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METHODS

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Design and analysis of multiple choice feeding preference data

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Abstract Traditional analyses of feeding experiments that test consumer preference for an array of foods suffer from several defects. We have modified the experimental design to incorporate into a multivariate analysis the variance due to autogenic change in control replicates. Our design allows the multiple foods to be physically paired with their control counterparts. This physical proximity of the multiple food choices in control/experimental pairs ensures that the variance attributable to external environmental factors jointly affects all combinations within each replicate. Our variance term, therefore, is not a contrived estimate as is the case for the random pairing strategy proposed by previous studies. The statistical analysis then proceeds using standard multivariate statistical tests. We conducted a multiple choice feeding experiment using our experimental design and utilized a Monte Carlo analysis to compare our results with those obtained from an experimental design that employed the random pairing strategy. Our experimental design allowed detection of moderate differences among feeding means when the random design did not.

Keywords Feeding preference · Hotelling's T^2

Introduction

Multiple-choice feeding experiments are widely used to test principles that are basic to trophic relationships in terrestrial and aquatic ecosystems (for a reviews see Peterson and Renaud 1989; Roa 1992). Peterson and Renaud (1989) recognized that most previous multiple-choice feeding-preference experiments suffered from inappropriate use of controls in estimating autogenic change in the experimental units, especially in cases where more than two food types were present. They suggested that a multivariate statistical solution would be desirable, but they did not provide such an approach to this problem.

Roa (1992) also pointed out the desirability of using a multivariate procedure in order to account for the dependencies among samples of various food types presented in a multiple-choice feeding experiment. He also recognized that another drawback of traditional multiple-choice feeding experiments was the method of using a constant correction factor to account for autogenic changes in experimental replicates that might have been masked by the consumption of the experimental foods. The consequence of using a constant correction factor was a misestimate of the error variance in the subsequent statistical analysis. His solution to this misestimation in the analysis was to randomly pair a single control food (without herbivore) with a single experimental food (with herbivore) for the purpose of applying a "correction" for autogenic change, and then to use a multivariate Hotelling's T^2 test to assess the correlated differences (preferences) among foods. The multivariate analysis accounted for the dependencies among the multiple experimental samples presented together to the consumer.

Manly (1993) described three problems with Roa's (1992) technique. First, the random assignment of control units to experimental units gives rise to many possible configurations of the data. In the six-replicate study of Roa (1992), 720 (6 factorial) different analyses could have been performed on one food alone. For the three foods in the Roa experiment, there would be 720^3 or 373,248,000 different possible pairings! As Manly (1993) pointed out, "the significance or otherwise of experimental results may depend on the particular pairing used." Secondly, Roa's (1992) randomization scheme loses the potential correlations that arise from the interactions among different food

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types within replicates and that are also inherent in the measurement process itself. For example, external influences such as temperature, time of day, experimenter's dexterity/experience, and equipment status will likely be uniformly applied to experimental and control units that are located in the same chamber. Separation of experimental and control samples, on the other hand, permits these external factors to affect them differentially. It is precisely because of these interactions that many authors have lamented the lack of a suitable multivariate analysis for these multiple-choice feeding experiments. Thirdly, Manly (1993) pointed out that a simple transformation of the measures used in the multivariate statistical test would allow for the inclusion of the sampling error in the estimates of the overall means. Roa's method assumed that the overall mean was known without any sampling error.

The solution proposed by Manly (1993) was to turn the test into a comparison of the means of two multivariate samples that may not necessarily have the same covariance structure. Using a test method described by Yao (1965), he reanalyzed Rao's data and arrived at a conclusion that contradicted Roa's.

