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PCA scores as a covariate to ANCOVA : a potentially useful statistical technique for equalizing microhabitat

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**PCA SCORES AS A COVARIATE TO ANCOVA:
A POTENTIALLY USEFUL STATISTICAL TECHNIQUE FOR EQUALIZING
MICROHABITAT**

**A Thesis
Presented to
The Faculty of the Department of Biology
San Jose State University**

**In Partial Fulfillment
of the Requirements for the Degree
Master of Science**

**by
Kirsten Rose Holmquist
December 1994**

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ABSTRACT

PCA SCORES AS A COVARIATE TO ANCOVA: A POTENTIALLY USEFUL STATISTICAL TECHNIQUE FOR EQUALIZING MICROHABITAT

by Kirsten R. Holmquist

In this paper, a new method is proposed for factoring microhabitat conditions out of statistical analyses for ecological field studies. Ideally, all measured sites should be identical except for differences in the independent variables (e.g., treatment and control). However, differences in microhabitat quality relative to the measured variable (e.g., abundance of a species) are common and may have adverse effects on analysis. If such differences in habitat occur, a method for factoring out the effects of habitat on the dependent variables would be beneficial. The proposed technique for filtering out microhabitat effects is to use scores from an ordination technique as a covariate to an Analysis of Covariance. This technique is examined by means of a Monte Carlo simulation. The simulation results indicate that microhabitat ordination scores do appear to effectively filter out microhabitat differences when used as a covariate to an Analysis of Covariance.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS	v
SUMMARY	1
1. INTRODUCTION	1
2. MONTE CARLO SIMULATION OF EXPERIMENTAL DATA	4
3. DESIGN OF SIMULATED EXPERIMENTS	7
4. RESULTS FOR THE SIMULATION STUDY	12
<i>Results for Scenario I</i>	
<i>Results for Scenario II</i>	
<i>Results for Scenario III</i>	
<i>Results for Scenario IV</i>	
5. DISCUSSION	22
REFERENCES	25

LIST OF ILLUSTRATIONS

Figure	Page
1. Results for Scenario I	13
2. Results for Scenario II	15
3. Results for Scenario III	17
4. Results for Scenario IV, sub-case I	20
5. Results for Scenario IV, sub-case II	21
6. Results for Scenario IV, sub-case III	23

**PCA scores as a covariate to ANCOVA:
a potentially useful statistical technique for equalizing microhabitat**

BY K. R. HOLMQUIST

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SUMMARY

In this paper, we propose a new method for factoring microhabitat conditions out of statistical analyses for ecological field studies. Ideally, all measured sites should be identical except for differences in the independent variables (e.g., treatment and control). However, differences in microhabitat quality relative to the measured variable (e.g., abundance of a species) are common and may have adverse effects on analysis. If such differences in habitat occur, a method for factoring out the effects of habitat on the dependent variables would be beneficial. The proposed technique for filtering out microhabitat effects is to use scores from an ordination technique as a covariate to an Analysis of Covariance. This technique is examined by means of a Monte Carlo simulation. The simulation results indicate that microhabitat ordination scores do appear to effectively filter out microhabitat differences when used as a covariate to an Analysis of Covariance.

Some key words: ANCOVA; Ecology; habitat ordination; microhabitat; Monte Carlo simulation; PCA; Spatial autocorrelation.

1. INTRODUCTION

In ecological studies, it is virtually impossible to ensure identical microhabitat conditions among sites for different levels of independent variables in a manipulated field experiment. Ideally, all sites should be identical except for differences in the independent variables. If the measured variable (e.g., abundance of species) is affected by changes in

microhabitat and sufficient microhabitat heterogeneity exists, treatment effects on the measured variable may be confounded (Legendre, 1993; Dutilleul, 1993).

