

# Quantifying diets for small pelagic fish: effects of weight versus occurrence methods and sampling effort

Erik Askov Mousing<sup>\*</sup>, Benjamin Planque, Per Arneberg, Vilde Regine Bjørdal, Felicia Keulder-Stenevik, Penny Lee Liebig, Herdis Langøy Mørk and Mette Skern-Mauritzen

Institute of Marine Research, Ecosystem Processes Group, Bergen, Vestland 5005, Norway

<sup>\*</sup> Corresponding author: tel: +4740427087; e-mail: [erik.askov.mousing@hi.no](mailto:erik.askov.mousing@hi.no).

In the Norwegian Sea, assessment of diet composition relies on annual cruise activity combined with visual identification and weight determination of prey from fish stomachs. This weight-based method is labour intensive, and suggestions to reduce cost include moving from the weight-based to the occurrence-based method and/or reducing sampling effort. Studies have suggested that the occurrence-based method may be more robust while providing similar results as the weight-based method. Here we re-analyse data from >14.000 stomachs for herring, mackerel, and blue whiting. We compare diet composition estimates and quantify the uncertainty using both methods. We also quantify the impact of reducing sampling effort (number of trawl stations and fish per station) on the diet uncertainties. Our results confirm that occurrence-based estimates are more precise than those based on weight. In addition, they are better at capturing year-to-year fluctuations. The occurrence-based method provides similar results to the weight-based method. Differences between methods arise primarily from disparities in the mean weight of prey in stomachs. Decreasing the number of stations sampled leads to increased uncertainty, while reducing the sampling effort from 10 to 5 fish per station has little impact on uncertainty estimates. These results provide quantified insights to guide future diet monitoring programmes.

**Keywords:** diet, Norwegian Sea, pelagic fish, sampling effort, stomach content.

## Introduction

Knowledge about diet composition is fundamental for understanding how species interactions and energy flow affect the structure and dynamics of ecosystems (Pascual and Dunne, 2006; McCann, 2007). Despite great potential in practical applications (Memmott, 2009), the use of diet information for supporting management decisions in the marine realm has thus far been limited (Skern-Mauritzen *et al.*, 2016). However, some recent applications of ecosystem models, relying on species interactions derived from diet studies, have been used to support decisions in fisheries management (Howell *et al.*, 2021).

The fish community in the Norwegian Sea is dominated by three pelagic species: Northeast Atlantic (NEA) mackerel (*Scomber scombrus*), Norwegian spring spawning (NSS) herring (*Clupea harengus*), and blue whiting (*Micromesistius poutassou*), which support the largest fisheries in the region (ICES, 2021). The three species feed in the Norwegian Sea during spring and summer before returning to overwintering areas at the edge of or outside the region (Skjoldal and Sætre, 2004). Their diets are dominated by mesozooplankton, with the calanoid copepod *Calanus finmarchicus* being particularly important for mackerel and herring. All three species are highly opportunistic feeders, and their diets can vary considerably in space and time. Thus, in addition to copepods, the diets of mackerel and herring include euphausiids, amphipods, and non-crustacean prey such as appendicularians, gastropods, and fish larvae (Dalpadado *et al.*, 2000; Gislason and Astthorsson, 2002; Dommasnes *et al.*, 2004; Prokopchuk and Sentyabov, 2006; Langøy *et al.*, 2012; Skaret *et al.*, 2015; Bachiller *et al.*, 2016; Allan *et al.*, 2021). Cope-

pods are less important for blue whiting, with larger prey such as amphipods, euphausiids, and appendicularians dominating their diet (Prokopchuk and Sentyabov, 2006; Langøy *et al.*, 2012; Bachiller *et al.*, 2016). Substantial dietary overlap is often observed between mackerel and herring, while there is some overlap in diet between mackerel and blue whiting, and to a lesser extent also between herring and blue whiting (Prokopchuk and Sentyabov, 2006; Langøy *et al.*, 2012; Bachiller *et al.*, 2016).

Different methods are available for diet determination, including visual and molecular analyses of stomach, intestine, or scat content, as well as chemical analyses of stable isotopes, fatty acids, and other biomarkers in the predator tissue (Traugott *et al.*, 2013; Nielsen *et al.*, 2018). While visual and molecular methods can give direct information on the prey ingested recently, analyses using biomarkers can provide information on diet over longer time periods as well as trophic level. Estimates of diet composition have been applied to answer several different research questions, including interspecific competition (Shaw *et al.*, 2008), predator–prey relationships (Cabral and Murta, 2002; Holt *et al.*, 2019), diet width and overlap (Langøy *et al.*, 2012; Knickle and Rose, 2014), as well as energetic-nutritional composition and input to quantitative food web analyses and ecosystem models (Nilsen *et al.*, 2020).

For pelagic fish in the Norwegian Sea, diet assessments have mostly been based on “conventional” visual inspection of gut content, assessing composition using relative weights (i.e. the weight-based method). This method provides diet estimates that are of direct relevance to estimating weight and energy consumption, but it is costly and labour intensive. Alternative

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methods based on visual inspection that are less expensive or demanding exist. Amundsen and Sánchez-Hernández (2019), in a review of six different diet assessment methods, concluded that the choice of method is dependent on the research question, and pointed out that while the occurrence-based method is by far the easiest and least labour intensive, it does not provide information on the relative importance of prey taxa at the individual level. At the population level, however, Baker *et al.* (2014) argued that calculating the frequency of occurrence by summing up present taxa over a collection of fish to represent a frequency distribution was more robust and retained information similar to the outputs from the more labour-intensive weight-based method.

The relevance of the occurrence-based method is amplified by the potential of molecular methods to increase diet estimate precision and open new avenues of research (Pompanon *et al.*, 2012; Nielsen *et al.*, 2018). Two recent studies using molecular techniques found herring larvae to be more common in the stomachs of mackerel than suggested in previous, visual-based studies (Skaret *et al.*, 2015; Allan *et al.*, 2021), probably due to fast degradation and the subsequent failure of visual detection. Similarly, jellyfish, which are easily digested and hard to detect using visual inspection, have been shown by the molecular method to be commonly ingested by herring in the Irish Sea and Gulf of Maine (Bowser *et al.*, 2013; Lamb *et al.*, 2017). For molecular methods, an occurrence-based method is currently the most suitable option due to challenges in quantifying biomass and/or abundance of prey (van der Loos and Nijland, 2021).

The performance of the weight-based and occurrence-based methods for estimating both diet composition and uncertainty has not been evaluated empirically on large spatiotemporal scales. The Institute of Marine Research (IMR) in Norway has collected stomach samples and estimated diet composition for several decades using the weight-based method. Diet composition estimates have multiple use cases (see above), but a major objective is to assess the relative importance of different prey groups in the diet and track potential inter-annual changes. For this assessment, a full or partial transition from the weight-based to the occurrence-based method could lead to reduced costs and greater compatibility with upcoming molecular methods. It should be noted, however, that the occurrence-based method may not be ideal for other use-cases such as estimating consumption rates or prey selection (Amundsen and Sánchez-Hernández, 2019) which should be considered when evaluating changes to the assessment procedure. A complementary way of reducing costs using either the weight-based or the occurrence-based methods is to decrease the sampling effort by reducing the number of trawl stations sampled and/or the number of fish sampled at each station. Transitioning from weight-based to occurrence-based methods and reducing sampling effort would require a prior assessment of the impact on the diet estimates and the continuation of the time-series.

