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CONCEPTS OF STATISTICAL DESIGN

TERMS

Population = all items we are interested in.

Experiment = set of conditions which can be repeated infinitely often.

x = numerically expressed outcome of experiment, a random variable.

$p(x_i)$ = probability of getting an x_i value.


Binominal probability function [Applies when: all items belong uniquely to one of only two categories which can be named as success and failure; probability of success is some number (p) and it is the same for all items; the success-nonsuccess of individual items is independent.

Let x be number of successes in n trials, then

$$p(x) = \left(\frac{n(n-1) \dots 1}{x(x-1) \dots 1 (n-x)(n-x-1) \dots 1} \right) p^x (1-p)^{n-x}$$

Discrete data = count numbers, binominal numbers, etc.

Continuous data = measurement values such as fish length.

Normal distribution =  total area equals 1. Any portion of area under curve is a number between 0 and 1. Tables and most statistical functions based on normal distribution of populations.

$$\text{Population mean} = \mu = \frac{\sum x_i}{N}$$

Population variance = average squared distance from μ .

$$\sigma_x^2 = \frac{\sum (x_i - \mu)^2}{N}$$

Standard deviation = square root of variance = $\sqrt{\sigma_x^2} = \sigma_x$

Working formula for variance =
$$\sigma_x^2 = \frac{\sum x_i^2 - \frac{(\sum x_i)^2}{N}}{N}$$

Transformations = changing measurement scales.

Random sample of size 2 with replacement and order from a population with 6 items has 30 possible combinations.

Random sample of size 2 without replacement and without order from a population of 6 items has 15 possible combinations. In fish and game this is usual case.

Whenever we sample and use sample quantities we are using statistics. Some statistics one generally is interested in are the estimates

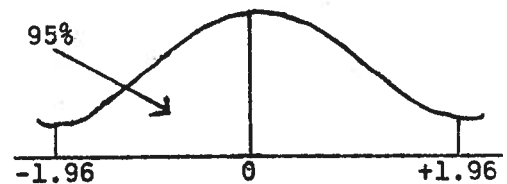
$$\hat{\mu} = \bar{x} = \frac{\sum x_i}{n}, \text{ and } \hat{\sigma}^2 = s^2 = \frac{(\sum x_i^2 - (\sum x_i)^2/n)}{n-1}$$

$Z \sim N(0, 1)$ Z is a random variable which has a normal distribution with mean = 0, and variance = 1.

$$Z = \frac{\bar{x} - \mu_{\bar{x}}}{\sqrt{\sigma^2/n}}$$

Confidence intervals using form of Z table

95% of area under curve is between -1.96 and +1.96. 95% confidence interval for population mean = $\bar{x} \pm (\sigma/\sqrt{n}) 1.96$. This says that the interval is one standard deviation times the Z value on either side of the mean.



EXAMPLE

Population = 2, 4, 6, 6, 8, 10

Sample = n = 2 without replacement no order

All possible samples = 2,4 4,6 6,6 6,8 8,10
2,6 4,6 6,8 6,10
2,6 4,8 6,10
2,8 4,10
2,10

Means of all possible samples = 3 5 6 7 9
4 5 7 8
 $\mu_{\bar{x}} = 6$ 4 6 8
5 7
6

All possible confidence intervals (90%) as constructed by example given below.

(.33, 5.67) (2.33, 7.67) (3.33, 8.67)
(1.33, 6.67) (2.33, 7.67) (4.33, 9.67)
(1.33, 6.67) (3.33, 8.67) (5.33, 10.67)
(2.33, 7.67) (4.33, 9.67)
(3.33, 8.67)

(4.33, 9.67) (6.33, 11.67)
(5.33, 10.67)

Assuming \bar{x} is normally distributed at 90% confidence our interval for μ is:

$$\bar{x} \pm \sqrt{\frac{N-n}{N-1} \frac{\sigma^2}{n}} \cdot 1.64, \text{ for example if } \bar{x} = 6, \text{ then } 6 \pm \sqrt{\frac{6-2}{6-1} \cdot \frac{6.67}{2}} \cdot 1.64 = 6 \pm 2.67$$

90% table value

at 90% confidence mean μ is between 3.33 and 8.67.

As can be seen from our population of sample means and their corresponding intervals, all intervals have $\mu = 6$ inside except for the intervals of the two extreme sample means of 3 and 9. Thus, our true confidence interval is 13/15 or 87%.

Of all possible samples of size 2 we can take from our original population of 6 items, 90% (actually 87%) of the time we will have a sample whose constructed confidence interval will contain μ .

SAMPLE SIZE

Formula for sample size using K table. K table can be found in Snedecor and Cochran, 6th Edition, page 113.

$n_1 = K (\sigma^2/\delta^2)$ This formula says that sample size = K value times the variance divided by desired interval width.

n_1 = sample size K = table value σ^2 = variance

δ = desired width of interval divided by 2

p = proportion of the time that the interval performs as requested

K table

1 - α

p		.99	.95	.90
	.8	11.7	7.9	6.2
	.9	14.9	10.5	8.6
	.95	17.8	13.0	12.8

EXAMPLE

From previous example on 6 items, with a standard deviation of $\sigma = .3\mu$ we wish to use a 90% confidence interval to be within $\delta = .1\mu$ of μ and are willing to accept that the sample size will be such that the constructed interval will perform as requested about 80% of the time.

$$n_1 = 6.2 (\sigma^2/\delta^2) = 6.2 (3)^2 = 56 \text{ observations}$$

EXAMPLE OF COMPUTING SAMPLE SIZE

These are measurements of zinc concentrations in rainbow trout gills in ppm.

