Feeding studies take guts – critical review and recommendations of methods for stomach contents analysis in fish

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Funding information
The work was supported financially by The Research Council of Norway (213610).

Abstract
Studies on the feeding ecology of fish are essential for exploring and contrasting trophic interactions and population and community dynamics within and among aquatic ecosystems. In this respect, many different methods have been adopted for the analysis of fish stomach contents. No consensus has, however, been reached for a standardised methodology despite that for several decades there has been an ongoing debate about which methodical approaches that should be preferred. Here, we critically review and scrutinise methods, addressing their strengths and weaknesses and emphasising inherent problems and possible pitfalls in their use. Although our critical assessment reveals that no completely ideal approach exists, appropriate and reliable procedures can be adopted through careful considerations and implementation. In particular, we advocate that different objectives require different methodical approaches and the choice of method should therefore be closely linked to the research questions that are addressed. For a standardisation of methods, we recommend a combination of the relative-fullness and presence–absence methods as the optimal approach for the commonly applied feeding studies addressing relative dietary composition in terms of prey diversity and abundance. Additionally, we recommend the gravimetric method for objectives related to the quantification of food consumption rates and the numerical method for prey selection studies. DNA-based dietary analysis provides a new and promising complementary approach to visual examination of stomach contents, although some technical challenges still exist. The suggested method standardisation facilitates comparisons across species, ecosystems and time and will enhance the applicability and benefits of fish feeding studies in trophic ecology research.

KEYWORDS
diet, food, foraging, method standardisation, prey consumption, trophic ecology
1 | INTRODUCTION

Food acquisition is crucial for heterotrophic organisms, providing energy for life maintenance, somatic growth and reproduction and constituting a key element in food-web ecology and species interactions like predation and resource competition. Hence, studies of the feeding ecology of animals are essential for understanding trophic interrelations and population and community dynamics within an ecosystem and for comparisons among systems. Stomach contents analyses are instrumental in this respect. Ever since trophic ecology theory was introduced in the early 1940s (Lindeman, 1942), much attention has been paid to the development of suitable methods to enable scientific progress in this field (Baker et al., 2014; Hynes, 1950; Hyslop, 1980 for fish; Pierce & Boyle, 1991 for marine mammals; Birkhofer et al., 2017 for invertebrates). In studies of aquatic animals such as fish, the possibilities for direct observation of feeding behaviour and prey choice are limited. Stomach contents analysis is therefore an important and universal means for detailed exploration of diet composition and feeding ecology. Fish are particularly benign organisms for dietary studies from stomach contents analysis as they can often be sampled in high numbers, usually swallow their prey whole and mostly have a well-defined stomach. Fish typically have key roles as consumers and top predators in the trophic network of aquatic ecosystems and their trophic ecology is an important parameter in most species descriptions (FishBase; Froese & Pauly, 2017). Consequently, a vast number of publications exist addressing aspects of fish feeding ecology from the analysis of stomach contents data (Braga et al., 2012; Simenstad & Cailliet, 2017) and many methods have been adopted in this respect (Baker et al., 2014; Hyslop, 1980; Manko, 2016). Over time a number of publications have addressed methodical approaches used in fish feeding studies (e.g., Ahlbeck et al., 2012; Baker et al., 2014; Berg, 1979; Buckland et al., 2017; Cortés, 1997; Hynes, 1950; Manko, 2016; Windell & Bowen, 1978), including a few method reviews that have become key references for stomach contents studies, in particular Hyslop (1980). However, despite these efforts, a consensus for a standardised methodology for stomach contents analysis has yet not been reached (Baker et al., 2014; Buckland et al., 2017).

