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A SIMPLE METHOD TO OBTAIN SERUM FROM SMALL FISH

It is desirable to obtain blood serum information from small fish due to their extensive use in pollutant and disease studies (Snieszko et al. 1969; Snieszko 1974; Mulcahy 1975). It is well known that gross observations cannot detect subtle changes in blood chemistry caused by environmental factors such as stress, diet, or inflammation (Mulcahy 1975) and some pesticides (Walker 1963).

Techniques to obtain fish blood for study have been described in reviews by Hesser (1960) and Blaxhall (1972). Cardiac and venous puncture are the most commonly used techniques for fish >150 mm, while severance of the caudal peduncle and insertion of a capillary tube to draw blood is usually employed for smaller fish. Fish <60 mm present problems because the quantity of blood obtainable is small (generally <0.2 ml), coagulation time is quick, and tissue fragments or clots can clog collecting tubes, causing loss of serum in the transfer from one container (or collecting tube) to another for centrifugation. In most cases anticoagulants are used to eliminate some of these problems.

Sodium oxalate, heparin, or dipotassium ethylenediaminetetraacetate (EDTA) are the most commonly used anticoagulants. Unfortunately, oxalate and EDTA anticoagulants can interfere with serum ion determinations, such as calcium, and produce misleading data (Tietz 1976). When many blood serum components are to be measured, especially on instrumentation such as an amino acid auto analyzer, a quantity of serum (at least 0.5 ml and preferably free of anticoagulant) must be obtained for the numerous tests these analyzers can do. Heparinized tubes, excellent for single serum component tests, are limited because the volume of serum they can obtain is generally not enough for use with sophisticated instrumentation. This note describes a simple method to obtain pooled serum samples, without anticoagulants, from fish <60 mm when heparinized tubes are not practical.

Materials and Methods

Small fish <60 mm fork length should be anesthetized, if desired, and blotted to remove excess water on the fish's body. A dry Kimwipe[1] is wrapped around the fish, covering the vent to prevent contamination of the sample, leaving approximately 2.5 cm of the tail exposed (Figure 1B). A small portion of the Kimwipe is allowed to overlap the fish's head. The caudal peduncle is severed with sharp scissors, leaving a slight point at the caudal region (Figure 1C). The fish is rapidly in-

[1]Reference to trade names does not imply endorsement by the University of Southern Mississippi or by the National Marine Fisheries Service, NOAA.

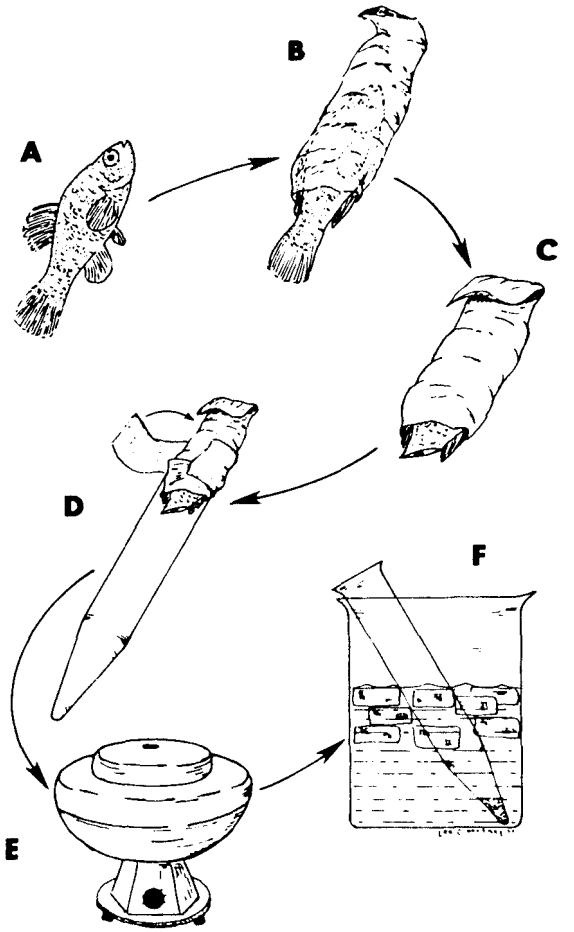

FIGURE 1.—Diagrammatic representation of the centrifuge method for extracting serum from small fishes. A. Blotted fish ready for bleeding. B. Fish is wrapped with Kimwipe, leaving overlap at top. Vent is also covered to prevent contamination of the sample. C. Caudal peduncle is cut at an angle with sharp scissors. D. Fish is fit snugly into a 15 ml centrifuge tube, using more or less Kimwipes to obtain a proper fit, and taped so the fish will not be pulled into the tube. E. Fish, in the tube, is spun at 400 *g* for 3 min. F. Covered tube with blood is kept in an ice water bath until another fish can be processed in the same tube.

serted, tail first, into a 15 ml Pyrex centrifuge tube and secured by taping the overlapping Kimwipe to the side of the tube (Figure 1D). (The fit must be snug but not too tight). Varying fish sizes can be compensated for by wrapping with more or less Kimwipes; fish will not be pulled into the tube if the wrapping is correct. Fish are spun in a centrifuge at 400 *g* for 3 min. After each fish is centrifuged, the tube of blood obtained (one tube for all fish) is covered and quickly placed in a beaker of ice water to inhibit evaporation of serum obtained and hemolysis of red blood cells. A total pooled sample of approximately 1 ml can be collected from 20 fish. The blood is allowed to clot and the serum drawn off. From 1 ml of pooled whole blood, 0.5 ml of serum can be obtained.