<u>Method I</u>		95% confidence interval calculations	
n = 5	10.39	$\bar{x} \pm s/\sqrt{n} \quad t(n-1) \quad \begin{matrix} \alpha/2 \\ \text{table value 95\% (upper 2.5\%),} \\ \swarrow 4 \text{ df} \end{matrix}$	
	16.50		
	10.85		
	9.36		
	8.00		
$\Sigma x_i = 55.1$		$\bar{x} = 11.02$	
$\Sigma x^2 = 649.53$		$s^2 = 10.58$	
		$11.02 \pm \sqrt{\frac{10.58}{5}} (2.776)$	
		$7.1 \leq \mu \leq 14.9$	

As can be seen there is a very wide range of values the mean could be at the 95% confidence level. We could detect a change of about $.7\mu$ in the population mean μ with this many observations.

How many fish would we have to measure to get a more precise estimate of the mean? Say if we wanted to detect a difference in the mean of $.15\mu$.

$(1 - \alpha) = .95$ this is same confidence level.

$$p = .8$$

$\delta = .15\mu$ population difference } δ is the population value
 $d = .15 (\bar{x})$ estimate difference } while d is the estimate

$$n_1 = K (s^2/d^2) = K \left(\frac{10.58}{(1.65)^2} \right) = 7.9 \left(\frac{10.58}{(1.65)^2} \right) = 7.9 \cdot 3.9$$

$$n_1 = 31$$

To adjust sample size estimate because we used sample estimates s and d of σ and δ we use the formula below:

$$n = n_1 \cdot \frac{df + 3}{df + 1} = 31 \cdot 33/31 = \underline{\underline{33}}$$

To detect a difference of $.15\mu$ we need a sample size of 33.

Example of comparing two methods of determining zinc content of rainbow trout gills:

<u>Method I</u>		<u>Method II</u>	
	10.39 ppm		11.61 ppm
	16.50		21.29
	10.85		13.52
	9.36		6.83
	8.00		13.38
Σx_I	= 55.10	Σx_{II}	= 66.63
\bar{x}_I	= 11.02	\bar{x}_{II}	= 13.33
Σx_I^2	= 649.5342	Σx_{II}^2	= 996.5199
s^2_I	= 10.583	s^2_{II}	= 27.152

Is there a difference in the means obtained from the two methods? Compare using unpaired t test and determine confidence interval for the difference between the two means.

↑ indicates an estimate

$$\underbrace{\mu_I - \mu_{II}}_{\text{population}} = \underbrace{\bar{x}_I - \bar{x}_{II}}_{\substack{\text{our} \\ \text{sample}}} = 11.02 - 13.33 = -2.31$$

Variance of the difference of two estimators is always the sum of variances of the individual estimators if the two are independent.

$$\text{Variance of } (\bar{x}_I - \bar{x}_{II}) = \frac{\sigma^2_I}{n_1} + \frac{\sigma^2_{II}}{n_2}$$

Confidence interval for differences between two means:

$$\bar{x}_I - \bar{x}_{II} \pm t_v \alpha/2 \sqrt{\frac{s_I^2}{n_1} + \frac{s_{II}^2}{n_2}}$$

$1 - \alpha$ = confidence level, in this case we used 90%

v = degrees of freedom -- must compute

$$v = \left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2} \right)^2 / \left[\frac{\left(\frac{s_1^2}{n_1} \right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2} \right)^2}{n_2 - 1} \right]$$

$$v = \left(\frac{10.583}{5} + \frac{27.152}{5} \right)^2 / \left[\frac{\left(\frac{10.583}{5} \right)^2}{4} + \frac{\left(\frac{27.152}{5} \right)^2}{4} \right] = 6.7 \text{ or } 7 \text{ degrees freedom}$$

In t table with 7 df and $\alpha/2 = .025$ (95% level) we get 2.365

$$\begin{aligned} \text{So: } \bar{x}_I - \bar{x}_{II} \pm t_v \alpha/2 & \sqrt{\frac{s_I^2}{n_1} + \frac{s_{II}^2}{n_2}} \\ -2.31 \pm 2.365 & \sqrt{\frac{10.583}{5} + \frac{27.152}{5}} \\ -8.81 \leq \mu_1 - \mu_2 \leq 4.19 \end{aligned}$$

The difference between the two means has a very wide range from -8.81 to +4.19.

Since zero is included within this interval we say that according to our two samples of 5 observations we could determine no difference in the two methods of sampling zinc concentrations.

How many observations would we have to make to get a better estimate -- say to detect a difference of 2 ppm? Use formula and K value for determining sample size.

$$1 - \alpha = .95$$

$$n_1 = K \frac{s^2 \text{ diff}}{d^2}$$

$$d = 2$$

$$s^2 \text{ diff} = s_I^2 + s_{II}^2 = 37.7$$

$$p = .8$$

$$n_1 = \frac{7.9 (37.7)}{4} = 74.45 \text{ or } 75 \text{ observations on each method}$$

$$n = n_1 \frac{df + 3}{df + 1} = 75 \cdot \frac{77}{75} = 77 \text{ observations}$$

EXAMPLE LAB PROBLEM

Given results of two methods of determining zinc concentrations in rainbow gills, find 90% confidence interval for $\mu_I - \mu_{II}$ and determine sample size for your own confidence level and d. For sample size problem I choose 95% level and a d = 5 units.

