

Testing for a change in diet using fatty acid signatures

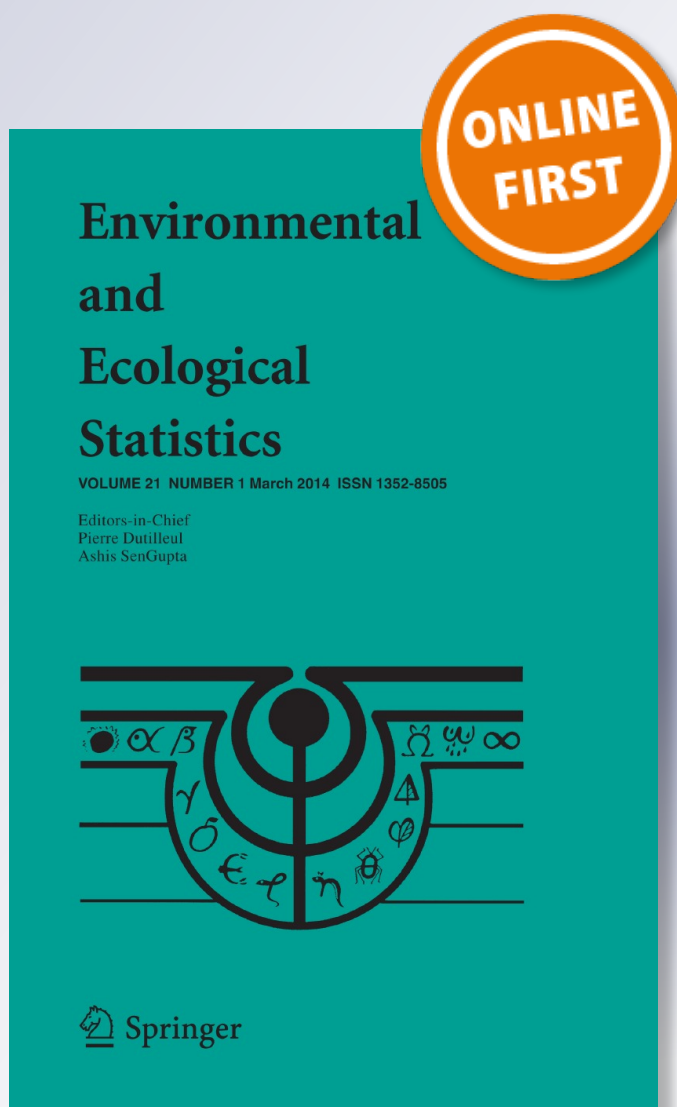
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Testing for a change in diet using fatty acid signatures

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Abstract Determining whether the diet of predators has changed is an important ecological problem and appropriate methodology is needed in order to test for differences or changes in diet. It is known that the fatty acid (FA) signature in a predator's adipose tissue predictably reflects the prey consumed and that, consequently, a change in the FA signatures can be largely attributed to changes in the predator's diet composition. The use of FA signatures as a means of detecting change in diet presents some statistical challenges however, since the FA signatures are compositional and sample sizes relative to the dimension of a signature are often small due to biological constraints. Furthermore, the FA signatures often contain zeros precluding the direct use of traditional compositional data analysis methods. In this paper, we provide the methodology to carry out valid statistical tests for detecting changes in FA signatures and we illustrate both independent and paired cases using simulation studies and real life seabird and seal data. We conclude that the statistical challenges using FA data are overcome through the use of nonparametric tests applied to the multivariate setting with suitable test statistics capable of handling the zeros that are present in the data.

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1 Introduction

Understanding predator diets and factors influencing what is consumed are central issues in understanding food web structure, the dynamics of predator–prey interactions, and impacts or roles of predation in ecosystems. Likewise, determining whether the diet of predators has changed is an important ecological problem and one that requires appropriate methodology to test for differences or changes in diets. For instance, it may be of interest to test for differences in diets of predator populations from one region to the next, between demographic groups, or over time perhaps in response to an environmental change. Relatively recent developments in biochemical methods, such as tissue fatty acids (FAs) and stable isotopes, provide valuable means to investigate diets of free-ranging animals (e.g., [Budge et al. 2006](#); [Iverson 2009](#); [Karnovsky et al. 2012](#)). FAs measured in the fat storage sites of predators are particularly sensitive to diet and can be used to study diets and trophic relationships in several ways, but the most common approach is using FAs qualitatively or semi-quantitatively to examine differences among predators in levels of specific FAs or in complete arrays of FAs ([Iverson 1993](#)). Since the FA signature of the predator is determined largely by its diet, a change in the FA signature can most often be attributed to changes in its diet composition. However, the means by which differences have been detected or assessed has varied substantially throughout the literature, and in some cases the biological relevance of an alleged change may be questionable. In this paper, we provide the methodology to carry out valid statistical tests for detecting changes in FA signatures, a problem that was motivated by the measurement of real-life predator FA signatures.

Fatty acids (FAs) are the main constituent of most lipids and, for most predators, the FAs of the prey are deposited in the adipose tissue of the predator with little modification (reviewed in [Iverson 2009](#)). The result is that the FA signature or profile (the distribution of all FAs) in the predator's adipose tissue reflects the prey consumed and a change in the FA signatures can most often be attributed to changes in diet composition. We note that since its introduction in [Iverson et al. \(2004\)](#), quantitative FA signature analysis (QFASA) has become an important method for quantitatively estimating diets of predators based on the FA profiles of their prey (e.g., [Iverson et al. 2004, 2006, 2007](#)). However, QFASA requires collection and analysis of an extensive prey database in addition to several other significant requirements, which may be onerous to meet, prior to being able to use the predator FAs alone to test for changes or differences in diet. Hence, here we focus solely on the latter, more qualitative and more common, use of FAs in diet studies. Since the components of the FA profile are proportions that sum to one, the data are termed compositional. While there are fairly standard statistical methods for handling compositional data (see [Aitchison 1986](#); [Pawlowsky-Glahn and Buccianti 2011](#)), the use of FA profiles creates some specific difficulties. The main challenge is that of sample size relative to the dimension of a profile. Approximately 70 FAs are routinely quantified in marine and

even terrestrial predators and their prey. Thus, due to sampling constraints, often the sample size is much less than the dimension of the data, precluding the use of standard multivariate techniques. Our procedures here are based on the ideas of permutation and randomization tests following the ideas of [Davison and Hinkley \(1997\)](#) but applied to a multivariate setting. We focus on developing testing methods that do not require the sample size to be larger than the dimension of the signatures.

