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Technical Note

Rapid Method for Immobilization and Collection of Sea Turtle Muscle Biopsies for Electrophoresis

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There is a growing interest in the use of electrophoretic methods in the study of population breeding structure and systematics of turtles (Smith *et al.* 1977; Frair 1982). For electrophoretic studies, blood can be readily sampled from turtles (Frair 1977; Owen and Ruiz 1980; Avery and Vitt 1984). To increase the range of enzymes available for study, a simple method was sought for the sampling of solid tissue, which can be more useful in electrophoretic studies, from turtles. Solid tissues such as liver and muscle usually are obtained through surgical manipulation or the killing of the animal; we here describe a non-destructive and non-surgical technique for the collection of sea turtle muscle tissue.

Both front flippers of the turtle must be restrained to prevent it injuring itself through flipper movement. The turtle can be restrained either while in its normal posture (as with a nesting turtle) without turning it on its back, or while on its back (as with turtles landed from boats). A restrained turtle is shown in Fig. 1. The restraining device consisted of a hook and two ropes. The hook (Fig. 1) consisted of a ring with two prongs made from steel rod 5 mm in diameter. Each prong was blunt-ended and recurved to fit over the posterior edge of the carapace. Hooks of different sizes were made to fit small immature turtles (curved carapace length, 40–80 cm) and breeding adults. Each rope was 2 m long with an eye splice at one end. A flipper was restrained by placing a running noose around the axillary area, then forming a half-hitch just proximal to the claw. The flipper was then tied firmly back to the hook attached to the posterior carapace. This achieved a dual result: as well as being immobilized the flipper was fully extended, allowing access to the upper limb.

This method seems a low-cost and non-injurious alternative to those employed elsewhere. In particular, it provides an alternative to the harmful practice of restraining sea turtles for research purposes by tying their flippers together with ropes threaded through holes cut in the flippers.

Muscle samples were taken with Tru-cut biopsy needles (Travenol Laboratories), according to the manufacturer's instructions. The muscles most conveniently sampled were the triceps brachii and the brachialis inferior. These muscle blocks were located by palpation above the elbow joint. Before sampling, local anaesthetic (xylocaine 2%) was injected intradermally in two or three equal doses around the site to be biopsied. Total volume of anaesthetic used was 0·5–1·0 ml, depending on the size of the turtle. From three to five samples could be obtained sequentially from the one site, causing only minor trauma to the turtle. On collection, samples

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were placed on strips of aluminium foil which were folded in such a way the consecutive samples were separated. These sample sets were placed immediately in plastic vials and frozen on dry ice for storage at −20°C. Individual samples weighed 10–30 mg and, when homogenized, provided 50–100 µl of clear supernatant suitable for electrophoresis.

Fig. 1. An adult loggerhead turtle restrained in preparation for taking a muscle biopsy. Arrow denotes the biopsy site.

The method was quick and caused minimal disturbance to the turtles. For example, a nesting turtle that had completed laying and was filling in its egg chamber was immobilized, sampled and released in less than 5 min. It would continue to complete its nesting cycle on release.

Biopsy sites on more than 25 nesting females were inspected during their subsequent renesting crawls approximately 2 weeks after the sampling. The puncture site could not be detected on any of them. In addition, none showed any signs of disability attributable to the biopsy. The method has been used successfully with several hundred turtles from all genera of the family Cheloniidae, ranging in size from immatures of 40 cm curved carapace length to breeding adults of both sexes. This technique should be applicable to obtaining muscle samples from a wide range of similar-sized animals.

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