Discussion

Contamination by tissue fluid, lymph, and cell debris is unavoidable but exists in any method which severs the caudal peduncle. Proper wrapping of fish and the use of sharp scissors help to reduce this contamination. The amount of lymph and intracellular fluid gained from the cutting action of the scissors and centrifugation is minimal and does not prejudice one's results significantly. Cellular debris from the actual wound is insignificant because the serum is separated from it before analysis. Careful placement of Kimwipes around the vent eliminates urine or feces contamination of the sample. This method has the advantage of simplicity, speed, and no anticoagulant contamination. Red blood cell hemolysis is minimal, and larger pooled blood samples can be obtained in a single container with little serum loss during unnecessary transfers when heparinized collecting tubes are unfeasible. This technique should be helpful in pathologic studies of small fish used in toxicity tests when it is desirable to monitor many blood serum parameters and where there is no objection to the use of pooled samples.

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STRANDING OF THE PILOT WHALE, *GLOBICEPHALA MACRORHYNCHUS*, IN FLORIDA AND SOUTH CAROLINA

An opportunity to observe the behavior of stranding pilot whales occurred in February 1977. Before dawn on the 6th, 175-200 pilot whales moved with the rising tide into the Fort George River, 1.5 km north of the mouth of the St. Johns River (lat. 30°25′ N, long. 81°29′ W), near Jacksonville, Fla. The weather was clear, calm, and cold; minimum air temperature was 0° C at Jacksonville Beach (Environmental Data Service 1977:6). Once inside the river mouth, the animals turned south into a small, shallow embayment (Figure 1). A chronology of the events that followed is presented below. Events of 6 February were summarized by Willard Patrick.[1]

6 February 1977.—Sometime prior to dawn the whales began moving onto the southeast shore (Figure 1, Site A), where they were stranded either by their movements or by the falling tide. Throughout the day, many of the whales were refloated repeatedly by Florida Marine Patrol (FMP) officers and local volunteers, but many were immediately stranded again. Some whales thrashed vigorously during attempts to refloat them. By 2100 h, 21 whales were dead on the beach and the remainder were milling around near the middle of the bay in water 1-2 m deep. During the night of 6-7 February, what was thought to be the remainder of the herd approached the surf zone at high tide and an estimated 25 whales moved into the ocean. Those whales not exiting through the surf are believed to have returned to the embayment although some may have stranded and died, then drifted out to sea.

7 February 1977.—At 0845 h, 23 whales, including the 21 from the previous day, were dead on the beach, most near Site A (Figure 1). Two groups of 40 to 60 whales were milling around in the bay, one group approximately in the center and one near Site B (Figure 1). Several smaller groups of up to five animals each were also sighted.

At about 1030 h, the large groups restranded at Sites A and B. Many of the animals near Site A were pushed off by volunteers; approximately 40 whales near Site B died within an hour.

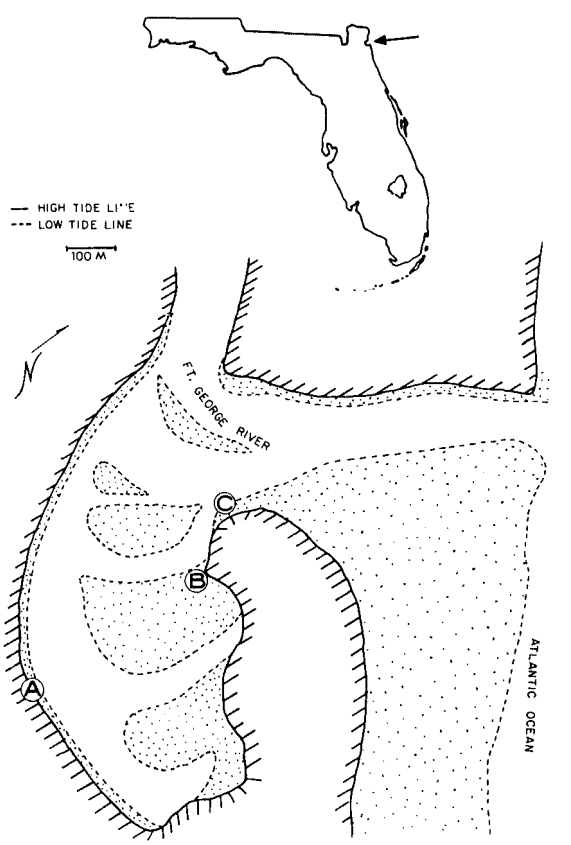

FIGURE 1.—Pilot whale stranding sites (A-C) in the Fort George River, Duval County, Fla., lat. 30°25′ N, long. 81°24′ W.

[1]Willard Patrick, Sergeant, Florida Marine Patrol, District 8, 4124 Boulevard Center Drive, Jacksonville, FL 32207, pers. commun. March 1977.