We propose a modification in the experimental design itself that overcomes the limitations of all previous methods (Peterson and Renaud 1989; Roa 1992; Manly 1993) that have been used to analyze feeding preference experiments involving more than two food types. Our experimental design combines, for each replicate observation, randomly arranged multiple food samples in experimental/control pairings within one chamber. The variance of the multivariate test statistic inherent in our design will be the one value unique to the set of measurements being examined, and not, therefore, a randomly chosen quantity from a large pool of possible values. By physically pairing the control and experimental samples of multiple food-types within each replicate chamber, the actual correlations within these chambers are maintained and are incorporated into a relatively uncomplicated multivariate analysis. For experiments consisting of two or three food types, there is no "order effect" issue, as each food is physically located next to each other. In cases with more than three food types, however, the order effects must be considered. These can be addressed by fundamental statistical design measures such as counterbalancing the orders of the food types among the replicates.

Our experimental design uses two concentric chambers, one inside the other (Fig. 1). The inner chamber is the experimental chamber, and is large enough to hold one sea urchin (or other consumer) and as many sample types of food that are to be tested. The outer chamber is the control chamber. It also contains the same types and amounts of food but no herbivore. Openings in both the inner and outer chambers are large enough to allow seawater flow but small enough to retain both the herbivore and any food debris within their respective chambers. This nested association of both the control and experimental chambers ensures that factors such as light intensity, day length, water temperature, and nutrients (especially those released as waste products of herbivore metabolism) jointly affect the control and experimental foods. [Nitrogen is frequently the limiting major nutrient for plant growth in marine waters as well as a major waste product of herbivore metabolism (Prince, personal observation; Ryther and Dunstan 1971)]. It is this joint effect on the control and experimental food samples that is enforced by the shared environment, which directly contributes to the intercorrelations that can now be incorporated into the analysis. This is the feature that distinguishes our method from both the artificially induced matched-sample test of Roa (1992) and the independent samples test of Manly (1993).

The statistical analysis is then performed on the difference values (weight change of controls vs weight change of experimentals) for each food type over all replicates, as in Roa (1992). For experiments comparing only two food types, the matched sample t -test is used. In the case of n food types, where n is greater than 2, the test of the null hypothesis, i.e., that there is no preferred food (i.e., that all corrected-means are equal), can be effectively carried out by means of the multivariate Hotelling's T^2 test by transforming the n mean values into $n-1$ values by, as one example, subtracting the nth corrected-mean from the preceding $n-1$ corrected-means. This is the transformation proposed by Manly (1993) as the solution to his third criticism of Roa's analysis (see above), and is simple to implement in standard statistical packages such as the SAS System's GLM/MANOVA procedure (SAS 1989). Post-hoc analyses for specific pairwise comparisons are easily conducted in the event of an overall significant result for the Hotelling's T^2 statistic (as suggested by Roa 1992). {Note, the output of the SAS MANOVA procedure includes a multivariate F and a Wilk's Λ which can be easily transformed into Hotelling's T^2 by a simple algebraic computation, e.g. Hotelling's $T^2=(n-1)\times[(1/\Lambda)-1]$, Littell et al. 1991, where n is the number of replicates.}

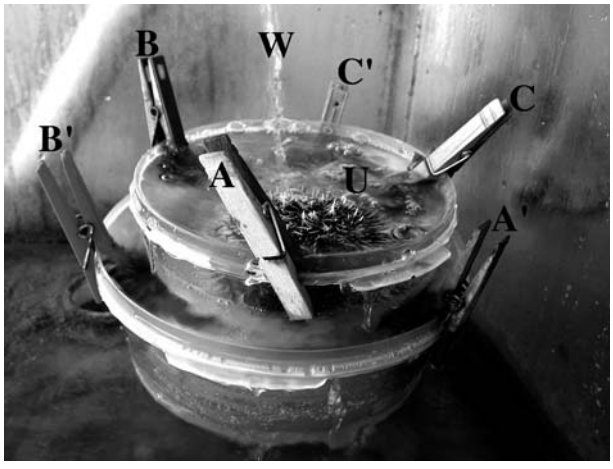

Fig. 1 Design for multiple choice feeding experiments showing experimental chamber (food types, *A–C* , with urchin) nested within the control chamber (food types, *A′–C′* , without urchin). *A, A′ Galaxaura oblongata*, *B, B′ Dictyota cervicornis* , *C, C′ Thalassia testudinum* , *U Tripneustes ventricosus* , *W* sea water