The lack of methodical consensus may chiefly be ascribed to the existence of several problems and challenges associated with the available methods for stomach contents analysis, which eventually may bias the outcome of such studies. An inherent problem with any method is that the contribution of slowly digested prey taxa will tend to be overestimated. More specifically, differential gastric evacuation rates of prey taxa due to, for example, differences in fat contents (Amundsen & Klemetsen, 1988; Elliott, 1972, 1991; Persson, 1979, 1981), energy levels (Jobling, 1980, 1987), or food particle sizes (Jobling, 1987; Legler et al., 2010) will lead to an overestimation of slowly digested prey, since these will tend to remain in the stomach for an extended time period (Baker et al., 2014; Hyslop, 1980). Furthermore, some indigestible remains, in particular hard body parts such as fish otoliths, crayfish gastroliths, chitinous head capsules of insects, mollusc shells and other exoskeleton or skeleton parts, are much easier to identify than the remains of softer prey types. Such indigestible remains may be preferentially retained in the stomach for prolonged periods of several weeks (dos Santos & Jobling, 1991; Jørgensen & Jobling, 1988) and their dietary role will inevitably tend to be overestimated in stomach contents analysis. On the other hand, digestion and fragmentation of prey, often combined with extensive mucus formation, can make the taxonomical identification difficult (Buckland et al., 2017). For the same reasons, it is usually difficult and highly time consuming to separate and handle prey items for mass or volume measurements, for example (Baker et al., 2014; Buckland et al., 2017), which are required by some methods (Hyslop, 1980). Typically, the potential problems and challenges for stomach contents analysis are of different importance among the various methodological approaches that are available, an aspect that needs careful consideration in the evaluation of different methods. Some methods are also far more time consuming than others, imposing important cost–benefit trade-offs that need to be considered in any method assessment.

Here, we provide a summary review and evaluation of available methods for stomach contents analysis, addressing their strengths and weaknesses and emphasising inherent problems and possible pitfalls in their use. In our assessment and recommendation of the methods, we advocate that different objectives require different methodical approaches. The choice of optimal methods for stomach contents analysis should therefore be closely linked to the key research questions that are addressed, an aspect that has been given modest attention in earlier reviews. The main goal of the present study is to summarise and scrutinise available methods and provide recommendations for a standardisation of optimal methods to be adopted for the main research objectives of fish feeding studies.

2 | KEY OBJECTIVES FOR STOMACH-CONTENT ANALYSES

Most of our current understanding of trophic ecology and interactions of fish populations comes from dietary studies based on the analysis of stomach contents, providing important information about many facets of the biology and ecology of fishes and aquatic ecosystems (Braga et al., 2012; Manko, 2016). Three main types of objectives can be identified from such studies, primarily being related to quantification of (1) relative diet composition; (2) prey selectivity; and (3) food consumption rate. The quantification of the relative diet composition (i.e., which prey taxa are utilised and how much does each prey type contribute to the overall diet) is the prime objective for most feeding studies of fish (Hyslop, 1980; Manko, 2016). Such studies provide essential information about the trophic ecology and dietary niche utilisation of the target species, including aspects such as basic feeding habits, resource utilisation and seasonal and ontogenetic diet shifts (Gerking, 1994; Sánchez-Hernández et al., 2019; Ward et al., 2006). Knowledge about these trophic relationships are important in order to understand the biology of a fish species and its ecological role in the aquatic system (Braga et al., 2012), including predator–prey
interactions and intra and interspecific competition which again are decisive for food-web topology and community dynamics.

Two variables are typically estimated for diet characterisations based on stomach contents analysis: frequency of occurrence and relative prey abundance (Amundsen et al., 1996; Hynes, 1950; Hyslop, 1980). The frequency of occurrence is defined as the number of stomachs in which each specific prey type is represented, expressed as a frequency (or percentage) of the total number of stomachs with prey. The relative prey abundance (or contribution to the overall diet) is defined as the proportion (or percentage) of the total number of stomachs with prey. The relative prey abundance is typically used for estimation of dietary niche overlap using indices such as Renkonen similarity or Schoener overlap (Colwell & Futuyma, 1971; Krebs, 1999; Schoener, 1979): 

\[ R_{ij} = p_i / m_i, \]  

or Ivlev’s (1961) electivity index: 

\[ E_i = (p_i - m_i) / (p_i + m_i), \]  

where \( p_i \) and \( m_i \) is the number of predators with prey type \( i \) in their stomach, \( m \) is the total number of predators with stomach contents, \( S_i \) the amount of stomach contents composed by prey type \( i \) and \( S \) the total amount of all prey types present in each stomach of the sample. The frequency of occurrence and in particular the relative abundance of prey (or a combination of both) are frequently used for quantitative description or graphical illustrations of the diet (Amundsen & Klemetsen, 1988; Greenwell et al., 2018; Hyslop, 1980; Lek et al., 2018). The relative prey abundance is the variable typically used to provide estimates of dietary niche width as calculated by for example Levins’ B (Levins, 1968) or Shannon’s H (Shannon, 1948) indices, where \( B = 1 / \Sigma p_i \) and \( H = - \Sigma p_i \log(p_i) \).

