CHAPTER

13

Stomach Content Analysis Techniques in Fishes

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In Ichthyology, fish ecology & fisheries resource management, the information on diet & food habits are valuable in the decision-making process related to natural resources (Kido, 1996). Fish gut content analysis provides an important insight into feeding patterns & quantitative assessment of feeding habits is an important aspect of fisheries management. Fish diet represents an integration of many important ecological components that includes behaviour, condition, habitat use, energy intake & inter & intra-specific interactions, etc. A valid description of fish diets & feeding habits also provides the basis for understanding trophic interactions in aquatic food webs. Conceptually, trophic relations of fishes begin with the food & feeding habits & gut content analysis can be used to evaluate the habitat preferences, prey selection, effects of ontogeny & developing conservation strategies (Chipps & Garvey 2007). A food habit study might be conducted to investigate the most frequently consumed prey or to determine the relative importance of different food types to fish nutrition & to quantify the ingestion rate of individual food types. All such questions demands information on fish diets & requires different approaches in how one collects & analyzes data. In summary, gut content analysis is used in the understanding of many aspects of fish ecology on individual, population & ecosystem levels. It helps us to study & elucidate specific problems of interactions, evolution, speciation, invasions & fishery management nature protection. As a result, stomach content studies could be incorporated into a variety of different research objectives. Consequently, the study of the gut content is not only way to know the diet but also superior source of information on many aspects of fish biology & ecology.

The study of the feeding habits of fish & other animals based on direct examination of stomach content has become a standard practice for many years (Hyslop, 1980). Recently, many other methodologies such as radioisotopes, stable isotope analysis, direct species observations & fatty acid analysis are currently being used (Braga *et al.*, 2012). These approaches have both positives (more accurate & can reveal even the items which cannot be identified by microscopic study) & negatives (expensive, complicated procedures). However, the direct gut content analysis carried commonly out through dissection or evacuation & examination of stomach contents is still the most used & easiest method with great potential & good enough for most biological/ecological studies (Manko, 2016). Other factors *viz.,* sampling location, time of day, prey availability & even the type of gear used collect the fishes need to be considered before initiating a diet study or analyzing existing diet data for a better understanding of diet data & for accurate interpretation of fish feeding habits (Zacharia, 2017).

Gut contents can be collected either from the live or fresh died or preserved fish. Irrespective of the method, investigators should ensure that, the gut removal technique effectively samples all food items in the gut or else data will be distorted toward food items that are more easily displaced from the stomach. Instead, live fish can also be forfeited & gut contents removed for analysis. When diet samples are not analysed forthwith, fish should be preserved immediately either by freezing or by fixing in formalin to avoid continued digestion of food contents (Chipps & Garvey 2007). Proper care should be taken to encounter more samples which include all size groups of the particular fish since the feeding behavior of juveniles & adults of many fish groups vary significantly. Sampled fish should be measured to its total length to the nearest 1 mm & weight to the nearest 0.1 g, then a make a longitudinal cut on the ventral side of the fish from just behind the isthmus of the gills posterior to the anal fin. Make two transverse cuts at each end of the first cut to open the coelom to expose the viscera & record the sex & maturity stage of the fish. Separate the digestive tract (esophagus, stomach & intestine) from other visceral organs, then judge the degree of distension of the stomach & classify as 'gorged, 'full', '3/4full', '1/2full' 1/4full & trace by eye estimation & note down the weight to the nearest 0.1 g. Stomach contents may be analysed immediately or preserved in 5% neutralized formalin to analyse later. For analysis, a longitudinal cut must be made across the stomach then sever the stomach (or foregut) from the hindgut to avoid the bias when both easily digested prey & resistant prey are present. In fish, Fishes which do not have a distinct stomach, the first half of the intestine can be dissected & the contents are transferred into a petri dish for further analysis. While analysing formalin preserved samples, keep gut contents out or in water on petri dishes for five minutes to remove excess formalin. Analyse the gut content to the genus & up to species level wherever possible by identify the large prey on eye observation & examination of small prey under binocular microscope.

