Design and analysis of multiple-choice feeding-preference experiments

Rubén Roa

Instituto de Fomento Pesquero (IFOP), Casilla 347, Talcahuano, Chile

Summary. A serious omission in ecological methodology is the absence of a rigorous statistical procedure to analyse multiple-choice feeding-preference experiments. A sample of 21 studies in the littoral marine context shows that results from such experiments are used to study a variety of conceptual issues, ranging from nutritional biology to ecosystem dynamics. A majority of such studies have been incorrectly analysed. The analytical problem has two facets: (1) lack of independence in the simultaneous offer of food types and (2) the existence of autogenic changes particular to each food type. Problem (2) requires the use of control arenas without the consumer. A recent advance allows the rigorous analysis of experiments with two food types offered simultaneously. Here I propose a method for the multiple-choice case. For the first problem I suggest the use of multivariate statistical analysis, providing both a parametric and a nonparametric procedure. The second problem is solved using basic statistical theory. I analyse data from an experiment with the sea urchin *Tetrapygus niger* feeding on three species of algae: *Ulva nematoidea*, *Gymnogongrus furcellatus*, and *Macrocystis pyrifera*. The parametric and nonparametric procedures yielded similar results, and showed that when offered the three species of algae *T. niger* does not feed at random but shows a preference for *U. nematoidea*. The method requires that the number of replicates in the treatment and control arenas be the same, and greater than the number of food types. The method is useful for other kinds of multiple-choice experiments.

Key words: Food preference – Habitat selection – Experimental design – Multivariate analysis – Sea urchins

Feeding-preference experiments, in which two or more food types are simultaneously offered to a consumer to assess its preference, are widespread in the ecological literature (see Table 1 for a sample of 21 studies done in the littoral marine context). Results are used to test a variety of important hypotheses in feeding ecology (see below), as well as to understand basic trophic relationships in all ecosystems (Peterson and Renaud 1989). Unfortunately, the analysis of feeding preference experiments has been flawed for two important reasons: incorrect use of univariate statistical analysis leading to violation of the assumption of independence of the errors, and incorrect use of controls leading to underestimation of error variances. These two problems are interrelated and have implications not only in statistical analysis but also in experimental design.

The problem is of general importance to ecological work since feeding-preference experiments are a useful tool in many contexts. A list of the conceptual issues involved in the use of results from feeding preference experiments of the studies in Table 1 demonstrates this: evolution of food preference related to availability of food instead of food quality (Arrontes 1990), vegetation structuring processes and herbivory (Barker and Chapman 1990), nutritional biology (Bonsdorff and Vahl 1982), dietary plasticity and prey population stability (Briscoe and Sebens 1988), organic matter cycling and conservation in mangrove ecosystems (Camilleri 1989; Poovachiranon et al. 1986), predation on the consumer and consumer feeding behavior (Hay et al. 1986), comparability of marine specialized herbivores to terrestrial insects in tolerance to plant chemical defenses (Hay et al. 1988; Paul and Van Alstyne 1988), effect of predator preference on other animals inhabiting the preferred prey (Holmlund et al. 1990), feeding behavior as an optimization strategy that maximizes growth and reproductive output (Vadas 1977; Larson et al. 1980), interspecific competition, coexistence and diet overlap (Morrisey 1988; Navarrete and Castilla 1988), ontogeny and the correspondence between food preference and growth (Pennings 1990), predator preference and prey population structure and dynamics (Robles et al. 1990; Vreeland and Lasker 1989; Watson and Norton 1987), predator preference and prey abundance and life history characteristics (Schiel 1982), and exploitation of avoided trophic niches (Wylie and Paul 1989).

In terms of the way the food types are offered to the consumer, there are two kinds of feeding preference experiments: simultaneous and separate offer. Separate-offer experiments do not address the problem of feeding preference because no food choice is provided to the consumer (Peterson and Renaud 1989). The interpretation of "preference rankings" from separate-offer experiments can be misleading because since no choice is given, a less preferred food type can be consumed at higher rates to compensate for a low nutritional value, as noted by Hay et al. (1988) and demonstrated for a parrotfish by Targett and Targett (1990). In this paper I do not address the design and analysis of separate-offer experiments, which is relatively straightforward, but only of "true feeding preference experiments" (Peterson and Renaud 1989), in which the food types are offered simultaneously to one or a group of consumers.

