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# Statistical Evaluation of Methodologies for Genetic Strain Evaluation in Small-to-Medium Sized Experimental Facilities

by

Zubaida U. Basiao

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
February 1994

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## ABSTRACT

A major goal of aquaculture genetics is to provide improved fish that will eventually benefit not only the private industry but also the small fish farmers. It is essential to develop strain testing methods that have sufficient statistical power to detect differences in strains. True differences that are not detected can mean millions of dollars of opportunity loss for the aquaculture industry. In this study, several strain testing procedures were evaluated in the context of their statistical power to detect economically important strain differences.

Two strains of Nile tilapia (Oreochromis niloticus) were reared under various experimental comparison procedures. The strains were size-matched (graded) and grown separately, together, and grown separately but with an internal reference fish (red tilapia) in each replicate cage or tank. Fish of mixed-sizes and ages (ungraded) were simultaneously grown separately, together, and separately but with an internal reference fish. On-farm, strain testing was also done in four rice-fish farms.

The power to detect true differences was low when strains were grown together and were of mixed sizes. Use of an internal reference fish was inadequate to remove environmental sources of variation when fish were of mixed sizes. Initial size differences resulted in apparent growth depensation under experimental conditions and growth compensation in rice-fish farms. The size-dependency of growth rates in fish may have important implications in strain testing and selection programmes in aquaculture. Large differences between two growth means are not always due to genetic variance, which is what is sought for strain testing or selection. Environmental variance (initial size differences) plays a major role in observed differences in growth rates of fish.

Size-grading or having almost the same common starting size among genotypes before strain testing may help minimize environmentally induced variation like initial size differences. This procedure is more powerful than mixed size rearing at detecting true differences.

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# CHAPTER 1

## GENERAL INTRODUCTION

There is a growing awareness that the application of genetic research to aquaculture will have significant impact in increasing aquaculture production (Wilkins and Gosling 1983; Gall and Busack 1986; Tiews 1987; Gjedrem 1990). Aquaculture genetics is a new discipline that is beginning to develop a body of mathematical theory that is distinct from other branches of genetics (e.g. population genetics, animal breeding, human genetics). There are number of problems, as for example the variable aquatic environments of fish, social interaction (interspecific and intraspecific competition and maternal effects), asynchronous spawning, high fecundities and difficulty of tagging new born individuals that are peculiar to aquaculture genetics.

In particular, in comparative strain testing and evaluation, no simple standardized and accepted procedures exist for use in aquaculture environments. A major constraint in strain comparison is how to provide adequate replication for statistical comparisons. Uraiwan and Doyle (1986) have shown that fish growth in aquaria, cage or pond environments is difficult to replicate even within a single breeding season at one location. The effects of uncontrolled environmental variables (such as water

temperature, water quality, population density and (diet) are widely recognized in aquaculture environments. Since it is not possible to control all potential sources of error in aquaculture situations (non-synchronous spawning, seasonal environmental change, random mortalities, and limited facilities) strain comparison and evaluation is difficult to undertake especially in developing countries.

A research objective of the SEAFDEC Binangonan Freshwater Substation, Philippines is to assess the genetic potential of several tilapia (Oreochromis niloticus) broodstocks and to develop strain comparison procedures for use in small-to-medium-sized facilities. My general objective in this thesis is to explore the statistical power of several possible strain testing procedures for detecting strain differences or estimating the magnitude of differences. A specific objective is to be able to propose an optimal experimental design for strain testing procedure that includes power analysis to detect a difference of a specified magnitude.

## Thesis Rationale

The power of a statistical test to detect strain differences when they exist, or to estimate the magnitude of differences, has major economic implications in aquaculture. This aspect of the problem is generally overlooked when designing aquaculture genetics

experiments. Most strain testing programmes in aquaculture and fisheries research in general are structured around formal tests of null hypotheses (Ho) of no difference (Dixon and Massey 1969; Cohen 1988; Shavelson 1988; Samuels 1989; Peterman 1990). In this decision-making process, most researchers concentrate on the error of falsely rejecting a true null hypothesis (Runyon 1977). In all such cases, researchers set up the null hypothesis (no effect) and design experiments that attempt to reject it. Some level of significance, alpha ($\alpha$), is reported when the Ho hypothesis is rejected. In cases where the Ho hypothesis is not rejected, the equally important concept of statistical power to detect a difference if one in fact exists is not reported. As it turns out, the latter type of error may be economically very important from an aquaculture perspective.

Within a hypothesis testing framework, two decisions can be made, either of which can be correct or wrong. The combination of these is shown in Table 1. (Shavelson 1988; Peterman 1990; Fairweather 1991; Toft and Shea 1983; and many other statistical references). If the null hypothesis is true and it is rejected, then a Type I or is committed. When the null hypothesis is not rejected and in reality there is no difference (say in the growth of two strains), then a correct decision is made. If, however, the null hypothesis (say that two strains are identical) is false and it is not rejected, then a Type II error is made. ß

Table 1. Decision problem in making inferences from sample data

| | True situation in population | |
|---|---|---|
| Decision | Ho is True | Ho is False |
| Do Not Reject Ho | Correct ($1-\alpha$) | Type II error ($\beta$) |
| Reject Ho | Type I error ($\alpha$) | Correct (Power) |

(beta) is the probability of making a Type II error. A correct decision, in this case, would be to reject a false null hypothesis.

The power of a test is the probability of not committing a Type II error (1-ß). Power indicates the probability that an experiment will detect the difference, if the difference truly exists. Alternatively, it can be viewed as the ability of a statistical procedure to determine whether a situation is different from the null hypothesis or that the null hypothesis is false (e.g. Toft and Shea 1983; Gerrodette 1987; Peterman 1990; Fairweather 1991).

Power analysis has been in use for a long time (Dixon and Massey, 1969; Zar 1984; Kraemer and Thiemann 1987; Cohen 1988). More recently, power analysis had been used by ecologists to detect community-wide patterns (Toft and Shea 1983); detect trends in marine mammal population (Gerrodette 1987); and estimate predator and prey relationship (Doherty and Sale 1985; Hall et al. 1990; Young 1990; Johnson et al. 1987). Fisheries scientists have likewise applied power analysis in fisheries and hatchery management (Peterman and Routledge 1983; Peterman 1989; Peterman and Bradford 1989; and Parkinson et al. 1988).

The magnitude of Type II error and the relative importance, for the future of aquaculture production, of Type I and Type II errors in a strain testing programme

needs to be evaluated. Type II errors have been ignored relative to Type I errors according to long-standing statistical tradition (Peterman 1990; Fairweather 1991). There are many situations in aquaculture research, however, in which the risk of a Type II error is of much greater concern than the risk of a Type I error. For instance, concluding that there is no difference between the growth rate of two strains of fish, when in fact the true difference was not detected in the experiment would be a costly error to commercial fisheries. Furthermore, the smaller the sample size and less-well conducted a statistical test, the greater likelihood that a Type II error will be committed. Thus developing countries are particularly vulnerable to the costly loss of opportunity presented by Type II errors in strain testing. On the other hand, mistakenly preferring one strain over another (Type I error) will be of no economic consequence if the strains are actually the same. It would be better to design tests that are efficient at estimating errors that have the greatest economic consequence.

In this thesis, the efficiency of several strain testing procedures on two Nile tilapia strains (Oreochromis niloticus) using small sample sizes are explored. The purpose of the experiment is not to establish whether or not differences exist for these particular strains, but to use the information to design

optimal experimental procedures for general use. The estimates of the error variances are used in proposing an experimental design for strain testing programmes that include power analysis.

## Status of aquaculture genetics in Southeast Asia

Although Southeast Asia has a long tradition of fish culture, the genetic aspects of managing aquaculture stocks do not receive as much attention as improving culture techniques, formulating cheap feeds, alleviating disease outbreaks and similar problems related to farm managements and production (see MacLean et al 1986; Hirano and Hanyu 1990 for the scanty literature on genetics in Southeast Asia).

It is only very recently that the potential of aquaculture genetics has been recognized and properly addressed in Southeast Asia. The International Development Research Centre of Canada (IDRC) has organized and developed aquaculture genetics programs in the form of an international network in Thailand, Indonesia, Philippines, India and China (Doyle and Newkirk 1987a,b). The objective of many of these projects under this network of Aquaculture Genetics Network in Asia (AGNA) is to develop and test locally adapted, genetically diverse strains of tilapia and carp, and to develop selection and strain comparison procedures in small-to-medium-sized facilities. The International Center for Living Aquatic

Resources Management (ICLARM) in collaboration with AKVAFORSK and several Philippine National Institutions is developing a strain of tilapia (the GIFT strain) using classical animal breeding procedures (Pullin et al 1991). The Overseas Development Agency (ODA) is using modern chromosome-manipulation technology to develop sex reversed fish and chromosomal "superior male" in the Philippines, and the European Community (EC) is involved in population genetics and gynogenesis in Thailand.

Southeast Asia has virtually become a multinational laboratory for the testing and application of aquaculture genetics. It is apparent that a revolution in genetic aquaculture has begun in Asia. These different procedures will soon be developing better strains (or claims will be made to that effect). It is vital that proper strain testing methodologies be developed that protect the best interest of the farmers and the farming system as a whole.

## Magnitude of expected differences between strains

In aquaculture decision-making the magnitude of difference that the researcher wants to detect can be decided a priori on purely economic grounds. Economic theory or results of previous strain-comparison studies can help indicate the expected magnitude of difference that would be important between the growth rates of fish. Genetic gains can be expected from selection on economically important traits like growth rate and age and

size at maturity. Initial results from genetic improvement programs with trout and salmon have indicated that growth-rate gains comparable to those obtained with livestock and poultry can be attained (Gall, 1988). Rainbow trout (Oncorhynchus mykiss) and Atlantic salmon (Salmo salar) have demonstrated improved growth rate performance by 5 and 7% per year, respectively (Gjerde 1986). Selected channel catfish (Ictalurus punctatus) are expected to grow 5.3% per year faster than unselected fish (Dunham and Smitherman 1983). A 10-year selection and breeding program with coho salmon have demonstrated a greater than 60% improvement in weight after four generations of selection (Hershberger et al. 1990). An IDRC supported project in Thailand has shown that after 5 generations of size-specific selection for growth in red tilapia, the selected line is 15.7% heavier and 7.8% longer than the control line (Jarimopas 1990). Progeny of O. niloticus (Chitralada strain) selected in Thailand for three generations grew an average of 36% faster than the control line (Uraiwan 1991). Initial results of an IDRC supported project in the Philippines have shown that after two generations of within family selection, selected tilapia gave higher growth rate than the control line in both tanks and cages (Abella et al 1990). The same study has shown that after 8 generations of within-family selection for growth, the selected fish were 8 to 37% heavier than the second generation random-bred control

line (Bolivar et al. In press)

## Implications of Type I and Type II errors for design of (1) experimental design of testing programmes, (2)national genetics programmes, and (3) fisheries management in general

Statistical power analysis should not be the only criterion for deciding on the design of strain-testing experiments, national genetics programmes, and fisheries management in general . However, it is essential to the design of sensitive tests, and can guide decision makers in making correct and economically appropriate interpretations of results.

Suppose in a strain testing experiment, the growth rates of two tilapia strains are statistically compared and statistically tested. The null hypothesis (Ho) is that the growth rate of strain A is the same as the growth rate of strain B. The alternative hypothesis is that strain A (say, an advertised superior strain or a selected strain ) grows faster than strain B. At an alpha level of .05 the null hypothesis is rejected. The investigators conclude that since the null hypothesis of no difference is rejected then the alternative hypothesis that strain A is better than strain B is then accepted. Since strains A and B are indeed different then the investigators made the correct decision. However, if in reality the two strains had been identical, then a Type I error would have been committed.

What would have been the cost of this Type I error?. There is no economic loss since the two strains are not different. Whichever strain is used by the farmers will not make any difference in terms of increased aquaculture production. The farmer may incur additional cost for the purchase of strain A seeds if they are successfully promoted as the superior strain by the salesman. However, there will be no change in biological yield whichever seed is chosen.

Suppose, however, that the strain testing experiment had had a different outcome. This time, the null hypothesis that the mean weights of the two strains are identical is not rejected at an alpha level of .05. The researcher, without estimating the power of his test, concludes that since the null hypothesis is not rejected, then the two strains have the same weight. As has been shown above, the differences between selected and control lines in selection experiments range from 5% to as much as 60%. Suppose, however, we take for our example a true difference of only 3%. In that case the null hypothesis of no difference is false, then the researcher made a Type II error for not rejecting the null hypothesis. His statistical test is not sensitive enough to pick up a significant difference of 3% between the two strains. A correct decision on the other hand would have been to reject the null hypothesis.

Since no difference in growth rates is established, farmers are advised to use either strain. What is the economic consequence of this decision? Suppose the annual national production is 100,000 mt. Then the undetected 3% difference is worth 3 million kilos or 6 million dollars, if a kilo of fish sells at two dollars. The difference that is lost could probably pay for the first year of a genetic program.

Time and money are wasted when a research program is not good and a test is not sensitive enough to detect a true difference. The opportunity lost will be much greater than the cost of the research itself if differences among strains really do exist.

I propose the inclusion of statistical power analysis in genetic programmes because of the economic consequences of error. Power should be calculated in choosing sample size and determining whether important differences are likely to be picked up. Post-hoc power analysis can be useful in doing a post-mortem on a performed test whenever the null hypothesis of no difference is not rejected. Power analysis can be used to judge whether the result can be interpreted with confidence or the test is too weak to detect an economically important difference (Toft and Shea 1983).

## Genotype x environment interaction (GxE)

Genotype x environment (G x E) interactions or the differential response of specific genotypes under different environmental conditions (Falconer 1989) have important implications for selection, strain testing, and aquaculture in general. There is so called weak G x E interaction when the relative performance of genotypes vary across environments but the rank order of genotypes is unchanged. Strong genotype x environment interaction is indicated when both the relative performance and rank order of genotypes vary. The presence of strong G x E means that the best strain in one environment may perform poorly in another.

An important implication of G x E interaction is development of multiple breeds specialized for diverse aquaculture systems, habitats and geographical locations (Doyle et al. 1991) using locally adapted and indigenous stocks. This concept is compatible with the diverse farming systems in Southeast Asia which is characterized by diverse cultural practices and heterogeneous environments. Genetic improvement through selection programmes that generate genetic diversity among breeds ( Moav et al. 1976; Wohlfarth et al. 1986; Wohlfarth and Moav 1991; Dunham et al 1990; Falconer 1990; Doyle et al. 1991; Romana and Doyle 1992) can be developed on-farm with the farmer's participation.

An appropriate breeding strategy in Southeast Asia may be to develop multiple breeds of fish for various types of habitat, farming system, or geographical location. This is possible if a strong genotype x environment interaction can be generated. When a genotype x environment interaction is ignored because of a weak test, the opportunity to develop, for instance, a strain of fish in a low-input environment is lost. Selecting for a strain of fish that performs well in a low-input and less managed environment is especially beneficial in developing countries where fish farms tend to be very variable and poorly controlled. There are many more situations where, if one puts an economic cost on the two types of errors, the impact of the Type II error is more costly.

Social interaction in communal and separate testing

Another practical question that I want to address is whether different genotypes of fish should be raised communally or separately during strain testing. Communal rearing, where all genotypes are mixed, was used by Donaldson et al. (1957) with cutthroat trout, Moav and Wohlfarth (1973; 1974), Wohlfarth et al.(1975) with common carp and Dunham et al. (1982) with catfish and McGinty (1983, 1987) with tilapia. Communal rearing increases the statistical precision of the contrast between genotypes. It eliminates environmental variation that occurs between

separate rearing units and also greatly reduces the number of replicates required for a test. However, social interactions, including competition within and between test strains can occur in communal rearing. In fish populations, the causes for differential growth rates may be both genetic and environmental but these differences can be confounded by social interaction, a widely recognized major source of growth rate variation (Magnuson 1962, Moav and Wohlfarth 1974; Purdom 1974; Yamagishi et al. 1974; Doyle and Talbot 1986a; Jobling and Reinesis 1986).

Results of separate rearing of test strains are in some ways more relevant to the actual farm situation, where strains are usually grown separately. A major drawback of separate testing is the need for a large number of containers to overcome the confounding of genetic variation with environmental differences between containers. A possible procedure to reduce environmental variation that occurs between separate rearing units is to use an internal reference population ( a third strain) to account for environmental differences (Kinkaid 1979; Klupp 1979; Moav and Hulata 1976; Basiao and Doyle 1990; Doyle et al. 1990).

Size-grading of fish

Environmental effects that cause phenotypic variation include maternal effects, asynchronous spawning and

different hatching time (Wohlfarth and Hulata 1970; Hulata et al 1974; 1976; Falconer 1989). In tilapia, mouthbrooding of eggs and larvae take about two weeks (Trewavas 1982).

Size-grading or "collimation" of fish before strain testing can minimize the non-genetic environmental variance in growth caused by variable egg quality and by asynchronous spawning (Doyle and Talbot 1986b). However, this may cause difficulties such as genotypes with exceptionally large or small growth rates being underrepresented, thus causing a reduction in variance, and, possibly, a bias.

Thesis structure:

Chapter 1. General Introduction: statement of thesis problem, rationale for claiming that statistical power analysis has important economic implications in aquaculture, other issues that need to be addressed in strain testing like genotype x environment interaction, social interaction, and size-grading of fish prior to testing.

Chapter 2. Power estimates of experimental designs in aquaculture. Calculations of power of different tests, power of performed tests and minimum number of samples to achieve a specified power are illustrated with examples.

Chapter 3. Estimates of statistical power of different strain comparison procedures. The relative power of the

different procedures is calculated and the information is used to design optimal large-scale experimental procedures.

Chapter 4. On-farm, non-experimental strain testing procedure. The power and associated error in the comparison of strains using scale circulus spacing measurement in rice paddies are covered in this chapter.

Chapter 5. Conclusion

## Chapter 2

## Power Estimates of Experimental Designs in Aquaculture

### Abstract

The power of a statistical test, defined as 1-ß, is the probability of rejecting the null hypothesis (Ho) of no difference, when it is in fact false and should be rejected. In aquaculture situation, the power of the test is the probability of detecting an economically important difference between say the growth of two fish strains when a difference does exist.

Power is a function of $\alpha$, $\sigma$, n, and $|\mu 1 - \mu 2|$. The calculations of power can be derived from standard equations and power tables. It is more appropriate to determine a-priori the power of a proposed test for decision-making as to number of replicates and samples to use. However, after an experiment is completed, it is likewise appropriate to estimate the power of a performed test when the Ho is not rejected. Post-hoc power analysis gives an estimate of the probability of having committed a Type II error (not rejecting a false Ho hypothesis). There are many situations in aquaculture where a Type II error is more important than a Type I error (rejecting a true Ho hypothesis).

## Introduction

The power of a statistical test is the probability of correctly detecting an effect, that is rejecting the null hypothesis (Ho) of no effect, when in fact there really is a difference (Dixon and Massey 1969; Zar 1984; Kraemer and Thiemann 1987; Cohen 1988; Shavelson 1988, Samuels 1989; Waples and Myers 1990). The alternative hypothesis (HA) is the hypothesis that there is a true difference of magnitude between say the growth of two fish strains. When HA is true and the Ho is not rejected, a Type II error (ß error) is committed. Power (1-ß) ranges between 0 and 1 and depends on the difference (d) we want to detect.

Power of tests can be calculated from standard equations and power tables (Dixon and Massey 1969; Zar 1984; Kraemer and Thiemann 1987; Cohen 1988; Shavelson 1988; Samuels 1989; Walpole and Myers 1990) but is rarely analyzed and reported by researchers in aquaculture (Trexler and Travis 1990; Trexler et al. 1990).

Power depends on $\alpha$, $\sigma$, n, and $|\mu 1 - \mu 2|$

The $\alpha$ level is a protection against Type I error ($\alpha$ error) and is under the control of the researcher. $\alpha$ is conventionally set at the conservative 0.05 and 0.01 levels. The null hypothesis of no difference is deemed to be rejected whenever the calculated P-value is less than

0.05 or 0.01. However, this protection for a Type I error is traded for vulnerability to Type II error. The probability of committing a Type II error (ß) is generally not known or specified. For a given sample size, n, the value of $\alpha$ is inversely related to the value of ß (Zar 1984). As the $\alpha$ level is decreased from 0.10 to 0.05 to 0.01, the probability of a Type I error is reduced while the chance of a Type II error is increased. Conversely, as the $\alpha$ is increased from 0.01 to 0.05 to 0.10, the chance of a Type I error is increased, but the probability of a Type II error is diminished.

Power is also highly dependent on $\sigma$ (the standard deviation of the data). $\sigma$ represents the background noise that tends to obscure a true difference between strains. A smaller $\sigma$ is desirable because the closer the samples approximate the relevant population mean value, the more reliable they are.

The choice of an appropriate sample size (number of replicates and/or of fish within replicates) is an essential component of any experimental design that is under the control of the researcher. The power of the test is likely to be insufficient to test hypotheses involving small differences between means, if the sample size is too small (Bros and Cowell 1987). The power of an experiment can be increased simply by increasing the replicates. While a large sample size is very desirable, a practical problem of increasing replicates in aquaculture is the

increase in environmental error as replicates are added. The environmental error is due to the qualitative changes in the aquaculture environment as new ponds are added in different parts of the field etc.

The power of a test also depends on the effect size (ES) or critical size. ES is the magnitude of departure from the null hypothesis (that is, the difference between strains) that researchers want to detect. In the growth comparison of two strains, the power of the test depends on the actual difference between the population means, that is, on $|\mu 1 - \mu 2|$. The larger the difference one is trying to detect or the stronger the effect, the more easily it can be detected, and the greater the power of a statistical test. Subtle differences are more difficult to detect. Cohen (1988) has devised a convention of "small, medium, and large" effect sizes related to estimates of variance. This is convenient to use when a researcher has no preliminary data to estimate the critical size. In aquaculture strain testing, it would often be sensible to decide a priori what minimum ES is economically important, and then design the test to detect an effect of that magnitude or larger.

