

Evaluating fish diet analysis methods by individual-based modelling

Ida Ahlbeck, Sture Hansson, and Olle Hjerne

Abstract: Knowledge of diet compositions is important in ecological research. There are many methods available and numerous aspects of diet composition. Here we used modelling to evaluate how well different diet analysis methods describe the “true” diet of fish, expressed in mass percentages. The methods studied were both basic methods (frequency of occurrence, dominance, numeric, mass, points) and composite indices (Index of Relative Importance, Comparative Feeding Index). Analyses were based on both averaged stomach content of individual fish and on pooled content from several fish. Prey preference, prey size, and evacuation rate influenced the performance of the diet analysis methods. The basic methods performed better than composite indices. Mass and points methods produced diet compositions most similar to the true diet and were also most robust, indicating that these methods should be used to describe energetic–nutritional sources of fish.

Résumé : Il importe de connaître la composition du régime alimentaire en recherche en écologie. De nombreuses méthodes sont disponibles pour déterminer la composition du régime alimentaire, qui comporte de nombreux aspects. Nous avons utilisé la modélisation pour évaluer comment différentes méthodes d’analyse du régime alimentaire décrivent l’alimentation « réelle » des poissons, exprimée en terme de pourcentages de la masse. Les méthodes étudiées reposent tant sur des paramètres de base (fréquence de l’occurrence, dominance, numérique, masse, points) que des indices composites (indice d’importance relative, indice comparatif d’alimentation). Les analyses sont basées sur le contenant stomacal moyen d’individus et sur le contenu stomacal combiné de plusieurs poissons. La performance des méthodes d’analyse de l’alimentation est influencée par les préférences des proies, la taille des proies et le taux d’élimination. La performance des méthodes reposant sur des paramètres de base s’avère supérieure à celle de méthodes faisant appel à des indices composites. Les méthodes reposant sur la masse et les points produisent les compositions du régime alimentaire qui s’approchent le plus du régime réel et sont également les plus robustes. Ces résultats indiquent que ces deux méthodes devraient être utilisées pour décrire les sources d’énergie–d’alimentation des poissons.

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Introduction

Knowledge of diet compositions is important in ecological research. As diet compositions show from where animals derive their sustenance, while simultaneously indicating potential food competitors and predator–prey interactions, they contribute to the understanding of ecosystem structure and population dynamics. Because of this, there are numerous articles on diets, of which many are based on analyses of stomach contents.

Diet analyses can be conducted in numerous ways, and methods have been reviewed and discussed by several authors (e.g., Hynes 1950; Hyslop 1980; Pierce and Boyle 1991). Opinions, however, diverge on the expediency of various methods (e.g., Hyslop 1980; Cortés 1997; Hansson 1998), which also depend on the objectives of the study (Hyslop 1980). The three main methods used are based on numbers, biomasses or volumes, and frequency of occurrence of prey. To compensate for assumed biases associated with

different methods, composite diet indices, integrating data from two or more methods, have been developed (e.g., Cortés 1997; Christensen 1978).

Several authors (e.g., Pinkas et al. 1971; Hynes 1950; Cortés 1997) have compared results from analysis methods based on mass, numbers, and frequency of occurrence of prey from in situ data and found poor agreement among methods. A problem when evaluating how well a method performs is that it is generally impossible to compare diet compositions derived from stomach content of naturally feeding fish with the actual true long-term diet of the fish. Long-term diets could be obtained by using laboratory and mesocosm experiments, but no such study has been presented where a range of methods are compared. We use modelling to compare how well diet compositions, derived from stomach content using 27 different analysis methods, agree with the long-term diet of fish. From these comparisons we evaluated the strengths and weaknesses of the different methods in relation to each other with the aim of finding one or more methods that preferably should be used for nutritional diet composition.

In the present study, diets are related to the energetic–nutritional composition, and the aim is to identify major energy sources of the fish, with no intention to determine fish electivity. We chose to express the true diets in terms of energetic–nutritional composition, as this is a common objective in diet analysis and is also used as basic input data in,

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for example, quantitative food web analyses and is frequently used in models such as Ecosim and Ecopath (Walters et al. 1997). The model was constructed to create reasonable prey composition and variation in stomachs, as well as certain true diets, to enable comparison between calculated and true diets and to identify variables critical to method performance. It is important to bear in mind that the objective of the model was not to realistically mimic foraging fish, but to generate stomach contents and diet compositions relevant to evaluate the performance of different diet analysis methods. Because of this, the parameters used in the model do not reflect, for example, natural prey capture success rates or prey preferences, but were chosen to create certain diet compositions (referred to as piscivorous, benthivorous, or size generalist) to allow evaluation of different analysis method's sensitivity to fish with different foraging strategies. From personal experience of analyzing many thousands of stomachs, we know that stomach contents in wild fish vary considerably, usually with only a few species from the known prey range in each stomach (S. Hansson, personal observation). This is mirrored in our simulated stomachs, where each prey type only occurs in a few percent of the stomachs (Fig. 1).

The great advantages of using modelling were that we obtained the true diets of the fish and were able to "create" fish with different types of feeding. Modelling also allowed for a larger number of replicates in a relatively short time. This evaluation of diet analysis methods was made with fish as a model species, but the results might also be applicable to other animals with similar variation in diet.

Materials and methods

Fish feeding modelling

An overview

To evaluate different methods used for fish diet analysis, an individual-based model of a foraging fish was constructed to create fish stomachs with varying content. In nature, a fish first encounters and then captures (or not) a prey, but we modelled this as a single process called prey capture. One time step in the model was called a predation cycle. In a predation cycle, the fish captured a prey and (or) digested the prey in its stomach by a prescribed amount (described below, Table 1). The predation cycles were running continuously. The fish could capture one prey per predation cycle, and the probability of capturing a particular prey differed among prey types and also depended on the size of the predatory fish (Table 1, columns 4–15). If the fish was unsuccessful long enough in one habitat, it switched to another habitat with a somewhat different prey assemblage. As long as there was room enough in the fish stomach, the captured prey entered the stomach, where it was digested and evacuated. This was repeated for 1000 predation cycles, the fish was then "caught" and the stomach content was analyzed. The stomach content of a sample of fish was analyzed with all the different diet methods, the results of which were compared with the true diet. The true diet was estimated as the integrated food consumption of 275 fish over a period of 60 000 predation cycles for each fish (described below). All methods were evaluated against a true diet based on mass percentages, even those methods not originally designed to describe diet-

ary nutritional value. Foraging was assumed to be the only activity of fish; no interactions with other fish were modelled. Modelling details are given below.

Fish characteristics

To investigate how the diet analysis methods perform for various types of predators, we modelled fish with different characteristics in terms of prey size preferences. Fish that primarily fed on (i.e., had high probability for) large prey were referred to as "piscivorous" (Table 1, columns 4–7), while fish that primarily fed on smaller prey were called "benthivorous" (Table 1, columns 8–11). These two types of fish fed on the same prey size assemblage (1–30 mass units; Table 1). We also simulated a "size generalist" fish with a larger prey size range that also fed on very small prey (0.1–30 mass units; Table 1, columns 12–15).

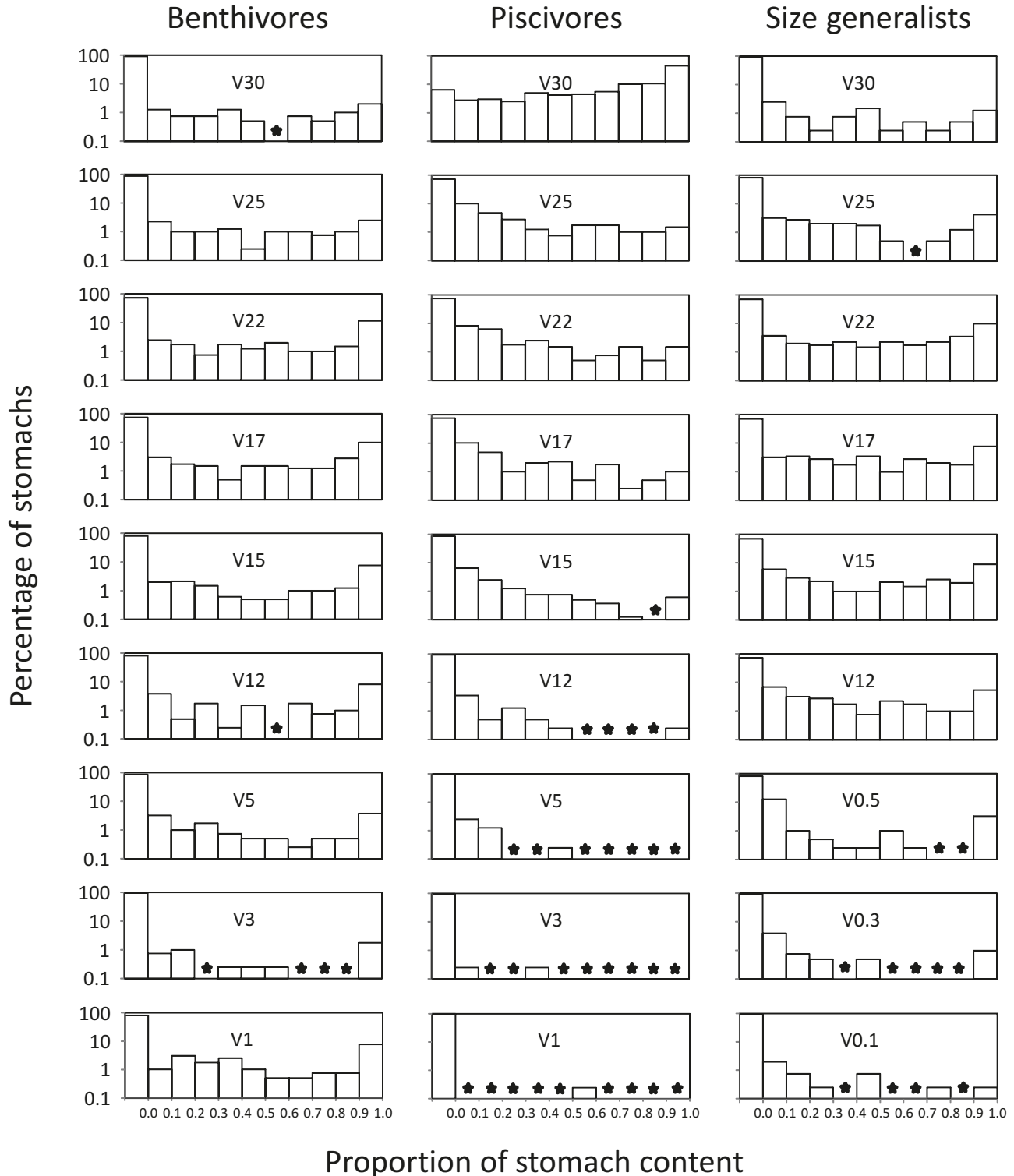
Furthermore, the piscivores and benthivores could forage in two alternative modes, continuously or periodically (discussed below). The size generalist only fed continuously.