In this study, we apply both the weight-based and the occurrence-based methods to a large data set of pelagic fish stomachs collected during two decades of trawl surveys in the Norwegian Sea. The objective is to compare estimates of diet composition for the major prey groups between the two methods, both in terms of the information they contain as well as to quantify the uncertainty of their estimates. In addition, we assess the impacts of reducing the number of stations and

reducing the number of fish sampled at each station on diet uncertainty estimates.

## Methods

### Data collection

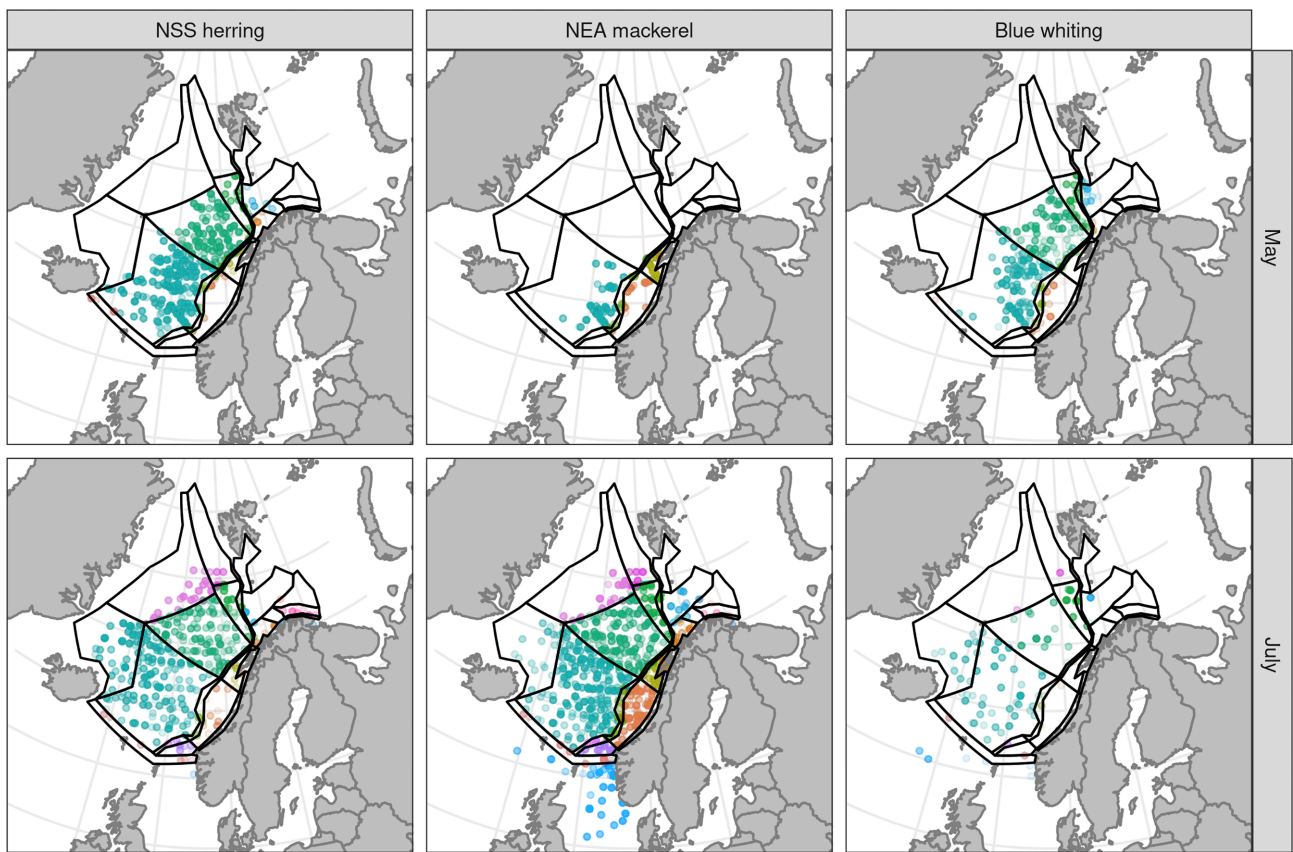
Data on stomach content for surveys in May [International Ecosystem Survey in the Nordic Seas (IESNS)] and July [International Ecosystem Summer Survey in the Nordic Seas (IESSNS)] for NSS herring, NEA mackerel, and blue whiting were extracted from the IMR database. In total, 36 surveys were included, with a total of 14462 sampled fish stomachs and 52643 individual prey registrations. A map of the trawl station locations, covering all years, is presented in Figure 1, and a summary is presented in Table 1. Onboard the ship, stomach samples were collected from ten randomly selected fish, which are usually representative of the catch of the pelagic fish species. Exceptions were torn stomachs, in which case the entire individual sample was replaced with a new randomly selected fish. Whole stomachs were removed by cutting off as far up the oesophagus and as close as possible to the appendix, respectively, to ensure that stomach contents were intact. Stomachs were individually packed in bags with station and sampling information and frozen for detailed analyses in the laboratory on land (see below). We refer to Salthaug *et al.* (2020) and Nøttestad *et al.* (2021) for a detailed description of the IESNS and IESSNS surveys, respectively.

### Laboratory analyses

Diet compositions were quantified in the laboratory through taxonomic classification of prey items and by visually inspecting the content of frozen stomach samples. Each stomach was defrosted and carefully opened with a scalpel or scissors. The contents were transferred to a glass petri dish, excluding as much stomach lining fat and mucus as possible. The contents were inspected under a stereomicroscope while taking care to prevent the sample from drying out by adding a few drops of fresh water. Prey items were separated from each other as much as possible and classified to the highest taxonomic resolution possible and noting down the digestive grade category.

The taxonomic classification of prey items was done following the IMR standard procedure for analysing pelagic fish diets outlined in the manuals for sampling fish and crustaceans, following Mjanger *et al.* (2019) for samples collected from 1996 to 2011 and Langøy Mørk (2020) for samples collected from 2012 to 2016. The procedure focuses on identifying and quantifying the most dominant and ecologically important taxa in more detail than less abundant taxa. Important taxa include calanoid copepods (*Calanus* spp., *Metridia* spp.), krill (*Meganycthyphanus* spp., *Thyssanoessa* spp.), hyperiid (*Themisto* spp.), and fish prey species. These prey items were identified to the species level where possible, with counts, developmental stage, and length measurements recorded for selected species. Other prey items may have been identified to a lower taxonomic resolution depending on the overall time required to process the entire sample.

After sorting, identifying, measuring, and counting, separate prey groups were transferred to pre-weighed aluminium foil trays before they were dried at 70°C for 24 h. The total



**Figure 1.** Map of the sampling region and locations of trawl stations in May and July for all years combined. Trawl locations are coloured according to the Atlantis regions (black polygons, Figure S1), representing the geographical regions used in the re-sampling procedure.

**Table 1.** Summary of included cruises per year and month, the total number of trawl stations, fish stomachs sampled, and prey taxa registered.

Species	Years	Month	No. of stations	No. of fish	No. of prey
NSS herring	1996, 2000–2003, 2005–2016	May	309	2 894	11 453
NES mackerel	2009–2016	May	76	703	3 459
Blue whiting	2000–2001, 2003, 2005–2006	May	243	1 856	5 723
NSS herring	2004–2007, 2009–2010, 2012–2016	July	373	3 015	9 770
NEA mackerel	2004–2007, 2009–2016	July	594	5 296	19 979
Blue whiting	2004–2007, 2009–2010, 2012–2013, 2016	July	93	699	2 259

weight of each prey item identified was then recorded as its dry weight in milligrams.

