CELLULASE ACTIVITY IN THE STOMACHS OF FRESHWATER FISHES FROM TEXAS

by
ROBERT R. STICKNEY
Department of Wildlife and Fisheries Sciences
Texas A&M University
College Station, Texas 77840

ABSTRACT

The occurrence of cellulase enzyme activity was examined in the stomachs of Texas fishes representing 26 species. Comparison of the results obtained from enzyme studies with food habits indicated that the two were independent. A hypothesis that the presence of certain enzymes might be correlated with black peritoneum in fish (McAllister 1959) must be rejected with regard to cellulase based on the results of this study. Unlike a previous study (Stickney and Shumway 1974) in which cellulase activity within species was always either present or absent, seven species examined during the present study were found to exhibit intraspecific differences in cellulase activity. Suggestions for further studies aimed at clarifying the mechanisms of cellulase occurrence and its potential importance to cultured species of fishes are presented.

INTRODUCTION

Digestion of cellulose by terrestrial animals such as cattle and termites has been well documented and is generally attributable to digestive system bacterial cellulase production. Cellulase activity has been detected in a variety of aquatic invertebrates (Crosby and Reid 1971, Halcrow 1971, Koningsor, *et al.* 1972, Lewis and Whitney 1968, Soedigdo, *et al.* 1970, Yokoe 1960, Yokoe and Yasumasu 1964) and in some cases activity continues after removal of digestive tract microorganisms (Yokoe and Yasumasu 1964) indicating *in situ* enzyme production independent of microflora. Vertebrates, on the other hand, do not seem to have the ability to form the enzyme but must depend upon digestive system bacteria for cellulase activity (Yokoe and Yasumasu 1964, Stickney and Shumway 1974).

If cellulase activity is adaptive in fishes, it would seem likely that some correlation between food habits and cellulase activity could be found. In a study of primarily estuarine and marine fishes (Stickney and Shumway 1974) no such correlation was apparent, although in that study few omnivores and no strict herbivores were included. One of the purposes for the present study was to expand the list of fishes examined for cellulase activity and to include species more highly dependent upon plant material for their food.

A second aspect of the study was to test a hypothesis presented by McAllister (1959) that the presence of black peritoneum in certain species of fishes is an adaptation which would prevent the destruction of photosensitive alimentary tract bacteria by eliminating the passage of light through the body wall. McAllister (1959) further suggested that cellulase producing bacteria may be one type protected in this manner. The present study included several species which exhibited black peritoneum.

The author is indebted to Dr. D. E. McAllister for his suggestions as to species with black peritoneum which might be locally available. The assistance in the collection of fish used in the study provided by William Neill, Richard Noble, Loren Skow, Scott Schofield, Raymond Germany and Timothy O'Keefe is gratefully acknowledged.

MATERIALS AND METHODS

Twenty-six species of freshwater fish were obtained from Texas waters in the vicinities of Bryan, Llano and Trinidad. Fish were collected by cast netting, seining and gill netting. All with the exception of *Tilapia aurea* are either native or well established introductions such as *Cyprinus carpio*. *T. aurea* was introduced in recent years into Trinidad Lake and has been able to overwinter by moving into the heated water effluent of a power generating plant located on the lake.

Small fish were frozen whole following capture, whereas the stomachs from larger fish were removed, then frozen. Attempts were made to check stomachs for cellulase activity within about 96 hours of capture, although in a few cases as much as 30 days passed between capture and analysis. Verification of the results obtained from stomachs which had received long term storage was made using fish of the same species which were run soon after capture.

While other methods for cellulase activity determination have been developed, the most common method employs viscometry. The method used in this study was modified from that described by Stickney and Shumway (1974). Following thawing the stomachs were opened and washed with a 0.1

M phosphate buffer solution at pH 6.8 to remove food material from the stomachs. The stomach tissue (or a portion of the stomach in the case of large fish) was homogenized in a tissue grinder with a small volume of phosphate buffer. The resulting homogenate was brought up to 5 ml with phosphate buffer in cases where the stomach was small (e.g., minnows) and 10 ml when a subsample from a large stomach was used (approximately 2 g samples). The homogenate was then centrifuged at 2500 rpm for 10 minutes.

The substrate used for testing for the presence of cellulase activity was 0.2 percent sodium carboxymethylcellulose (Na-CMC) solution which was prepared using the phosphate buffer as the solvent. Five ml of the Na-CMC solution were added to an Ostwald viscometer and 0.5 ml of the phosphate buffer was introduced and mixed with the Na-CMC solution. The viscometer was then placed in a water bath at 30 C and allowed to incubate for 10 minutes. The flow rate of this control solution was then determined and recorded. The solution was then removed from the viscometer and replaced with 5 ml of Na-CMC solution and 0.5 ml of the centrifuged stomach extract. This solution was also allowed to incubate in the water bath for 10 minutes and the flow rate was determined at 10 minute intervals for an additional 30 minutes.