Generating variation in the diet

At the start of a simulation the length of each predatory fish was determined at random within a length range of 15 to 25 length units, with equal probability for each length within the range. The probabilities to capture different prey were set for the predator type (piscivorous, benthivorous, or size generalist) but were also affected by the size of the fish. Large fish had higher capture probabilities for larger prey than small fish, and capture probabilities for intermediate-sized fish were derived by linear interpolation (Table 1, columns 4–15). By including size dependence in the diet, we increased the heterogeneity in stomach content. It also allowed for some realism, as real samples seldom consist of equally sized fish. Large fish, with more stomach content, may influence the results more than smaller fish when stomach contents are pooled before analysis. To compensate for this, stomach content data can be scaled to fish size, and such weighting was explored in our analyses.

Diet variability within a fish type was further increased by having two alternative habitats with somewhat different prey availability and prey capture probabilities (Table 1, prey types 1–10), habitat A harbouring larger prey and habitat B harbouring smaller prey, representing idealized cases of offshore–pelagic and inshore–littoral habitats. In simulations with the size generalist, three small prey were included that were not used in the other simulations (Table 1, prey types 11–13). Each habitat had eight prey types, of which six occurred in both habitats, while two were unique to each habitat (Table 1, columns 4–15). The 13 prey species had different masses and were evacuated at different rates (Table 1, columns 2–3). Two of the prey types had the same mass but different evacuation rates, and two prey types had the same evacuation rate but different masses. At the start of a simulation, each fish was positioned randomly in one of the two habitats, where it started to forage. The fish stayed in a habitat until it failed to capture prey for seven (habitat A) or five (habitat B) predation cycles; this ensured similar amount of time spent in each habitat having one habitat slightly more profitable than the other (lower probability for capture failure; Table 1).

Fig. 1. The bars show the percentage of stomachs (logarithmic y axis) with a certain proportion of a prey type (indicated by its volume: V30–V0.1 in panel rows; Table 1) in the stomach content for the benthivore, piscivore, and size generalist fish (panel columns). The first bar in each panel shows the percentage of fish stomachs that contained nothing of that prey type, the second bar shows the percentage of fish stomachs with a proportion of >0 to 0.1 of that prey type, etc. A star indicates that there were no stomachs with that proportion of the prey type.



Foraging and evacuation

Foraging was modelled as predation cycles, where a fish could capture and consume at maximum one prey per cycle. A captured prey could only be consumed if there was room enough in the stomach. A stomach could hold at most a prey

mass equivalent to fish length³/100 (Fulton's condition factor (Heincke 1908) was used as a proxy for the mass a stomach could contain). "Continuous feeding" meant that the fish ate and evacuated prey continuously. When a fish had eaten a prey, it was stored in the stomach and successively digested

Table 1. Parameters describing prey and predation characteristics and probabilities to capture certain types of prey for different types of fish, in different habitats and for different fish sizes.

Prey type	Mass of prey (mass units)	Predation cycles in stomach	Probability											
			Piscivore				Benthivore				Size generalist			
			Habitat A		Habitat B		Habitat A		Habitat B		Habitat A		Habitat B	
			Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25
0	0	0	0.402	0.395	0.599	0.647	0.649	0.605	0.369	0.377	0.536	0.51	0.38	0.392
1	30	10	0.337	0.387	NP	NP	0.036	0.043	NP	NP	0.015	0.029	NP	NP
2	25	9	0.052	0.044	NP	NP	0.045	0.052	NP	NP	0.034	0.048	NP	NP
3	22	8	0.052	0.044	0.044	0.041	0.051	0.059	0.016	0.021	0.053	0.067	0.022	0.030
4	17	7	0.050	0.042	0.046	0.042	0.057	0.064	0.019	0.024	0.072	0.078	0.031	0.036
5	15	6	0.051	0.042	0.046	0.041	0.066	0.073	0.022	0.027	0.097	0.102	0.041	0.047
6	15	5	0.015	0.012	0.055	0.049	0.022	0.024	0.030	0.037	0.036	0.034	0.063	0.065
7	12	5	0.020	0.017	0.054	0.047	0.034	0.037	0.033	0.040	0.070	0.058	0.087	0.079
8	5	3	0.021	0.017	0.053	0.046	0.040	0.043	0.037	0.045	NP	NP	NP	NP
9	3	2	NP	NP	0.052	0.044	NP	NP	0.042	0.049	NP	NP	NP	NP
10	1	1	NP	NP	0.051	0.043	NP	NP	0.432	0.380	NP	NP	NP	NP
11	0.5	3	NP	NP	NP	NP	NP	NP	NP	NP	0.087	0.074	0.103	0.094
12	0.3	2	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	0.120	0.112
13	0.1	1	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	0.153	0.145

Note: In each predation cycle, a fish tries to capture a prey, and the outcome is determined by a random number generator. Probabilities to fail in capturing a prey (prey type 0) and probability to capture different prey are shown in columns 4–15. Two alternative habitats, A and B, with different prey availability and different prey capture probabilities were used to increase diet variability. In simulations with the size generalist, three small prey were included that were not used in the other simulations (prey types 11–13). Each habitat had eight prey types, of which six occurred in both habitats, while two were unique to each habitat. The probabilities shown are for fish sized 15 and 25 length units; for intermediate-sized fish, probabilities are derived by linear interpolation between these values. NP indicates non-present prey. Column 3 shows the number of predation cycles that a prey remains in the stomach.

by an amount dictated by the assumed gut evacuation rates (Table 1, column 3). After this a new predation cycle was initiated. When caught, these fish contained prey at different stages of digestion. In “periodic feeding”, no evacuation took place during feeding. These fish types foraged until their stomach was at least 70% full and then switched from feeding to evacuation (evacuating prey from the stomach according to the assumed gut evacuation rates; Table 1, column 3). The fish did not resume feeding until the stomach was empty. These fish could only be caught during the time spent foraging (in nature fish can be expected to be more exposed to fishing gear during its active feeding phase) and then contained undigested prey. If such fish were caught during the evacuation phase, diet analysis would resemble those of trap- or gillnet-caught fish with continuous feeding (described below).

Gut evacuation in real fish results from both digestion of food and the transport of food remains into the intestine. In our model, these two processes were combined and referred to as evacuation. Gut evacuation rates have been described as both linear and exponential (Jobling 1986), so we have included both in our model. When linear evacuation rates were used, the mass of each prey was reduced by a constant amount in each predation cycle (Table 1, column 3). With exponential evacuation, a certain fraction of the still not evacuated mass of each prey was removed in each predation cycle, which can be expressed as

$$\text{mass}_{t+1} = \text{mass}_t \times e^{-k}$$

where mass was the mass of an individual prey before (t) and after ($t + 1$) a predation cycle, and k was the evacuation rate. The evacuation rates were set to evacuate prey in a certain number of cycles, mainly in relation to its size (Table 1, column 3). For linear evacuation, an individual prey disappeared from the stomach when its mass reached zero, while exponentially evacuated prey disappeared when the remains were $\leq 1\%$ of the original mass. Total evacuation time for each prey type was identical for linear and exponential evacuation. We assumed that the evacuation rates were independent of the total stomach content (e.g., Temming and Herrmann 2003).

Scenarios

We simulated scenarios with piscivorous and benthivorous fish as both continuous and periodic feeders, but the size generalists were only simulated as continuous feeders, as simulations were time consuming. Those five scenarios were run with exponential or linear evacuation, resulting in 10 main scenarios. In all scenarios, stomach contents were analyzed both instantaneously and after a period of stomach content evacuation (described below), resulting in 20 scenarios. However, the effective numbers of scenarios were 18, because by definition the gut evacuation type did not matter for instantaneous sampling in periodic feeders, since evacuation did not start before the stomach analysis in these cases (Fig. 2).

Sensitivity analysis

Analysis of the different scenarios represented a form of sensitivity analysis of the diet analysis methods. In addition to these main scenarios, we analyzed the sensitivity further,

by additional parameter changes. In terms of prey capture probabilities, the main scenarios were supplemented with scenarios of high probability for failing to capture a prey (“High fail”), low probability for failing to capture a prey (“Low fail”), all prey having identical capture probabilities (“Generalist 1”), normal distribution of prey capture probabilities (the middle-sized prey most common; “Generalist 2”), and increasing capture probability with decreasing prey size (“Generalist 3”) for continuous feeders (see Table 2). In the main scenarios, the evacuation rates were closely coupled to prey size. To test for effects of variation in evacuation rates due to, for example, evacuation rates influenced by prey tissue composition, we ran simulations with two different versions of randomly increased or decreased evacuation rates for continuously feeding piscivores (“Random piscivore 1” and “Random piscivore 2”) and benthivores (“Random benthivore 1” and “Random benthivore 2”) with exponential evacuation (Table 3). The evacuation rates were $k_{\text{random}} = k_{\text{original}} \cdot [1 + \text{Rand}(0.5)]$.