Materials and methods

Multiple-choice feeding experiment to test proposed experimental design

Sea urchins [*Tripneustes ventricosus* (Lamarck)] with a mean test diameter of 75.6±2.6 mm, 20 (SD, *n* ; range: 71–80 mm) were collected from the local waters of St. Ann's Bay, Jamaica and starved for 5 days prior to the feeding experiments. Sea weeds [*Dictyota cervicornis* Kuetzing and *Galaxaura oblongata* (Ellis & Solander) Lamouroux] and sea grass (*Thalassia testudinum* Banks ex König) were collected at the start of the experiment and each separated into 4–5 g portions (wet weight determined after spinning 20 times at approximately 120 rpm in a salad spinner-see Prince and LeBlanc 1992). The foods were distributed randomly and at an equal distance from each other in both experimental and control chambers (with and without sea urchin, respectively) and secured to the chamber wall by plastic clips (Fig. 1). The volumes of the inner (with urchin) and outer chamber were 1.1 and 1.8 l respectively. Seawater (28°C) flowed into the inner chamber, and holes (3 mm in diameter) in the chamber walls allowed for water flow between chambers but retained plant fragments. Wet weight of the foods was recorded at the start of the feeding experiment and after a 12 h interval.

Results

Table 1 shows percent consumption of the three foods presented to starved sea urchins over a 12-h period. Each number represents the 12-h change in control food weight (expressed as the percent of initial amount) minus the 12-hour change in the weight of a paired experimental food (expressed as the percent of initial amount). A Hotelling's T^2 analysis shows a significant difference among the means (P <0.0001; T^2=35.03, F =16.59, df =2, 18). Post-hoc comparisons showed a significant feeding preference for *Dictyota* verses that for either *Galaxaura* or *Thalassia* [P <0.01, F =14.44, df =1, 19; P <0.0001, F =34.72, df =1, 19 respectively) but no feeding preference was found between *Galaxaura* and *Thalassia* [P =0.079; F =3.43 df =1, 19].

Table 1 The data from a 20-replicate multiple food choice experiment. The data elements represent measurements of amount of food eaten, corrected for autogenic change, expressed as a percentage of initial quantities

Replicate	*Dictyota*	*Galaxaura*	*Thalassia*
1	79.40	31.25	6.72
2	71.21	2.32	8.84
3	80.19	0.19	25.48
4	8.22	15.18	6.77
5	2.17	0.09	4.40
6	54.17	2.72	20.45
7	84.44	4.26	11.02
8	64.40	−2.04	6.52
9	−9.84	−8.16	6.52
10	66.96	54.06	8.65
11	8.70	2.27	4.15
12	85.42	0.00	8.51
13	22.22	8.15	−2.36
14	80.48	23.37	0.45
15	74.60	52.27	8.89
16	6.52	2.08	−7.68
17	67.99	38.64	−9.61
18	82.51	17.78	−1.62
19	6.57	0.38	−2.08
20	28.98	85.15	2.46
Mean	48.27	16.50	5.32
SD	34.29	24.28	8.30
Correlation matrix			
	1	0.22	0.32
		1	−0.17
			1

A simulation study to compare methods of design and analysis of multiple choice feeding preference experiments

We conducted a simulation experiment in order to assess the advantage of our design which preserves the actual correlation structure among the data elements (by nesting experimental/control chambers) to a design that does not (Roa's1992, method of randomly pairing experimental and control observations). To simulate the conditions of our nested-chamber experiment (Method 1), 1,000 sets of a 20×3 matrix of data points were constructed as if drawn from a parent population having an inter-column correlation matrix equivalent to the correlation matrix of the data set from our feeding experiment. To simulate the conditions imposed by Roa's (1992) manipulation (Method 2), the data points of each of the 1,000 sets of data from Method 1 were reordered. This reordering within each of the three food types was done so that the intercolumn correlation coefficients averaged out to zero but the mean consumption associated within each food type was unchanged. The data sets in each of the two methods, therefore, reflected the same amount of simulated consumption for each food type.

