Efficacy of Rotenone to Collect Bluegill for Stomach Content Analysis

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Abstract: In 1980 and 1981, bluegill, *Lepomis macrochirus,* were collected concurrently and in adjacent littoral areas of West Point Lake, a 10,480-ha reservoir (Alabama-Georgia), by seining (S) and by poisoning with rotenone (R). A statistical comparison of total stomach content volume and individual food item volume for S and R fish was conducted to determine if rotenone caused either gorging or regurgitation of food as reported for some piscivorous fishes. Stomach contents of 744 R and 1,121 S fish were examined. Data were paired by date, site, and fish size. Although significant differences in total stomach content volume for R and S fish were detected in the majority of comparisons, the number of incidences in which a larger food volume occurred was about evenly divided between R and S fish. Similar results were observed when comparisons were made on the basis of individual food items. There is no evidence to indicate that bluegill collected with rotenone gorged, regurgitated, or selectively fed on moribund food organisms available to them.

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Fish food habit studies necessitate collection of large numbers of fish for stomach examination (Hoffman 1978). Methods routinely used to collect fish are: seining, gill netting, electrofishing, trapping, and angling (Lagler 1978). All of these methods, however, are time consuming and labor intensive and the number of fish collected is frequently small. The fish toxicant, rotenone, has been used extensively for fish population studies (Krumholz 1948, Carter 1957, Lambou and Stern 1957). The prevailing view among fishery scientists is that fish collected with rotenone should not be used for food habit studies. Carter (1957) warned against using piscivorous fish collected with rotenone because unaffected fish, especially larger

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ones, tend to gorge on affected food organisms prior to the time they themselves are affected by rotenone. After gorging, many of them regurgitated. No such observations have been made on planktivorous or insectivorous fishes feeding on stressed zooplankton or benthic macroinvertebrates. This study was conducted to determine if the use of rotenone to collect bluegill (*Lepomis macrochirus*) for food habit studies biased results. It was part of a larger research effort to determine food availability and bluegill food selectivity.

Methods

Study Site

The study was conducted at West Point Reservoir, an impoundment of the Chattahoochee River on the Alabama-Georgia border. The reservoir, impounded in the fall of 1974, extends from the dam, 5.2 km north of West Point, Georgia, to Franklin, Georgia. It has a surface area of 10,480 ha at full pool and a shoreline length of 845 km.

Sampling and Analyses

Three sites were randomly selected and sampled weekly (Timmons et al. 1978). In 1980, 49 sites were sampled from 9 May to 29 August; in 1981, 54 sites were sampled from 9 May to 3 October. Two methods were used to obtain fish. For fish collected with rotenone (R), a net (30.5 m × 2.7 m with a 0.5-cm bar mesh) was used to enclose a semicircular 0.01-ha area of the littoral zone. Sufficient rotenone was distributed and thoroughly mixed within the enclosed area to provide at least 1 ppm concentration (Timmons et al. 1978). Fish were collected as they surfaced; small fish were immediately stunned by placing in ice water to prevent regurgitation (Doxtater 1963) and later preserved in 10% formalin solution while larger fish were placed on ice. The net was pulled ashore to collect any remaining fish. The operation was usually completed in 30 minutes or less. While the rotenone sample was being taken, seining(s) was carried out in a nearby littoral area to obtain fish to serve as a control. Seining was continued until at least 10 fish were obtained.

In the laboratory, fish were measured to the nearest millimeter (total length) and weighed to the nearest gram. Stomach contents of each fish were then removed and blotted dry. Volume of the contents was measured by displacement in a centrifuge tube graduated to 0.01 mm^3 (Laevastu 1965). Contents were transferred to a Sedgewick-Rafter cell and identified to the lowest taxon possible (Edmondson 1959, Pennak 1978, Lemkuhl 1979, Ruttner-Kolisko 1974) with a dissecting or compound microscope equipped with an ocular micrometer. Volume of larger food items was measured by displacement while the volume of smaller items was estimated using the cubic standard unit method described by Welch (1948). With this method, the shape of an organism was related to a common geometric object and the volume calculated mathematically. A total of 376 R fish and 617 S fish were examined in 1980; in 1981, the numbers of R and S fish examined were 368 and 504, respectively for a total of 1,865 fish.