Recently, Peterson and Renaud (1989) found a rigorous procedure to analyse feeding preference experiments in which two food types are offered to a consumer. The rigorous procedure to analyse multiple-choice experiments is still lacking. Peterson and Renaud (1989) stated that to find such a procedure is "one of the biggest current challenges in ecological methodology" (p. 86). In this paper I propose an experimental and statistical procedure that appears to solve both the problems of lack of independence and of misuse of controls. I illustrate the method using data from an experiment on sea urchins feeding on algae.

The current situation

The problem of lack of independence

Recently, Hay et al. (1988) correctly pointed out that in feeding preference experiments where two or more food types are offered simultaneously to a consumer, it is incorrect to use ANOVA (or any univariate analysis) to support significant differences in the consumptions on the different food types. This is because the treatments, the different foods, are not independent: the consumption of one food type is certainly dependent on the presence of the others if there is a preference. Non-parametric analyses which have been used also require the treatments to be independent.

In the littoral marine context several studies have used ANOVA, non-parametric rank tests, or approximate χ^2 tests to analyse data from simultaneous-offer feeding-preference experiments. Table 1 shows a sample of 21 such studies. In those studies using ANOVA (7, 33% of the sample in Table 1), the error terms are not independent of the treatments, so that the estimation of treatment variance by the among treatment mean square is incorrect, and the F-test of significance can be seriously impaired (Sokal and Rohlf 1969; Underwood 1981). Studies using Wilcoxon's test (2, 9.5% of the sample) failed to note that the test assumes not only independence within the samples but also mutual independence

Table 1. A sample of simultaneous-offer feeding-preference experiments in littoral marine ecology, and the analysis performed

Paper	Consumer-Food Type	Choice	Analysis
Arrontes (1990)	Isopods-Algae	Double	χ^2
Barker and Chapman (1990)	Snails-Algae	Multiple	No formal
Bonsdorff and Vahl (1982)	Sea urchins-Algae	Multiple	ANOVA
Briscoe and Sebens (1988)	Sea urchins-Algae, mussels	Double	ANOVA
Camilleri (1989)	Crabs-Seagrass	Multiple	No formal
Hay et al. (1986)	Sea urchins-Algae	Multiple	ANOVA
Hay et al. (1988)	Fishes, Polychaetes-Algae	Multiple	No formal
Holmlund et al. (1990)	Fishes-Algae	Multiple	Corrected t tests
Klinger (1982)	Sea urchins-Artificial food	Multiple	ANOVA
Larson et al. (1980)	Sea urchins-Algae	Multiple	ANOVA?
Morrisey (1988)	Snails-Microalgae	Double	χ^2
Navarrete and Castilla (1988)	Crabs-Invertebrates	Double Multiple	Likelihood ratio
Paul and Van Alstyne (1988)	Snails-Algae	Double	Likelihood ratio
Pennings (1990)	Sea hares-Algae	Multiple	MANOVA
Poovachiranon et al. (1986)	Amphipods-Seagrass	Multiple	ANOVA
Robles et al. (1990)	Lobsters-Mussels	Double	χ^2
Schiel (1982)	Sea urchins-Algae	Multiple	ANOVA
Vadas (1977)	Sea urchins Algae	Multiple	ANOVA
Vreeland and Lasker (1989)	Polychaetes-Gorgonians	Double	Likelihood ratio
Watson and Norton (1987)	Snails-Algae	Double	Wilcoxon's
Wylie and Paul (1989)	Fishes-Coral	Double	Wilcoxon's

between the two samples (Conover 1981). Studies using approximate χ^2 distributions [these include studies using the log-likelihood ratio, called *G*-Test in Sokal and Rohlf (1969)] (6, 29% of the sample) also violated the assumption of independent observations inherent in the χ^2 test (Conover 1981). I do not mean that all results and interpretations from the feeding preference experiments cited above are incorrect, since many of them show fairly obvious results, robust to many errors. It may be expected however, that a correct design and analysis would yield somewhat different results, particularly in relation to food types for which consumers do not show a large degree of preference or rejection.

