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A Radioisotopic Method of Measuring Food Evacuation Time in Fish[1]

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ABSTRACT

Pinfish (*Lagodon rhomboides*) were fed cerium-144 labeled food to determine if movement of the label could be used to estimate food evacuation time. Radioactivity was retained by the fish until their gastrointestinal tracts were empty. Replicate measurements showed that individual fish may have significantly different evacuation rates. Cerium evacuation and serial slaughter methods provided similar estimates of evacuation time; for the same precision fewer fish are needed for the cerium method. From these experiments, it appears that ^{144}Ce can be used as a tag to measure food evacuation time.

The most frequently used method of measuring food evacuation is by serial slaughter (Windell 1967). This method, which requires sacrificing a large number of experimental organisms, does not give a measure of variation in any one individual. In order to determine the evacuation rate in individuals, Seaburg and Moyle (1964) used a stomach pump which permitted reuse of animals, but required numerous feedings and considerable effort. As indicated by Windell (1967) there is a definite need for improved methodology in this field. One improvement is the use of food, tagged with a nondigestible tracer. Lawrence (1971) colored food and fed it to transparent fish larvae. He was then able to observe the movement and evacuation time of the colored food items. Radioactive cesium has been used as a tracer (Kevern 1966); however, it is readily assimilated which makes it undesirable for evacuation studies. We propose the use of radioactive cerium as a tag and believe that it offers the following advantages: (1) it can be used on nontransparent fish; (2) the procedure negates sacrificing the fish; (3) the exact amount of tag

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remaining at various times after feeding can easily be measured; (4) cerium is poorly assimilated; and (5) it will readily attach to any desired food particle by adsorption.

Elements in the rare earth series including cerium, scandium and others, behave chemically in a manner which makes all of them potentially useful tags for food evacuation studies. These elements occur mainly in a particulate state, primarily as hydroxides of the type $R(OH)_3$. They will readily attach to particles by adsorption which simplifies food tagging and, because of their particulate nature, are poorly assimilated by animals (Palumbo 1963). To test whether retention of one of the rare earth isotopes in the gut was similar to food retention, we performed comparative experiments using ^{144}Ce and the more traditional serial slaughter method. We chose ^{144}Ce because it has a reasonably long half life (282 days) and because its radioactive decay products are easily measured on standard laboratory detection equipment. In addition, the cost of this radionuclide is less than for other isotopes considered.

MATERIALS AND METHODS

Evacuation time for a radioactive food tag was measured in pinfish (*Lagodon rhomboides*, 7–20 g). Measurements were made during early summer using approximate natural photoperiod and temperature (24 C and 14 hr light). Laboratory acclimation was approximately 2 wk. Food (small fish and shrimp) was tagged either individually, by using a hypodermic syringe to inject 25 μCi $^{144}CeCl_3$ dissolved in HCl, or as a group, by holding the organisms for 12 hr in sea water containing 200 μCi ^{144}Ce/liter. All food items were rinsed in uncontaminated seawater before they were offered to the fish. The experimental fish were held in 20-liter tanks, starved long enough to have empty gastrointestinal (GI) tracts, and then allowed to feed on tagged food until they were satiated. Radioactivity in the fish which had eaten tagged food was measured in a whole-animal scintillation counter. Periodic remeasurements were made until less than 1% of the original radioactivity remained. For radiation measurements, fish were placed in glass jars with a volume of seawater approximately 10 times that of the fish. Three replicate measurements were made and all fish returned to their holding tank within 10 min. Background radiation of water in the holding tank did not increase following feeding or evacuation.

To trace passage of food and tag, GI tracts of some fish were examined. Tracts cleared of all visible particles were sagittally sectioned, and the radiation in the sections was measured to help locate any residual ^{144}Ce. Evacuated fecal particles were also checked for presence of radioactive tag.

Five pinfish were used to determine individual variation in ^{144}Ce evacuation time. Variation within individuals was investigated by testing three additional pinfish each three times.