Total volume of stomach contents and volumes of individual food items for R fish were compared to S fish, with a Chi-square contingency table analysis (Steel and Torrie 1980). To determine whether rotenone caused gorging or regurgitation fishes were grouped into: 1) length groups (nearest 1.0 cm TL), and 2) functional size groups of small (<7.5 cm), intermediate (7.6-14.9 cm) and large (>15.0 cm) individuals to facilitate comparison, (Swingle 1956). Food items were grouped either as individual items (e.g. fish eggs, chironomid larvae), or grouped items (e.g. microcrustaceans, Megaloptera). Total stomach content volumes and volumes of individual food items of R and S fish were then compared for each date, site, and fish size category. Combinations used for comparison were: 1) date, site, centimeter length group, individual food items; 2) date, site, centimeter length group, grouped food items; 3) date, site, functional-size fish group, individual food items; and 4) date, site, functional-size fish group, grouped food items.

After the calculations were completed, counts were made of the number of significant comparisons ($P \leq 0.05$), nonsignificant comparisons ($P > 0.05$), and the number of single observations, in which only an R or an S observation was available (no pair). Results of the enumeration were tabulated to determine if fish collected with rotenone had stomach content volumes significantly different from those collected with the seine.

Results and Discussion

Of the 4 different sets of combinations tested, only 2 could be used because combinations 1 and 2 had a high percentage of single observations (Table 1). Combinations 3 and 4 dealt with functional size fish groups. Only 2 of the size groups (small and intermediate) could be evaluated because of the small number of comparisons (4 for combination 3 and 3 for combination 4) available. To determine if differences existed in stomach content volumes of R and S fish, the number of paired comparisons that yielded significantly different results were counted for small and intermediate size fish. Comparisons based on volumes of individual food

Table 1. Number of paired comparisons of the stomach contents of rotenone collected (R) and seined (S) fish and single observations (R or S) for the four different combinations tested. The percent of paired or single observations within each combination is also given.

Combination		Paired comparisons of R and S		Single comparisons of R and S	
No.	Description	*N*	%	*N*	%
1	Indiv. food items by date, site and length group	321	46.9	364	53.1
2	Grouped food items by date, site and length group	325	47.2	363	52.8
3	Indiv. food items by date, site and functional size group	159	79.1	42	20.9
4	Grouped food items by date, site and functional size group	159	79.1	42	20.9

Table 2. Number and percent of comparisons in which rotenone collected (R) or seined (S) fish had the higher ($P < 0.05$) total stomach content volume or in which there were no significant differences ($P > 0.05$).

	Small (<7.5 cm)						Intermediate (7.6–14.9 cm)					
	Rotenone		Seine		Not significant		Rotenone		Seine		Not significant	
Combination	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
3 (Individual Food Items)	21	61.8	13	38.2	23	40.4	39	45.3	47	54.7	4	4.3
4 (Grouped Food Items)	22	56.4	17	43.6	17	30.4	36	46.2	42	53.8	11	12.2

items for small and intermediate bluegills yielded significant ($P \leq 0.05$) differences for 34 pairings (59.6%) and 88 pairings (95.7%), respectively. Comparisons based on volumes of grouped food items yielded significant ($P < 0.05$) differences for 39 pairings (69.6%) and 79 pairings (87.8%), respectively.

In each significantly different pairing it was noted whether R or S fish had a larger total stomach content volume (Table 2). This would indicate whether observed differences were caused by the R fish having higher total stomach content volumes owing to gorging, or lower volumes owing to regurgitation (Carter 1957). When considering individual food items and small bluegill, R fish had higher total stomach content volumes in 21 pairings (61.8%) compared to 13 (38.2%) for S fish; for intermediate size bluegill, R fish had 39 pairings (45.3%) with higher volume compared to 47 (54.7%) for S fish. When considering grouped food items and small bluegill there were 22 pairings (56.4%) in which R fish had higher total stomach content volumes compared to 17 (43.6%) for S fish. For intermediate bluegill, R fish had 36 pairings (46.2%) with higher total stomach content volumes while S fish had 42 (53.8%) pairings. Thus, instead of having all R fish with constantly higher total stomach content volumes, which would indicate gorging, or low total stomach content volumes, which would indicate regurgitation, results indicated that the number of occurrences of high or low total stomach content volume was divided almost evenly between R fish and S fish. There is no evidence to indicate that rotenone caused gorging or regurgitation among affected bluegills.