Some of the authors in Table 1 (Barker and Chapman 1990; Hay et al. 1988; Holmlund et al. 1990; Pennings 1990) were aware of the problem of lack of independence in their experimental design. Barker and Chapman (1990) and Hay et al. (1988) did not perform a formal analysis and based their interpretations in obvious results. Holmlund et al. (1990) used the procedure of Peterson and Renaud (1989) for paired comparisons in a multiple-choice experiment, correcting the α-level for multiple use of data as in Sokal and Rohlf (1969). This is a possible solution for the analysis of multiple-choice feeding-preference experiments but has two serious disadvantages, one theoretical and the other practical. First, it does not allow a formal and simultaneous analysis of the covariances among the consumptions on the different food types, and second, computational work grows exponentially as the number of food types increases. Finally, Pennings (1990) used the analytical procedure that I propose here (MANOVA, see below), but he did not explain the theory that supports his decision to use multivariate analysis nor did he advance a solution for the problem of misuse of controls.

The problem of controls

Controls must be included in a feeding preference experiment when the duration of the feeding trial is long enough for autogenic changes to occur in the variable which measures the consumption of the consumer (mass, area, numbers, etc.) (Peterson and Renaud 1989). By "autogenic change" is meant all changes in the food that occur independent of the action of the consumers, changes which are to be measured by the controls.

Peterson and Renaud (1989) reviewed the literature on marine herbivore preference using seaweeds as foods. They found that in the best of the cases, the controls were misused by computing a constant of autogenic change for each food type and subtracting these changes from those in the treatment arenas. This constant comes from a single control or from the mean of replicated controls, not always equal in number to the treatment replicates. When a single control is used, no variance of autogenic change can be recorded, and when the control is replicated, the use of a single mean for each prey causes the loss of error variance. In both cases the error variance is underestimated, so that more null hypotheses are rejected than expected by chance (at the specified level of significance) when the null hypothesis of no effect is true. That is, more significant preferences are found which are not real.

Peterson and Renaud (1989) proposed a method for the rigorous analysis of feeding preference experiments in which two food types are offered to a consumer. Their method is based on computing the differences in the variable which measures consumption and autogenic change between the two food types and using a *t*-test to compare this difference, treatment *versus* control. I use a different approach to analyse feeding preference experiments in which more than two food types are offered to a consumer in the presence and in the absence of autogenic change of the food types.

The recommended analysis

The case of no autogenic change

There are several cases in which there is no reason to suspect autogenic changes in the food types. These are cases in which the time scale of the experiment is too short for those changes to occur. There is another major class of multiple-choice experiments in which autogenic changes are unimportant and controls need not be included: habitat selectivity, in which individuals select for refuges or food patches by occupying them. Nevertheless, the selection among several alternative habitats is dependent on the presence of the multiple choice, thus the assumption of independence between the error and the treatment effects is not satisfied for univariate analysis.

I suggest the use of multivariate analysis techniques. Let consider first a parametric approach, and assume that $p(p > 1)$ food or habitat types are offered to a consumer and it exerts a preference by somehow evaluating the different food or habitat types. The response variable in such an experiment is a vector of consumption or selectivity of dimension p, one for each replicate, in which the components (the different foods or habitats) are correlated. These components can be the difference between initial and final masses, areas or numbers, after a certain period of consumption, or a variable which measures habitat selectivity. The vector of responses is assumed to have a multivariate normal distribution with vector of mean responses μ and variance-covariance matrix Σ which is unknown. The mean responses are then assumed not to be independent. We can now test the null hypothesis that there is no food or habitat preference of the consumer. This hypothesis is technically equivalent to testing whether the components of the mean vector are all equal to a constant k, where

$$k = (1/pn) \Sigma_i \Sigma_j x_{ij}, \quad i = 1, p, \quad j = 1, n$$

where p is the number of food or habitat types, n is the number of replicates, and x_{ij} are the consumptions or selectivities measured in each cell. Hence we can write the null hypothesis H_0, as

$$H_o : \begin{bmatrix} \mu_1 \\ \vdots \\ \mu_p \end{bmatrix} = \begin{bmatrix} k \\ \vdots \\ k \end{bmatrix}$$

The test statistics to test this hypothesis is the one-sample Hotelling's T^2, which has the form

$$T^2 = n(\mathbf{X} - \boldsymbol{\mu}_0)' \mathbf{S}^{-1} (\mathbf{X} - \boldsymbol{\mu}_0)$$

where n is the number of replicates, $\mathbf{S}^{-1}$ is the inverse of the sampling variance-covariance matrix, and $\mathbf{X} - \boldsymbol{\mu}_0$ is the vector of differences between the sample means and the hypothesized constants (Morrison 1967). This statistic is the multivariate analogue of the familiar t. Indeed, T^2 reduces to $t^2 = F$ when $p = 1$. When the null hypothesis is true,

$$F = \frac{n - p}{p(n - 1)} T^2$$

has the F distribution with p and $n - p$ degrees of freedom. Note that an important requisite for the use of this test statistic is that n must be greater than p, that is, the experiment must be designed so that there are more replicates than food or habitat types. The greater this difference the better the test is at detecting small but significant differences among means, since as the sample increases, so does the power of the test.