For two independent samples, effect size index (d) is defined as the difference in population means expressed relative to the common population standard deviation (Cohen 1988; Shavelson 1988; Samuels 1989).

Effect size index (d) = $|\mu 1 - \mu 2|/\sigma$

where d = ES index for tests of means in standard unit, $\mu 1$ and $\mu 2$ = population means expressed in raw (original measurement units, and $\sigma$ = the common standard deviation. The means of two populations can be estimated from some pilot or preliminary studies such as those described here while $\sigma$ can be calculated from an ANOVA table.

There is no standard method for setting effect size in strain testing experiments, or in other aquaculture research in general. Theory or pilot studies may, however, indicate the magnitude of difference that exists between say the growth rates of two strains of fish. An on-going within-family selection in the Philippines has shown that after 8 generation of selection, the selected tilapia line is 7 to 37% heavier than the second generation random-bred control line (Bolivar et. al. In press). The estimated annual production of tilapia in the Philippines is 70,000 mt.(Guerrero pers. communication). In the above mentioned within-family selection, an 8% difference means an additional 10 million dollars per annum if a kilo of tilapia sells for 2 dollars.

It is difficult to determine a priori how large or small an effect size or difference is to be expected. The effect size should be determined on the basis of the minimum difference expected to have important economic difference.

Power Analysis in strain testing

In principle, the computation of power and the required number of samples (replicates) are straightforward once the critical size effect is specified and the $\alpha$ level is specified for a particular design. Power and Sample Tables (Dixon and Massey 1969; Zar 1984; Kraemer and Thiemann 1987; Cohen 1988; Shavelson 1988; Samuels 1989; Walpole and Myers 1990) are available for the purpose.

Suppose that in a strain testing experiment, two fish strains of size $n_1$ and $n_2$ are randomly drawn from two populations with means $\mu_1$ and $\mu_2$ and known common variances $\sigma_1^2$ and $\sigma_2^2$.

Consider testing the null hypothesis

Ho: $\mu_1 = \mu_2$

against the alternative hypothesis

HA: $\mu_1 > \mu_2$

with $\sigma$ known at level $\alpha$ in a one sided test.

The Ho is rejected if

$$\frac{(\bar{y}_1 - \bar{y}_2)}{\sigma\sqrt{1/n_1 + 1/n_2}} \geq z_\alpha$$

where n is the number in each sample

i.e. reject Ho if $(\bar{y}_1 - \bar{y}_2) \geq z_\alpha \sigma\sqrt{2/n}$

Suppose we want power for a difference $\mu_1 - \mu_2 = e$

Power = Prob(rejecting Ho when $\mu_1 - \mu_2 = e$)

$1 - \beta$ = Prob($(\bar{y}_1 - \bar{y}_2) > z_\alpha \sigma\sqrt{2/n}$ when $\mu_1 - \mu_2 = e$)

= Prob($(\bar{y}_1 - \bar{y}_2 - e)/\sigma\sqrt{2/n} \geq z_\alpha \sigma\sqrt{2/n} - e/\sigma\sqrt{2/n}$)

therefore,

$$\beta = \text{Prob}((\bar{y}_1 - \bar{y}_2) < z_\alpha \sigma\sqrt{2/n} \text{ when } \mu_1 - \mu_2 = e)$$

$$= \text{Prob}((\bar{y}_1 - \bar{y}_2 - e)/\sigma\sqrt{2/n} < z_\alpha \sigma\sqrt{2/n} - e/\sigma\sqrt{2/n} \text{ when } \mu_1 - \mu_2 = e)$$

Under the alternative hypothesis $\mu_1 - \mu_2 = e$, the statistic $(y_1-y_2-e)/\sigma\sqrt{2/n}$ is the standard normal variable Z.

Therefore,

$$\beta = \text{Prob}(Z < z_\alpha \sigma\sqrt{2/n} - e/\sigma\sqrt{2/n})$$

We need

$$z_\alpha \sigma\sqrt{2/n} - e/\sigma\sqrt{2/n} \geq z_\beta \Longleftrightarrow (z_\alpha + z_\beta)\sigma/e \geq \sqrt{n/2} \Longleftrightarrow$$

$n \geq 2(z_\alpha + z_\beta)^2/(e/\sigma)^2$ where $(e/\sigma)^2$ is written as d by Cohen (1988) and Shavelson (1988), giving the formula for the choice of sample size as

$$n \geq 2(z_\alpha + z_\beta)^2/(d)^2$$

Suppose we choose d = 1.00, $\alpha$ = 0.05 and Power = .99, the sample size is calculated as

$$n \geq 2(1.65 + 2.33)^2/1^2 = 31.60$$

The entry in Cohen's sample size table (Table 2.1) is 32. Areas under the Normal Curve (Table 2.2) are used to locate the $z_\alpha$ and $z_\beta$ values to substitute in the formula $n \geq 2(z\alpha + z\beta)^2/(d)^2$

Power calculations in Analysis of Variance

We know from statistical theory that if the Ho is true then the groups MS (mean square), as well as the error MS will be an estimate of $\sigma^2$, the variance common to all k populations. The variance-ratio of MSgroups/MSerror

## Table 2.1. Sample size Table

| | $a_1$ = .01 ($a_2$ = .02) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 547 | 138 | 62 | 36 | 24 | 17 | 13 | 10 | 7 | 5 | 4 |
| .50 | 1083 | 272 | 122 | 69 | 45 | 31 | 24 | 18 | 12 | 9 | 7 |
| .60 | 1332 | 334 | 149 | 85 | 55 | 38 | 29 | 22 | 15 | 11 | 8 |
| 2/3 | 1552 | 382 | 170 | 97 | 62 | 44 | 33 | 25 | 17 | 12 | 9 |
| .70 | 1627 | 408 | 182 | 103 | 66 | 47 | 35 | 27 | 18 | 13 | 10 |
| .75 | 1803 | 452 | 202 | 114 | 74 | 52 | 38 | 30 | 20 | 14 | 11 |
| .80 | 2009 | 503 | 224 | 127 | 82 | 57 | 42 | 33 | 22 | 15 | 12 |
| .85 | 2263 | 567 | 253 | 143 | 92 | 64 | 48 | 37 | 24 | 17 | 13 |
| .90 | 2605 | 652 | 290 | 164 | 105 | 74 | 55 | 42 | 27 | 20 | 15 |
| .95 | 3155 | 790 | 352 | 198 | 128 | 89 | 66 | 51 | 33 | 23 | 18 |
| .99 | 4330 | 1084 | 82 | 272 | 175 | 122 | 90 | 69 | 45 | 31 | 23 |
| | $a_1$ = .05 ($a_2$ = .10) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 189 | 48 | 21 | 12 | 8 | 6 | 5 | 4 | 3 | 2 | 2 |
| .50 | 542 | 136 | 61 | 35 | 22 | 16 | 12 | 9 | 6 | 5 | 4 |
| .60 | 721 | 181 | 81 | 46 | 30 | 21 | 15 | 12 | 8 | 6 | 5 |
| 2/3 | 862 | 216 | 96 | 55 | 35 | 25 | 18 | 14 | 9 | 7 | 5 |
| .70 | 942 | 236 | 105 | 60 | 38 | 27 | 20 | 15 | 10 | 7 | 6 |
| .75 | 1076 | 270 | 120 | 68 | 44 | 31 | 23 | 18 | 11 | 8 | 6 |
| .80 | 1237 | 310 | 138 | 78 | 50 | 35 | 26 | 20 | 13 | 9 | 7 |
| .85 | 1438 | 360 | 160 | 91 | 58 | 41 | 30 | 23 | 15 | 11 | 8 |
| .90 | 1713 | 429 | 191 | 108 | 69 | 48 | 36 | 27 | 18 | 13 | 10 |
| .95 | 2165 | 542 | 241 | 136 | 87 | 61 | 45 | 35 | 22 | 16 | 12 |
| .99 | 3155 | 789 | 351 | 198 | 127 | 88 | 65 | 50 | 32 | 23 | 17 |
| | $a_1$ = .10 ($a_2$ = .20) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 74 | 19 | 9 | 5 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| .50 | 329 | 82 | 37 | 21 | 14 | 10 | 7 | 5 | 4 | 3 | 2 |
| .60 | 471 | 118 | 53 | 30 | 19 | 14 | 10 | 8 | 5 | 4 | 3 |
| 2/3 | 586 | 147 | 65 | 37 | 24 | 17 | 12 | 10 | 6 | 4 | 3 |
| .70 | 653 | 163 | 73 | 41 | 27 | 19 | 14 | 11 | 7 | 5 | 4 |
| .75 | 766 | 192 | 85 | 48 | 31 | 22 | 16 | 13 | 8 | 6 | 4 |
| .80 | 902 | 226 | 100 | 57 | 35 | 26 | 19 | 14 | 10 | 7 | 5 |
| .85 | 1075 | 269 | 120 | 67 | 43 | 30 | 22 | 17 | 11 | 8 | 6 |
| .90 | 1314 | 329 | 146 | 82 | 53 | 37 | 27 | 21 | 14 | 10 | 7 |
| .95 | 1713 | 428 | 191 | 107 | 69 | 48 | 35 | 27 | 18 | 12 | 9 |
| .99 | 2604 | 651 | 290 | 163 | 104 | 73 | 53 | 41 | 26 | 18 | 14 |

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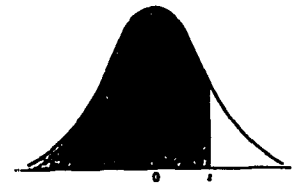


## Table 2.2. Areas under the normal curve

| z | .00 | .01 | .02 | .03 | .04 | .05 | .06 | .07 | .08 | .09 |
|---|---|---|---|---|---|---|---|---|---|---|
| −3.4 | .0003 | .0003 | .0003 | .0003 | .0003 | .0003 | .0003 | .0003 | .0003 | .0002 |
| −3.3 | .0005 | .0005 | .0005 | .0004 | .0004 | .0004 | .0004 | .0004 | .0004 | .0003 |
| −3.2 | .0007 | .0007 | .0006 | .0006 | .0006 | .0006 | .0006 | .0005 | .0005 | .0005 |
| −3.1 | .0010 | .0009 | .0009 | .0009 | .0008 | .0008 | .0008 | .0008 | .0007 | .0007 |
| −3.0 | .0013 | .0013 | .0013 | .0012 | .0012 | .0011 | .0011 | .0011 | .0010 | .0010 |
| −2.9 | .0019 | .0018 | .0017 | .0017 | .0016 | .0016 | .0015 | .0015 | .0014 | .0014 |
| −2.8 | .0026 | .0025 | .0024 | .0023 | .0023 | .0022 | .0021 | .0021 | .0020 | .0019 |
| −2.7 | .0035 | .0034 | .0033 | .0032 | .0031 | .0030 | .0029 | .0028 | .0027 | .0026 |
| −2.6 | .0047 | .0045 | .0044 | .0043 | .0041 | .0040 | .0039 | .0038 | .0037 | .0036 |
| −2.5 | .0062 | .0060 | .0059 | .0057 | .0055 | .0054 | .0052 | .0051 | .0049 | .0048 |
| −2.4 | .0082 | .0080 | .0078 | .0075 | .0073 | .0071 | .0069 | .0068 | .0066 | .0064 |
| −2.3 | .0107 | .0104 | .0102 | .0099 | .0096 | .0094 | .0091 | .0089 | .0087 | .0084 |
| −2.2 | .0139 | .0136 | .0132 | .0129 | .0125 | .0122 | .0119 | .0116 | .0113 | .0110 |
| −2.1 | .0179 | .0174 | .0170 | .0166 | .0162 | .0158 | .0154 | .0150 | .0146 | .0143 |
| −2.0 | .0228 | .0222 | .0217 | .0212 | .0207 | .0202 | .0197 | .0192 | .0188 | .0183 |
| −1.9 | .0287 | .0281 | .0274 | .0268 | .0262 | .0256 | .0250 | .0244 | .0239 | .0233 |
| −1.8 | .0359 | .0352 | .0344 | .0336 | .0329 | .0322 | .0314 | .0307 | .0301 | .0294 |
| −1.7 | .0446 | .0436 | .0427 | .0418 | .0409 | .0401 | .0392 | .0384 | .0375 | .0367 |
| −1.6 | .0548 | .0537 | .0526 | .0516 | .0505 | .0495 | .0485 | .0475 | .0465 | .0455 |
| −1.5 | .0668 | .0655 | .0643 | .0630 | .0618 | .0606 | .0594 | .0582 | .0571 | .0559 |
| −1.4 | .0808 | .0793 | .0778 | .0764 | .0749 | .0735 | .0722 | .0708 | .0694 | .0681 |
| −1.3 | .0968 | .0951 | .0934 | .0918 | .0901 | .0885 | .0869 | .0853 | .0838 | .0823 |
| −1.2 | .1151 | .1131 | .1112 | .1093 | .1075 | .1056 | .1038 | .1020 | .1003 | .0985 |
| −1.1 | .1357 | .1335 | .1314 | .1292 | .1271 | .1251 | .1230 | .1210 | .1190 | .1170 |
| −1.0 | .1587 | .1562 | .1539 | .1515 | .1492 | .1469 | .1446 | 1423 | .1401 | .1379 |
| −0.9 | .1841 | .1814 | .1788 | .1762 | .1736 | .1711 | .1685 | .1660 | .1635 | .1611 |
| −0.8 | .2119 | .2090 | .2061 | .2033 | .2005 | .1977 | .1949 | .1922 | .1894 | .1867 |
| −0.7 | .2420 | .2389 | .2358 | .2327 | .2296 | .2266 | .2236 | .2206 | .2177 | .2148 |
| −0.6 | .2743 | .2709 | .2676 | .2643 | .2611 | .2578 | .2546 | .2514 | .2483 | .2451 |
| −0.5 | .3085 | .3050 | .3015 | .2981 | .2946 | .2912 | .2877 | .2843 | .2810 | .2776 |
| −0.4 | .3446 | .3409 | .3372 | .3336 | .3300 | .3264 | .3228 | .3192 | .3156 | .3121 |
| −0.3 | .3821 | .3783 | .3745 | .3707 | .3669 | .3632 | .3594 | .3557 | .3520 | .3483 |
| −0.2 | .4207 | .4168 | .4129 | .4090 | .4052 | .4013 | .3974 | .3936 | .3897 | .3859 |
| −0.1 | .4602 | .4562 | .4522 | .4483 | .4443 | .4404 | .4364 | .4325 | .4286 | .4247 |
| −0.0 | .5000 | .4960 | .4920 | .4880 | .4840 | .4801 | .4761 | .4721 | .4681 | .4641 |

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## Table 2.2. Areas under the normal curve

| z | .00 | .01 | .02 | .03 | .04 | .05 | .06 | .07 | .08 | .09 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | .5000 | .5040 | .5080 | .5120 | .5160 | .5199 | .5239 | .5279 | .5319 | .5359 |
| 0.1 | .5398 | .5438 | .5478 | .5517 | .5557 | .5596 | .5636 | .5675 | .5714 | .5753 |
| 0.2 | .5793 | .5832 | .5871 | .5910 | .5948 | .5987 | .6026 | .6064 | .6103 | .6141 |
| 0.3 | .6179 | .6217 | .6255 | .6293 | .6331 | .6368 | .6406 | .6443 | .6480 | .6517 |
| 0.4 | .6554 | .6591 | .6628 | .6664 | .6700 | .6736 | .6772 | .6808 | .6844 | .6879 |
| 0.5 | .6915 | .6950 | .6985 | .7019 | .7054 | .7088 | .7123 | .7157 | .7190 | .7224 |
| 0.6 | .7257 | .7291 | .7324 | .7357 | .7389 | .7422 | .7454 | .7486 | .7517 | .7549 |
| 0.7 | .7580 | .7611 | .7642 | .7673 | .7704 | .7734 | .7764 | .7794 | .7823 | .7852 |
| 0.8 | .7881 | .7910 | .7939 | .7967 | .7995 | .8023 | .8051 | .8078 | .8106 | .8133 |
| 0.9 | .8159 | .8186 | .8212 | .8238 | .8264 | .8289 | .8315 | .8340 | .8365 | .8389 |
| 1.0 | .8413 | .8438 | .8461 | .8485 | .8508 | .8531 | .8554 | .8577 | .8599 | .8621 |
| 1.1 | .8643 | .8665 | .8686 | .8708 | .8729 | .8749 | .8770 | .8790 | .8810 | .8830 |
| 1.2 | .8849 | .8869 | .8888 | .8907 | .8925 | .8944 | .8962 | .8980 | .8997 | .9015 |
| 1.3 | .9032 | .9049 | .9066 | .9082 | .9099 | .9115 | .9131 | .9147 | .9162 | .9177 |
| 1.4 | .9192 | .9207 | .9222 | .9236 | .9251 | .9265 | .9278 | .9292 | .9306 | .9319 |
| 1.5 | .9332 | .9345 | .9357 | .9370 | .9382 | .9394 | .9406 | .9418 | .9429 | .9441 |
| 1.6 | .9452 | .9463 | .9474 | .9484 | .9495 | .9505 | .9515 | .9525 | .9535 | .9545 |
| 1.7 | .9554 | .9564 | .9573 | .9582 | .9591 | .9599 | .9608 | .9616 | .9625 | .9633 |
| 1.8 | .9641 | .9649 | .9656 | .9664 | .9671 | .9678 | .9686 | .9693 | .9699 | .9706 |
| 1.9 | .9713 | .9719 | .9726 | .9732 | .9738 | .9744 | .9750 | .9756 | .9761 | .9767 |
| 2.0 | .9772 | .9778 | .9783 | .9788 | .9793 | .9798 | .9803 | .9808 | .9812 | .9817 |
| 2.1 | .9821 | .9826 | .9830 | .9834 | .9838 | .9842 | .9846 | .9850 | .9854 | .9857 |
| 2.2 | .9861 | .9864 | .9868 | .9871 | .9875 | .9878 | .9881 | .9884 | .9887 | .9890 |
| 2.3 | .9893 | .9896 | .9898 | .9901 | .9904 | .9906 | .9909 | .9911 | .9913 | .9916 |
| 2.4 | .9918 | .9920 | .9922 | .9925 | .9927 | .9929 | .9931 | .9932 | .9934 | .9936 |
| 2.5 | .9938 | .9940 | .9941 | .9943 | .9945 | .9946 | .9948 | .9949 | .9951 | .9952 |
| 2.6 | .9953 | .9955 | .9956 | .9957 | .9959 | .9960 | .9961 | .9962 | .9963 | .9964 |
| 2.7 | .9965 | .9966 | .9967 | .9968 | .9969 | .9970 | .9971 | .9972 | .9973 | .9974 |
| 2.8 | .9974 | .9975 | .9976 | .9977 | .9977 | .9978 | .9979 | .9979 | .9980 | .9981 |
| 2.9 | .9981 | .9982 | .9982 | .9983 | .9984 | .9984 | .9985 | .9985 | .9986 | .9986 |
| 3.0 | .9987 | .9987 | .9987 | .9988 | .9988 | .9989 | .9989 | .9989 | .9990 | .9990 |
| 3.1 | .9990 | .9991 | .9991 | .9991 | .9992 | .9992 | .9992 | .9992 | .9993 | .9993 |
| 3.2 | .9993 | .9993 | .9994 | .9994 | .9994 | .9994 | .9994 | .9995 | .9995 | .9995 |
| 3.3 | .9995 | .9995 | .9995 | .9996 | .9996 | .9996 | .9996 | .9996 | .9996 | .9997 |
| 3.4 | .9997 | .9997 | .9997 | .9997 | .9997 | .9997 | .9997 | .9997 | .9997 | .9998 |

all k populations. The variance-ratio of MSgroups/MSerror follows the F distribution being defined by the numerator and denominator degrees of freedom ($v_1$ and $v_2$, respectively). If, however, Ho is false and the k population means are not equal, then the groups MS will be greater than the error MS and the variance-ratio follows instead the noncentral F distribution, defined by $v_1$ and $v_2$ and a third quantity known as the noncentrality parameter ($\Gamma$). The power of ANOVA testing is estimated by calculating phi ($\phi$) which is related to non-centrality parameter ($\Gamma$) by

$\phi = \Gamma/\sqrt{(v_1 + 1)}$ where $v_1$ is df of numerator in F-test, $v_1 = k-1$

and $\Gamma^2 = n\sum_{i=1}^{k} (\mu_i - \bar{\mu})^2/\sigma^2$ where $\mu_i$ = mean of ith group (Scheffe p. 39, rule 1, p 40 section 2.8, p62-64)

Hence $\phi = \sqrt{n\sum_{i=1}^{k} (\mu_i - \bar{\mu})^2/k\ \sigma^2}$

and since $\sigma^2$ is estimated by $s^2$

$\phi = \sqrt{n\sum_{i=1}^{k} (\mu_i - \bar{\mu})^2/k\ s^2}$

A-priori power calculations

It is desirable to investigate the power of the proposed test. When the estimated power of the proposed test is so low, researchers must make decisions to run the experiment with many more data or perhaps, not run the experiment at all. In aquaculture situation, when the minimum difference between say the growth of two fish strains is considered economically important, the

opportunity loss for not running a strain testing experiment with many replicate cages will be much greater than the cost of the research itself.

A priori power estimates are usually done by specifying a minimal detectable difference ($\delta$). In specifying $\delta$, we assume

$\mu_1 = \delta/2$, $\mu_2 = -\delta/2$, $\mu_3 = \ldots = \mu_k = 0$. It is also usually assumed that $\Sigma\mu_i = 0$.