The scale on which the experimental manipulations are made creates a problem in design. For example, assume that one wishes to assess the effect of a treatment on the abundance of a species in a two level design (i.e., treatment versus control). Also, assume that, independent of the treatment, better habitat conditions will mean higher population abundance. If treatment and control manipulations can be done on a small scale, a randomized block design will remove the variation among sites from the analyses. However, if the treatment and control are conducted over larger areas (e.g., effect of off-road vehicles versus undisturbed sites), a block design is not possible.

In situations where large scale manipulations have been made, microhabitat heterogeneity effects on measured variables can confound the results of an experiment in four ways. Consider situations where a treatment may have a potentially adverse effect on abundance.

In case one, the microhabitat variation among sites is extensive in both treatment and control areas, but it is not different between treatment and control areas per se. Furthermore, shifts in microhabitat quality within an area are continuous. The net effect of the microhabitat variation is to increase the variation in the measured variable among sites and thus reduce the power of a test to resolve differences between the levels of the independent variable. If there are no real differences between the control and treatment, this type of microhabitat variation will not adversely affect the outcome of the analysis. However, if real differences do exist between treatment and control, this type of variation may contribute to a Type II statistical error.

Case two is similar to case one in that microhabitat variation is extensive among treatment and control areas but equivalent between them. However, shifts in microhabitat

are discontinuous, creating multiple distinct associations within areas. The variation in the measured variable among sites is greater than in case one, further reducing test power. Again, Type II statistical error would be exaggerated.

In case three, microhabitats within the control areas are of better quality than those within treatment areas. The net effect is that microhabitat differences will tend to exaggerate differences in the measured variables between treatment and control areas. If no real differences exist between treatment and control, this type of microhabitat variation would contribute to a Type I statistical error. If real differences do exist, a subsequent analysis would tend to inflate those differences.

Case four is similar to case three, but the differences between treatment and control microhabitat quality are reversed. Microhabitat differences will tend to minimize differences in the measured variable for this case. If the treatment has an effect, subsequent analyses may fail to detect a difference (Type II error). The failure to detect abundance changes will depend on the magnitude of the microhabitat differences. Type I error is still of concern as well.

In this study, a possible solution for minimizing the confounding effects of microhabitat is presented and evaluated. The solution proposed combines two standard analyses. First, an ordination technique such as Principal Components Analysis (Tabachnick & Fidell, 1989) is used to provide quantitative measures of microhabitat characteristics that are independent of treatment and control. Then, if the dependent variable is linearly associated with habitat quality, an Analysis of Covariance (Tabachnick & Fidell, 1989) can be used to test for differences between independent variables with ordination scores as covariates. The Analysis of Covariance (ANCOVA) could potentially remove the effects of the microhabitat variation captured by the Principal Components Analysis (PCA) from the analysis.

This study evaluates the PCA/ANCOVA technique's ability to distinguish real differences in the independent variable from differences due to confounding microhabitat variation. This evaluation is done in the context of assessing the effect of a treatment on a single dependent variable in a two level design. Large scale replication of treatment application is not modeled for this initial exploratory work. The standard analysis for such a design would be the two-sample independent t-test (Sokal & Rohlf, 1981). The proposed technique was evaluated by comparing the Type I and Type II error rates for all four cases to those for the t-test. A Monte Carlo simulation is used to create populations with known abundance distributions and habitat characteristics. The relationship between microhabitat characteristics and abundance can then be controlled. If the PCA/ANCOVA combination demonstrates reduced error rates with respect to the t-test when evaluating this simulated experimental data, the proposed technique's potential will be validated as a potential statistical analysis tool.

2. THE MONTE CARLO SIMULATION OF EXPERIMENTAL DATA

A potential relationship between habitat and a measured variable, species abundance, was simulated with a Monte Carlo model. The simulation ensures that the population abundance values have the inexactness associated with real sampling but also conform to a known relationship with the PCA scores. Population abundance was the sole dependent variable with one independent variable and two levels, control and treatment. The simulation was coded in Borland C++ 4.02. The uniform random number generator used as a basis for the Monte Carlo draws was developed from Knuth (1981). Random number generators for other distributions were taken from Press et al. (1988).