Fish diets are measured in a variety of ways. Gut contents analysis are broadly classified into qualitative & quantitative methods. The qualitative analysis involves a complete identification of the organisms in the gut contents. Only with extensive knowledge & with the aid of good references it is possible to identify prey & other food particles from digested, broken & fine comminuted materials. Quantitative methods of analysis are classified in to three types, *viz.,* numerical, gravimetric & volumetric. Quantitative methods are most discussed problematics in the gut content analysis. Many authors examined these methods, compared & employed the best one for application in the various scenarios & for highlighting different aspects of feeding ecology (Hynes, 1950; Pillay, 1952; Hyslop, 1980; Cortes 1997; Hansson 1998; Liao *et al*., 2001; Chipps & Garvey, 2007; Ahlbeck *et al.,* 2012; Baker *et al.,* 2014 & Manko, 2016). Generally, on the basis of grading & comparing food contents in the fish diets, one presume that some food is more important than others to the growth, survival, recruitment, size structure, condition, reproductive success, or other aspects of the ecology of the fishes, thus it is crucial to describe the true importance of food contents (Bowen, 1996).

1) Numerical methods

The numerical methods are centered 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 & these can be classified under four distinct heads *viz.,* a) Frequency of occurrence, b) Number c) Dominance & d) Point methods.

a) Frequency of occurrence:

Recording the presence or absence of each food item across all individuals is the simplest way to reveal the relative importance of different food items & to judge the dietary composition of a fish population. The importance is inferred from the proportion of total guts containing each food item (Baker et al. 2014). Each food item occurred in number of stomachs is recorded & expressed as a percentage of the total number of fish stomachs examined.

Frequency of occurrence,

$$\% O_i = \frac{N_i}{N} \times 100$$

Where: % O is the frequency of occurrence of given food i

N_i is the number of stomachs containing prey i

N is the total number of stomachs with some food

Frequency of occurrence method exhibits what organisms are being foraged upon, the advantages are food items are readily classifiable, rapid & requires the minimum of apparatus. However, frequency of occurrence furnish little indication of the relative importance or bulk of each food category present in the stomach.

b) Number method:

The number method is based on the counts of food items in the gut content. The number of individuals of each food category in each stomach are recorded & expressed as a percentage of the total number of food items in all fish stomachs examined or as a proportion of the food items of each stomach of fishes examined, which raised to the total percentage composition (Hynes, 1950). The numerical method is easy & relatively fast.

Percentage by number,

$$\% O_i = \frac{N_i}{N_t} \times 100$$

Where: % N_i is the percentage of food item i

N_i is the number of particular food item i

N_t is the total number of food (gut content) items

This method has been applied successfully by many workers in studies on feeding habit of fishes *viz.,* plankton feeders & piscivorous, where prey items of different species are in the same size range & the ease of counting individual of countable prey or their appendages like head capsules, carapace, other body parts etc. (Beyerle & Williams, 1968; Guma'a, 1978). In contrast, this method is not practicable & will not yield correct evaluations when the food do not appear in separate units (like detritus, macro algae, comminuted plant matter), the food is masticated or fast digestible because of its nature. (Hyslop, 1980; Scharf *et al.,* 1997; Legler *et al.,* 2010; Ahlbeck *et al.,* 2012; Baker *et al.,* 2014; Zacharia, 2017).