Now $\sum_{i=1}^{K} (\mu_i - \bar{\mu})^2 = \delta^2/4 + \delta^2/4 = \delta^2/2$

Then $\phi = \sqrt{n\delta^2/2k\sigma^2}$ since $s^2$ is an estimate of $\sigma^2$

$\phi = \sqrt{n\delta^2/2ks^2}$

when k = 2 (two-sample t test),

$\phi = \sqrt{n\delta^2/4s^2}$

Post-hoc power analysis

The power of a test is more appropriately estimated prior to collecting data for hypothesis testing. However, after an experiment is completed and the Ho hypothesis is not rejected, it is desirable to estimate the probability of having committed a Type II error. If the test has been done, we have to estimate

$\sum_{i=1}^{K} (\mu_i - \bar{\mu})^2$ or equivalently $\Gamma$ (non-centrality parameter).

In one-way ANOVA

$$E(\text{MS groups}) = \sigma^2 + \frac{n\sum_{i=1}^{k} (\mu_i - \mu)^2}{k - 1} \quad \text{(p 59 Scheffe)}$$

Hence

$$\phi^2 = \frac{n}{k}\sum_{i=1}^{k} (\mu_i - \bar{\mu})^2/k\,\sigma^2 = (k-1)[E(\text{MS groups}) - \sigma 2]/k\,\sigma 2$$

If E(MS group) is replaced by its estimate MS group and $\sigma^2$ by $s^2$ then

$\phi$ = (k-1)(groups MS - s2)/ks2 (Zar 1984)

If k = 2, MS groups = $n(\bar{y}_1 - \bar{y}_2)^2/2 = nd^2/2$

where $d = \bar{y}_1 - \bar{y}_2$

So for k = 2,

$$\phi = \sqrt{\frac{nd^2/2 - s^2}{2s^2}} = \sqrt{\frac{nd^2 - 2s^2}{4s^2}} \quad \text{(Zar 1984)}$$

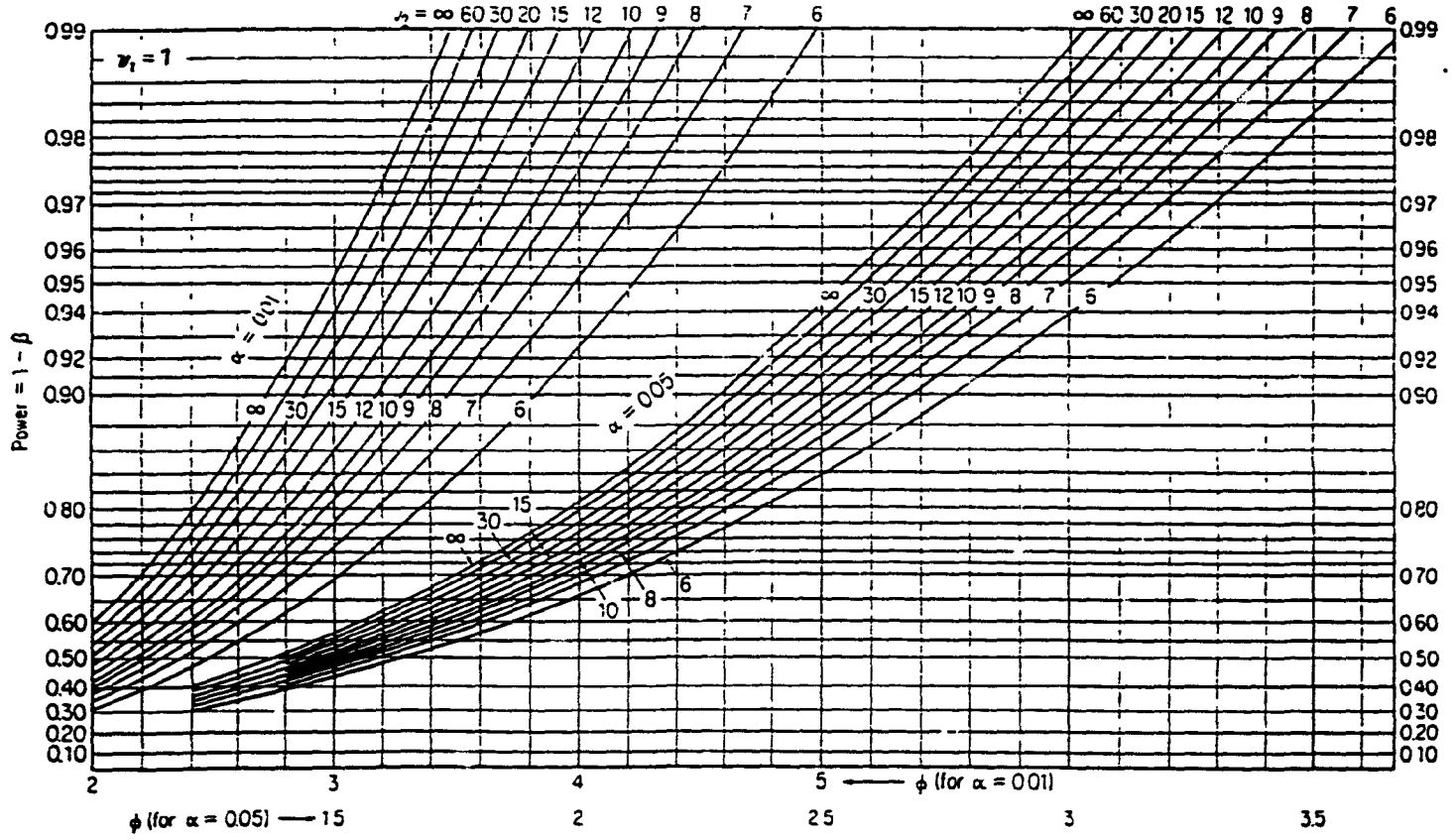
Once $\phi$ has been obtained, the power of the tests can be determined from Fig. 2.1 which is composed of several pages, each with a different v1 (i.e. group DF (degrees of freedom)) found at the upper left of the graph. Each of the curves on the graph is for a different $v_2$ (i.e. error DF). The power of the test is determined by locating the point at which the calculated $\phi$ intersects the curve for the given $v_2$ and reads horizontally to either the left or right axis depending on the specified level of $\alpha$.

Sample size calculations

Zar (1984) estimates the required sample size of two samples by an iterative process using

$n \geq 2\ sp^2/\delta^2(t_{\alpha,v} + t_{\beta(1),v})^2$ where $\delta$ $(\mu_1 - \mu_2)$ is the population difference we want to detect; $sp^2$ is the within population variability which can be based on a

**Figure 2.1. Power and sample size in analysis of variance**



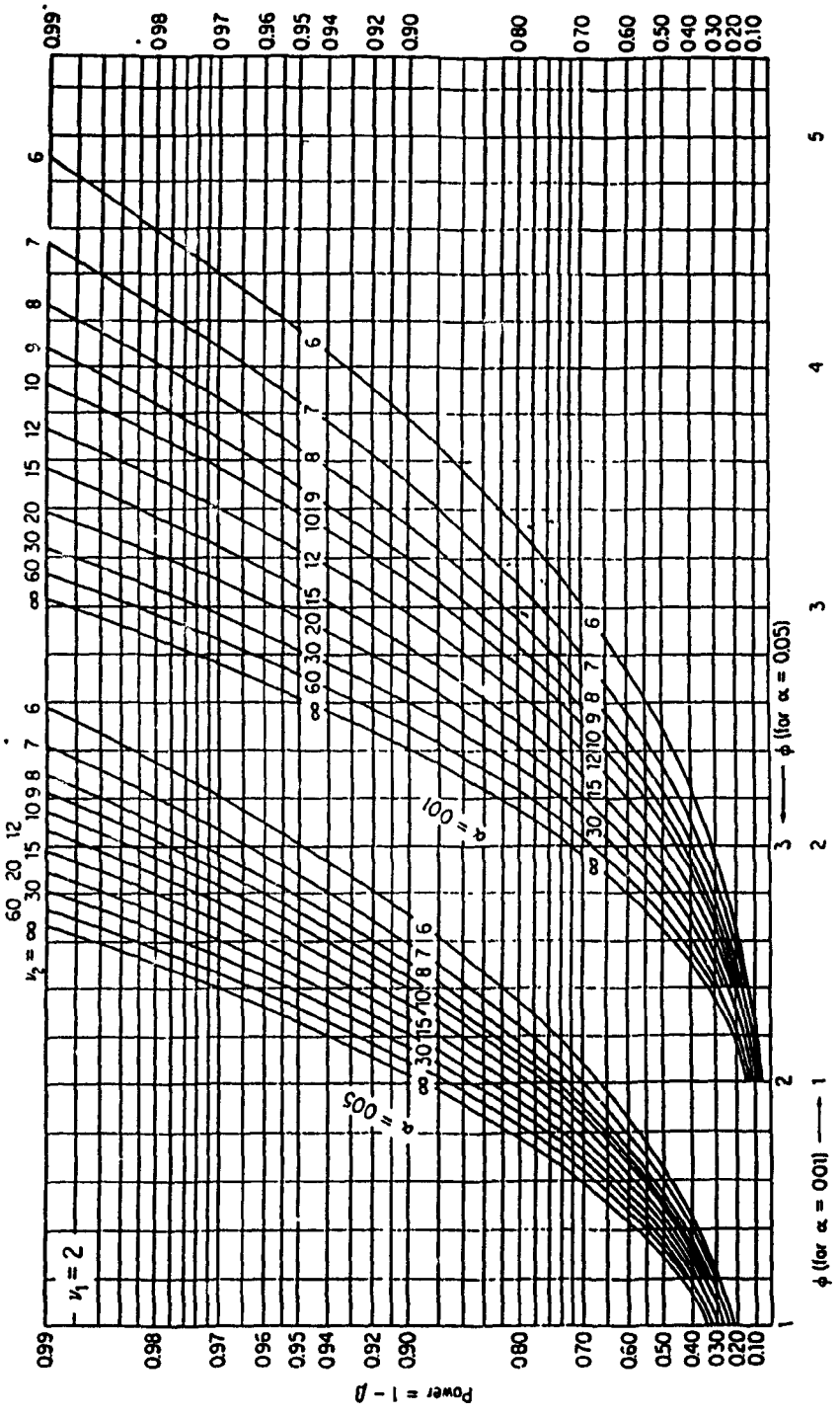
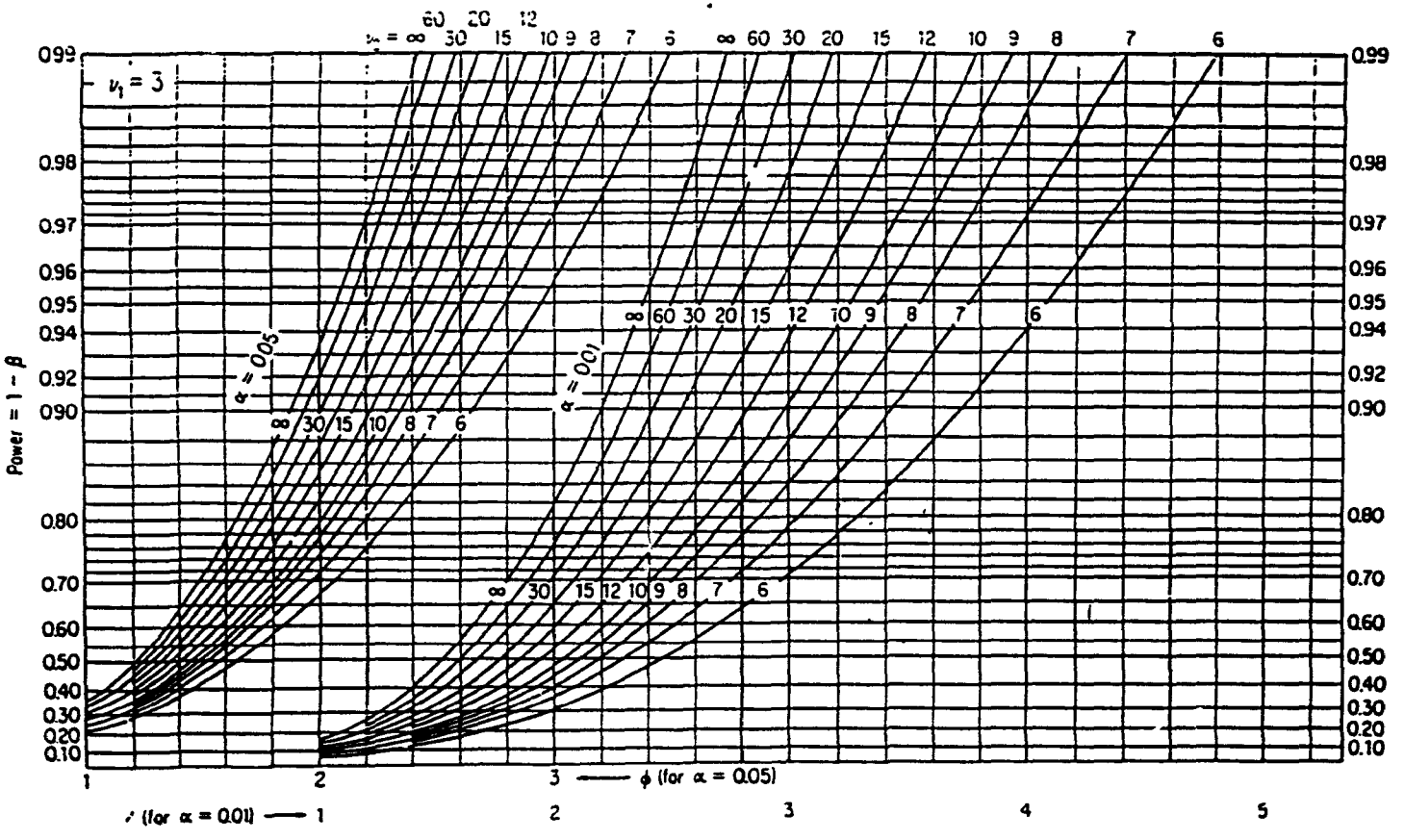



Figure 2.1. Power and sample size in analysis of variance

**Figure 2.1. Power and sample size in analysis of variance**




**These graphs were taken from Pearson and Hartley (1951, Biometrika 38:112-130) with permission of the Biometrika Trustees.**

previous study; $t_{\alpha,v}$ and $t_{\beta(1)v}$ are obtained from the critical value of the t distribution, where $t_{\beta(1)}$ is the critical value of t when a one-sided test is performed (Table 2.3); v = 2(n-1) degrees of freedom. Each iterative step will bring the estimate of n closer to the final result (declared when two successive iterations do not change the value of n rounded to the next highest integer).

The sample size required in ANOVA is also calculated by an iterative process using the formula $\phi = \sqrt{n\delta^2/2ks^2}$ (Zar 1984) where n = number of replicates, $\delta$ = smallest difference between the two most different population means, k = number of population means, $s^2$ is the estimate of the within population variability which we can obtain from an ANOVA table of a similar study.

Examples of power and sample size calculations

Example 1. Estimation of required sample size for a two-sample t test.

Results of the preliminary experiment I conducted will be used to illustrate the calculation. The estimates of mean and variances for two tilapia strains were obtained when the two strains were graded and reared separately in cages.

$\mu$nifi = 42.950 mm (estimates of population mean of NIFI)

# Table 2.3. Critical values of the t distribution

| ν | α(2): 0.50<br>α(1): 0.25 | 0.20<br>0.10 | 0.10<br>0.05 | 0.05<br>0.025 | 0.02<br>0.01 | 0.01<br>0.005 | 0.005<br>0.0025 | 0.002<br>0.001 | 0.001<br>0.0005 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.000 | 3.078 | 6.314 | 12.706 | 31.821 | 63.657 | 127.321 | 318.309 | 636.619 |
| 2 | 0.816 | 1.886 | 2.920 | 4.303 | 6.965 | 9.925 | 14.089 | 22.327 | 31.599 |
| 3 | 0.765 | 1.638 | 2.353 | 3.182 | 4.541 | 5.841 | 7.453 | 10.215 | 12.924 |
| 4 | 0.741 | 1.533 | 2.132 | 2.776 | 3.747 | 4.604 | 5.598 | 7.173 | 8.610 |
| 5 | 0.727 | 1.476 | 2.015 | 2.571 | 3.365 | 4.032 | 4.773 | 5.893 | 6.869 |
| 6 | 0.718 | 1.440 | 1.943 | 2.447 | 3.143 | 3.707 | 4.317 | 5.208 | 5.959 |
| 7 | 0.711 | 1.415 | 1.895 | 2.365 | 2.998 | 3.499 | 4.029 | 4.785 | 5.408 |
| 8 | 0.706 | 1.397 | 1.860 | 2.306 | 2.896 | 3.355 | 3.833 | 4.501 | 5.041 |
| 9 | 0.703 | 1.383 | 1.833 | 2.262 | 2.821 | 3.250 | 3.690 | 4.297 | 4.781 |
| 10 | 0.700 | 1.372 | 1.812 | 2.228 | 2.764 | 3.169 | 3.581 | 4.144 | 4.587 |
| 11 | 0.697 | 1.363 | 1.796 | 2.201 | 2.718 | 3.106 | 3.497 | 4.025 | 4.437 |
| 12 | 0.695 | 1.356 | 1.782 | 2.179 | 2.681 | 3.055 | 3.428 | 3.930 | 4.318 |
| 13 | 0.694 | 1.350 | 1.771 | 2.160 | 2.650 | 3.012 | 3.372 | 3.852 | 4.221 |
| 14 | 0.692 | 1.345 | 1.761 | 2.145 | 2.624 | 2.977 | 3.326 | 3.787 | 4.140 |
| 15 | 0.691 | 1.341 | 1.753 | 2.131 | 2.602 | 2.947 | 3.286 | 3.733 | 4.073 |
| 16 | 0.690 | 1.337 | 1.746 | 2.120 | 2.583 | 2.921 | 3.252 | 3.686 | 4.015 |
| 17 | 0.689 | 1.333 | 1.740 | 2.110 | 2.567 | 2.898 | 3.222 | 3.646 | 3.965 |
| 18 | 0.688 | 1.330 | 1.734 | 2.101 | 2.552 | 2.878 | 3.197 | 3.610 | 3.922 |
| 19 | 0.688 | 1.328 | 1.729 | 2.093 | 2.539 | 2.861 | 3.174 | 3.579 | 3.883 |
| 20 | 0.687 | 1.325 | 1.725 | 2.086 | 2.528 | 2.845 | 3.153 | 3.552 | 3.850 |
| 21 | 0.686 | 1.323 | 1.721 | 2.080 | 2.518 | 2.831 | 3.135 | 3.527 | 3.819 |
| 22 | 0.686 | 1.321 | 1.717 | 2.074 | 2.508 | 2.819 | 3.119 | 3.505 | 3.792 |
| 23 | 0.685 | 1.319 | 1.714 | 2.069 | 2.500 | 2.807 | 3.104 | 3.485 | 3.768 |
| 24 | 0.685 | 1.318 | 1.711 | 2.064 | 2.492 | 2.797 | 3.091 | 3.467 | 3.745 |
| 25 | 0.684 | 1.316 | 1.708 | 2.060 | 2.485 | 2.787 | 3.078 | 3.450 | 3.725 |
| 26 | 0.684 | 1.315 | 1.706 | 2.056 | 2.479 | 2.779 | 3.067 | 3.435 | 3.707 |
| 27 | 0.684 | 1.314 | 1.703 | 2.052 | 2.473 | 2.771 | 3.057 | 3.421 | 3.690 |
| 28 | 0.683 | 1.313 | 1.701 | 2.048 | 2.467 | 2.763 | 3.047 | 3.408 | 3.674 |
| 29 | 0.683 | 1.311 | 1.699 | 2.045 | 2.462 | 2.756 | 3.038 | 3.396 | 3.659 |
| 30 | 0.683 | 1.310 | 1.697 | 2.042 | 2.457 | 2.750 | 3.030 | 3.385 | 3.646 |
| 31 | 0.682 | 1.309 | 1.696 | 2.040 | 2.453 | 2.744 | 3.022 | 3.375 | 3.633 |
| 32 | 0.682 | 1.309 | 1.694 | 2.037 | 2.449 | 2.738 | 3.015 | 3.365 | 3.622 |
| 33 | 0.682 | 1.308 | 1.692 | 2.035 | 2.445 | 2.733 | 3.008 | 3.356 | 3.611 |
| 34 | 0.682 | 1.307 | 1.691 | 2.032 | 2.441 | 2.728 | 3.002 | 3.348 | 3.601 |
| 35 | 0.682 | 1.306 | 1.690 | 2.030 | 2.438 | 2.724 | 2.996 | 3.340 | 3.591 |
| 36 | 0.681 | 1.306 | 1.688 | 2.028 | 2.434 | 2.719 | 2.990 | 3.333 | 3.582 |
| 37 | 0.681 | 1.305 | 1.687 | 2.026 | 2.431 | 2.715 | 2.985 | 3.326 | 3.574 |
| 38 | 0.681 | 1.304 | 1.686 | 2.024 | 2.429 | 2.712 | 2.980 | 3.319 | 3.566 |
| 39 | 0.681 | 1.304 | 1.685 | 2.023 | 2.426 | 2.708 | 2.976 | 3.313 | 3.558 |
| 40 | 0.681 | 1.303 | 1.684 | 2.021 | 2.423 | 2.704 | 2.971 | 3.307 | 3.551 |
| 41 | 0.681 | 1.303 | 1.683 | 2.020 | 2.421 | 2.701 | 2.967 | 3.301 | 3.544 |
| 42 | 0.680 | 1.302 | 1.682 | 2.018 | 2.418 | 2.698 | 2.963 | 3.296 | 3.538 |
| 43 | 0.680 | 1.302 | 1.681 | 2.017 | 2.416 | 2.695 | 2.959 | 3.291 | 3.532 |
| 44 | 0.680 | 1.301 | 1.680 | 2.015 | 2.414 | 2.692 | 2.956 | 3.286 | 3.526 |
| 45 | 0.680 | 1.301 | 1.679 | 2.014 | 2.412 | 2.690 | 2.952 | 3.281 | 3.520 |
| 46 | 0.680 | 1.300 | 1.679 | 2.013 | 2.410 | 2.687 | 2.949 | 3.277 | 3.515 |
| 47 | 0.680 | 1.300 | 1.678 | 2.012 | 2.408 | 2.685 | 2.946 | 3.273 | 3.510 |
| 48 | 0.680 | 1.299 | 1.677 | 2.011 | 2.407 | 2.682 | 2.943 | 3.269 | 3.505 |
| 49 | 0.680 | 1.299 | 1.677 | 2.010 | 2.405 | 2.680 | 2.940 | 3.265 | 3.500 |
| 50 | 0.679 | 1.299 | 1.676 | 2.009 | 2.403 | 2.678 | 2.937 | 3.261 | 3.496 |