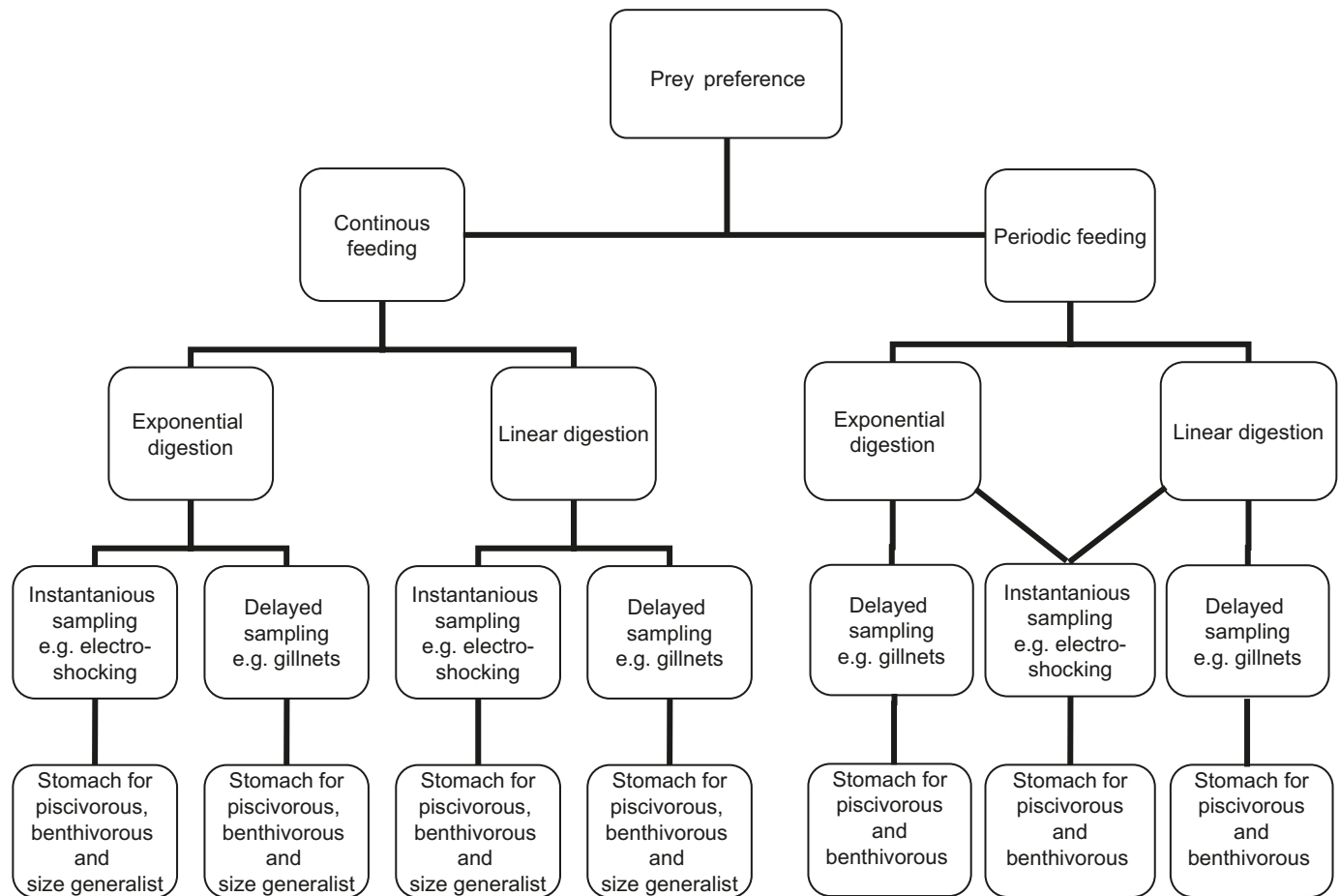
Heavily digested prey can be difficult to identify, and to explore effects of this, a simulation in which prey could be identified at a mass of 10% of its original mass was run in addition to the main scenario where 1% of original prey mass was used (this simulation, “Identification level”, was run only for the continuously feeding benthivorous fish with exponential evacuation). Two alternative predator stomach sizes (fish length³/100 and fish length³/10) were used to investigate the effect of prey numbers in the stomach (this simulation, “Stomach size”, was run only for the periodically feeding benthivorous fish with exponential evacuation). We also tested how different habitat utilization (i.e., number of predation cycles spent in each habitat) affected the method results (this simulation, “Cycles”, was run only for the continuously feeding benthivorous fish). Finally, alternative prey size distributions between habitats were explored by removing prey types 5 and 10 from habitat A instead of prey types 9 and 10 and removing prey types 2 and 6 from habitat B instead of prey types 1 and 2 (this simulation, “Prey availability”, was run only for the continuously feeding benthivorous fish). As the simulations were time consuming, we had to restrict the number of simulations for some scenarios of the sensitivity analysis (i.e., running them only for one prey preference, one feeding mode, and one evacuation type).

Results from these additional simulations will only be discussed in relation to the sensitivity analysis, as they did not influence conclusions substantially.

Fish's true diet

Knowing the long-term “true” diets was critical to our analyses. In the true diet, the “life time” consumption of the fish was registered, as opposed to the stomach content, which presents the prey composition at a certain time, described above. True diets were obtained by simulating 60 000 predation cycles (initial simulations showed this to be sufficient to obtain stable diet composition) for 25 fishes from each of the 11 fish sizes (15, 16, ..., 25 length units), and the accumulated consumption was recorded for each of these individuals. For each of the 25 individuals in each size class, the diet composition was calculated as the mass percentages of different prey, and then the average mass percentages were calculated for the size class. The true diet was then estimated as

Fig. 2. Diagrammatic representation of possible fish and sampling characteristics used to derive simulated stomach contents. All these assumptions were used for piscivores and benthivores, but for size generalists we did not analyze scenarios with periodic feeding. In total, this resulted in 18 sets of scenarios.



the average of the average mass percentages across all 11 size classes. This procedure gave fish of different size, with different amounts of consumed food, the same influence on the true diet. Since prey capture probabilities and evacuation rates varied among fish types, true diets were calculated separately for each fish type, as described above. The results from all diet analysis methods were compared with the true diets. To visualize the modelled diet compositions and enable comparison with real diet samples, we show the true diet composition per fish type (Fig. 3). Diet compositions vary widely depending on fish species and even vary within species depending on ontogeny (e.g., International Council for the Exploration of the Sea 1997). The diet compositions generated by our program fit well within the variation of real life data.

Sampling fish for stomach analysis

A sample of fish for stomach analysis consisted of 20 fish caught after their 1000th predation cycle. Twenty fish can be seen as a small sample size, but it is a realistic sample size in situ samples after stratifying a larger data set into, for example, size classes, sampling sites, season, and year. To explore the variation in diets derived with a certain method, we analyzed 20 samples of 20 fish each for each scenario (i.e., for all combinations of prey preference, feeding mode,

evacuation type, and sampling method; Fig. 2). To describe diet variation over, for example, larger areas or longer time periods, 20 samples of fish is not an unrealistic assumption and will give a reasonable estimate of variation in different methods. Hence, 400 fish in total (20 samples \times 20 fish per sample) with non-empty stomachs were analyzed for each scenario.

Two alternative sampling methods were applied. Depending on catch method used in situ, fish can be sampled and analyzed almost instantaneously (e.g., electrofishing, explosives, or angling) or collected over a somewhat longer time (e.g., traps or gillnets) with the risk of fish regurgitating or digesting some of the stomach content for hours before stomach content is analyzed. To simulate stomach sampling where fish were collected instantaneously, the stomach content used was that present after the 1000th predation cycle but before the gut evacuation in this cycle had commenced. To simulate a situation where the fish could evacuate some of its stomach content after it was caught but before stomach analysis was made, the fish was allowed to evacuate its stomach content during a randomly determined period (0 to 5 cycles, even distribution) before the stomach content was analyzed. If the stomach was empty when the fish was caught, an additional fish was sampled, until 20 fish with non-empty stomachs were caught.

Table 2. Prey capture probabilities (columns 2–20) for the for the high fail, low fail, and generalist 1–3, in different habitats and for different fish sizes, in the sensitivity analysis.

Prey type	High fail				Low fail				Generalist 1				Generalist 2				Generalist 3			
	Habitat A		Habitat B		Habitat A		Habitat B		Habitat A		Habitat B		Habitat A		Habitat B		Habitat A		Habitat B	
	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25	Size 15	Size 25
0	0.790	0.781	0.690	0.695	0.153	0.135	0.096	0.103	0.455	0.455	0.455	0.455	0.445	0.403	0.411	0.444	0.536	0.510	0.381	0.393
1	0.014	0.015	NP	NP	0.051	0.075	NP	NP	0.068	0.068	NP	NP	0.021	0.042	NP	NP	0.015	0.029	NP	NP
2	0.023	0.024	NP	NP	0.077	0.102	NP	NP	0.068	0.068	NP	NP	0.048	0.069	NP	NP	0.034	0.048	NP	NP
3	0.036	0.036	0.013	0.016	0.101	0.124	0.036	0.054	0.068	0.068	0.068	0.068	0.076	0.095	0.040	0.060	0.053	0.067	0.021	0.030
4	0.045	0.044	0.017	0.020	0.124	0.146	0.047	0.068	0.068	0.068	0.068	0.068	0.103	0.121	0.058	0.081	0.072	0.078	0.031	0.036
5	0.060	0.057	0.022	0.026	0.155	0.166	0.058	0.076	0.068	0.068	0.068	0.068	0.138	0.155	0.076	0.101	0.097	0.102	0.041	0.047
6	0.023	0.019	0.035	0.035	0.076	0.058	0.117	0.108	0.068	0.068	0.068	0.068	0.052	0.038	0.116	0.101	0.036	0.034	0.063	0.065
7	0.037	0.031	0.041	0.041	0.119	0.088	0.132	0.118	0.068	0.068	0.068	0.068	0.062	0.043	0.100	0.083	0.070	0.058	0.087	0.078
8	0.047	0.040	0.050	0.051	0.143	0.107	0.150	0.136	0.068	0.068	0.068	0.068	0.055	0.035	0.085	0.065	0.087	0.074	0.103	0.094
9	NP	NP	0.057	0.059	NP	NP	0.170	0.156	NP	NP	0.068	0.068	NP	NP	0.067	0.044	NP	NP	0.120	0.112
10	NP	NP	0.068	0.071	NP	NP	0.194	0.180	NP	NP	0.068	0.068	NP	NP	0.047	0.021	NP	NP	0.153	0.145

Note: In each predation cycle, a fish tries to capture a prey, and the outcome is determined by a random number generator. Probabilities to fail in capturing a prey (prey type 0) and probabilities to capture different prey are shown in columns 2–20. Two alternative habitats, A and B, with different prey availability and different prey capture probabilities were used to increase diet variability. Each habitat had eight prey types, of which six occurred in both habitats, while two were unique to each habitat. The probabilities shown are for fish sized 15 and 25 length units; for intermediate sized fish, probabilities are derived by linear interpolation between these values. NP indicates non-present prey. Prey are evacuated from the stomachs at the same rate as in the main scenarios (Table 1).

Table 3. Number of cycles that the prey remained in the stomach for the simulations with random evacuation rates in the piscivorous and benthivorous fish with continuous exponential evacuation.

Prey type	Random piscivore 1	Random piscivore 2	Random benthivore 1	Random benthivore 2
1	8	15	8	15
2	12	11	12	11
3	11	6	11	6
4	7	7	7	7
5	8	7	8	7
6	7	5	7	5
7	4	10	4	10
8	3	5	3	5
9	3	2	3	2
10	1	1	1	1

Methods evaluated

Stomach content was analyzed in four different basic ways: frequency of occurrence, dominance method, numeric methods, and mass-based methods. An alternative to using prey masses could be to base the analyses on prey volumes (Hynes 1950; Swedberg and Walburg 1970). We have not explored this alternative, since specific densities of different prey types can be expected to be similar and hence also the outcome of the comparison of methods. Stomach content was also manipulated to mimic data uncertainty and approximate methods. Furthermore, diets were also derived from different composite indices, which combine different basic data: Percent Index of Relative Importance and Comparative Feeding Index. Analyses were made from individual stomachs, after merging stomach content, pooled, and after accounting for the size of the analyzed fish, pooled adjusted, as described in Hyslop (1980). Abbreviations used below are explained in Table 4.