The differences in size of food items are not considered in the number method, similarly to the frequency of occurrence. This method overestimates small prey items taken in large numbers & underestimates large food items like the gut contents of a carnivore which may consist of only one large sized fish & a couple of small larvae. Thus, the number method has very limited use in the studies on the food of fishes other than plankton feeders, when food consists of significantly variable prey (food) size (Ahlbeck *et al.,* 2012).

c) Dominance method:

This method is a further improvement of the occurrence method. The number of fish in which each food item occurs as the dominant food material is expressed as a percentage of the total number of fishes examined (Hynes, 1950). The dearth of the quantities of the food items present in the stomach should be eliminated (Zacharia & Abdurahiman, 2004), but the dominance method gives substantially the same result as in the occurrence method. It is applicable only to count food occurring in discrete units when the dominance is derived from numbers. Therefore, it is questionable if it makes sense to use this method in practice. This method gives only a very rough picture of the dietary of a fish & the food items which are less dominant due to environmental reasons may escape attention. Therefore the dominance of particular item is calculated according to equation if used.

$$\text{Dominance of food, } D_i = \frac{N_{di}}{N} \times 100$$

Where: N_{di} is the number of fish in which food item i dominates

N is the number of fish examined

d) Points (Numerical) Method:

This method is an improvement on the number method where consideration is given to the bulk of the food items. Food items are classified as very common, common, frequent, rare, etc., based on rough counts & judgments by eye. Due importance is also given to the size of the food item during random classification & food contents of all stomachs are tabulated. Different classes are allotted a certain number of points & the summations of the points for each food item are reduced to percentages to show the percentage composition of diet.

2) Volumetric methods

Volume of food is considered as a more satisfactory method by many workers for quantitative analysis of gut contents. The volume of each food item or of the total food of each fish is given in this method. The volume forms a very suitable means of assessment especially in the case of herbivorous & mud feeding fishes where the numerical methods are inaccurate (Hynes, 1950). Even in cases where the numerical methods are appropriate, volume has been considered as an essential aspect to be quantified with, & in all improved numerical methods the volume of the food items is taken in to consideration in some way or other. The volume of specific food item is expressed as the individual food item volume percentage of the total volume of digestive tract contents.

$$\text{Percentage by volume, } \%V_i = \frac{V_i}{V_t} \times 100$$

Where: $\%V_i$ is the ratio of the food item i

V_i is the volume of food item i

V_t is the total volume of food (gut content)

a) Eye estimation method: - Eye estimation is probably the simplest & easiest way of ascertain the volume of food items with only little effort when comparing with other volumetric methods though it suffers from several weaknesses. This method of analysis is subjective in nature & the investigators personal bias is likely to influence the results greatly. This limitation can be minimised by experience/training gained by the analysis of large samples & repeated evaluation of estimated values in the same sample. Eye estimation is a substitute approach to the numerical method when analysing diet with food items *viz.,* plant material & debris which cannot be counted.

b) Points (Volumetric) method: - Points method is a contrast to the eye estimation method. Instead of direct estimation of the volume by sight as in the former method, each food content in the stomach is allotted a certain number of points based on its volume. While allotment of points both the length of the fish & the fullness of the stomach are taken into account by certain workers. Total 16 points are given to the highest volume of the diet component & every other constituent is awarded 16, 8, 4, 2, 1 & 0 points depending on the volume relative to the component with the highest volume. Percentage volumes within each subsample were calculated as:

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

Where: α is the percentage volume of the prey component α

Point's method is more convenient for analyzing herbivores & omnivorous fish diet, where measuring volumes of microscopic organisms such as diatoms & filamentous algae are very difficult.

c) Displacement method:

This method is probably the most accurate one to estimate the volume. The volume of each food item, or of the total food of each fish is expressed as a percentage of the total weight of the fish. The volume of each food content is measured by displacement of water in a graduated container such as a cylinder with the smallest possible diameter for accuracy & could be used for calculation of these ratios (Hynes, 1950). The displaced volume of water is equal to that of the food item. Alternately, volume of the food contents may be measured by allowing them to settle in a graduated measuring cylinder (Hyslop, 1980).

Displacement method is eminently suited in the estimation of the food contents of carnivorous fishes eating larger preys rather than for small/ rare occurring food & the differential rate of digestion of the food items may sometimes affect the accuracy of the observations. However, if fishes caught immediately after feed on, this complication can be overcome. A knowledge of the volumes of the different size groups of the food items may be of great help in estimating of the volume if the whole item is created by semi-digested fragments (Zacharia, 2017). Other problems *viz.,* water trapped within the food may cause large errors in the estimate, food items may change their volume differently in preservation media & presence of large volumes of mucus in some species could make this method more difficult (Baker *et al.,* 2014).