Volumes or individual food items and grouped food items from stomachs of R and S fish were also compared to determine if R fish were actively selecting moribund food organisms that might have been affected by the rotenone. The number of comparisons was recorded in which either R or S fish had significantly higher volumes for a particular food item. Differences occurred almost evenly for all food items compared (Tables 3, 4), indicating that selective feeding on dead or dying organisms did not occur among the R fish.

In order to determine which food items contributed most to the differences in total stomach content volume, the number of pairings between R and S fish in which significant differences in volume of a particular food item occurred was recorded.

Table 3. The percent of comparisons using individual food items for which differences ($P < 0.05$) occurred in volume of a food item between fish collected with rotenone and seine. The number of comparisons in which rotenone collected (R) or seined (S) fish had higher ($P < 0.05$) stomach content volumes.

	Small (<7.5 cm)			Intermediate (7.6–14.9 cm)		
		Number			Number	
Food item	% Sig.	R	S	% Sig.	R	S
Alonella sp.	5.9	1	1	2.3		2
Cyclops sp.	11.8	3	4	1.2	1	
Diaphanosoma sp.	2.9	1		1.2	1	
Diaptomus				1.2		1
Ilyocryptus sp.	2.9	1				
Scapholeberis sp.	2.9		1			
Brachionus sp.				1.2		1
Ostracoda	11.8	2	2	2.3	1	1
Chironomid larvae	41.2	8	6	51.2	20	24
Chironomid pupae	5.9	1	1	18.6	8	8
Chironomid adults	20.6	3	4	26.7	13	10
Ceratopogonid larvae	2.9	1		2.3	1	1
Stratiomyid larvae	2.9	1		1.2		1
Tabanid larvae				2.3	1	1
Unidentified dipterans	5.9		2	16.3	5	9
Caenis sp. nymphs	5.9		2	7.0	2	4
Siphlonurid nymphs				1.2	1	
Coenagrionid nymphs				4.7	1	3
Macromiid nymphs				1.2	1	
Unidentified odonate nymphs				1.2	1	
Dytiscid larvae	2.9	1				
Adult beetles				8.1	3	4
Adult wasps				8.1	4	3
Helicopsychid larvae				1.2	1	
Trichopteran larvae	2.9	1		16.3	8	6
Adult lepidopterans				2.3	2	
Spiders				5.8	2	3
Ants				12.8	5	6
Hydracarina	2.9	1		1.2	1	
Fish eggs	23.5	3	5	22.1	8	11
Fish larvae	5.9	1	2	14.0	6	6
Unidentified aquatic vegetation	29.4	5	5	51.2	23	21
Oedogonium sp.				14.0	7	5
Spirogyra sp.				14.0	7	5
Detritus	23.5	2	6	45.3	17	22
Sand and silt	11.8	2	2	19.8	11	6
Oligochaetes	2.9		1	4.7	2	2
Leeches				1.2		1
Asiatic clams				2.3	2	

When considering individual food items and small fish (Table 3), the major food items were aquatic vegetation (29.4%), chironomid larvae (41.2%), adult chironomids (20.6%), detritus (23.5%), and fish eggs (23.5%); whereas for intermediate fish, the major items were chironomid larvae (51.2%), aquatic vegetation (51.2%), adult chironomids (26.7%), detritus (45.3%), and fish eggs (22.1%).

When considering grouped food items and small fish (Table 4), the major food items were microcrustaceans (38.5%), fly larvae (46.2%), fish eggs and larvae (25.6%), aquatic vegetation (33.3%), and detritus (30.8%). The main items for intermediate size fish were fly larvae (53.8%), fish eggs and larvae (34.6%), aquatic vegetation (59.0%), detritus (50.0%) and bottom substrates (23.1%). Therefore, the major food items that could be affected by rotenone were composed mainly of microcrustaceans, dipterans, and fish larvae.

