or difficulty localizing individuals is a sure sign that something is wrong with a tag or it has fallen off. In many cases, tags can be reused, so effort should be made to find lost tags. Finding and disabling these tags will also improve ability for tracking other individuals in the study area. To find a lost tag, follow the localization steps, but searchers will undoubtedly have to do go into the marsh on foot and it may require more than one receiver actively searching a small area. When the signal strength is approaching maximum values at minimum gains, the tag is within 1-2 m of the antennae.

6.6 Data recording

All localization data should be entered into a spreadsheet program as timely as possible. The following information is typical: tag ID; band number; date; time (GMT); UTM; station number; frequency; gain; average signal strength; estimated accuracy; direction of signal; comments.

7. Geolocators

Geolocators can be useful to identify migration routes and wintering grounds, provided that individuals can be reliably recaptured with equipment intact and functional (e.g., Stutchbury et al. 2009). Harness and attachments are very similar, if not identical to, radio-transmitter attachments. However, recapture effort for the year following geolocator deployment must be increased substantially in order to guarantee retrieval of the unit.

Average cost for one geolocator tag is ~$250 to $350. Geolocators are also available with a radio beacon that can be activated automatically in the following season to aid in unit recovery. If geolocators are desired for LEBI work, the beacon option is strongly recommended (cost ~$550 to $750 per unit). However, given that there is no information available on site fidelity or survivorship of Least Bitterns, and very limited information on the success of trapping efforts, it is recommended that geolocators not be deployed until a successful trapping methodology has been established.

<u>Suppliers:</u>

- British Antarctic Survey
- Lotek Wireless, Inc.

8. Tissue Sampling

8.1 Feather collection

Feather collection is simple, relatively non-invasive, and samples can be stored for an extended period of time until funding, time, or resources permit analysis.

Feather collection can aid in the identification of wintering grounds through stable isotope analysis (Hobson and Wassennaar 2008), and population genetics by analyzing tissues of the calamus of feather shafts or other small tissue samples (Kerr et al 2007, 2009).

Preliminary results from specimens at the Royal Ontario Museum suggest that LEBI collected in Canada winter primarily along the Gulf Coast of the United States (Nick Bartok pers. comm.). Feather collection and analysis beyond those available through museum specimens may not yield more useful information. That said, the museum samples represent a large cross-section of time periods and may not necessarily represent the current condition.

All efforts should be made to keep the feather free from contamination. Latex gloves should be worn or sterile tweezers used to extract the feather. Hold the feather tightly as close to the body as possible and give it a firm quick tug perpendicular to the feather tract. From 3-5 feathers per individual should be sufficient. Place the feather in a small coin envelope. Mark the date, collector's name, location, and band number of the individual directly on the envelope.

Ideally, the collected feather will have a complete calamus (quill) and any reminisce of the feather sheath if possible. Sometimes moulting feathers will contain a sheath that is full of blood that may be beneficial for genetic or contaminant analysis.

Moult considerations: LEBIs have a complete basic moult strategy in which adult (after-hatch year; AHY) birds replace all their feathers once a year, primarily on the breeding grounds. Hatch-year (HY) or second-year (SY) LEBIs have a preformative moult (post-juvenal) that occurs on the non-breeding grounds and includes all body feathers and some proximal flight feathers (some rectrices and tertials). For this reason, feather sampling for the identification of wintering grounds using stable isotopes will only be useful if body feathers or newly-grown rectrices are collected from known SY birds. Body feathers of other ages may be collected for contaminant or genetic analyses. See Pyle (2008) for more details on age determination and moult patterns.

8.2 Blood sampling

Blood collection carries with it inherent risks and should only be conducted if deemed necessary to fill critical information gaps. Blood sampling is most commonly used for genetic analysis, monitoring of stress hormones, and measuring potential contaminant burdens. Most analyses require only small quantities that can be obtained relatively quickly and easily. See Fair et al. (2010) before conducting any blood work.

Concern that blood sampling may have an adverse effect on the welfare of sampled individuals may make researchers and conservation managers reluctant to participate in such studies. In determining research and management priorities for endangered species, any deleterious effects of blood sampling on the survival of even a few individuals must be given serious consideration.