Another issue that arises when dealing with FA signatures is the presence of zeros. In general, zeros in compositional data are problematic since the techniques recommended in [Aitchison \(1986\)](#) involve transformations using logarithms. [Martín-Fernández et al. \(2011\)](#) distinguish between the types of zeros and refer to rounded zeros as either small values that have been rounded to zero, or as proportions that fall below detection limit and are consequently recorded as zero. Rounded zeros are generally less difficult to handle than essential zeros, defined as the “absolute absence of the part in the observation” ([Martín-Fernández et al. 2003](#)). Zeros in the FA signatures may result from some FAs being present in such small amounts that they are not detected by the measuring device, or they may truly be zero. The proper treatment of the zeros depends on their type which is, unfortunately, generally unknown in practice. Hence we consider two different methods of dealing with them. The first approach treats the zeros as below-detection values and involves replacing the zeros by a small value and then using a test statistic based on Aitchison’s methods. We have chosen to use the multiplicative replacement strategy discussed in [Martín-Fernández et al. \(2003\)](#) and in [Martín-Fernández and Thio-Henestrosa \(2006\)](#) while more recent approaches are discussed in [Palarea-Albaladejo and Martín-Fernández \(2007\)](#), [Palarea-Albaladejo and Martín-Fernández \(2008\)](#), [Martín-Fernández et al. \(2011\)](#) and [Palarea-Albaladejo and Martín-Fernández \(2013\)](#).

Replacement strategies are not recommended for handling essential zeros. We have thus chosen to examine an alternative method which is well-suited for essential zeros in the FA signatures. This second approach involves designing a test statistic that allows for components to be truly zero, but which adheres, at least from a practical perspective, to fundamental compositional data analysis principles such as scale invariance and subcompositional coherence. This approach has the advantage that it could be applied to the problem of testing for a difference in diet using the QFASA diet estimates (not considered here) since the diet estimates typically contain many essential zeros. Note that treatment of essential zeros associated with estimating the diet of predators using FA signatures is discussed in [Stewart and Field \(2011\)](#) and in [Stewart \(2013\)](#).

2 Methods

We begin with the case of comparing independent FA signatures followed by comparing paired FA signatures. The cases are considered separately since the testing procedures differ. The performance of the testing procedures is assessed through simulation studies using simulated predators called pseudo-predators. Pseudo-predators were first introduced in [Iverson et al. \(2004\)](#) to assess QFASA performance. Our modified algorithm for generating pseudo-predators along with the data set that we will use to create them is also discussed in more detail in Sect. 3.

2.1 Independent Samples of Predators

Suppose we have two independent samples of predator FA signatures, say \mathbf{y}_{1i} , $i = 1, \dots, n_1$ and \mathbf{y}_{2i} , $i = 1, \dots, n_2$, each of dimension m . In the absence of knowing the true species proportions in the diet of the two populations, we test for a difference in the location of the FA signatures and make the assumption that this is equivalent to testing that the diets are the same, which is ultimately of interest. While the diet of the predators varies across seasons and regions, changes in the diet will be reflected in the FA signatures. However, the prey FA signatures consumed by the predators also vary over time and space, and differences in the FA signatures do not always translate directly to differences in the diet. This potential limitation of working at the FA level, is discussed further in Sect. 5.

To determine whether there is a difference in the FA signatures, we require a test statistic that measures the distance between the data and the null hypothesis of no difference in the location of the FA signatures. Note that in terms of variability in the FA signatures, for our practical examples (for instance, the real-life data in Sect. 4) the dispersion is generally similar for both samples. However, unequal dispersion is not as critical in the nonparametric setting.

We are mainly interested in the more difficult case in which n_1 and n_2 are small relative to m since otherwise standard multivariate techniques suitable for compositional data may be used. Aitchison (1986) describes such methods in detail. Essentially the methods involve transforming the data and then proceeding as if the transformed data are multivariate normal. These methods require sample sizes to be larger than m and that no zeros be present, though rounded zeros could be modified as previously discussed.

For our procedures the sample sizes n_1 and n_2 need not be larger than m . Our testing method is based on the nonparametric permutation test for the comparison of two means discussed in Davison and Hinkley (1997). With their approach, a P value is computed by pooling the n_1 and n_2 observations and considering permutations of the concatenation of the two random samples. For each permutation, the first n_1 components of the concatenated vector give the first sample and the remaining n_2 observations the second sample. An appropriate test statistic is calculated for R such permutations. The P value is computed by comparing the R test statistics to the observed test statistic.

It is straightforward to extend the algorithm to the multivariate case and all that is required is a suitable test statistic that measures the “distance” between the two compositional samples. We consider two measures of distance but, in both cases, the overall test statistic is

$$T = \sum_{i_1=1}^{n_1} \sum_{i_2=1}^{n_2} \text{dist}(\mathbf{y}_{1i_1}, \mathbf{y}_{2i_2}) \quad (1)$$

where “dist” denotes one of these two measures.