## Table 2.3. Critical values of the t distribution

| ν | α(2): 0.50<br>α(1): 0.25 | 0.20<br>0.10 | 0.10<br>0.05 | 0.05<br>0.025 | 0.02<br>0.01 | 0.01<br>0.005 | 0.005<br>0.0025 | 0.002<br>0.001 | 0.001<br>0.0005 |
|---|---|---|---|---|---|---|---|---|---|
| 52 | 0.679 | 1.298 | 1.675 | 2.007 | 2.400 | 2.674 | 2.932 | 3.255 | 3.488 |
| 54 | 0.679 | 1.297 | 1.674 | 2.005 | 2.397 | 2.670 | 2.927 | 3.248 | 3.480 |
| 56 | 0.679 | 1.297 | 1.673 | 2.003 | 2.395 | 2.667 | 2.923 | 3.242 | 3.473 |
| 58 | 0.679 | 1.296 | 1.672 | 2.002 | 2.392 | 2.663 | 2.918 | 3.237 | 3.466 |
| 60 | 0.679 | 1.296 | 1.671 | 2.000 | 2.390 | 2.660 | 2.915 | 3.232 | 3.460 |
| 62 | 0.678 | 1.295 | 1.670 | 1.999 | 2.388 | 2.657 | 2.911 | 3.227 | 3.454 |
| 64 | 0.678 | 1.295 | 1.669 | 1.998 | 2.386 | 2.655 | 2.908 | 3.223 | 3.449 |
| 66 | 0.678 | 1.295 | 1.668 | 1.997 | 2.384 | 2.652 | 2.904 | 3.218 | 3.444 |
| 68 | 0.678 | 1.294 | 1.668 | 1.995 | 2.382 | 2.650 | 2.902 | 3.214 | 3.439 |
| 70 | 0.678 | 1.294 | 1.667 | 1.994 | 2.381 | 2.648 | 2.899 | 3.211 | 3.435 |
| 72 | 0.678 | 1.293 | 1.666 | 1.993 | 2.379 | 2.646 | 2.896 | 3.207 | 3.431 |
| 74 | 0.678 | 1.293 | 1.666 | 1.993 | 2.378 | 2.644 | 2.894 | 3.204 | 3.427 |
| 76 | 0.678 | 1.293 | 1.665 | 1.992 | 2.376 | 2.642 | 2.891 | 3.201 | 3.423 |
| 78 | 0.678 | 1.292 | 1.665 | 1.991 | 2.375 | 2.640 | 2.889 | 3.198 | 3.420 |
| 80 | 0.678 | 1.292 | 1.664 | 1.990 | 2.374 | 2.639 | 2.887 | 3.195 | 3.416 |
| 82 | 0.677 | 1.292 | 1.664 | 1.989 | 2.373 | 2.637 | 2.885 | 3.193 | 3.413 |
| 84 | 0.677 | 1.292 | 1.663 | 1.989 | 2.372 | 2.636 | 2.883 | 3.190 | 3.410 |
| 86 | 0.677 | 1.291 | 1.663 | 1.988 | 2.370 | 2.634 | 2.881 | 3.188 | 3.407 |
| 88 | 0.677 | 1.291 | 1.662 | 1.987 | 2.369 | 2.633 | 2.880 | 3.185 | 3.405 |
| 90 | 0.677 | 1.291 | 1.662 | 1.987 | 2.368 | 2.632 | 2.878 | 3.183 | 3.402 |
| 92 | 0.677 | 1.291 | 1.662 | 1.986 | 2.368 | 2.630 | 2.876 | 3.181 | 3.399 |
| 94 | 0.677 | 1.291 | 1.661 | 1.986 | 2.367 | 2.629 | 2.875 | 3.179 | 3.397 |
| 96 | 0.677 | 1.290 | 1.661 | 1.985 | 2.366 | 2.628 | 2.873 | 3.177 | 3.395 |
| 98 | 0.677 | 1.290 | 1.661 | 1.984 | 2.365 | 2.627 | 2.872 | 3.175 | 3.393 |
| 100 | 0.677 | 1.290 | 1.660 | 1.984 | 2.364 | 2.626 | 2.871 | 3.174 | 3.390 |
| 105 | 0.677 | 1.290 | 1.659 | 1.983 | 2.362 | 2.623 | 2.868 | 3.170 | 3.386 |
| 110 | 0.677 | 1.289 | 1.659 | 1.982 | 2.361 | 2.621 | 2.865 | 3.166 | 3.381 |
| 115 | 0.677 | 1.289 | 1.658 | 1.981 | 2.359 | 2.619 | 2.862 | 3.163 | 3.377 |
| 120 | 0.677 | 1.289 | 1.658 | 1.980 | 2.358 | 2.617 | 2.860 | 3.160 | 3.373 |
| 125 | 0.676 | 1.288 | 1.657 | 1.979 | 2.357 | 2.616 | 2.858 | 3.157 | 3.370 |
| 130 | 0.676 | 1.288 | 1.657 | 1.978 | 2.355 | 2.614 | 2.856 | 3.154 | 3.367 |
| 135 | 0.676 | 1.288 | 1.656 | 1.978 | 2.354 | 2.613 | 2.854 | 3.152 | 3.364 |
| 140 | 0.676 | 1.288 | 1.656 | 1.977 | 2.353 | 2.611 | 2.852 | 3.149 | 3.361 |
| 145 | 0.676 | 1.287 | 1.655 | 1.976 | 2.352 | 2.610 | 2.851 | 3.147 | 3.359 |
| 150 | 0.676 | 1.287 | 1.655 | 1.976 | 2.351 | 2.609 | 2.849 | 3.145 | 3.357 |
| 160 | 0.676 | 1.287 | 1.654 | 1.975 | 2.350 | 2.607 | 2.846 | 3.142 | 3.352 |
| 170 | 0.676 | 1.287 | 1.654 | 1.974 | 2.348 | 2.605 | 2.844 | 3.139 | 3.349 |
| 180 | 0.676 | 1.286 | 1.653 | 1.973 | 2.347 | 2.603 | 2.842 | 3.136 | 3.345 |
| 190 | 0.676 | 1.286 | 1.653 | 1.973 | 2.346 | 2.602 | 2.840 | 3.134 | 3.342 |
| 200 | 0.676 | 1.286 | 1.653 | 1.972 | 2.345 | 2.601 | 2.839 | 3.131 | 3.340 |
| 250 | 0.675 | 1.285 | 1.651 | 1.969 | 2.341 | 2.596 | 2.832 | 3.123 | 3.330 |
| 300 | 0.675 | 1.284 | 1.650 | 1.968 | 2.339 | 2.592 | 2.828 | 3.118 | 3.323 |
| 350 | 0.675 | 1.284 | 1.649 | 1.967 | 2.337 | 2.590 | 2.825 | 3.114 | 3.319 |
| 400 | 0.675 | 1.284 | 1.649 | 1.966 | 2.336 | 2.588 | 2.823 | 3.111 | 3.315 |
| 450 | 0.675 | 1.283 | 1.648 | 1.965 | 2.335 | 2.587 | 2.821 | 3.108 | 3.312 |
| 500 | 0.675 | 1.283 | 1.648 | 1.965 | 2.334 | 2.586 | 2.820 | 3.107 | 3.310 |
| 600 | 0.675 | 1.283 | 1.647 | 1.964 | 2.333 | 2.584 | 2.817 | 3.104 | 3.307 |
| 700 | 0.675 | 1.283 | 1.647 | 1.963 | 2.332 | 2.583 | 2.816 | 3.102 | 3.304 |
| 800 | 0.675 | 1.283 | 1.647 | 1.963 | 2.331 | 2.582 | 2.815 | 3.100 | 3.303 |
| 900 | 0.675 | 1.282 | 1.647 | 1.963 | 2.330 | 2.581 | 2.814 | 3.099 | 3.301 |
| 1000 | 0.675 | 1.282 | 1.646 | 1.962 | 2.330 | 2.581 | 2.813 | 3.098 | 3.300 |
| ∞ | 0.6745 | 1.2816 | 1.6449 | 1.9600 | 2.3263 | 2.5758 | 2.8070 | 3.0902 | 3.2905 |

These tables were taken from Jerrold H. Zar, BIOSTATISTICAL ANALYSIS, 2e, Copyright 1984, pp.484-484. Reprinted by permission of Prentice-Hall, Englewood Cliffs, NJ.

$\mu$com = 41.236 mm (estimates of population mean of COM)

sp2 = 75.86

$\delta$ = 42.950 - 41.236 = 1.714

We wish to test at the 0.05 level of significance, with a 90% chance of detecting a true difference between the population means as small as 1.714.

Let us suppose that sample size of 100 will be required. Then v = 2(n-1) = 2(100-1) = 198, $t_{0.05(2),198}$ = 1.972, $\beta$ = 1-power = 1 - 0.90 = 0.10, $t_{0.10(1),198}$ = 1.286. Values for t$\alpha$ and t$\beta$ are obtained from the critical values for the t distribution.

$$n \geq 2sp^2/\delta^2(t_\alpha+t_\beta)^2$$

$$n \geq 2(75.86)/(1.714)^2(1.972 + 1.286)^2$$

$$n \geq 51.641(10.614) = 548$$

Let us now use n= 548 to determine v=2(n-1) = 1094 $t_{0.05(2)}$1094 = 1.962, $t_{0.10(1),1094}$ = 1.282.

$$n \geq 2(75.86)/(1.714)^2(1.962+1.282)^2 = 547$$

Let us now use n = 547 to determine v=2(n-1) = 1092 $t_{0.05(2)}$1092 = 1.962, $t_{0.10(1)}$1092 = 1.282.

$$n \geq 2(75.86)/(1.714)^2(1.962+1.282)^2 = 547.$$

Therefore, we conclude that each of the two fish strains should have 547 replicate cages if we want a power of 0.90 to detect a difference of 1.714 mm between the two population means.

Cohen (1988) estimates sample size by calculating effect size (d) expressed in standard deviation units (d) = $|\mu 1 - \mu 2|\sigma$ and uses his sample size table (Table 2.1) to locate

the required sample size for a specified $\alpha$ and power. I will illustrate this calculation using the above example.

$\mu$nifi = 42.950 mm (mean estimate of NIFI)

$\mu$com = 41.236 mm (mean estimate of COM)

$\sigma$ = 8.71 (estimate of common standard deviation)

$d = |42.950 - 41.236|/8.71 = 0.19 \approx 0.20$ mm

Since we are not predicting the direction of the tests, this example is a two-tailed t test. At $\alpha = 0.05$ and a specified power of 0.90, Table 2.1 shows that 526 replicate cages are required of each strain. This is a little less than the 547 replicate cages obtained by using Zar's formula. The difference is presumably from rounding d= 0.19 to 0.20. A less stringent $\alpha = 0.10$, requires 429 replicate cages of each strain.

If the desired power is only 0.70, at $\alpha = 0.05$, the required replicate cages is only 310, at $\alpha = 0.10$, replicate cages is 236.

Example 2. Power of a two-sample t test after it has been performed. I will illustrate post-hoc power calculation using data from the preliminary strain testing experiments I conducted where two tilapia strains were graded and grown separately but with red tilapia as an internal control in each replicate. The power of a performed t test is estimated by calculating $\phi$.

$\phi = \sqrt{nd^2 - 2sp^2/4sp^2}$ where $d^2 = (\bar{y}_1 - \bar{y}_2)^2$

$\mu$nifi = 44.57 mm (mean estimates of NIFI strain)

$\mu$com = 39.09 mm (mean estimates of COM strain)

d = 44.57 - 39.09 = 5.48 mm

$d^2$ = 30.03

$sp^2$ = 8.122

n = 4 replicates

$\phi = \sqrt{4(30.03)-2(8.122)/4(8.122)} = 1.79$ and by consulting Fig. 2.1 for v = 6, the estimated power is estimated to be 0.45. I used $\phi$ to calculate post-hoc power analysis to show another method of calculation depending on what source is available.

Power of performed two-sample t test can also be estimated using Cohen's Power Table (Table 2.4). We only need to calculate the effect size (d) expressed in terms of standard deviation units, specify $\alpha$ and consult the Power Tables.

Example 3. Data is from Bolivar et al. In press.

Eight full-sib families were studied to determine the response to selection in NIle tilapia (Oreochromis niloticus) that had been subjected to 8 generations of within-family selection for growth. The response was measured by comparing the growth performance of the 8th selected generation and a 2nd generation random-bred control line grown together in one tank. AT 20 weeks, all the selected lines except for one family were

## Table 2.4. Power Table

Power of t test of $m_1 = m_2$ at $a_2 = .05$

| | | d | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | $d_c$ | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| 8 | 1.07 | 05 | 07 | 09 | 11 | 15 | 20 | 25 | 31 | 46 | 60 | 73 |
| 9 | 1.00 | 05 | 07 | 09 | 12 | 16 | 22 | 28 | 35 | 51 | 65 | 79 |
| 10 | .94 | 06 | 07 | 10 | 13 | 18 | 24 | 31 | 39 | 55 | 71 | 84 |
| 11 | .89 | 06 | 07 | 10 | 14 | 20 | 26 | 34 | 43 | 61 | 75 | 87 |
| 12 | .85 | 06 | 08 | 11 | 15 | 21 | 28 | 37 | 46 | 65 | 80 | 90 |
| 13 | .81 | 06 | 08 | 11 | 16 | 23 | 31 | 40 | 50 | 69 | 83 | 93 |
| 14 | .78 | 06 | 08 | 12 | 17 | 25 | 33 | 43 | 53 | 72 | 86 | 94 |
| 15 | .75 | 06 | 08 | 12 | 18 | 26 | 35 | 45 | 56 | 75 | 88 | 96 |
| 16 | .72 | 06 | 08 | 13 | 19 | 28 | 37 | 48 | 59 | 78 | 90 | 97 |
| 17 | .70 | 06 | 09 | 13 | 20 | 29 | 39 | 51 | 62 | 80 | 92 | 98 |
| 18 | .68 | 06 | 09 | 14 | 21 | 31 | 41 | 53 | 64 | 83 | 94 | 98 |
| 19 | .66 | 06 | 09 | 15 | 22 | 32 | 43 | 55 | 67 | 85 | 95 | 99 |
| 20 | .64 | 06 | 09 | 15 | 23 | 33 | 45 | 58 | 69 | 87 | 96 | 99 |
| 21 | .62 | 06 | 10 | 16 | 24 | 35 | 47 | 60 | 71 | 88 | 97 | 99 |
| 22 | .61 | 06 | 10 | 16 | 25 | 36 | 49 | 62 | 73 | 90 | 97 | 99 |
| 23 | .59 | 06 | 10 | 17 | 26 | 38 | 51 | 64 | 75 | 91 | 98 | * |
| 24 | .58 | 06 | 10 | 17 | 27 | 39 | 53 | 66 | 77 | 92 | 98 | |
| 25 | .57 | 06 | 11 | 18 | 28 | 41 | 55 | 68 | 79 | 93 | 99 | |
| 26 | .56 | 06 | 11 | 19 | 29 | 42 | 56 | 69 | 80 | 94 | 99 | |
| 27 | .55 | 06 | 11 | 19 | 30 | 43 | 58 | 71 | 82 | 95 | 99 | |
| 28 | .54 | 07 | 11 | 20 | 31 | 45 | 59 | 73 | 83 | 96 | 99 | |
| 29 | .53 | 07 | 12 | 20 | 32 | 46 | 61 | 74 | 85 | 96 | 99 | |
| 30 | .52 | 07 | 12 | 21 | 33 | 47 | 63 | 76 | 86 | 97 | * | |
| 31 | .51 | 07 | 12 | 21 | 34 | 49 | 64 | 77 | 87 | 97 | | |
| 32 | .50 | 07 | 12 | 22 | 35 | 50 | 65 | 78 | 88 | 98 | | |
| 33 | .49 | 07 | 13 | 22 | 36 | 51 | 67 | 80 | 89 | 98 | | |
| 34 | .48 | 07 | 13 | 23 | 37 | 53 | 68 | 81 | 90 | 98 | | |
| 35 | .48 | 07 | 13 | 23 | 38 | 54 | 70 | 82 | 91 | 98 | | |
| 36 | .47 | 07 | 13 | 24 | 39 | 55 | 71 | 83 | 92 | 99 | | |
| 37 | .46 | 07 | 14 | 25 | 39 | 56 | 72 | 84 | 92 | 99 | | |
| 38 | .46 | | 14 | 25 | 40 | 57 | 73 | 85 | 93 | 99 | | |
| 39 | .45 | | 14 | 26 | 41 | 58 | 74 | 86 | 94 | 99 | | |
| 40 | .45 | 07 | 14 | 26 | 42 | 60 | 75 | 87 | 94 | 99 | | |
| 42 | .43 | 07 | 15 | 27 | 44 | 62 | 77 | 89 | 95 | 99 | | |
| 44 | .42 | 07 | 15 | 28 | 46 | 64 | 79 | 90 | 96 | * | | |
| 46 | .41 | 08 | 16 | 30 | 48 | 66 | 81 | 91 | 97 | | | |
| 48 | .41 | 08 | 16 | 31 | 49 | 68 | 83 | 92 | 97 | | | |

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significantly heavier than the control line.

What was the power of the hypothesis tests in the selected family that was not significantly different from the control line?

n of the selected family = 30

n of the control family = 19

Since $n_1$ and $n_2$ are not equal, we must calculate the harmonic mean of the two sample sizes (Cohen 1988).

$n = 2n_1n_2/n_1 + n_2 = 2(30)(190/30 + 19 = 23$

$\mu sel$ = 27.7 gm (mean estimate of selected line)

$\mu con$ = 25.5 gm (mean estimate of control line)

$s^2sel$= 38.58 (variance estimate of selected line)

$s^2con$= 27.28 (varianc estimate of control line)

sp2 = pooled variance estimate = $v_1s^2_1+v_2s^2_2/v_1+v_2$

where $v_1$ and $v_2$ are degrees of freedom of $n_1$ and $n_2$.

$sp^2 = 29(38.58) + 18(27.28)/29 + 18 = 34.25$

$d = \mu sel - \mu con = 27.7 - 25.5 = 2.2$

Since d = 2.2 and $s^2p = 34.25$,

$$\phi = \sqrt{nd^2 - 2sp^2/4sp^2} = \sqrt{23(2.2)^2 - 2(34.25)/4(34.25}$$

$= 0.559$

and, by consulting Fig. 1 for v=47, the estimated power is < 0.10. It means that the chance of committing a Type II error is more than 90%. There was a very high error of not being able to detect a true difference between this particular selected family and control line. If the number of samples have been increased to around 150 fish of each group, the chance of detecting the 2.2 gm

difference would have been around 80%.

Example 4. I will use data from the study of Jarimopas (1990) to illustrate an example of power calculation in a size-selected mass selection for growth in the Thai red tilapia. In the hypothesis test, the Ho was rejected and it was concluded that the growth performance of the selected line of red tilapia was significantly better than the control line after 5 generations of size-selection for growth.

$\mu$sel = 10.90 cm (mean estimate of selected line)

$\mu$con = 10.04 cm (mean estimate of control line)

$s^2$sel = 1.44 (variance estimate of selected line)

$s^2$con = 1.39 (variance estimate of control line)

nsel = ncon = 400

$s^2p = 399(1.39) + 399(1.44)/399+399 = 1.415$

d = 10.90 - 10.04 = 0.86 cm

$\phi = \sqrt{400(0.86)^2 - 2(1.415)/4(1.418)} = 7.19$

Since the calculated $\phi$ was 7.19 with v = 399, the estimated power of the test is > 0.99. The conclusion of Jarimopas (1990) that there was response to size-selection for growth after 5 generations was based on a very high power of her test.

Example 5. Estimate of power where the Ho was not rejected. Huang and Liao (1990) did not find any significant response to bidirectional mass selection for

growth in the Nile tilapia (Oreochromis niloticus).

$\mu$high = 69.09 gm (mean estimate for body weight)

$\mu$low = 65.48 gm (mean estimate for body weight)

$s^2$high = 940 (variance estimate)

$s^2$low = 1410 (variance estimate)

nhigh = 209

nlow = 236

n = 2(209)(236)/209+236 = 222

$sp^2$ = 208(940) + 235(1410)/208 +235 = 224

d = 69.09 - 65.48 = 3.61

$\phi = \sqrt{222(3.61)^2 - 2(224)/4(224)} = 1.652$

This value of $\phi$ with v = 443 and $\alpha$ = 0.05 is associated with an estimated power of 0.60. The authors did not find any significant difference between body weights of the high and low lines of Nile tilapia using mass selection. The power of the test to detect a response to selection if there was indeed a response was only about 0.60. If the power had been 0.90 it would have been more valid for the authors to conclude that there was no response to mass selection in tilapia. As the analysis showed, the chance of not detecting (Type II error) a significant difference between the high and low lines of tilapia was 40%.

Example 6. Data is from Basiao and Doyle (1990a)

ANOVA of mean growth of 10 full-sib families from each of three strains in the presence of an internal reference fish.

| Source | DF | MS | F-ratio |
|---|---|---|---|
| Strain | 2 | 18.457 | 3.871 |
| Error | 27 | 4.768 | |

The conclusion from this study was that three strains of Nile tilapia in the presence of an internal reference fish in each replicate grew at different rates in a crowded environment.