In general, birds are fairly resilient to blood loss since they do not exhibit respiratory acidosis (when, under abnormal breathing, the amount of acid in the blood increases) and thus do not typically go into shock when blood is lost (Sturkie 1986). A large and growing number of studies have found a lack of effect of blood sampling on survival or return rates (Franks 1967; Raveling 1970; Wingfield and Farner 1976; Bigler et al. 1977; Stangel 1986; Arctander 1988; Colwell et al. 1988; Dufty 1988; Stangel and Lennartz 1988; Hoysak and Weatherhead 1991; Lubjuhn et al. 1998; Arden et al. 2004; Perkins et al. 2004; Schmoll et al. 2004), bird behaviour (Utter et al. 1971; Wingfield and Farner 1976; Frederick 1986; Arctander 1988), and even nest success and related breeding behaviours (Wingfield and Farner 1976; Wingfield 1985; Arctander 1988; Hoysak and Weatherhead 1991; Perkins et al. 2004; and Schmoll et al. 2004). There is little information, however, on the specific effects of blood collection on LEBI, and there are always unexpected risks.

The American Ornithologists' Union (1988) recommends that no more than 10-20% of the total blood volume be collected. Total blood volume for birds is usually about 6-8 ml per 100 g body mass (Sturkie 1986). As such, LEBI weighing ~50 to 90 g are expected to have a blood volume between 3 and 7 ml. Similarly, another general rule of thumb is that no more than 2% of the body weight of the animal be collected in any 14-day period, or no more than 1% at any one time (McGuill and Rowan 1989). For a 100-g bird, the maximum would be approximately 20-30 capillary tubes of blood. These limits apply whether blood is collected for DNA analysis, or whether plasma is harvested for hormone, metabolite studies, etc.

A common procedure to assess physical condition and stress levels in birds is blood sampling for analysis of the hormone corticosterone (e.g., Marra and Holberton 1998). To collect blood samples for measurement of corticosterone, two samples are taken from a bird after capture. An initial blood sample for baseline corticosterone (e.g., pre-disturbance) is taken immediately after the bird is captured (within 3 min).

First, the area around the brachial vein on the wing is cleaned with alcohol. A sterile 26 ½ gauge syringe needle is used to make a tiny puncture in the vein, and a 50 *u*L heparinized capillary tube is used to collect the blood droplets that emerge. When 20-30 *u*L of blood have been collected, a small piece of sterile cotton ball is placed on the puncture, and the wing is gently held shut in a normal position for 1 minute. The bird is then banded aged, sexed, measured and weighed and held in a ventilated cloth bag until 30 minutes post-capture. A second blood sample is taken from the other wing, after the rest period, to measure the induced response (an increase in corticosterone) after a stressor stimulus (the capture event). The bird is then released. More detail on sampling with capillary tubes is provided in Appendix 1.

9. Permit Requirements

- Federal migratory bird banding permit from Environment Canada with special authorization for capture, banding, colour-banding, and telemetry/geolocators and special authorization for a SARA-listed species
- Canadian Wildlife Service – federal scientific collection permit for feather/blood samples and any dead birds or addled eggs that are found incidentally
- Industry Canada approval of frequencies for radio transmitters
- Canadian Wildlife Service - National Wildlife Area research permit (if applicable)
- Parks Canada Agency research permit (if applicable)
- Provincial research permit for work on Crown Lands or provincial parks (if applicable)
- Provincial Species at Risk research permit (if applicable)
- Animal Care Committee approval (not essential, but recommended)
- Boat operator's certificate(s) for relevant field staff

Permission, ideally in written form, will also be needed to access any privately-owned properties.

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Appendix 1. Details on blood sampling procedures from the brachial vein.