Microcrustaceans are very sensitive to rotenone and can be killed by concentrations as low as 0.5 ppm (Kiser et al. 1963, Anderson 1970). The most sensitive genera are *Daphnia, Diaptomous* and *Cyclops* (Hamilton 1941, Brown and Ball 1943). Other forms affected by rotenone are *Diaphanosoma, Bosmina, Chydorous,* and *Ceriodaphnia* (Smith 1941, Kiser et al. 1963). In this study, comparisons of stomachs with high volumes of microcrustaceans revealed an almost even distribution between the R and S fish (Tables 3 and 4). Even though these forms might be affected by rotenone and thus, become more susceptible to predation during the rotenone collection of fish, our results show that they were not taken selectively by the bluegill.

The even split between R and S fish in terms of higher stomach volumes of chironomids (Table 3) indicated that the method of collection had no effect on consumption of these organisms. Chironomids have high resistance to rotenone, probably because of their ability to burrow into the substrate (Brown and Ball 1943). Almquist (1959) found that a 1.0 ppm concentration of 5% rotenone emulsion was

Table 4. The percent of comparisons using grouped food items for which differences ($P < 0.05$) occurred in volume of a food item group between fish collected with rotenone and seine. The number of comparisons in which rotenone collected (R) or seined (S) fish had higher ($P < 0.05$) stomach content volumes.

		Small Number			Intermediate Number	
Food Item	% Sig.	R	S	% Sig.	R	S
Microcrustacea	38.5	7	8	12.8	4	6
Diptera	46.2	10	8	53.8	19	23
Ephemeroptera	5.1		2	7.7	4	2
Odonata				9.0	3	4
Coleoptera	2.6	1		9.0	4	3
Hymenoptera				9.0	4	3
Trichoptera	7.7	1	2	14.1	5	6
Lepidoptera				2.6	2	
Arachnida				6.4	2	3
Formicidae				15.4	5	7
Hydracarina	5.1	2		1.3	1	
Fish eggs and larvae	25.6	4	6	34.6	12	15
Aquatic vegetation	33.3	8	5	59.0	29	17
Detritus	30.8	3	9	50.0	18	21
Bottom substrates	10.3	2	2	23.1	12	6
Aquatic worms	2.6		1	6.4	2	3
Asiatic clams				2.6	2	

lethal to chironomid larvae, however, the time required to kill the animals was more than 20 hours. Cushing and Olive (1956) found that chironomid larvae could tolerate rotenone in excess of 1.0 ppm.

Most immature aquatic insects can tolerate rotenone concentrations as high as 3.0 ppm (Engstrom-Heg et al. 1978). The concentration of rotenone used in this study (1.0 ppm) represents a level that has been reported to have no effect on bottom invertebrates (Brown and Ball 1943, Leonard 1938). Hooper (1948) found no apparent effect of rotenone on the mayfly *Caenis* sp. McGonigle and Smith (1938) stated that bottom organisms were not affected by a rotenone concentration that was toxic enough to kill the brook trout *Salvelinus fontinalis* and the juvenile salmon, *Salmo salar.* Claffey and Ruck (1966) observed that the concentrations (1-2 ppm) of rotenone used to kill fish did not affect food organisms such as the damselfly (*Agrion*), dragonfly (*Anax*), mayfly (*Siphlonurus*) nymphs, and the caddisfly (*Phryganea*) larvae. Our results (Tables 3 and 4) confirm that fish are apparently more sensitive to rotenone than immature aquatic insects that they use as a food source. Other bottom dwelling invertebrates such as oligochaetes appear to be more tolerant to rotenone than chironomids (Cushing and Olive 1956). In our study, both small and intermediate size bluegill collected with rotenone and by seining, fed on larval fish. However, there was no evidence that gorging took place, because most of the ingested larval fish were partially digested.

The bluegill is an insectivore and in contrast to piscivorous species such as the largemouth bass (*Micropterus salmoides*) did not exhibit the gorging or regurgitation behavior described by Carter (1957) during the rotenone sampling carried out in this study. Bluegill collected with rotenone could be used for food habit analysis without introducing unnecessary bias into the study. The same may be true for other planktivorous and insectivorous fishes, as well as for young fish that feed exclusively on zooplankton or insects early in the life cycle.

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