<u>Method I</u>		<u>Method II</u>	
	10.391		33.240
	11.639		21.067
	14.588		23.934
	19.313		20.156
	20.767		27.200
Σx_I	= 76.698	Σx_{II}	= 125.597
Σx_I^2	= 1260.509	Σx_{II}^2	= 3267.657
s_I^2	= 20.998	s_{II}^2	= 28.184
\bar{x}_I	= 15.340	\bar{x}_{II}	= 25.119

$$\bar{x}_I - \bar{x}_{II} = -9.78$$

$$\bar{x}_I - \bar{x}_{II} \pm t_v \alpha/2 \sqrt{\frac{s^2_I}{n_1} + \frac{s^2_{II}}{n_2}} \quad v = \left(\frac{s^2_I}{n_1} + \frac{s^2_{II}}{n_2} \right)^2 \left/ \left[\left(\frac{s^2_I}{n_1} \right)^2 + \left(\frac{s^2_{II}}{n_2} \right)^2 \right] \right.$$

$v = 7.83$ or 8 degrees of freedom

$$-9.78 \pm 1.86 (3.136)$$

$$-15.613 \leq \mu_I - \mu_{II} \leq -3.947$$

at the 10% significance level there is a difference between two means since zero is not in interval -15.613 to -3.947 of the 90% confidence level. Still there is quite a wide range for difference.

The sample size needed to detect a 5 ppm difference at the 5% significance level (or to have a confidence interval of width $\pm d$ at the 95% confidence level) would be obtained as follows:

$$1 - \alpha = .95 \quad d = 5 \quad p = .8 \quad \text{from table K} = 7.9$$

$$n_1 = \frac{K s^2_{\text{diff}}}{d^2} \quad s^2_{\text{diff}} = 49.182$$

$$n_1 = \frac{(7.9)(49.182)}{25} = 15.54 \text{ or } 16 \text{ observations}$$

$$n = n_1 \frac{df + 3}{df + 1} = 16 \cdot 18/16 = 18 \text{ observations after correcting for using sample variance.}$$

In the previous example on the zinc in rainbow trout gills, we can remove some of the variability by comparing gills on same fish. Using paired observations.

<u>Method I</u>	<u>Method II</u>	<u>Difference within pairs</u>
19.751 ppm	22.054 ppm	- 2.303
6.882	6.849	0.143
23.111	25.192	- 2.081
15.455	20.021	- 4.566
13.375	15.546	- 2.171
		$\Sigma x_{diff} = -10.978$

$$\bar{x}_{diff} \pm s/\sqrt{n} \quad t_{(n-1)}^{\alpha/2}$$

$$\Sigma x_{diff}^2 = 35.216416$$

$$s^2 = 2.7783$$

$$-2.196 \pm (0.75) (2.78) \quad \sqrt{95\% \text{ table value}}$$

$$\bar{x}_{diff} = -2.196$$

$$-4.26 \leq diff \leq -.01 \quad \text{zero is not in}$$

the interval, therefore methods are not the same.

However, .01 is pretty close to zero. If at the other extreme of -4.26 we have a 25% difference from mean, the difference is between zero and 25%.

How do you know when to pair? If \bar{x}_1 and \bar{x}_2 are independent, then the variance of $(\bar{x}_1 - \bar{x}_2) = \frac{\sigma^2}{n_1} + \frac{\sigma^2}{n_2}$ and we used unpaired tests.

If not independent, the variance of the difference is as follows:

$$V(\bar{x}_1 - \bar{x}_2) = V(\text{mean difference}) = \frac{\sigma_1^2}{n} + \frac{\sigma_2^2}{n} - 2 \frac{\text{cov}(x_1 x_2)}{n}$$

In other words, we are able to reduce the variation of the difference due to covariance between paired observations. Limits on covariance are:

$$\text{cov}(x_1 x_2) \leq \sqrt{\sigma_1^2 \sigma_2^2}$$

He did not go into covariance in any more detail. Covariance between two variables, say length and weight of fish, is estimated by:

$\text{cov}(x,y) = s_{xy} = [\sum(x_1 - \bar{x})(y_1 - \bar{y})]/(n-1)$ and is a measure of linear association between x and y . Thus, the higher the positive linear association between pairs of observations, the smaller the variance of the difference. Hence, one would use a paired test if the experimenter can find or create pairs of items which have a high positive correlation. If pairs do not behave the same when there is no treatment difference, then it does not help to pair.

ANALYSIS OF VARIANCE

140	120	100	80	60	# of fish/raceway
raceways of fish					

Fed same amount of feed in each raceway. Find the effect of density on fish growth.

<u>Fish lengths by raceway</u>					
10.5	10.3	11.5	11.9	12.6	$H_0: \mu_{140} = \mu_{120} = \dots = \mu_{60}$
10.4	10.8	11.3	12.5	13.3	$H_a: \text{at least one inequality}$
10.8	11.5	10.8	12.0	14.0	
<u>9.7</u>	<u>10.6</u>	<u>11.6</u>	<u>12.1</u>	<u>13.5</u>	
T_i 41.4	43.2	45.2	48.5	53.4	

$N = 20$

A. O. V. Table

Source	df	ss	ms	F
Total	19	25.5455		
Between trout or Density	4	22.5180	5.6295	27.9
Within treat.	15	3.0275	0.2018	$p < .005$

When looking at F and put confidence level on this AOV we make assumptions:

- (1) $\sigma_1^2 = \sigma_2^2 = \sigma_3^2 \dots = \sigma_5^2$
- (2) normal distribution of length
- (3) individual fish within treat. are independent

EXAMPLE LAB PROBLEM

Fish growth under three treatments

	I	II	III	
	2.180	1.452	2.436	$H_0: \mu_1 = \mu_2 = \mu_3$
	1.630	1.862	2.470	
	1.622	2.993	2.388	$H_a: \text{at least one inequality}$
	2.122	2.201	3.717	
	2.179	2.086	2.874	
T_1	= 9.7332	10.594	13.885	
\bar{x}	= 1.947	2.1188	2.777	
Σx_1^2	= 19.291	23.729	39.813	
s_1^2	= 0.086	0.3206	0.3137	

Source	df	ss	ms	F
Total	14	4.8014		
Treatment	2	1.920	0.960	4.00 Significant at 5% level
Error	12	2.881	0.240	