All glassware was rinsed in dilute HCl between uses to assure destruction of any enzyme which might have been present. The glassware was then rinsed several times with tap water and finally rinsed with distilled water and dried at 100 C for 24 hours before reuse.

RESULTS AND DISCUSSION

An increase in flow rate with time, indicating a reduction in the viscosity of the Na-CMC solution as cellulose is converted to glucose by cellulase, was used to confirm the presence of cellulase activity. Examples of changes in flow rate (Δt) plotted against time for species exhibiting high, low and zero cellulase activity are presented in Figure 1.

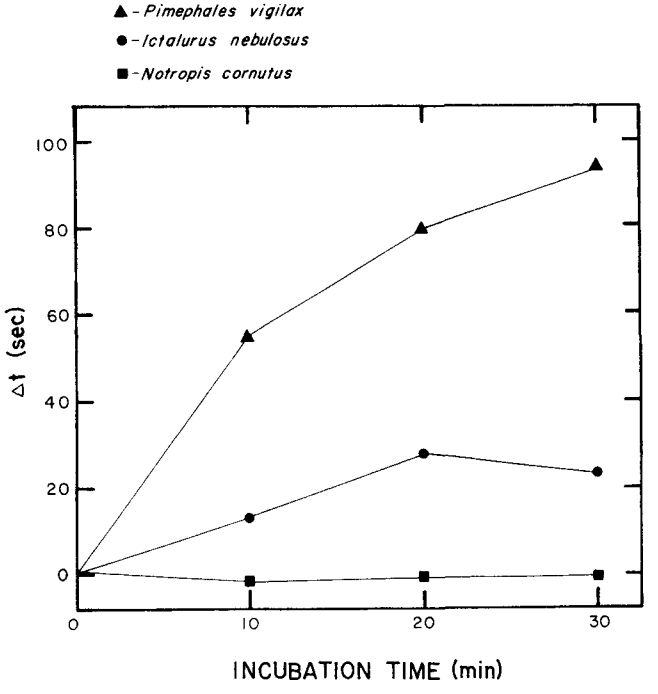

Figure 1. Change in flow rate (Δt) with time for species exhibiting high cellulase activity (*Pimephales vigilax*), low cellulase activity (*Ictalurus nebulosus*) and no cellulase activity (*Notripis cornutus*).

Of the 26 species of fish examined two exhibited high cellulase activity, 11 showed low activity and six showed no activity. In seven species activity was present at low levels in some individuals and absent in others. A previous study of primarily estuarine and marine fish (Stickney and Shumway 1974) revealed that all of the 62 species examined were either positive or negative with regard to cellulase activity. That same study demonstrated that for *Ictalurus punctatus* cellulase activity persisted following a period of starvation but the enzyme was destroyed when the fish were exposed to antibiotics. Thus, cellulase activity was related to digestive tract microflora. In addition, the *I. punctatus* examined in the earlier study were reared indoors in well water and fed a pelleted diet which showed no cellulase activity. It was concluded that the fish harbored cellulase producing bacteria which were not brought in with the food but must have been part of a persistent intestinal flora.

Two additional species of ictalurids were included in the present study: *I. melas* and *I. nebulosus*. Both showed results similar to those recorded from *I. punctatus* (Table 1, Stickney and Shumway 1974). The probability that in some cases cellulase activity is related to its presence within the food rather than to an established gut flora is indicated by the seven species in which some individuals demonstrated activity and some did not.

Table 1. Classification, number of individuals examined and occurrence of cellulase activity in Texas freshwater fishes.