Each scenario (i.e., each of the four cases already described) was modeled under a number of treatment effects on the population abundance. The amount of abundance

variation unexplained by microhabitat was also varied to explore the limits of the ANCOVA technique. Each combination of scenario, unexplained variation level, and treatment effect was simulated 1000 times. This number of runs gives a resolution of 0.001 for the Type I/II error rates. The analysis of each of the scenarios given a particular treatment effect and unexplained variation level proceeded in the following manner.

For each scenario, the amount of population variation unexplained by microhabitat was varied to simulate different strengths of relationship between microhabitat and abundance. The variation unexplained by the habitat characteristics was measured as a percentage of the variation explained (Sokal & Rohlf, 1981). Three levels of unexplained variation were simulated: 1) 15% of explained variation, 2) 25%, and 3) 50%.

The treatment effect on the population was also varied to explore the error rates for the analyses. To evaluate Type I error for all cases, the simulation generated population distributions in which no real differences existed between treatment and control. The evaluation of Type II error is more complicated, because it depends on the degree of real difference between treatment and control. The smallest possible effect a treatment can have on a population is to create a real difference between treatment and control populations of one individual.

To explore Type II error, the real difference between treatment and control populations was initially set at this smallest possible value of one individual. The error rate for the ANCOVA and the Student-t test was determined. The real difference was then increased and the error rate determined for this new treatment effect. This process was continued until both tests could determine the correct answer reliably. In ranges of effect where one or both techniques experienced difficulties determining the correct answer, the treatment effect was evaluated at every integer value. The exact effects

explored varied with the scenario and the control-treatment relationship. The bounds will be given in the description for each scenario.

The simulation embodied, as much as possible, operational field conditions. For instance, each PCA score was presumed to be calculated from four habitat measurements. The range of the PCA score deviates was restricted from -3.0 to 3.0. This range was established from PCA scores generated from real habitat data. Taken with the population abundance, five measured variables were used altogether. For multivariate analyses, ecologists typically take at least five measurements per variable used in analysis. Therefore, twenty-five sets of paired habitat-population abundance measurements were simulated for each theoretical field experiment.

The PCA scores and the population abundance values were randomly specified in the course of the simulation. The PCA scores were chosen from a uniform random distribution. A linear equation was used to generate expected population abundance from habitat quality represented by PCA scores. The treatment effect on abundance was factored into this equation. The expected value was used as the mean in a random normal draw to get the measured abundance.

The population abundance random draws were also based on field experience. For the abundance deviates, a mean and standard deviation are required. The low end of the expected value for abundance was set at thirty individuals. This number is reasonable for many species such as plants, inverts, etc. Ten individuals (or less) would be more reasonable for large vertebrates. This number, however, would not have allowed the same exploration of treatment effect because populations expire at zero individuals. In so far as the results can be replicated, however, setting the low end at ten has no discernible effect on the results of the statistics (unpublished data). The range of the population means, however, was adjusted to attain certain levels of unexplained variation for the ANCOVA.

The standard deviation was set to one. This standard deviation results in simulated population values that have a variation range of length 6-8 centered on the expected value. This variation can represent as much as 25% of the expected value for the population abundance. This variability reflects various sampling errors and variation not due to measured habitat attributes.

3. DESIGN OF SIMULATED EXPERIMENTS

The four scenarios describe specific relationships between the control and treatment habitat. These scenarios specify particular levels of alpha (within-group variation) and beta (between-group variation) diversity. Each scenario, then, put further constraints on the habitat PCA scores. The scenarios are arranged such that, with minor exceptions, the alpha diversity is decreasing and the beta diversity is increasing.

It is important to note that a particular percent unexplained variation in abundance is achieved by varying the alpha diversity. As the unexplained variance decreases, the alpha diversity increases. This reduction in alpha diversity is important because the Student t-test is expected to be sensitive to the level of alpha diversity in the population. An increase in alpha diversity means an increase in the variation for the measured variable within a treatment area. This increase in variation within a level of the independent variable translates into reduced the power for an analysis such as the t-test. The t-test would be expected to do increasingly poorly as unexplained variation decreases.

