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A Saline-flushing Technique for Determining the Diet of Seed-eating Birds

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Stomach flushing has been used to determine the diets of many avian species. It has been widely used to sample the food habits of large, hardy birds such as penguins (Randall and Davidson 1981, Horne 1985, Gales 1987) and other seabirds (Wilson 1984, Ryan and Jackson 1986, Wilson et al. 1989). Applications of stomach flushing to smaller birds generally have been limited to nongranivorous species. Brensing (1977; cited in Ford et al. 1982) used warm tap water forced through plastic tubing to flush the crops of more than 2,100 migrant passerines of 35 species (mostly insectivores), with only one mortality. Ford et al. (1982) used a similar procedure to recover food from crops and, sometimes, gizzards of 157 passerines (28 insectivorous and honey-eating species). Although 13 birds required reflushing, all birds eventually regurgitated some of the stomach contents, and no deaths occurred. Major (1990) flushed the stomachs of more than 300 White-fronted Chats (*Ephthianura albifrons*) with no deaths. Zach and Falls (1976), however, reported heavy mortality of Ovenbirds (*Seiurus aurocapillus*) when saline flushing was attempted. Moody (1970) described a saline-flushing technique for insectivorous birds in which gizzard contents were intentionally forced out through the cloaca. This method was used to void the digestive tracts of 72 swallows, with 8% mortality. Moody's attempts to use the method on granivorous birds were unsuccessful because the muscular gizzards impeded the flow of the saline solution. When Laursen (1978) used this method to flush the digestive tracts of 396 migrant passerines, 14 birds died during flushing, and the flushing removed only one-half of the initial stomach contents.

Our analyses of grit use by captive House Sparrows (*Passer domesticus*; Best and Gionfriddo 1994, Gionfriddo and Best 1995) required a method of removing all grit from the gizzards of live birds. We developed a saline-flushing technique that enables researchers to recover the food and grit in gizzards of live granivorous birds. Although our method was used in the laboratory, it can be adapted for use in the field. The procedure requires two workers and involves anesthetizing the birds and allowing their recovery before they are released into the wild or used in experiments. It is highly efficient in voiding the gizzard, and the mortality rate is low. We used the method only with House Sparrows, but believe that, with minor modification, it would be effective with other avian granivores with well-developed, muscular gizzards.

Before flushing the gizzard, we anesthetized each bird by injecting it with a mixture of ketamine hydrochloride (Vetalar; Fort Dodge Laboratories, Fort Dodge, Iowa; diluted to 10 mg/ml) and diazepam (Valium; Elkins-Sinn, Inc., Cherry Hill, New Jersey; 5 mg/ml). Diazepam reduced wing fluttering and facilitated recovery from ketamine hydrochloride. For each bird, the anesthetic was prepared by drawing two to three drops of diazepam into a 1.0-cc plastic, disposable syringe equipped with a disposable (26 gauge, 1.0 cm) hypodermic needle and then reinjecting the diazepam into its vial, leaving a tiny residue (less than one drop) in the syringe. Ketamine hydrochloride (0.15 cc) was then drawn into the syringe and mixed with the diazepam. The mixture then was injected into the bird's pectoral muscle, and the bird was restrained or placed in a holding cage for 2 to 3 min to allow the drugs to take effect.

To flush the gizzard, we used a 10-ml Cornwall syringe pipet (Thomas Scientific, Swedesboro, New Jersey) with a ball-tipped, straight intubation needle (18 gauge, 7.6 cm long). The bird was placed on its back, its head extending over the edge of the table or work surface. The tip of the intubation needle was placed in the bird's mouth and then carefully passed through the esophagus to the gizzard. This process was facilitated by gently holding the bird's head and slightly stretching its neck. We sometimes had to maneuver delicately the tip of the intubation needle to enter the opening to the gizzard. The syringe pipet and the bird were then carefully raised to a vertical position, with the bird's beak pointing downward. The syringe pipet's plunger was then depressed 20 times to pump 30 cc of saline solution (0.9% sodium chloride irrigation, USP; Travenol Laboratories, Inc., Deerfield, Illinois) from a nearby bottle to the gizzard, in 1.5-cc injections. After the first 10 injections, we paused for 15 to 30 s to make certain that the bird was not having difficulty breathing. Gizzard contents were forced out through the esophagus and mouth by hydraulic pressure and gravity. A funnel with filter paper was placed below the bird to recover grit and food flushed from the gizzard. If the bird showed signs of having inhaled the saline solution (gasping, sputtering), flushing was stopped temporarily to allow recovery. Usually a 1-min rest was sufficient. When flushing was completed, the bird was placed in a pa-

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per bag or a holding cage for 0.5 to 2 h to permit recovery from anesthesia. Most birds recovered fully in less than 1 h.

The effectiveness of the saline-flushing technique was validated in two ways. In developing the method, we tested several combinations of injection volume (1.0, 1.5, or 2.0 ml), number and timing of injections (5 injections, pause, 5 injections; 10 consecutive; or 10, pause, 10), and total injected volume (10, 15, 20, 30, or 40 ml). For each combination, the gizzards of 10 or more House Sparrows euthanized 1 h after flushing were removed, and the gizzard contents examined with a microscope. Material that had been flushed from each of these gizzards and collected also was examined. All procedures we tested that had total injected volumes of 15 ml or more flushed nearly all grit and food particles from gizzards. Procedures incorporating the "10 injections, pause, 10 injections" protocol had the combined advantages of a large number of injections, a large total injected volume, and a low incidence of birds' having breathing difficulties. The only procedure tested that achieved 100% removal of food and grit particles was flushing gizzards with 30 ml of saline solution delivered as "10 injections, pause, 10 injections" (at 1.5 ml/injection). We selected this as the most efficient and humane of the procedures that we tested.