3. Gravimetric method

The gravimetric method consists of the estimation of the mass of each food item, or of the total food content of each fish, which is usually expressed as a percentage of the total weight of the fish as in other quantitative methods. Gut content may be expressed as wet, dry or ash-free dry weight (Hyslop, 1980). The wet weight of the food is measured after removing excess water by blotting with tissue paper to diminish the prejudice caused by measuring food items with water trapped between the food pieces. Contrarily this issue could be evaded by measuring the dry mass of food in the gut content. Dry weight estimation is more time taking & is usually employed where accurate determinations of calorific intake is required. Dried food contents can be weighted when they are big enough to be handled individually & the accurate weighing of small quantities of food matter is extremely difficult & impracticable in studies of large collections (Bowen, 1996). Hence this method is generally employed only in conjunction with other methods to demonstrate seasonal variations in the intensity of feeding. Dry weight is estimated after drying to constant mass by oven-drying at 60 - 105°C for 48 hours. Food samples are cooled down in a vacuum desiccator & then weighed in case of very precise results are needed.

$$\text{Percentage by weight,} \quad \%W_i = \frac{W_i}{W_t} \times 100$$

Where: $\%W_i$ is the percentage of food item i

W_i is the weight of food item i

W_t is the total weight of food (gut content)

Table 1. Example of results obtained using different methods of estimation of stomach contents for two *Epinephelus diacanthus*

E. diacanthus 1 (Ed1). 1 *Nemipterus* spp., 6 cm long, weight 10g, volume 12 ml, 1 *Loligo* spp., 3 cm long, weight 8g, Volume 10ml, 9 *Acetes* sp. each 2.5 cm long, weight 350 mg each, Vol. 2ml.

E. diacanthus 2 (Ed2). 2 *Nemipterus* spp., 5 cm long, weight 8g each, volume 24 ml, 5 *Acetes* sp. each 2 cm long, weight 180 mg each, Vol. 1ml.

Food	Method	Fish			%	Total of which % expressed
		Ed1	Ed2	Total		
Nemipterus spp.		1	1	2	40	
Loligo spp.	Occurrence	1	0	1	20	All food occurrences
Acetes sp.		1	1	2	40	
Nemipterus spp.		1	2	3	16.7	
Loligo spp.	Numerical	1	0	1	5.57	All food organisms
Acetes sp.		9	5	14	7.8	

Nemipterus spp.		1	1	2	100	
Loligo spp.	Dominance	1	0	1	50	All fish
Acetes sp.		1	1	2	100	
Nemipterus spp.		12	24	36	73.5	
Loligo spp.	Total volume	10	0	10	20.4	Total food volume
Acetes sp.		2	1	3	6.1	
Nemipterus spp.		50	96	73	73	
Loligo spp.	% volume	41.7	0	20.9	20.9	Food volume
Acetes sp.		8.3	4	6.1	6.1	
Nemipterus spp.		10	16	26	68.5	
Loligo spp.	Gravimetric	8	0	8	211	Total weight of food
Acetes sp.		3.1	0.9	4	0.5	

Food analysis indices

A. Simple indices:

1. Index of fullness (ISF):

Index of fullness express the ratio of food weight to body weight. This index is extensively employed & it could be applied to the food in the stomach, or to that in the whole digestive tract. (The ratio of corresponding volume can also be used.) It is usually asserted as parts per 10,000 (%00, or parts per decimal) & calculated using formula:

$$\text{Index of Stomach Fullness, (ISF)}, = \frac{W_g}{W_f} \times 1000$$

Where: % W_g is the weight of the stomach contents (g)