Frequency of occurrence (FO)

The proportion of stomachs containing each prey type was calculated and expressed as a percentage of the total number of stomachs.

$$\text{Diet}_{i}^{\text{FO}} = \frac{N_{\text{fish},i}}{N_{\text{fish}}} \times 100$$

Dominance method (DM)

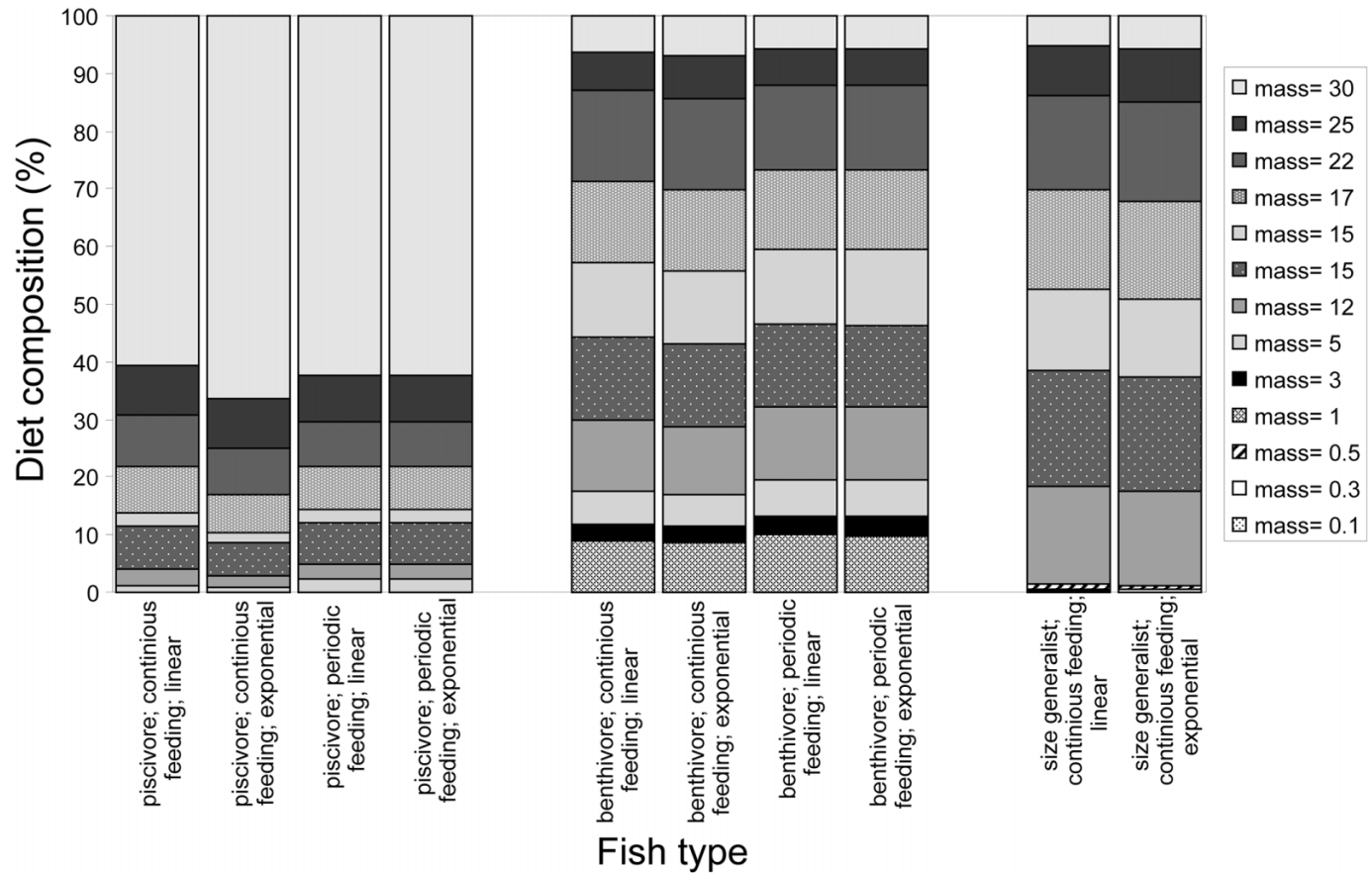
The proportion of stomachs dominated by mass by a certain prey type was calculated and expressed as a percentage of the total number of stomachs.

$$\text{Diet}_{i}^{\text{DM1}} = \frac{N_{\text{fish},i}^{\text{w}}}{N_{\text{fish}}} \times 100$$

Numeric method (NM1–NM3)

In this method, the diet was expressed as the numeric percentage of each prey type relative to the total number of prey. This diet could be derived in three different ways. In numerical method 1 (NM1), prey percentages were first calculated for each fish separately and then averaged over all individuals in the sample (Hyslop 1980). This meant that each fish impacts the result equally much.

Fig. 3. The true diet compositions of the different fish types used in the 18 scenarios expressed as mass percentages of the 13 prey types (masses 30–0.1 arranged top to bottom; also see Table 1).



$$\text{Diet}_i^{\text{NM1}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{N_{ij}}{N_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

In numerical method 2 (NM2), the stomach contents of all fish in the sample were pooled before percentages of different prey types were calculated (Hyslop 1980). The impact of each fish on the result differed among fish and was proportional to the number of prey in the stomach.

$$\text{Diet}_i^{\text{NM2}} = \frac{\sum_{j=1}^{N_{\text{fish}}} N_{ij}}{\sum_{j=1}^{N_{\text{fish}}} N_{\bullet j}} \times 100$$

Numerical method 3 (NM3) was basically the same as NM2, but before pooling prey from different stomachs, prey numbers were divided by the mass (length³) of the fish. This reduced the greater impact that larger fish, with on average more prey in their stomach, may have had.

$$\text{Diet}_i^{\text{NM3}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{N_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{N_{\bullet j}}{L_j^3}} \times 100$$

Mass method (MM1–MM6)

As with the NM, diets based on prey mass could be calculated in different ways. In mass method 1 (MM1), mass percentages of the digested (*w*) prey items found in the stomach were first calculated for each fish separately and then averaged over all individuals in the sample. This meant that each fish impacted the result equally.

$$\text{Diet}_i^{\text{MM1}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{w_{ij}}{w_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

In mass method 2 (MM2), the stomach contents of all fish in the sample were pooled before mass percentages of different prey were calculated (Hyslop 1980). The impact of each fish on the result differed among fish and was proportional to the mass of prey in the stomach.

$$\text{Diet}_i^{\text{MM2}} = \frac{\sum_{j=1}^{N_{\text{fish}}} w_{ij}}{\sum_{j=1}^{N_{\text{fish}}} w_{\bullet j}} \times 100$$

Mass method 3 (MM3) was basically the same as MM2, but before pooling prey from different stomachs, prey masses were divided by the mass (length³) of the fish (adjusted pooled analysis; Hyslop 1980).

Table 4. Abbreviations used in method equations.

$\text{Rand}(\mu, \sigma)$	= normally distributed random number; mean = μ and standard deviation = σ
N_{fish}	= no. of fish examined
N_{prey}	= no. of prey types
$N_{\text{fish},i}$	= no. of fish that have prey of type i in the stomach
$N_{\text{fish},iw}$	= no. of fish in which prey of type i dominates (i.e., has the largest mass) the stomach content, based on the masses of digested prey
N_{ij}	= no. of prey type i in the stomach of fish j
$N_{\bullet j}$	= total no. of prey in the stomach of fish j
L_j	= length of fish j
w_{ij}	= mass of prey type i in the stomach of fish j
$w_{\bullet j}$	= total mass of prey in the stomach of fish j
\tilde{w}_{ij}	= approximate mass of prey type i in the stomach of fish j ; $w_{ij} \times \text{Rand}(1, 0.15)$
$\tilde{w}_{\bullet j}$	= the sum of approximate prey masses in the stomach of fish j
W_{ij}	= mass of prey type i in the stomach of fish j , based on original, undigested masses
$W_{\bullet j}$	= total mass of prey in the stomach of fish j , based on original, undigested mass of each prey
\tilde{W}_{ij}	= approximate original mass of prey type i in the stomach of fish j ; $W_{ij} \times \text{Rand}(1, 0.15)$
$\tilde{W}_{\bullet j}$	= the sum of approximate original prey masses in the stomach of fish j
p_{ij}	= points allotted to prey type i in the stomach of fish j
$p_{\bullet j}$	= total no. of points allotted to prey in the stomach of fish j
p_i	= points allotted to prey i after merging the stomachs of all N_{fish} in the sample
Diet_i^X	= percentage (%) of prey i in the diet, estimated using method X

$$\text{Diet}_i^{\text{MM3}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{w_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{w_{\bullet j}}{L_j^3}} \times 100$$

As a modification of the three methods described above, the original prey masses (W), before digestion had started, were used for the calculations (MM4–MM6). MM4 hence corresponded to MM1, only with original prey mass instead of digested prey mass. Further, MM5 corresponded to MM2 and MM6 corresponded to MM3 in the same way.

$$\text{Diet}_i^{\text{MM4}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{W_{ij}}{W_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

$$\text{Diet}_i^{\text{MM5}} = \frac{\sum_{j=1}^{N_{\text{fish}}} W_{ij}}{\sum_{j=1}^{N_{\text{fish}}} W_{\bullet j}} \times 100$$

$$\text{Diet}_i^{\text{MM6}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{W_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{W_{\bullet j}}{L_j^3}} \times 100$$

Approximate mass method (AMM1–AMM6)

Determining the mass of different prey is time consuming and can be associated with substantial errors if a large proportion of the stomach content cannot be apportioned to prey types. To evaluate the possible impact of errors in the mass

partitioning among prey in stomachs, we introduced an error in the mass data. This was done by multiplying the actual prey masses by a random number with an average of 1 and a standard deviation of 0.15. Based on many years of experience in stomach analyses, we consider this degree of uncertainty in prey mass determination realistic. The approximate mass methods AMM1–AMM3 corresponded to the MM1–MM3 described above, respectively, but associated with errors.

In approximate mass method 1 (AMM1), digested mass percentages were calculated, multiplied by the error value, and averaged over all individuals in the sample. Each fish impacted the result equally:

$$\text{Diet}_i^{\text{AMM1}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{\tilde{w}_{ij}}{\tilde{w}_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

In approximate mass method 2 (AMM2), the digested stomach contents of all fish in the sample were pooled before percentages of different prey were calculated and the error was imposed. The impact of a fish was proportional to its contribution to the total mass of prey:

$$\text{Diet}_i^{\text{AMM2}} = \frac{\left(\sum_{j=1}^{N_{\text{fish}}} w_{ij} \right) \times \text{Rand}(1, 0.15)}{\sum_{i=1}^{N_{\text{prey}}} \left[\left(\sum_{j=1}^{N_{\text{fish}}} w_{ij} \right) \times \text{Rand}(1, 0.15) \right]} \times 100$$

In approximate mass method 3 (AMM3), the size of the fish was taken into account before pooling mass data.

$$\text{Diet}_i^{\text{AMM3}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{\tilde{w}_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{\tilde{w}_{\bullet j}}{L_j^3}} \times 100$$

In this study, the original masses of each prey type were defined, but in practice back-calculated original prey masses are associated with errors. To test for effects of this, the estimated masses of different prey prior to digestion were associated with errors (AMM4–AMM6).

