# AN IMPROVED X-RAY TECHNIQUE FOR INVESTIGATING INGESTION OF LEAD BY WATERFOWL

FRANK MONTALBANO, Florida Game and Fresh Water Fish Commission, Okeechobee, FL 33472
TOMMY C. HINES, Florida Game and Fresh Water Fish Commission, Gainesville, FL 32611

*Abs act*: This study compares 3 techniques for their effectiveness in detecting lead shot in waterfowl gizzards. X-rays of gizzard contents are more accurate than either X-rays of whole gizzards or manual examination of contents. Manual examination missed 24% and X-rays of whole gizzards missed at least 28%. The most accurate technique appears to be X-ray of contents with manual verification of all positive X-ray signatures.

Proc. Ann. Conf. S.E. Assoc. Fish & Wildl. Agencies 32: 364-368

Detrimental effects of ingested lead shot pellets upon waterfowl populations have been documented for at least 50 years (Wetmore 1919). Bellrose (1976:99) estimated that 2 to 3% of the fall and winter continental waterfowl population dies from lead ingestion each year. The significance of this mortality increases as the status of the waterfowl resources deteriorates (Bellrose 1975). To alleviate this waste of waterfowl, the U.S. Department of the Interior proposed restrictions on the use of lead shot for taking ducks, geese, swans (Anatidae), and coots (*Fulica americana*).

The burden for collecting evidence to support identification of areas with elevated lead ingestion rates fell to the state wildlife agencies. As a result, the Florida Game and Fresh Water Fish Commission's Division of Wildlife developed a program to identify the principal areas within the state contributing to lead poisoning in waterfowl.

Studies of lead ingestion rates have employed a number of techniques, including direct examination of gizzard contents (Bellrose 1959, Stutzenbaker 1975:3), fluoroscopic examination of live-trapped ducks (Bellrose 1959) and fluoroscopic and radiographic examination of gizzards (Lewis and Legler 1968, Bellrose 1959, USDI 1976:30).

Experience from the direct examination of contents of 77 Florida duck (*Anas platyrhynchos fulvigula*) gizzards for ingested lead during 1975 and 1976 led project personnel to question the accuracy of the technique. The similarities in size, and in some cases color, between ingested pellets and various seeds and grit commonly found in the gizzards made identification of ingested pellets difficult.

K. Moore (personal communication), of the California Department of Fish and Game, evaluated the results of fluoroscopic examinations of three lots totaling 4,700 gizzards to determine inaccuracies inherent in that technique. Whole gizzards were fluoroscoped and estimates of ingested lead were recorded. Gizzards were then opened and gizzard contents were fluoroscopically examined. In all 3 lots, additional ingested lead was identified by examining gizzard contents only. Moore indicated that the source of error seemed to be masking of small, well-eroded pellets by the fluoroscopic signature from tightly-packed gizzard contents.

In light of Moore's findings and our experience with direct examination of gizzard contents, we concluded that fluoroscopic examination of gizzard contents would provide the most reliable indication of ingestion rates. However, a preliminary evaluation revealed several disadvantages. The Phillips Super 70 X-ray machine utilizes an auxiliary tele-display of the fluoroscopic image. The image was of poor resolution and covered a narrow field of view requiring excessive manipulation of specimens.

As a result of uncertainty concerning the reliability of various techniques, we designed a study to determine the most accurate and applicable technique of quantifying the occurrence of ingested lead shot in duck gizzards.

We thank K. C. Moore of the California Fish and Game Department for his suggestions regarding X-ray techniques, J. R. Brady for his assistance in fabricating materials, T. M. Goodwin for assistance in specimen preparation, and A. Moreland and his staff at the University of Florida, Animal Resources Department for their assistance in providing radiographic services. R. Scheaffer of the University of Florida Department of Statistics provided suggestions on treatment of data and furnished the statistical analysis. Student Assistant V. Toy assisted with the literature review and compilation of references. We are also indebted to M. Olinde and L. Perrin for critical review of the manuscript. J. Baker, S. Nesbitt, and P. Moler served as cooperators in the manual examination in X-ray technique comparison.

