

Differential Digestibilities of Channel Catfish, Bluegill, and Gizzard Shad: *In vitro* Standards for Gastric Digestion by Seabirds

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Abstract.—I determined relative rates of digestion of three fish species *in vitro* by mimicking the gastric juices of piscivorous birds. Bluegill (*Lepomis macrochirus*) were digested more slowly than Channel Catfish (*Ictalurus punctatus*) and Gizzard Shad (*Dorosoma cepedianum*). Digestion kinetics of Bluegill were linear; those of Channel Catfish and Gizzard Shad nonlinear. These digestibility data may be used to predict *in vivo* digestibility, to correct estimates of food habits of individuals in field studies where gut contents are sampled, or to estimate nutritional contribution of different fish to the diets of birds. Received 20 December 1991, accepted 8 March 1992.

Key words.—Bluegill, Channel Catfish, *Dorosoma cepedianum*, fish, Gizzard Shad, *Ictalurus punctatus*, *in vitro* digestion, *Lepomis macrochirus*, seabirds.

Colonial Waterbirds 15(2): 257-260, 1992

Birds digest prey items at variable rates depending on the prey's chemical composition, physical structure, and size as well as physiological limitations such as their own rate of enzyme synthesis and kinetics, passage rate, and metabolic demand (Robbins 1983, Karasov 1990). Variation in rate and extent of digestion of each food item results in differential contribution of that item to the overall nutrition of the animal. In field studies, differential digestion of food poses methodological problems: estimation of food habits from gut contents may be biased if the disappearance rates of food from the esophagus, proventriculus, and ventriculus are not known (Swanson and Bartonek 1970).

Currently, seabird diets and digestive capacities are of interest because of competition between birds and humans for fishery resources. In particular, wintering populations of Double-crested Cormorants (*Phalacrocorax auritus*) are of concern at catfish ponds, commercial fisheries, and gamefish hatcheries, where they may consume mixed diets of cultivated and wild fish (Craven and Lev 1985, Bayer 1989). As part of a study to assess the relative contributions of fish species to energy budgets of Double-crested Cormorants wintering in the Mississippi Delta, I determined *in vitro* digestibility reference values for three fish species commonly consumed in southern states (Bivings *et al.* 1989, Campo *et al.*

1988): Gizzard Shad (*Dorosoma cepedianum*), Channel Catfish (*Ictalurus punctatus*), and Bluegill (*Lepomis macrochirus*). These results may be used as standards for comparison with *in vivo* determinations of digestibility (Tilley and Terry 1963).

METHODS

A one-stage *in vitro* protein digestibility technique was used to estimate the rate of gastric digestion of fish (Bigg and Fawcett 1985, Jackson *et al.* 1987). Frozen fish, 10 to 15 cm long, were thawed, towel-dried, weighed (± 0.01 g), and measured for total length (± 1 mm). A single fish of a species was bound in a plastic net bag (mesh size 10 \times 3 mm) and suspended head-first in a 600 ml beaker containing 360 ml of an aqueous solution of 0.5% HCl, 0.6% Na₂CO₃, and 1% pepsin (materials from Sigma Chemical Co., St. Louis, MO). Some fish had to be folded in the bag to ensure that the tail was immersed in the solution. The HCl concentration was adjusted to give the solutions an initial pH of 1.5 (within the range of 0.9-2.9 given for the gastric pH of Great Cormorants, *P. carbo*, van Dobben 1952, and raptorial birds, Duke *et al.* 1975). These beakers were placed in a water bath at 38-40° C. Eight replicates per species were tested.

Immediately after immersion in the solution and at 1-hour intervals thereafter, each sample was removed from its beaker, drained of extra solution, and weighed to the nearest 0.5 g with a Pesola scale. Masses were recorded until samples lost $\geq 95\%$ of original mass. Measurements were not made overnight (between 2000 and 0800). The pH was kept between 1.5 and 2.5 throughout the experiments by adding HCl. The optimal pH for this pepsin product is 2.0 (Sigma Chemical Co., St. Louis, MO).

Dry matter and organic matter contents were determined for 8 samples of each fish species (Associa-

tion of Official Agricultural Chemists 1991). Whole fish were partially dried at 60° C until they reached constant mass. To prevent formation of a paste due to high fat content, fish were ground in dry ice in a mill (C. W. Brabender Instruments). Replicate samples were oven-dried at 105° C for 8 h to obtain an estimate of dry matter as a percent of fresh mass. Although some lipids may vaporize at this temperature, possibly biasing the estimates of dry matter, dry matter must be determined at a temperature above vaporization of water at standard pressure. Thus I had to use a high temperature for the procedure. Samples were burned at 500° C for 3 h to obtain an estimate of organic matter content as a percentage of dry matter.