Data from the ANOVA table can be used to calculate the power of this performed ANOVA.

k = 3 strains

groups MS = 18.457

$s^2 = 4.768$

$$\phi = \sqrt{(k-1)(\text{groups MS} - s^2)/ks^2}$$

$$= \sqrt{(3-1)(18.457 - 4.768)/3(4.768)} = 1.38$$

This value of $\phi$ with $v_1 = 2$ and $v_2 = 27$ at $\alpha = 0.05$, has an estimated power of only 0.48. The comparison experiment has shown that the Israel strain was the fastest growing, followed by the NIFI strain and the CLSU strain. However, the authors did not conclude that the

Israel strain was superior to the other two strains.

Example 7. Sample size calculation in ANOVA

Suppose we want to repeat the above experiment (Example 6) and wish to test at the 0.05 level of significance, with an 80% chance of detecting a true difference as small as 2 mm between the two most different population means. The estimated within population variability ($s^2$) based on the previous study was 4.768.

Let us guess that 15 replicate cages will be required. Then k=3, $v_1$=k-1= 2, $v_2$=3(n-1) = 42.

$$\phi = \sqrt{15(2)^2/2(3)(4.768)} = 1.40$$

Fig. 1 gives us an estimated power of about 0.60. Since we want a power of 0.80, then we can try to increase our number of cages to 20. Then $v_2$ = 3(n-1)=57.

$$\phi = \sqrt{20(2)^2/2(3)(4.768)} = 1.7$$

With $v_2$= 57 and $\alpha$ = 0.05, the estimated power is about 0.80. The test will have a 20% chance of not detecting a true difference of 2 mm between the two most different means. There would be more confidence to conclude that one strain is superior to the other two strains.

Power and sample size in nested analysis of variance

An ANOVA experimental design that is referred to as crossed is one where all possible combinations of levels in the factors exist. In aquaculture research, some

experimental designs, however, may have some different levels of one factor occurring in combination with the levels of one or more other factors, and, other different levels occur in combinations with others. An important consideration is that, the subordinate level of classification must always be randomly chosen (Sokal and Rolf 1969). The subordinate level is always a Model II (random effects). The higher level of classification may be Model I (fixed effects) or Model II (random effects). A nested anova is a mixed model when the higher level of classification is Model I. It is a pure model when the higher level of classification is Model II.

In the present study, the experimental design when two tilapia strains were graded and grown separately in cages/tanks was a simple example of a nested ANOVA. The graded fish were nested within cages/tanks and the cages/tanks were likewise nested within strains. It was a mixed model, since, the strain effect was a fixed effect and the cage/tank effect was a random effect. In testing the variance-ratio for strain effect, the subgroups MS (cage{strain} nesting term ) was used as denominator instead of the error MS.

The power of a nested ANOVA is given by the formula $\phi = \sqrt{(k' - 1)(\text{factor MS})/k's^2}$ (Zar 1984) where

$k'$= the number of levels of the factor

$v_1 = k' - 1$

$s^2$= the appropriate mean square that is used as the denominator of the F-ratio used to test that factor in the ANOVA, and $v_2$ is the degree of freedom associated with $s^2$.

The power of a performed nested ANOVA is calculated from the formula

$\phi = \sqrt{(k' - 1)(\text{factor MS} - s2)/k's^2}$ and the minimum number of data per level that would be needed to achieve a specified power is estimated from

$\phi = \sqrt{n'\delta^2/2k's^2}$

Example 8. Power of a performed nested ANOVA when two strains were graded and grown separately in cages.

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 108.954 | 1.437 |
| Cage{Strain} | 6 | 75.802 | 3.437 |
| Error | 144 | 22.749 | |

k' = 2 (factor strain)

$v_1 = 2 - 1 = 1$

$s^2$ = 75.802 since the factor strain was tested using the nesting term (subgroups MS) in the F-ratio.

$$\phi = \sqrt{(k' - 1)(\text{factor MS} - s^2)/k's^2}$$

$$= \sqrt{(2-1)(108.954 - 75.802)/2(75.802)} = 0.47$$

Since Fig. 2.1 gives only a value of $\phi$ as low as 1, we can assume that the estimated power of the test with $\phi$

= 0.47 was < 0.10. There was more than 90% chance of not detecting a true difference between the two strains.

Suppose we wish to repeat the experiment with a power of 0.90 to detect a small difference of 3 mm between the two strains at $\alpha$ 0.05. We then use the formula

$$\phi = \sqrt{n'\delta 2/2k's2}$$

Let us guess that 50 cages of each strain will be needed. $\phi = \sqrt{50(3)^2/2(2)(75.802)} = 1.2$

Fig. 2.1 gives a power estimate of <0.10. We need to increase our replicate cages since we want a very high power of 0.90. We might try 100 cages which give us a power of only 0.65. If we consider the 3 mm difference to be economically important, we might decide to increase the number of cages to 180 which give an estimated power of 0.90. The probability of a Type II error will only be 0.10. That is for every 10 experiments, there is only 1 chance that a true difference of 3 mm will not be detected.

The cost of not being able to detect a true difference of 3 mm if it is considered economically important may be more expensive than the cost of running the strain testing experiment using 180 cages.

Power calculation

Power calculation is as straightforward as how a significance level ($\alpha$) is derived, but researchers are trained to deal with the traditional significance level ($\alpha$

= 0.05 and 0.01) in statistical testing, not with power. In aquaculture strain testing, researchers should be encouraged to incorporate power analysis in the design of experiments. This is because of the high risk of not being able to detect (Type II error) an economically important difference between two strains of fish. Geneticists should be encouraged to do post hoc power analysis before making conclusions about the lack of response to selection, or low heritability of a trait. The statistical test may just have low power to detect a small but economically important response to selection.

In aquaculture, the effect size can be set a priori on economic grounds and $\alpha$ is also under the control of the researcher which can be set at 0.10 rather than the traditional 0.05 or 0.01 level. The sample size, necessary to generate a high probability (high power) of detecting the effect size set a priori, can be derived from Sample Size Tables or computed by iteration. A test with a power of 0.80 has an 80% chance of detecting a true difference if it exists. The probability of a Type II error is 0.20 when the power is 0.80. When the Type II error is 0.20, it means that there is only a 20% chance of not detecting the difference if it exists. A test with a power of 0.20 has a 20% chance of detecting a true difference and 80& chance of not detecting a true difference. The higher the power of a test, the higher

is the chance of detecting a difference if it exists. In strain testing it is very essential to have a high chance (probability) of detecting an econmically important difference say in the growth of two strains of fish.

# CHAPTER 3

## ESTIMATES OF STATISTICAL POWER OF DIFFERENT STRAIN COMPARISON PROCEDURES


### ABSTRACT

Recent interest in the genetic improvement of economically important traits like growth rate has created a need for more information on many aspects of genetic selection of domesticated aquaculture species. My main objective is to obtain rough estimates of mean and error variances from a small sample size under various strain comparison procedures, to design optimal experimental strain testing procedures that include statistical power analysis. A small but economically important difference that was not detected in the growth of two fish strains can mean millions of dollars of opportunity loss for the aquaculture industry.

Two Nile tilapia strains were size-matched (graded) and grown separately, communally, and grown separately with an internal reference fish (red tilapia) in cages and tanks. Likewise, fish of mixed-sizes (ungraded) were simultaneously compared separately, communally, and

separately but with an internal reference fish (red tilapia ) in cages and tanks.

Results suggest that initial size differences can be magnified under competition when strains are of mixed sizes and grown together in cages. The COM strain showed a significantly higher (P=0.034) absolute growth than the NIFI but only when the initial length was used as a covariate. The COM strain was initially bigger (37.80 mm) than the NIFI strain (36.19 mm). Distribution of the COM strain was negatively skewed (-0.667), while the NIFI strain was positively skewed (0.377). The absolute and corrected growth rates of the COM strain was significantly higher (P=0.015 and P=0.004, respectively) than the NIFI strain when red tilapia was used as an internal control in each cage and all three strains were of mixed-sizes. The growth variance observed between the two strains is due mainly to environmental variation. Grading fish appears to eliminate or minimize the initial size advantage of some individual fish.

# INTRODUCTION

In strain testing and other aquaculture situations, Type I error is not economically important, since if two strains of fish are really the same and they were just mistakenly taken to be different, the farmer will neither lose nor benefit with whatever choice of strain he makes. The risks of a Type II error (failing to reject a false null hypothesis) usually has far more economic consequence than a Type I error (rejecting a true null hypothesis). Failing to detect a small but economically important difference in growth rates between two fish strains (Type II error) can mean an opportunity loss of millions of dollars. Since power is the probability of not making a Type II error, high power is desirable in strain testing experiments.

The objective of my experiment is not to establish whether or not differences exist for the particular strains that I have used, but to use the preliminary information to design optimal experimental procedures for general use. I use the estimates of the error variances based on small sample sizes under various comparison procedures in proposing an experimental design for strain testing programmes that include statistical power analysis. The question of power must be considered in planning a strain testing experiment and not after the the study is done and the results are known.

# MATERIALS AND METHODS

## Test populations

Two strains of Oreochromis niloticus were used as test fish. The Chitralada or NIFI strain is the second generation offspring of a stock that was obtained from the National Inland Fisheries Institute (NIFI) in 1987. This strain is referred to as NIFI throughout this thesis. The second strain is also the second generation offspring of an O. niloticus stock that was obtained from a commercial hatchery in Bay, Laguna, Philippines. This strain is referred to as COM. A red tilapia strain is used as an internal reference population in some comparison procedures. This is a third generation offspring of a population that was obtained from Bioresearch, a commercial farm in the Philippines. It is a three-way cross of O. niloticus, O. mossambicus and O. hornorum (Ang, pers. comm.).

## Spawning protocol

A large population of each strain (150 females and 50 males) was mass spawned over the course of three weeks in each of three 10 x 5 x 1-m outdoor tanks. Breeders were fed ad libitum with commercial diet (42% protein) three times daily. The breeders were transferred to other tanks and the fry were left in each spawning tank for 4 weeks of initial growth period. Fry were fed ad libitum with

commercial diet (26%) and with the natural food available in each tank.

## Experimental design of comparison procedures

### Size-grading

At the end of the initial growth period, each population was divided into two groups. One group was size graded based on individual standard length measurements to obtain a sample of animals all the same size. This size-grading technique, or size matching, minimizes the non-genetic environmental variance in growth caused by variable egg quality and by asynchronous spawning (Doyle and Talbot 1986b). However, grading may cause difficulties such as genotypes with exceptionally large or small growth rates being underrepresented, thus causing a reduction in variance, and, possibly, a bias. Uniformly-sized test fish close to modal size, which gave the greatest number of individuals of similar size, were chosen as the initial length.

The other group was left ungraded to obtain a sample of animals of mixed sizes and mixed ages (within the 3-week spawning period).

### Location

Strain testing was done simultaneously in floating hapa cages set in Laguna Lake, Philippines and in indoor polyethylene tanks. My objective was to see how changing

the type of environment might influence the power of the tests.

To obtain estimates of the NIFI and COM population means and their common standard deviation ($\sigma$), three strain testing procedures were evaluated using small sample sizes in four different aquaculture situations.

Comparison of two strains grown separtely

One practical question that I wanted to address is whether mixed genotypes of fish should be tested together or separately. In actual farm situations, strains are usually grown separately. A major disadvantage of separate testing, however, is the need for a large number of containers to overcome the confounding of genetic variation with environmental differences between containers.

Size-graded strains were grown separately in eight 1 x 1 x 1 m hapa cages set in Laguna Lake and, simultaneously, in eight 250 litre indoor aerated polyethylene tanks of standing water. Each unit was stocked with 20 fingerlings.

Likewise, ungraded test strains were grown separately in eight 1 x 1 x 1 m hapa cages set in Laguna Lake and in eight 250 litre indoor aerated polyethylene tanks of standing water. Each experimental unit was stocked with 20 fingerlings.

Comparison of two strains grown together

Communal testing reduces the number of replicates required for a test to achieve a given power. Pairing in an experimental design can serve to reduce bias, increase precision or both (Samuels 1989). An effectively paired experiment is more efficient than an unpaired experiment with the same number of replicates.

Ten individuals of each size-graded test strain were paired by standard length and grown together in four 1 x 1 x 1 m hapa cages set in Laguna Lake and in four 250 litre indoor aerated polyethylene tanks of standing water. The right and left pectoral fins were fin-clipped to distinguish the NIFI from the COM strain.

Likewise, fin-clipped ungraded test strains were grown together in four 1 x 1 x 1 m hapa cages set in Laguna Lake and in four 250 litre indoor aerated polyethylene tanks of standing water. Each unit was stocked with 10 fingerlings of each strain.

Use of an internal reference fish

A possible procedure for reducing environmental variation that occurs between separate rearing units is to use an internal reference population (a third strain) to account for environmental differences (Kincaid 1979; Klupp 1979; Moav and Hulata 1976; Basiao and Doyle 1990; Doyle et al 1990).

A red tilapia population was used as an internal statistical control to minimize replicate variance (Basiao and Doyle 1990) and as a measure of environmental quality within each container. Each size-graded test strain was matched with a reference fish of exactly the same size and grown together in eight 1 x 1 x 1 m hapa cages set in Laguna Lake, and in eight 250 litre indoor aerated polyethylene tanks of standing water. Each unit was stocked with 10 fingerlings of the test strain and 10 red tilapia fingerlings.

Likewise each ungraded test strain was grown with an internal reference fish (red tilapia) in eight 1 x 1 x 1 m hapa cages set in Laguna Lake and in eight 250 litre indoor aerated polyethylene tanks of standing water. Each experimental unit was stocked with 10 fingerlings of the test strain and 10 red tilapia fingerlings.

In all comparison procedures, fish that were grown in the tanks were fed ad libitum three times a day with commercial food pellet (26% protein). Feces and debris were removed and one third of the water was changed daily in the tanks. Fish in hapa cages were not fed commercial food.

## Measurements

The major experiment was conducted over a period of 120 days. I measured the initial and final standard lengths and weights of all individuals. Standard length

was used as the basis for the measure of growth because of the higher measurement variation associated with sampling wet weights of small fish than with sampling lengths. Three growth parameters were used in the analysis of data:

(1) Absolute growth (DL) is defined as the difference between the mean final length and the mean initial length of each group of fish after 120 days.

DL = Len120 - Len0 where, Len120 = mean final standard length and Len0 = mean initial standard length.

(2) Corrected growth (CORDL) is the correction made by transforming the observed gain in length (DL) of each strain (Moav et al 1975):

$$CORDL = DL - b(Len0 - meanlen)$$

where

CORDL = corrected gain in length; DL = observed gain in length = final length - inital length; b = coefficient of linear regression of DL on initial length; Len0 = initial length; meanlen = mean initial length of the two strains.

(3) Specific growth rates (SG) = instantaneous or incremental growth rate were calcaulated from the formula

$$SG = \log_e Len120 - \log_e Len0$$ where

Len120 = mean log final standard length

Len0 = mean log initial standard length

## Statistical Analyses

### Strains grown separately

Analysis of variance - or ANOVA - is the most commonly used tool for determining whether genetic strains of fish are different from each other. I used the one-way analysis of variance (ANOVA) to test hypotheses about the growth differences between the NIFI and COM strains when they were graded and grown separately. The model I used was:

(1) $DLij = \mu + Si + Eij$

$SGij = \mu + Si + Eij$

where DLij represents the mean absolute increase in growth of fish of the ith test strain, in the jth replicate; SG is the specific growth; $\mu$ is the grand mean of the test fish; Si is the ith strain effect and Eij is the random error term.

I also performed a nested analysis of variance since it was possible to estimate individual fish growth within each replicate because all the fish started with the same initial size. Nested ANOVA was appropriate to use when strains were grown separately because the replicates (tanks or cages) were nested within each strain. The model used was:

(2) DLijk = $\mu$ + Si + cage/tank{Si} + error

where

DLijk is the kth fish in the jth cage or tank nested within the ith strain and the cage/tankj{i} is a random factor.

In a nested ANOVA that involves combinations of fixed and random factors, the expected mean squares for certain effects are different from those for fully fixed or random designs. For this particular strain testing, appropriate error for testing strain differences is the "nesting term" (cage/tank{strain}, rather than the expected mean square error.

For fish of mixed-sizes (ungraded) and mixed-ages, two other models were used:

(3) CORDLij = $\mu$ + Si + Eij

where CORDL is the corrected DL

(4) DLij = $\mu$ + Si + ßLij + SLij + Eij (initial length as covariate), where DLij represents the mean growth of fish of the ith test strain, in the jth replicate; $\mu$ is the grand mean of the test fish; Si is the ith strain effect, Lij is the mean initial length of fish of the ith test strain, from the jth replicate; SLij is the interaction term; and Eij is the random error.

When the interaction term was found nonsignificant, the model followed was:

(5) Dlij = $\mu$ + Si + ßLij + Eij

Strains reared together

One way to compare two strains when they are reared together is by using a factorial model. In this strain testing procedure where the two strains were grown together in each replicate cage/tank, I used a two-way model. Each strain is exposed to the same replicate cage/tank (factorial design). I used two statistical models for graded (size-matched) fish :

(6) $DLij = \mu + Si + Rj + Eij$

$SGij = \mu + Si + Rj + Eij$

where DLij represents the mean absolute growth of fish of the ith test strain, from the jth replicate cage/tank; $\mu$ is the grand mean of the test fish; SG is the specific growth rate; Si is the ith strain effect, Rj is the replicate cage/tank effect and the Eij is the random error.

(7) $DLijk = \mu + Si + Rj + SRij + Eijk$

where DLijk is the growth of the kth individual fish of the ith strain in the jth replicate cage/tank; $\mu$ is the grand mean; Si is the fixed strain effect, Rj is the replicate cage/tank random effect and Eijk is the random error. For testing strain effect, I used the expected mean square of the interaction term (SRij) in the denominator

of the F-ratio instead of the expected mean square error, because the replicate cage/tank was a random factor.

For ungraded fish initial length was used as a covariate and two statistical models were used:

(8) $DLij = \mu + Si + \beta Lij + Rj + (SR)ij + Eij$

where DLij represents the mean growth of fish of the ith test strain, from the jth replicate; $\mu$ is the grand mean of the test fish; Si is the ith strain effect, Lij is the mean initial length of fish of the ith test strain, from the jth replicate; Rj is the replicate cage/tank effect; SLij is the interaction term and Eij is the random error. When the interaction term was found nonsignificant, the model followed was:

(9) $DLij = \mu + Si + \beta Lij + Rj + Eij$

The corrected growth (CORDL) was also used in the analysis of ungraded fish

(10) $CORDLij = \mu + Si + Eij$

Internal reference fish (strains reared separately)

The use of a concomitant variable can add greatly to the power of a data analysis, even if the relationship between the covariate and the dependent variable is not of primary interest. Two models were used to test the fish growth data using growth of the reference population as

the covariate to control for environmental and population density variation among replicates.

(11) $\Delta Lij = \mu + Si + \beta Rij + (SR)ij + Eij$

where Rij is the mean absolute growth of the covariate (red tilapia). When the interaction term was nonsignificant, the conventional analysis of covariance (ANCOVA) model used was:

(12) $DLij = \mu + Si + \beta Rij + Eij$

All statistical analyses were performed with the SYSTAT statistical package (Wilkinson 1988).

## RESULTS

My objective in doing pilot strain testing experiments, was not to establish strain differences between NIFI and COM, but to use the information to illustrate the concept of power analysis in aquaculture situations and to obtain estimates of population means and common variances. I will, nevertheless, present the results of the different statistical analyses for each particular strain testing procedure that was evaluated.