Similarly, prey abundance is also typically used for estimation of dietary niche overlap using indices such as Renkonen similarity or Schoener overlap (Colwell & Futuyma, 1971; Krebs, 1999; Schoener, 1970), which are equivalent measures most simply calculated as: 

\[ O_{jk} = \Sigma \text{minimum } p_{jk}, \]  

where \( O_{jk} \) is the dietary niche overlap between species \( j \) and \( k \) and \( p_{jk} \) and \( p_{kj} \) are the relative prey abundance of prey type \( i \) in the stomachs of the respective species. The overlap is considered biological significant when the index value exceeds 0.60 (or 60%; Wallace, 1981). Several other niche-width and overlap or similarity indices are also available (Krebs, 1999), typically with the index calculation being based on the relative prey abundance as the dietary contribution variable. Prey abundance is also a key variable in Bolnick et al. (2002)’s methodical approach for quantifying individual specialisation and the within and between-individual components (WIC and BIC) to the total niche width of a population (TNW). For a graphical evaluation of feeding strategy and individual specialisation, Amundsen et al. (1996) introduced a new parameter, the specific prey abundance \( s_{ij} \), estimated by: 

\[ s_{ij} = \Sigma S_i / \Sigma T_i, \]  

where \( T_i \) is the total stomach contents of predators with prey \( i \) in their stomach. This parameter is closely linked to prey abundance and frequency of occurrence as the product of prey-specific abundance and frequency of occurrence equals prey abundance (Amundsen et al., 1996): 

\[ p_i = s_i f_i. \]

As evident from the examples above, quantification of the relative diet composition is key for a number of research questions. Another important objective for dietary studies of fish is related to prey preferences and selection by the target species, although this is less commonly addressed than dietary composition and relative prey importance. In addition to analysis of stomach contents, such studies require a detailed account of the relative availability of different prey species in the environment. Since most fish species consume their prey individual by individual, prey selectivity studies are usually based on carefully counting each prey organism both from the stomach and environmental samples. Several indices have been developed for the calculation of prey preferences from the proportion of each prey type \( i \) in the stomachs (\( p_i \)) v. in the environment (\( m_i \)), including single ratios such as the forage ratio (Edmondson & Winberg, 1971; Strauss, 1979): 

\[ R_{ij}^p = p_i / m_i, \]

or Ivlev’s (1961) electivity index: 

\[ E_i = (p_i - m_i) / (p_i + m_i), \]  

as well as other, more sophisticated approaches; for example Manly’s a (Chesson, 1978; Manly et al., 1972). Selectivity studies can also be expanded to contrast, for example, prey size between prey samples from the stomachs v. the environment (Rincón & Lobón-Cerviá, 1999; Sánchez-Hernández & Cobo, 2015).

Stomach-content analyses of fish are also adopted for bioenergetic studies through the estimation of food consumption rates. Such studies are typically using stomach-content mass and estimates of gastric evacuation rates to calculate the daily food consumption rate, as exemplified by the Baikov/Eggers method (Eggers, 1977, 1979): 

\[ C_{24} = 24 \cdot S \cdot R, \]  

where \( S_{24} \) is the daily consumption rate, \( S \) the mean mass of stomach contents and \( R \) the instantaneous gastric evacuation rate (Amundsen et al., 2007; Amundsen & Klemetsen, 1986, 1988; Modica et al., 2014). Hence, stomach-content analyses of fish are used for highly different purposes and objectives, which call for different methodological approaches. Consequently, the choice of methods for stomach-content analysis requires careful considerations of which principle research questions that are addressed.

3 | METHODS FOR STOMACH-CONTENT ANALYSIS

The methods that are used for stomach-content analysis of fish comprise three main approaches: presence–absence, numerical and bulk, the latter including the gravimetric, mass reconstruction, volumetric, point and relative-fullness methods (Hyslop, 1980). More recently, DNA-based dietary analysis has also been adopted (Jakubavičiūtė et al., 2017; Kress et al., 2015; Valentini et al., 2009). The easiest way to assess the dietary composition of a fish population is to record the presence or absence of each prey taxon across all sampled individuals and express their frequency of occurrence in the stomachs as an indicator of prey importance. Other available approaches are typically more laborious and time consuming. The numerical approach is based on detailed counts of all individuals of each prey taxa represented in the stomachs, whereas the bulk approaches quantify the prey composition in terms of mass or volume. With the gravimetric method, each prey type in the stomach is weighed separately for a calculation of their relative contribution to the diet. The reconstruction method is also typically mass-based, but here the relative prey importance is deducted from a back-calculation of the original mass of each prey item present in the stomachs. With the volumetric method, the volume of each prey item or prey type is measured for calculation of their relative contribution to the stomach contents. The point and
relative fullness methods are also volumetric approaches, but these are based on a visual estimation of the relative prey composition in the stomachs, the former using a points system and the latter a percentage scale to score the contribution of each prey type. The use of DNA metabarcoding constitutes a promising addition to these traditional methods, although some technical challenges still exist.