Aitchison (1992) argues that a measure of distance between two compositional vectors must satisfy various intuitive criteria including the principles of scale invariance and subcompositional coherence. While scale invariance ensures that the ratio of parts

is preserved, subcompositional coherence is described in [Bacon-Shone \(2011\)](#) as the principle that inferences about subcompositions should remain the same, regardless of whether or not the inference is carried out on the full composition or a subcomposition. In dealing with distance measures, for instance, we require that the distance between two full compositions be no less than the distance between two corresponding subcompositions. Aitchison's distance satisfies both of these principles and is defined as follows:

$$\text{AIT}(\mathbf{y}_{1i_1}, \mathbf{y}_{2i_2}) = \left(\sum_{j=1}^m \left\{ \log [y_{1i_1j} / g(\mathbf{y}_{1i_1})] - \log [y_{2i_2j} / g(\mathbf{y}_{2i_2})] \right\}^2 \right)^{1/2} \quad (2)$$

where $g(\mathbf{y}) = (y_1 \cdots y_m)^{\frac{1}{m}}$ represents the geometric mean ([Bacon-Shone 2011](#)).

Since this test statistic involves the logarithmic function, in order to apply this test, the rounded zeros in the FA signatures need first be modified using a replacement strategy and the details of this may be found in [Sect. 4](#).

When essential zeros are present, finding a test statistic that allows for them (without having to alter the zeros) and satisfies the principles of scale invariance and subcompositional coherence is not a trivial task, in part because the principles implicitly require that ratios of components be considered where one component (possibly the denominator) could be zero. Our second test statistic does not require that the zeros be modified and its development was the result of the ideas presented in [Greenacre \(2011a,b\)](#). [Greenacre \(2011b\)](#) proposed measuring subcompositional incoherence by examining how similar distances between components in the full composition were to distances between the corresponding components in subcompositions. He remarked that a method may be, for practical purposes, close enough to being subcompositionally coherent that the advantages of the method, particularly if it solves the problem of zeros for example, may outweigh slight incoherence. Here we consider our proposed distance measure to be coherent if distances between full compositions are as least as large as the distances between corresponding subcompositions ([Egozcue and Pawlowsky-Glahn 2011](#)).

According to [Greenacre \(2011a\)](#), the chi-square distance used in correspondence analysis between two rows in a matrix of compositional data has the property that if the data are power transformed (i.e. \mathbf{Y}^γ , for a matrix \mathbf{Y} of FA signatures) and then re-closed, it will converge to the AIT distance in [Eq. 2](#) as the power, γ , tends to zero and provided certain scaling factors are used. This latter result essentially stems from the Box–Cox transformation (see [Greenacre 2010](#)). We have used these facts and constructed a test statistic (or, equivalently, a distance measure) based on the chi-square distance measure which we have found empirically to converge to the AIT distance measure when the data are power transformed (and re-closed) as the power tends to zero. Our distance measure can therefore be made to become arbitrarily close to the AIT distance measure which is known to satisfy the scale invariance and subcompositional coherence principles.

We define our chi-square distance based measure between two FA signatures \mathbf{y}_{1i_1} and \mathbf{y}_{2i_2} as follows:

$$CS(\mathbf{y}_{1i_1}, \mathbf{y}_{2i_2}) = 1/\gamma \sqrt{2m} \left(\sum_{j=1}^{n_{FA}} \frac{(z_{1i_1j} - z_{2i_2j})^2}{c_{i_1i_2j}} \right)^{1/2}, \tag{3}$$

where \mathbf{z}_1 and \mathbf{z}_2 are the power transformed data, reclosed, and

$$c_{i_1i_2j} = \begin{cases} 1 & \text{if } z_{1i_1j} = z_{2i_2j} = 0 \\ z_{1i_1j} + z_{2i_2j} & \text{otherwise.} \end{cases}$$

Note that the distance measure in Eq. 3 can handle essential zeros in the FA signatures and that if both z_{1i_1j} and z_{2i_2j} are zero, the corresponding component in the sum is also zero.

The power transformation, γ , where $0 < \gamma \leq 1$, is chosen so that our distance measure is practically subcompositionally coherent. More specifically, to determine γ we consider a sequence of γ values starting with $\gamma = 1, \gamma = 1/2, \gamma = 1/3$, etc. The data are power transformed by γ and re-closed, and all possible pairwise distances are computed from the full (transformed) compositions. In a different context, Greenacre (2011b) found that two-part subcompositions appear to be the most sensitive to subcompositional incoherence. Therefore for each two-part subcomposition, pairwise distances are similarly computed. As an alternative, we compute the proportion of times that the distance between the full compositions is greater than or equal to the corresponding two-part subcomposition and our measure of incoherence for that two-part subcomposition is one minus this value. Since this measure is computed for each two-part subcomposition, we use an average value over all possible two-part subcompositions. This procedure is repeated with decreasing γ values until the measure is close to zero (we used $\leq 1 \times 10^{-6}$ in our simulations). Our chosen γ is then fixed in Eq. 3. Note that for our purposes it is not known which size subcomposition is most sensitive and we therefore need to make the assumption that for our choice of power transformation, the stress would be close to zero regardless of the subcomposition size used to determine γ . If γ is small, this should be a reasonable assumption since we know that the CS distance converges to the AIT distance as γ tends to 0.

The multivariate permutation test (MPT) is summarized below, where ‘‘dist’’ refers to either AIT or CS defined in Eqs. 2 and 3, respectively.