The first scenario is modeled such that microhabitat variation is extensive, continuous, and well-matched between control and treatment sites. The large microhabitat variation within a study area means this scenario has high alpha diversity. The similarity of microhabitats between study areas for treatment and control means this scenario has low beta diversity. PCA scores for this scenario ranged between -3.0 and 3.0

for both control and treatment habitat. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to fifteen, eight, and four respectively. The largest of the treatment effects was 40% of the regression mean abundance for 15% unexplained variation, 30% of the mean for 25% unexplained variation, and 20% of the mean for 50% unexplained variation. The population abundance values and their relationship to the habitat PCA scores would look something like the following:

The second scenario is modeled such that the habitat variation is extensive, discontinuous, and well-matched between control and treatment sites. For this scenario, study areas still have high microhabitat diversity, but gaps in microhabitat quality create multiple distinct associations within study areas. Consequently, this scenario has lower alpha diversity than the first scenario. The differences in microhabitat quality for treatment and control are still minor so this scenario has low beta diversity as well. PCA scores for this scenario fell into three ranges to simulate an idealized multiple association scheme. The ranges were -3.0 to -2.0, -0.5 to 0.5, and 2.0 to 3.0. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to twelve, seven, and four respectively. The largest of the treatment effects was 25% of the regression mean abundance for 15% unexplained variation, 20% of the mean for 25% unexplained variation, and 15% of the mean for 50%

unexplained variation. The population abundance values and their relationship to the habitat PCA scores would look something like the following:

The third scenario is modeled such that the control sites' habitat quality is much better than the treatment sites' habitat. The microhabitat variation within a study area is small which means this scenario has low alpha diversity. However, the microhabitat is now completely different between study areas for treatment and control which means this scenario has high beta diversity. PCA scores for this scenario ranged between -3.0 to -2.0 for treatment measurements and between 2.0 to 3.0 for control measurements. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to seven, five, and three respectively. The largest of the treatment effects was 75% of the regression mean abundance for 15% unexplained variation, 60% of the mean for 25% unexplained variation, and 40% of the mean for 50% unexplained variation. The population abundance values and their relationship to the habitat PCA scores would look something like the following:

The fourth scenario is modeled such that the treatment sites' habitat is better than the control sites' habitat. Like the third scenario, this scenario has low alpha diversity and high beta diversity. Because the scope of the statistical problems for these last two cases depends on the magnitude of the differences between control and treatment sites, several sub-cases of this scenario were evaluated to track the development of error rates for both techniques. The between-site diversity was progressively increased (with a corresponding decrease in within-site diversity) for the three cases.

For the first sub-case, the microhabitat was modeled such that both treatment and control contained average quality sites. However, all poor quality microhabitat was associated with control study areas, and all the best quality microhabitat was associated with treatment study areas. PCA scores ranged between -3.0 to 2.0 for control measurements and between -2.0 to 3.0 for treatment measurements. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to seven, four, and one and a half respectively. The largest of the treatment effects was 40% of the regression mean abundance for 15% unexplained variation, 25% of the mean for 25% unexplained variation, and 10% of the mean for 50% unexplained variation.

For the second sub-case, the microhabitat quality was modeled such that the best control site was of lesser quality than the worst treatment site. However, the difference in

microhabitat quality between those two sites was minor. PCA scores ranged between -3.0 to 0.0 for control measurements and between 0.0 to 3.0 for treatment measurements. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to seven, five, and three respectively. The largest of the treatment effects was 60% of the regression mean abundance for 15% unexplained variation, 40% of the mean for 25% unexplained variation, and 35% of the mean for 50% unexplained variation.