3.1 | The presence–absence method

The presence–absence method (also referred to as the frequency of occurrence method) simply relies on the positive identification of a prey or an identifiable part of the prey to provide an accurate recording of which prey taxa are present in the diet, without taking the amount or relative contribution of each prey type into account. The number of stomachs in which each prey taxon occurs is expressed as a ratio of the total number of (usually the non-empty) stomachs examined, thereby providing an estimate of the frequency of occurrence for each prey type. The number of occurrences of all prey types may also be summed and scaled down to a percentage or ratio basis to provide an occurrence-based estimate of their relative importance (Hynes, 1950). The presence–absence method does not require any time-consuming measures and is thus relatively quick and easy to use and can be executed with far less effort and cost than most other, typically more laborious approaches. Baker et al. (2014) and Buckland et al. (2017) strongly recommended the use of the presence–absence method, arguing that it provides the most robust and interpretable measure of diet composition, in particular due to a number of problems identified with the use of the numerical and bulk methods for quantification of the gut contents. From model simulations and comparisons of several diet analysis methods, Ahlbeck et al. (2012) also found that this method performed surprisingly well despite its simplicity. However, they concluded that the presence–absence approach was in general more variable and less robust than the mass and points methods and resulted in a large overestimation of the contribution of small prey (Ahlbeck et al., 2012). The presence–absence method has similarly been criticised for providing no, or misleading indication of the relative amounts of the different prey types that have been eaten (Hyslop, 1980). The method further tends to exaggerate the importance of incidental prey and prey taxa that are slowly digested or resistant to digestion due to hard body parts (Pierce & Boyle, 1991). This problem is particularly large for prey types that have indigestible and easily identifiable remains that can be retained in the stomachs for extended periods (dos Santos & Jobling, 1991). Prey types that are subject to a generalised feeding strategy (i.e., have a high frequency of occurrence, but a low abundance in the stomachs; Amundsen et al., 1996), will similarly tend to be overestimated. Most importantly, however, the presence–absence method does not provide any estimate of the relative contribution or importance of the various prey types; estimates that are needed for the calculation of for example dietary breadth and trophic niche overlap between species or groups of fish and for addressing the significance of individual feeding specialisation in fish (Bolnick et al., 2002; Jirka & Kraft, 2017; Sánchez-Hernández & Cobo, 2018).

3.2 | The numerical method

The numerical method is based on the counting of all prey items present in the sampled stomachs. The total number of individuals of each prey type is recorded and expressed as a frequency of the total number of prey individuals of all food types (Hynes, 1950). The method requires that prey items can easily be identified and counted; as for example, when prey individuals are not heavily fragmented during digestion or they have resistant and easy identifiable body parts such as head capsules, carapaces or shell. Under most circumstances the numerical approach is highly laborious, but the method may still work reasonably well for piscivorous and benthivorous fish species utilising relatively large prey specimens (Ahlbeck et al., 2012). The method is particularly suitable when the utilised prey taxa are of similar size and has typically been applied in prey selectivity studies, where compositional data from counts of prey individuals in stomach and environmental samples are contrasted (Amacker & Alford, 2017; Lazzaro, 1987; Worischka et al., 2015). However, the method faces serious problems if the food does not appear in discrete units (e.g., detritus or pieces of plant material) or if some of the prey types are easily digested or fragmented (Ahlbeck et al., 2012; Baker et al., 2014; Legler et al., 2010; Scharf et al., 1997). Moreover, the method can give rather meaningless outcomes if the predator is feeding on prey types of highly different body sizes, such as a combination of zooplankton and fish or large invertebrate prey, as numerical estimates under such situations strongly will overemphasise the importance of small prey items taken in large numbers (Hyslop, 1980).