Multivariate permutation test (MPT)

1. Compute the test statistic, $T = \sum_{i_1=1}^{n_1} \sum_{i_2=1}^{n_2} \text{dist}(\mathbf{y}_{1i_1}, \mathbf{y}_{2i_2})$.
2. for $r = 1, \dots, R$
 - (a) Pool the two samples.
 - (b) Permute the $n_1 + n_2$ observations to obtain: $\mathbf{y}_i^{*r}, i = 1, \dots, n_1 + n_2$.
 - (c) Let $\mathbf{y}_{1i}^{*r} = \mathbf{y}_i^{*r}, i = 1, \dots, n_1$ and $\mathbf{y}_{2i}^{*r} = \mathbf{y}_i^{*r}, i = n_1 + 1, \dots, n_1 + n_2$.
 - (d) Compute the test statistic, $T^{*r} = \sum_{i_1=1}^{n_1} \sum_{i_2=1}^{n_2} \text{dist}(\mathbf{y}_{1i_1}^{*r}, \mathbf{y}_{2i_2}^{*r})$.
3. Compute the P value

$$p^{\text{MPT}} = \frac{\#\{T^{*r} \geq T\}}{R}.$$

The multivariate permutation test was assessed through simulations by examining (1) the probability of concluding that the diets differ when they do not (the probability of committing a type I error) and (2) the probability of concluding that the diets differ when they do (the power of the test).

2.2 Paired samples of predators

We now consider the analysis of paired samples of FA signatures, where a predator is sampled at two different time points, say \mathbf{y}_{Bi} and \mathbf{y}_{Ai} , $i = 1, \dots, n$. Our method tests for a change in the location of the FA signatures and is intended for the more challenging, but often realistic, setting in which $n < m$. Recall that when the sample sizes are small, standard multivariate techniques, including those discussed in [Aitchison and Ng \(2003\)](#) for paired compositional data, are not useful.

For this case, we apply an extension to the univariate matched-pair randomization P value discussed in Problem 7 (p. 186) of [Davison and Hinkley \(1997\)](#). Their univariate method involves re-sampling under the null hypothesis of no difference by computing $d_i^* = s_i d_i$, $i = 1, \dots, n$ where d_i are the computed differences in the original data set and s_i are independent and equally likely to be $+1$ and -1 . We use the $*$ notation to denote the samples from this null resampling model. For each generated sample, a suitable test statistic is computed. In the multivariate setting, we have chosen to compute $\mathbf{d}_i^* = s_i \mathbf{d}_i$, where \mathbf{d}_i is a vector containing the m differences between the i th before and after FA signature.

As in the independent case, we compute the differences using two different approaches. While both take into account the compositional nature of the data, the second method treats the zeros as essential zeros and does not require the use of a replacement method to deal with them. In both cases, our test statistic can be written as

$$T = \left(\sum_{j=1}^m \bar{d}_j^2 \right)^{1/2} \tag{4}$$

where $\bar{d}_j = 1/n \sum_{i=1}^n d_{ij}$, $j = 1, \dots, m$ and d_{ij} denotes the difference, computed using one of the two methods discussed below, between the j th FA for the i th individual.

Our first approach treats the zeros in the FA signatures as rounded zeros and involves modifying the zeros, log-ratio transforming the data and then computing relative differences using methods in [Aitchison \(1986\)](#) and [Aitchison and Ng \(2003\)](#). More specifically, let \mathbf{y}_{B-m} denote a “before” FA signature without the last FA component and similarly for the “after” FA signature. For $i = 1, \dots, n$ we then compute the differences

$$\mathbf{d}_i^{\log} = \mathbf{z}_{Ai}^{\log} - \mathbf{z}_{Bi}^{\log} \tag{5}$$

where

$$\mathbf{z}_{Bi}^{\log} = \log \left(\frac{\mathbf{y}_{Bi-m}}{y_{Bim}} \right) \text{ and } \mathbf{z}_{Ai}^{\log} = \log \left(\frac{\mathbf{y}_{Ai-m}}{y_{Aim}} \right).$$

Note that we could have opted to use an alternative transformation such as the centered log-ratio transformation (on which Aitchison's distance is based) or the isometric log-ratio transformation (see Egozcue and Pawlowsky-Glahn 2011).

Our alternative method of computing differences is based on Eq. 3 and the differences are calculated as follows:

$$\mathbf{d}_i^{\text{CS}} = 1/\gamma\sqrt{2m}\mathbf{C}^{-1/2} \left(\mathbf{z}_{Ai}^{\text{CS}} - \mathbf{z}_{Bi}^{\text{CS}} \right) \tag{6}$$

where \mathbf{z}_A^{CS} and \mathbf{z}_B^{CS} are the re-closed power transformed data and \mathbf{C} is a $m \times m$ diagonal matrix with diagonal elements $c_{ij} = z_{Aij} + z_{Bij}$, provided one of z_{Aij} and z_{Bij} are non zero. If both are zero, c_{ij} is set to 1 so that, in this case, $d_{ij}^{\text{CS}} = 0$

The choice of γ is determined using an algorithm similar to that used in the independent case where the distances $\text{CS}(\mathbf{y}_{Ai}, \mathbf{y}_{Bi}) = \left(\sum_{j=1}^m \left(d_{ij}^{\text{CS}} \right)^2 \right)^{1/2}$, $i = 1, \dots, n$ are compared to the distance between the corresponding two-part subcompositions, when the data are power transformed.

Our multivariate randomization P value is computed using the algorithm below, where \mathbf{d}_i can be either $\mathbf{d}_i^{\text{log}}$ or \mathbf{d}_i^{CS} .

Multivariate randomization test (MRT)

1. Compute the differences \mathbf{d}_i , $i = 1, \dots, n$.
2. Compute the test statistic

$$T = \left(\sum_{j=1}^m \bar{d}_j^2 \right)^{1/2}$$

using differences in 1.

