A Suggested Method for Quantifying Gut Contents in Herbivorous Fishes

Robert S. Jones

Division of Biosciences and Marine Studies
University of Guam

Review of the literature (general reviews of methods of stomach analyses are found in Hynes, 1950 and Pillay, 1953) has revealed that quantitative analysis of gut contents of animals feeding on multicellular benthic algae is virtually unknown. Even when readily identifiable, the algae are not easily separated for quantitative determinations. Some of the methods commonly found in other works follow:

1. A generic or specific list of the algal components found in a series of stomachs from a fish species may be given. This is probably the least useful but most frequent method employed. A few filaments of one alga appearing in only one or two stomachs would be given equal weight with an alga found in great abundance in all stomachs.

2. The percent of "filamentous algae" in a stomach sample may be visually approximated. This method is useful in so far as one can determine whether or not a fish is strictly a herbivore or to what degree it is dependent upon plant material for food. The disadvantage of this method is the failure to separate the algal food material into component taxa.

3. The frequency of occurrence of each algal taxon in a series of fish stomachs may also be estimated. A certain amount of reliable information may be gained from this, but all too often a diminutive alga may appear in 100 percent of the fish and yet represent only a small biomass, perhaps having no nutritive significance at all.

Attempts to find a more precise method than those given above have proven difficult. Physical separation of the various algal taxa is virtually impossible due to their frequent microscopic size.

A direct filament count was considered to be most feasible. A fragment of a multicellular alga is not as clearly defined a unit as is a small animal swallowed intact by a carnivore. However, it is a reasonable assumption that an entire thallus need not be ingested for it to be considered significant enough to be counted. Hence, each algal filament or fragment bitten off by a fish may be viewed as a single countable unit for measuring abundance. An additional complication is encountered here in fishes because there is often some pharyngeal mastication that further divides an algal filament into smaller pieces. In this respect, genera such as the "jointed" *Jania* and the easily fragmented *Lyngbya* would provide incorrect counts. Finally, the morphological difference in size of the various algae makes a one-to-one count of a piece of *Pocockiella* (large) to a piece of *Lyngbya* (small) somewhat ludicrous in terms of biomass. A direct count of algal filaments may therefore be virtually meaningless.

As a compromise the following procedure is suggested: The esophagus and stomach to the region of the pyloric caeca is removed from each fish. The excised portion of the gut is split open and the contents washed into a petri dish. This material is thoroughly mixed with tap water and all clumps broken up with a dissecting needle. Three drops of this slurry are taken from three different parts of the petri dish and each drop is placed on a glass slide. The material is partially spread with a dissecting needle and is additionally spread in placing the coverglass. The homogeneity of the samples can be questioned, but the random manner of preparation is considered relatively free from bias. Therefore, the ratio of algal taxa on the slides is believed to be representative of the ratio of algal taxa in the stomach. Results from preparations done in triplicate are found to be more reliable than those obtained from a single slide preparation. Estimated error is about 5-10 per cent

Each of the three slides is examined under the low power of a compound microscope equipped with a 1 mm ocular grid. The slides are manipulated with a stage micrometer. Nine areas on each slide are studied (Fig. 1 a). The grid is positioned over each one of these points. The grid itself has nine vertical and nine horizontal lines each 0.1 mm apart. The central vertical and horizontal lines each make eight points of intersection with the lines in the opposite plane plus one intersection with each other (Fig. 1 b). This results in 17 points of intersection. If an alga falls on any one or more of these points the number of intersections is counted and recorded for that genus.

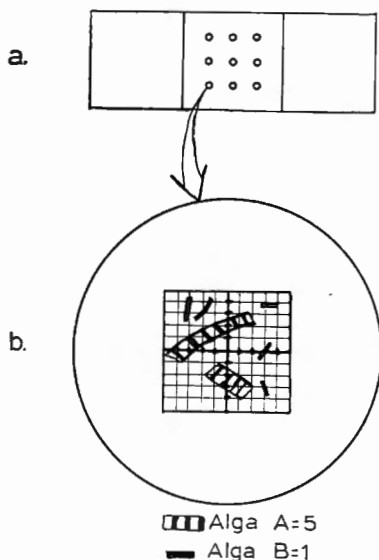Thus there are 17 points of intersection per grid counted, nine grids per slide

Fig. 1. Stomach Analysis Technique.
    a. Microscope slide and coverglass with sampling points shown as circles.
    b. One of the sampling points enlarged to show the ocular grid and method of counting.

and 27 grids in three slides. The result is a total of 459 points of intersection examined per fish. The principle behind the technique is similar to determining area under a grid by square counting. This method considers the cross sectional area occupied by each algal genus. The more points that are considered, the more accurate the count. Figure 1 b shows a hypothetical example of this technique.

The following relationship may be calculated from these counts:

$$\frac{\text{\# intersections with alga A}}{\text{intersections with all algae}} = \text{Abundance of alga A in the fish stomach relative to the other algae}$$

$$\text{Abundance} \times 100 = \text{Relative Abundance of A}$$

The advantage of the direct count method is that it allows for agreement between two researchers using the same material. Though some variability is to be expected from preparation of the slides, using three slides increases the probability of having representative material. The data obtained are relative and not based directly on the total biomass of each alga in the stomach.

Literature Cited

Hynes, H. B. N. 1950. The food of freshwater sticklebacks (*Gastrerosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. J. Anim. Ecol. 19:36–58.

Pillay, T. V. R. 1953. A critique of the methods of study of food of fishes. J. Zool. Soc. India 4(1952):185–200.

. . .