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CULTURING, A METHOD USED TO IDENTIFY ALGAE INGESTED BY TILAPIA

By CHARLES S. MANOOCH, III [1]

ABSTRACT

Bold's Basal and Gorham's media were used to culture algae removed from the digestive tracts of Blue Tilapia, *Tilapia aurea* (Steindachner). Nine fish representing three-size categories collected from Lake Parker, Florida, were used in the study. Samples extracted from three areas of the gut were introduced to the culture media within twenty-four hours after collection. Microscopic examination of the cultured materials was conducted over a four-week period to enable the completion of reproductive cycles and excystment of algal cells.

Twenty-one taxa of algae were identified by sampling the culture vessels. Planktonic green algae were the dominant foods of tilapia at the time of sampling. Species of *Scenedesmus, Pediastrum, Ankistrodesmus* and chlorococcoid algae appeared in all specimens. Colonial chlorophytes, pennate diatoms, flagellated unicells, and remains of filamentous algae occurred less frequently. *Spirulina sp.* was the only blue-green occurring in significant quantities. Two rotifers, two ostracods, and a cladoceran were the only zoplankters observed.

Both, type of media used and region of gut sampled, produced slight quantitative and qualitative differences in data obtained. Maximum taxonomic diversity was encountered in the anterior samples cultured in Bold's Basal medium. Bacterial conditions in the digestive systems had no inhibitory effect on culturing algae.

The method of culturing ingested materials definitely has a future in specialized fisheries research programs. It would be particularly useful in studying dietary habits of juvenile fish and other small aquatic organisms (crustaceans and mollusks).

INTRODUCTION

Blue tilapia, *Tilapia aurea* (Steindachner) has been described as the fastest spreading exotic fish in South Florida (Buntz and Manooch, 1968). In the Lakeland area concentration of the species has created a unique local sport fishery (Buntz and Manooch, 1969).

Although some work has been conducted on various life history aspects of the Florida population, no research has been initiated on the dietary requirements. Such a study is essential since food habits of tilapia are not only interspecifically different, but also habitat depedendent. Lowe (1955) found *Tilapia melanopleura* and *T. zilli* eat higher aquatic plants while other species are mainly algal feeders. In lakes, tilapia fed primarily on phytoplankton; yet in ponds, the same species utilized filamentous algae, insect larvae, tadpoles, and even eggs and fry of other fish. In aquaria tilapia are often omnivorous, eating a variety of food items. The ecological impact of this introduced species cannot be fully evaluated until a food habits study is made. The mutual relationship and effect of two organisms occupying the same environment depends considerably on whether or not there exists a competition for food.

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Obviously this factor could have a tremendous influence on the native fish populations.

Another interesting problem related to tilapia nutrition is to what extent are the fish able to utilize algal cells. There is little evidence that herbaceous fish have an endocommensal bacterial fauna which can break down cellulose. Apparently they have to rely on mechanical breakdown of plant materials, hence the development of pharyngeal teeth in tilapia. Fish (1951, 1955) found *T. esculenta,* although a plankton feeder unable to break down the cell walls of blue-green and green algae, presumably owing to the absence of cellulases. *Microcystis, Botryococcus,* and such green algae as *Pediastrum* and *Scenedesmus* were unaffected by gastric juices and could be cultured from the excrement of the fish. Apparently some species of tilapia must consume a large volume of algal material daily as a result of conversion inefficiency.

Perhaps one reason a food habits study has not been conducted in Florida is that fishery biologists have not been provided with a technique of studying adequate quantities of fresh algal material. An inspection of a blue tilapia intestine is rather discouraging as the contents resemble organic muck in appearance and consistentcy.

The main objective of this paper is to introduce a method which may assist researchers to identify algae consumed by fish and other aquatic organisms.

