TECHNIQUES

A Non-destructive Source of DNA from Hatchling Freshwater Turtles for Use in PCR Base Assays

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In recent years, a number of turtle species have been designated at risk. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) currently lists five turtle species: leatherback turtle (*Dermochelys coriacea*; endangered), eastern spiny softshell turtle (*Apalone spinifera*; threatened), Blanding's turtle (*Emydoidea blandingii*; threatened), spotted turtle (*Clemmys guttata*; vulnerable), and wood turtle (*Clemmys insculpta*; vulnerable), with seven additional species yet to be assessed.

Molecular genetics has provided an increasingly valuable tool in gathering information germane to conservation status and management plans (Lambert and Millar 1995; Moritz 1995; Prior et al. 1997). The development of highly variable genetic markers (for review see O'Reilly and Wright 1995) and the polymerase chain reaction (PCR; Mullis et al. 1986) allows examination of population genetic structure at fine spatial and temporal scales as well as examination of relatedness and parentage, using very small amounts of DNA.

In adult turtles, DNA can be obtained non-destructively from blood samples. However, hatchling freshwater turtles are often too small to be effectively sampled by drawing blood. Toe clipping, which yields DNA in amphibians (Gonser and Collura 1996), is undesirable in turtles as claws are used in mating for both titillation and mounting. Blood has been collected from both the dorsal cervical sinus and the occipital sinus in sea turtle hatchlings (Wibbels et al. 1998 and references therein), however these sinuses either do not exist or are insufficiently developed in freshwater turtles to use as a blood sampling site. Until now, DNA could be obtained from hatchling freshwater turtles in two ways: sacrifice the hatchlings (see Galbraith 1993) or raise hatchlings in captivity until they are large enough for blood sampling (Mockford et al. 1999). We describe here a non-destructive source of DNA from hatchling turtles for use in PCR-based assays.

As part of the monitoring program of the Nova Scotia population of Blanding's turtle (*Emydoidea blandingii*) newly emerged hatchlings are given nest-specific marks (Herman et al., in press). Marking is done by notching up to three marginal scutes (Standing et al. 1995), from each of which a small fragment of carapace (approximately 2 mm^2) is removed. The underside of these carapace fragments is not yet ossified bone, and serves as our source of DNA.

Scutes are notched using a nail clipper and resulting fragments are placed in 1 ml of 70% ethanol at collection and stored at –20°C for up to one year. Nail clippers are rinsed in 100% ethanol and allowed to air dry between samples. Prior to extraction, individual fragments are removed from the ethanol and rinsed in 50µl high TE (100mM Tris-base, 40mM EDTA, pH 8.0) for approximately one minute, then placed in 25µl extraction buffer (10mM Tris-base [pH 8.3], 50mM KCl, 0.8% Tween 20) to which 2.5µl of Proteinase K (1mg/ml) has been added. The extraction buffer is incubated at 45°C overnight, and subjected to centrifugation at 11000 g for 1 minute. 20µl of the supernatant is pipetted into a new tube. We use the undiluted product of this crude extraction in PCR reactions for microsatellite assays.

While this crude extraction can be used directly, the DNA is likely contaminated with proteins (A260/A280 < 1.0) and may not be stable for long term storage. Where longer storage is desired DNA can be precipitated in 1/10 volume 3M sodium acetate and 2 volumes cold 100% ethanol. Following precipitation and resuspension in 25µl TE the average yield per fragment was 3.02µg (SE 1.02, N = 12; Beckman DU-64 spectrophotometer) with an A260/A280 of 1.56 (SE 0.22, N = 12). The comparison of absorbance at 260nm and at 280nm provides a measure of the purity of the DNA; pure DNA = 1.8.

Seventy-eight percent of samples were successfully PCR amplified in the first attempt from the crude extraction, and this did not improve following precipitation. Subsequent PCR reactions of the remaining samples required adjustment of the DNA concentration. This protocol has been used in a variety of tissue types including muscle tissue (McConnell et al. 1995) and fish eyeballs (Ruzzante et al. 1996) with similar results (D. Cook, pers. comm.).

As molecular genetics continue to be integrated into conservation biology the need for non-destructive sampling techniques increases. The technique described here has been used to sample hatchlings as small as 6.1 g with carapace lengths as short as 30 mm. While we have only used this technique on samples from Blanding's turtles, similarity in carapace anatomy suggests that this technique will work with many freshwater turtle species.

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Comparing the Use of Pitfall Drift Fences and Cover Boards for Sampling the Threatened Sand Skink (*Neoseps reynoldsi*)

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The sand skink (*Neoseps reynoldsi* Stejneger) is a fossorial lizard found only in xeric upland habitats (sandhill, scrub, scrubby flatwoods) in central Florida, USA. It is a small species, adults typically are only 100–130 mm in total length, and it displays a variety of morphological characters probably related to a fossorial existence. Among these characters are small limbs, lateral grooves to accept limbs during subsurface locomotion ("sand swimming"), wedge-shaped head, subterminal jaw, and small eyes.