W_f is fish body weight

2. Index of Preference, Index of Selection, Forage ratio (FR):

Most of the fishes have a degree of selection for the food organisms available in their habitat, so that some are consumed large in numbers, others moderately, some not at all. A quantitative index of such variances called as the forage ratio. The forage ratio developed by Savage (1931) used the percentage of quantity of food item i in the gut as a percentage of the total gut content & the relative quantity of the same food item in the environment as a proportion (percentage) of the total abundance of available food in the habitat. The lower limit for this index is 0 & upper limit is indefinitely large.

$$\text{Forage ratio,} \quad FR_i = \frac{r_i}{p_i}$$

Where: r_i is the percentage weight of the food item i in the stomach

p_i is the percentage weight of the food item i in the habitat

3. Index of electivity (E), Index of selection, Ivlev's Forage Ratio:

The Electivity index proposed by Ivlev (1961) is slightly different quantitative measure of selection which has been widely used in comparing the feeding habits of fishes. This index uses the relative abundance of food item i in the stomach as a percentage of the total gut content & the relative abundance of the same food item in the habitat as a proportion (percentage) of the total abundance of available food in the habitat. Electivity index was developed to describe the electivity as a degree of selection of particular prey species by predator studied & to avoid the weakness of forage ratio (FR) resulting from the 0 to infinity range. The index has possible range from -1 to +1. Negative values indicated as avoidance or inaccessibility of the food item, zero representing random selection form the environment & positive values indicate active selection.

Ivlev's index of electivity, $$E_i = \frac{r_i - p_i}{r_i + p_i}$$

Where: r_i is the percentage weight of the food item i in the stomach

p_i is the percentage weight of the food item i in the habitat

Though the Electivity index initially assumed as unbiased & relatively independent of sampling size were later, after empirical & theoretical re-evaluations declared as invalid by Strauss (1979). He revealed similar to FR this index also significantly biased when the size samples from the stomach & from the environment are unequal, it is dependent upon sample size (both relative & absolute) & is also not useful for food item not dominant in the environment. This flaw will influence the results concerning rare food items, even though large number of samples are analysed (Lechowicz, 1982). Another issue is the extreme values *ie.,* -1 & +1. The -1 value (total avoidance) can be obtained only in such case, when the food item does not occur in the fish stomach, but occurs in the environment irrespective of how scare or abundant it is. In contrast +1 (the maximal positive selection) can be obtained only in the case when the food item do not occur in the environment, but occurs in the fish stomach, irrespective of how large or small is its proportion (Straus, 1979).

B. Compound indices:

Food content analysis can give us data which helps to resolve more complex questions of fish ecology. This vital information provides in-depth insight to the fish feeding ecology, resources availability & demands, potential competition & other aspects of fish ecology & biology, consumption or predation (Liao *et al.,* 2001). Individual food content provide unique information about relative importance of particular food. They also offer the possibility to express the ratio of individual food item in the diet & some authors employ the percentage by number (%N), weight (%W), volume (%V), & occurrence (%O) to express the relative importance of prey items. %W (or %V) has been the most accepted index among others, to describe prey importance & its relationships with fish well-being & food availability (Hartman & Brandt, 1995; Persson & Hansson, 1999). However many others opined these information do not always indicate the real importance of particular food *viz.,* from the nutritional value point of view. Therefore In an attempt to receive more complex & objective information, & to avoid information loss, researchers developed combine two or more measures into a single index with a belief is that compound indices capture more information than do single component measures (Cortes, 1997; Chipps & Garvey, 2007; Gelwick & Matthews, 2006).