The model that would represent the above example would be as follows:

$$x_{ij} = \mu + t_i + e_{ij}$$

In words this means that any observation (x_{ij}) can be represented by a grand

mean (μ) plus the treatment effect (t_i) plus the error (variation) as-

sociated with an individual in a given treatment. In the above example

$$\hat{\mu} = 2.280, t_1 = -0.333, t_2 = -0.161, t_3 = 0.497, e_1 = 0.233, e_2 = -0.317,$$

$$e_3 = -0.325, e_4 = 0.175, e_5 = 0.232, e_6 = -0.667, e_7 = -0.257, e_8 = 0.974,$$

$$e_9 = 0.082, e_{10} = -0.033, e_{11} = -0.341, e_{12} = 0.307, e_{13} = -0.389, e_{14} = 0.940,$$

$$e_{15} = 0.97.$$

$$t_i - \hat{\mu} = \text{treatment effect}$$

$$e_{ij} = x_{ij} - \bar{x}_i$$

$$\sum e_{ij}^2 = \text{error sum of squares in AOV table or } 2.881$$

EXAMPLE 2

Vegetative plots in a forestry study

Control (treat. 1)		Burn (treat. 2)		Spray (treat. 3)		
plot 1	plot 2	plot 1	plot 2	plot 1	plot 2	
Transect counts	30	25	16	21	18	32
	34	23	20	23	20	30
Total	64	48	36	44	38	62
	112		80		100	
N = 12,		G or grand total = 292		$\Sigma x^2_{ijk} = 7484$		

A. O. V. Table

Source	df	ss	ms	F
Total	11	378.67		
Treatment	2	130.67	65.33	0.876 (computed by dividing treat/plots within treatment or 65.33/74.6)
Plots within treatment	3	224.00	74.6	
Transects within plots	6	24	4.0	

We divided by plots within treatments because we randomized plots.

$$\text{Model: } \Sigma x_{ijk} = \mu + t_i + \beta_{ij} + e_{ijk}$$

E = average value symbol

V = variance

$$E(\beta_{ij}) = 0$$

$$V(\beta_{ij}) = \sigma^2_p$$

$$E(e_{ijk}) = 0$$

$$V(e_{ijk}) = \sigma^2_t$$

$$i = 1, \dots, a$$

a = treatment

$$j = 1, \dots, b$$

b = plots = experimental units

$$k = 1, \dots, n$$

n = transects within each plot,
n is subsample within each plot.

A. O. V. Table for Model

Source	df	ss
Total	abn - 1	$\Sigma x_{ijk}^2 - x^2 \dots / abn$
Treatment	a - 1	$\Sigma x_i^2 \dots / bn - x^2 \dots / abn$
Plots within treat.	a (b-1)	$\Sigma x_{ij}^2 \dots / n - \Sigma x_i^2 \dots / bn$
Transects within plot	ab (n-1)	$\Sigma x_{ijk}^2 - \Sigma x_{ij}^2 \dots / n$

., ..., ..., all mean sum over that unit. If a dot replaces an i then i's are summed out.

$$E (\text{plots within treatment mean squares}) = \sigma_t^2 + n\sigma_p^2$$

$$E (\text{transects within plot mean squares}) = \sigma_t^2$$

$$E (\text{treatment mean squares}) = \sigma_t^2 + n\sigma_p^2 + nb \frac{\sum ti^2}{a-1}$$

$$\bar{x}_{i..} = i^{\text{th}} \text{ treatment mean}$$

$$\text{Variance } (\bar{x}_{i..}) = \frac{\sigma_p^2}{b} + \frac{\sigma_t^2}{bn} = \frac{\sigma_t^2 + n\sigma_p^2}{bn} = \frac{E (\text{plot within treat. m.s.})}{bn}$$

$$\text{Estimate of S.E. (standard error) of each treat. mean} = \frac{\sqrt{\text{plots within treat. m.s.}}}{bn}$$

*Ratio of variance of $\bar{x}_{i..}$ for n transects per plot to variance of $\bar{x}_{i..}$

$$\text{for one transect per plot} = \frac{\sigma_p^2 + \sigma_t^2/n}{\sigma_p^2 + \sigma_t^2}$$

If the above ratio is a large value it doesn't help to take more transects.

Small ratio value says we get help with more transects.

Plugging in our numbers for the CONTROL, BURN, SPRAY EXPERIMENT we get the following: $\frac{35.33 + 4/n}{35.33 + 4}$

If we increase n or transects we get 4/n close to zero. Best we can get is ratio of 0.9. We can make a 10% improvement in variance with many more transects per plot. Standard square root of .9 is .95. Therefore, we only realize a 5% improvement in standard error with infinitely more transects per plot.

INDIVIDUAL COMPARISONS OR SIMULTANEOUS COMPARISONS OF MEANS

Simultaneous confidence level for all statements that could be made. (Orthogonal comparison type)

(A) Look at all pairwise comparisons of the means

$\mu_i - \mu_j$ We need equal sample size (n_i)

$$\bar{x}_i - \bar{x}_j \pm Q_{\alpha, k, df} \sqrt{\frac{\text{Error mean square}}{n}}$$

$Q_{\alpha, k, df}$ = table value of Studentized range at the α level,
with k the number of treatments and df degrees
of freedom of error mean square.

(B) Look at any or all statements of the type $\sum a_i \mu_i$

$$\sum a_i \bar{x}_i \pm K \sqrt{\frac{\sum a_i^2}{n_i} \text{ Error m.s.}}$$

Case 1. If all the statements of interest have $\sum a_i = 0$,

$$\text{then } K = \sqrt{(k-1) F_{k-1, df}^{\alpha}}$$

Case 2. If any of the statements have $\sum a_i \neq 0$, then

$$K = \sqrt{k F_{k, df}^{\alpha}}$$

EXAMPLE OF PAIRWISE COMPARISONS

Stem length of browse plants

SHEEP Stocking Rate		DEER Stocking Rate	
Heavy	Light	Heavy	Light
5	8	6	2
15	7	9	0
14	10	7	5
7	3	2	3
12	5	11	2

Is there a difference in the mean stem length of browse utilized between the four treatments? Is there a difference between the deer and sheep means, between heavy and light stocking rates? Is the sample size large enough?