The sand skink is a federally threatened species, with poorly-understood patterns of distribution and abundance among habitats (Campbell and Christman 1982a; Christman 1992; Cooper 1953; McCoy et al. 1999; Myers and Telford 1965; Telford 1959; U.S. Fish and Wildlife Service 1993). The development of precise methods for recognizing patterns of distribution and abundance is vital to its recovery (U.S. Fish and Wildlife Service 1993). A first step in developing such methods is to compare how well different sampling techniques detect its presence and accurately reflect its abundance. We compared two common herpetological sampling techniques, pitfall drift fences and cover boards.

Recent studies of the sand skink have used pitfall drift fences almost exclusively (e.g., Andrews 1994; McCoy and Mushinsky 1994; McCoy et al. 1999; Mushinsky and McCoy 1995). Pitfall drift fences are effective in capturing individuals, but they are relatively expensive to construct and maintain. Cover boards (Grant et al. 1992), on the other hand, are relatively inexpensive to construct and maintain, but are not as effective as pitfall drift fences in capturing individuals. Although individuals occasionally may be found under cover boards and other kinds of ground cover, they are not constrained to remain there. The fact that they do not constrain individuals may not necessarily be a shortcoming of cover boards, however, in many studies of the sand skink.

We compared the relative effectiveness of the two sampling techniques at seven sites, all of which were in scrub habitat and were known from preliminary surveys to harbor the sand skink (McCoy et al. 1999). We had little a priori information about the abundance at the seven sites, which made it difficult to decide how many cover boards and pitfall drift fences to install. We settled on a density of 1 cover board per 100 m^2 (a total of four cover boards at each site), based on previous studies. This density is lower than the density recommended for intensive field studies (e.g., Fellers and Drost 1994), but higher than the density employed in previous comparative studies (e.g., Campbell and Christman 1982b; Grant et al. 1992). We installed pitfall drift fences at a four-fold higher density than cover boards (a total of 16 at each site). Although matched numbers of cover boards and pitfall drift fences at each site would have provided a parsimonius experimental design, we installed additional pitfall drift fences to provide the higher level of trapping intensity that we thought would be required to estimate overall activity and reflect total abundance accurately (see below).

A pitfall drift fence consisted of a 2 m length of aluminum flashing with two 3.8 L buckets countersunk at each end, so that individuals diverted by the fence fell into the buckets. The combined lengths of the 16 drift fences at a site approximately equals the combined lengths of the four drift fences in a standard pitfall drift fence array (Campbell and Christman 1982b), but the number of pitfall traps is quadrupled. We assumed that the increase in the number of traps per meter of drift fence would increase the chance of capturing individuals of the small sand skink. Pitfall drift fences were centered on 16 stakes, evenly spread over a 20 m x 20 m area. Drift fences were buried to a depth of 30 cm, to ensure that individuals would not pass under them. Buckets were countersunk to depths between 3 cm and 8 cm below ground surface. The bottom of each bucket was perforated with at least ten 0.32 cm holes to promote drainage, and a lid was positioned over the bucket on clothespin supports to provide shade. We placed a layer of sand, at least 5 cm deep, in each bucket to provide substrate for burrowing.

A cover board consisted of a 1.27 cm-thick piece of plywood, 60 cm x 60 cm, which is intermediate in size to those used in most previous studies, 30 cm x 30 cm to 66 cm x 133 cm (Fellers and Drost 1994; Grant et al.1992). One cover board was placed at random within each quarter of the grid created by the pitfall drift fences at a site. The area where a cover board was placed was gently raked to bare sand and then leveled, so that the cover board rested on the ground at all points. In a few places where roots were at the surface, sand was added to create a uniform sandy surface under a cover board.

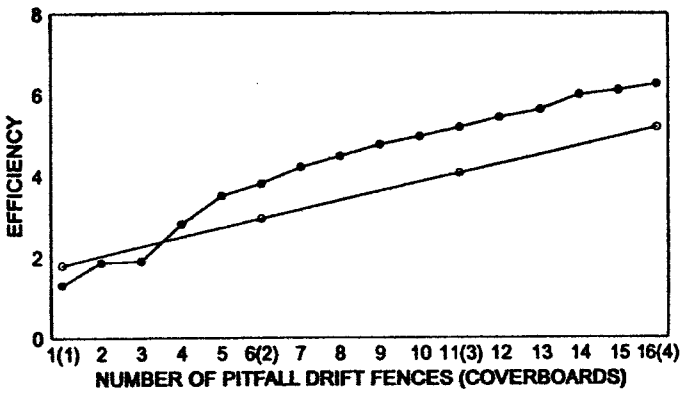

FIG. 1. Efficiency of detection (reciprocal of number of days to detect presence x 100) for 1–4 cover boards (open circles) and 1–16 pitfall drift fences (closed circles).