Scientific Name	*Common Name*	*Number of individuals examined*	*Cellulase activity*
Lepisosteus oculatus (Winchell)	Spotted gar	3	−
Lepisosteus osseus (L.)	Longnose gar	2	±
**Dorosoma cepedianum* (Lesueur)	Gizzard shad	5	±
**Dorosoma petenense* (Gunther)	Threadfin shad	6	±
Cyprinus carpio Linnaeus	Carp	2	−
Notemigonus crysoleucas (Mitchill)	Golden shiner	5	+
Notropis cornutus (Mitchill)	Common Shiner	2	−
Notropis lutrensis (Baird & Girard)	Red shiner	3	++
**Pimephales promelas* Rafinesque	Fathead minnow	3	+
Pimephales vigilax (Baird & Girard)	Bullhead minnow	1	++
Ictiobus bubalus (Rafinesque)	Smallmouth buffalo	3	±
Moxostoma congestum (Baird & Girard)	Gray redhorse	2	+
Ictalurus melas (Rafinesque)	Black bullhead	1	+
Ictalurus nebulosus (Lesueur)	Brown bullhead	3	+
**Fundulus notatus* (Rafinesque)	Blackstripe topminnow	3	−
**Gambusia affinis* (Baird & Girard)	Mosquitofish	2	+
Labidesthes sicculus (Cope)	Brook silverside	3	+
Morone chrysops (Rafinesque)	White bass	1	+
Lepomis cyanellus Rafinesque	Green sunfish	6	±
Lepomis humilis (Girard)	Orangespotted sunfish	4	±
Lepomis macrochirus Rafinesque	Bluegill	5	±
Lepomis megalotis (Rafinesque)	Longear sunfish	2	+
Micropterus salmoides (Lacepede)	Largemouth bass	4	+
Pomoxis nigromaculatus (Lesueur)	Black crappie	1	−
Aplodinotus grunniens Rafinesque	Freshwater drum	2	+
**Tilapia aurea*	Tilapia	6	−

++ Cellulase activity high + Cellulase activity low − Cellulase activity absent
± Cellulase activity present in some individuals, absent in others
* indicates fish with black peritoneum

Table II. Food habits of fishes from Texas which were evaluated for cellulase activity.

Scientific name	*Food habits*
Fish with high levels of cellulase activity	
Notropis lutrensis	Algae, insects, crustaceans
Pimephales vigilax	Algae, gastropods, amphipods, insects
Fish with low levels of cellulase activity	
Notemigonus crysoleucas	Algae (including phytoplankton), small crustaceans (including zooplankton), insects
Pimephales promelas	Algae, insects, helminths, small crustaceans, detritus
Moxostoma congestum	Zooplanktonic crustaceans, oligochaetes, gastropods, insects, detritus (food habits based on related species)
Ictalurus melas	Plant detritus, small crustaceans, insects, fish, frogs
Ictalurus nebulosus	Plant material, insects, molluscs, fish eggs, fish
Gambusia affinis	Algae, copepods, mosquito larvae & pupae, small fish
Labidesthes sicculus	Small crustaceans (feed at the surface)
Morone chrysops	Insects, fish (feed near the surface)
Lepomis megalotis	Small crustaceans, insects, helminths, gastropods (food habits based on *L. macrochirus*)
Micropterus salmoides	Adults feed primarily on fish, crayfish and frogs
Aplodinotus grunniens	Aquatic insects, molluscs, amphipods
Fish with cellulase present in some individuals, absent in others	
Lepisosteus osseus	Fish primarily
Dorosoma cepedianum	Phytoplankton and zooplankton
Dorosoma petenense	Phytoplankton and zooplankton
Ictiobus bubalus	Algae, duckweed, zooplankton, aquatic insects
Lepomis cyanellus	Insects, amphipods
Lepomis humilis	Aquatic insects, crustaceans
Lepomis macrochirus	Small crustaceans, insects, helminths, gastropods
Fish with no cellulase activity	
Lepisosteus oculatus	Primarily fish; also insects, amphipods and crayfish
Cyprinus carpio	Phytoplankton, plant detritus, zooplankton, benthos
Notropis cornutus	Algae, rotifers, small crustaceans, insects
Fundulus notatus	Zooplankton, benthos, mosquito larvae (based on other species of *Fundulus*)
Pomoxis nigromaculatus	Crustaceans, aquatic insects, minnows and other small fish
Tilapia aurea	Algae, zooplankton, benthos

Food habits of *T. aurea* from McBay (1961), Yashouv (1969), Yashouv and Chervinski (1960, 1961).
Food habits of all other species obtained from Carlander (1969) and Eddy and Underhill (1974).

Species within the same family did not always demonstrate similar cellulase activity patterns. This result corresponds with that obtained by Stickney and Shumway (1974). However, within genera most species showed similar results in terms of activity. The most obvious exception was the genus *Notropis* where *N. lutrenis* demonstrated a high level of activity and *N. cornutus* showed no activity (Table 1). The general food habits of the species examined are presented in Table 2. Dissimilarities in the food habits of *N. lutrensis* and *N. cornutus* do not appear to be great enough to explain the difference in enzyme activity although the specific food habits of the fish captured were not determined. Both species were collected from the same sampling area, so food quality or availability may not have been a mitigating factor. A second genus which showed a difference in cellulase activity between species was *Lepisosteus*. Of two *L. osseus* examined, one showed activity and the second did not. All three *L. oculatus* tested were negative for cellulase activity.