DESCRIPTION OF STUDY LAKE

Lake Parker is a 2,291-acre lake which borders the city of Lakeland in central Florida. An irregular shoreline consisting of about 9 miles is surrounded by residential development on three sides and reclaimed phosphate mined land on the north. An electro-power plant discharges cooling water into the southeastern portion. Aquatic vegetation is primarily marginal emergent represented by cattail, *Typha sp.,* maiden cane, *Panicum sp.,* and alligatorweed, *Alternanthera philoxeroides,* and two floating species, water hyacinth, *Eichhorina crassipes,* and water lettuce, *Pistia stratiotes.*

The lake was totally renovated in 1960 and restocked with largemouth bass, *Micropterus salmoides,* bluegill, *Lepomis macrochirus,* and redear sunfish, *Lepomis microlophur.* Blue tilapia were first verified in 1965 after being illegally introduced. Since renovation, eutrophic conditions have become manifest, accelerating the production of forage fish. Gizzard and threafin shad, *Dorosoma cepedianum* and *D. petenese,* respectively, comprise approximately 68% of the total weight (George Horel, unpublished data). Planktonic green and blue-green algae attain dense growths in early spring and the warmer months.

METHODS

A. *Collection of Fish and Extraction of Food Materials*

Nine *T. aurea* representing three-size categories were collected from Lake Parker in February, 1971 (Table 1). Fish were obtained by electro-fishing gear. Water temperature at time of collection was 68°F. Specimens ranged from 140 mm to 376 mm total body length. Immediately after collection, the fish were placed on ice and air shipped to Raleigh, N. C., for study at North Carolina State University.

After individual statistics had been recorded, contents of the digestive tracts were examined macroscopically for larger food items. Sand particles were the only large objects observed. Samples taken from anterior, mid, and posterior areas of the gut were cultured separately. The anterior section included the esophagus and anterior half of the stomach. The mid area was defined as the intestine immediately behind the stomach. The posterior portion was the intestine just anterior of the anus. Approximately 2 ml of material extracted from each area was divided equally between the two-test media. In most instances, the intestines were found to be full of food. The anterior portion of several

TABLE 1. Individual statistics of nine *T. aurea* collected from Lake Parker, Florida.

Group	Size Category	Sex	Wgt(g)	TL(mm)	SL(mm)
1	<6	F	69	147	116
1	<6	F	81	150	117
1	<6	F	63	140	113
2	7-10	M	202	205	158
2	7-10	M	150	194	141
2	7-10	M	126	183	139
3	>10	F	680	296	229
3	>10	M	953	339	265
3	>10	M	1179	376	291

fish had been diluted and slightly flushed by the melting ice during shipment. Perhaps a piece of cotton inserted in the buccal cavity would prevent the loss of ingested materials.

The fish were divided into the following size categories:
Group 1 (<6″)—Fish from 140 mm-150 mm in total body length.
Group 2 (7-10″)—Fish from 183 mm-205 mm in total body length.
Group 3 (>10″)—Fish from 296 mm-376 mm in total body length.

B. *Preparation of Media and Conditions of Culturing*

Ingredients used in preparing Bold's Basal and Gorham's media are listed in Table 2. Purification of media was obtained by milipore filtration. Sterile conditions were obtained by autoclaving the culture vessels (test tubes) at 15 PSI for 15 minutes. The media were then added to the prospective tubes under a positive pressure transfer hood. The orifices of all culture vessels were flamed each time the lids were removed. Air was constantly filtered to prevent atmospheric contamination.

In general a temperature of 20°C and a light source of from 800-1,200 foot candles is used in culturing (Prescott, 1958). In this study a walk-in growth chamber was utilized. Temperatures were controlled at 19-20°C and the cultures were exposed to a 1,000 foot candle light source 12 hours a day.

For identification, subsamples were removed from each tube with a sterile pipette and placed on a slide for microscopic examination. Individual cells can be isolated from the cultures by the drop dilution technique or by spraying the liquid media on agar plates.

RESULTS

The following food categories were identified by sampling the culture media:

A. *Algae*

Chlorophyta. Planktonic chloroccalean algae were by far the dominant food of blue tilapia at the time of collection. Species of *Scenedesmus* and *Pediastrum* were cultured from all samples and *Ankistrodesmus* occurred in 77.8% of the cultures and all fish (Tables 3 and 4). Frequency occurrences of other representatives may be seen by reviewing Table 3.

Chrysophyta. Pennate diatoms were observed in 44.4% of the culture vessels and 77.7% of the specimens (Tables 3 and 4). One genus, *Tabellaria,* was identified.

Cyanophyta. Low diversity of blue-green algae was of particular interest. Evidently the February sample date was too early to include *Anabaena* and *Microcystis,* two genera which are abundant in Lake

TABLE 2. Ingredients used to prepare (a) Bold's Basal and (b) Gorham's media.