3. for $r = 1, \dots, R$
 - (a) For the i th observation, randomly select +1 or -1 and call this s_i^{r*} , $i = 1, \dots, n$.
 - (b) Compute $\mathbf{d}_i^{*r} = s_i^{r*}\mathbf{d}_i$, $i = 1, \dots, n$.
 - (c) Compute $T^{*r} = \left[\sum_{j=1}^m \left(\bar{d}_j^{*r} \right)^2 \right]^{1/2}$.
4. Compute the P value

$$p^{\text{MRT}} = \frac{\#\{T^{*r} \geq T\}}{R}.$$

3 Simulation study

We carried out simulations to assess the performance of the MPT and MRT in terms of the probability of committing a type I error and power when we are ultimately interested in determining whether the diets differ.

We used simulated predators to evaluate our methods. These simulated predators were also used in [Iverson et al. \(2004\)](#) to assess the methods of QFASA. To create a pseudo-predator, we require a prey database containing samples of prey FA signatures from each species of prey that could potentially be a part of the predator's diet. The prey database that we use to generate the pseudo-predators was collected along the Scotian Shelf off eastern Canada (see [Budge et al. 2002](#)). This prey database contains 28 species and 2110 FA signatures (specimens) in total. Refer to Online Resource 1 for these species and their sample sizes.

Not all of the FAs contribute equal information about diet and we have chosen to use a subset of 40 FAs from the 65 FAs in the full prey base. These 40 FAs include FAs that arise only from diet as well as those that arise from both diet and biosynthesis. This particular subset of FAs, along with any of the data sets used in this paper, are available from the authors.

We then formed a pseudo-predator by choosing a true diet and sampling proportionately from the prey database with replacement from the species in the diet. For example, if the true proportion of Cod in the diet was 0.5 and the total number of prey FA signatures used in the simulations was 50, then we would sample 25 Cod signatures with replacement. To account for the predator eating small amounts of prey not considered to be part of the diet, noise is added to the pseudo-predator by sampling from species not in the specified diet. Our simulations actually make use of the updated pseudo-predator generating algorithm found in [Stewart and Field \(2011\)](#) which incorporates fat content of the prey. Prey with a higher fat content contribute a larger proportion to the FA signature of the predator than those with a lower fat content and these pseudo-predators are designed to take into account this additional variable. Generated samples of pseudo-predators then constitute our samples of compositional data.

We examined two sets of diets in our simulation studies consisting of species of fish that seals on the east coast of Canada (especially grey seals, *Halichoerus grypus*) are known to eat. For simulations based on the first set of diets (Table 1), we used Diet 1 throughout as the diet of the first sample of seals (in the independent case) or as the “before” seals (in the paired case). To compute $P[\text{Type I Error}]$, Diet 1 was also used as the diet of the second sample of seals (or the “after” sample in the paired case). To illustrate the power of our tests, we chose the second sample of seals to have one of diets two to five, where each of these diets gradually gets “farther” from Diet 1 in terms of distance. In [Bowen and Harrison \(2007\)](#), seasonal and interannual variability in the diet of grey seals near Sable Island was studied through faecal samples. The second set of diets (Table 2) used in our simulations studies reflect four of these estimated diets. Note that while [Bowen and Harrison \(2007\)](#) obtained estimates of diet for years 1991–1998 and for the winter, spring and fall seasons, it was not practical to study the power between all combinations of diets since obtaining results is highly computationally intensive. Instead, we computed the power for diets with the smallest within season relative distance and similarly for within years. In particular, the distance between the fall 1992 and fall 1995 diets was the smallest using the CS distance with $\gamma = 1/3$ when compared to all other within season comparisons and the distance between winter 1995 and spring 1995 was the smallest for all within year comparisons. Power calculations were then computed for these pairs diets. Note that we chose to use $\gamma = 1/3$ for effect

Table 1 Diets used in simulations for assessing the power of the multivariate permutation and randomization tests

Species	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Northern Sandlance	35	38	42	45	50
Redfish	0	0	0	0	0
Capelin	0	0	0	0	0
Atlantic Cod	30	27	24	20	15
Silver Hake	15	12	10	10	5
American Plaice	0	0	0	0	0
Yellowtail Flounder	10	13	14	15	20
Longhorn Sculpin	0	0	0	0	0
Other	10	10	10	10	10
Total	100	100	100	100	100

Table 2 Estimated diets of grey seals near Sable Island in fall 1992, fall 1995, winter 1995 and spring 1995 used in simulations for assessing power of the multivariate permutation and randomization tests

Species	Fall 1992	Fall 1995	Winter 1995	Spring 1995
Northern Sandlance	67	60	40	25
Redfish	0	0	0	0
Capelin	0	0	0	7
Atlantic Cod	18	17	13	29
Silver Hake	6	10	3	7
American Plaice	3	5	15	11
Yellowtail Flounder	5	4	16	12
Longhorn Sculpin	0	0	9	4
Other	1	4	4	5
Total	100	100	100	100

size/distance calculations between true diets because this value lay within the values typically observed in the data.

For both the independent and paired cases, we chose $\alpha = 0.01, 0.05$ and 0.1 and examined two sample sizes, namely $n_1 = n_2 = 10, 25$ (or simply $n = 10, 25$ in the paired setting) since results become computationally intensive to obtain as the sample size increases. Furthermore, standard multivariate techniques can be applied when n_1 and n_2 are large. We set $M = 500$ for samples sizes of 10 but, for the independent case only, used a reduced number of simulations of only $M = 200$ for sample sizes of 25 because results took much longer to obtain for this case and at this sample size. Note that this difference in number of replicates means, for example, that if the power is 0.75, the standard error of our estimate of power is approximately $\sqrt{0.75 \times 0.25/200} = 0.031$ when $M = 200$ but only 0.019 when $M = 500$.