### Descriptive Statistics

Tables 3.1 to 3.4 show the mean values of the absolute growth, absolute growth corrected for initial

Table 3.1. Mean DL (absolute growth), CORDL (corrected growth for initial length), SG (specific growth) of graded/ungraded strains grown together and separately in cages, N= number of replicates

Graded in cages

| | Strains | N | Initial length (mm) | Final lenth (mm) | DL | SG |
|---|---|---|---|---|---|---|
| Separate | NIFI | 4 | 32.063 | 75.013 | 42.950 | 0.708 |
| SD | | | 0.125 | 2.134 | 2.130 | 0.024 |
| Skewness | | | 1.155 | 0.288 | 0.391 | 0.492 |
| | COM | 4 | 32.063 | 73.299 | 41.236 | 0.689 |
| SD | | | 0.125 | 1.833 | 1.890 | 0.022 |
| Skewness | | | 1.155 | 0.027 | 0.065 | 0.080 |
| Together | NIFI | 4 | 35.400 | 73.797 | 38.397 | 0.612 |
| SD | | | 0.000 | 0.944 | 0.944 | 0.011 |
| Skewness | | | 0.000 | 0.887 | 0.887 | 0.882 |
| | COM | 4 | 35.400 | 73.528 | 38.128 | 0.609 |
| SD | | | 0.000 | 2.378 | 2.378 | 0.027 |
| Skewness | | | 0.000 | 0.480 | 0.480 | 0.467 |

Ungraded in cages

| | Strains | N | Initial length | Final length | DL | CORDL | SG |
|---|---|---|---|---|---|---|---|
| Separate | NIFI | 4 | 36.466 | 77.134 | 40.668 | 40.898 | 0.624 |
| SD | | | 0.904 | 1.578 | 0.919 | 0.957 | 0.011 |
| Skewness | | | 0.355 | -0.283 | -0.927 | -0.089 | -1.084 |
| | COM | 4 | 37.379 | 78.631 | 41.252 | 41.564 | 0.619 |
| SD | | | 0.939 | 3.081 | 3.134 | 2.916 | 0.038 |
| Skewness | | | 0.975 | -0.986 | -0.690 | -0.828 | -0.233 |
| Together | NIFI | 4 | 36.187 | 75.238 | 38.452 | 37.563 | 0.596 |
| SD | | | 1.543 | 2.614 | 3.580 | 2.071 | 0.054 |
| Skewness | | | 0.377 | 0.995 | 0.212 | 1.150 | -0.260 |
| | COM | 4 | 37.799 | 78.463 | 40.664 | 37.563 | 0.608 |
| SD | | | 0.939 | 3.145 | 2.759 | 2.071 | 0.028 |
| Skewness | | | -0.667 | 0.861 | 0.354 | -0.307 | -0.243 |

Table 3.2. Mean DL (absolute growth), SG (specific growth), CORDL (corrected for initial length) of graded/ungraded strains grown together and separately in tanks

Graded in tanks

| | Strains | N | Initial length (mm) | Final lenth (mm) | DL | SG |
|---|---|---|---|---|---|---|
| Separate | NIFI | 4 | 32.063 | 83.937 | 51.874 | 0.802 |
| SD | | | 0.125 | 0.774 | 0.846 | 0.010 |
| Skewness | | | 1.155 | 0.263 | 0.205 | -0.002 |
| | COM | 4 | 32.063 | 82.876 | 50.813 | 0.791 |
| SD | | | 0.125 | 2.372 | 2.494 | 0.027 |
| Skewness | | | 1.155 | -0.951 | -0.971 | -1.005 |
| Together | NIFI | 4 | 35.400 | 82.242 | 46.842 | 0.702 |
| SD | | | 0.000 | 3.271 | 3.271 | 0.033 |
| Skewness | | | 0.864 | 0.864 | 0.864 | 0.848 |
| | COM | 4 | 35.400 | 82.851 | 47.451 | 0.708 |
| SD | | | 0.000 | 2.785 | 2.785 | 0.028 |
| Skewness | | | -0.000 | -0.993 | -0.993 | -1.004 |

Ungraded in tanks

| | Strains | N | Initial length | Final length | DL | CORDL | SG |
|---|---|---|---|---|---|---|---|
| Separate | NIFI | 4 | 37.287 | 85.890 | 48.603 | 51.091 | 0.695 |
| SD | | | 0.637 | 3.341 | 3.635 | 3.101 | 0.040 |
| Skewness | | | 0.347 | 0.846 | -1.081 | -0.897 | 1.146 |
| | COM | 4 | 35.647 | 83.804 | 48.157 | 46.628 | 0.712 |
| SD | | | 0.334 | 1.684 | 1.772 | 2.045 | 0.020 |
| Skewness | | | 0.734 | 0.274 | 0.150 | 0.346 | -0.204 |
| Together | NIFI | 4 | 37.960 | 82.894 | 44.935 | 44.907 | 0.650 |
| SD | | | 0.257 | 4.416 | 4.414 | 4.352 | 0.044 |
| Skewness | | | 0.996 | 0.575 | 0.675 | 0.584 | 0.761 |
| | COM | 4 | 38.106 | 86.555 | 48.449 | 48.342 | 0.683 |
| SD | | | 0.996 | 4.898 | 4.486 | 4.334 | 0.041 |
| Skewness | | | -0.516 | 0.493 | 0.064 | -0.425 | -0.686 |

Table 3.3. Mean DL (absolute growth), SG (specific growth) of graded/ungraded strains grown with a red internal control in cages; RN= (red tilapia with NIFI strain), RC= (red tilapia with COM strain), N= (number of replicates)

Graded strains and grown with red internal control in cages

| Strains | N | Initial length (mm) | Final lenth (mm) | DL | SG |
|---|---|---|---|---|---|
| NIFI | 4 | 35.500 | 80.067 | 44.567 | 0.677 |
| SD | | 0.000 | 5.101 | 5.101 | 0.053 |
| Skewness | | 0.000 | -0.143 | -0.143 | -0.159 |
| RN | 4 | 35.500 | 83.710 | 48.209 | 0.713 |
| SD | | 0.000 | 6.095 | 6.095 | 0.060 |
| Skewness | | 0.000 | 0.337 | 0.337 | 0.305 |
| COM | 4 | 35.500 | 74.588 | 39.088 | 0.618 |
| SD | | 0.000 | 1.760 | 1.760 | 0.020 |
| Skewness | | 0.000 | 0.235 | 0.235 | 0.207 |
| RC | 4 | 35.500 | 80.649 | 45.149 | 0.683 |
| SD | | 0.000 | 4.782 | 4.782 | 0.050 |
| Kurtosis | | 0.000 | -1.541 | -1.541 | -1.516 |

Ungraded and grown with red as internal control in cages

| Strains | N | Initial length (mm) | Final length (mm) | DL | CORDL | SG |
|---|---|---|---|---|---|---|
| NIFI | 4 | 36.896 | 78.776 | 41.879 | 40.961 | 0.632 |
| SD | | 0.563 | 1.293 | 1.579 | 2.051 | 0.022 |
| Skewness | | 0.244 | -0.052 | -0.300 | -0.178 | -0.458 |
| RN | 4 | 37.595 | 84.032 | 46.437 | 46.143 | 0.669 |
| SD | | 1.315 | 5.750 | 4.668 | 2.543 | 0.035 |
| Skewness | | 0.447 | 0.803 | 0.697 | -0.052 | 0.273 |
| COM | 4 | 37.919 | 82.924 | 45.005 | 44.958 | 0.652 |
| SD | | 1.307 | 2.301 | 1.809 | 1.827 | 0.023 |
| Skewness | | 0.750 | -0.692 | 0.272 | 0.356 | 0.720 |
| RC | 4 | 37.382 | 83.468 | 46.085 | 46.302 | 0.667 |
| SD | | 1.282 | 7.377 | 6.789 | 6.725 | 0.063 |
| Skewness | | -0.935 | 0.204 | 0.109 | -0.389 | -0.292 |

Table 3.4. Mean DL (absolute growth), SG (specific growth) of graded/ungraded strains grown with red as internal control in tanks. RN= (red tilapia with NIFI strain), RC= (tilapia red with COM strain), N = number of replicates

Graded strains and grown with red internal control in tanks

| Strains | N | Initial length (mm) | Final lenth (mm) | DL | SG |
|---|---|---|---|---|---|
| NIFI | 4 | 35.500 | 86.081 | 50.581 | 0.738 |
| SD | | 0.000 | 5.081 | 5.081 | 0.048 |
| Skewness | | 0.000 | 0.815 | 0.815 | 0.776 |
| RN | 4 | 35.500 | 85.079 | 49.579 | 0.728 |
| SD | | 0.000 | 1.453 | 1.453 | 0.014 |
| Skewness | | 0.000 | 0.349 | 0.349 | 0.342 |
| COM | 4 | 35.500 | 85.511 | 50.011 | 0.735 |
| SD | | 0.000 | 2.067 | 2.067 | 0.020 |
| Skewness | | 0.000 | 1.028 | 1.028 | 1.022 |
| RC | 4 | 35.500 | 83.875 | 48.375 | 0.716 |
| SD | | 0.000 | 1.955 | 1.955 | 0.019 |
| Skewness | | 0.000 | -0.276 | -0.276 | -0.289 |

Ungraded strains and grown with red as internal control in tanks

| Strains | N | Initial length (mm) | Final length (mm) | DL | Cordl | G |
|---|---|---|---|---|---|---|
| NIFI | 4 | 37.632 | 89.934 | 52.302 | 56.814 | 0.725 |
| SD | | 1.129 | 5.590 | 6.544 | 4.746 | 0.073 |
| Skewness | | 0.923 | 0.592 | 0.330 | 0.284 | 0.014 |
| RN | 4 | 37.224 | 85.533 | 48.280 | 48.053 | 0.691 |
| SD | | 0.876 | 1.989 | 7.081 | 6.773 | 0.076 |
| Skewness | | -0.132 | -0.025 | -0.132 | -0.538 | 0.086 |
| COM | 4 | 35.840 | 85.505 | 50.663 | 46.504 | 0.734 |
| SD | | 0.732 | 6.633 | 4.505 | 5.439 | 0.051 |
| Skewness | | -0.736 | -0.395 | 0.058 | -0.047 | 0.185 |
| RC | 4 | 37.325 | 86.578 | 49.252 | 49.525 | 0.701 |
| SD | | 0.651 | 3.024 | 3.647 | 2.850 | 0.043 |
| Skewness | | -0.432 | 0.928 | 0.884 | -0.680 | 0.819 |

length differences, and specific growth together with standard deviations and skewness of distributions of the NIFI and COM strains compared under various testing procedures and environmental situations. The strain that was initially large showed larger final size and absolute growth (Table 3.5). When the strains were ungraded and grown separately in cages, the NIFI showed a slightly higher specific growth rate than the COM strain although the COM strain was initially larger. The difference in the specific growth rates between the two strains was not however statistically significant. On the other hand, when red tilapia was used as an internal control in tanks and the fish were ungraded, the COM strain showed higher specific growth rate than the NIFI strain, although the NIFI strain was initially larger. The difference was, likewise, not statistically significant.

Strains reared separately

In both cages and tanks, no significant strain difference in any of the growth parameters was detected when the two strains were initially matched by size at the start of the experiment (Tables 3.6a to 3.7b). In the strain testing procedures where strains were of mixed sizes, and grown separately in cages, the specific growth did not show any signficant difference between NIFI and COM strains (Tables 3.8a).

Table 3.5. Initial size difference (DI), final length difference (DFL), absolute growth difference (DG) between the COM (C) and NIFI (N) strains in various strain testing procedures. The strain in parenthesis was the bigger strain

Strains grown separately

| | Cages | | | Tanks | | |
|---|---|---|---|---|---|---|
| | DI | DFL | DG | DI | DFL | DG |
| Graded | 0 | 1.714 (N) | 1.714 (N) | 0 | 1.061 (N) | 1.061 (N) |
| Ungraded | 0.913 (C) | 1.497 (C) | 0.584 (C) | 1.640 (N) | 2.086 (N) | 0.446 (N) |

Strains grown together

| | Cages | | | Tanks | | |
|---|---|---|---|---|---|---|
| | DI | DFL | DG | DI | DFL | DG |
| Graded | 0 | 0.269 (N) | 0.259 (N) | 0 | 0.609 (C) | 0.609 (C) |
| Ungraded | 1.612 (C) | 3.225 (C) | 2.212 (C) | 0.146 (C) | 3.661 (C) | 3.512 (C) |

Strains grown separtely but with red as internal control

| | Cages | | | Tanks | | |
|---|---|---|---|---|---|---|
| | DI | DFL | DG | DI | DFL | DG |
| Graded | 0 | 5.479 (N) | 5.479 (N) | 0 | 0.057 (N) | 0.057 (N) |
| Ungraded | 1.023 (C) | 4.148 (C) | 3.126 (C) | 1.792 (N) | 4.429 (N) | 1.639 (N) |

Table 3.6a. ANOVA results when strains are graded and grown separately in cages, N = 4 replicates each strain

mo dl = constant + strain + error

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 5.876 | 1.449 |
| Error | 6 | 4.054 | |

$R^2$= .195

mo sg = constant + strain + error

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 0.0007 | 1.365 |
| Error | 6 | 0.0005 | |

$R^2$= .185

Table 3.6b. Nested ANOVA of graded strains and grown separately in cages, N = 152 individual fish

mo dl = constant + strain + cage{strain} + error

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 108.954 | 1.437* |
| Cage{strain} | 6 | 75.802 | 3.332** |
| Error | 144 | 22.749 | |

* The strain effect was tested by using the expected mean square of the cage{strain} as denominator for the F-ratio.

**P level = 0.004

Table 3.7a. ANOVA when strains were graded and grown separately in tanks, N = 4 replicates each strain

| mo dl = constant + strain + error | | | $R^2$ = .098 |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 2.250 | 0.649 |
| Error | 6 | 3.468 | |

| mo sg = constant + strain + error | | | $R^2$ = .088 |
|---|---|---|---|
| Strain | 1 | 0.0002 | 0.578 |
| Error | 6 | 0.0004 | |

Table 3.7b. Nested ANOVA when strains were graded and grown separately in tanks, N= 157 individual fish

mo dl = constant + strain + tanks{strain} + error

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 44.975 | .651* |
| Tanks{strain} | 6 | 68.816 | .932 |
| Error | 149 | 73.820 | |

$R^2$=.045

*The expected mean square of the tanks{strain} was used as the denominator in the F-test for strain effect.

Table 3.8a. ANOVA results when strains were ungraded and grown separately in cages, N= 4 replicates each strain

| mo dl = constant + strain + error | | | $R^2$=.021 |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 0.683 | 0.128 |
| Error | 6 | 5.633 | |
| **mo cordl = constant + strain + error** | | | **$R^2$ = .030** |
| Strain | 1 | 0.886 | 0.188 |
| Error | 6 | 4.710 | |
| **mo sg = constant + strain + error** | | | **$R^2$ = .010** |
| Strain | 1 | 0.00005 | 0.059 |
| Error | 6 | 0.00076 | |

Table 3.8b. ANOVA results when strains were ungraded and grown separately in tanks, N= 4 replicates each strain

| mo dl = constant + strain + error | | | $R^2$ = .008 |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 0.398 | 0.049 |
| Error | 6 | 8.179 | |
| **mo cordl = constant + strain + error** | | | **$R^2$ = 0.490** |
| Strain | 1 | 39.828 | 5.773* |
| Error | 6 | 6.899 | |
| **mo sg = constant + strain + error** | | | **$R^2$ = .096** |
| Strain | 1 | 0.0006 | 0.634 |
| Error | 6 | 0.0010 | |

* P = .053

The corrected growth of the NIFI strain was slightly higher (P = 0.053) than the COM strain when both strains were of mixed sizes and reared separately in tanks (Table 3.8b). The growth rate distribution of the NIFI strain was negatively skewed while distribution of the COM strain was positively skewed (Table 3.2).

Strains reared togther

No significant strain difference in any of the growth parameters was detected when the strains were size-graded and grown together in both tanks and cages (Tables 3.9a to 3.10b). However, when the two strains were of mixed sizes and reared together in cages, the COM strain showed a significanty higher (P=0.034) absolute growth than the NIFI strain, but only when the initial length was used as a covariate (Table 3.11a). However, neither the corrected absolute growth nor the specific growth were significantly higher for the COM strain. The COM strain was initially bigger than the NIFI strain (Table 3.1).

Use of internal reference fish

No significant strain difference was detected when all three strains were size-graded and tested in tanks and cages (Table 3.12a and 3.12b).

The absolute growth and corrected growth of the COM strain was significantly higher (P=0.015 and P=0.004)

Table 3.9a. Two-way ANOVA when strains were graded and reared together in cages, N= 4 replicates each strain

| mo dl= constant + strain + cage + error | | | $R^2$ = .457 |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 0.145 | 0.041 |
| Cage | 3 | 2.967 | 0.830 |
| Error | 3 | 3.577 | |
| mo sg = constant + strain + cage + error | | | $R^2$ = .461 |
| Strain | 1 | 0.00002 | 0.045 |
| Cage | 3 | 0.00037 | 0.806 |
| Error | 3 | 0.00046 | |

Table 3.9b. Two-way ANOVA when strains were graded and reared together in cages, N= 74 individual fish

| mo dl = constant + strain + cage + strain*cage + error | | | |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 1.725 | 0.065* |
| Cage | 3 | 27.945 | 1.045 |
| Strain*cage | 3 | 32.683 | 1.222 |
| Error | 66 | 26.744 | |
| $R^2$=.093 | | | |

*The strain*cage expected mean square was used to test for strain effect

Table 3.10a. Two-way ANOVA when strains were graded and reared together in tanks, N = 4 replicates

| mo dl= constant + strain + tank + error | | | $R^2$= .622 |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 0.743 | 0.105 |
| Tank | 3 | 11.392 | 1.612 |
| Error | 3 | 7.065 | |
| mo sg = constant + strain + tank + error | | | $R^2$ = .618 |
| Strain | 1 | 0.0001 | 0.0996 |
| Tank | 3 | 0.0001 | 1.5816 |
| Error | 3 | 0.0007 | |

Table 3.10b. Two-way ANOVA when strains were graded and reared together in tanks, N= 72 individual fish

| mo dl = constant + strain + tank + strain*tank + error | | | |
|---|---|---|---|
| Source | DF | MS | F |
| Strain | 1 | 7.737 | 0.111* |
| Tank | 3 | 111.235 | 2.319 |
| Strain*tank | 3 | 69.560 | 1.450 |
| Error | 71 | 47.958 | |

*Effect of strain was tested by using the expected mean square of the strain*tank as denominatopr in the F-test.

Table 3.11a. Two-way ANOVA when strains were ungraded and grown together in cages, N= 4 replicates each strain

mo dl = constant + strain + cage + error

| Source | DF | MS | F |
|---|---|---|---|
| Strain | 1 | 9.793 | 1.773 |
| Cage | 3 | 14.907 | 2.699 |
| Error | 3 | 5.522 | |

$R^2$ = 0.767

mo dl = constant + strain + len0 + cage + error

| | | | |
|---|---|---|---|
| Strain | 1 | 20.495 | 28.259* |
| Len0 | 3 | 15.116 | 20.843** |
| Cage | 3 | 16.227 | 22.374*** |
| Error | 2 | 0.725 | |

$R^2$=0.980

mo cordl = constant + strain + cage + error

| | | | |
|---|---|---|---|
| Strain | 1 | 14.585 | 5.720 |
| Cage | 3 | 10.388 | 4.074 |
| Error | 3 | 2.550 | |

$R^2$=0.857

mo sg = constant + strain + cage + error

| | | | |
|---|---|---|---|
| Strain | 1 | 0.0003 | 0.136 |
| Cage | 3 | 0.0017 | 0.842 |
| Error | 3 | 0.0020 | |

$R^2$=0.470

*P = 0.034; **P = 0.045; ***P = 0.043

Table 3.12a. ANCOVA results when strains are graded/ungraded and grown with an internal reference fish in hapa cages. Red = internal reference fish as covariate, N= 4 replicates each strain

| | | | |
|---|---|---|---|
| | | Graded in cages | |
| mo dl = constant+strain +red | | | $R^2$ = 0.724 |
| Source | DF | MS | F |
| Strain | 1 | 27.847 | 3.419 |
| Red | 1 | 46.649 | 5.728 |
| Error | 5 | 8.144 | |
| mo sg = constant + strain + red | | | $R^2$ = 0.719 |
| Strain | 1 | 0.0031 | 3.404 |
| Red | 1 | 0.0052 | 5.583 |
| Error | 5 | 0.0009 | |
| | | Ungraded in cages | |
| mo dl = constant + strain + red | | | $R^2$ = 0.792 |
| Source | DF | MS | F |
| Strain | 1 | 20.485 | 13.397* |
| Red | 1 | 9.647 | 0.054 |
| Error | 5 | 1.529 | |
| mo cordl = constant + strain + red | | | $R^2$ = 0.886 |
| Strain | 1 | 31.128 | 24.905** |
| Red | 1 | 16.382 | 13.107 |
| Error | 5 | 1.250 | |
| mo sg = constant + strain + red | | | $R^2$ = 0.396 |
| Strain | 1 | 0.00085 | 1.876 |
| Red | 1 | 0.00067 | 1.468 |
| Error | 5 | 0.00045 | |

*P = .015; **P = .004

Table 3.12b. ANCOVA results when strains are graded/ungraded and grown with an internal reference fish in tanks. Red = internal reference fish as covariate, N=4 replicates each strain

| | | | | |
|---|---|---|---|---|
| | | Graded in tanks | | |
| mo dl = constant + strain + red | | | | $R^{2=}$ 0.028 |
| Source | DF | MS | F | |
| Strain | 1 | 1.017 | 0.058 | |
| Red | 1 | 2.371 | 0.134 | |
| Error | 5 | 17.649 | | |
| mo sg = constant + strain + red | | | | $R^2$ = 0.025 |
| Strain | 1 | 0.000 | 0.045 | |
| Red | 1 | 0.000 | 0.118 | |
| Error | 5 | 0.002 | | |
| | | Ungraded in tanks | | |
| mo dl = constant + strain + red | | | | $R^2$ = 0.154 |
| Source | DF | MS | F | |
| Strain | 1 | 12.081 | 0.367 | |
| Red | 1 | 24.799 | 0.754 | |
| Error | 5 | 32.909 | | |
| mo cordl = constant + strain + red | | | | $R^2$ = .780 |
| Strain | 1 | 249.306 | 15.377* | |
| Red | 1 | 75.220 | 4.639 | |
| Error | 5 | 16.213 | | |
| mo sg = constant + strain + red | | | | $R^2$ = .566 |
| Strain | 1 | 0.000 | 0.003 | |
| Red | 1 | 0.019 | 6.436 | |
| Error | 1 | 0.003 | | |

*P= 0.011

than the NIFI strain when red tilapia was used as an internal control fish in each cage and all three strains were of mixed sizes (Table 3.12a). The COM strain was again initially bigger. The distribution of growth rates differed between NIFI and COM strains. NIFI was negatively skewed while the COM strain was positively skewed (Table 3.3). The corrected growth of the NIFI strain was significantly higher (P=0.011) than the COM strain when the two strains were of mixed sizes and red tilapia was used as an internal control in tanks (Table 3.12b).

The ANCOVA of specific growth did not show significant difference between the two strains.

## DISCUSSION

It is relatively difficult to design an optimal experiment for comparative strain testing in aquaculture environments. The effects under study (say, growth differences) in an aquaculture situation tend to be masked by fluctuations and large uncontrolled variations which are characteristic of aquaculture environments.
Extraneous factors (like initial size advantage ) can cause significant differences in means, even though there may really be no differences in the means under study. Likewise, extraneous factors can mask or obscure a real difference that exists.

It was shown in the present study that the strain that was initially large also showed larger final size and growth rate (Table 3.5). In contrast, growth compensation seemed to occur under rice-fish farm conditions (Chapter 4 of this thesis). The final size difference between the NIFI and COM strain was not as large as the initial size difference. Presumably, in the highly structured environment of the rice fish farms, there was less competition. The faster growth of the smaller strain may be due to availability of the right size of food particles or feeding microhabitats in the rice paddies, or simply to the maturation of the initially larger fish which allowed the smaller ones to catch up.