Σx_i	53	33	35	12
\bar{x}_i	10.6	6.6	7.0	2.4
Σx_i^2	639	247	291	42
s_i^2	19.3	7.3	11.5	3.3

A. O. V. Table

To Test Difference Between Treatments

Source	df	ss	ms	F
Total	19	1219 - 884.45 = 334.55		
Treatment	3	168.95	56.317	5.44
Within treat.	16	165.6	10.35	

Table 10% level F = 4.07

There is a difference between 4 treatments at 10% significance level.

To test sheep vs deer, use this formula for testing difference between two means:

$$\Sigma a_i \bar{x}_i \pm t^{\alpha/2} \sqrt{\Sigma \left(\frac{a_i^2}{n_i} \right)} \text{ Error m.s.}$$

a's are orthogonally picked numbers in this case to add to zero and compare sheep to deer. $a_1 = \frac{1}{2}$, $a_2 = \frac{1}{2}$, $a_3 = -\frac{1}{2}$, $a_4 = -\frac{1}{2}$

n_i = number of observations which went to make up mean and in this case $n_i = 5$.

$$\Sigma a_i \bar{x}_i \pm t_{\alpha/2} \sqrt{\Sigma \left(\frac{a_i^2}{n_i} \right)} \text{ Error m.s.}$$

$$\underbrace{\frac{1}{2} (10.6) + \frac{1}{2} (6.6) - \frac{1}{2} (7.0 - \frac{1}{2} (2.4))}_{\text{equals: } \Sigma a_i \bar{x}_i} \pm t_{16}^{.05} \sqrt{1/5 \text{ Error m.s.}}$$

$$\Sigma a_i \bar{x}_i = \text{sheep mean} - \text{deer mean} = 8.6 - 4.7 = 3.9$$

$$3.9 \pm t_{16}^{.05} \sqrt{1/5 \text{ e.m.s.}} \quad \text{e.m.s.} = 10.35 \text{ (see AOV table)}$$

$$3.9 \pm 1.745 \sqrt{1/5 \cdot 10.35}$$

$$3.9 \pm 1.746 (1.439) = 3.9 \pm 2.51$$

$1.45 \leq \mu \leq 6.4$ This says that at the 90% confidence level the mean difference between means is between 1.45 and 6.4. Since zero is not included or found in this interval, there is a difference between deer and sheep.

Sample size needed in the deer vs sheep problem:

$$n_1 = K \frac{s^2}{d^2}$$

n_1 = sample size

K = table value given $1 - \alpha$, d and p

s^2 = variance

$$s^2 \text{ of difference} = V(\Sigma a_i \bar{x}_i) = \frac{\Sigma a_i^2}{n} \sigma^2$$

n drops out of this formula because we are interested in the variance per one observation not in the variance of means. Therefore, $s^2 = (\Sigma a_i^2) (\text{e.m.s.})$

$$n_1 = \frac{(6.2)(10.35)}{4} = 16$$

$$1 - \alpha = .9$$

$$d = 2$$

$$p = .9$$

In order to detect a difference between treatments of 2 units at the 90% level of confidence and probability of .9 we need 16 observations in each treatment.

From fish feeding raceway density example, we can look at effect due to linear regression.

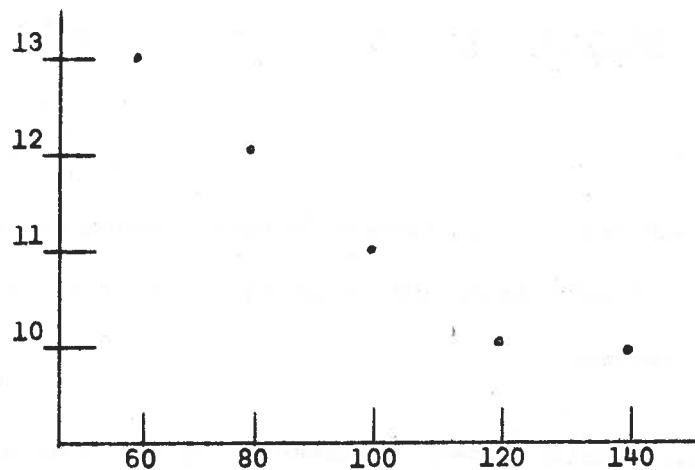
	No. Fish/Raceway				
x	140	120	100	80	60
mean length \bar{y}	10.35	10.80	11.30	12.125	13.35
4 fish per treatment n = 20					

A. O. V. Table

Source	df	ss
Total	19	25.5455
Density	4	22.5180
linear regression 1		21.462
quad reg. 1		1.004

$$\text{Linear regression s.s.} = \frac{\left[\sum x_i y_i - \left(\frac{\sum x_i \sum y_i}{n} \right) \right]^2}{\sum x^2 - \frac{(\sum x)^2}{n}}$$

$$\text{Linear regression s.s.} = 21.462$$



Linear regression accounts for 21.462 out of 22.5180 of the sum of squares difference between density effect.