## MATERIALS AND METHODS

### X-ray of While Gizzards vs. X-ray of Gizzard Contents

A sub-sample of 175 gizzards was selected for evaluation of the whole gizzard X-ray technique (Lewis and Legler 1968). Data cards were completed, specimen numbers assigned, gizzards placed on 43.2 x 35.6 cm trays, and radiographs shot on 43.2 x 35.6 cm casettes using a Phillips Super 70 X-ray machine at 1/30 second, mAs 33, KV35, focus 1,2. Radiographs were interpreted and the number of ingested shot in each gizzard cavity was estimated and recorded. Gizzards in the sub-sample were then prepared and re-examined using a combination of the X-ray and manual examination of gizzard contents.

Another sub-sample of 669 gizzard contents was selected, a data card was completed for each gizzard, and gizzards were opened and contents washed into polystyrene petri dishes (66 mm x 15 mm). Petri dishes were placed on pressboard trays. A standardized numbering system was utilized to correlate specimens and individual data cards with completed radiographs. The presence of pellet entry wounds on gizzard linings was noted to aid in the differentiation between ingested and penetrating shot.

X-rays were shot as previously described, and examined for the bright signatures indicating the presence of shot. The number of shot per gizzard as determined from X-rays alone was recorded. Verification of the amount of lead in the gizzard contents was completed using a combination of the X-ray and manual examination of those showing any indication of lead. Pellets which were shiny, and conspicuously flattened or burred, or surrounded by a feather wad, were categorized as penetrating shot. Notations made at the time of the gizzard opening on the presence of entry wounds on gizzard linings were considered diagnostic when difficulty was encountered in differentiating between ingested and penetrating shot.

### Manual Examination vs. X-ray of Contents

A sub-sample was selected, prepared and X-rayed using previously described techniques to obtain a set of 300 lead-free gizzard contents placed in dishes. Number 12 lead shot was sandpapered to simulate the size and texture of eroded pellets found in gizzards and some actual ingested lead was used. Shot was introduced into gizzard contents at the rate of 13% in 2 lots, and 16% in the third lot. Two, 4 and 5%, respectively, were seeded with 2 or more shot pellets, while the remaining seeded dishes received 1 shot each. Seeding rates approximated ingestion rates for Florida as determined by preliminary analysis of other gizzard contents.

Following seeding of specimens, gizzard contents were X-rayed as previously described. The X-rays were stored for later analysis. Lots of approximately 100 seeded gizzard contents were provided to each of 3 cooperators, who were asked to examine them manually for the presence of shot. One cooperator had previously manually examined several thousand gizzard contents for another study, a second had limited experience, while the third had no previous experience. After completion of manual examination of contents, cooperators examined x-rays of gizzard contents and estimated and recorded the number of simulated ingested pellets.

Analysis of Differences

Agreement between the X-ray of whole gizzards and X-ray of gizzard contents techniques was tested using Fleiss' Kappa statistic (Fleiss, 1973:146-147), with the combined techniques of X-ray examination of gizzard contents and manual examination of contents furnishing an estimate of true ingestion percentages.

Computation of Fleiss' Kappa statistic (K) for each technique is:

$$K = \frac{Po-Pc}{1-Pc}$$

where Po denotes the proportion of the specimens for which the 2 rating techniques agree, and Pc denotes the proportion of agreements expected solely on the basis of chance. If $Po<Pc$, indicating less than chance agreement, then $K<0$. If $Po = Pc$, $K = 0$, and if $Po = 1$, indicating perfect agreement, then $K = 1$.

For testing the difference between the two methods, the statistic

$$\frac{Ko-Ki}{\sqrt{V(Ko)+V(Ki)}} =$$

is computed, where Ko and Ki represent Fleiss' Kappa values for whole gizzard X-rays and X-rays of gizzard contents respectively, and V(Ko) and V(Ki) represent the variance of Ko and Ki respectively.

A chi-square test was applied to the data generated by the comparison between manual examination and X-ray of contents techniques to determine if there were real differences in accuracy between the two techniques.

## RESULTS

### Whole Gizzard X-ray vs. X-ray of Contents

The comparison of the whole gizzard technique vs. X-ray of contents technique indicated that the X-ray of contents detected more shot. Of the 669 gizzards examined by the latter method, 7.8% of the positive specimens went undetected as opposed to 28.3% undetected by the whole gizzard technique (Table 1).