The saline-flushing technique also was validated incidentally in many of our grit-use experiments with captive House Sparrows. We flushed the gizzards of birds before experiments in which the birds were given access to one or more types of "experimental" grit. When gizzard contents were examined after the birds were euthanized at the ends of these experiments, particles of "natural" grit picked up by the birds before capture and not removed by the flushing procedure rarely were found (Gionfriddo and Best unpubl. data).

Since its development, we have used this technique to void the gizzards of 974 House Sparrows. Its advantages include the ability to flush thoroughly all grit and food particles from the gizzard by repeatedly injecting a metered volume of solution. Diet and grit analyses may be seriously biased if the gizzard contents are not completely voided. The syringe pipet can be adjusted to deliver any volume from 0.5 to 10.0 cc per injection and, with intubation needles available in various lengths and shapes, can accommodate birds of different sizes. Avian gastric morphology varies greatly among species, however, and must be carefully considered when determining the feasibility of stomach flushing and when selecting the length and curvature of the intubation needle. Another major advantage of stomach-flushing methods in general is their nondestructive nature. Birds may be processed and released, or sampled repeatedly in experiments. Recaptures and resightings of treated birds suggest that there are no lasting ill effects of stomach flushing (Moody 1970, Ford et al. 1982, Major 1990).

Several limitations are associated with the use of this saline-flushing method. In our work with House Sparrows since the development of the technique, death occurred in 80 (8.2%) instances, either during flushing or within 24 h, usually resulting from inhalation of saline solution. Many (26) of these deaths occurred during a two-day period when we used the method outdoors during relatively cold (<10°C) weather. Hypothermia may have contributed to the unusually high mortality rate (22.6%). Of the 974 House Sparrows on which we used this flushing method, 12 were killed when the ball-tipped intubation needle punctured the esophageal or proventricular wall. No known anesthetic overdoses occurred. Saline flushing caused gizzard contents to be forced out through the cloaca (as in Moody's [1970] method), rather than the mouth, in 26 birds.

Other limitations associated with saline flushing include biases associated with differential digestive rates for various food items and the identification of fragmented food items (Rosenberg and Cooper 1990). These biases, however, are common to all methods involving the analysis of stomach contents.

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Intersexual Comparison of Plasma Osmolytes, Kidney Size, and Glomerular Number and Size in Pekin Ducks (*Anas platyrhynchos*)

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Increased sodium chloride (NaCl) intake resulted in sexually disparate changes in body mass and plasma osmolality (Osm_{pl}; Hughes et al. 1992) and plasma concentrations of the osmoregulatory hormones arginine vasotocin ($[AVT]_{pl}$) and angiotensin II ($[AII]_{pl}$; Zenteno-Savin 1991) in Pekin ducks (*Anas platyrhynchos*). During gradual increase in drinking water NaCl concentration ([NaCl]), both sexes maintained body mass and changed Osm_{pl}, $[AVT]_{pl}$, and $[AII]_{pl}$ similarly until they drank 300 mM NaCl; at higher [NaCl], males became more dehydrated and their Osm_{pl}, $[AVT]_{pl}$, and $[AII]_{pl}$ increased significantly more than in females. Kidney mass tended to be smaller in male ducks than in female ducks (Hughes et al. 1989, 1992).

In this study, we reexamined body mass, hematocrit (Hct), plasma ion concentrations, and Osm_{pl} of male and female Pekin ducks rapidly acclimated (over three weeks) to 225 mM NaCl and following longer exposure to this [NaCl] (four and seven months). Finally, the ducks were euthanized and dissected to obtain organ masses. We found female ducks had larger kidneys, so we counted glomeruli (nephrons) in kidneys of both sexes to determine if the kidneys of females actually contained more nephrons. We also measured glomerular diameters to determine if glomerular sizes were equally represented in both sexes.

Methods and materials.—Twelve Pekin ducks (6 male, 6 female) were reared for six months in adjacent enclosures on *ad libitum* tap water and duck pellets (Buckerfield's, Abbottsford, British Columbia; sodium, $[Na^+]$, potassium, $[K^+]$, and chloride, $[Cl^-]$, concentrations, 83, 153.5, and 99 $mM \cdot Kg^{-1}$, respectively). They were then acclimated in three equal weekly increments to 225 mM NaCl. Drinking this solution at the minimum rate measured for Pekin ducks (Hughes et al. 1991) should replace at least one-half of the Na^+ in the extracellular fluid each day. This Na^+ intake should have been sufficient to increase the secretory potential of the salt glands allowing Osm_{pl} to be maintained (Hughes et al. 1992). At the end of each week, birds were weighed and 5.0 mL blood samples were taken. The birds were held on 225 mM NaCl for seven months and blood samples were taken at the end of the fourth and seventh months. Triplicate Strumia microhematocrit tubes were immediately filled from each blood sample and centrifuged simultaneously with the remaining blood for 3 min at 15,600 × g. After the last blood sampling, four female and three male ducks were intravenously infused with Alcian blue for 30 min to stain the glomeruli (Bankir and Hollenberg 1983) and then euthanized with a lethal dose of sodium pentobarbitol. The heart, liver, kidneys, and salt, adrenal, and Harderian glands were removed and weighed with a Mettler top-loading balance with an accuracy of 1 mg. Experiments conformed to guidelines of the Canadian Council on Animal Care.

All analyses were done at least in duplicate. De-