EXAMPLE OF EXPERIMENT WITH FISH AND ZINC

Observations expressed as log of zinc in mg/g in bone of fish

Concentrations of Zinc in Water

	2.4	1.2	0.3	0.15	0
	2.51	2.43	2.42	2.39	2.20
	2.96	2.57	2.53	2.40	2.38
	2.59	2.65	2.41	2.50	2.35
	2.70	2.42	2.32	2.23	2.33
Σx_i	10.76	10.07	9.68	9.52	9.26
\bar{x}_i	2.69	2.52	2.42	2.38	2.31
Σx_i^2	29.060	25.389	23.448	22.695	21.456

H_0 : no difference in zinc in bone by concentration

A. O. V, Table

Source	df	ss	ms	F
Total	19	0.573		
Treatment	4	0.341	0.08525	5.5
Within treat.	15	0.232	0.01547	

significant
at 1% level

$$\text{Total ss} = \sum x_{ij}^2 - \frac{G^2}{N} = 122.048 - 121.475 = 0.573$$

$$\text{ss treatments} = \frac{\sum t_i^2}{n} - \frac{G^2}{N} = 121.816 - 121.475 = 0.341$$

$$\text{ss due to linear regression} = \frac{\left[\sum x_i y_i - \frac{(\sum x_i)(\sum y_i)}{n} \right]^2}{\sum x_i^2 - \frac{(\sum x_i)^2}{n}}$$

$$\text{Since } y_i = 0 \therefore \text{ss} = \frac{(\sum a_i \bar{x}_i)^2}{\sum a_i^2} = \frac{(0.583)^2}{4.032} = \frac{0.3408}{1.008} = 0.338$$

(i.e. in this case $\sum a_i = 0$)

Almost all the sums of squares of the treatment effect can be accounted for by linear regression (0.338 out of 0.341). An alternate to looking at linear regression is just a straight mean comparison, for example:

$$\begin{array}{c} \nwarrow \quad \swarrow \quad \leftarrow \text{indicates estimate} \\ (\mu_{2.4} - \mu_0) = 0.380 \text{ difference} \end{array}$$

$$0.380 \pm t_{15}^{90} \text{ df } \sqrt{\frac{\sum a_i^2}{n_i} \text{ e.m.s.}} = 0.380 \pm 1.753 \sqrt{(1.008)(0.01547)}$$

$$0.5989 \geq \mu_{2.4} - \mu_0 \geq 0.1611 = 0.380 \pm 0.2189$$

Be careful in looking at the results of the above difference because the numbers still represent logs. Even though it looks like small differences by converting back to real concentrations we can get an

entirely different picture. An example of this can be shown by looking at the 90% confidence level for $\mu_{2.4}$ (mean of the zinc concentration of 2.4).

$$\mu_{2.4} \pm t_3^{.05} \frac{\sqrt{\sigma^2}}{n} = 2.69 \pm 2.353 (0.0981) = 2.69 \pm 0.232$$

$2.922 \geq \mu_{2.4} \geq 2.458$ Converting back to real concentrations from logs we really have $835 \geq \mu_{2.4} \geq 287$. Where the logs look fairly close we can see we have greater than a twofold difference.

EXPERIMENTAL DESIGN

Example: People on treadmill - how long can they run? Have a before figure for time they can stay on treadmill, then have three treatments one of which is a control. Rerun on treadmill and observe response to treatments.

Pretreatment data: 2.0, 3.1, 4.3, 2.4, 4.7, 3.2, 3.5, 2.2, 2.0, 5.0, 3.7, 1.9, 3.3, 4.4, 4.0.

Case 1: Assigning individuals by Complete Randomized Method

$n_1 = 5,$	$n_2 = 5,$	$n_3 = 5$
A	B	C
3.5	3.2	3.1
2.4	2.0	4.3
4.4	3.3	2.2
4.0	4.7	3.7
5.0	2.0	1.9

One way or Complete Randomized A. O. V. Table

Source	df	ss	ms
Total	14	15.157	
Treatment	2	2.241	1.121
Error	12	12.916	1.076

Case 2: Arrange individuals by blocks. Assign three lowest to block 1 and so on. Randomize the A, B, C assigned to each block. Using this new design we are trying to take out some of the variation in the error term - making it smaller.

Randomized Block Design

BLOCK 1		
C	A	B
1.9	2.0	2.0

BLOCK 2		
A	B	C
2.2	2.4	3.1

BLOCK 3		
C	A	B
3.2	3.3	3.5

BLOCK 4		
C	B	A
3.7	4.0	4.3

BLOCK 5		
C	A	B
4.4	4.7	5.0

A. O. V. Table for Randomized Block Design

Source	df	ss	ms
Total	14	15.157	
Treatment	2	0.037	
Reps or Blocks	4	14.297	
Error	8	0.823	0.103

Using a Randomized Block Design we have improved our error estimate by a factor of 10. Thus, we will be able to detect a smaller treatment difference.

EXAMPLE: Pothole Blasting Experiment (See Fig. 1)

Different charges of powder, 25, 50, 75 and 150 were used to blast potholes for ducks. Then duck visits to the different potholes were noted. We want to know which type of pothole attracted most ducks, and which type of pothole gave the best return when cost was considered.

Duck Visits/Hour Over 3 Years						
	Powder Charge:	25	50	75	150	Σ
Block A		0.43	0.81	1.75	3.69	6.68
Block B		0.51	0.87	2.20	2.98	6.56
Block C		<u>0.88</u>	<u>0.93</u>	<u>2.84</u>	<u>2.61</u>	7.26
Σ		1.82	2.61	6.79	9.28	

A. O. V. Table				
Source	df	ss	ms	F
Total	11	13.7532		
Blocks	2	0.0701	0.035	0.167
Treatment	3	12.4282	4.1427	20.92*
Error	6	1.2549	0.2092	

*Highly significant treatment effect

To look and see if blocking helped us in the error term we can make a comparison with complete random using the following formula:

$$\frac{s^2_{cr}}{s^2_{rb}} = \frac{(b-1) ms_{block} + b(a-1) ms_{error}}{(ab-1) ms_{error}} = \frac{2(0.035) + 3(3)(0.2092)}{(12-1)(0.2092)} = \frac{1.953}{2.30} = .85$$

cr = complete random rb = randomized block s^2 = variance b = blocks
a = treatments

We lost by blocking by 15%. Gains would be increases over a ratio value of 1.