When the null hypothesis of no preference is rejected, it is necessary to ascertain which food or habitat has been preferred and which rejected. Those with means greater than the constant k of no preference are suspected to be preferred, and the opposite for foods or habitats with means less than k. An approximate solution is to compute simultaneous confidence intervals for linear combinations of the means, using

$$\left[(1/n) \, \mathbf{a}' \mathbf{S} \mathbf{a} \, \frac{(n-1)p}{n-p} F_{\alpha; p, n-p} \right]^{1/2}$$

around $\mathbf{a}' \mathbf{x}$, where $\mathbf{a}$ is a vector of coefficients of the form

$$\mathbf{a}' = [0 \ 0 \ \dots \ 1 \ \dots \ 0 \ 0],$$

and $a'x$ has the normal distribution with mean $a'\mu$ and variance-covariance matrix $a'\Sigma a$ (Morrison 1967). The position of 1 in the vector a depends on the response variable (in our case food or habitat type) whose simultaneous confidence interval is looked for (Morrison 1967). So that a simultaneous confidence interval for a food type i can be computed as

$$x_i \pm \left[(1/n)s_i^2 \frac{(n-1)p}{n-p} F_{\alpha;\, p,\, n-p} \right]^{1/2}$$

Those food or habitat types whose simultaneous confidence interval do not include the constant of no preference k contributed to the rejection of the null hypothesis.

The method outlined above has two assumptions: the replicates of each observation vector are (1) independently drawn (2) from a normal multivariate population. The fulfilment of assumption (1) is a matter of experimental design. Regarding assumption (2), a necessary but not sufficient condition for multivariate normality is univariate normality of the components of the observation vector. Hence a partial test of assumption (2) is to perform univariate normality tests for each food or habitat type, available on most statistical packages. Data transformations must be tried if univariate normality is not satisfied for any food or habitat type. Specific tests for multivariate normality are those of Koziol (1982), based on the empirical distribution function of generalized distances of the observation vectors, and Machado (1983), based on multivariate measures of skewness and kurtosis.

When the distributional assumption (multinormality) cannot be fulfilled or the mean vector and the variance-covariance matrix cannot be adequately estimated, a nonparametric test based on ranks is available, in which only the first assumption need be satisfied. If the data consists of p food types and n independent replicates, then Quade (1979)'s test statistic

$$T_1 = \frac{(n-1)B_1}{A_1 - B_1}$$

has an approximate F distribution with $p-1$ and $(p-1)(n-1)$ degrees of freedom, and where

$$A_1 = \Sigma_i \Sigma_j (s_{ij})^2,$$

$$B_1 = (1/n) \Sigma (s_i)^2,$$

and

$$s_{ij} = Q_j [R(X_{ij}) - (p+1)/2]$$

In this latter expression $R(X_{ij})$ is the rank of the jth observation of the ith food or habitat type, and Q_j is the rank of the difference between the greatest and smallest value in the jth replicate (see also Conover 1981, p.295). Because $(p+1)/2$ is the mean rank of the food types, the value between the widest parentheses is the centered rank of each observation. Hence s_{ij} is a weighted-centered rank. Furthermore A_1 is akin to a total sum of squares and B_1 to a treatment sum of squares. Conover (1981, p. 299) recommends the use of this test when the number of correlated variables p is less than 5, and Friedman's test when p is ≥ 5. I refer the reader to Conover's textbook for an account of Friedman's test.

When the null hypothesis of no preference has been rejected using the nonparametric procedure, a multiple comparison test can be used to ascertain which food or habitat types are preferentially consumed or occupied. Let i and j denote two different food or habitat types; then they are considered different if the inequality

$$|s_i - s_j| > t_{\alpha/2} \left[\frac{2n(A_1 - B_1)}{(n-1)(p-1)} \right]$$

is satisfied for $t_{\alpha/2}$ with $(n-1)(p-1)$ degrees of freedom, using the same α-level as in Quade's test.