For the third sub-case, the habitat quality for treatment sites was modeled to be much better than control sites. The microhabitat variation within a study area is small, however, the microhabitat is now completely different between study areas for treatment and control. PCA scores ranged between -3.0 to -2.0 for control measurements and between 2.0 to 3.0 for treatment measurements. To get 15%, 25%, and 50% unexplained variation in abundance, the slope of the linear equation relating abundance to habitat was set to seven, five, and three respectively. The largest of the treatment effects was 75% of the regression mean abundance for 15% unexplained variation, 60% of the mean for 25% unexplained variation, and 40% of the mean for 50% unexplained variation. The population abundance values and their relationship to the habitat PCA scores for each of the three sub-cases would look something like the following:

The simulation and design described above generated theoretical experimental data where the answer is known. The ability of different statistical techniques to discern the correct answer can now be directly evaluated. The simulated wildlife-habitat values were analyzed by both a Student t-test and an ANCOVA to independently determine if population differences exist. A nominal level of 0.05 was set for rejection of the null hypothesis. Each technique's Type I and II error rates were then compared to test relative efficacy.

4. RESULTS FOR THE SIMULATION STUDY

4.1 Results for Scenario I

Figure 1 illustrates the error domains of the two techniques for the case where microhabitat variation is high but similar between treatment and control. The Student t-test exhibits a large Type II error domain, whereas the PCA/ANCOVA does not.

For the 15% level of unexplained variation shown in Figure 1a, the Student t-test correctly distinguishes a real difference between the treatment and control when the treatment effect is greater than twenty-five. For instance, a negative treatment effect on mean population abundance of twenty-five individuals results in the correct conclusion being drawn for over 90% of the one thousand simulated experiments. However, as the real difference between treatment and control diminishes, the percent of conclusions made correctly diminishes to effectively zero.

For the 50% level of unexplained variation shown in Figure 1c, the t-test correctly distinguishes differences of eight or greater. Again, as the real difference diminishes, the percent of correct conclusions dwindles to effectively zero. The 25% level of unexplained variation is intermediate between these two cases.

Fig. 1. Results for Scenario I where habitat variation is extensive and continuous but control and treatment sites are of similar quality. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

The PCA/ANCOVA, however, adjusts correctly for that habitat generated abundance variation. The ANCOVA is a conservative for the minimal treatment effect of a mean change of one individual but still returns the correct answer 90% of the time even then. The weakest population-habitat relationship results were indistinguishable from the results for the strongest relationship. For large population changes, the two techniques are equivalent.

Type I error would occur only at a treatment effect of zero. For this scenario, both techniques demonstrate the ability to correctly determine that no real difference exists between treatment and control. The two techniques do equally well at making this determination. The ability to draw the correct conclusion was unaffected by the amount of unexplained variation.

4.2 Results for Scenario II

Figure 2 illustrates the error domains of the two techniques for the case where microhabitat variation is discontinuous but similar between treatment and control. The Student t-test once again exhibits a large Type II error domain, and the PCA/ANCOVA does not. The t-test results are similar to scenario I, but the Type II error rate is accentuated by the discontinuity in habitat quality.

For the 15% level of unexplained variation shown in Figure 2a, the Student t-test correctly distinguishes a real difference between the treatment and control when the treatment effect is greater than twenty. However, as the real difference between treatment and control diminishes, the percent of conclusions made correctly plummets. At a treatment effect of ten individuals, the percent of correctly made conclusions is already zero.

Fig. 2. Results for Scenario II where habitat variation is extensive and discontinuous but control and treatment sites are of similar quality. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

For the 50% level of unexplained variation shown in Figure 2c, the t-test correctly distinguished differences of six or greater. Again, as the real difference diminishes, the percent of correct conclusions plummets to zero. The 25% level of unexplained variation is intermediate between these two cases.

The PCA/ANCOVA, however, again adjusts correctly for that habitat generated abundance variation. The ANCOVA remains conservative for the minimal treatment effect of a mean change of one individual but still returns the correct answer 90% of the time even then. The weakest population-habitat relationship results were indistinguishable from the results for the strongest relationship. For large population changes, the two techniques are equivalent.