### Data preparation

Stomach content data was extracted for each individual survey and combined to comprise data sets for May and July, individually. Additional taxonomic information for the prey taxa was added using the “taxize” package v0.9.99 (Chamberlain and Szöcs, 2013) based on the Taxonomic Serial Number (TSN). The IMR database generally conforms to the default ITIS classification (Integrated Taxonomic Information System; <https://www.itis.gov/>), but includes a few custom categories. In these cases, taxonomic information was added manually to each record. Undetermined prey items (“Indeterminatus”) were removed prior to the statistical analyses.

In general, prey taxa had been identified to a relatively low taxonomic resolution. The major exceptions were *Calanus*

spp. and *Themisto* spp., where a high proportion of the registered taxa were identified to the species level (80% of all species-level registrations). This distribution was not surprising given how the samples were processed (see above) and posed a challenge to how diet composition should be synthesized across multiple taxonomic levels. Most taxa, however, were identified to at least the order level, and the registrations were therefore aggregated at this level or higher for subsequent analyses. This method was recommended by Buckland *et al.* (2017) for comparisons across large data sets due to all factors impacting taxa identification. In addition, the current literature on diet composition in the Norwegian Sea has also focussed on aggregations at higher taxonomic levels ( $\geq$  family, although in some cases including genera or specific species such as *C. finmarchicus*; see Introduction for references), and we therefore argue that our choice of the order level of aggregation is to a large degree comparable to earlier studies.

Trawl locations were grouped into geographical regions (Figure 1) based on the regions defined in the Nordic and Barents Seas Atlantis model (NoBa Atlantis; Hansen *et al.*, 2016). NoBa Atlantis defines 60 regions that are relatively homogeneous in terms of hydrography and bathymetry and that have been reviewed by a panel of experts in oceanography, demersal and pelagic fish, benthos, and marine mammals. In total, trawl stations were distributed across 22 Atlantis regions. For NEA mackerel, several stations were positioned south of the defined Atlantis regions (south of 61°N), but as these were predominantly found in the northern North Sea, a new region was defined, including all unassigned stations.

### Statistical analyses

All statistical analyses were performed in the free and open-source statistical software R v3.6.0 (R Core Team, 2019). See the supplementary material for a list of specific packages and versions used in this study. Analyses were performed for both the May and July surveys individually. For the analyses of the impact of station and fish sample size, the results are only presented for the July 2010 survey, as this survey contains a reasonably high number of fish and station registrations for all three species (Supplementary Tables S1–S2).

### Weight-based and occurrence-based estimates of relative contribution to the diet

Many methods for diet assessment have been developed, and we refer to Amundsen and Sánchez-Hernández (2019) and Ahlbeck *et al.* (2012) for a thorough review. A comprehensive evaluation of all the various methods was not the aim of the current work, and we focussed on two methods: (1) weight-based assessment (a.k.a. the mass or gravimetric method), which is based on visual prey detection and subsequent weighing of stomach content; and (2) occurrence-based assessment (a.k.a. the presence–absence method), which depends on visual prey detection only. We re-analysed existing data using these two methods.

For the weight-based method, we applied the pooled method [Equation (1); method MM2 in Ahlbeck *et al.* 2012] where the stomach contents of all fish are pooled prior (either across all years or within specific years) to calculating relative importance. The contribution of each fish to the final index is proportional to the total weight of prey in its stomach. The index (RW) is used to reflect the proportional weight of prey in the diet at the population level.

$$RW_i = \frac{\sum_j w_{ij}}{\sum_j w_{.j}} \quad (1)$$

Where  $w_{ij}$  is the mass of prey type  $i$  in the stomach of fish  $j$  and  $w_{.j}$  is the total mass of all prey in the stomach of fish  $j$ . This equation can be re-written in the following way:

$$RW_i = \frac{n_i \bar{w}_i}{\sum_i n_i \bar{w}_i} \quad (2)$$

Where  $n_i$  is the number of stomachs that contain prey  $i$  and  $\bar{w}_i$  is the mean weight of prey  $i$  across all stomachs that contain this prey.

For the occurrence-based assessment, we calculated the frequency of occurrence [FO; Equation (3)]. FO provides a frequency distribution, i.e. the proportion of fish containing each prey type. This proportion does not provide a measure of the relative importance of each prey type. To calculate the relative

occurrence of each prey group [RO; Equation (5)], FO was divided by the total occurrence of all prey types [TO; Equation (4)].

$$FO_i = \frac{n_i}{N} \quad (3)$$

$$TO = \frac{\sum_i n_i}{N} \quad (4)$$

$$RO_i = \frac{FO_i}{TO} = \frac{n_i}{\sum_i n_i} \quad (5)$$

Where  $N$  is the total number of fish stomachs. We can see that the RO estimate in Equation (5) is analogue to the special case of the RW estimate [Equation (2)] when the mean weights of every prey ( $\bar{w}_i$ ) consumed are identical.

### Uncertainty estimates for RO and RW

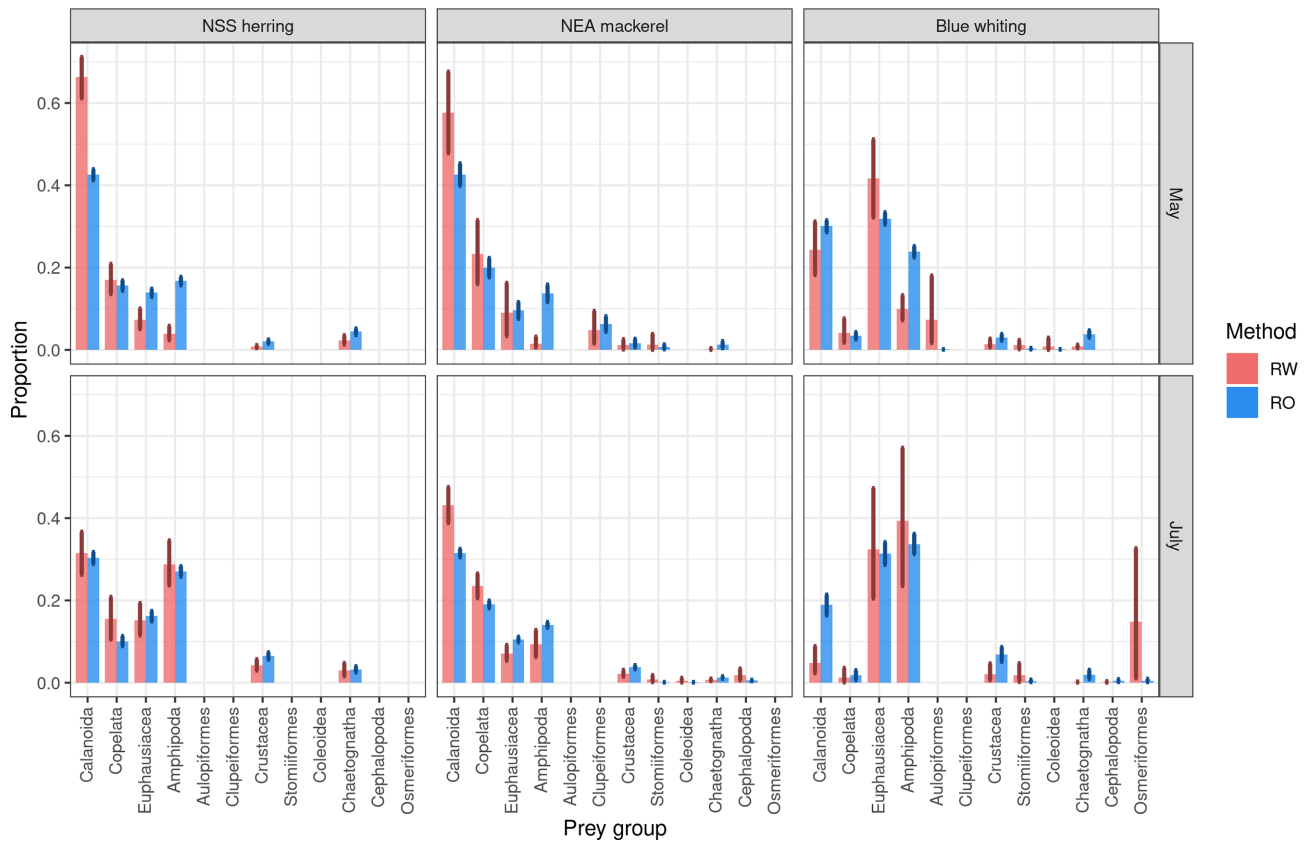
Equations (2) and (5) provide point estimates of the relative proportion in weight or occurrence of each prey group. To derive uncertainty estimates for these two quantities, we used a bootstrapping re-sampling procedure (Efron, 1992; Tibshirani and Efron, 1993). The general principle of bootstrapping is to compute the statistics of interest (here, RW and RO) from a large number of pseudo-samples. The resulting distributions are approximations of the underlying distributions of the statistics in the original sample. From these distributions, uncertainty estimates such as the 95% CI can be derived.