While details of our simulation algorithms are provided in Online Resource 2, note that the n_1 and n_2 pseudo-predators are both sampled from the same prey database

Table 3 Type I error simulation results associated with the MPT and MRT for three significance levels and sample size $n = 10$, computed using two different test statistics

Test	Test statistic	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.1$
MPT	CS	0.012	0.052	0.110
	AIT	0.014	0.042	0.088
MRT	CS	0.008	0.052	0.090
	LOG	0.014	0.062	0.114

and may have some prey signatures in common. Consequently, the samples may not be entirely independent and in practice the $P[\text{Type I Error}]$ could be slightly larger than what is presented in our results section. Also, when the data are paired we need to be able to simulate paired samples of pseudo-predators. While there may be various ways of accomplishing this, we have chosen to adjust our “after” predators as follows: let

$$\mathbf{y}_A^* = (1 - \lambda)\mathbf{y}_A + \lambda\mathbf{y}_B,$$

where \mathbf{y}_B and \mathbf{y}_A are the “before” and “after” pseudo-predators and \mathbf{y}_A^* is our adjusted “after” predator. In our simulations, we set λ equal to 0.5.

The MPT and MRT type I error simulation results for sample sizes of 10 are displayed in Table 3 while the power results for this sample size are demonstrated graphically in Figs. 1 and 2. Results for sample sizes of 25 are given in Online Resource 3 since conclusions were, in essence, the same for this sample size. We use the notation AIT to denote the test statistic based on Aitchison’s distance (Eq. 2), CS to denote the test statistics based on Eqs. 3 and 6, and LOG for the test statistic based on Eq. 5.

In terms of type I error, the MPT and MRT with either test statistic appear to perform well as the targeted significance levels (0.01, 0.05 or 0.1) are within plus or minus two standard deviations our estimate of the probability of type 1 error. Figure 1 displays the approximate probability of concluding that two diets differ when, in fact, they do for the MPT at sample sizes of 10 and for various combinations of diet where, for example, D1/D3 denotes the power when Diet 1 and Diet 3 from Table 1 are considered to be the two true underlying diets. (F92, F95, W95, and S95 denote the fall 92, fall 95, winter 95 and spring 95 diets respectively given in Table 2). Figure 2 similarly illustrates the power for the MRT when $n = 10$. The effect size (or distance between the two diets) is computed using Eq. 3 with $\gamma = 1/3$.

Perhaps the most noticeable feature of the graphs is the drop in power when the fall 92 and fall 95 diets are used even though the CS distance between them (with $\gamma = 1/3$) is approximately the same as the distance between Diets 1 and 5. With the exception of this drop, the power results suggest overall that the MPT and MRT are reasonably powerful even at small sample sizes, with power increasing with effect size and sample size as expected. One reason for the apparent discrepancy in power for the fall 92 and fall 95 diets could be related to the choice of γ used in calculating the effect size since effect size varies with γ . In fact, using $\gamma = 1/10$, the effect size for these diets is in between the effect size for D1/D2 and D1/D3. The results also show

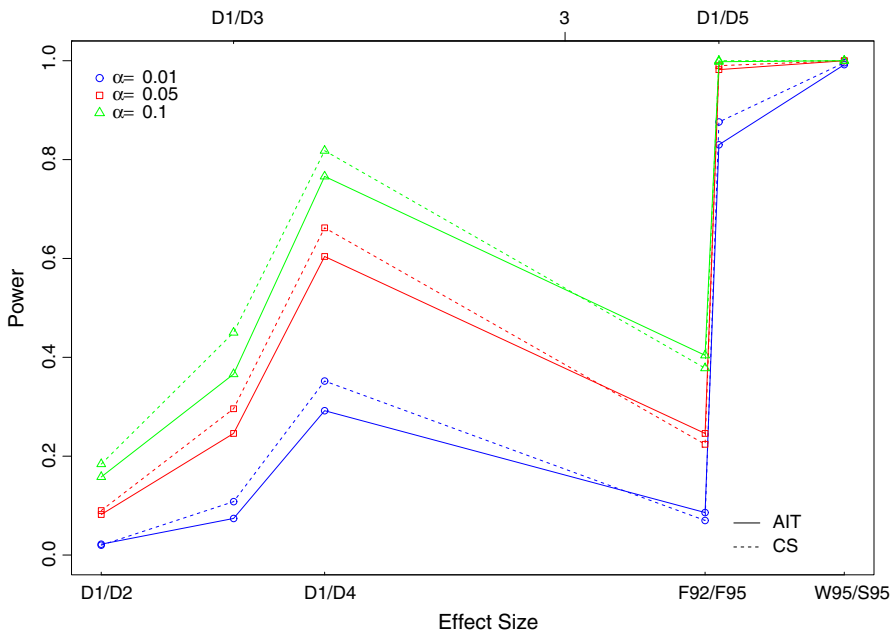


Fig. 1 Power simulation results associated with the MPT for samples of size 10, computed using two different test statistics. Effect size is the CS distance between the two diets (with $\gamma = 1/3$) where, for example, the distance between Diet 1 and Diet 2 is denoted as D1/D2. F92, F95, W95, and S95 denote the fall 92, fall 95, winter 95 and spring 95 diets respectively

that while power is comparable for both test statistics, it is generally higher when the CS distance is used and the difference is particularly noticeable for the MRT (Fig. 2).