Again, Type I error would occur only at a treatment effect of zero. For this scenario, both techniques demonstrate the ability to correctly determine that no real difference exists between treatment and control. The two techniques do equally well at making this determination. The ability to draw the correct conclusion was unaffected by the amount of unexplained variation.

4.3 Results for Scenario III

Figure 3 illustrates the error domains of the two techniques for the case where control microhabitat quality is better than the treatment microhabitat quality. The Student t-test and the PCA/ANCOVA exhibit a Type II error domain, but the t-test also exhibits a Type I error domain. The t-test cannot determine when there is no treatment effect (Type I). It also cannot distinguish between two populations for which treatment effect is confounded by microhabitat effect. If the treatment has a positive effect on abundance when treatment microhabitat has a negative effect, the t-test can have trouble determining

Fig. 3. Results for Scenario III where control habitat quality is much better than treatment. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

if a real difference occurred between treatment and control. The microhabitat effect can neutralize the treatment effect.

For the 15% level of unexplained variation shown in Figure 3a, the t-test is unable to accurately detect treatment derived changes in mean abundance for a range of five individuals. The range is centered on the treatment effect which equalizes the mean populations for the control and treatment sites.

For the 50% level of unexplained variation shown in Figure 3c, the t-test is unable to accurately detect treatment derived changes in mean abundance for a range of three individuals. The range is again centered on the treatment effect which equalizes the mean populations for the control and treatment sites. The 25% level of unexplained variation is intermediate between these two cases.

The PCA/ANCOVA, however, was able to determine correctly when no treatment effect exists, but this technique now has significant Type II error limits. The ANCOVA has trouble accurately detecting treatment effects of up to eight or nine individuals. It has less than an even chance of giving the correct answer for treatment effects up to five individuals. The weakest population-habitat relationship results were still indistinguishable from the results for the strongest relationship. As will be demonstrated in Scenario IV, the extent of the critical range for the ANCOVA is driven by the magnitude of the beta (between-site) diversity.

4.4 Results for Scenario IV

Figures 4-6 illustrate the error domains of the two techniques for the cases where treatment microhabitat quality is better than the control microhabitat quality. The Student t-test and the PCA/ANCOVA exhibit a Type II error domain, but the t-test also exhibits a

Type I error domain. For this scenario, three sub-cases were modeled in order to explore the development of the errors for the two tests.

The t-test results for all three sub-cases display Type I and Type II errors. The t-test cannot determine when there is no treatment effect (Type I). It also cannot distinguish between two populations for which treatment effect is confounded by microhabitat effect. If the treatment has a negative effect on abundance and treatment microhabitat has a positive effect, the t-test can have trouble determining if a real difference occurred between treatment and control. The microhabitat effect can neutralize the treatment effect.

The PCA/ANCOVA, however, now has problems of its own. Depending on the sub-case, ANCOVA results can display significant Type II error limits. The degree of unreliability for the ANCOVA is driven by the magnitude of the beta diversity. The percent unexplained variation once again does not seem to be a major factor driving the error rate for this technique.

Figure 4 illustrates the sub-case where the treatment habitat quality is better than control but both have average quality sites. The PCA/ANCOVA is accurate. It is conservative for the minimal treatment effect of a mean change of one individual but still returns the correct answer more than 85% of the time even then. The t-test has less than an even chance of detecting treatment derived changes in mean abundance for a range of four (Fig. 4c) to thirteen (Fig. 4a) individuals. The range is centered on the treatment effect that equalizes the mean populations for the control and treatment sites. The t-test also is correct less than 40% of the time when no treatment effect occurs.

Figure 5 illustrates the sub-case where the worst treatment habitat quality is only slightly better than the best control quality. The PCA/ANCOVA still does well. It is inaccurate for the minimal treatment effect of a mean change of one individual,

Fig. 4. Results for Scenario IV, sub-case I, where treatment habitat quality is generally better than control, but both have average quality sites. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

Fig. 5. Results for Scenario IV, sub-case II, where the worst treatment habitat quality is only slightly better than the best control quality. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

conservative for a mean change of two individuals with better than 85% accuracy, and highly accurate otherwise. The t-test has less than an even chance of detecting treatment derived changes in mean abundance for a range of three (Fig. 5c) to seven (Fig. 5a) individuals. The range is again centered on the treatment effect that equalizes the mean populations for the control and treatment sites. The t-test also is completely unable to determine when no treatment effect occurs.