3.3 | The gravimetric method

In gravimetric analysis of stomach contents, the mass of each prey type is measured in terms of wet or dry mass and the relative prey importance is expressed as a fraction of the total weight of all prey. As the actual mass measurements are highly accurate, the gravimetric method has been considered as the method with the highest precision of the estimated diet composition (Hyslop, 1980). However, high precision measurement does not necessarily reflect the true precision of the method. Firstly, it is often difficult to physically separate the various prey types with high accuracy, especially if the digestion process has come far. Furthermore, there is often much mucus present in the sampled stomachs, which can be difficult to separate from the prey items before weighing and thus may bias the mass measurements. Additionally, the method is laborious and time consuming, especially if there are many different prey types in each stomach that need to be separated and weighed. As with the other methods, the gravimetric method may overestimate the relative importance of slowly digested prey. However, if the evacuation rates for different prey types are available and taken into account, for example by the calculation of consumption rates, the relative contribution of each prey type may be more accurately estimated, but this is again a highly time-consuming process if all different prey types should be addressed.
separately. Hence, this approach is most suitable when quantification of food and energy consumption is the key objective and the various prey taxa only need to be separated among the typically few categories that show differential digestion rates; i.e., fish prey and prey with high fat or energy contents relative to most other prey (Amundsen & Klemetsen, 1988; Gillum et al., 2012; Jobling, 1980; Persson, 1979).

3.4 | The reconstruction method

With the reconstruction method, the relative prey importance and dietary composition are estimated from a back-calculation of the original mass of each prey item based on identification and size measurements of undigested body remains in the stomachs (Hartman & Brandt, 1995; Overton et al., 2009; Scharf et al., 1997). For each prey taxon, the reconstructed masses are estimated from the lengths of undigested parts of the consumed organisms, using pre-established size relationships of these body parts relative to the whole body of the organism (Alonso et al., 2002; Hartman & Brandt, 1995; Overton et al., 2009). Such datasets are often not available and the implementation of this method may therefore require large efforts. For instance, to provide accurate prey-size relationships, it is for each prey taxon necessary to make systematic measurements on a number of specimens of different sizes (Manko, 2016; Pope et al., 2001). Despite these highly laborious requirements, the reconstruction method has been recommended and advocated as the most robust and reliable approach to quantify the relative composition of the diet in terms of biomass or energy flow (Baker et al., 2014; Buckland et al., 2017). However, we dispute this conclusion, arguing that there are some severe pitfalls and biases in the use of the reconstruction method. As earlier emphasised, all available methods may to some, usually moderate, extent overestimate the role of slowly digested prey types. For the reconstruction approach, in contrast, there will typically be a chief overestimation of slowly digested prey as this effect is multiplied during the procedure with back-calculations to the original mass of the prey. This bias is especially large when the back-calculation procedure is based on small indigestible prey remains, such as a fish otolith or the head capsule of an insect, from which the original prey mass easily may be scaled up 1–3 orders of magnitudes. As pointed out earlier, such indigestible remains may be retained in the stomachs for extended periods of time (dos Santos & Jobling, 1991; Jørgensen & Jobling, 1988). As a consequence, the mere presence of such remains in the stomachs can lead to an overestimation of their importance with any available method, but through back-calculation to the original size of the prey using the reconstruction method (e.g., from a small otolith remain to the fully sized fish prey), this overestimation will easily be taken completely out of proportion and lead to profound bias.

3.5 | The volumetric method

The volumetric method is similar to the gravimetric method in that it provides a measurement of the bulk of prey, but in terms of volume rather than weight. Different means have been used to measure the volume of each prey taxon present in the stomachs, with the displacement technique being the most commonly applied approach (Hyslop, 1980; Manko, 2016). The volumetric method is most useful when the prey items are large-sized (e.g., fish prey), but in contrast hardly processable for small prey types like zooplankton. The method also suffers from similar challenges as the gravimetric and numerical methods in the practical execution, such as difficulties in separating the various prey types and problems with mucus formation in highly digested samples. When carefully applied under suitable circumstances the method may provide robust measurements, but it is typically laborious and time-consuming (Buckland et al., 2017; Jobling et al., 2001) and will therefore rarely constitute a cost-efficient alternative.

