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Technical Notes

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METHODS OF STOMACH CONTENT ANALYSIS OF FISHES

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The study of the feeding habits of fish and other animals based upon analysis of stomach content has become a standard practice (Hyslop 1980). Stomach content analysis provides important insight into fish feeding patterns and quantitative assessment of food habits is an important aspect of fisheries management. Lagler (1949) pointed out that the gut contents only indicate what the fish would feed on. Accurate description of fish diets and feeding habits also provides the basis for understanding trophic interactions in aquatic food webs. Diets of fishes represent an integration of many important ecological components that included behavior, condition, habitat use, energy intake and inter/intra specific interactions. A food habit study might be conducted to determine the most frequently consumed prey or to determine the relative importance of different food types to fish nutrition and to quantify the consumption rate of individual prey types. Each of these questions requires information on fish diets and necessitates different approaches in how one collects and analyzes data. Here, we outline qualitative and quantitative techniques used to describe food habits and feeding patterns of fishes. For a better understanding of diet data and for accurate interpretation of fish feeding patterns, time of day, sampling location, prey availability and even the type of collecting gear used need to be considered before initiating a diet study or analyzing existing diet data.

Stomach contents can be collected either from the live or fresh died fish. Regardless of the method, investigators should ensure that the removal technique effectively samples all items in the gut. Other wise data will be skewed toward items that are more easily displaced from the stomach. Alternatively, live fish can be sacrificed and stomach contents removed for analysis. If fish are to be sacrificed, they should be preserved immediately either by freezing or by fixing in formalin. Stomach contents will continue to digest, rendering rapid preservation of the fish or removed contents necessary to prevent loss of resolution. As in most fish groups feeding behavior of juveniles and adults vary distinctly attention should be taken to encounter more samples which will include all size groups of the particular fish. The specimens either from live or preserved should be measured to its total length to the nearest 1mm and weight to the nearest 0.1 g. Cut open the fish and record the sex and maturity stage of the fish. Remove the stomach and preserve them in 5% neutralized formalin for further analysis. For the analysis, a longitudinal cut must be made across the stomach and the contents are transferred into a Petri dish. The contents then keep for five minutes to remove excess formalin and then examine under binocular microscope. Identify the gut content up to the genus and if possible up to species level depending up on the state of digestion. Various taxa digest at different rates. As such, all recently consumed taxa may be present in the foregut but only resistant items remain in the hindgut. To avoid bias when both easily digested prey and resistant prey are present, only the immediate foregut (e.g., stomach) should be sampled.

Prey items in fish stomachs are often not intact. Hard parts such as otoliths, scales, cleithra or backbones have diagnostic, species specific characteristics useful for identifying prey. Alternatively, partially digested prey may be identified using unique biochemical methods such as allozyme electrophoresis, or immunoassays. An important fact assessed by the examination of the stomach is the state or the intensity of feeding. This is judged by the degree of distension of the stomach or by the quantity of food that is contained in it. The distension of the stomach is judged and classified as ‘gorged or distended’, ‘full’, ‘3/4full’, ‘1/2full’ etc by eye estimation.

Fish diets can be measured in a variety of ways. Methods of gut contents analysis are broadly divisible into two, *viz.*, qualitative and quantitative. The qualitative analysis consists of a complete identification of the organisms in the gut contents. Only with extensive experience and with the aid of good references it is possible to identify them from digested, broken and finely comminuted materials. Quantitative methods of analysis are three types, *viz.*, numerical, gravimetric and volumetric. All these types of analysis are widely employed by different workers. The following outline of methods is based mainly on the reviews by Hynes (1950), Pillay (1952), Windell (1968), Hyslop (1980) and Chipps *et a*l (2002).

1) Numerical methods

The numerical methods are based on the counts of constituent items in the gut contents. The numerical methods have been adapted in different ways to assess the relative importance of food items and these can be classified under four distinct heads, viz., a) Occurrence, b) Dominance, c) Number and d) Point (Numerical) methods.

a) Frequency of Occurrence. Stomach contents are examined and the individual food organisms sorted and identified. The number of stomachs in which each item occurs is recorded and expressed as a percentage of the total number of stomachs examined.

$$\text{Frequency of Occurrence, } O_i = \frac{J_i}{P}$$

Where, J_i is number of fish containing prey **i** and P is the number of fish with food in their stomach.

