THE ESTIMATION OF ORGANIC CARBON IN THE STOMACH CONTENTS OF SOME MARINE FISHES

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ABSTRACT

The method, which was originally developed for the determination of organic carbon in marine muds, has been adopted for the estimation of organic carbon in stomach contents of marine fishes. Analysis of the stomach contents of four species with different feeding habits has been given and working instructions have been provided for the determination of organic carbon. The same method has also been used for the determination of organic carbon in the whole fish. The method gave consistent results throughout the analysis. The ratio of body carbon to food carbon in a zooplankton feeder and a carnivore were nearer to 1, but in phytoplankton and detritus feeders these ratios were between 5 and 7.

INTRODUCTION

In a recent communication (Qasim, 1972), the importance of chemical analysis of food of fishes has been pointed out. As one of the possible approaches, the determination of organic carbon has been suggested for expressing the food in terms of energy units. This paper gives the working instructions for the analysis of organic carbon in the stomach contents of fishes, which has been adapted from the method developed by Wakeel and Riley (1957) for marine muds. The advantage of this method is that it requires no special apparatus.

FOOD ANALYSIS

The stomach portions of about 20 or more frozen or refrigerated fishes are removed (Pl. I, Figs. a—d) and their contents are emptied carefully in watch glasses and lumped together in bowls containing melting ice. The total quantity is then shaken thoroughly and two or more subsamples are taken from it for qualitative analysis. These are carefully examined under a microscope and the analysis is recorded as shown below for four species, each of a different feeding habit (Pl. I, Figs. e—h). If desired, the organisms present in the stomach can be counted and their total volume determined. These can then be expressed quantitatively.

1. Sardinella longiceps

Date of collection — 18-4-1972; place — Manassery (Cochin); time — 11.30 a.m.; number of specimens analysed — 146; size range — 151-190 mm; craft — country (non-mechanised); colour of stomach contents — dark green pulpy mass; general composition — detrital material most abundant, silt and small yellowish orange particles common; identifiable components of the food were as follows:

Phytoplankton : *Pleurosigma normani* (very abundant), *Nitzschia closterium* (fairly common), *Navicula* sp., *Peridinium steinii, Thalassiosira subtilis, Surirella fluminensis* (fairly common), *Cyclotella* sp. (abundant), *Coscinodiscus* sp. (abundant), *Thalassiosira decipiens, Melosira sulcata, Biddulphia mobiliensis, Dinophysis caudata, Amphiprora gigantea, Asteromphalus wyvillei, Peridinium oceanica, Thalassionema nitzschioides* (present).

Zooplankton: tintinnids and remains of copepods.

2. Mugil macrolepis

Date of collection — 27-5-1972; place — Malippuram fish farm (Kerala); time — 9.00 a.m.; number of specimens analysed — 40; size range — 168-205 mm; method of collection — cast net; colour of stomach contents — dark grey; general composition — major portion of the contents included fine silt and shiny sand particles with large detrital aggregates; identifiable components were as follows:

Diatoms: *Pleurosigma elongatum, Navicula hennedyii* (abundant), *Navicula* sp., *Nitzschia closterium* (very abundant), *Cocconeis* sp., *Amphiprora alata* (abundant), *Surirella fluminensis, Amphiprora gigantea.* Blue-green alga : *Cynechocystis* sp.,

Zooplankton remains were not identifiable and formed a very small part.

3. Rastrelliger kanagurta

Date of collection — 24-5-1972; place — Narakkal landing site; time — 11.00 a.m.; number of specimens analysed — 30; size range — 192-233 mm; craft — country (non-mechanised); colour of stomach contents — yellowish grey pulpy mass; general composition — detrital material formed a substantial portion of the food; identifiable components of the food were as follows:

Phytoplankton : *Peridinium depressum, Fragilaria oceanica* (abundant), *Ornithocercus magnificus* (abundant), *Pleurosigma elongatum, Biddulphia mobiliensis, Thalassiosira subtilis, Biddulphia aurita, Coscinodiscus gigas, Thalassiothrix frauenfeldii, Diplopsalis lenticula, Triceratium favus, Coscinodiscus radiatus, Ceratium breve, Planktoniella sol, Rhizosolenia styliformis, Ditylum brightwelli, Thalassionema nitzschioides.*

Zooplankton : tintinnids, decapod larvae, fish eggs, *Acartia spinicauda* (male and female), *Pseudodiaptomus mertoni* (male and female), *Oithona* sp., (very abundant), *Euterpina acutifrons* (female), *Acartia* copepodites, *Paracalanus aculeatus* (male and female) copepod nauplii, *Temora turbinata* (female), *Acrocalanus monachus* (female), *Acartia centrura* (female), *Pseudodiaptomus serricaudatus* (female).