Comparison of Tables 1 and 2 does not demonstrate any consistent positive correlation between cellulase activity and food habits. While none of the fish examined were strict herbivores, many feed on algae and other plant material to a greater or lesser extent. Cellulase activity in these species would be of potential usefulness in making more of the energy in the ingested material available to the fish. However, species such as *T. aurea* which consume large amounts of plant material demonstrated no cellulase activity, whereas predatory fish often demonstrated activity. The earlier statement that the establishment of cellulase producing microflora is related to the food ingested, and may sometimes be a function of a recent meal appears to be supported. Species which do not demonstrate cellulase activity either do not consume invertebrates harboring cellulase producing bacteria, the bacteria do not become established in the intestinal tract of those species, or the enzyme is destroyed during digestion.

Six of the species examined had black peritoneum (Table 1). Of these none showed high cellulase activity, *Pimephales promelas* and *Gambusia affinis* showed low activity, *Dorosoma cepedianum* and *D. petenense* had individuals showing both positive and negative activity and *Fundulus notatus* and *T. aurea* showed no activity. Thus, the distribution of activity was nearly equally spread among the activity categories. The presence of black peritoneum does not seem to be protective of the cellulase enzyme system although the theory may apply to one or more other enzyme systems.

Several additional studies are suggested from the results presented here. For example, food habit studies of the fish should be made in the area of capture and correlated with cellulase activity. In addition, the presence or absence of cellulase activity should be determined among the various invertebrate food organisms found in the diets of fish exhibiting cellulase activity. Studies as to the nutritional importance of cellulase activity in fishes of aquacultural interest would demonstrate the utility of providing cellulose as an ingredient in diet formulations. Another aspect would concern the ability of these species to utilize cellulose if the enzyme activity could be induced and maintained in species which do not presently show activity. In addition, it may be possible to enhance activity in fish which have been shown to possess a low level of activity.

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AERIAL SURVEILLANCE TO MONITOR WATER QUALITY IN CATFISH PONDS

by
H. RANDALL ROBINETTE and DON FINNIE
Department of Wildlife and Fisheries
FRANK D. WHISLER and JUDY YOUNG
Department of Agronomy
W. FRANK MILLER and TIM CANNON
Department of Forestry
Mississippi Agricultural and Forestry Experiment Station
Mississippi State University
Mississippi State, Mississippi 39762

ABSTRACT

Remotely sensed data and ground truth data were collected simultaneously from 16 experimental ponds during 6 days in June and July, 1974. Color infrared images were taken with hand-held 35mm cameras from single engine aircraft. Numerical color values for pond color were obtained by visually matching the pond color with a Munsell Color System chip which had a standardized numerical value assigned to it. Ground truth data involved the determination of 14 chemical, physical, and biological parameters. Regression analysis indicated a significant correlation ($P < .01$) existed only between total and inorganic solids and the Munsell Color System. There was evidence to suggest that the color—inorganic solid relationship was masked by the organic solids present.

INTRODUCTION

A new rapid technique for monitoring water quality is needed by fish culturists for two reasons; to monitor and maintain the new Environmental Protection Agency water quality standards and to be able to predict the onset of low oxygen conditions. The Federal Water Pollution Control Act Amendments of 1972 requires the states, beginning in 1975, to submit annual reports to the EPA which locate point sources of pollution, describe existing and anticipated water quality, and contain proposals for point and nonpoint source control. A small but vital part of the overall pollution problem will be fish culture systems as they will soon fall under EPA guidelines. The fish culturist will be responsible for monitoring and maintaining EPA standards for his particular systems effluent.

Secondly, thousands of acres are now in aquaculture production in the U. S. However, due to several high-risk problems, maximum growth of aquaculture systems has not been realized. One of the primary problems is maintaining water quality; namely, preventing oxygen depletions. At present, most fish farmers are not equipped to deal effectively with oxygen depletion because, more often than not, this problem is discovered too late to remedy. Since even seemingly identical ponds are seldom if ever identical in terms of water quality, the farmer cannot monitor one pond and expect it to be representative of every pond on his farm.

Clearly then, there is a need for a technique which will allow us to monitor water quality rapidly on a large scale. Such a technique might involve aerial surveillance, thus "remote sensing".

Most prediction techniques which utilize remotely sensed data collected during aerial surveillances involve the use of expensive instruments. In a study to determine various pollutants in lakes