3.6 | The point method

The labour and time consumption involved in processing large amounts of material using the numerical, gravimetric or volumetric methods have led to the development of techniques for subjective estimation of the dietary composition, such as the point and relative-fullness methods (Hynes, 1950; Hyslop, 1980). The point method, originally proposed by Swynnerton and Worthington (1940), ranks the prey in the stomachs by means of a subjective scale (common, rare, etc.), where each food category is awarded points proportional to their estimated contribution to the stomach volume. Hynes (1950) suggested some modifications and practical adjustments in respect to the allocation of points and further advocated taking the stomach fullness into account as suggested by Frost (1943). After a consideration of various occurrence, numerical, gravimetric and volumetric methods, Hynes concluded that the point method was the most satisfactory approach being rapid and easy with few shortcomings and without the unwarranted impression of accuracy given by for example the numerical and gravimetric methods (Hynes, 1950). Based on these recommendations, the point method has commonly been adopted in fish feeding studies and Ahlbeck et al. (2012) concluded from their modelling evaluations that the point and the mass methods were most robust and produced diet compositions most similar to the true diet. Severe criticism and objections to the point method have however also been raised, the main concern being that the method is subjective and approximate relative to the numerical and gravimetric approaches (Hyslop, 1980; Windell & Bowen, 1978). Marrero and Lopez-Rojas (1995) similarly showed from a simulation test using several analysers that the outcome of the point method varied considerably. From the same test, they found that the percentage method (i.e., the relative-fullness method) provided more consistent results than the point method (Marrero & Lopez-Rojas, 1995), as the percentage approach is quantitatively more intuitive and easier to interpret than the point method.

3.7 | The relative-fullness method

The relative-fullness method resembles the point method but the relative prey contribution in the stomach is visually scored in percentage rather than in points. With this method, the total fullness of all
stomach contents is first visually assessed and usually expressed on a scale from empty (0%) to full (100%). The fullness contribution of each prey category is then assigned summing up to the total stomach fullness. The practical execution of this method is simple and fast relative to other approaches (Hyslop, 1980). As with the point method, the relative fullness method has been criticised for being highly subjective (Baker et al., 2014; Windell & Bowen, 1978), as the visual estimation of the bulk of food does not have the measurement precision of the gravimetric and volumetric methods. However, to our knowledge, this consideration and the consistency of diet estimates resulting from the relative-fullness method have not been further scrutinised and tested. We therefore performed a series of blind tests contrasting dietary composition outcomes from analyses of the same stomach-content materials by different analysers, including both experienced and quite inexperienced personnel. As it turns out, no significant differences were found between the outcome of the various examiners in any of the blind-test scenarios (Figure 1), demonstrating a high consistency and reliability of this method. Combined with the ease with which this approach allows for efficient processing of large sample sizes, this strong consistency makes the relative-fullness method highly reliable and appropriate for stomach-content analysis of the relative prey composition, as illustrated by the outcome of the blind tests (Figure 1). Manko (2016) similarly pointed out that when strictly defined and rigidly enforced, this method will provide results with a high accuracy as for example demonstrated for terrestrial herbivores (Sparks & Malechek, 1968).

3.8 | DNA-based diet analyses

Dietary studies using DNA metabarcoding is rapidly proliferating, opening new techniques for the exploration of trophic interactions and population and community dynamics (Jakubavičiūtė et al., 2017; Kress et al., 2015; Valentini et al., 2009). Hence, DNA-based analysis of stomach contents is a promising approach, especially from the possibility of providing a high taxonomic resolution of prey (Jakubavičiūtė et al., 2017; Riccioni et al., 2018). However, there are still some shortcomings of this approach. One handicap of DNA-based diet analysis is linked to the fact that sequence data for many prey groups are currently unavailable in public gene databases, resulting in imprecise identification of prey taxa (Harms-Tuohy et al., 2016; Jakubavičiūtė et al., 2017; Sakaguchi et al., 2017). However, the availability of such data is rapidly increasing, thereby enhancing the usefulness of this approach. DNA degradation during digestion is another limiting factor for DNA barcoding of stomach contents (Kress et al., 2015; Sakaguchi et al., 2017). Other constraints relate to the situation that the results mainly are limited to species richness and frequency of occurrence of prey due to the semi-quantitative nature of the metabarcoding