In approximate mass method 4 (AMM4), original mass percentages, multiplied by the error value, were averaged over all individuals in the sample. Each fish impacted the result equally:

$$\text{Diet}_i^{\text{AMM4}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{\tilde{W}_{ij}}{\tilde{W}_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

In approximate mass method 5 (AMM5), the original stomach contents of all fish in the sample were pooled before percentages of different prey were calculated and the error was imposed. The impact of a fish was proportional to its contribution to the total back-calculated mass of prey:

$$\text{Diet}_i^{\text{AMM5}} = \frac{\left(\sum_{j=1}^{N_{\text{fish}}} W_{ij} \right) \times \text{Rand}(1, 0.15)}{\sum_{i=1}^{N_{\text{prey}}} \left[\left(\sum_{j=1}^{N_{\text{fish}}} W_{ij} \right) \times \text{Rand}(1, 0.15) \right]} \times 100$$

In approximate mass method 6 (AMM6), the size of the fish was taken into account before pooling mass data.

$$\text{Diet}_i^{\text{AMM6}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \frac{\tilde{W}_{ij}}{L_j^3}}{\sum_{j=1}^{N_{\text{fish}}} \frac{\tilde{W}_{\bullet j}}{L_j^3}} \times 100$$

Points method (PM1–PM2)

In this method, points were allocated to prey types in relation to their percentages of the total prey mass in the stomach (Swynnerton and Worthington 1940; cited in Hynes 1950). This allowed for less careful mass determinations than required for the mass methods (MM1–MM6). In our evaluation, points were allotted according to Table 5. We have used an approximately logarithmic scale, as, for example, Hynes (1950) and Christensen (1978) have both previously used logarithmic scales.

These points were then treated in the same way as mass data, both averaging over all individuals in the sample (each fish impacts the result equally, PM1) and pooling the stomach content of all fish before percentages were calculated and points were allocated (PM2).

$$\text{Diet}_i^{\text{PM1}} = \frac{\sum_{j=1}^{N_{\text{fish}}} \left(\frac{p_{ij}}{p_{\bullet j}} \times 100 \right)}{N_{\text{fish}}}$$

$$\text{Diet}_i^{\text{PM2}} = \frac{P_i}{\sum_{x=1}^{N_{\text{prey}}} P_x} \times 100$$

Table 5. Point allocated to the mass percentages of prey in the points method.

Percentage of mass in stomach (%)	Points
0	0
>0–1	0.5
>1–2	1.5
>2–4	3
>4–8	6
>8–16	12
>16–32	24
>32–64	48
>64–99	82
>99	100

Percent Index of Relative Importance (%IRI1–%IRI6)

This composite measure combined three of the methods mentioned above (Cortés 1997). Separately for each prey type *i*, diet percentages derived from the FO method ($\text{Diet}_i^{\text{FO}}$) were multiplied by the sum of diet percentages derived with one of the MM methods ($\text{Diet}_i^{\text{MMx}}$) and one of the NM methods ($\text{Diet}_i^{\text{NMx}}$). To calculate the percentages of prey types in the diet, the product calculated for each prey type was divided by the sum of all such products. The performance of the %IRI method was explored using six alternative combinations of MM and NM:

$$\text{Diet}_i^{\% \text{IRI1}} = \frac{\text{Diet}_i^{\text{FO}} (\text{Diet}_i^{\text{NM1}} + \text{Diet}_i^{\text{MM1}})}{\sum_{x=1}^{N_{\text{prey}}} \text{Diet}_x^{\text{FO}} (\text{Diet}_x^{\text{NM1}} + \text{Diet}_x^{\text{MM1}})} \times 100$$

and then for %IRI2, as for %IRI1 but using $\text{Diet}_i^{\text{NM2}}$ and $\text{Diet}_i^{\text{MM2}}$; for %IRI3, as for %IRI1 but using $\text{Diet}_i^{\text{NM3}}$ and $\text{Diet}_i^{\text{MM3}}$; for %IRI4, as for %IRI1 but using $\text{Diet}_i^{\text{NM1}}$ and $\text{Diet}_i^{\text{MM4}}$; for %IRI5, as for %IRI1 but using $\text{Diet}_i^{\text{NM2}}$ and $\text{Diet}_i^{\text{MM5}}$; and for %IRI6, as for %IRI1 but using $\text{Diet}_i^{\text{NM3}}$ and $\text{Diet}_i^{\text{MM6}}$.

Comparative Feeding Index (CFI1–CFI2)

A Comparative Feeding Index (cf. Christensen 1978) was calculated by multiplying diet percentages derived from the FO method ($\text{Diet}_i^{\text{FO}}$) and percentages from one of the PM methods ($\text{Diet}_i^{\text{PMx}}$). To calculate the percentages of prey types in the diet, the product calculated for each prey type was divided by the sum of all such products.

$$\text{Diet}_i^{\text{CFI1}} = \frac{\text{Diet}_i^{\text{FO}} \times \text{Diet}_i^{\text{PM1}}}{\sum_{x=1}^{N_{\text{prey}}} \text{Diet}_x^{\text{FO}} \times \text{Diet}_x^{\text{PM1}}}$$

$$\text{Diet}_i^{\text{CFI2}} = \frac{\text{Diet}_i^{\text{FO}} \times \text{Diet}_i^{\text{PM2}}}{\sum_{x=1}^{N_{\text{prey}}} \text{Diet}_x^{\text{FO}} \times \text{Diet}_x^{\text{PM2}}}$$

Evaluation of methods

To evaluate the performance of each method, the diet com-

position for each of the 20 samples of a scenario, each containing 20 fish with non-empty stomachs, was calculated. The similarity between each sample and the “true” diet was then calculated using Schoener’s similarity index (Schoener 1970):

$$\text{Similarity} = \sum_{i=1}^{N_{\text{prey}}} \min(\text{Diet}_i, \text{True}_i)$$

where Diet_i and True_i were the percentages (%) of prey type i in the diet estimated by a particular method and in the true diet, respectively. This index produces a value of 0% at total dissimilarity and 100% at total similarity between the diet estimate and the true diet.

The average similarity across the 20 samples of the scenario was calculated for each diet analysis method, and then the average similarity was calculated across all 18 scenarios (Fig. 4): Further, we derived two measures of robustness of the methods across the 18 scenarios:

$$(a) \quad \text{variation}_a = \text{average}[\text{st. dev}(\text{similarity})]$$

where st. dev was the standard deviation of the 20 similarity indices calculated from each of the 20 samples of each scenario derived by a certain method. The average of these standard deviations was then calculated across the 18 scenarios. A high value on variation_a implies that the method generally produced substantial differences in diet estimates among samples of fish with the same characteristics and sampling method. The second measured is as follows:

$$(b) \quad \text{variation}_b = \text{st. dev}[\text{average}(\text{similarity})]$$

and measures the variation in the average similarity over the 20 samples among the 18 scenarios. A high value of variation_b implies that the method produces substantial differences in diet estimates among different scenarios (i.e., types of fish or sampling methods).

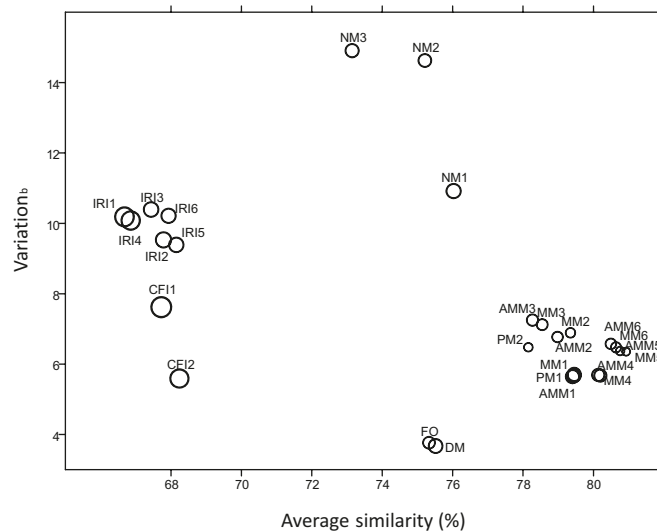
The best diet analysis methods should have high values for similarity and low values for variation_a and variation_b . All simulations were done in MATLAB 7.5.0 (The MathWorks Inc., Natick, Massachusetts).

Results and discussion

The basic methods (FO, DM, NM, MM, AMM, and PM) produced diet estimates that were on average more similar to the true diets than were the estimates of the composite indices (%IRI and CFI; Fig. 4). Unsurprisingly, the mass-based methods, traditionally used for energetic–nutritional diet composition, were the ones that described diets most accurately; it is, however, noteworthy that some non-mass-based methods were also describing diets well.

There were some general patterns of prey over- and under-estimation for the different methods. Small and rare prey were substantially overestimated by some methods, whereas the percentages of large and common prey were less biased (Fig. 5). Individually analyzed mass-based methods (MM1, MM4, AMM1, AMM4, and PM1) generally overestimated small prey (Figs. 5a, 5c), but large overestimations of small prey were also derived with FO, NM, and occasionally with DM (Figs. 5a, 5c).