This method demonstrates what organisms are being fed upon, but it gives no information on quantities or numbers and doest not take in to consideration the accumulation of food organisms resistant to digestion. For instance, three organisms in a stomach, say, prawn, rotifers and diatoms, present in the ratio of 1:200:2000 would all be treated by this method as 1:1:1 with reference to the stomach in question. This method holds good even when there is differential distribution of various food organisms in the water for the same reason that it is not biased by size or numbers of organism comprising the food. Many have used this method as an indicator of inter-specific competition while some utilized this method to illustrate the seasonal changes in diet composition.

b) Number method. The number of individual of each food type in each stomach is counted and expressed as a percentage of the total number of food items in the sample studied, or as a percentage of the gut contents of each specimen examined, from which the total percentage composition is estimated.

$$\text{Percent by number, } N_i = \frac{N_i}{\sum_{i=1}^{Q} N_i}$$

Where, N_i is the number of food category **i**

This method has been employed successfully by several workers in studies on the food of plankton feeding fishes where the items can be counted with ease. In the basic number method, no allowance is made for the differences in size of food items. So in the studies on the food of fishes other than plankton feeders, the number method has very limited use. The counting of comminuted plant matter in the stomach of fish is impracticable and will not yield correct evaluations. So also in the analysis of the gut contents of a carnivore which may consist of only one large sized fish and a couple of small larvae, the counting are of little value computations. These are summed to give totals for each kind of food item in the whole sample, and then a grand total of all items. The quotient of these gives the percentage representation, by number, of each type of food item.

c) Dominance method. Essentially the dominance method is a partial improvement of the occurrence method, *viz*., the lack of consideration of the quantities of the food items present in the stomach, sought to be remedied. The stomach contents comprising the main bulk of the food materials present, is determined and the number of fish in which each such dominant food material is present is expressed as a percentage of the total number of fishes examined. The percentage composition of the dominant food materials can also be expressed by this method as in the occurrence method.

Though in an analysis of dominance the bulk of the food material is taken in to account, it can yield only a very rough picture of the dietary of a fish. More over, items which are less dominant due to environmental reasons may escape notice. Though this defect can also be remedied to a certain extent by the examination of large samples spread over a long period of time, a system of assay that takes in to account the relative importance of food constituents will obviously be more suitable in gut content analysis.

d) Points (Numerical) Method. The points method is an improvement on the numerical method where consideration is given to the bulk of the food items. The simple form of points method is the one in which the counts are computed falling a certain organisms as the unit. In a more modified form, the food items are classified as 'very common', 'common', 'frequent', 'rare', etc., based on rough counts and judgments by the eye. In this arbitrary classification the size of the individual organisms is also given due consideration. The contents of all stomachs are then tabulated and as a further approximation, different categories are allotted a certain number of points and the summations of the points for each food item are reduced to percentages to show the percentage composition of the diet. This method is essentially a numerical one; the volume being only a secondary consideration and it is only in the counts that a certain amount of accuracy can be claimed.

2) Volumetric methods

Many workers consider the volume as a more satisfactory method for quantitative analysis of gut contents. As Hynes (1950) pointed out, volume forms a very suitable means of assessment, this is especially so in the case of herbivorous and mud feeding fishes where the numerical methods "become meaningless as well as inaccurate". Even in cases where the numerical methods are suitable, volume has been considered as an essential factor to be reckoned with, and in all improved numerical methods the volume of the food items is taken in to consideration in some way or other. The chief methods that are employed in assessing the volume of food items in the gut contents of fishes are:

a) Eye estimation method: - This is probably the simples and easiest means of determining the volume of food constituents. In this method the contents of each sample is considered as unity, the various items being expressed in terms of percentage by volume as estimated by inspection. This method of analysis is subjective in nature and the investigators personal bias is likely to influence the results very greatly. This defect can be minimized to a great extent by the examination of large samples conducted over a long period.

b) Points (Volumetric) method: - This method is a variation of the eye estimation method. Here instead of directly assessing the volume by sight as in the previous method, each food item in the stomach is allotted a certain number of points based on its volume. Certain workers have taken into account both the size of the fish and the fullness of the stomach in the allotment of points. The diet component with highest volume was given 16 points. Every other component was awarded 16, 8, 4, 2, 1 and 0 points depending on the volume relative to the component with the highest volume. Percentage volumes within each subsample were calculated as:

$$? = \frac{\text{Number of points allocated to component } ?}{\text{Total points allocated to sub sample}} \text{ X } 100$$

Where,
? is the percentage volume of the prey component ?