Asynchronous spawning and maternal effects that give rise to initial size advantage to some individuals prior to strain testing are some of the widely recognized sources of common environmental variation in fish growth (Doyle and Talbot 1986b; Hulata et al. 1976; Wohlfarth and Hulata 1970). In my study, this was shown when the NIFI and COM strains were of mixed-sizes and mixed-ages and were grown together in cages. The COM strain showed a significantly higher growth rate than the NIFI strain only because it was initially bigger (Tables 3.1 and 3.11). Furthermore, fish growth can respond sensitively to differences in population density (Backiel and Le Cren 1967), available food (Magnuson 1962), food size (Nakamura and Kasahara 1956), water temperature (Brett 1979), and

size hierarchies (Brown 1946; 1957; Purdom 1974, Keen 1982).

Strains were of mixed-sizes and ages

Significant differences between the absolute growth rates of the NIFI and COM strains were observed only when the two strains were of mixed sizes and reared together in cages set in Laguna Lake, and when each strain was reared together with an internal control fish (red tilapia) in each cage. In both situations, the strain with the bigger initial size showed higher absolute growth rate. This result seems to be a case of a difference forced by competition rather than a measurement-scale effect since it only occurred when fish were reared together. Purdom (1974) has noted that 70% or more of the variance in growth rate in fish can be behavioral in origin. Skewed distributions were also noted in the present study. A similar skewed distribution was reported by Nakamura and Kasahara (1955) in the common carp. The reasons given include the establishment of social hierarchy and competition for food.

Mixed-sized (ungraded) strains that were reared together showed significant differences in absolute growth only when the initial size was used as a covariate. In all cases, however, the strain that was initially largest grew fastest (Table 3.5). The COM strain was initially bigger than the NIFI strain when they were grown together

in cages. Their initial size difference was 1.612 mm. The strain that was initially larger also had the larger final size and absolute growth. The final length difference was 3.225 mm, while difference in absolute growth was 2.212 mm. Likewise, when red tilapia was used as a covariate in cages and all three strains were of mixed sizes the COM strain was initially bigger than the NIFI strain. The initial size difference was 1.023 mm. The COM strain had higher final size and absolute growth. Difference in final length was 4.148 mm and absolute growth difference was 3.126 mm. Moav and Wohlfarth (1974) found similar magnification of inter-group differences in common carp due to competition. In rice-fish farms (Chapter 4 of this thesis), the large initial size differences between the NIFI and COM strains in farms 3 (6.37 mm) and 4 (10.61 mm.) had also induced significant strain differences. However, in rice-fish farms, the initially smaller NIFI strain grew faster. This significant strain effect was removed by using initial length as a covariate in farm 3 but not in farm 4, however.

Competition induced effects (or growth depensation at least ) were more apparent in the cage environment than in tank environment. Fish that were reared in tanks were fed in excess with commercial feed (26% protein during the strain testing period) whereas fish in cages set in Laguna Lake were not given supplemental food. The experiments in cages in Laguna Lake was performed between

10 October 1991 to 10 February 1992. In earlier studies, it has been noted that phytoplankton biomass and primary productivity of waters are relatively low between August and February ( Nielsen et al 1981; Basiao and San Antonio 1986). Competition for food was probably more intense in the cage environment due to the limited food resource available in the confined cages. Rearing was also performed in fine-meshed hapa cages to prevent the of unwanted wild fry entry into the cage .

Power considerations, mixed sizes grown together

If the true situation is that the NIFI and COM strains are identical (true Ho) the results suggest that rearing the two strains together when they are of mixed sizes can induce or force a spurious "significant" difference due to competition, which is considered an environmental artifact. This would have led to a Type I error (strains are taken to be different when they are identical). I noted previously that Type I error is theoretically of no economic consequence, since if two strains are really the same a farmer will neither benefit nor lose by preferring one over another. However, the competitive magnification of small differences might not be an appropriate selection procedure. Even if competitiveness

is inherited to some degree, selection for this factor might increase the level of interaction in the strain and not the average performance of the fish (Purdom 1974). The following is an example of a post hoc power analysis when mixed sizes were grown together in cages and initial length was used as a covariate in the analysis.

$\mu$nifi =38.452 mm (estimate of mean absolute growth)

$\mu$com =40.664 mm (estimate of mean absolute growth)

$\sigma$ = 0.851 (common standard deviation)

Step 1. Specify $\alpha$ = 0.05

Step 2. Calculate effect size (d) = $\left|\frac{\mu\text{nifi} - \mu\text{com}}{\sigma}\right|$

$$d = |38.452 - 40.664|/0.851 = 2.60$$

Step 2. Use Table 3.13 (Cohen 1988)

The power value for this large effect size (2.60) is greater than .995. If there were true difference between the two strains, a small number of replicates (cages=4) would be enough to detect the difference because of the large magnitude of difference between the two strains. This large effect size was due to the initial size difference of the two strains and not a true genetic difference. No significant effect was detected when the the corrected absolute change in length and specific

## Table 3.13. Power table

Power of t test of $m_1 = m_2$ at $a_2 = 05$

| | | d | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | $d_c$ | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| 8 | 1.07 | 05 | 07 | 09 | 11 | 15 | 20 | 25 | 31 | 46 | 60 | 73 |
| 9 | 1.00 | 05 | 07 | 09 | 12 | 16 | 22 | 28 | 35 | 51 | 65 | 79 |
| 10 | .94 | 06 | 07 | 10 | 13 | 18 | 24 | 31 | 39 | 55 | 71 | 84 |
| 11 | .89 | 06 | 07 | 10 | 14 | 20 | 26 | 34 | 43 | 61 | 75 | 87 |
| 12 | .85 | 06 | 08 | 11 | 15 | 21 | 28 | 37 | 46 | 65 | 80 | 90 |
| 13 | .81 | 06 | 08 | 11 | 16 | 23 | 31 | 40 | 50 | 69 | 83 | 93 |
| 14 | .78 | 06 | 08 | 12 | 17 | 25 | 33 | 43 | 53 | 72 | 86 | 94 |
| 15 | .75 | 06 | 08 | 12 | 18 | 26 | 35 | 45 | 56 | 75 | 88 | 96 |
| 16 | .72 | 06 | 08 | 13 | 19 | 28 | 37 | 48 | 59 | 78 | 90 | 97 |
| 17 | .70 | 06 | 09 | 13 | 20 | 29 | 39 | 51 | 62 | 80 | 92 | 98 |
| 18 | .68 | 06 | 09 | 14 | 21 | 31 | 41 | 53 | 64 | 83 | 94 | 98 |
| 19 | .66 | 06 | 09 | 15 | 22 | 32 | 43 | 55 | 67 | 85 | 95 | 99 |
| 20 | .64 | 06 | 09 | 15 | 23 | 33 | 45 | 58 | 69 | 87 | 96 | 99 |
| 21 | .62 | 06 | 10 | 16 | 24 | 35 | 47 | 60 | 71 | 88 | 97 | 99 |
| 22 | .61 | 06 | 10 | 16 | 25 | 36 | 49 | 62 | 73 | 90 | 97 | 99 |
| 23 | .59 | 06 | 10 | 17 | 26 | 38 | 51 | 64 | 75 | 91 | 98 | * |
| 24 | .58 | 06 | 10 | 17 | 27 | 39 | 53 | 66 | 77 | 92 | 98 | |
| 25 | .57 | 06 | 11 | 18 | 28 | 41 | 55 | 68 | 79 | 93 | 99 | |
| 26 | .56 | 06 | 11 | 19 | 29 | 42 | 56 | 69 | 80 | 94 | 99 | |
| 27 | .55 | 06 | 11 | 19 | 30 | 43 | 58 | 71 | 82 | 95 | 99 | |
| 28 | .54 | 07 | 11 | 20 | 31 | 45 | 59 | 73 | 83 | 96 | 99 | |
| 29 | .53 | 07 | 12 | 20 | 32 | 46 | 61 | 74 | 85 | 96 | 99 | |
| 30 | .52 | 07 | 12 | 21 | 33 | 47 | 63 | 76 | 86 | 97 | * | |
| 31 | .51 | 07 | 12 | 21 | 34 | 49 | 64 | 77 | 87 | 97 | | |
| 32 | .50 | 07 | 12 | 22 | 35 | 50 | 65 | 78 | 88 | 98 | | |
| 33 | .49 | 07 | 13 | 22 | 36 | 51 | 67 | 80 | 89 | 98 | | |
| 34 | .48 | 07 | 13 | 23 | 37 | 53 | 68 | 81 | 90 | 98 | | |
| 35 | .48 | 07 | 13 | 23 | 38 | 54 | 70 | 82 | 91 | 98 | | |
| 36 | .47 | 07 | 13 | 24 | 39 | 55 | 71 | 83 | 92 | 99 | | |
| 37 | .46 | 07 | 14 | 25 | 39 | 56 | 72 | 84 | 92 | 99 | | |
| 38 | .46 | 07 | 14 | 25 | 40 | 57 | 73 | 85 | 93 | 99 | | |
| 39 | .45 | 07 | 14 | 26 | 41 | 58 | 74 | 86 | 94 | 99 | | |
| 40 | .45 | 07 | 14 | 26 | 42 | 60 | 75 | 87 | 94 | 99 | | |
| 42 | .43 | 07 | 15 | 27 | 44 | 62 | 77 | 89 | 95 | 99 | | |
| 44 | .42 | 07 | 15 | 28 | 46 | 64 | 79 | 90 | 96 | * | | |
| 46 | .41 | 08 | 16 | 30 | 48 | 66 | 81 | 91 | 97 | | | |
| 48 | .41 | 08 | 16 | 31 | 49 | 68 | 83 | 92 | 97 | | | |

growth rate were used in the two-way ANOVA (Table 3.11a).

Strains were size-graded

Size grading is routinely practised in fish hatcheries on the assumption that small fish will grow better once the larger fish are removed. Evidence supporting this assumption comes from fish behaviour and social hierarchy studies conducted under laboratory conditions (Keenleyside and Yamamoto 1962; Yamagishi 1962; Fenderson and Carpernter 1971; Li and Brocksen 1977).

I have explored a possible way of reducing the effects of initial size difference between the two strains by size-matching or size-grading based on individual standard length measurements prior to st~ain testing. The idea is to minimize the non-genetic environmental variance in growth caused by variable egg quality and by asynchronous spawning (Doyle and Talbot 1986b). In common carp, differential growth rates were attributed to maternal effects and different hatching time (Hulata et al. 1974; 1976). Wohlfarth and Hulata (1970) found that a 24-hour spawning time difference in common carp resulted in size adavantage to the fry that is a day older. Intra-population competition for food, especially for some limiting component, confers an advantage to the larger older fry. The relative size of common carp fry immediately after hatching is a major determinant of further growth rate (Nakamura and Kasahara 1956, 1961). It

was shown in common carp fry, that for an individual fish, growth rate and chance of survival increase with its size relative to its competitors. The problem with size-grading is that it may bias the genetic sample of the populations being compared. The magnitude of the bias depends on the ratio of genetic variance to phenotypic variance for growth at the time of size-matching. In the present study, fish were collected over a 3-week period. This collection period introduces a lot of environmental variation (phenotypic variation) reflected in the significant differences obtained in some testing procedures when both strains were of mixed-sizes and ages.

Power considerations of size-grading

Size-grading appears to control a Type I error (two strains are considered different when they are identical) by eliminating or reducing the environmental variance due to competition that can induce a non-genetic significant difference when two strains are identical. Likewise, size-grading can also be a powerful procedure to detect strain differences if they exist. The risk of the more economically important Type II error (not able to detect a difference that exists) is minimized by size-grading the fish prior to testing. The initial size advantage of an individual fish that can mask or obscure a real difference that exists is eliminated by size-grading. Basio and Doyle (1990) and Romana-Eguia and Doyle (1992) reported

that were size-graded prior to the comparison experiments. The follwing is an example of power calculation when strains are graded and reared separately in cages (ANOVA result was not significant).

$\mu$nifi = 42.950 mm (estimates of mean change in length)

$\mu$com = 41.236 mm (estimates of mean change in length)

$\sigma$ = 2.013 mm (common standard deviation)

Step 1. Specify $\alpha = 0.10$

Step 2. Calculate effect size (d) = $\left|\frac{\mu\text{nifi}-\mu\text{com}}{\sigma}\right|$

$$d = |42.950 - 41.236|/2.013 = 0.85 \text{ mm}$$

Step 3. For a two-tailed test at $\alpha = .10$, Table 3.14 shows that the number of replicate cages required to detect an effect size of 0.85 mm with a desired power of 0.80 is 20 of each strain. If the desired power is only 0.50 at $\alpha = .10$, the number of replicates required is only 9 of each strain.

If the two tilapia strains were really different, the experimental design of size grading the fish and rearing them separately in cages would require about 20 replicate cages of each strain to have an 80% chance of detecting an effect size of 0.85 mm. If an aquaculturist thinks this effect size is economically important, he should consider

## Table 3.14. Sample size table

| | $\alpha_1 = .01$ ($\alpha_2 = .02$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 547 | 138 | 62 | 36 | 24 | 17 | 13 | 10 | 7 | 5 | 4 |
| .50 | 1083 | 272 | 122 | 69 | 45 | 31 | 24 | 18 | 12 | 9 | 7 |
| .60 | 1332 | 334 | 149 | 85 | 55 | 38 | 29 | 22 | 15 | 11 | 8 |
| 2/3 | 1552 | 382 | 170 | 97 | 62 | 44 | 33 | 25 | 17 | 12 | 9 |
| .70 | 1627 | 408 | 182 | 103 | 66 | 47 | 35 | 27 | 18 | 13 | 10 |
| .75 | 1803 | 452 | 202 | 114 | 74 | 52 | 38 | 30 | 20 | 14 | 11 |
| .80 | 2009 | 503 | 224 | 127 | 82 | 57 | 42 | 33 | 22 | 15 | 12 |
| .85 | 2263 | 567 | 253 | 143 | 92 | 64 | 48 | 37 | 24 | 17 | 13 |
| .90 | 2605 | 652 | 290 | 164 | 105 | 74 | 55 | 42 | 27 | 20 | 15 |
| .95 | 3155 | 790 | 352 | 198 | 128 | 89 | 66 | 51 | 33 | 23 | 18 |
| .99 | 4330 | 1084 | 482 | 272 | 175 | 122 | 90 | 69 | 45 | 31 | 23 |
| | $\alpha_1 = .05$ ($\alpha_2 = .10$) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 189 | 48 | 21 | 12 | 8 | 6 | 5 | 4 | 3 | 2 | 2 |
| .50 | 542 | 136 | 61 | 35 | 22 | 16 | 12 | 9 | 6 | 5 | 4 |
| .60 | 721 | 181 | 81 | 46 | 30 | 21 | 15 | 12 | 8 | 6 | 5 |
| 2/3 | 862 | 216 | 96 | 55 | 35 | 25 | 18 | 14 | 9 | 7 | 5 |
| .70 | 942 | 236 | 105 | 60 | 38 | 27 | 20 | 15 | 10 | 7 | 6 |
| .75 | 1076 | 270 | 120 | 68 | 44 | 31 | 23 | 18 | 11 | 8 | 6 |
| .80 | 1237 | 310 | 138 | 78 | 50 | 35 | 26 | 20 | 13 | 9 | 7 |
| .85 | 1438 | 360 | 160 | 91 | 58 | 41 | 30 | 23 | 15 | 11 | 8 |
| .90 | 1713 | 429 | 191 | 108 | 69 | 48 | 36 | 27 | 18 | 13 | 10 |
| .95 | 2165 | 542 | 241 | 136 | 87 | 61 | 45 | 35 | 22 | 16 | 12 |
| .99 | 3155 | 789 | 351 | 198 | 127 | 88 | 65 | 50 | 32 | 23 | 17 |
| | $\alpha_1 = .10$ ($\alpha_2 = .20$) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 74 | 19 | 9 | 5 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| .50 | 329 | 82 | 37 | 21 | 14 | 10 | 7 | 5 | 4 | 3 | 2 |
| .60 | 471 | 118 | 53 | 30 | 19 | 14 | 10 | 8 | 5 | 4 | 3 |
| 2/3 | 586 | 147 | 65 | 37 | 24 | 17 | 12 | 10 | 6 | 4 | 3 |
| .70 | 653 | 163 | 73 | 41 | 27 | 19 | 14 | 11 | 7 | 5 | 4 |
| .75 | 766 | 192 | 85 | 48 | 31 | 22 | 16 | 13 | 8 | 6 | 4 |
| .80 | 902 | 226 | 100 | 57 | 36 | 26 | 19 | 14 | 10 | 7 | 5 |
| .85 | 1075 | 269 | 120 | 67 | 43 | 30 | 22 | 17 | 11 | 8 | 6 |
| .90 | 1314 | 329 | 146 | 82 | 53 | 37 | 27 | 21 | 14 | 10 | 7 |
| .95 | 1713 | 428 | 191 | 107 | 69 | 48 | 35 | 27 | 18 | 12 | 9 |
| .99 | 2604 | 651 | 290 | 163 | 104 | 73 | 53 | 41 | 26 | 18 | 14 |

investing in 40 cages.

Strains grown together

Another question which I wanted to address is whether strains should be reared together in a common environment or reared separately during strain testing. Communal testing was first demonstrated as an effective and convenient method of testing different genetic groups of common carp in earthen ponds (Wohlfarth and Moav 1975) and in cages (Wohlfarth and Moav 1991). It has also been tried on channel catfish (Dunham et al. 1982), tilapia (McGinty 1984, 1985, 1987), coho salmon (Busack and Riddel 1985) and largemouth bass (Williamson and Carmichael 1990).

Communal rearing is viewed as more efficient than separate rearing because the strains being compared share a single experimental unit and are thus subjected to common environmental experiences. However, competition may occur in communal rearing. And it may be more intense if the strains being compared are of mixed-sizes.

Results from the present study showed significant strain differences in absolute growth rates when the initial length of the NIFI and COM strains was used as a covariate. This was apparent in the procedure where both strains were of mixed sizes and were communally grown in cages (Table 3.12a). The initial size advantage of the COM strain influenced the absolute growth rate difference.

This is clearly a case of an environmentally induced difference which was not observed when the two strains were size-matched and grown communally in cages. In the common carp, the bias due to initial size difference is corrected by transforming the observed weight gain of each group (Moav et al. 1975). In the catfish, the problem of initial size difference is overcome by correcting the effect of the initial weight on final weight, or the fry and young fingerlings can be reared to a common starting size (Dunham et al. 1982).

Fingerlings are forced to a common size by the manipulation of feed and/or stocking rate. The growth rate was corrected for the initial size difference (Moav et al. 1975) but the correction was not enough to remove the initial size adavantage of the bigger fish

It is apparent from these studies that communal rearing is only efficient than separate rearing when the strains have a common starting size. In this study, the NIFI and COM strains were size-matched (size-graded) to a common starting standard length when compared communally in tanks and cages. In contrast, to the procedure where the strains were of mixed sizes and communally reared, no significant difference in growth rates was found in the size-matched (size-graded) fish.

Power considerations, strains grown together

The following is an example of power calculation when strains were graded and grown together in cages. Two-way ANOVA did not show any strain effect.

$\mu$nifi = 38.397 mm (estimates of change in length)

$\mu$com = 38.128 mm (estimates of change in length)

$\sigma$ = 1.89 mm (common standard deviation)

Step 1. Set $\alpha$ = .10

Step 2. Calculate effect size (d)= $\frac{|\mu\text{nifi} - \mu\text{com}|}{\sigma}$

$$d = |38.397 - 38.128|/1.89 = .14 \text{ mm}$$

Step 3. Use Table 3.14

The number of cages required to generate a power of .80, given the observed magnitude of the strain difference, would be around 1000 cages. If only a power value of .50 is desired, the number of replicates would be about 500 cages. A small effect size requires a large number of replicates. A strain testing design that would require around 1,000 cages to generate an 80% chance of detecting an effect size of 0.14 mm is impractical to implement and not worth considering unless the opportunity cost is very high.

Size grading the fish and growing them together in cages appears to be an efficient design to detect the real

difference between two fish strains when such differences exist.

Use of an internal reference fish

The use of supplementary observations to reduce error (Cox 1958) is a powerful method that is underexploited in aquaculture research. A strain testing situation has been considered, in which, in addition to the two strains that were reared separately, a third strain (red tilapia) was used as a concomitant variable or covariate for each replicate cage or tank.

In this preliminary study, use of red tilapia as a covariate when the fish were of mixed-sizes and tested in hapa cages in the lake showed significant growth rate differences. This is again a case of an environmentally induced variance due to initial size difference. As in the significant differences obtained in the other procedures, the strain that was initially large showed higher absolute growth rate but not specific growth (Table 3.5). The method of Wohlfarth and Moav (1975) for correcting the initial size difference has been adopted, but the correction was not effective in removing the effect of initial size difference.

Power considertions, use of internal reference fish

Power calculation when an internal reference fish was used as a covariate and all three strains were of the

same initial sizes will be illustrated. ANCOVA did not show significant strain effect (Table 3.12a) with this procedure.

$\mu$nifi = 44.567 mm (estimates of change in length)

$\mu$com = 39.088 mm (estimates of change in length)

$\sigma$ = 2.85 mm (common standard deviation)

Step 1. Specify $\alpha$ = .10

Step 2. Calculate effect size (d)

d = |44.567 - 39.683|/2.85 = 1.71 mm

Step 3. Use Sample Size Table

For a desired power of .80 at an $\alpha$ = .10, the required replicate to detect an effect size of 1.71 is 7 cages of each strain. The experimental design of using an internal reference fish and size grading the test strains before strain testing seems to be an efficient and economical procedure to detect differences when real differences exists.

Example of power calculation when the two strains were of mixed sizes and ages and grown with an internal control fish in cages. ANCOVA showed a significant strain effect.

$\mu$nifi= 41.879 mm (estimates of mean change in length)

$\mu$com = 45.005 mm (estimates of mean change in length)

$\sigma$ = 1.236 mm (common standard deviation)

Step 1. Specify $\alpha$ = .05

Step 2. Calculate effect size (d)

$d = |41.879 - 45.005|/1.236 = 3.126$ mm

Step 3. Use Power Table (Table 3.14) to determine the power of the test. The power of the test for a large effect size of 3.126 mm was greater than .995. Large effect sizes have greater power and require a smaller number of replicates. However, this observed size effect was only an environmentally induced difference that was due to the mixed initial sizes and ages of the fish. If there were a real genetic difference between the two strains, use of an internal reference fish would have been a very efficient procedure to detect differences.