Figure 6 illustrates the sub-case where the treated site habitat quality is much better than the control. The ANCOVA has trouble accurately detecting treatment effects of up to eight or nine individuals. It has less than an even chance of giving the correct answer for treatment effects up to five individuals. The t-test is unable to accurately detect treatment derived changes in mean abundance for a range of three individuals. The range is centered on the treatment effect that equalizes the mean populations for the control and treatment sites. The t-test also is completely unable to determine when no treatment effect occurs.

5. DISCUSSION

The analysis results suggest that the PCA scores are generally successful in filtering microhabitat effects within ANCOVA. The Student t-test is unable to make this adjustment and hence is prone to Type II error. The ANCOVA, however, has an additional source of error from regression that becomes significant if the control and treatment sites are too disparate in microhabitat quality. Of course, if the population does not respond to the chosen habitat measurements, the power of the t-test would be unaffected by microhabitat. The microhabitat differences would not be introducing ambiguity, so no need would exist to compensate for those differences.

Fig. 6. Results for Scenario IV, sub-case III, where treatment habitat quality is much better than control. Level of unexplained variation in abundance varies from (a) 15%, (b) 25%, to (c) 50%.

It may, at first glance, seem counter-intuitive that the power of the t-test improves with increasing unexplained variation. However, the alpha (within-site) abundance diversity is creating the variation which reduces the power of the test. The larger the diversity, the less power the t-test has to distinguish real differences caused by treatments. Since the unexplained variation was increased by decreasing the alpha diversity, the t-test results are actually as expected. The abundance alpha diversity generated by habitat alpha diversity confounds this technique.

Scenario I and II, in particular, demonstrate the weakness of the Student t-test. Because the t-test is confounded by alpha diversity and the ANCOVA is not, the ANCOVA is a more reliable technique for assessing population changes. The ANCOVA would be the best technique to use to identify population changes of unknown magnitude. It would also be advisable for managing negative treatment effects on small populations that could collapse before the critical range for the t-test was exceeded.

Scenario III and IV demonstrate that ANCOVA cannot compensate for all evils. Control and treatment sites that are very badly matched in terms of habitat quality will greatly increase the effect of the regression error on the conclusions derived from ANCOVA. The regression error greatly expands the minimum detectable difference for the ANCOVA. The Student t-test, however, has difficulty determining when the population has changed if the two populations become equalized by the treatment. It also cannot tell when the populations have not been affected by the treatment. The last is of concern because the point at which the Student-t would experience difficulty would be unpredictable.

The ANCOVA is subject to two preconditions that limit its use, however. The relationship between PCA scores and population abundance must be a linear one. The ANCOVA is likely to be sensitive to violations of this precondition. Also, the rate

relationship for the linear equation must remain unchanged by the treatment. A simple test exists to ensure that this precondition is satisfied (Zar, 1984). The latter precondition is of much less concern because of this test. After all, if the rate relationship has changed, a treatment effect is demonstrated without needing recourse to the ANCOVA.

Based on the results, general recommendations can be made for when the ANCOVA/PCA technique would be the most useful. The Student t-test works perfectly fine when the population is indifferent to the pre-existing microhabitat differences. The ANCOVA should only be used if the population is responding to those differences. If the rate relationship between population abundance and habitat quality changes, one can conclude that a treatment effect exists without using ANCOVA. And, finally, the ANCOVA can compensate for a lot of habitat quality variation, but as the difference in habitat quality increases, so too does the minimum detectable difference. For sites that are extremely different, the ANCOVA will be unable to detect smaller changes in abundance.

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