This method is quite useful for analyzing omnivorous and herbivores where measuring volumes of microscopic organisms such as diatoms and filamentous algae are very difficult.

c) Displacement method: - The displacement method is probably the most accurate one for assessing the volume. The volume of each food item is measured by displacement in a graduated container such as a cylinder with the smallest possible diameter for accuracy. This method is eminently suited in the estimation of the food of carnivorous fishes. But the differential rate of digestion of the food items may sometimes affect he accuracy of the observations. However, if the collections are made when the fish are on feed, this defect can be easily overcome. A knowledge of the volumes of the different size groups of the food items ay be of great help in estimating the volume of the whole item form the semi digested fragments

3. Gravimetric method

The gravimetric method consists of the estimation of the weight of each of the food items, which is usually expressed as percentages of the weight of the total gut contents as in other quantitative methods.

$$\text{Percent by weight, } W_i = \frac{W_i}{\sum_{i1}^{Q} W_i}$$

Where, W_i is the weight of the prey **i**

Generally the wet weigh of the food after removing superfluous water buy pressing it dry between filter papers is taken for this purpose. Dry weight estimation is more time consuming and is usually employed where accurate determinations of calorific intake is required. The limitation of weight as a criterion of analysis has already been referred in the consideration of the method of assessing the condition of feed. Besides these, the accurate weighing of small quantities of food matter is extremely difficult and impracticable in studies of large collections. This method is, therefore generally employed only in conjunction with other methods to demonstrate seasonal variations in the intensity of feeding.

Food analysis indices

A. Simple indices

1) Index of fullness. This is measured as the ratio of food weight to body weight as an index of fullness, which is very widely employed. (The ratio of corresponding volume can also be used.) This index can be applied to the food in the stomach, or to that in the whole digestive tract. It is usually expressed as parts per 10,000 (%00, or parts per decimile); that is:

$$\text{Fullness index} = \frac{\text{weight of the stomch contents x 10,000}}{\text{weight of fish}}$$

2) Index of consumption. Some authors have used not the actual weight (or volume) of the stomach contents, but their reconstructed weight: i.e. their estimated weight at time of ingestion. When reconstructed weights are used in the formula above, the index obtained has been distinguished as the index of consumption

$$\text{Consumption index} = \frac{\text{reconstructed weight of stomach contents x 10,000}}{\text{weight of fish}}$$

Reconstructed weights are estimated form the lengths of relatively indigestible parts of the organisms consumed- for example shells, chitin, bones, otoliths, scales or stomachs. For accuracy it is necessary to make systematic measurements on whole specimens of various sizes, for each of the food species consumed.

3) Index of selection or forage ratio. Most fishes have a scale of preference for the organisms in their environment, so that some are consumed in large numbers, others moderately, some not al all. A quantitative index of such differences called as the forage ratio. A study of the quantities of different organisms available to the fish is made, and also of the various items in their stomachs; then;

Selection index = forage ratio = $\frac{s}{b}$

Where, s = percentage representation by weight, of a food organism in the stomach and b = percentage representation of the same organism in the environment. The lower limit for this index is 0; its upper limit is indefinitely large.

4) Index of electivity, Ivlev (1961) proposed a somewhat different quantitative measure of selection which has been widely used as mean of comparing the feeding habits of fishes and other aquatic organisms with the availability of potential food resources in natural habitats. The relationship is defined as

Electivity index = E = $\frac{s - b}{s ? b}$

The index has a possible range of -1 to +1, with negative values indicating avoidance or inaccessibility of the prey item, zero indicating random selection form the environment, and positive values indicating active selection.

5) Manly-Chesson index

When given a variety of prey types, most fishes select some food categories over others. To measure this selectivity, a variety of indices have been developed that incorporate measures of prey use and prey availability. While prey use can be easily determined from gut content analysis, accurate description of prey availability can be problematic. What we quantify as prey availability may be quite different than what fish perceive under natural conditions. Furthermore, because different prey can occupy different habitats, a single sampling technique may not adequately quantify the relative abundance of different prey items in the environment. This is important because we cannot use volumetric estimates of zooplankton abundance (e.g. no/L) and area densities of benthic invertebrates (e.g., no/m^2) as a simultaneous measure of prey availability. Only in cases where prey is collected with the same gear type, such as open water zooplankton, can we begin to compare use versus availability.