In conventional bootstrapping, each pseudo-sample is obtained by re-sampling the original sample with replacement. The pseudo-sample therefore has the same size as the original sample. In the current context, the original sampling protocol was hierarchical, with individual prey items sampled from individual fish, themselves sampled from individual trawl stations, themselves sampled within individual geographical regions, themselves sampled within individual years. One particularity of this sampling scheme is that the sample size of prey-per-fish and fish-per-station can vary. Prey identification is as much as possible a census, i.e. all identifiable prey items are registered and weighed. The number of fish sampled by station is a census when ten fish or less are collected. When more than ten fish are collected, only ten individuals are randomly selected for prey identification and weighing.

To derive the 95% CI for the estimates of RW and RO, we used a stratified bootstrapping procedure that mimicked the original sampling design. First, individual trawl stations were re-sampled within each geographical region. Second, individual fish were re-sampled within each station. Third, individual prey items were re-sampled within each fish. By following this procedure, bootstrapped samples did not always have the exact same number of prey items as in the original sample. However, variations in the size of re-sampled diet matrices were relatively small and should not significantly affect the resulting 95% CI for the RW and RO estimates. All bootstrap estimates were computed from 1000 pseudo-samples.

### Effect of station sample size and fish sample size on estimations uncertainties

We adapted the above bootstrapping procedure to assess the effects of station sample size and fish stomach sample size on the uncertainty estimates of RW and RO. To study the effect of station sample size, we repeated the bootstrapping procedure while gradually reducing the maximum number of stations per year from 40 to 30, 20, 15, 10, and finally 5. Similarly, to study



**Figure 2.** Mean proportion and 95% CI of the 12 most dominant prey groups in May and July across all years for the relative weight (RW; red) and relative occurrence (RO; blue) methods.

the effect of fish sample size, we repeated the bootstrapping procedure while gradually decreasing the maximum number of fish per station from 10 (as in the actual sampling design) to 8, 4, 2, and 1 fish. The point estimates of RW and RO are expected to vary little with the decreasing number of stations or fish sampled, but it is expected that the 95% CI should become broader (i.e. less certain) with decreasing sample size.

#### Time-series similarities, signal strength, and consistency

Correlation analyses were used to quantify the internal consistency of individual diet composition time-series derived from the RW and RO methods, as well as to measure the similarity between corresponding RW and RO time-series. Internal consistency was assessed by calculating the Pearson product moment correlations between resampled time-series of the same diet, where each time-series calculated using the RW or RO method was correlated to all other time-series for that respective prey group. The median and 95% CI were derived from the resulting distributions of correlation coefficients for each prey group. A positive and high correlation value is indicative of high consistency within a diet time-series using that method, i.e. the bootstrapped time-series for one prey and one diet metric.

The similarity between the RO and RW estimates was investigated in a similar fashion by calculating the Pearson product moment correlations between resampled time-series of RO versus RW estimates for the same prey. A positive and high correlation value is indicative of high similarity between the

bootstrapped time-series for RO and RW estimates for the same prey. For both internal consistency and similarity, correlation coefficients were only calculated if the two resampled data sets being compared had four or more overlapping years.

Lastly, we performed signal-to-noise analyses of each method to assess if interannual patterns in the RO and RW estimates for each prey group could be detected across the sampled period. This was done by calculating the ratio between the within-year variance and the between-year variance. A ratio  $>1$  indicates that the variance between years is greater than within years and that interannual changes in diet can be detected.

## Results

### Overall diet composition

The relative importance of the most common prey groups in May and July across all years is presented in Figure 2. Both weight- and occurrence-based methods identify Calanoida as the main prey group for NSS herring, with a relatively higher contribution in May compared to July. In July, RO and RW estimates are statistically similar for all prey groups, whereas in May, the relative contribution of Calanoida is much higher when based on weight (0.66) than on occurrence (0.43). Oppositely, the relative contributions of Amphipoda and Euphausiacea in May are greater when estimated with RO (0.17 and 0.14, respectively) than with RW (0.04 and 0.07, respectively). Calanoida is also identified as the main prey group for

NEA mackerel in May and July, and in both months the estimated relative contribution is larger when using RW (0.58 and 0.43, respectively) compared to RO (0.43 and 0.32, respectively). In contrast, the contribution of Amphipoda to the mackerel diet was higher based on the RO estimates (0.14 and 0.14, respectively) than on the RW estimates (0.02 and 0.09, respectively). For other prey groups, there are no noticeable differences in the estimated diet contribution between the two methods. For blue whiting, Euphausiacea and Calanoida are the main prey groups in May according to both methods. For Euphausiacea, the RO estimate (0.32) is less than the RW estimate (0.42), while the opposite is true for Amphipoda (0.24 and 0.10, respectively), which are estimated to contribute a similar proportion as Calanoida (0.30) using the RO method. For July, both methods identify Amphipoda (0.39 and 0.33 for the RW and RO methods, respectively) and Euphausiacea (0.32 and 0.31) as the main prey groups for blue whiting, with similar contributions to the diet. The contribution of Calanoida is significantly greater using the RO estimate (0.19) than the RW estimate (0.05), while the highly uncertain contribution of Osmeriformes estimated by RW decreases to near zero when estimated with RO (compared to 0.15 using the RW method).

The confidence intervals of occurrence-based estimates are considerably narrower than those of weight-based estimates. This pattern is particularly evident for the blue whiting in July, where RW estimates for Amphipoda (0.24–0.57), Euphausiacea (0.21–0.47), and Osmeriformes (0.01–0.33) display very broad confidence intervals, while the confidence intervals of RO estimates are much narrower (0.31–0.36, 0.29–0.34, and 0.001–0.01, respectively). The case of Osmeriformes is caricatural: with only a few fish stomachs containing large weights of Osmeriformes, the weight-based estimates are high but highly uncertain, whereas the occurrence-based estimates are low and more precise.

### Inter-annual variation in diet composition

Interannual variations in the relative importance of the most dominating prey groups in May and July are presented in Figures 3 and 4. Only prey groups with data available for four or more years and for at least one fish species were included. For some prey groups there were an insufficient number of recorded years to assess the similarity in the time-series trajectories between the RW and RO methods (i.e. <4; see Methods).