4 Real-life data

4.1 Independent samples of seabird FA signatures

To illustrate the MPT procedure on real-life data, we examined captive seabird data obtained from islands in the southeastern Bering Sea and used in Iverson et al. (2007) to validate QFASA. The data consists of 20 Red-legged Kittiwake FA signatures and 26 Common Murre FA signatures. Both samples of seabirds were split evenly into two groups. For the Kittiwake seabirds, from hatching until day 15, both groups were fed herring and silverside and then from day 16 until day 42 the first group was fed silverside only and the second group only smelt. On day 42 the FA signatures were sampled, providing us with two independent samples of FA signatures on which we can apply and assess our MPT. A similar experiment was carried out using the Common Murre seabirds and the MPT was applied to this data set as well. Only 40 FAs of the original 68 determined to be associated with diet were used in the analysis. As discussed in more detail below, the seabird samples contain zeros which we first treat as rounded zeros and, in which case, they need to be appropriately modified in order

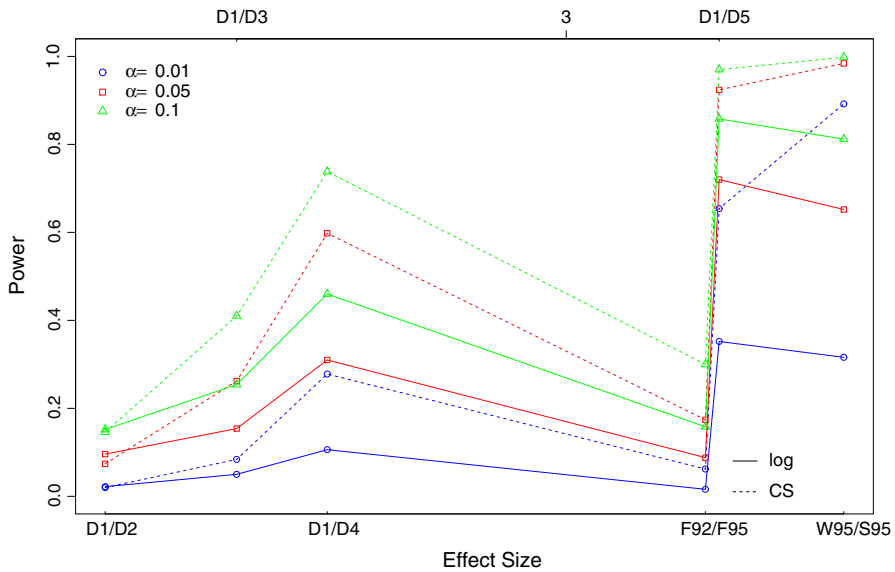


Fig. 2 Power simulation results associated with the MRT for samples of size 10, computed using two different test statistics. Effect size is the CS distance between the two diets (with $\gamma = 1/3$) where, for example, the distance between Diet 1 and Diet 2 is denoted as D1/D2. F92, F95, W95, and S95 denote the fall 92, fall 95, winter 95 and spring 95 diets respectively

to use the AIT test statistic. Following the recommendations in [Martín-Fernandez and Thio-Henestrosa \(2006\)](#), we have used a multiplicative replacement strategy where the modified FA signature, say \mathbf{R} , is given by

$$r_j = \begin{cases} \delta_j & \text{if } y_j = 0, \\ \left(1 - \sum_{k|y_k=0} \delta_k\right) y_j & \text{if } y_j > 0 \end{cases} \quad (7)$$

and δ_j is a small value determined through a sensitivity analysis. We have chosen to use the same value of δ_j for all FAs and will, from here on in, refer to this value as simply δ . We compare the results with the CS based test statistic in which we assume that the zeros are essential zeros and do not modify them.

Tables 4 and 5 provide the captive seabird diets along with the MPT P values for each sample. For two FAs, all observations were zero in all four data sets and we chose to remove these FAs prior the analysis. Without their removal, the % of zeros in three of the data sets were slightly $>10\%$, the maximum percentage of zeros that should occur when applying the multiplicative replacement strategy according to [Martín-Fernandez and Thio-Henestrosa \(2006\)](#). (The removal of these FAs is not necessary prior to using the CS test statistic, however, since we were interested in comparing the results of both test statistics it seemed reasonable to use the same FAs in the analysis). It is important to note that because the multiplicative replacement approach replaces zeros by a fixed amount, as the percentage of zeros in the data increases, so does the potential for issues such underestimation of the variability in the data. In

Table 4 True diets and MPT P values (computed using two different test statistics) for two independent samples of Red-legged Kittiwakes

Diet 1, $n_1 = 10$		Diet 2, $n_2 = 10$		Test statistic	P value
Day 0–15	Day 16–42	Day 0–15	Day 16–42	CS	<0.002
Herring/Silverside	Silverside	Herring/Silverside	Smelt	AIT	<0.002

Table 5 True diets and MPT P values (computed using two different test statistics) for two independent samples of Common Murres

Diet 1, $n_1 = 13$		Diet 2, $n_2 = 13$		Test statistic	P value
Day 0–15	Day 16–42	Day 0–15	Day 16–42	CS	<0.002
Silverside	Silverside	Silverside	Smelt	AIT	<0.002

general, more recent approaches such as those discussed in [Palarea-Albaladejo and Martín-Fernández \(2013\)](#) (including a replacement strategy based on the log-normal distribution) hold better statistical properties when the percentage of zeros is large. These methods, however, require parameter estimation and for small sample sizes such as ours may perform poorly.

The CS test statistic was computed with $\gamma = 1/5$ for the Red-legged Kittiwakes and $\gamma = 1$ for the Common Murres as these values were found to be optimal in terms of minimizing subcompositional incoherence. For both types of seabirds, the CS based P values were <0.002 (based on 500 permutations).

To obtain the AIT test statistic results, the remaining zeros (ranging from 1.2 to 12.1 % of the data) needed to be modified. In order to select δ we carried out a brief sensitivity analysis and attempted to determine a value such that results (i.e. P values) became unstable. The smallest non zero value in all four data sets was about 0.0001 and we found, however, that for both types of seabirds the P value was <0.002 for all δ values that we examined between 0.00001 and 0.0001. We therefore opted to simply use $\delta = 0.00005$, or one half of the smallest non zero value. The corresponding P value was <0.002 for both types of seabirds.