For each species, the mean (\pm SE) proportion of fish mass that remained at each hourly interval was calculated. An initial lag time, during which little or no digestion occurred, was identified for each species. Linear and nonlinear regressions were compared to describe the relationship of mean mass remaining and hours in the digestion solution (Sokal and Rohlf 1981). Values collected during the lag time or after the 12-hour interruption overnight were not included in the regressions. Linear and nonlinear regressions that related digestion rates among species to mean wet mass, dry matter, or organic matter were compared.

RESULTS

Body sizes, dry matter, and organic matter content of fish used in these tests are given in Table 1. Gizzard Shad and Channel Catfish lost a mean of 95% original mass within 28 hours of placement in the artificial digestion liquor (Fig. 1). I terminated the experiment after 34 h, but before Bluegill lost 95% original mass. Gizzard Shad, Catfish and Bluegill lost an average of 50% original mass in 4.5, 8, and 23 h, respectively (Fig. 1).

Digestion responses differed among species (Fig. 1). After a lag time of 2 hours, the digestion response for Bluegill (y) as a function of time (x) fit the linear equation,

$$y = 104.3 - 2.7x \quad (r = 0.99, df = 7, P < 0.01).$$

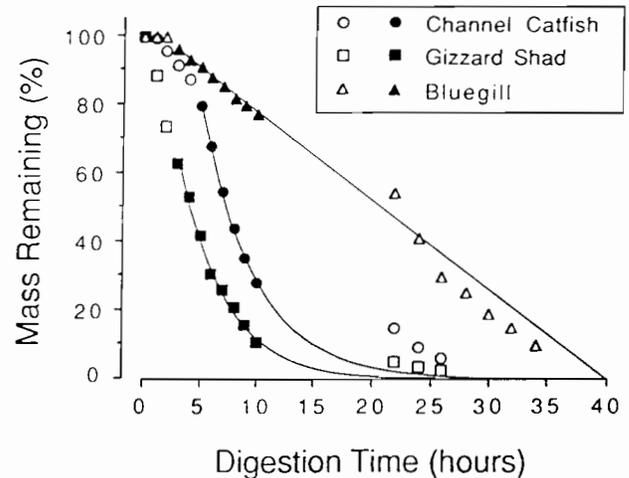


Figure 1. Mean percent of original mass remaining for each of three fish species during *in vitro* digestion trials. Black symbols indicate values used to calculate digestion curves. White symbols indicate values obtained during the lag time or after a 12-hour interruption in data collection. Standard error is omitted to reduce clutter.

Channel Catfish and Gizzard Shad digested more rapidly than Bluegill, with digestion responses that were nonlinear. Channel Catfish had a lag time of 4 hours, after which digestion fit the exponential equation,

$$y = 234.4 * 10^{(-0.099 * x)} \quad (r = 0.99, df = 5, P < 0.01).$$

Gizzard Shad had a lag time of 1 h, after which digestion fit the equation,

$$y = 130.2 * 10^{(-0.041 * x)} \quad (r = 0.99, df = 8, P < 0.01).$$

The digestion curves for Channel Catfish and Gizzard Shad did not differ ($F_{1,18} = 2.27, P = 0.15$). Among species, mean digestion rates were not explained by wet mass, dry matter or organic matter content of fish.

Although digestion responses of Channel Catfish and Gizzard Shad did not differ, there were differences in appearances

Table 1. Mean (\pm SE) length, mass, dry matter (DM as % fresh mass), and organic matter (OM as % DM) content of fish used in the *in vitro* digestibility determinations (N = 8 per species).

	Length (cm)		Mass (g)		DM (%)		OM (%)	
	\bar{x}	(SE)	\bar{x}	(SE)	\bar{x}	(SE)	\bar{x}	(SE)
Channel Catfish								
(<i>Ictalurus punctatus</i>)	15.5	(0.46)	30.2	(1.94)	13.7	(0.01)	76.9	(0.01)
Gizzard Shad								
(<i>Dorosoma cepedianum</i>)	12.8	(0.88)	19.0	(3.08)	15.6	(0.02)	74.2	(0.02)
Bluegill								
(<i>Lepomis macrochirus</i>)	12.9	(0.35)	39.6	(2.76)	24.2	(0.02)	72.0	(0.01)

of fish shortly after immersion. In the first 3 hours after artificial "ingestion" in the digestion liquor, Bluegill showed no effects of digestion, while Channel Catfish lost superficial skin, and Gizzard Shad lost substantial skin and muscle tissue. Bluegill were still relatively intact after 24 h of sampling. After 24 h, skulls and spines remained in the Channel Catfish bags; a few ribs remained in the Gizzard Shad bags; and meaty heads, spines, ribs and tail rays remained in the Bluegill bags.