Change size, sizes pothole
and waterfowl use

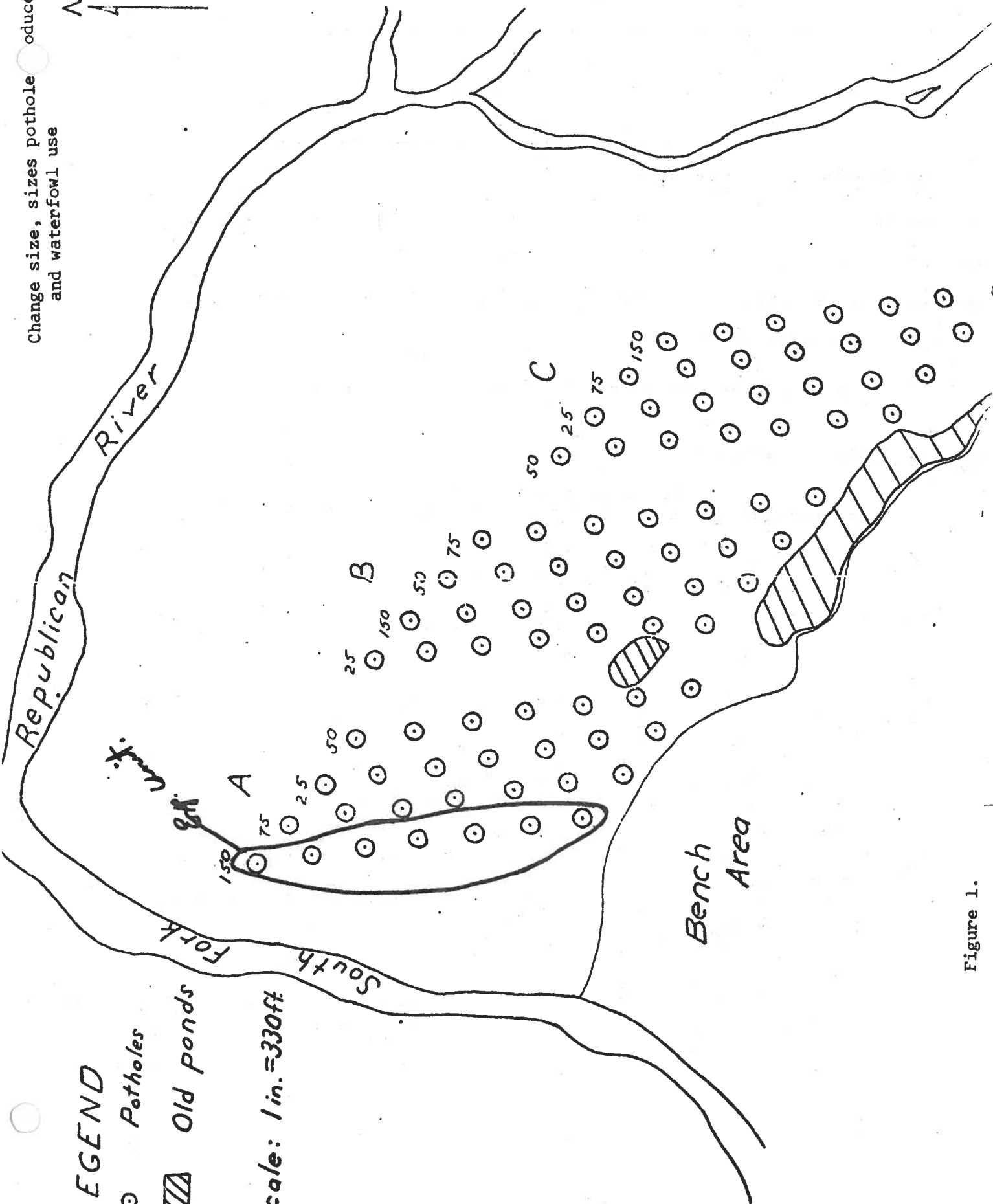


Figure 1.

Considering cost which is best size of charge for duck potholes?

	Charge Size			
	25	50	75	150
Avg cost/pothole	4.87	7.11	11.47	18.81
Avg size in 100 sq ft	2.01	2.93	5.70	8.51
Avg cost/100 sq ft	2.423	2.427	2.012	2.210
Mean duck visit/hr/charge	0.607	0.870	2.263	3.093
Cost/duck visit/hr/100 sq ft	8.03	8.17	<u>5.70</u>	8.52

The cost indicates that the 75 charge is the best buy

HANDLING MISSING OBSERVATIONS

Example of Missing Data
(See previous example of treadmill randomized block design)

	Blocks					
	I	II	III	IV	V	Σ
Treat A	2.0	2.2	3.3	4.3	4.7	16.5
B	2.0	*	3.5	4.0	5.0	14.5
C	<u>1.9</u>	<u>3.1</u>	<u>3.2</u>	<u>3.7</u>	<u>4.4</u>	<u>16.3</u>
	5.9	5.3	10.0	12.0	14.1	47.3

* We need to put a number in this missing space which will make our error mean square as small as possible. Use this formula:

$$X = \frac{bB + aT - S}{(a-1)(b-1)}$$

$$X = \frac{5(5.3) + 3(14.5) - 47.3}{(3-1)(5-1)}$$

$$X = \frac{22.7}{8} = \underline{\underline{2.8}}$$

b = number of blocks

B = block total where missing observations occur

a = number of treatments

T = treatment total where missing observations occur

S = grand total without adding missing observations

A. O. V. Table on Missing Data Example

Source	df	ss	ms
Total	13	14.54	
Treatment	2	0.12	
Blocks	4	13.69	
Error	7	0.73	0.104

Compare this table with the complete randomized AOV table in earlier example. Error terms are quite close. We didn't lose too much with one missing cell.