1. Substituting observed values of the Fleiss Kappa statistic for the X-ray of whole gizzards,

$$Ko = \frac{.8923-.8175}{1-.8175}$$

$$Ko = .4099$$

and for the X-ray of gizzard contents,

$$Ki = \frac{.9342-.6880}{1-.6880}$$

$$Ki = .7891$$

provides Ko and Ki values for testing and 2 methods. Computation of the test statistic

$$= \frac{.4099-.7891}{.001193+.001475}$$

indicated that X-ray of gizzard contents gives significantly greater accuracy ($P < 0.01$) than X-ray of whole gizzards.

### X-ray vs. Manual Examination

When the accuracy of X-ray was compared to manual examination, the combined data for 3 observers examining approximately 100 gizzards per observer indicated that manual examination accounted for 76% of the seeded shot while X-ray accounted for 100 percent (Table 2).

A chi-square test ($X = 11.35$) indicated that the differences between the 2 techniques was highly significant ($P < .005$). We had hypothesized that more experienced observers

Table 1. Comparison of X-ray of whole gizzard versus X-ray of contents to detect lead shot.

| *Method of analysis* | *No. of specimens* | *Specimens containing ingested shot (A)*[a] | *Positive specimens not identified (B)* | *% of positive specimens undetected by technique (B/A)* |
|---|---|---|---|---|
| Whole gizzard X-ray | 715 | 46 | 13 | 28.3 |
| X-ray of contents | 669 | 116 | 9 | 7.8 |

[a]As determined by examination of whole gizzard X-ray with verification.

would be as accurate as the X-ray, but these data indicated no differences between observers (Table 2).

## DISCUSSION

The data presented in this report demonstrated that X-ray of gizzard contents is more accurate than either whole gizzard X-ray or manual examination of gizzard contents.

Table 2. Comparison of X-ray and manual examination of gizzards for occurrence of lead shot.

| | *Shot detected by X-ray* | *Shot detected manually* | *Shot actually present* |
|---|---|---|---|
| Observer No. 1 (96) | 13 | 9 | 13 |
| False positives | | (1) | |
| Observer No. 2 (103) | 16 | 12 | 16 |
| False positives | (1) | | |
| Observer No. 3 (102) | 13 | 11 | 13 |
| False positives | (2) | | |
| Totals | 42 | 32 | 42 |

It appears that the primary sources of error in whole gizzard X-rays comes from small, well-eroded pellet fragments whose X-ray signatures are masked by that from grit in the gizzard. Error in manual examination of gizzard contents stems primarily from difficulty in differentiating between small, eroded pellets and seeds and grit. Both techniques led to underestimates of the ingested lead in gizzards.

Verification of the occurence of ingested lead through a combination of examination of X-rays of gizzard contents and manual examination of contents revealed that some errors occur in the examination of X-rays of gizzard contents technique. Nearly 8% of the specimens containing ingested lead were not detected, basically as a result of interpreting real positive signatures as the false positive signatures. A compensating source of error resulted from the inability of observers to differentiate between penetrating shot and ingested shot in interpreting X-rays, resulting in an over-estimate of ingested shot. In the seeded shot experiment, 3 out of 300 (1%) false positives were recorded. In our experience, this and other errors can be reduced by manually examining the gizzard contents that produced a positive signature on the X-ray. Also, if an inexperienced observer will verify a number of questionable X-ray signatures by manually examining the contents, the accuracy with which subsequent X-rays can be read will improve.

It is obvious that Bellrose's data (1959) which still forms the basis for the USDI's (1976) determination that lead ingestion constitutes an unacceptable drain on continental waterfowl populations, were based upon techniques which significantly *understimate* the occurrence of ingested lead in those populations. Similarly, the technique (manual examination of contents) recommended by the U.S. Fish and Wildlife Service (Robert I. Smith, personal communication) for detecting ingested shot in gizzards also appears to *underestimate* significantly the true frequency of occurrence.

Thus, the conclusion that lead ingestion constitutes an unacceptable drain on continental duck populations (USDI 1976) may be based on a *conservative* estimate of the magnitude of that problem.

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