(a)

Macroelements	Concentration (g./400 ml distilled water)
$NaNO_3$	10.0
KH_2PO_4	7.0
$MgSO_4.7H_2O$	3.0
$CaCl_2.2H_2O$	1.0
NaCl	1.0

Minor (trace) elements	Ingredients	Concentration [1]
1. EDTA stock soln.	EDTA	50.0 g.
	KOH	31.0 g.
2. H-Fe stock soln.	$FeSO_4.7H_2O$	4.98 g.
3. H-Boron stock soln.	H_3BO_3	11.42 g.
4. $H\text{-}H_5$ stock soln.	$ZnSO_4.7H_2O$	8.82 g.
	$MnCl_2.4H_2O$	1.44 g.
	MoO_3	0.71 g.
	$CuSO_4.5H_2O$	1.57 g.
	$CO(NO_3)_2.6H_2O$	0.49 g.

Preparation: ten ml of each macroelement and one ml of each minor element for each 100 ml of distilled water.

(b) Ingredient	Concentration
$NaNO_3$	400 mg/1
K_2HPO_4	39 mg/1
Fe citrate	6 mg/1
$MgSO_4.7H_2O$	75 mg/1
$CuCl_2$	27 mg/1
$Na_2SiO_3.9H_2O$	58 mg/1
citric acid	6 mg/1
EDTA	1 mg/1
Na_2CO_3	20 mg/1

[1] In element No. 1 concentration dilute to 1-liter distilled H_2O and in Nos. 2, 3, and 4, concentrations dilute to 1- liter acified H_2O.

Parker during the warmer months. *Spirulina* was the only genus identified; however, it occurred in all of the fish (Table 4). One unidentified Cyanophyte was cultured from the anterior gut of a large tilapia (Table 3).

B. *Higher Plants*

Remains of vascular plants were found in four of the tilapia. Unfortunately, only very small pieces of stem were observed. It is difficult to say whether these plant materials were selectively eaten or consumed incidentally.

C. *Invertebrates*

A first day examination of the anterior gut sample of three fish (representative of each size group) produced no invertebrates. After all 54 culture vessels had been subsampled, only six tubes were found to contain animal materials. Live ciliates occurred in one, a cladoceran in one, ostracods in two, a live nematode in one, and a rotifer, *Kertella sp.*, in each of two tubes. There was no correlation between size of fish and the frequency occurrence of invertebrates (Table 4).

TABLE 3. Materials identified by sampling cultures representing three sizes of Tilapia and three areas of digestive tract.

Food Item	Control		Size Range (mm) 140-150									183-205								296-376								Freq. Occ. (%)
			1			2			3			4			5			6			7			8			9		
	B	G	A[1]	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	A	M	P	
Ankistrodesmus			X	X		X	X	X	X	X			X		X	X	X	X	X	X		X	X	X	X	X	X	X		77.8
Botryococcus				X		X	X		X									X			X									22.2
Chlamydomonas															X							X								7.4
Chlorella-like			X			X	X	X	X		X		X	X	X	X			X	X			X	X		X	X		X	63.0
Chlorococcoid	X		X					X	X	X	X	X	X	X	X		X		X	X			X		X	X	X		X	63.0
Closterium									X				X												X					11.1
Eudorina			X																		X									7.4
Euglenoid									X										X						X	X				14.8
Filamentous remains				X	X										X		X													14.8
Flagellated unicells			X		X			X	X									X			X	X					X		X	33.3
Golenkina									X		X				X	X		X				X	X							25.9
Oocystis				X																										3.7
Pandorina									X			X						X				X								14.8
Pediastrum spp.			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100.0
Pennate diatoms			X	X	X	X	X	X	X				X		X				X			X	X							44.4
Plant remains									X						X				X								X			14.8
Plantosphaeria												X																		3.7
Scenedesmus spp.			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100.0
Selenastrum								X																						3.7
Sphaerocystis																									X					3.7
Spirulina			X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X		X		88.9
Staurastrum			X		X		X																		X					14.8
Tabellaria							X																							3.7
Tetracystis									X																					3.7
Unid. detritus			X			X			X		X		X		X		X		X		X			X	X		X	X		55.5
Unid. Cyanophyte																									X					3.7
Ciliata																									X					3.7
Cladocera				X																										3.7
Ostracoda										X																X				7.4
Nematoda															X															3.7
Rototaria					X																						X			7.4
Total items/area	1	0	11	9	8	8	9	9	16	6	7	6	9	5	13	6	6	9	9	7	7	9	9	11	8	7	9	5	5	

[1] Indicates: A—anterior, M—mid, and P—posterior, areas of digestive tract.