Patterns of interannual variability in May (Figure 3a) are similar ( $r > 0.5$ ) between the RO and RW methods across most prey and fish species. Both methods show a decline in the importance of Calanoida and an increase in the importance of Copelata for NSS herring and, to a lesser extent, NEA mackerel. Other prey groups show no clear long-term trend but exhibit similar interannual changes for both methods. For example, for blue whiting in May, a steep decline in the importance of Calanoida with a concomitant increase in the importance of Euphausiacea is observed from ~2005 to 2010, followed by opposite trends until ~2015, using both methods ( $r > 0.72$  and  $r > 0.75$  for the similarity between the RW and RO estimates). In general, the similarity between the two methods is supported by the correlations between time-series based on each method. Of the 19 time-series for which similarity could be assessed, 5 time-series show a strong positive relationship ( $r > 0.7$ ), 8 time-series show a moderate positive

relationship ( $0.70 > r > 0.5$ ), and 6 time-series exhibit weak relationships.

In general, annual diet composition estimates are less precise for the weight-based method than for the occurrence-based method, which is consistent with the general pattern across all years (Figure 2). Although the amplitude of interannual variations may be larger for RW than RO estimates, the results of the signal-to-noise analysis indicate interannual variations are better detected using the RO method (Figure 3b). Interannual variations are detected for all prey groups using both methods (F-ratio > 1.0), but with the RO method outcompeting the RW method for almost every prey–predator combination. The occurrence-based method also outperforms the weight-based method for time-series consistency for all but one prey–predator combination (Figure 3c).

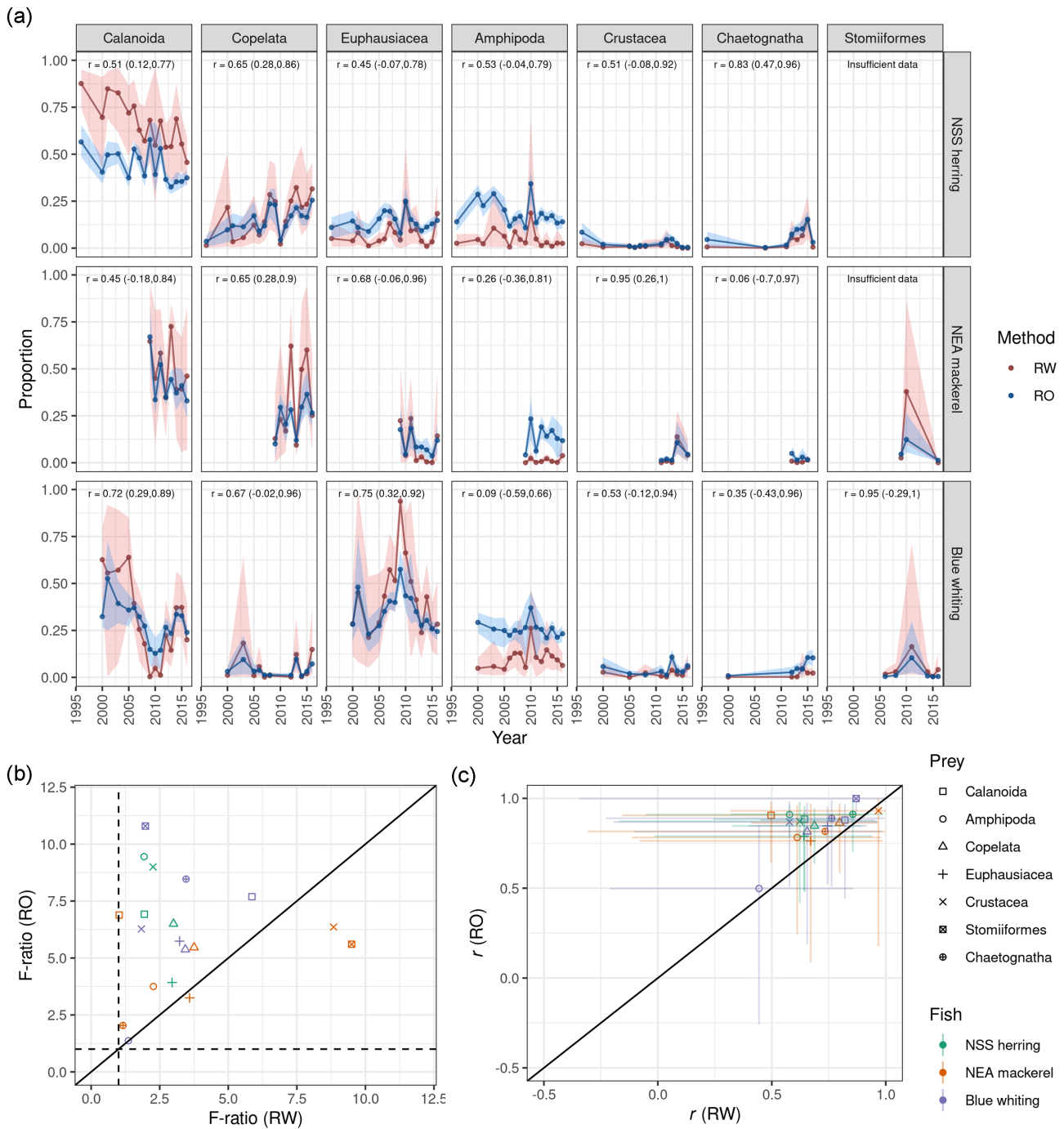
For July (Figure 4a), interannual changes are generally similar between the two methods, except for a few groups. For example, the RW-based time series indicates an increasing trend for Amphipoda in the diet of NSS herring that is not detected using the RO method. The RW- and RO-based trends in Euphausiacea's contribution to the diet of blue whiting are also largely decoupled. Of the 18 time-series for which the similarity in the trajectories between the RW and RO methods could be assessed, 9 time-series show a strong positive relationship ( $r > 0.7$ ), 3 time-series show a moderately positive relationship ( $0.7 > r > 0.5$ ), and the remaining time-series exhibit lower values. The signal-to-noise analyses (Figure 4b) show that interannual changes can be detected using both methods for all prey–predator combinations except for NSS herring preying on Calanoida using both the RW and RO methods and for blue whiting preying on Euphausiacea using the RW method. In 12 out of 20 instances, the occurrence-based method outcompetes the weight-based method in detecting interannual variations. The occurrence-based method also outcompetes the weight-based method for time-series consistency for most prey–predator combinations (Figure 4c).

For both May and July (Figures 3 and 4), the general pattern is that occurrence-based time-series of diet contribution are more precise, have a higher signal-to-noise ratio, and a higher consistency than weight-based time-series. There is some degree of similarity in the patterns of interannual fluctuations in diet between the two methods, with about two-thirds of the time-series displaying a moderate to strong positive correlation ( $r > 0.5$ ).

### Effect of station and fish sample size on estimate uncertainty

The effect of station sample size on the estimate uncertainty is illustrated for July in the year 2010 in Figure 5. Decreasing the number of stations sampled leads to broader confidence intervals for both the RW and RO methods. In general, the largest increase in uncertainty is associated with decreasing the station sample to <20 stations. In most cases, however, decreasing from 40 stations down to 20 also leads to a visible increase in uncertainties. The confidence intervals are generally broader for estimates using RW compared to the RO estimates, to the point that uncertainties in RW estimates based on full sampling (40 trawl stations) can in several instances be larger than the corresponding uncertainty for the RO estimates based on five stations only.