4. Nemipterus japonicus

Date of collection — 27-5-1972; place — Indo-Norwegian Project (Cochin); time — 2.30 p.m.; number of specimens analysed — 49; size range — 125-196 mm; craft — mechanised vessels; colour and composition of stomach contents — greyish pulpy material with larger organisms (crustaceans and young fishes) dispersed in it; identifiable components were as follows:

Gammarids (very abundant), fish larvae, juvenile fish (fragments), alima larva of *Squilla*, small *Squilla*, bivalve shells, mysids, small crabs, sand and silt, unidentifiable animal remains (numerous).

PROCEDURE

Preparation of samples

The stomach contents are transferred to suitable glass bowls and the extra water is removed by using a fine pipette. The moist food is then exposed to an infra-red lamp for drying. The drying operation can also be undertaken successfully in an oven running at a temperature of 60-70° C, or under an ordinary electric lamp, but the time taken should not exceed 2-3 hours. After drying, the contents form flakes which are ground into fine powder by using a pestle and mortar and stored in properly stoppered specimen tubes until analysed. In April and May 1972, the colour of the powdered food material was found to be light grey in the oil sardine, dark grey in mullet, very dark grey in mackerel and greyish white in *Nemipterus*.

Preparation of reagents

(a) *Chromic acid* — Dissolve 13 g of chromium trioxide in minimum quantity of water (5-8 ml). Add about 900 ml of concentrated sulphuric acid. Special care should be exercised to make sure that all glasswares used during the analysis are of either Pyrex, Borosil or Corning make. Allow the solution to cool down to room temperature and then add some more concentrated sulphuric acid to bring the solution to 1 litre.

(b) *Ferrous ammonium sulphate* — Dissolve 19.65 g of ferrous ammonium sulphate (0.2N) in 200 ml of distilled water containing 5 ml of concentrated sulphuric acid. Bring the solution to 250 ml by adding some more distilled water.

(c) *Ferrous-phenanthroline indicator* (0.025M) — Dissolve 0.337 g of 1-10 phenanthroline monohydrate (C_{14} H_8 N_2. H_2O) in 25 ml of 0.695% ferrous sulphate solution. If ferrous ammonium sulphate is used, instead of ferrous sulphate, dissolve 0.245 g in 25 ml of distilled water and then add 0.337 g of 1-10 phenanthroline monohydrate. The colour of the indicator is brick red.

Determination of sample

From the dried food sample, weigh 50 mg and transfer it into a hard glass boiling tube (20 x 2.5 cm). Add 30 ml of the prepared chromic acid by using a pipette. Shake the tube gently and cover it with aluminium foil. Heat the tube in a bath of boiling water for about 1 hour until the sample is digested. For routine analysis, 5-10 boiling tubes can be heated together tied with a rubber band. Cool and pour the contents of the boiling tube into a conical flask containing 200 ml of distilled water. Add 4-6 drops of the indicator, depending upon the quantity of carbon present in the sample. This can easily be judged from the colour of the samples which, when rich in carbon, has a dark green tint. Titrate with 0.2 N ferrous ammonium sulphate solution until a brick red colour appears. This colour will persist for about 1 minute and will then change to permanent bluish green.

Determination of blank

With each batch of sample 2blank determinations should be carried out by following exactly the procedure described above, except that no sample (dried food material) is added.

Calculation

1 ml of 0.2 N ferrous ammonium sulphate is equivalent to 0.6 mg of carbon. As an example, if the titration value for the blank is x and the titration value for the sample is y, then x-y is the volume of ferrous ammonium sulphate which is equivalent to the chromic acid reacted. If 5 or 10 ml chromic acid is used for the blank, instead of the usual 30 ml, the titration values should be multiplied by 6 and 3 respectively.

It is desirable to reject the solution of ferrous ammonium sulphate after each set of analysis. The prepared solution should preferably be used within 3 to 4 hours.

Results of Analysis

Table 1 gives the values of organic carbon in the stomach contents of four species. The consistency of results obtained in each fish clearly indicates the reliability of the method used. Table 1 also gives the values of organic carbon in the whole fish. The preparation of the samples of whole fish is by