TABLE 4. Frequency occurrence of food materials sampled from cultures of three size categories of *Tilapia aurea*.

Size Range (mm)	Ankistrodesmus	Botryococcus	Chlamydomonas	Chlorella-like	Chlorococcoid	Closterium	Eudorina	Euglenoid	Filamentous remains	Flagellated unicells	Golenkina	Oocystis	Pandorina	Pediastrum spp.	Pennate diatoms	Plant remains
140-150	X	X		X	X		X		X	X		X		X	X	
140-150	X	X		X	X					X				X	X	
140-150	X	X		X	X	X		X		X	X		X	X	X	X
Freq. Occ. (%)	100.0	100.0	0.0	100.0	100.0	33.3	33.3	33.3	33.3	100.0	33.3	33.3	33.3	100.0	100.0	33.3
183-205	X			X	X	X							X	X	X	
183-205	X		X	X	X				X		X			X	X	X
183-205	X	X		X	X			X		X	X		X	X	X	X
Freq. Occ. (%)	100.0	33.3	33.3	100.0	100.0	33.3	0.0	33.3	33.3	33.3	66.7	0.0	66.7	100.0	100.0	66.7
296-376	X	X	X	X	X		X			X	X		X	X		
296-376	X			X	X	X		X						X	X	X
296-376	X			X	X					X				X		
Freq. Occ. (%)	100.0	33.3	33.3	100.0	100.0	33.3	33.3	33.3	0.0	66.7	33.3	0.0	33.3	100.0	33.3	33.3
Total Freq. Occ. (%)	100.0	55.5	22.2	100.0	100.0	33.3	22.2	33.3	22.2	66.6	44.4	11.1	44.4	100.0	77.7	44.4

Size Range (mm)	Plantosphaeria	Scenedesmus supp.	Selenastrum	Sphaerocystis	Spirulina	Staurastrum	Tabellaria	Tetracystis	Unid. detritus	Unid. Cyanophyte	Ciliata	Cladocera	Ostracoda	Nematoda	Rototaria
140-150		X			X	X			X						X
140-150		X	X		X	X	X		X			X			
140-150		X			X			X	X				X		
Freq. Occ. (%)	0.0	100.0	33.3	0.0	100.0	66.7	33.3	33.3	100.0	0.0	0.0	33.3	33.3	0.0	33.3
183-205	X	X			X				X						
183-205		X	X		X				X					X	
183-205		X			X				X						
Freq. Occ. (%)	33.3	100.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	33.3	0.0
296-376		X			X				X						
296-376		X		X	X	X			X	X	X		X		
296-376		X			X				X						X
Freq. Occ. (%)	0.0	100.0	0.0	33.3	100.0	33.3	0.0	0.0	100.0	33.3	33.3	0.0	33.3	0.0	33.3
Total Freq. Occ. (%)	11.1	100.0	11.1	11.1	100.0	33.3	11.1	11.1	100.0	11.1	11.1	11.1	22.2	11.1	22.2

TABLE 5. Analysis of student's t-test for diversity of food items correlated to area of digestive tract cultured.

Comparison	Class	Ma [1]	Mp	d [2]	Σd	$\bar{d}$	N	Degrees of freedom	t	t.05
Ant. & Pos.	1	11.7	8.0	3.7	9.0	3.0	3	2	5.820*	4.303
	2	9.3	6.0	3.3						
	3	9.0	7.0	2.0						
		Ma	Mm							
Ant. & Mid.	1	11.7	8.0	3.7	6.7	2.23	3	2	3.005 N.S.	4.303
	2	9.3	8.0	1.3						
	3	9.0	7.3	1.7						
		Mm	Mp							
Mid. & Pos.	1	8.0	8.0	0.0	2.3	0.77	3	2	1.060 N.S.	4.303
	2	8.0	6.0	2.0						
	3	7.3	7.0	0.3						

[1] Average number of food items per region of digestive tract indicated: a—anterior, m—mid, and p—posterior.
[2] Difference in values.