The effect of fish sample size on the estimate uncertainty is illustrated for July in the year 2010 in Figure 6. Reducing the

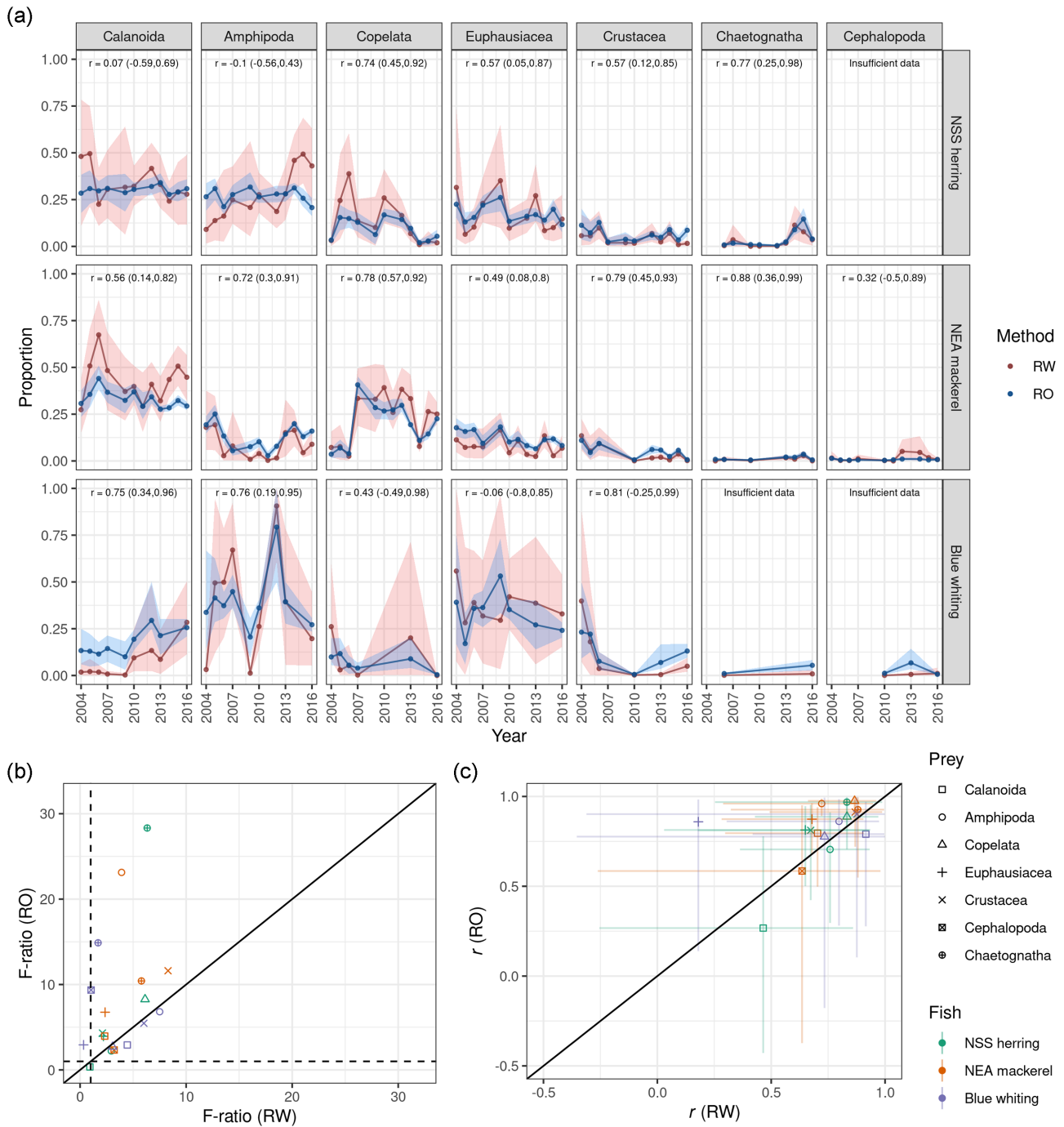


**Figure 3.** Interannual changes in May: **(a)** time-series of the mean proportion and 95% CI of the dominant prey groups in May for the relative weight (RW; red) and relative occurrence (RO; blue) methods. Numbers are the median correlation coefficients and 95% CI between RW and RO estimates across all resampled diets; **(b)** time-series signal-to-noise ratios for RW and RO estimates. Dashed lines represent the threshold below which interannual changes cannot be detected; **(c)** time-series consistency for RW and RO estimates with 95% CI. In (a) and (b), the RO method outcompetes the RW method if the points are above the 1:1 line, and vice versa.

number of fish sampled per station has, surprisingly, a minor impact on the uncertainty. For the RW estimates, the effect is often negligible when reducing from 10 to 5 fish per station, while there are noticeable increases in uncertainty when reducing sampling from 5 to 1 fish. For the RO estimates, the confidence intervals are nearly identical for the whole range of fish sampled, although a small increase in uncertainty is evident for some groups when reducing sampling effort to three fish per station or less.

## Discussion

In this study, we applied the weight-based (RW) and occurrence-based (RO) methods to estimate diet proportions of the dominating prey groups for NSS herring, NEA mackerel, and blue whiting in the Norwegian Sea. In addition, through stratified re-sampling, we derived the 95% CI around the estimates of relative prey group contribution. Overall, we show that the two methods have a large degree of similarity, although with some significant differences (discussed below).