Like diet and overlap indices, there is much controversy over which index is best. Comparisons of different indices have revealed that the Manly-Chesson (Chesson 1983) and the Linear index (Strauss 1979) are good choices for quantifying prey preference. The Manly-Chesson index is frequently used to quantify prey preference and can be calculated for two scenarios

a) Constant prey abundance – used when the number of prey eaten is very small relative to its total population or when prey is replaced as in laboratory studies. The equation for the Manly-Chesson index under constant prey abundance is,

$$?_i ? \frac{r_i}{n_i} \frac{1}{? \ (r_j / n_j)}$$

Where $?_i$ = Manly's alpha for prey type i

r_i, r_j = Proportion of prey type **i** or j in the diet

n_i, n_j = Proportion of prey type **i** or j in the environment

m = Total number of prey types

Values of $?_i$ are normalized so that $\overset{m}{?} \, ?_i = 1.0$

Prey preference is indicated when $?_i$ values are greater than 1/m. Conversely, $?_i$ values Less than 1/m imply that prey species **i** is avoided in the diet because it is used in lower proportion than its availability in the environment.

b) Variable prey abundance – used when the number of prey eaten is large relative to its total population in the environment or, in experimental studies, when prey are not replaced after being eaten. The Manly-Chesson index for variable prey populations is calculated using the equation,

$$?_i = \frac{\log p_i}{\overset{m}{\underset{j?1}{?}} \log p_j}$$

Where $?_i$ = Manly's alpha for variable prey populations

p_i, p_j = Proportion of prey i or j remaining at the end of the experiment (= ei/ni)

Where,

ei = Number of prey type i remaining at the end of experiment

ni = Number of prey type i at the beginning of the experiment

m = Total number of prey types

In practice, indices such as the Manly-Chesson can be used to test for differences in prey selectivity providing important information about preferred (or vulnerable) prey types.

Compound indices

In an attempt to consolidate the desirable properties of individual diet measures (e.g., Ni, Wi. Foi), compound indices were developed that combine two or more measures into a single index. The belief is that compound indices capture more information than do single component measures (Chipps et al 2002).

1) Index of Preponderance: - (Natarajan and Jhingran, 1961)

This index gives a summary picture of frequency of occurrence as well as bulk of various food items. It provides a definite and measurable basis of grading the various food elements. The bulk of food items can be evaluated by 1) Numerical 2) volumetric and 3) Gravimetric methods. As the numerical method is not suited to the index with the frequency of occurrence it magnifies the importance of smaller organisms which may appear in enormous numbers. Therefore either volumetric or gravimetric are best to assess the food items quantitatively. If we V_i and O_i are the volume and occurrence index of food item i. then,

$$\text{Index of preponderance } I_i = \frac{V_i O_i}{? \; V_i O_i} ? \, 100$$

Example: The ‘Index of Preponderance’ of food items of *Catla catla* (Ham.) is given in the table 1 with rankings in brackets.

Index of Preponderance (Natarajan and Jhingran, 1961) of adult Catla

Food items	Percentage of occurrence (O_i)	Percentage of volume (V_i)	$V_i O_i$	$\frac{V_i O_i}{? \; V_i O_i} ? \, 100$
Crustaceans	24.5	57.1	1398.95	64.50 (1)
Algae	27.3	24.0	655.20	30.06 (2)
Plants	6.4	8.2	52.48	2.41 (3)
Rotifers	10.8	2.4	25.92	1.19 (4)
Insects	3.6	6.0	21.60	0.99 (5)
Protozoa	0.6	0.3	0.18	0.01 (8)
Molluscs	….	….	……	…...
Polyzoa	….	….	……	. ….
Detritus	10.0	1.3	13.00	0.60 (6)
Sand and mud	16.8	0.7	11.76	0.54 (7)
?	100	100	2179.09	100

According to the index crustacea and algae constitute 1 and 2 ranks in *Catla catla*. While third, fourth and fifth places are held by plants, rotifers and insects. In grading the food elements accidental and incidental inclusions like sand, mud, etc., may be left out of consideration.