1. Index of Preponderance:

The index developed by Natarajan & Jhingran (1961) gives a single value for each attribute based on frequency of occurrence & bulk of various food items. Index of Preponderance provides a definite & measurable basis of grading the various food elements. The bulk of food items could be assessed by 1. Numerical 2. Volumetric & 3. Gravimetric methods. As the numerical method is biased with the frequency of occurrence, since it magnifies the importance of smaller organisms which may appear in enormous numbers, either volumetric or gravimetric are best to measure the food items quantitatively.

$$\text{Index of preponderance, } IP_i = \frac{V_i O_i}{\text{“}V_i O_i} \times 100$$

Where: V_i is percentage of the volume of food item i

O_i is the percentage of occurrence of given food item i

A comparison of the values obtained permits a ranking of the food items in order of mathematical dominance as an expression of the importance within the diet & authors of Index of Preponderance are convinced it has enormous advantages particularly when studying fish diet in open waters where animals have ingress to various organisms (Mohan & Sankaran, 1988). They also consider it to be an objective & suitable measure of food dominance within the diet. On the other side, the Index of Preponderance technique does not discriminate between the importance of food items by weight or occurrence & it is not suitable for dietary comparisons (Marshall & Elliot, 1997).

Table 2. Example of the Index of Preponderance of food items of *Catla catla* with rankings in brackets

Food items	Percentage of Occurrence (O_i)	Percentage of Volume (V_i)	$V_i O_i$	$\frac{V_i O_i}{\Sigma V_i O_i} \times 100$
Algae	30.5	25.2	768.6	37.13 (II)
Crustaceans	21.8	50.5	1100.9	53.17 (I)
Plants	10.5	13.2	138.6	6.69 (III)
Rotifers	6.5	1.6	10.4	0.50 (VI)
Insects	4.2	7.2	30.4	1.47 (IV)
Detritus	7.3	0.9	6.57	0.32 (VII)
Protozoa	1.2	0.6	0.72	0.03 (VIII)
Molluscs	-	-	-	-
Sand & mud	18	0.8	14.4	0.69 (V)
Σ	**100**	**100**	**2070.43**	**100**

As per the results of the Index of Preponderance, crustaceans & Algae comprise 1st & 2nd ranks in *Catla catla*. Followed by plants, insects, rotifers & other food items. Accidental & incidental inclusion of sand, mud etc. may be left out of consideration while grading the food items.

2. Index of Relative Importance (IRI):

This index is widely used in fish diet studies. While calculating IRI, the percentage of frequency of occurrence of each food item (%O) is multiplied by the sum of the percentage by volume (%V), or weight (%W) & percentage by number (%N) to evaluate the relationship of the various food items found in the stomach (Pinkas *et al.,* 1971). IRI is a composite index employed to describe fish diets & ascertain the relative importance of common food categories (Pinkas *et al.,* 1971; Prince, 1975). The three standard dietary measures are used to compute the IRI as follows:

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

or

$IRI_i = (\%N_i + \%W_i)\ \%O_i$

Where: $\% N_i$ is the percentage of specific food category by number

$\% V_i$ is the percentage by volume

$\% O_i$ is the frequency of occurrence

% Wi is the percentage by weight

Table 3. Example of the Index of relative importance of food items of *Priacanthus hamrur* with rankings in brackets

Food items	$\% N_i$	$\%W_i$	$\%O_i$	$(\%N_i + \%W_i)\ \%O_i$	% IRI
Nemipterus spp.	10.50	28.12	9.87	381.18	5.65 (II)
Acetes sp.	65.2	27.39	63.50	5879.47	87.22 (I)
Cynoglossus sp.	6.30	14.58	9.20	192.10	2.85 (III)
Loligo spp.	4.70	10.59	6.15	94.03	1.39 (V)
Shrimps	4.80	6.80	4.53	52.55	0.78 (VI)
Bregmaceros sp.	8.50	12.52	6.75	141.89	2.11 (IV)
Σ	**100**	**100**	**100**	**6736.33**	**100**

According to the Index of relative importance *Acetes* sp. formed the bulk of the food. Followed by *Nemipterus* spp. & *Cynoglossus* sp. formed 2nd & 3rd most important pry. Though *Bregmaceros* sp. (8.5% N) ingested more than *Cynoglossus* sp. (6.3% N), ranked 4th in prey importance (2.11% (IRI). *Loligo* spp. & other shrimps were the least preferred prey formed only 1.39% (IRI) 0.78% & (IRI) importance.

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