The brachial vein is located on the underside of the wing. To take a blood sample from the brachial vein, it is necessary to restrain the bird with one hand only, as the other hand needs to be free to manipulate the needle. The bird is held so that it is upright and with its ventral surface uppermost. It is necessary to retain sufficient control of the wings to prevent the bird from either escaping or flicking its wings while sampling, without putting undue pressure on the chest. The state of the bird is continually monitored throughout the sampling procedure and immediately halted if the bird starts to show any sign of respiratory distress or shock. The bird is held in the “bander’s grip” with the bird’s neck between your fingers, and its back along your palm. The bird’s wing that is to be sampled for blood is held between the index and second fingers, with fingers three and four restraining the wing on the opposite side. The index finger goes underneath the wing and holds it away from bird’s body to expose the brachial vein. The second blood sample is taken from the other wing, and the bird is held the opposite way round so as to access the other wing.

To take a blood sample, the needle is held so that it enters the vein at a 45° angle. The aim is to prick the vein without going straight through it, using a controlled sharp stabbing motion. The emerging blood forms a droplet if the vein has been pierced correctly. The blood is then taken up by applying a heparinised capillary tube to the blood droplet, holding the capillary tube so that it is horizontal or pointing slightly downwards from the drop of blood so that it draws the blood up by capillary action. Then, the capillary tube is emptied into a small plastic vial, ensuring that the blood is delivered to the bottom of the vial rather than becoming lodged part way up. After sufficient blood has been drawn up, a small piece of cotton batton is placed over the vein and the bird’s wing gently closed over it, back in place next to its body. This gentle pressure is sufficient to promote rapid clotting. The bird is held in this position for a minute before checking to ensure that bleeding has ceased. These bags are placed in a secure, quiet location in the shade and sheltered from direct effects of weather, with plenty of air circulation around the bag. After collecting blood from the second wing and ensuring the blood has clotted, the bird is released at the site of capture.

Used needles and capillary tubes are immediately disposed of in a sharps bin, and caps are put on the blood storage vials to prevent spillage. Blood samples are kept on ice in the field, until they are prepared for storage.

Preparing and storing blood samples

Blood is stored on ice until plasma recovery within 4 hours of sampling. To recover the plasma, blood is centrifuged at 6000 rpm for 5 minutes, and the plasma is drawn off and stored at -22^{o}C. Corticosterone concentration is determined in the lab using I125 Coat-A-Count Rat Corticosteroid Radioimmunoassay Kits (Diagnostic Products Corp., Los Angeles, California).

Centrifuged samples are checked to ensure that the blood cells are thoroughly compacted at the bottom of the tube and are properly separated from the plasma. The

plasma appears as a straw coloured layer overlying the layer of compacted red cells. If the plasma appears pink or red, then haemolysis has occurred. Slightly haemolysed (pink) samples need not be discarded, but if any sample appears very dark this will be noted, in case the pigment cross-reacts in the assay. If one of the samples is shaken or jogged accidentally, then the samples will be given another quick spin (about one minute) in the centrifuge.

The plasma fraction is drawn off and transferred to a small, labelled storage vial. It is best to choose small, thin storage vials to minimise the surface area of the plasma as well as the volume of air above it, as large air spaces above the plasma promotes freeze drying during storage, thus artificially concentrating the plasma. To reduce the chance of error, the centrifuged blood sample and the plasma storage vial that is intended for it are picked up together, and the labels on both checked to ensure that they correspond before transferring the plasma to the storage vial. When drawing plasma up, the sample vial is held close to the horizontal, and the needle of the syringe enters the plasma fraction with the slanting orifice of the needle facing downwards, to facilitate collection of as much as possible of the plasma volume whilst minimising the risk of disturbing the red cells. The tip of the needle is kept 1-2 mm away from the red blood cell layer while drawing plasma up to avoid accidentally drawing up the blood cells. The plasma is ejected into the storage vial, taking care to deliver it to the bottom of the vial rather than squirting it down the sides. Several syringes may be needed to collect all of the plasma. If blood cells are drawn up into in the syringe by accident, then the contents of the syringe are squirted back into the sample vial and the sample should be given a pulse in the centrifuge to separate the plasma from the blood cells again. Once the sample has been transferred to the storage vial successfully, the vial is capped at once to prevent evaporation or spillage. The syringe is then rinsed several times in distilled water before moving on to the next sample.

Plasma samples will be kept cool, placed on ice if the room is warm, and subsequently stored in a -20°C freezer until it is convenient to carry out the assay.