In all cases, the P values support the alternative hypothesis that the diets have changed which is known to be the case for this experiment.

4.2 Paired samples of seal FA signatures

Paired data consisting of before and after seal FA signatures were collected by Margi Cooper (Dalhousie University) for each of two separate experiments, one conducted in the fall of 1999 and the other in the spring of 2000, and the FA signatures of seals before and after a feeding experiment were recorded. For this experiment, of interest is whether or not the FA signatures have changed to reflect the known change in the diet and we may apply our MRT.

The data consists of 8 seals for the 1999 data set and 10 seals for the 2000 data sets. The 40 FAs used in the seabird data were also extracted from the original 69

Table 6 MRT P values (computed using two different test statistics) for two before and after seal feeding experiments

	n	MRT P values	
		CS	AIT
Fall 1999	8	0.006	0.01
Spring 2000	10	0.002	<0.002

paired FAs. Some zeros occurred in these data sets as well preventing the direct use of Aitchison's methods. Before applying Eq. 5, the zeros were modified using the multiplicative replacement strategy given in Eq. 7.

Results of the before and after seal experiments are displayed in Table 6. For the CS test statistic, the optimal values of γ were determined to be 1/2 for the 1999 data and 1 for the 2000 data.

The 1999 "after" data set contained the highest percentage of zeros at about 3.4%. The smallest non zero FA occurred in the "before" year 2000 data set and was approximately 1.2×10^{-6} . Note that one FA was zero for all seals in the 1999 data sets but not in the 2000 data sets. We did not remove this FA since, overall, the percentage of zeros was still small. In order to choose δ a sensitivity analysis was carried out with δ ranging from 1×10^{-8} to 1×10^{-5} . For the 1999 data set, the corresponding P values ranged from <0.002 to 0.024 and for the 2000 data set from <0.002 to 0.012. Using $\delta = (1.2 \times 10^{-6}) / 2$, we obtained the P values shown in Table 6.

For both data sets the P values are very small and we may conclude that the FA signatures of the seals changed significantly during the feeding experiments.

5 Discussion

The use of FAs offers a promising approach to the study of food webs, predator–prey interactions and overall ecosystem dynamics. A key feature of FAs is that, unlike other dietary nutrients, they are generally not broken down during digestion and FAs in the predator can resemble FA patterns in the prey consumed. We can therefore use FAs to detect changes in or differences between the diets of predators. Although FA signatures (the distribution of all FAs) are known to reflect changes in diet, using them to test for a difference in diet is challenging from a statistical point of view. Managing samples sizes that are much less than the dimension of the FA signature is a substantial difficulty. We have successfully dealt with this issue through the use of nonparametric procedures with test statistics carefully chosen to handle the compositional nature of the data as well as zeros that may arise. We considered both the independent and paired settings and the procedures were validated through a simulation study and real-life examples. Results from the simulation study indicate that applying Aitchison's method in permutation/randomizations tests for differences in the location of the signatures lead to reasonably powerful tests at relatively small sample sizes. This is also the case if the CS distance measure is used. Furthermore, the CS distance measures appears to be slightly more powerful but it is only needed when there are essential zeros in the data. The real-life examples consisting of seabird and seal data also present support for the practical usefulness of the developed statistical methodology for detecting changes

in FA signatures. As compositional data arise in many applications, our methods can be extended to other problematic applications involving small sample sizes and potentially problematic zeros.

A practical limitation of our procedures is that the analysis is carried out at the FA level and a significant difference in the FA signatures may not always relate to a difference in the diets. For instance, if the FA signatures are found to be significantly different, interpretation issues may arise if the prey themselves differ between the two predator population samples. This can occur, for example, if sampling is done at different times of the year and/or in different regions. Consider two populations of seals eating only one species, say sandlance, from two different regions. If the FA signatures of sandlance from the two regions are different then so will be the seal FA signatures and we may correctly conclude that the seal FA signatures are different even though the seals have the same diet. That is, both populations are eating sandlance. In these cases, a difference in FA signatures does not necessarily imply a difference in diet. However, provided the FA sampling is done in the same area and same season, a change in FA signatures should indicate a change in diet composition.

Iverson et al. (2004) provide a method (called QFASA) of estimating the diet of a predator based on the mean FA signature of the various prey species potentially in the predator's diet. While we could alternatively apply our procedures to diet estimates obtained through QFASA, to more directly address the problem of testing for a difference in diet, there are significant additional challenges to overcome when working at the diet estimate level. For QFASA, in addition to building a comprehensive and reliable prey base which can sometimes represent an enormous undertaking, there are also additional requirements such as the development of calibration factors needed to account for the effects of predator metabolism on FA deposition in lipid stores. Currently these coefficients are estimated through studies in which a predator is fed a constant diet over a long term and any differences between the FAs in the predator and the prey are then assumed to be attributed to metabolic processing. However, such studies require carefully controlled long-term captive studies, and depending on the predator, may be difficult to undertake. An estimate of the fat content of each prey species is required as well in order to account for prey of differing lipid contents (and thus relative FA contributions) and yet species variability in fat content can be large. Thus, estimation procedures then need to account for the variability due to the calibration factors and fat content. Further challenges include the selection of FA subset applied in QFASA and the issue of estimating the contribution of prey species with similar FA signatures. A major statistical challenge when working at the diet estimate level is the proper management of the abundance of essential zeros that typically occur, though our proposed distance measure would be helpful here.

With the aforementioned challenges in mind, using FAs alone to determine whether the diet has changed or whether diets differ clearly has great appeal and our proposed tests are applicable to many real-life situations. Using the statistical procedures we outline here, it is possible to examine empirically determined differences among predators in levels of specific FAs or in complete arrays of FAs, which can be used to indicate differences in diet(s) among individuals, populations, and species.

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