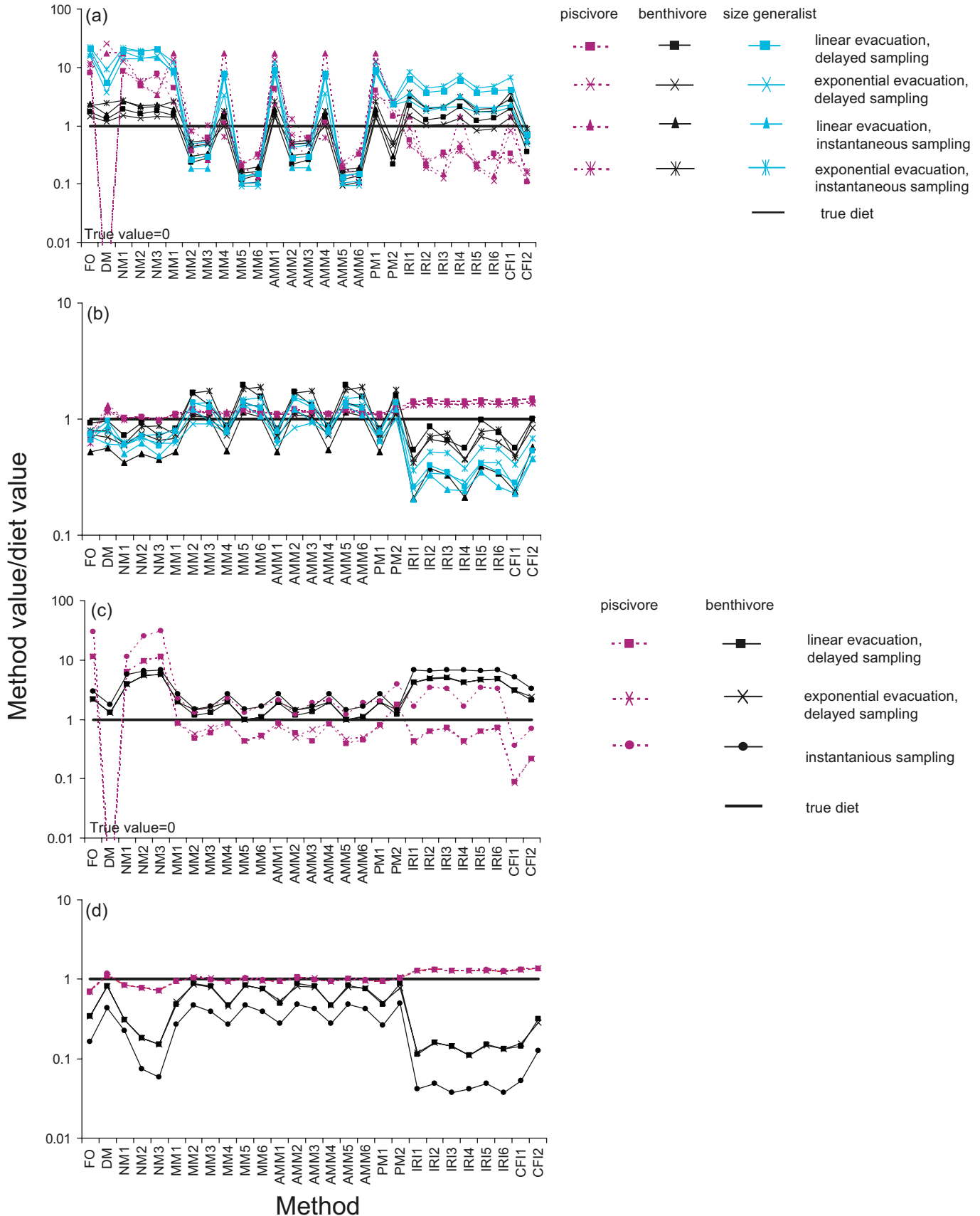
Fig. 4. The average similarity of the diet analysis methods over the 18 scenarios. The sizes of the symbols are proportional to variation_a . A high value on variation_a (larger symbols) implies that a method produced substantial diet differences among samples of fish with the same characteristics and sampling method (e.g., among samples of piscivorous fish with instantaneous sampling). A high value on variation_b implies that a method produced substantial diet differences among different fish types (i.e., between all piscivorous fish, benthivorous fish, and size generalist fish). The smaller the symbols are and the closer a method is positioned to the lower right corner, the better is its performance.



Mass method, approximate mass method, and points method

As expected, given that the true diets were expressed in mass, the mass-based methods (MM, AMM, PM) were the methods that on average produced the highest similarities, with low variation both among scenarios (variation_b , Fig. 4) and among samples (variation_a , Fig. 4). A low variation_b means that the method performed equally well for different predator types and sampling techniques, hence being a good method to use if little is known about the characteristics of the fish. A low variation_a means that the method gave similar results for each of the 20 fish samples, thus implying that the method is feasible to use with a relatively small sample size. Although the difference in performance among the mass-based methods was small, with no great distinction among them, some patterns did emerge. The MM and AMM using back-calculated prey masses (MM4–MM6, AMM4–AMM6) generally had slightly higher similarities than the corresponding methods with digested prey masses (MM1–MM3, AMM1–AMM3; Fig. 4). The MM and AMM with pooled analyses and back-calculated prey masses (MM5–MM6, AMM5–AMM6) gave the highest similarities to the true diets, as they increased the diet percentage of large prey (Figs. 5b, 5d). The MM and AMM with individual analyses (MM1, MM4, AMM1, AMM4) performed almost as well (Fig. 4) and were slightly more robust (lower among scenario variation, variation_b ; Fig. 4). The pooled analyses based on digested prey masses (MM2–MM3, AMM2–AMM3) had both slightly lower similarities and slightly higher variation_b than the individually analyzed methods (Fig. 4). The AMM follows the MM closely, indicating that the error induced did

Fig. 5. The over- and under-estimations of the smallest and the largest prey made by the methods compared with the true diet: (a) small prey in continuously feeding fish; (b) large prey in continuously feeding fish; (c) small prey in periodically feeding fish; (d) large prey in periodically feeding fish. The value of the y axis is the ratio between the estimated and the true mass percentages of a prey type in the diet.



not affect the method to any great extent. The individually analyzed PM1 had, as could be expected, higher similarity than the pooled version, PM2 (Fig. 4), the reason being that in PM1 the approximate points estimates were averaged over 20 stomachs, while in the pooled analysis the approximate points estimates were made directly from the pooled stomach content.

The true diets were calculated as the average of the original prey masses of individual fish (i.e., in the same way as the individually analyzed mass methods). Not surprisingly then, MM4 and AMM4 were among the most similar to the true diets; however, MM5–MM6 and AMM5–AMM6 were, despite this, slightly more similar to the true diets, as they generally overestimated the largest, and energetically most important, prey rather than underestimated these prey, as MM1, MM4, AMM1, and AMM4 did.

If we look at the results in more detail, we can see differences between feeding modes and evacuation types. In simulations of continuous feeding, the individually analyzed mass-based methods (MM1, MM4, AMM1, AMM4, and PM1), in contrast with pooled analyses, generally greatly overestimated small prey (Fig. 5a) and underestimated large prey (Fig. 5b). The reason for this is that stomachs containing only a few small prey are given the same importance as stomachs filled with large prey. Hence, for continuously feeding fish, it could be better to use individual analysis rather than pooled analyses, since the overestimation of small prey by individually analyzed methods (Figs. 5a, 5b) could compensate for the fast evacuation rate of small prey. In simulations of periodic feeding, on the other hand, small prey were not overestimated to the same extent by individually analyzed mass-based methods, since stomachs containing only a few prey were more unlikely, as evacuation did not start before stomachs were filled to at least 70%. In simulations of periodic feeding where small prey could be numerous, the pooled mass-based methods produced diets more similar to the true diets (Fig. 6), as they generally overestimated the small prey to a lesser degree and underestimated large prey to a lesser degree than the individually analyzed methods (Figs. 5c, 5d). For the size generalist, however, the pooled methods generally produced better results than individually analyzed methods even for continuous evacuation, since the smallest prey contributed very little to the total diet by mass and were overestimated to a lesser degree by pooled methods.

In simulations of fish with exponential evacuation rates, methods using original prey masses generally produced diets closer to the true diets than corresponding methods using digested prey masses for both continuous and periodic feeding (Fig. 6). This is due to the fact that a large fraction of the prey was evacuated shortly after ingestion in exponential evacuation. For continuous feeding, original prey masses resulted in a slightly greater overestimation of large prey and a greater underestimation for small prey than if digested prey mass methods were used (Figs. 5a, 5b). This is because, for example, the largest prey could have been partly digested in the stomach at the sampling (1000th cycle), and their diet percentage could therefore have increased when converted to original mass, but the smallest prey had no potential to increase their percentage in the diet, as it was completely digested under one predation cycle. Also in simulations of periodic feeding, small prey got a higher estimate by using

digested masses (Figs. 5c, 5d) for the same reason as explained above. Hence, original prey masses should be used in exponentially evacuating fish.

Frequency of occurrence, dominance, and numeric methods

FO, DM, and NM produced average similarities closer to the mass-based methods than the composite indices (Fig. 4) despite the fact that they did not consider mass properties. The methods with lowest among scenario variation (variation_b) were FO and DM (Fig. 4). FO generally produced high similarities with the true diets for all predator types and hence had a low variation_b. However, its performance (similarity) relative to the other methods was more variable, being most similar or least similar to the true diets depending on fish type (Fig. 6). NM had the highest among scenario variation (variation_b; Fig. 4) performing well in some scenarios and poorly in others, also in relation to the other methods (Fig. 6).

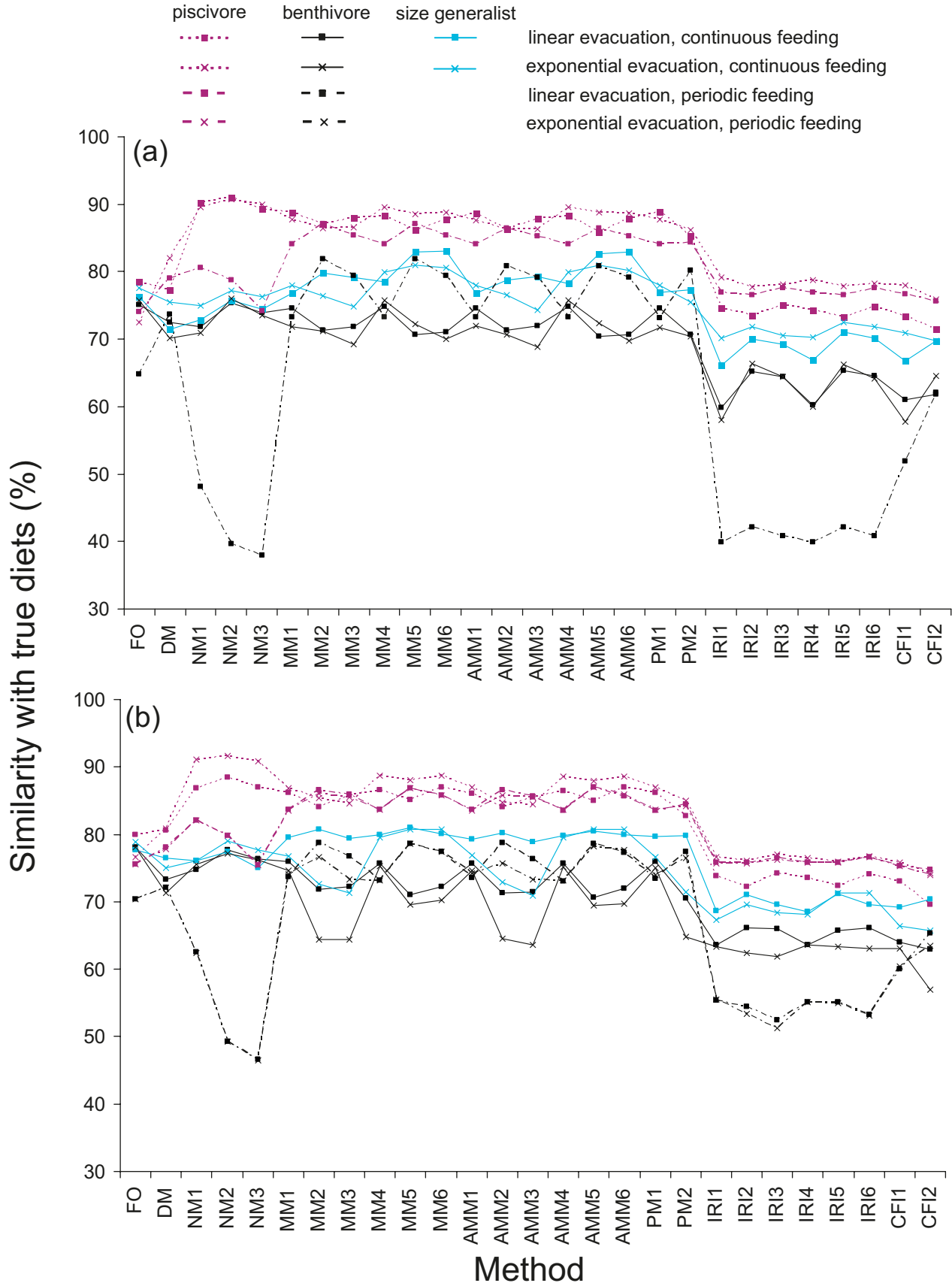
The large overestimation of small prey derived with FO and NM, both in simulations of continuously and periodically feeding fish (Figs. 5a, 5c), can be explained by both methods giving the same “importance” to all prey irrespective of their mass; hence, the diet contribution of small prey became overestimated. For the same reason, these methods underestimated the diet contribution of larger prey (Figs. 5b, 5d). This caused FO to produce lower similarities to the true diets in piscivorous fish than did other methods (Fig. 6). Because of the overestimation of small prey (Figs. 5a, 5c), FO also had a somewhat lower similarity relative to the MM, AMM, and PM methods for the size generalist, especially for linear evacuation (Fig. 6).