An analysis of the distribution of different food items indicates no major differentiation in diet correlated with size of *T. aurea*. The group 1 fish reveal a diversity value slightly greater than the other two groups; however, this is largely due to additional planktonic Chlorophytes (Tables 3 and 4).

The area of digestive system sampled produced significant quantitative differences in data obtained. The t-test was used to compare differences of mean diversity values from three areas of gut, using two comparisons simultaneously. The results, shown in Table 5, are significant at the 0.05 level of probability from Snedecor and Cochran (1967). The anterior gut revealed the highest diversity value and the posterior the lowest (Table 5). Future food habit studies should include the anterior portion so that the maximum diversity of algae is cultured.

The Bold's Basal medium cultures yielded a slightly greater variety of organisms than did the Gorham's medium. Both should be used; however, since the growth of any blue-greens present would be optimum in Gorham's medium.

Another interesting observation was that algal growth in the 54 culture vessels was not inhibited by bacterial infestations. This was surprising considering the source of the "seed" materials.

SUMMARY

1. Twenty-one taxa of algae were identified by culturing ingested materials with Bold's Basal and Gorham's media.

2. Bacterial infestations had no inhibitory effect on culturing algae.

3. Anterior gut samples generally yielded the highest diversity of food items and the posterior samples the lowest.

4. Bold's Basal medium cultures produced a slightly greater variety of organisms than did the Gorham's.

5. Planktonic chlorophytes were the dominant foods of *Tilapia aurea* at the time fo collection.

6. Conversely, invertebrates were insignificant as revealed from an initial examination of extracted materials.

7. Culturing contents of digestive systems could be useful to researchers studying food habits of juvenile fish and other small aquatic organisms. In these cases where digestive tracts are very small, the entire system could be introduced to the medium, thereby, eliminating tedious dissections and extraction of foods.

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EFFECTS OF INCREASED TEMPERATURE ON POST-LARVAL AND JUVENILE ESTUARINE FISH[1]

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ABSTRACT

We simulated thermal increases encountered by postlarval and juvenile estuarine fishes entrained in power plant cooling systems. Three methods were used to measure the effects of thermal shock on these fishes: (1) critical thermal maximum (CTM); (2) changes in routine oxygen consumption; and (3) survival after exposure to sudden increases in temperature for various periods of time.

For menhaden, spot, and pinfish acclimated at 15° C, CTM values were 29.4, 31.0, and 31.0 respectively. Oxygen consumption of menhaden, spot, and pinfish, increased as we raised the temperature in 5° increments from the environmental temperature, indicating that additional energy expenditures are necessary to maintain the fish at the elevated temperatures. At temperatures of 15° C above the normal environmental temperature, all of the menhaden, spot, and pinfish died within 5 to 10 minutes. Young striped killifish acclimated at 22° C survived at 39° C for more than 30 minutes but all died at 40° C.

INTRODUCTION

Most S.E.S. (steam electric stations) in the United States use open circuit or once-through cooling systems. River, stream, lake, or estuary water is used to condense steam, and then the heated coolant is returned to the source some distance from the intake site. The amount of cooling water used by these plants is in the order of 0.1 to 3 billion gallons of water per day per plant depending on the size of the plant (Mihursky and Cronin 1967). Destruction of a high percentage of the entrained organisms in the large volume of cooling water needed may have a significant effect on aquatic population levels, especially in estuarine nursery areas.

In this investigation we are interested in the effects of a sudden increase in temperature (thermal shock) on postlarval fish that may be entrained in power plant cooling systems. At the present time information is conflicting on whether or not organisms can survive passage through cooling systems. Kerr (1953) found that small yearling salmon could survive a 16° F temperature rise in a S.E.S. condenser with no fatalities after five days and that small yearling striped bass could withstand the 16° F temperature rise with a survival rate of 94% after five days. He concluded that yearling striped bass and king salmon passing through the condenser system tested (entrainment time 3.5 to 5 min.) would have a high rate of survival. Adams (1969) found natural growth of several species of shellfish in the discharge canal of a California S.E.S. Since the net flow in this particular canal was always outward he concluded that the free-swimming stages of these bivalves had passed through the condenser system of the plant. Mihursky and Cronin (1967), however, reported up to 95% destruction of zooplankton

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