2) Index of Relative Importance (IRI):- Leo Pinkas et al (1971)

This index is an integration of measurement of number, volume and frequency of occurrence to assist in evaluating the relationship of the various food items found in the stomach. It is calculated by summing the numerical and volumetric percentages values and multiplying with frequency of occurrence percentage value.;

Index of relative importance, $IRI_i = (\% N_i + \% V_i) \, \% O_i$,

Where, N_i , V_i and O_i represent percentages of number, volume and frequency of occurrence prey **i** respectively.

Example:. Index of Relative Importance of pelagic preflexion summer flounder, *Paralichthys dentatus* larvae (Grover, 1998).

Prey	% N_i	% V_i	% O_i	(% N_i +% V_i) % O_i	%IRI
Tintinnids	28.7	3.3	37.6	1203.2	19.3
Copepod nauplii	20.0	10.2	41.2	1244.24	20.0
Copepodites	16.0	61.4	30.0	2322	37.3
Calanoids	0.6	4.9	2.0	11	0.2
Cyclopoids	0.6	2.0	2.4	6.24	0.1
Copepod eggs	16.0	1.2	34.8	598.56	9.6
Bivalve larvae	12.1	14.8	28.0	753.2	12.1
Invertebrate eggs	3.7	0.9	11.6	53.36	0.9
Other	2.3	1.3	9.2	33.12	0.5

In pelagic preflexion summer (*Paralichthy dentatus)* larvae, copepodites composed the bulk of the diet (61.4% Vol, 37.3 % IRI) and formed the most important prey. Copepod nauplii, the second most important prey, composed 20.0% (N and IRI). Tintinnids, despite being the most abundantly ingested prey (28.7% N); ranked third in importance at 19.3% (IRI). Bivalve larvae and copepod eggs were the only other prey that accounted for >1% of the diet, and together they composed 21.7% (IRI).

Diet overlap indices

Niche overlap indices tabulated in the form of matrices are often used to measure the magnitude of resource overlap among different species. Although sometimes used to infer competition, we should recognize that high resource overlap between two species may not indicate competitive bottlenecks. Rather, it may be indicative of high resource abundance such as seasonal peaks in prey availability.

a) Morista's index

When stomach data are represented in prey numbers or only prey numbers are available, Morista's index has been recommended as the most robust index.

Morista's index is calculated using the equation,

$$M = \frac{2\sum p_{ij} p_{ik}}{\sum^{n} p_{ij}\left[(n_{ij}-1)/(N_j-1)\right] + \sum^{n} p_{ik}\left[(n_{ik}-1)/(N_k-1)\right]}$$

Where, M =Morista's index of niche overlap between species *j* and *k*

p_{ij} =Proportion resource *i* is of the total resources used by species *j*

p_{ik} =Proportion resource *i* is of the total resources used by species *k*

n_{ij} =Number of individuals of species *j* that use resource category *i*

n_{ik} =Number of individuals of species *k* that use resource category *i*

N_j, N_k =Total number of individuals of each species in sample

b) Horn's index

If stomach data are not expressed as prey numbers (e.g., biomass or volume), then Horn's index is recommended and is calculated as,

$$H = \frac{\sum (p_{ij} + p_{ik})\log(p_{ij} + p_{ik}) - \sum p_{ij}\log p_{ij} - \sum p_{ik}\log p_{ik}}{2\log 2}$$

Where H =Horn's index of overlap between species j and k

p_{ij} =Proportion resource *i* is of the total resources used by species j

p_{ik} =Proportion resource *i* is of the total resources used by species k

c) Schoener's index

Basically this index was used to study the diet overlap of terrestrial animals. Later many fishery biologists have used this index to compare the dietary overlap of the two fish species or of the two size/age categories or of the two different habitats. Percentage values of weight of the prey or Index of Relative Importance can be used to compare the diets.

$$S_{io} = 1\text{-}0.5\sum_{j=1}^{n} \left| p_{xi} - p_{y_i} \right|$$

Where,

p_{xi} = the proportion of the prey i in the diet of fish species x (or size class x);

p_{y_i} = The proportion of prey i in the diet of two species y (or size class y);

and j = the numbers of prey categories.

An overlap value of S_{io} ? 0.6 (Schoener, 1970) is considered as biologically significant.

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