EXAMPLE: Analyzing Blood Samples of Deer

Send four different deer blood samples to three different laboratories. Want to see if there is a difference between laboratories. Two results from each lab, use sum.

		Labs			Total
		I	II	III	
Deer 1	8)	19	9)	10)	63
	11)		12)	13)	
2	14)	33	10)	9)	76
	19)		13)	11)	
3	20)	36	22)	24)	129
	16)		25)	22)	
4	19)	32	19)	17)	104
	13)		17)	19)	
Total		120	127	125	

A. O. V. Table

Source	df	ss	ms	F
Total	23	596.00		
Lab	2	3.25	1.63	$\frac{1.63}{14.90} = 0.11$
Deer	3	434.33	144.77	
I Error (Lab x deer)	6	89.417	14.90	$\frac{14.90}{5.75} = 2.59$
II Error (samples within deer within lab)	12	69.	5.75	

The lab x deer F value is significant at 0.1 level but not at a lower level. No difference in labs with F of 0.11.

EXAMPLE: Three treatments on 3-day experiment with three different periods observed. Set up latin square type design.

Day				
	I	II	III	Total
Period 1	A	B	C	
	.194	.730	1.187	2.111
2	C	A	B	
	.758	.311	.589	1.658
3	B	C	A	
	.369	.558	.311	1.238
Total	1.321	1.599	2.087	5.007

Treatments are A, B, C.

Σ trt A = .816 B = 1.688 C = 2.503

$$\text{Total ss} = \Sigma x_i^2 - \frac{G}{N} = 3.541957 - 2.78556 = 0.7564$$

A. O. V. Table

Source	df	ss	ms	F
Total	8	0.7564		
Treatment	2	0.4745	0.2372	8.69
Day	2	0.1002	0.0501	1.84
Period	2	0.1271	0.0635	2.33
Error	2	0.0546	0.0273	

Slight difference is in treatment effect. Test to see if treatment difference due to linear regression.

$$\text{ss for linear regression} = \frac{(\sum a_i \bar{x}_i)^2}{\frac{\sum a_i^2}{n}}$$

$$\text{mean treatment } A/3 = \bar{x}_1 = .272$$

$$B/3 = \bar{x}_2 = .563$$

$$C/3 = \bar{x}_3 = .834$$

$$a_1 = -1$$

$$a_2 = 0$$

$$a_3 = 1$$

$$n = 3$$

ss for linear regression = 0.4737. Almost all of the treatment can be accounted for by linear regression.

ARRANGEMENT OF EXPERIMENTAL UNITS AND DESIGN

EXAMPLE: Have three species of fish (rainbow, brown and brook) in hatchery and test all species with and without a special diet. Weigh the fish three times for three observations per treatment.

- I. With completely randomized design you have six treatments (A, B, C, D, E, F).

A. O. V. Table for Complete Random Design

Source	df	ss	ms
Total	17	96.00	
Treatment	5	78.67	
Species	2	20.33	10.17
Diet	1	56.89	
Sp x Diet	2	1.45	
Error	12	17.33	1.44

II. With randomized complete block. Have same treatments only three replicates.

Source	df	ss	ms
Total	17		
Treatment	5		
Species	2		
Diet	1		
Sp x Diet	2		
Blocks	2		
Error	10		

LABORATORY EXERCISE (6-16-72)

Using factorial design we have three treatment factors. Factor I = 3 areas, Factor II = 3 ages, Factor III = male or female. For example, say we are interested in stomach content of deer in three areas, at three ages, and for male and female. Take two samples from each.

AGE	SEX	AREAS							
Factor II	Factor III			Factor I					
		1		2		3		Totals	
1	M	25	40	28	42	30	27	192	428
	F	55	36	40	30	40	35	236	
2	M	50	40	46	52	48	58	294	600
	F	58	56	53	45	50	44	306	
3	M	50	70	74	62	92	80	428	812
	F	68	52	78	66	62	58	384	
Totals		306	294	319	297	322	302		
		600		616		624			

Also sum out totals of M and F and totals of both M and F within each factor 1 x 2 block

A. O. V. Table

Source	df	ss	ms	F
Total	35	8921.56		
Treatment	17	7741.56	455.38	6.95
Factor I	2	24.89	12.45	0.19
Factor II	2	6166.23	3083.12	47.03*
Factor III	1	4.00	4.00	0.06
I x II	4	428.44	107.11	1.63
II x III	2	330.66	165.33	2.52
III x I	2	386.00	193.00	2.94
I x II x III	4	401.34	100.34	1.53
Error	18	1180.	65.56	

*Age is the most significant factor. There is some interaction effect, however.

SPLIT PLOT DESIGN

EXAMPLE: Have a browse experiment with rodents. Have one plot with exclosure to keep rodents out and a control where rodents have access. Have six transects and measure browse different years.

Years:	EXCLOSURE			CONTROL		
	1957	1960	1964	1957	1960	1964
Transect 1	6.8	11.1	1.7	21.5	16.1	14.1
2	9.4	4.2	6.4	21.4	23.4	23.4
3	0.0	0.1	0.0	22.3	14.4	16.7
4	18.0	16.2	16.8	22.9	23.6	20.8
5	10.3	11.5	9.1	24.2	17.0	11.7
6	33.7	34.3	28.7	8.7	9.1	10.4

A. O. V. Table

Source	df	ss	ms
Total	35	2794.1	
Treatment	1	296.9878	
Years	2	64.8067	
Trt x years	2	11.4822	
Trans. within trt	10	2266.179	
Trans. within years by treat interaction > 20		154.64	

To test for difference in treatment effect you would use the transects within treatments for error term to divide into ms of treatment for an F value. Also if you test for a difference in the treatment x years interaction using the transect x years x treatment interaction for the error term you will be able to tell if there is a treatment effect.