The NM methods gave high similarities relative to the other methods in simulations of piscivorous and benthivorous fish with continuous feeding and delayed sampling, but gave somewhat lower similarities for the size generalist, particularly for linear evacuation (Fig. 6), again owing to the overestimation of small prey. The NM methods were hence sensitive to the prey size composition. As prey size range was larger in the size generalist compared with the piscivore and benthivore, it resulted in a decreased similarity for the NM compared with the other methods (Fig. 6), as the smallest prey now contributed even less to the total prey mass but still had the same “importance” in numbers.

The NM and FO methods also had lower similarities (Fig. 6) and higher variation_a (Fig. 4) in simulations of periodically feeding fish, as small prey, that did not dominate in terms of mass, were nevertheless often numerous in stomachs of that kind of fish. During simulations of continuous evacuation, on the other hand, small prey disappeared from the stomachs quickly because of their high evacuation rate and the overestimations of small prey by NM; therefore, FO were to some extent compensated for during these conditions. Similarities determined by NM and FO increased in all simulations of delayed catch (Fig. 6b) compared with simulations of instantaneous catch for the same reason. However, periodic feeding still had lower similarity than continuous feeding.

NM performed opposite to the mass-based methods when it came to using pooled or individual analysis in the different feeding modes. Pooled NM methods were somewhat better than the individual NM for simulations of continuous feeding

Fig. 6. The performance of all methods relative to each other for piscivorous, benthivorous, and size generalist fish types for (a) instantaneous sampling (e.g., electro fishing) and (b) delayed sampling (e.g., gillnets or traps). During periodic feeding, the instantaneous sampling produces identical results for digested and back-calculated prey mass, as no evacuation has occurred when the fish is caught.



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(Fig. 6), as the overestimation of small prey relative to larger prey was reduced in this way (Figs. 5a, 5b), but during simulations of periodic feeding, individual NM was better (Fig. 6). The DM showed a stable similarity index across fish types (variation_b; Fig. 4) and usually performed moderately compared with the other methods (Fig. 6). It often overestimated small prey in simulations of continuous feeding, especially for piscivores and size generalists, but in some cases small prey were instead estimated to zero (Fig. 5a). The smallest prey very seldom dominate the stomach content of individual fish, but when they did it gave a large overestimation, as those prey contributed little to the total diet.

Index of Relative Importance and Comparative Feeding Index

The composite indices performed consistently poor in relation to the other methods (Fig. 4, Fig. 6). The %IRI had high among scenario variation (variation_b; Fig. 4), being sensitive to fish characteristics, and also high among sample variation (variation_a; Fig. 4) as did CFI (variation_a; Fig. 4), indicating that larger samples are needed to produce a robust diet composition estimate with these methods.

The composite indices generally overestimated small prey in the size generalist and the benthivorous fish (Figs. 5a, 5c). The methods showed slightly opposite patterns for the estimation of large prey depending on fish type, both in simulations of continuous and periodic feeding (Figs. 5b, 5d). The %IRI consists of three component methods (FO, NM, and MM), which in the combination of the %IRI ($\%IRI = FO \times (\%NM + \%MM)$) gave lower similarities than the component methods did when used separately (Fig. 4). To assess why the %IRI behaved this way, the component methods were removed one at a time and any two of the remaining methods were combined ($\%NM + \%MM$ or $FO \times \%NM$ or $FO \times \%MM$) and thereafter compared with each other and with the %IRI. The combination $\%NM + \%MM$ performed considerably better than the combinations involving multiplication in all fish types. This indicates that the multiplication of methods is problematic. The CFI is a multiplication of FO and PM, again resulting in diets less similar to the true diets than the component methods arrived at when used separately. Hence, multiplication of methods, also discussed by Hyslop (1980), should be avoided, since this tends to amplify the over- or under-estimation of prey.

Analyzing pooled samples commonly produced lower among sample variation (variation_a) than analyses based on individual fish for all 27 methods (Fig. 4). However, %IRI and CFI had higher variation_a than the other methods both as pooled and individually analyzed, having about 50% larger variation than the mass-based methods (Fig. 4). Variation_b was more variable, but was higher in the pooled methods of NM, MM, AMM, and PM.

Results of sensitivity analysis

The parameterization of the model fish was made to produce certain diet compositions and stomachs with realistic variation in prey composition and not in an effort to mimic the reality of fish foraging. To evaluate if the parameterization influenced the model outcome and hence our conclusions, we evaluated the diet analyses methods for a wide range of parameter values. This resulted in fish and environ-

ments with different characteristics (Table 1). As discussed in the Materials and methods section, we extended the exploration of the sensitivity of our findings in the main scenarios by using additional differences in parameter settings in the model (Table 6). The additional prey capture probabilities (Table 2) produced virtually the same results as the main scenarios (Table 6), with basic methods (FO, NM, MM, AMM, PM) producing results more similar to the true diets than the composite indices (%IRI, CFI). DM varied some in relation to the other methods.

In addition to the effect of evacuation rates explored in the simulations of periodic versus continuous feeding, simulations with two different versions of random evacuation rates were explored for the piscivorous and benthivorous fish with continuous exponential evacuation (Table 3). In simulations of the benthivorous fish, the pooled mass-based methods using digested prey masses (MM2–MM3, AMM2–AMM3, PM2) in one case (random benthivore 1; Table 3) produced similarities as low as those produced by the %IRI and CFI (Table 6). The explanation is that smaller prey could remain longer in the stomach than larger prey when evacuation was random and the use of digested prey masses could then bias the results substantially. In simulations of the piscivorous fish, the method's performances in relation to each other remained similar to simulations with original evacuation rates as long as the largest prey were having longer passage times than smaller prey. However, when the largest prey had a shorter passage time (random piscivore 1; Table 3), the NM had decreased similarities, as the largest prey become underestimated. NM, MM2–MM3, AMM2–AMM3, and PM2 were hence sensitive to evacuation rates.

Prey distributions between habitats, time spent in different habitats, and alternative least possible prey identification mass (1% or 10% of original mass) were explored, as were two alternative predator stomach sizes (Table 6). None of the results from these simulations influenced conclusions substantially. As discussed above, the way the fish were sampled affects the order of the methods just slightly.

In relation to previous findings

In nature there are many factors that influence fish diet composition and generate variation in stomach content among individuals (e.g., the size of the fish or when and where it feeds). A consequence of this is that large numbers of fish may have to be analyzed to derive a representative average diet. At the same time, each stomach analysis can be very time consuming, particularly if there are difficulties in identifying and counting prey because they have been heavily masticated or digested. Hence, easy and fast methods may be preferable, as they will allow for more fish to be examined. It is therefore encouraging to see that the basic methods perform well, including the approximate points method.

Different methods provide different insight into the feeding of animals. For example, mass-based methods can provide information about impact on prey population or dietary nutritional values, numerical methods can describe feeding behaviour of individuals by showing number of prey taken, and FO shows population-wide food habits by showing how many individuals of a population feeds on what prey species (Pierce and Boyle 1991; Cortés 1997). In this study we used them all with the objective to describe the diet in terms of

Table 6. The different simulations run in the sensitivity analysis.

Simulation	Parameter changed	Range of change	Result compared with main scenarios
Generalist 1	Prey probabilities	See Table 2	Similar to benthivore
Generalist 2	Prey probabilities	See Table 2	Similar to benthivore
Generalist 3	Prey probabilities	See Table 2	Similar to benthivore
High fail	Prey probabilities	See Table 2	Similar to benthivore
Low fail	Prey probabilities	See Table 2	Similar to benthivore
Random piscivore 1	Prey evacuation rates $k_{\text{random}} = k_{\text{original}} \cdot [1 + \text{Rand}(0.5)]$	See Table 3	Decreased NM compared with piscivore
Random piscivore 2	Prey evacuation rates $k_{\text{random}} = k_{\text{original}} \cdot [1 + \text{Rand}(0.5)]$	See Table 3	Similar to piscivore
Random benthivore 1	Prey evacuation rates $k_{\text{random}} = k_{\text{original}} \cdot [1 + \text{Rand}(0.5)]$	See Table 3	Decreased MM2–MM3, AMM2–AMM3, and PM2 compared with benthivore
Random benthivore 2	Prey evacuation rates $k_{\text{random}} = k_{\text{original}} \cdot [1 + \text{Rand}(0.5)]$	See Table 3	Similar to benthivore
Identification level	Prey identification level	Identification of prey at 10% instead of 1%	Similar to benthivore
Stomach size	Predator stomach size	From $L^3/100$ to $L^3/10$	Similar to benthivore
Cycles	No. of predation cycles in each habitat	From 50%/50% to 30%/70%	Similar to benthivore
Prey availability	Available prey types in each habitat	Prey types 5, 10 (Habitat A) and 1, 6 (Habitat B) missing instead of prey types 1, 2 (Habitat A) and 9, 10 (Habitat B)	Similar to benthivore

Note: “Parameter changed” indicates which parameter was changed, “Range of change” indicates how much the parameter was changed, and “Result compared with main scenarios” indicates how this change affected the performance of the method in relation to the main scenarios.

energetic–nutritional composition, as this is a common objective in diet analysis and also basic input data in, for example, quantitative food web analyses and is frequently used in models such as Ecosim–Ecopath (Walters et al. 1997).

Hyslop (1980) concluded in a review of methods for diet analysis that calculations based on prey mass give the best results, while he criticized the points method for being too subjective. Our findings are reasonably consistent with this. The mass-based methods produced diets that were consistently more similar to the true diets than the other methods. However, we conclude that the PM with an approximately logarithmic scale (e.g., Hynes 1950 and Christensen 1978 previously used logarithmic scales) mirrors the diet well (Fig. 4). This method has the advantage of being faster than MM.