FIGURE 1 | Blind tests of stomach contents analysis using the relative-fullness method and expressing the dietary composition in terms of prey abundance (%). Comparisons of the result outputs of (a) two experienced examiners (A and B; experience from analysing >> 1000 stomachs) analysing the same stomach contents samples from Arctic charr Salvelinus alpinus and (b), (c) an inexperienced examiner (C1; basic training but no further experience with stomach contents analysis), the same examiner re-doing the analysis c. 1 year later (C2; modest experience from analysing >500 stomachs), and an highly experienced examiner (D; experience with analysing >> 1000 stomachs) analysing the same stomach contents samples from Arctic charr (b) and brown trout Salmo trutta L. (c). The similarity between examiners has been contrasted by the Renkonen–Schoener similarity index (%) and was always highly significant with similarity index values consistently >87%: (a) A v. B = 94.8%; (b) C1 v. C2 = 91.1%, C1 v. D = 90.3%, C2 v. D = 87.7%; (c) C1 v. C2 = 91.5%, C1 v. D = 90.0%, C2 v. D = 88.0%. Similarly, a statistical testing of the pairwise blind tests using the Kolmogorov–Smirnov test revealed no significant differences (P > 0.05). A, □ B, □ C1, □ C2 and □ D.
analysis of stomach contents (Harms-Tuohy et al., 2016; Riccioni et al., 2018). Secondary prey (i.e., prey of the primary prey) may also be detected from the barcoding, resulting in an overestimation of prey richness (Harms-Tuohy et al., 2016; Sakaguchi et al., 2017). Nevertheless, DNA metabarcoding provides a new and promising complementary approach to traditional morphological methods and the combination of visual and metabarcoding analyses of stomach contents will likely prove to be a powerful tool (Jakubavičiūtė et al., 2017; Riccioni et al., 2018). For fluid-feeding parasitic fishes like such as catfishes in the family Trichomycteridae with mucophagic, lepidophagic or hematophagic diets that cannot be visually identified, the molecular approach is a particularly useful alternative (Bonato et al., 2018).

4 | COMPARISON AND CHOICE OF METHODS

The presence–absence method is the simplest and least laborious and time-consuming approach for stomach-content analysis (Baker et al., 2014; Buckland et al., 2017). The strength of this method is related to the cost-efficient documentation of the diversity and frequency of occurrence of prey utilised by the predator population. However, the method also faces some shortcomings that make the sole use of this approach less suitable than credited by Baker et al. (2014) and Buckland et al. (2017). In particular, the presence–absence method fails to provide information about prey importance in terms of their relative contribution to the overall amount of food, which is a key objective for most feeding studies and a requirement for calculations of dietary indices; e.g., niche width and diet overlap. Hence, the inclusion of a numerical or bulk approach is required for most dietary studies.

The numerical method may under certain circumstances be a suitable option for quantification of relative prey importance, although in general this approach is too laborious to constitute a cost-efficient alternative. The method also suffers some serious shortcomings, such as when there are large size differences among prey, or some of the prey types are easily digested or fragmented. Nevertheless, for prey-selectivity studies, the numerical method will usually be the optimal approach as most fish species tend to select and consume their prey specimen by specimen and their feeding preferences may thus conveniently be scrutinised by contrasting the numerical composition of prey in the stomachs with similar compositional data from the environmental prey community. However, such data are not easily retrievable, especially when the predator is utilising different feeding habitats or consuming prey of highly different sizes, but for prey selection studies of planktivorous and benthivorous fish, for example, and their respective zooplankton and benthic prey communities, this approach has been successfully adopted in many studies (Amacker & Alford, 2017; Lazzaro, 1987; Sánchez-Hernández et al., 2011; Worischka et al., 2015).

For most feeding studies, the gravimetric method will typically be a more suitable approach than the numerical, as weighing of bulks of prey belonging to the same taxa generally is easier to perform than counting all individual prey. However, the weighing of each present prey type is also a tedious process and thus highly time consuming when a high diversity resolution is required. Moreover, even though the mass measurement is an accurate quantification, the actual precision may often be overrated as it is strongly influenced by any difficulties in physically separating the different prey items and taxa before weighing due to the effect of digestion, fragmentation and mucous formation (Baker et al., 2014; Hyslop, 1980). Hence, the gravimetric method appears to be most suitable when a high prey diversity resolution is less important (or can be achieved through the support of other methods). This is particularly the case when quantification of food consumption rate is the key objective rather than a detailed characterisation of the dietary composition and larger bulks or even all stomach contents thus can be jointly weighed for each fish.