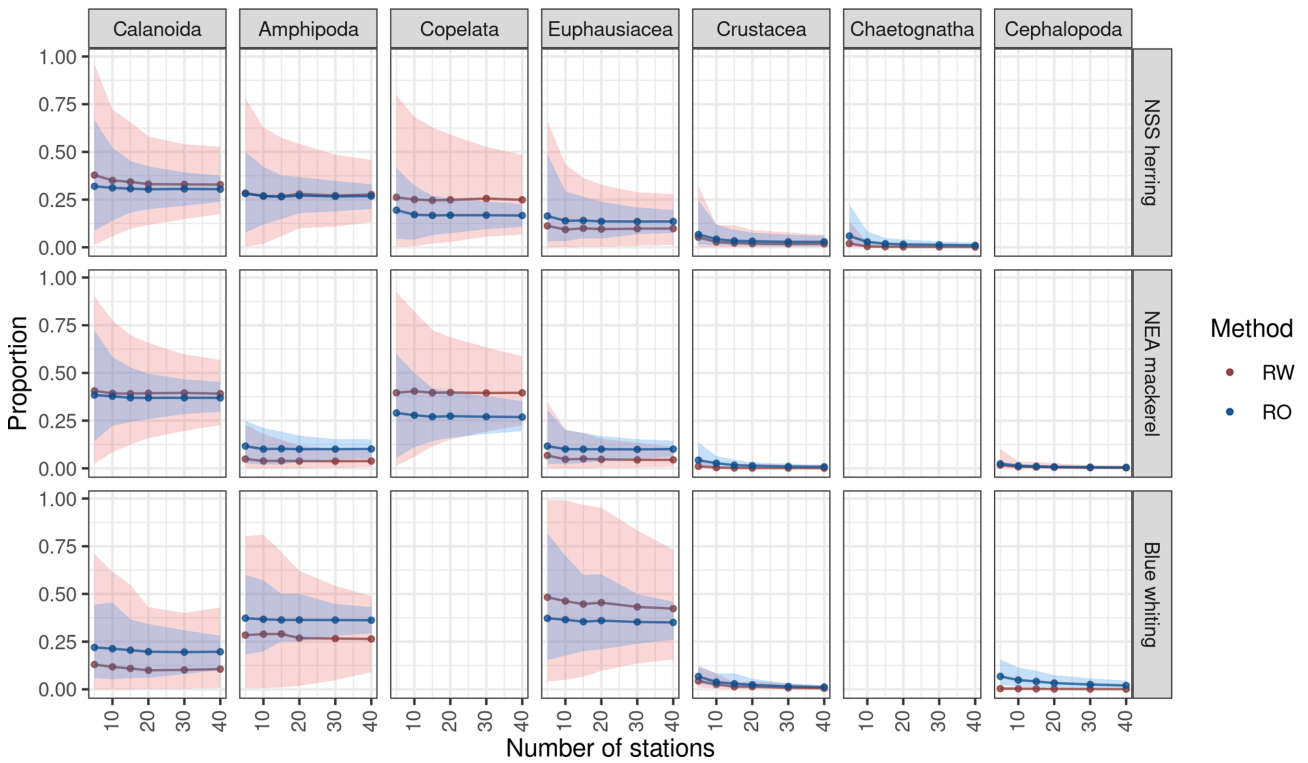


**Figure 4.** Interannual changes in July: **(a)** time-series of the mean proportion and 95% CI of the dominant prey groups in May for the relative weight (RW; red) and relative occurrence (RO; blue) methods. Numbers are the median correlation coefficients and 95% CI between RW and RO estimates across all resampled diets; **(b)** time-series signal-to-noise ratios for RW and RO estimates. Dashed lines represent the threshold below which interannual changes cannot be detected; **(c)** time-series consistency for RW and RO estimates with 95% CI. In (a) and (b), the RO method outcompetes the RW method if the points are above the 1:1 line, and vice versa.

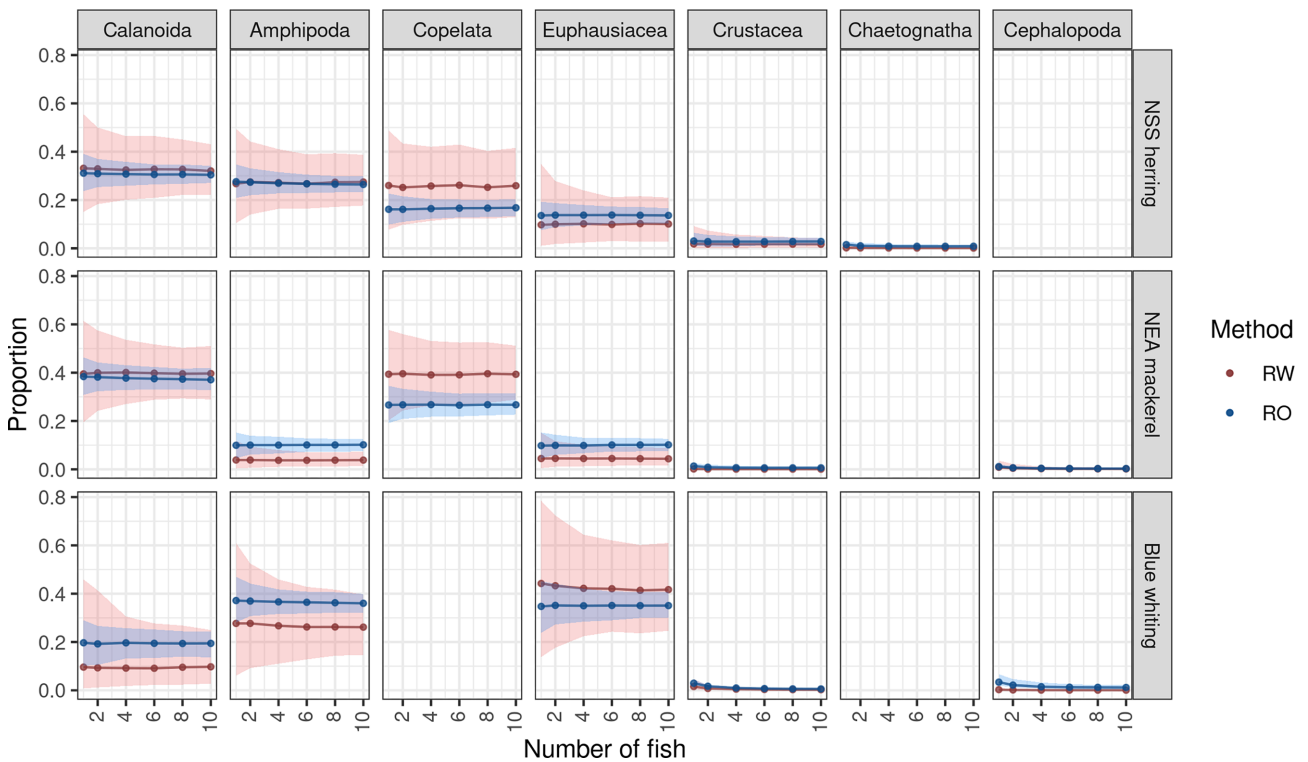
In addition, overall, the confidence intervals of the estimates using RO are generally much narrower than for the estimates resulting from using RW. This pattern of higher confidence in the estimates using the RO versus the RW method is a common feature across all analyses conducted. Our results are in line with the conclusions of Baker *et al.* (2014) and Buckland *et al.* (2017) that the occurrence-based method is more robust while retaining information similar to the output of the more labour-intensive weight-based method.

There are, however, noticeable differences between the results of the two methods. In May, the contribution of Amphipoda to the diet of herring, mackerel, and blue whiting is significantly greater when using the RO method versus the RW method. The opposite is true for the contribution of Calanoida to the diet of herring and mackerel. The proportion of Euphausiacea in the diet of herring is larger while in the diet of blue whiting it is smaller when using the RO method. In July, the most noticeable differences are for Calanoida (RO < RW)





**Figure 5.** Effect of the number of sampled stations in July 2010 on the 95% *CI* of the mean proportion of the major prey groups for the relative weight (RW; red) and relative occurrence (RO; blue) methods.



**Figure 6.** Effect of the number of fish sampled at each station in July 2010 on the 95% *CI* of the mean proportion of the major prey groups for the relative weight (RW; red) and relative occurrence (RO; blue) methods.

and Amphipoda (RO > RW) in the diet of mackerel and for Calanoida (RO > RW) and Osmeriformes (RO < RW) in the diet of blue whiting. The case of Osmeriformes is illustrative of how the two methods differ. There are only a few stomach samples in which Osmeriformes are present, and in these samples, the weight of Osmeriformes is high. The contribution of Osmeriformes to the diet in terms of occurrence is therefore low, while it is significantly higher in terms of biomass. Also, because only a few samples contribute to the high biomass, resampling leads to high variations in the estimates of biomass (which depend on whether these few samples are resampled or not) and therefore large confidence intervals.

Overall, the diet proportions for blue whiting in July have the broadest confidence intervals. This likely results from the low number of trawl stations in a relatively large area (Figure 1) and is in line with the increased confidence intervals as the number of stations declines (Figure 5). Compared to NEA mackerel in May, which exhibited comparatively narrower confidence intervals while being represented by a similar number of observations, the area was much smaller and covered fewer geographical regions.

The reason for the differences between the two methods can be attributed to the estimated mean weights of prey,  $\bar{w}_i$ 's. As seen from Equations (2) and (5), when the mean weights of all prey are identical, the two methods return the same point estimates. On the other hand, when the mean biomass of prey  $i$  in the stomachs is greater than the average biomass of other prey, then  $RW_i$  is expected to be higher than  $RO_i$ , and vice-versa. Uncertainties in the RW and RO estimates arise from the variability in prey occurrence and weight across samples and from the sampling effort. Since both estimates are based on the same sampling effort and both also include the variability in prey occurrence [ $n_i$  in Equations (4) and (5)], the differences in the uncertainty between the RO and RW estimates can be attributed to the variability in prey weight. This highlights that under the current sampling scheme, the mean weights of prey are often estimated with high uncertainties, and these uncertainties propagate into the weight-based diet proportion estimates.