The case of autogenic change

When the food types can suffer autogenic change on the time scale of the experiment, controls must be included. In these cases, the variable which measures consumption has two supposedly independent sources of variation, other than the error variance: the pattern of autogenic change, and the pattern of consumption by the consumer. What we need is a variable showing only the pattern of consumption, to identify a feeding preference. But that variable must not lose the variance due to autogenic change, because that variance is part of the error in the treatment arenas. So the question is: how to eliminate the pattern of autogenic change to analyse the consumer-related variation *without loss of error variance*? Here I show that by randomly subtracting control data from treatment data, *datum by datum*, we eliminate the pattern of autogenic changes and we add an estimate of the autogenic variance. The method consists of random allocation of the values recorded for autogenic change in the control replicates to the treatment replicates for each food type. Random pairs are formed and the value for the control replicate is subtracted from that recorded in the corresponding treatment replicate. What results is a new variable, the random difference between treatment and control, for each food type. The new set of variables reflects only the pattern of consumption because the pattern of autogenic change has been eliminated but the variance of this pattern has been added to the variance of the pattern of consumption.

To show the formal basis of this procedure, I reproduce basic results of statistical theory, starting with the definition of the variance of a random variable, as

$$VAR(X) = E(X^2) - [E(X)]^2$$

where the operator E refers to the expectation of X (Morrison 1967; Lindgren 1976). So, if we look for the variance of the subtraction of two random variables, we have

$$VAR(X_1 - X_2) = E[(X_1 - X_2)^2] - [E(X_1 - X_2)]^2$$
$$= VAR(X_1) + VAR(X_2) - 2COV(X_1, X_2)$$

But if the two random variables are independent, then the term $COV(X_1, X_2)$ is zero, and the variance of the difference between two random independent variables reduces to the sum of the variances. This result is shown by Lindgren (1976, pp 137–8).

Since the changes recorded in the control and treatment arenas are independent by vitue of the experimental design, our new variable, the randomized difference between treatment and control has a mean which is the difference between treatment and control *but has a variance which is the sum of the variances recorded in the control and treatment arenas*.

One condition must be met to use this procedure: the number of replicates in the control and treatment arenas must be the same.

After applying this random subtraction procedure to eliminate autogenic changes from the data without underestimating the error variance, the analysis can proceed as in the case of no autogenic change illustrated above.

An example with sea urchins feeding on algae

In spring and fall 1989 I performed feeding-preference experiments with two size classes of the sea urchin *Tetrapygus niger* feeding on six species of algae. To illustrate the method I pooled data from the two seasonal trials and from preliminary experiments. Exploratory analysis showed no consistent variation among the different sources of data either in the pattern of consumption or the pattern of autogenic change. Hence any significant variation will only increase the variability in the data,

but will not introduce bias, making the results more conservative, i.e. reducing the statistical power of the analysis. I had a total of six treatment and control replicates, thus the mathematics of the parametric method allowed me to test for preferential consumption among a maximum of five food types. I selected three food types to illustrate the method. These three food types. *Ulva nematoidea* (Chlorophyceae), *Gymnogongrus furcellatus* (Rhodophyceae), and *Macrocystis pyrifera* (Phaeophyceae), were chosen because they showed no consistent variation in autogenic change among the different sources of control replicates. Moreover, three food types and six replicates provides sufficient statistical power to test for preferential consumption and to illustrate the effect of reducing the number of replicates on statistical power. I show the results of the analysis only for one size class of sea urchins.

The sea urchins were collected from a subtidal population at Bahía Coliumo (36°32'S; 72°56'W) and starved for 3 days previous to the experiments. Preliminary observations showed that in 3 days most of the urchins had emptied their guts. Groups of five urchins of less than 6 cm test diameter were put in tanks (33 l) with continuous flow (about 3 l/min) of sea water at ambient photoperiod and sea-water temperatures and salinities, and with 8–11 g of algae collected on the day of the experiment. Data is initial minus final wet (blotted) mass after 24 h. After finishing the experiments the sea urchins were returned to the sea at suitable habitats. Table 2 shows the data, the random pairs formed with control and treatment data for each food type, and the new data, the randomized difference of treatment and control. Note in Table 2 that for each food type the mean in the new data is the difference between the means of treatment

and control, that is, the pattern of autogenic change has been eliminated from the pattern of consumption, but note also that for each food type the variance of the new data is greater than the variance of treatment, that is, we have not underestimated the error variance. That the final variance for each food type is not exactly the sum of the variances of treatment and control, as predicted by the theory (see above), reflects the sampling nature of those variances. The constant of no preference, k, is the mean of the new data, what should be expected if the sea urchins fed at random among the three food types, i.e., showed no preference. This constant was 0.543 g/5 ind/day. To center the data around zero I subtracted this constant from each datum of the new data, so that the constant of no preference was zero.