Once the basic framework of the two data sets was in place, a further manipulation was introduced to assess the power of the two methods to detect differences in feeding preference among the food types. The magnitudes of the differences among the three food types in both methods were manipulated to reflect a progressive increase in feeding preference (Table 2). Condition 1 had no difference in feeding preference among the three food types (all three column means set at zero—see Table 2), Condition 2 had a small difference among food types (means set to 0, 0.5, and 1), Condition 3 a larger difference (means set to 0, 1, and 2), and Condition 4 a pronounced difference in feeding preference among food types (means set to 0, 2, and 4).

Table 2 shows the consequences of the loss of the inter-correlation among the food types on the power of the two methods to detect a difference among means. When there is no difference among the three means (Condition 1), both

Table 2 Percentages of statistically significant results in a simulation study analyzing 1,000 sets of data for a feeding preference experiment with three food types. In Method 1, the data were generated to reflect the correlation among the measurements of the three food types as obtained by our feeding experiment. In Method 2, measurements among the three food types were randomly paired, as described by Roa (1992). Four different conditions representing an increasing divergence between means were tested (see text). Alpha was set at 0.05

Condition	Means	Method 1	Method 2
1	(0, 0, 0)	5.4	4.1
2	(0, 0.5, 1)	14.2	8.4
3	(0, 1, 2)	52.8	35.6
4	(0, 2, 4)	99.1	95.9

methods have a false positive rate close to alpha (0.05), with Method 1 being somewhat closer to the expected value of 0.05 (alpha). As the difference among the means increases (Conditions 2–4), and especially at the levels of a moderate difference among means (Conditions 2 and 3), Method 1 is consistently more prone to detect that difference. In the situation with a major difference in feeding preference, Condition 4, even the less powerful test is almost certain to declare a significant effect.

Discussion

One of the principle advantages of the nested configuration for the experimental and control chambers for determining feeding preference is that it preserves the inter-correlation among food types that are lost in Roa's (1992) randomization process. In addition, both experimental and control chambers experience identical environmental conditions in each replicate and both receive any nutrients released by the herbivore. Furthermore, unlike the procedures proposed by Manly (1993), the data generated by our protocol allow for the use of a multivariate analysis that is easy to implement with standard statistical software. All analyses can be performed on simple differences and percents that are easily computed from the data, avoiding the need for extensive data manipulation/randomization techniques. Standard MANOVA analyses, moreover, include the availability of multiple-comparison testing procedures.

Our design could be applied to other herbivore feeding choice situations in both the aquatic and terrestrial environments where autogenic changes in food weight occur during the progress of the experiment. For example, field experiments examining the affect of herbivory on attached algal presence and/or biomass generally require replicating two or more separate conditions; one with the food exposed, the second protects the food from herbivory by a net exclosure, and the third partially encloses the food to account for shading by the netting (Carpenter 1986). Another approach involves attaching experimental foods to submerged lines allowing grazing while caged (control) foods are attached to separate lines (Hay 1981; Schiel 1982; Lewis 1986; Coen and Tanner 1989). Grand means are calculated to account for autogenic change in the control and the experimental weight is corrected accordingly. A more appropriate design would physically associate experimental foods with their control counterparts, then replicating these pairs or triads the appropriate number of times. Statistical analysis would proceed as above. Our nested experimental design could also be used when using changes in leaf weight to analyze the feeding preference of beetles for leaves from different tree species.

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