DISCUSSION

The *in vitro* test for digestibility used in the current study is an approximation of *in vivo* digestion. This method assumes that only exogenous chemical digestive processes are responsible for food digestion (i.e., enzymatic digestion by the bird) and does not account for endogenous digestion within the fish or the effect of refluxing digesta within the gut of the bird. It provides an estimate of the rate of digestion (how fast fish degrade in a proteolytic environment), but not necessarily the extent of digestion (the types of amino acids that are released). The method could be improved if enzymes or acids that closely approximate those in the stomach and intestines of seabirds were commercially available, or if patterns of gut motility could be mimicked in a shaker bath (Jackson *et al.* 1987, Duke *et al.* 1989). However, the method is rapid and repeatable, thus enables direct comparisons among prey species. For example, the relative digestion rates of prey that vary in size, structure, or seasonal condition can be quantified with large samples and the potential contribution of different fish to the diet of seabirds examined.

The estimates of *in vitro* digestion responses can be used in two ways (Swaigood and Castagnani 1991). First, differential digestion of the three fish species may be factored into food habits studies where gut contents are sampled to estimate how much of each species is eaten per unit time. For example, an index to time-since-ingestion by fish species may be constructed, based on appearance and mass of fish at known intervals in the laboratory. The index may be applied to samples collected in the field to estimate the length of time fish have remained in the

stomach or to estimate total amount (number, mass) of that fish species in the diet.

Second, *in vitro* digestibility trials may be used in estimating the nutritional contribution of a fish species to the diet of the bird. For example, the energy content of the digestible fraction of the fish may be calculated by subtracting that of the non-digestible component (the residue) from that of the whole fish. A similar approach could be applied to other nutritional determinations such as nitrogen or calcium.

Differences in digestive kinetics between the linear decline of Bluegill and the exponential declines of Channel Catfish and Gizzard Shad could have several explanations. The bone and scale structures differed among the fish; their presence may contribute to linear digestive responses. For example, the heavy bones and scales of Bluegill could have hindered physical access of pepsin to fish proteins or perhaps ions contained in bones and scales could have altered activity of pepsin by buffering the solutions. The nonlinear kinetics of the Channel Catfish and Gizzard Shad suggest that the pepsin enzyme is allosteric (Engel 1977). The pepsin used in this study could have multiple subunits that simultaneously bind and hydrolyze several molecules of fish protein, thereby contributing to exponential digestion. If true, then the molecular structures of Channel Catfish and Gizzard Shad proteins may differ from those of Bluegill.

In mammals, digestibility of foods determined *in vitro* is generally different from that determined *in vivo* and corrective equations are required to relate one measure to the other (Robbins 1983). If the *in vitro* estimates for fish digestibilities correctly predict the relative *in vivo* responses of birds, then these data suggest that a fish-eating bird would digest Gizzard Shad and Channel Catfish faster than Bluegill in these size classes. If true, the ranking of fish digestion rates suggests a digestive component of food selection. Assuming all other factors (such as foraging effort) are equal, fish-eating birds such as Double-crested Cormorants may select the most easily processed fish of those available, i.e., the species or size class that digests the most rapidly. The hypothesis could be tested with captive birds.

The final evaluation of *in vitro* digestibility measurements requires digestion trials with birds. Although these are cumbersome and may be difficult to conduct, controlled feeding trials are needed to determine how well the *in vitro* estimates approximate *in vivo* measures. Sources of variation due to species, age, reproductive status or other factors related to the bird may affect its ability to digest fish in ways that cannot be predicted by the composition or structure of the fish alone.

ACKNOWLEDGMENTS

I appreciate the contributions of J. Estes (Florida Game and Fresh Water Fish Commission), who provided Gizzard Shad and Bluegill; P. Nol and M. A. Cone (University of Florida Office of Sponsored Research), who assisted in the laboratory. Thanks are due to M. L. Avery, G. Duke, M. Erwin, J. F. Glahn, H. M. Tiebout III, and an anonymous reviewer for reviewing earlier drafts of the manuscript.

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