Evacuation time for nonradioactive food was determined by the serial slaughter technique so that comparisons could be made between the two methods. Fifty-five pinfish were fed to satiation with commercial food. Gastrointestinal contents of five fish were removed at 2- to 3-hr intervals, dried, and weighed. Content at any interval was expressed as the geometric mean of one plus the percent of dry body weight in the alimentary tract. These means were converted to logarithms and regressed on time after feeding (Brett and Higgs 1970).

RESULTS AND DISCUSSION

Dissection of the fish showed that as ^{144}Ce evacuation neared completion, recognizable food had disappeared from the gut. Fish which still contained 5–10% of the original radioactivity also contained remains of food particles. However, in fish retaining 1% or less of the original ^{144}Ce, the alimentary tracts contained ample fluid but no recognizable particles. High radioactivity in fecal particles indicated the tag was being evacuated in the feces. Contamination of water in the holding tanks was not a problem because the cerium remained attached to the feces and settled out of the water.

Radioactive cerium, when evacuated, may

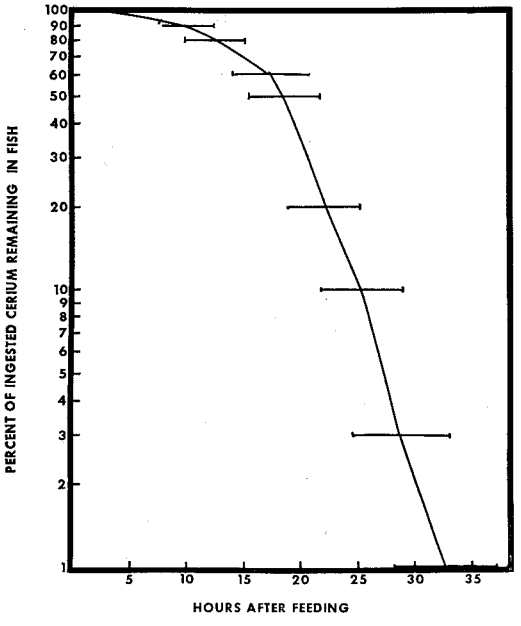

FIGURE 1.—Retention of a ^{144}Ce tag by pinfish at 24 C. Standard errors are shown ($n = 5$).

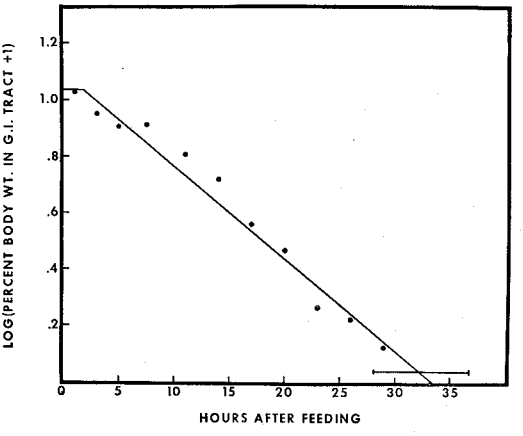

FIGURE 2.—Gastrointestinal evacuation of commercial food by pinfish at 24 C using the serial slaughter method. Log $Y = 1.037 - 0.033\ (X - 2)$; where $Y = \text{Log}_{10}$ (1+ % dry body weight in GI tract); and $X =$ hours since feeding (except $X - 2 \geqslant 0$). A 95% confidence interval is shown around the estimate of time to reach 1% of the amount ingested.

not be attached to the same particle as it was when ingested because of changes in pH between the stomach and intestine. Acidic conditions in the stomach may dissolve some of the tag; but, when the pyloric sphincter opens and pH near the food becomes more alkaline, the tag should readsorb to available surfaces including, to some extent, the adjacent walls of the GI tract. Sections of the alimentary tract of pinfish showed that the minimal cerium retained was primarily adjacent to the pyloric sphincter.