The volumetric method suffers similar problems as the gravimetric method. In addition, the prey volume is generally more difficult to measure than the mass and is particularly difficult to apply for small-sized prey; e.g., zooplankton, or when the stomach contents are highly digested or fragmented. As with the numerical and gravimetric methods, the volumetric approach is very laborious and time-consuming and will therefore rarely constitute a cost-efficient alternative for stomach-content analysis. In essence, our considerations about the numerical, gravimetric and volumetric methods are in accordance with Swynnerton and Worthington (1940)’s consideration that, “Individual species comprising the food may be counted, or they may be weighed wet or dry, or their volume may be measured. The several processes take much time and it is doubtful how far results justify the labour except in special detailed work”.

The point and relative-fullness methods were developed to compensate for the tedium involved with the use of the numerical, gravimetric and volumetric methods (Hynes, 1950). Despite their subjective approach, these visually based methods can provide robust estimates of the relative diet composition when carefully executed (Ahlbeck et al., 2012). The relative-fullness method is particularly robust as the comparative assessment of the relative abundance of different prey types is rather intuitive and easy to execute, as firmly demonstrated by our method testing (Figure 1). Hence, the comparison and ranking of the different prey types within each stomach and the assessment of their relative contribution can be executed with a pertinent consistency and reliability relative to other bulk and numerical methods, especially taking their inherent problems and pitfalls into consideration. Similar as for all other available methods, the relative-fullness method is not flawless, particularly due to its subjective nature. Skillfully and carefully executed, however, the method has an adequate precision compared with other bulk approaches and emerges as the most time-efficient and useful approach for an estimation of the relative importance of the different prey types in stomach-content analysis in fish. More so, the execution of this method does not require much extra time relative to the presence-absence method, which, in practice, is also an implicit part of the relative-fullness approach. Hence, a combination of these two methods emerges as the optimal approach for stomach-content analysis in studies addressing dietary composition and relative prey abundance, which is the principal objective of most feeding studies of fish. Nonetheless, as for any new method.
implementation, a method scrutiny in terms of blind testing is advisable in order to secure that the execution is consistent and reliable.

DNA metabarcoding of stomach-content samples is a promising method that is rapidly progressing and emerging as a powerful tool, especially for an enhanced taxonomic resolution of prey. Despite some technical challenges, the barcoding approach constitutes a strong complementary tool for visual approaches like the presence–absence and relative-fullness methods. It should also be emphasised that diet-tracing techniques other than stomach-content analysis are increasingly utilised, including stable isotopes and biomarkers like fatty acids (Nielsen et al., 2018). These approaches are contributing to major advances in trophic ecology and studies integrating multiple methods may prove to be particularly useful (Kainz et al., 2017; Nielsen et al., 2018; Nolan & Britton, 2018). However, despite the advanced analytical processes involved in the performance of these methods (Nielsen et al., 2018), visual dietary analysis still prevails as a fundamental basis for such studies, thus emphasising the importance of optimising and standardising the choice of methods utilised for stomach-content analysis.

5 | CONCLUSIONS AND RECOMMENDATIONS

In conclusion, we emphasise that none of the available methods for stomach-content analysis are perfect as all suffer some weaknesses and constraints. However, with careful selection and use of methods, the pitfalls and limitations can chiefly be bypassed and avoided. For a standardisation of methods for stomach-content analysis we recommend a combination of the presence–absence and the relative-fullness methods as the optimal approach for studies addressing research objectives and questions related to dietary composition and relative prey importance. The presence–absence method can be executed as an implicit part of the relative-fullness method and both approaches are time-efficient and relatively easy to perform and together they provide a solid and reliable estimation of the diet composition in terms of both relative prey abundance and frequency of occurrence. We furthermore recommend the numerical method (when practicable) as the optimal approach for prey selectivity studies, whereas the gravimetric method is recommended for quantification of food consumption rates. Our recommendations facilitate efficient and standardised procedures for stomach-content analysis and will hopefully lead to a harmonisation of methods used by the scientific community, thereby making trophic studies of fish more comparable across species, ecosystems and time and enhance their usefulness and benefits in ecological research and meta-analyses.

ACKNOWLEDGEMENTS

Sincere thanks are due to Cesilie Bye, Karin Strand Johannessen and Runar Kjaer for their skilful laboratory assistance.

CONTRIBUTIONS

P.A.A. conceived the general ideas and initiated the paper and has lead the writing process. J.S.H. provided substantial contribution and support in the conceptual developments and writing of the paper.

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