Using the MGLH module of SYSTAT (Wilkinson 1988) I tested the null hypothesis of no preference, that is

$$H_0: \begin{bmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix},$$

where 1 stands for *U. nematoidea*, 2 for *G. furcellatus*, and 3 for *M. pyrifera*. Hotelling's T^2 was 15.437 with 3 and 3 degrees of freedom, with a P value of 0.025. Hence there is strong evidence against the null hypothesis of no preference. The centered mean for *U. nematoidea* is 0.91, for *G. furcellatus* is −0.45, and for *M. pyrifera* is −0.45, so it is clear that the first alga is significantly preferred over the other two. In cases where the interpretation regarding which difference caused the rejection of the null hypothesis is not so clear, it is convenient as an approximation to compute simultaneous confi-

Table 2. Raw data from a multiple-choice feeding-preference experiment with the sea urchin *Tetrapygus niger* and three species of algae: *Ulva nematoidea* (Un), *Gymnogongrus furcellatus* (Gf), *Macrocystis pyrifera* (Mp)

Replicates	Treatment			Control		
	Un	Gf	Mp	Un	Gf	Mp
1	2.21	0.15	0.21	0.87	−0.23	0.14
2	1.73	−0.06	−0.09	0.64	0.04	0.05
3	1.30	−0.08	0.19	−1.61	0.11	−0.07
4	2.27	0.31	−0.10	−0.84	0.06	−0.10
5	1.08	0.65	0.25	1.22	0.38	−0.04
6	0.81	−0.40	0.42	0.43	−0.31	0.34
Mean	1.57	0.09	0.15	0.12	0.01	0.05
Variance	0.36	0.13	0.04	1.21	0.06	0.03
1	2−1	4−5	1−4	0.86	−0.07	0.31
2	6−3	2−2	3−5	2.42	−0.10	0.23
3	3−4	6−3	5−3	2.14	−0.51	0.32
4	5−2	3−4	6−1	0.44	−0.14	0.28
5	1−6	5−6	4−2	1.78	0.96	−0.15
6	4−5	1−1	2−6	1.05	0.38	−0.43
Mean				1.45	0.09	0.09
Variance				0.61	0.26	0.09

Data is initial minus final (blotted) mass (g) after 24 h in the presence (treatment) and the absence (control) of 5 urchins. The bottom-left block shows the random pairs of treatment minus control. The bottom-right block shows the new data. The mean of the data in this latter block is the constant of no preference $k = 0.543$

dence intervals for each food type (see above). Small sample size ($n = 6$) precluded application of Koziol (1982)'s generalized distances and Machado (1983)'s statistics of skewness and kurtosis to test the assumption of multivariate normality. Nevertheless, the fact that the nonparametric Quade's test yielded similar results ($A_1 = 182$, $B_1 = 114.3$, $T_1 = 8.45 > F_{0.05; 2, 10} = 4.10$) supports the conclusion for this particular example.

Discussion

T. niger smaller than 6 cm test diameter from Bahía Coliumo, Central Chile have a clear preference for *U. nematoidea* when offered this alga together with *G. furcellatus* and *M. pyrifera*. This statistically rigorous finding forms a safe starting point for other studies. For example, in analysing the natural diet of small *T. niger* I (Roa 1990) found that most of the diet in terms of proportion in the gut contents was crustose calcareous algae and *U. nematoidea*, the preferred food. However, in analysing the distribution of the sea urchin and its food, the same study shows that subtidal populations of small-sized sea urchins do not co-occur with *U. nematoidea* but inhabit rocks covered with crustose calcareous algae. This finding leads to the suggestion that the sea urchin could reach its preferred food by feeding on drift algae. This is in contradiction to previous reports (Contreras and Castilla 1987) which showed that *T. niger* does not feed on drift algae but grazes over the bottom, and could lead to a new evaluation of the role of *T. niger* in littoral habitats of central Chile.