The results of experiments using the ^{144}Ce tag (Fig. 1) and the serial slaughter technique (Fig. 2) gave similar estimates of the time required to reach 1% of the amount ingested (32.7 and 32.4 hr, respectively). Although different foods were used in the two determinations, this is not a serious drawback since Windell (1967 and 1972) found various low fat foods were evacuated at similar rates. Previous research (Peters, Kjelson, and Boyd in press) established that food ingested by pinfish in nature is evacuated at approximately the same rate as commercial food used in these experiments. Evacuation of the commercial food at 24 C was inconsequential during the first 2 hr, i.e., our regression estimate of the amount of food in the alimentary tract (9.9% body wt) at 2 hr represented 100% of the amount ingested. Assumption of a lag before the onset of evacuation increased the fit of the evacuation model for three species including pinfish (Peters et al. in press).

The serial slaughter method used 11 times as many fish, so its estimate of evacuation time was more precise than the value obtained with the ^{144}Ce method. From the regression (serial slaughter) analysis the 95% confidence interval around the time to 1% of the ingested food remaining in the gut was 27.9–36.9 hr. A necessary sample size comparison (Quenouille 1950) indicated that the ^{144}Ce tagging method could produce as precise an estimate of evacuation with approximately 26 fish as was obtained with 55 fish using the serial slaughter method. Much of the variation in the ^{144}Ce method is apparently due to individual differences between the fish tested. Replicate feedings and measurements on specific pinfish showed that even under apparently constant conditions individual fish

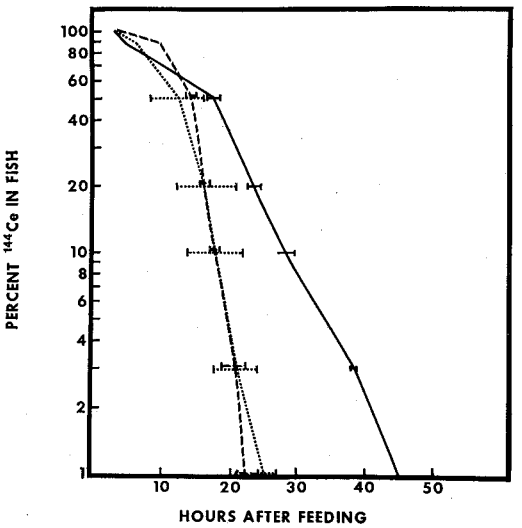

FIGURE 3.—Variation in ^{144}Ce retention by individual pinfish. Each curve represents the mean and standard error from three experiments on a single pinfish.

of the same species may have significantly different evacuation rates (Fig. 3). These differences are apparently characteristic of individual fish, as there were no obvious differences attributable to other factors such as amount of food consumed, fish size, or temperature.

From these tests it appears that ^{144}Ce can be used as a tag to measure food evacuation time in fish. The tagging method gave the same time estimate as serial slaughter; however, it required fewer fish for the same precision. Therefore, when fish are difficult to obtain or holding facilities are limited, use of the tagging method described here will be advantageous. Use of the radioactive tag permits repeated determinations on the same fish but requires less effort than earlier methods (e.g., Seaburg and Moyle 1964). Replicate measurements can show variation within individual fish and changes in evacuation rate as a fish grows or its diet is changed. Also, use of the tagging method allows measurement of other parameters (e.g., oxygen consumption) either during or following the evacuation period.

Because radioactive cerium is unassimilated it can be used not only for determining food evacuation but also to assess digestibility of food. Pappas, Tiemeier, and Deyoe (1973) used Cr_2O_3 in estimating digestion and absorption of protein, fat and carbohydrate; they utilized neutron activation analysis to determine the chromium concentration in the gut contents. Since their method requires a nuclear reactor, it is unavailable to many biologists. Cowey and Sargent (1972) suggested ^{144}Ce should be tested as a substitute for chromic oxide in fish digestion studies. Since radioactive cerium is easily analyzed in samples and is very poorly assimilated by fish, the substitution seems appropriate.

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