The interannual pattern showed a generally smaller amplitude using the RO versus the RW method. However, in most cases where using the RW method resulted in a large change in the relative proportion between years, these estimates were associated with large uncertainties. Langøy *et al.* (2012) investigated the relative diet proportions of NSS herring and NEA mackerel in July 2004 and 2006. While the authors used a different regional grouping than in our study, their results from the Atlantic region are like the values reported in this study (years 2004 and 2006 in Figure 4). For example, for NSS herring in 2006, the estimates of Calanoida and Amphipoda are both greater using the RO method versus the RW method. In addition, Langøy *et al.* also report significantly smaller relative proportions of Appendicularia using the RO method versus the RW method, which is also evident in our study (here aggregated as Copelata). However, our study reveals that a main driver of this difference is the uncertainty originating from the weight-based estimates. That is, as for the Osmeriformes example above, Copelata is present in relatively few stomachs, but when it is present, the weight is high. Thus, the high relative contribution of Copelata in July 2006 resulting from the RW estimate is dependent on these individuals being caught. The occurrence-based estimates in most cases levels

out large interannual changes in diet composition compared to the weight-based method.

The interannual patterns also reveal that the overall differences between the methods (Figure 3 and 4) is not a systematic bias (i.e. Calanoida and Amphipoda are not always lower/higher when using the RO method compared to the RW method). The Amphipoda for NSS herring in July illustrates this case, where the occurrence-based estimates show little interannual variation, whereas the weight-based estimates show an increasing trend, with estimates being lower using the RW method in the beginning of the time series and higher in the end of the time-series compared to using the RO method. This signifies that overall, the Amphipoda has not become more frequently preyed upon during the period, but when it is present, it has contributed more to the overall weight in the stomach. However, whether this constitutes a real change is hard to assess given the large uncertainties associated with the weight-based estimates.

While the differences between the weight-based and occurrence-based methods can be explained to a large degree by variability in prey weight, shifting from RW to RO-based assessment will have implications for our understanding of the relative importance of prey. According to our current understanding, Calanoida is considered the most important prey group of NSS herring and NEA mackerel (Dalpadado *et al.*, 2000; Gislason and Astthorsson, 2002; Dommasnes *et al.*, 2004; Prokopchuk and Sentyabov, 2006; Langøy *et al.*, 2012; Bachiller *et al.*, 2016). From our study, we see that shifting to the RO method will still support this general understanding. However, it also points to the greater importance of larger prey types such as Amphipods and Euphausiacea. Conversely, for blue whiting, estimating diet proportions using the RO method will not change the general understanding of the higher importance of larger prey types (Prokopchuk and Sentyabov, 2006; Langøy *et al.*, 2012; Bachiller *et al.*, 2016), but it will lead to a higher relative importance of smaller prey types such as Calanoida.

There is a long history of diet method studies discussing “the best method” to arrive at a standard protocol for diet composition studies. Agreement on a standard protocol would allow easier comparisons across studies and aggregation of data to allow analyses on broader spatiotemporal scales (Buckland *et al.*, 2017). Early studies have emphasized the weight-based method (Hyslop, 1980) as it provides relatively objective estimates of prey group importance and is a direct proxy for the energetic-nutritional composition, which is often the purpose of diet analyses (Ahlbeck *et al.*, 2012; Amundsen and Sánchez-Hernández, 2019). However, while weight measurements can be performed with high accuracy, the estimates can be biased due to difficulties in identifying and separating macerated and/or partially digested prey groups. This bias led Baker *et al.* (2014) and Buckland *et al.* (2017) to conclude that the occurrence-based method was a more robust and unambiguous method and showed that the results resulting from the occurrence-based method were similar to those of the weight-based method.

Other recent studies have reached different conclusions. Amundsen and Sánchez-Hernández (2019) recommended combining the occurrence-based and relative fullness (Swynerton and Worthington, 1940) methods for assessing relative dietary composition. Conversely, Ahlbeck *et al.* (2012) found that the weight-based method outperformed the occurrence-based method and advocated for the weight-based method

when energetic-nutritional composition was the objective of diet analysis. A major challenge for reaching consensus and making general recommendations is that the “true diet” is unknown. Ahlbeck *et al.* (2012), through their modelling study, had access to a simulated “true diet”, which is a great advantage of their approach. Yet, the uncertainty then propagates to how well the model simulates reality.

When comparing methods based on an incomplete understanding, the resulting diet composition, regardless of the accuracy of the assessment, cannot be expected to produce an exact picture (Buckland *et al.*, 2017). It implicitly follows that it is not possible to compare methods from the perspective of “which method is closer to the truth”. Hence, in the context of the current study, it is not possible to conclude if the changes resulting from shifting from the RW method to the RO method are a better representation of the true diet. Accepting that diet composition estimates are biased and represent only part of the truth, we can however conclude that the estimates resulting from using the RO method instead of the RW method are more robust and less affected by sampling bias. The reduction in sampling bias from using the RO method also means that potentially fewer stations, as well as fewer fish per station, can be sampled for a similar confidence level compared to using the RW method.

### Concluding remarks

With few notable exceptions, the two methods deliver similar pictures of the mean contributions of prey groups to the diets of herring, mackerel, and blue whiting and of the year-to-year fluctuations in diets. Our results show that the occurrence-based method results in more precise estimates than the weight-based method. With lower uncertainties, the occurrence-based method also provides more consistent estimations of year-to-year fluctuations in diet proportions, together with a higher signal-to-noise ratio. The occurrence-based method is therefore more sensitive to detecting year-to-year changes in diet. The differences in diet contributions between the two methods can be attributed to the uneven distribution of mean weight-per-stomach across the different prey. The greater uncertainties associated with the weight-based method can also be attributed to large uncertainties in mean weight estimates.

Shifting from the RW method (the current method) to the RO method is straightforward since the data required to compute RO is readily available. This would result in lower costs and efforts and more robust estimates of diet contributions, although these cannot be directly interpreted in terms of mass or energy. Shifting back from the RO method to the RW method requires estimates of the mean prey biomass in the stomachs, and the empirical data used here shows that these estimates are currently highly uncertain. Reducing the sampling effort by decreasing the number of fish-per-station to five individuals appears to have a minimal impact on diet uncertainty estimates, while the effect of reducing the number of stations appears more substantial.

Here, we have quantified the impacts of changing the diet estimation method or the sampling effort. Our conclusions are only appropriate if the objective is to assess the diet contribution of major prey groups, as has been performed here. If the objective is to assess diet diversity, food consumption rates, and/or prey selection, the results and recommendations could be different (Amundsen and Sánchez-Hernández, 2019)

and should be based on additional dedicated analyses. In addition, if occurrence-based estimates were to become the only ones available, any procedure currently relying on diet composition expressed as weight or energy would need to be complimented by some assumptions or estimates of the mean weight (or energy content) of the prey items in the stomachs.

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### Supplementary data

Supplementary material is available at the ICESJMS online version of the manuscript.

### Conflict of interests statement

The authors have no conflicts of interest to declare.

### Author contributions statement

All co-authors contributed to developing the idea for the study. VRB, FKS, PLL, and HLM performed the laboratory analyses. EAM and BP conducted the analyses and wrote the first draft of the manuscript. All co-authors contributed feedback to the final manuscript.

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### Data availability statement

The data underlying this article are available from the Norwegian Marine Data Centre at <https://doi.org/10.21335/NMDC-720668044> (Mousing *et al.*, 2022) and the R scripts for replicating the analyses are available at <https://git.imr.no/ecosystem-processes/public/network-dynamics/major-prey-weight-vs-freq> under the tag—“v2.0”.

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