Despite our focus on diet composition expressed in mass, the FO and the NM performed surprisingly well. The NM methods were, however, more variable than the mass-based methods, strongly influenced by feeding mode and prey traits. In nature, digestion rates are influenced by a number of factors like periods of food deprivation (Elliott 1972; cf. Hyslop 1980), temperature (Reimers 1957), and prey qualities like energy content (Jobling 1980) and hard or indigestible body parts (Hess and Rainwater 1939). These factors may then particularly impact diets calculated from frequency of occurrence and numbers in the stomachs. The FO has earlier been criticized for ignoring the relative amounts of prey (Hyslop 1980) and exaggerating the importance of incidental prey and prey with a long passage time due to hard body parts (Pierce and Boyle 1991). This is in accordance with our simulations, as seen in the simulations of the piscivorous, periodically feeding fish. In this type of fish, small prey were rare and there was no digestion during feeding, making small prey stay as long as large prey in the stomach, resulting in

overestimation of small prey (Fig. 5c), as they are “weighted” the same irrespective of their number or mass because FO only measures presence or absence, making FO perform poorly (Fig. 6). The NM methods have been criticized for overestimating small prey taken in large numbers (Hyslop 1980), and not surprisingly this is confirmed by our simulations, as the NM methods performed worse in the size generalist fish, where prey size differences were large, and in fish with periodic feeding, when small prey were present in larger numbers in the stomach.

Fish with large numbers of prey in the stomach, like zooplanktivores, were not simulated in this study. The diet of a zooplanktivorous fish could, however, be seen as an extrapolation of the periodically feeding benthivorous fish, indicating that the NM would perform poorly for zooplanktivorous fish. This is also emphasized by Hyslop (1980). Prey with long passage time will also be overestimated by the NM according to Hyslop (1980). This is shown in the simulations of the periodically feeding fish where the smaller prey got overestimated because of the absence of evacuation during feeding in this scenario. In practice, a problem with NM is the difficulty of counting the number of prey when prey are thoroughly masticated, digested, or do not appear in discrete units like detritus (Hyslop 1980). The simulations comparing possible prey identified at a level of 1% of original prey mass versus 10% of original prey mass addresses some of these problems. We found that there was no difference in the relative performance of the methods between these two identification thresholds. Generally speaking, the FO and the NM methods are easy to use, fast, and do not require time consuming mass determinations or other measurements of prey. However, these methods had on average lower similarity indices and (or) were less robust (Fig. 4, Fig. 6), making other methods more reliable.

Hyslop (1980) further debates the reliability of indices like %IRI in his review. An index incorporating values from different methods should be more representative according to some authors (Windell 1971; Cortés 1997), as several measurements are incorporated. Hyslop seems sceptical towards this, pointing out the increasing number of error sources. Bigg and Perez (1985) also uphold the shortcomings of such methods because of biases from food types comprising less than 1% of the component methods. Bigg and Perez (1985) compared the %IRI and the simple volumetric method and found them to perform similarly, but when doing the same comparison using non-trace data (data disregarding body parts that are more resistant to digestion like squid beaks), the %IRI method gave deviating results compared with the volumetric method. Hansson (1998) criticizes the %IRI method for producing variable results depending on the taxonomic resolution. Our simulations show that the %IRI method, together with the CFI method, generally produces diets that deviate more from the true diet compositions than the other methods, likely because of the multiplication of diet percentages derived from other diet analysis methods (discussed above).

In this model, foraging is assumed to be the fish's only activity. In real life, this is of course not the case. For example, social interactions like inter- and intra-specific competition will occur. During competition, fish have been shown to either specialize or generalize to decrease dietary overlap (e.g., Wiens 1993; Gabler and Amundsen 2010). This will be a problem for methods like the NM and FO that diverge in performance during different scenarios; however, the mass-based methods (MM, AMM, PM) are quite stable and should be able to predict diets accurately even if fish pursue different feeding strategies concerning prey choice or feeding mode. Another phenomenon occurring in nature is ontogenetic diet shifts. This is not addressed in this study because the diet difference between the smallest and largest fish (15–25 units) are not large enough to represent an ontogenetic shift. Rather the simulations are aimed at mirroring a sample of fish with comparable diets, as in situ samples of fish are often divided into size classes where a relatively homogeneous diet is expected. Even if ontogenetic diet shifts are not modelled in this study, one of the mass-based methods should be able to predict diet composition well even in samples with fish on both sides of a diet shift.

The aim of this study was to explore how characteristics of fish and sampling methods influenced the performance of different diet analysis methods when compared with a “true” diet of the fish. Prey preference, prey size, and evacuation rates are important factors influencing the performance of the diet analysis methods. Basic methods (FO, DM, NM, MM, AMM, and PM) performed on average better than composite indices (%IRI and CFI). FO, DM, and NM described the true diet surprisingly well despite it being expressed in mass; however, FO and NM varied in performance (similarity with true diet) in relation to other methods, producing diet estimates most similar or least similar to the true diet depending on fish type. All MM, AMM, and PM methods produced on average diet compositions most similar to the true diet and were most robust (lowest variation_a and variation_b). This shows that mass-based methods should be used for diet studies aiming at describing the energetic–nutritional sources

of fish and the quantitative energy flow in food webs. Some potential biases were noted in MM1, MM4, AMM1, AMM4, and PM1, resulting in the overestimation of small prey types. If there are not enough resources for rigorous weighing or identification or if large or many samples are required to describe diet variation over large areas, long time periods, or for a wide size range of fish, the faster PM could be used.

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References

- Bigg, M.A., and Perez, M.A. 1985. Modified volume: a frequency–volume method to assess marine mammal food habits. *In* Marine mammals and fisheries. Edited by J.R. Beddington, R.J.H. Beverton, D.M. Lavigne, and D. Martin. Allen & Unwin, London, UK. pp. 277–283.
- Christensen, M.S. 1978. Trophic relationships in juveniles of the species of sparid fishes in the South African marine littoral. *Fish Bull.* **76**(2): 389–401. Available from <http://fishbull.noaa.gov/76-2/christensen.pdf> [accessed 5 July 2011].
- Cortés, E. 1997. A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. *Can. J. Fish. Aquat. Sci.* **54**(3): 726–738. doi:10.1139/cjfas-54-3-726.
- Elliott, J.M. 1972. Rates of gastric evacuation in brown trout, *Salmo trutta* L. *Freshw. Biol.* **2**(1): 1–18. doi:10.1111/j.1365-2427.1972.tb01575.x.
- Gabler, H.-M., and Amundsen, P.-A. 2010. Feeding strategies, resource utilisation and potential mechanisms for competitive coexistence of Atlantic salmon and alpine bullhead in a sub-Arctic river. *Aquat. Ecol.* **44**(2): 325–336. doi:10.1007/s10452-009-9243-x.
- Hansson, S. 1998. Methods of studying fish feeding: a comment. *Can. J. Fish. Aquat. Sci.* **55**(12): 2706–2707. doi:10.1139/f98-158.
- Heincke, F. 1908. Bericht über die Untersuchungen der Biologischen Anstalt auf Helgoland zur Naturgeschichte der Nutzfische. Die Beteiligung Deutschlands an der Internationalen Meeresforschung, **4/5**: 67–155.
- Hess, A.D., and Rainwater, J.H. 1939. A method for measuring the food preferences of trout. *Copeia*, **1939**(3): 154–157. Available from <http://www.jstor.org/stable/1436810> [accessed 5 December 2010].
- Hynes, H.B.N. 1950. The food of fresh-water sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*), with a review of methods used in studies of the food of fishes. *J. Anim. Ecol.* **19**(1): 36–58. doi:10.2307/1570.
- Hyslop, E.J. 1980. Stomach content analysis — a review of methods and their application. *J. Fish Biol.* **17**(4): 411–429. doi:10.1111/j.1095-8649.1980.tb02775.x.
- International Council for the Exploration of the Sea. 1997. Report of the study group on multispecies model implementation in the Baltic. 4–10 December 1996, ICES, Copenhagen, Denmark. CM 1997/J:2.
- Jobling, M. 1980. Gastric evacuation in plaice, *Pleuronectes platessa* L.: effects of dietary energy level and food composition. *J. Fish Biol.* **17**(2): 187–196. doi:10.1111/j.1095-8649.1980.tb02752.x.
- Jobling, M. 1986. Mythical models of gastric emptying and implications for food consumption studies. *Environ. Biol. Fishes*, **16**(1–3): 35–50. doi:10.1007/BF00005158.

- Pierce, G.J., and Boyle, P.R. 1991. A review of methods for diet analysis in piscivorous marine mammals. *In* *Oceanogr. Mar. Biol. Rev.* 29. *Edited by* M. Barnes. Aberdeen University Press, Aberdeen, UK. pp. 409–486.
- Pinkas, L., Oliphant, M.S., and Iverson, I.L.K. 1971. Food habits of albacore, bluefin tuna, and bonito in California waters. *Fish Bull.* 152: 1–105.
- Reimers, N. 1957. Some aspects of the relation between stream foods and trout survival. *Calif. Fish Game*, 43(1): 43–69.
- Schoener, T.W. 1970. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology*, 51(3): 408–418. doi:10.2307/1935376.
- Swedberg, D.V., and Walburg, C.H. 1970. Spawning and early life history of the freshwater drum in Lewis and Clark Lake, Missouri River. *Trans. Am. Fish. Soc.* 99(3): 560–570. doi:10.1577/1548-8659(1970)99<560:SAELHO>2.0.CO;2.
- Swynnerton, G.H., and Worthington, E.B. 1940. Notes on the food of fish in Haweswater (Westmorland). *J. Anim. Ecol.* 9(2): 183–187. doi:10.2307/1454.
- Temming, A., and Herrmann, J.-P. 2003. Gastric evacuation in cod: prey-specific evacuation rates for use in North Sea, Baltic Sea and Barents Sea multi-species models. *Fish. Res.* 63(1): 21–41. doi:10.1016/S0165-7836(03)00041-9.
- Walters, C.J., Christensen, V., and Pauly, D. 1997. Structuring dynamic models of exploited ecosystems from trophic mass-balanced assessments. *Rev. Fish Biol. Fish.* 7(2): 139–172. doi:10.1023/A:1018479526149.
- Wiens, J.A. 1993. Fat times, lean times and competition among predators. *Trends Ecol. Evol.* 8(10): 348–349. doi:10.1016/0169-5347(93)90216-C. PMID:21236192.
- Windell, J.T. 1971. Food analysis and rate of digestion. *In* *Methods for assessment of fish production in fresh waters*. 2nd ed. *Edited by* W.E. Ricker. Blackwell Scientific Publications, Oxford and Edinburgh, UK. pp. 215–226.