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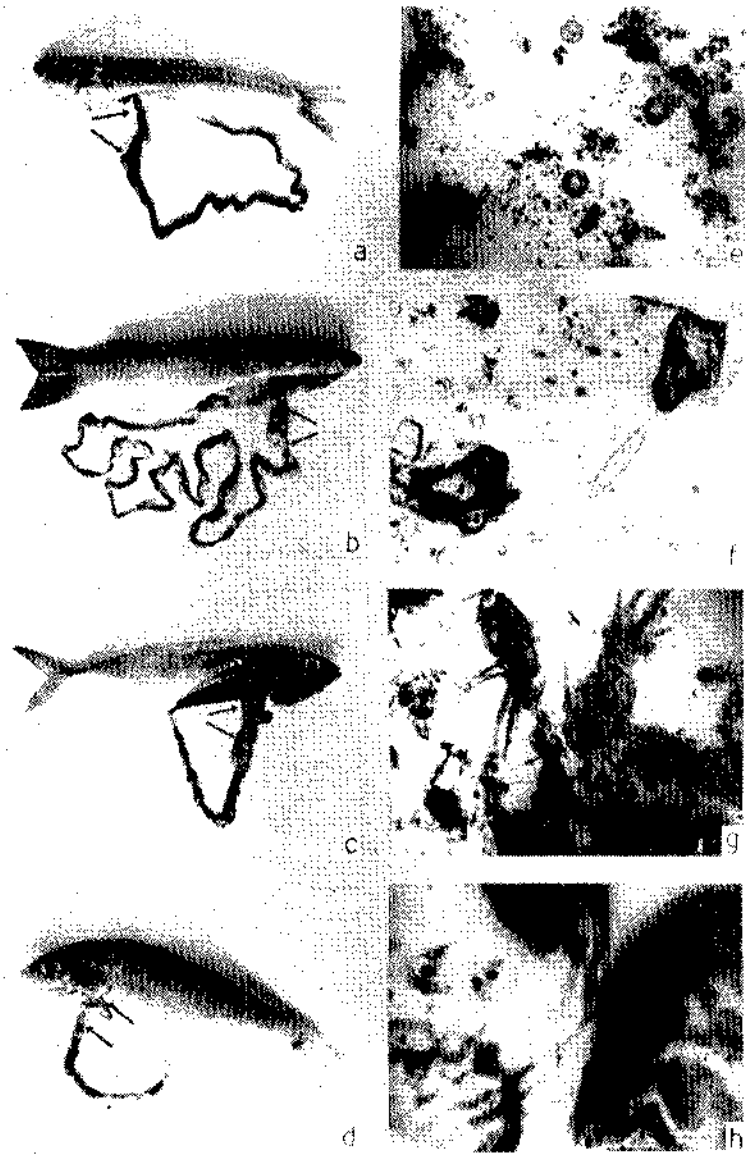

Figs. a-d Dissected fishes showing their entire guts. The arrows indicate stomach portions which were removed and their contents examined. *a. Sardinella longiceps. b. Mugil macrolepis. c. Rastrelliger kanagurta. d. Nemipterus japonicus*

Figs. e-h. The stomach contents of the four species as seen under a microscope. *e. Sardinella longiceps. f. Mugil macrolepis. g. Rastrelliger kanagurta. h. Nemipterus japonicus.*

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TABLE 1. *Total organic carbon in the stomach contents of four species of fishes in relation to their body carbon together with other relevant data*

Species	Feeding habit	Date of collection	Total specimens used for analysis	Organic carbon in stomach-contents (% dry wt)	Organic carbon in body (% dry wt)	Ratio body carbon: food carbon	Moisture content of fish %
Sardinella longiceps	Phyto-	21-12-71	41	7.94	—	—	
	plankton	24- 3-72	105	7.08	46.74	6.60	
	feeder	5- 4-72	114	7.44	44.34	5.96	71.15
		10- 4-72	101	7.26	43.14	5.94	
		18- 4-72	146	6.96	43.92	6.31	
Mugil macrolepis	Detritus	24- 5-72	30	7.74	51.00	6.59	
	feeder	27- 5-72	40	7.68	50.34	6.55	66.60
Rastrelliger kanagurta	Phyto-and	15- 5-72	22	30.05	36.60	1.22	
	zooplankton	24- 5-72	50	29.52	37.00	1.25	73.25
	feeder	25- 5-72	18	30.00	37.02	1.23	
Nemipterus japonicus	Carnivore	15- 5-72	16	26.64	33.18	1.25	
		27- 5-72	50	28.20	35.04	1.24	76.00

macerating the gutted fish, in an electric tissue grinder. Alternatively, a kitchen meat mincer can also be used. The minced material thus obtained is dried under the infra-red lamp exactly like the food samples. It is further treated and analysed like the food samples. The consistency in the values of organic carbon in the whole fish (Table 1) indicates that the method can be successfully applied to the estimation of body carbon. Table 1 also gives the ratio of the body carbon to food carbon, and the moisture content of each fish. The latter was determined from the loss in weight of several ungutted specimens of each species, before and after drying to constant weight.

It is interesting to note that the ratios of body carbon to food carbon in *R. kanagurta* (phyto-and zooplankton feeder) and in *N. japonicus* (carnivore) are approximately 1, whereas in *S. longiceps* (phytoplankton feeder) and *M. macrolepis* (detritus feeder) these are between 5 and 7. The ratios pertain to April and May 1972 when the samples were obtained. It would be interesting to know whether the ratio in the same species would change seasonally, depending upon the quality of food consumed and whether it would also be influenced by such biological features as maturity, spawning and growth. If, however, the ratios do not fluctuate widely, it would be even more interesting to investigate what are the factors which lead to their constancy.

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