To be effective, multivariate analysis requires relatively large samples. In comparative studies – the traditional field of applications of multivariate analysis – this is normally not a serious problem. However, experimental researchers cannot afford to replicate without limit, so that a very careful balance must be reached to attain power without an excessive burden of replication. For the parametric analysis a minimum limit for the number of replicates exists, and is $p + 1$. But this limit will usually be too small to detect significant departures from the null hypothesis. For instance, deletion of one replicate in our example of sea urchins feeding on 3 species of algae produces no evidence against the null hypothesis of no preference ($p > 0.10$) in one case (deletion of replicate 2 in Table 2), only weak evidence against the null hypothesis ($0.10 > P > 0.05$) in 3 cases (deletion of replicates 1, 3 and 4, one at a time), and strong evidence against the null hypothesis ($P < 0.05$) in only two cases (deletion of replicates 5 and 6, one at a time). Hence the chance of having sufficient power to reject the null hypothesis of no preference using only 5 replicates was 0.33. Thus it is convenient to exceed p as much as possible. There is no fixed rule for knowing in advance the number of replicates, but in a simulation study intended to show the sampling stability of variable loadings in linear discriminant analysis (which is structurally similar to multivariate analysis of variance), Williams and Titus (1988) recommended that 3 times as many samples as variables measured for each group of individuals should be obtained. If we ex-

tend this recommendation to our experimental case, it would be safe to use 3 times as many replicates as food or habitat types are offered to the consumer.

The mathematics of the nonparametric approach allows a minimum number of replicates of only 2, whatever the number of food or habitat types. However this approach will usually need more replicates than the parametric procedure because of the reduction in statistical power inherent in using ranks instead of the raw data. Furthermore, the statistic T_1 only approximates the F distribution, and does it better as the sample size increases. Hence I strongly recommend the use of the number of replicates appropriate for the parametric procedure, regardless of whether the parametric or the nonparametric procedure will be applied. Otherwise, a statistical power analysis must be performed to ascertain the level of type II error when the null hypothesis of no preference is not rejected.

The method proposed here does not have stringent assumptions. First, one-sample Hotelling's T^2 assumes independence between the replicates and multivariate normal distribution for the variables under study, but does not require variance homogeneity, since we are just testing that a certain normal random vector has a given constant form (the no preference form). Second, Quade's test only assumes independence between the replicates. But if the purpose is to test a more complex hypothesis, for example that two size classes of a certain consumer have different food or habitat preferences, then the test statistic is the two-sample Hotelling's T^2, which requires not only multivariate normality but also homogeneity of the variance-covariance matrices for the preference vectors of the two size classes.

A critical assumption of the method proposed here affects the cases in which autogenic change of the food types is important. The use of the randomized difference between treatment and control assumes that the pattern of autogenic change is the same in the absence and the presence of the consumer, that is, that the variance of autogenic change recorded in the control replicates is an unbiased estimate of the variance for autogenic change in the treatment arenas. Departures from this assumption cause a misestimation of the error variance. This problem has already been noted by Peterson and Renaud (1989), and they showed some useful defences against unpredictable violations of this assumption.

References

Arrontes J (1990) Diet, food preference and digestive efficiency in intertidal isopods inhabiting macroalgae. J Exp Mar Biol Ecol 139:231–249

Barker KM, Chapman ARO (1990) Feeding preferences of periwinkles among four species of *Fucus*. Mar Biol 106:113–118

Bonsdorff E, Vahl O (1982) Food preference of the sea urchins *Echinus acutus* and *E. esculentus*. Mar Behav Physiol 8:243–248

Briscoe CS, Sebens KP (1988) Omnivory in *Strongylocentrotus droebachiensis* (Müller) (Echinodermata: Echinoidea): predation on subtidal mussels. J Exp Mar Biol Ecol 115:1–24

Camilleri J (1989) Leaf choice by crustaceans in a mangrove forest in Queensland. Mar Biol 153–159

Contreras S, Castilla JC (1987) Feeding behavior and morphological adaptations of two sympatric sea urchins species in central Chile. Mar Ecol Prog Ser 38:217–224

Conover WJ (1981) Practical Nonparametric Statistics. John Wiley & Sons. NY, Chichester, Brisbane, Toronto

Hay ME, Lee RR, Guieb RA, Bennett MM (1986) Food preference and chemotaxis in the sea urchin *Arbacia punctulata* (Lamarck) Philippi. J Exp Mar Biol Ecol 96:147–153

Hay ME, Renaud PE, Fenical W (1988) Large herbivore versus small sedentary herbivores and their resistance to seaweed chemical defenses. Oecologia 75:246–252

Holmlund MB, Peterson CH, Hay ME (1990) Does algal morphology affect amphipod susceptibility to fish predation? J Exp Mar Biol Ecol 139:65–83

Klinger TS (1982) Feeding rates of *Lytechinus variegatus* Lamarck (Echinodermata: Echinoidea) on differing physiognomies of an artificial food of uniform composition. In: Lawrence JM (ed) International Echinoderms Conference, Tampa Bay. A.A. Balkema, Rotterdam, pp 29–32

Koziol JA (1982) A class of invariant procedures for assessing multivariate normality. Biometrika 69:423–427

Larson BR, Vadas RL, Keser M (1980) Feeding and nutritional ecology of the sea urchin *Strongylocentrotus drobachiensis* in Maine, USA. Mar Biol 59:49–62

Lindgren BW (1976) Statistical Theory, 3rd Edn. Macmillan Publishing Co., New York

Machado SG (1983) Two statistics for testing for multivariate normality. Biometrika 70:713–718

Morrisey DJ (1988) Differences in effects of grazing by deposit-feeders *Hydrovia ulvae* (Pennant) (Gastropoda: Prosobranchia) and *Corophium arenarium* Crawford (Amphipoda) on sediment microalgal populations. I. Qualitative differences. J Exp Mar Biol Ecol 118:33–42

Morrison DF (1967) Multivariate Statistical Methods. McGraw-Hill, London, New York

Navarrete SA, Castilla JC (1988) Foraging activities of Chilean intertidal crabs *Acanthocyclus gayi* Milne-Edwards et Lucas and *A. hassleri* Rathbun. J Exp Mar Biol Ecol 118:115–136

Paul VJ, Van Alstyne KL (1988) Use of ingested algal diterpenoids by *Elysia halimedae* Macnae (Opistobranchia: Ascoglossa) as antipredator defenses. J Exp Mar Biol Ecol 119:15–29

Pennings SC (1990) Size-related shifts in herbivory: specialization in the sea hare *Aplysia californica* Cooper. J Exp Mar Biol Ecol 142:43–61

Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. Oecologia 80:82–86

Poovachiranon S, Boto K, Duke N (1986) Food preference studies and ingestion rate measurements of the mangrove amphipod *Parhyale hawaiensis* (Dana). J Exp Mar Biol Ecol 98:129–140

Quade D (1979) Use of weighted rankings in the analysis of complete blocks with additive block effects. J Am Stat Assoc 74:680–683

Roa R (1990) Efecto del tamaño y la estacionalidad sobre la preferencia alimentaria y la dieta natural del erizo *Tetrapygus niger* Molina de Chile centro sur. M. Sc. thesis, Departamento de Oceanología, Universidad de Concepción, Chile

Robles C, Sweetnam D, Eminike J (1990) Lobster predation on mussels: shore-level differences in prey vulnerability and predator preference. Ecology 71:1564–1577

Schiel DR (1982) Selective feeding by the echinoid, *Evechinus chloroticus*, and the removal of plants from subtidal algal stands in Northern New Zealand. Oecologia 54:379–388

Sokal RR, Rohlf FJ (1969) Biometry. W.H. Freeman and Co., New York

Targett TE, Targett NM (1990) Energetics of food selection by the herbivorous parrotfish *Sparisoma radians*: roles of assimilation efficiency, gut evacuation rate, and algal secondary metabolites. Mar Ecol Prog Ser 66:13–21

Underwood AJ (1981) Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr Mar Biol Annu Rev 19:513–605

Vadas RL (1977) Preferential feeding: An optimization strategy in sea urchins. Ecol Monogr 47:337–371

Vreeland HV, Lasker HR (1989) Selective feeding of the polychaete *Hermodice canunculata* Pallas on Caribbean gorgonians. J Exp Mar Biol Ecol 129:265–277

Watson DL, Norton TA (1987) The habitat and feeding preferences of *Littorina obtusata* (L.) and *L. mariae* Sacchi et Rastelli. J Exp Mar Biol Ecol 112:61–72

Williams BK, Titus K (1988) Assessment of sampling stability in ecological applications of discriminant analysis. Ecology 69:1275–1285

Wilkinson L (1988) SYSTAT: The System for Statistics. Evanston, Illinois, SYSTAT Inc

Wylie CR, Paul VJ (1989) Chemical defenses in three species of *Sinularia* (Coelenterata, Alcyonacea): effects against generalist predators and the butterflyfish *Chaetodon unimaculatus* Bloch. J Exp Mar Biol Ecol 129:141–160