This research has shown that some statistical tests and designs can give misleading results if caution is not taken during strain testing. For instance, use of just the absolute increase in growth can give misleading results due to initial size difference between two strains. Likewise, communal rearing which is considered a very efficient method to remove environmental differences can give inaccurate results when strains under consideration are of mixed sizes and ages. Use of an internal control fish as a covariate to remove environmental error becomes inadequate when the initial

sizes of the fish are different. Size-matching or the size-grading of fish appears to be the most efficient method of minimizing one environmental source of variation in strain testing. The risks of both Type I and the more important Type II error are reduced. Further investigations similar to those described in my thesis are required on a larger scale and in a more typical commercial aquaculture situations.

## Chapter 4

## ON-FARM, NON-EXPERIMENTAL STRAIN TESTING PROCEDURE

### ABSTRACT

Conclusions from environmentally controlled strain testing studies may have little bearing on the performance of the fish under farm conditions. It becomes important to evaluate the relative performance of fish in actual farm situations. My objectives for testing two tilapia strains in rice farms are: (1) to obtain estimates of means and error variances in rice-paddies and to use the information for power analysis, (2) to test the efficiency of the scale circulus spacing (CIRC) technique as a growth estimator.

The NIFI and COM strains were compared in four rice-fish farms. These tilapia strains were individually tagged and measured, and were grown together with the farmer's own tilapia strain in each farm. The CIRC technique developed by Doyle et al (1987) was used to estimate growth. Absolute and specific growths were also calculated.

The correlation between growth rates and CIRC in farms 1 and 2 range between 0.450 and 0.542. Non-significant correlations were found in farms 3 and 4 (0.194,-0.267 respectively). Initial size-difference

growth rate among strains that appear to have no genetic basis as shown in farms 3 and 4. The significant strain effect in farm 3 was removed when initial size was used as a covariate. In farm 4, on the other hand, use of initial size as a covariate did not remove the significant strain effect. The exaggeration of initial size differences by competitive social interactions could make strain evaluation difficult if the initial size differences are not genetically based.

Size-grading or having almost the same size range among genotypes may help minimize environmentally induced variation like initial size differences.

# INTRODUCTION

The relationship between circulus spacing and growth rate on fish scales has been known for a long time (Graham 1929; Gray and Setna 1931; Bhatia 1931, 1932). These studies have shown that circulus spacing on fish scales can potentially serve as a record of variations of growth rate and that circulus spacing depends on growth conditions. Recently, a simple technique based on circulus spacing to estimate current growth rate of individual fish without disturbing the culture systems in which they grow has been developed by Doyle et al. (1987) and Talbot et al. (1988). The growth rate of a fish is estimated from the spacing between calcified bony ridges (circuli) on scales. The technique was used by McNaughton (1986) to compare the growth rates of several carp culture systems in Indonesia and by Matricia (1989) to compare the performance of different tilapia colour morphs that were grown together in "uncontrolled", non-experimental aquaculture systems in Indonesia. The technique was also used by Nicholas (1987) to test the relationship between initial larval size and early growth rate in tilapia and by Kamonrat and Doyle (1989) for estimating heritability of tilapia growth rates in laboratory culture.

Comparative strain testing is usually conducted in experimentally controlled environments quite different from the environment in which the studied fish are

actually grown. Conclusions from experimental studies may have little bearing on the performance of the fish in actual aquaculture conditions. It is therefore important to evaluate the relative performance of fish strains under actual farm situations. The objectives in doing a preliminary strain testing in rice-paddies are to obtain estimates of means and error variances in rice-paddies and to validate the efficiency of the CIRC technique in estimating growth rate of fish in artisanal environments.

The reason for using rice-paddies is the growing interest in rice-fish culture activities not only in the Philippines but also in China, Thailand, India, and Indonesia (Grover 1976; Middendorp and Verreth 1986; Kangmin 1988; Sollows and Tongpan 1986; Bailey and Skladany 1990; Fagi and Suriapermana 1992; Leelapatra et al 1992; de la Cruz et al 1992).

## MATERIALS AND METHODS

### Rice-fish farm/strain comparison experiment

Fish used were two and a half month old fingerlings of the NIFI and COM strains that were spawned at the SEAFDEC Binangonan Freshwater Station. The four rice-paddies are located at Triala, Guimba Nueva Ecija on the Island of Luzon, Philippines.

The study was conducted from 9 August to 13 November 1991. Individual initial length measurements were taken

before stocking. The NIFI and COM strains that were communally raised with the farmers fish were individually floy tagged for identification. Each rice-paddy was stocked with 35 NIFI and 35 COM fingerlings. The fish were harvested by cast net and lift nets after the harvest of rice. Individual final length measurements were taken and scales were collected from all the fish that were identified from each farm. Additional information regarding the rice-fish paddies and management by the farmers was recorded.

Scale collection and measurement

The technique develop by Doyle et al. (1987) for scale collection and growth estimation was followed. One scale was removed with forceps from each side of the body from the caudal area of each fish, just below where the lateral line intersects an imaginary vertical line between the insertions of the dorsal and anal fins (Fig.4.1) and immediately preserved in 10% buffered formalin.

The scales were wet-mounted and viewed with a projection microscope (Leitz-Promar), which projected the image of the scale onto a table for convenient measurement with calipers. Measurements were taken from one scale per fish. Regenerated scales which were detected by the large irregular focus were not measured. Such scales are unsuitable for measuring growth because they tend to have

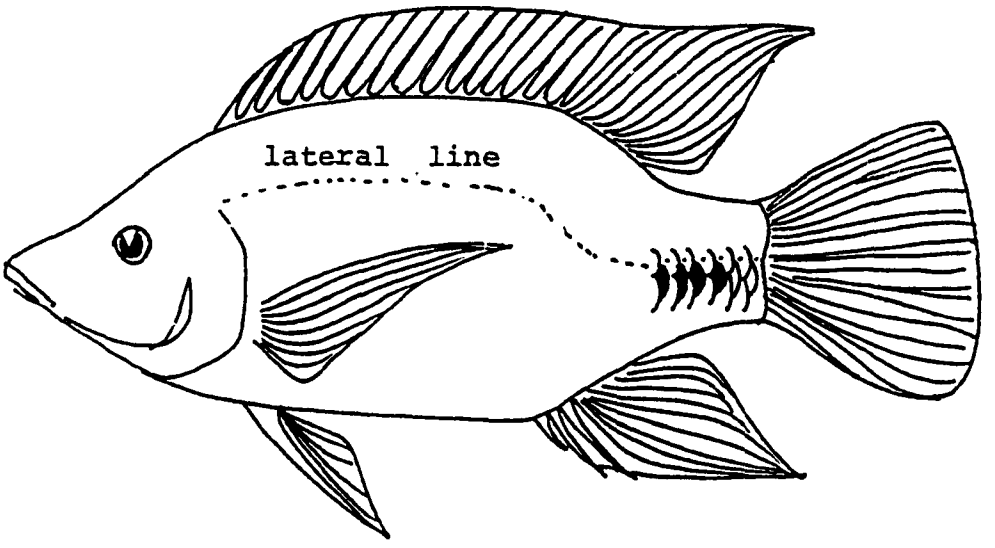



**Fig. 4.1. Position of scales used in this study (Kamonrat 1987)**

wider circulus spacing than original scales on the same fish (Sire 1986). The scale measurement that was taken to represent very recent or "current" growth, was the 3 outermost, and therefore most recent circulus spaces at the scale margin (Fig.4.2). In this study, CIRCM is an average of 5 measurements on the central part of the anterior edge of one scale.

Growth measurements and Analyses

Since all the experimental fish were individually tagged, it was possible to measure the individual growth rates accurately. Absolute growth rate (change in length) is measured as

(1) DL = final length - initial length

and specific growth is measured as the natural logarithm of proportional growth in length (Ricker 1979).

(2) G = ln (final length/initial length)

CIRCM = circulus spacing at the scale margin

Farms and strains were coded for analysis of variance. One-way ANOVA as implemented in the MGLH module of the SYSTAT statistical package (Wilkinson 1986) was used.

(3) $DL_{ijk}/CIRCM_{ijk} = \mu + Sj + error$

where

DL = absolute growth rate for the kth fish of the jth strain at the ith farm, $\mu$ is the grand mean, Sj is the jth

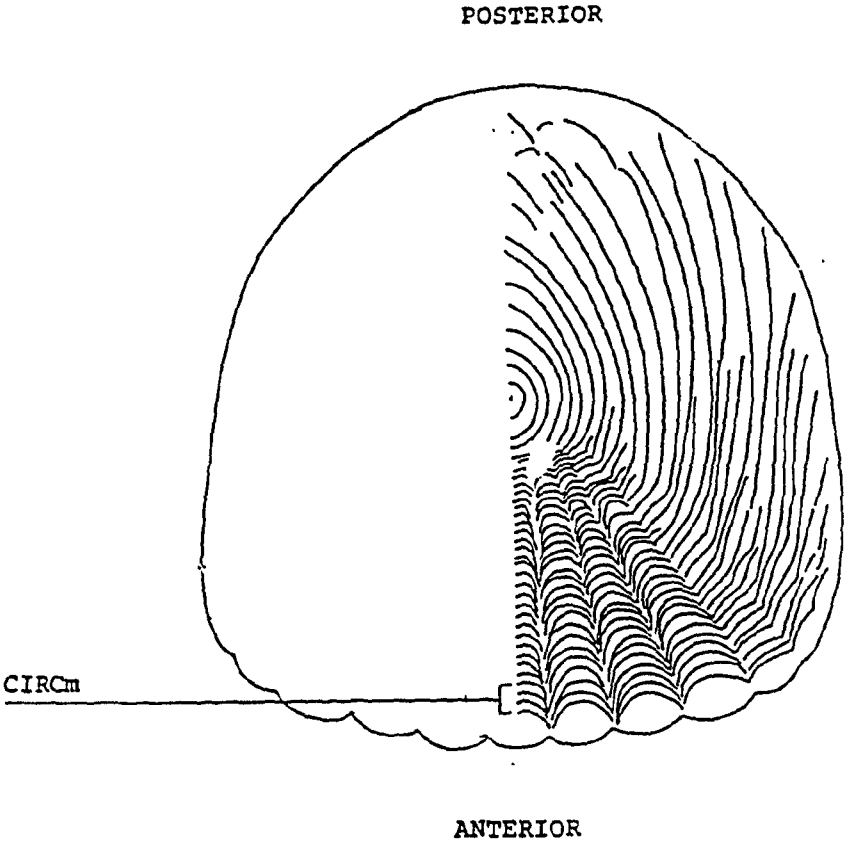



**Fig. 4.2. Diagram of a tilapia scale showing the CIRCm measurement used to represent recent growth.**

strain.

CIRCM = is the circulus spacing at the scale margin of the kth fish of the jth strain at the ith farm. When the initial length (len0) was used as a covariate, the ANCOVA (analysis of covariance) model used was:

(4) DLijk/CIRCMijk = $\mu$ + Sj + ßLen0 + error

Two-way ANOVA as implemented in the MGLH module of the SYSTAT statistical package (Wilkinson 1988) was likewise performed

(3) DLijk/Gijk/CIRCMijk = $\mu$ + Fi + Sj + (FS)ij + Eijk

where

DL = absolute growth rate for kth fish of the jth strain at the ith farm

G = specific growth rate for the kth fish of the jth strain at the ith farm

CIRCM = circulus spacing at the scale margin of the kth fish of the jth strain at the ith farm

$\mu$ = is the grand mean

F = is the fixed farm effect

S = is the fixed strain effect

FS = is the interaction term

E = is the random error term

To confirm the suitability of CIRCM as an estimate of growth rate, Pearson correlation coefficients were calculated for CIRCM versus standard initial length,

increase in standard length (DL) versus CIRCM and specific growth (SG) versus CIRCM. The correlation coefficients between initial length and growth were also calculated.

## RESULTS

The estimates of the population means of NIFI and COM strains together with their standard deviations when compared in four rice-paddies are given in Table 4.1. The initial number of fish stocked in each farm was 35 fingerlings for each strain. The lowest recovered number of fish was COM strain in farm 4. Only 11 fish out of 35 were recovered after 96 days of culture. It was difficult to assess whether the low recovery of fish was due to mortality or due to loss of the floy tags from the fish.

The fish increased in length an average of 6.7 mm to 21.7 mm across farms. Specific growth ranged from 0.088 to 0.279, with the lowest growth for COM strain in farm 4 and the highest for the NIFI strain in farm 2. CIRCM ranged from 66.79 to 75.75.

### Circulus spacing as a measure of growth

The Pearson correlation coefficients for the relationship between CIRCM and increase in length and specific growth are given in Table 4.2a. The r values

Table 4.1. Mean, standard deviation (parenthesis), Len0= mean of individual initial length (mm), Len96= mean of individual final length (mm), change in length (DL), specific growth (G), CIRCM (microns), N = number of fish

| Farm | Strain | N | Len0 | Len96 | DL | G | CIRCM |
|---|---|---|---|---|---|---|---|
| 1 | NIFI | 18 | 73.55 | 94.50 | 20.94 | 0.25 | 68.55 |
| | | | (7.41) | (6.66) | (6.09) | (0.08) | (7.67) |
| | COM | 15 | 73.87 | 93.47 | 19.60 | 0.24 | 66.79 |
| | | | (6.33) | (7.53) | (7.23) | (0.09) | (5.79) |
| 2 | NIFI | 16 | 68.06 | 89.81 | 21.75 | 0.28 | 68.40 |
| | | | (8.39) | (9.40) | (9.74) | (0.13) | (11.07) |
| | COM | 22 | 69.77 | 87.68 | 17.91 | 0.23 | 68.60 |
| | | | (5.19) | (8.24) | (7.02) | (0.08) | (7.04) |
| 3 | NIFI | 17 | 65.94 | 80.12 | 14.18 | 0.20 | 75.75 |
| | | | (6.50) | (2.57) | (6.10) | (0.09) | (7.44) |
| | COM | 16 | 72.31 | 80.56 | 8.25 | 0.11 | 75.72 |
| | | | (6.13) | (4.89) | (5.77) | (0.08) | (8.49) |
| 4 | NIFI | 18 | 63.39 | 76.83 | 13.44 | 0.19 | 69.68 |
| | | | (5.02) | (5.11) | (3.17) | (0.05) | (8.62) |
| | COM | 11 | 74.00 | 80.82 | 6.73 | 0.09 | 74.69 |
| | | | (5.75) | (4.62) | (2.94) | (0.04) | (6.61) |

Table 4.2a. Pearson correlation coefficient (with N in parenthesis) for the relationship between circm and initial standard length, circm and change in length (DL), and circm and specific growth (G)

| Farm | All fish pooled | NIFI | COM |
|---|---|---|---|
| | | CIRCM vs SIZE | |
| 1 | -0.425 (33)** | -0.329 (18) | -0.611 (15)** |
| 2 | -0.447 (38)*** | -0.528 (16)* | -0.316 (22) |
| 3 | -0.235 (33) | -0.116 (17) | -0.413 (16) |
| 4 | 0.290 (29) | 0.245 (18) | -0.137 (11) |
| | | CIRCM vs DL | |
| 1 | 0.509 (33)*** | 0.562 (18)** | 0.453 (15) |
| 2 | 0.450 (38)*** | 0.827 (16)*** | -0.097 (22) |
| 3 | 0.194 (33) | 0.068 (17) | 0.367 (16) |
| 4 | -0.267 (29) | -0.114 (18) | 0.039 (11) |
| | | CIRCM vs G | |
| 1 | 0.542 (33)*** | 0.563 (18)** | 0.519 (15)* |
| 2 | 0.495 (38)*** | 0.844 (16)**** | 0.075 (22) |
| 3 | 0.196 (33) | 0.078 (17) | 0.378 (16) |
| 4 | -0.303 (29) | -0.180 (18) | 0.045 (11) |

*Significant at 0.05 P level
**Significant at 0.02 P level
***Significant at 0.01 P level
****Significant at 0.001 P level

Table 4.2b. Pearson correlation coefficient matrix where all fish are pooled by farm. Len0= mean individual initial length (mm); Len96= mean individual final length (mm); DL= mean absolute growth; G= mean specific growth; N= (number of individuals).

| | Len0 | Len96 | Dl | G | CIRCM |
|---|---|---|---|---|---|
| | | Farm 1 (33) | | | |
| Len0 | 1.000 | | | | |
| Len96 | 0.549*** | 1.000 | | | |
| DL | -0.458*** | 0.491*** | 1.000 | | |
| G | -0.653**** | 0.273 | 0.971 | 1.000 | |
| CIRCM | -0.425** | 0.063 | 0.509*** | 0.542*** | 1.000 |
| | | Farm 2 (38) | | | |
| Len0 | 1.000 | | | | |
| Len96 | 0.431*** | 1.000 | | | |
| DL | -0.350* | 0.694**** | 1.000 | | |
| G | -0.491*** | 0.572**** | 0.986 | 1.000 | |
| CIRCM | -0.447*** | 0.090 | 0.450*** | 0.495*** | 1.000 |
| | | Farm 3 (33) | | | |
| Len0 | 1.000 | | | | |
| Len96 | -0.383* | 1.000 | | | |
| DL | -0.844**** | 0.172 | 1.000 | | |
| G | -0.877**** | 0.106 | 0.997 | 1.000 | |
| CIRCM | -0.235 | -0.098 | 0.194 | 0.196 | 1.000 |
| | | Farm 4 (29) | | | |
| Len0 | 1.000 | | | | |
| Len96 | 0.802**** | 1.000 | | | |
| DL | -0.718**** | -0.160 | 1.000 | | |
| G | -0.304**** | -0.293 | 0.986 | 1.000 | |
| CIRCM | 0.290 | 0.182 | -0.267 | -0.303 | 1.000 |

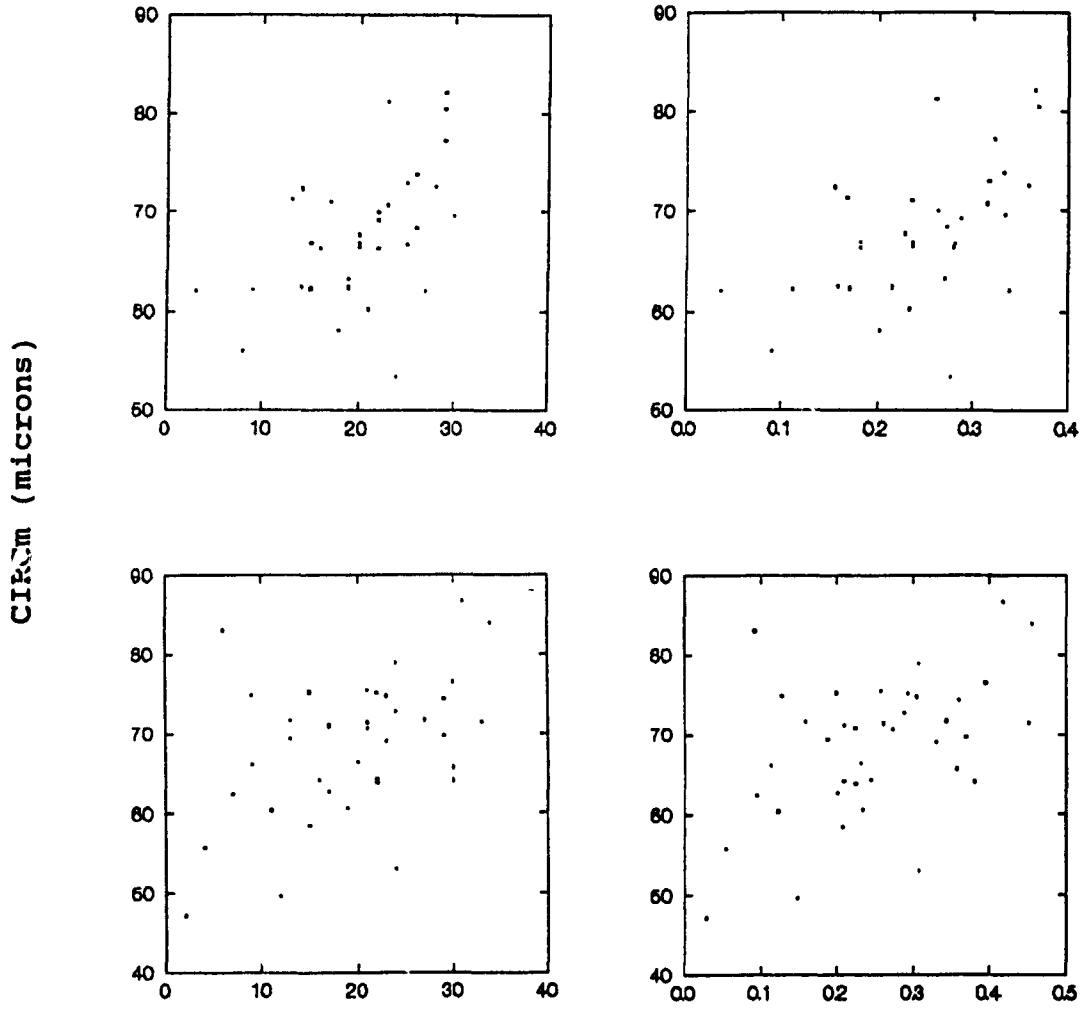
*Significant at 0.05 P level
**Significant at 0.02 P level
***Significant at 0.01 P level
****Significant at 0.001 P level

for farms 1 and 2 are highly significant ($P<0.01$) when all the fish are pooled. When correlation was done by farm and by strain, the NIFI strain showed significant r values in farm 1 ($r= .562$, $P=0.02$) and farm 2 ($r = .827$, $P= 0.001$). The COM strain showed a significant r value for specific growth in farm 1 ($r =.519$, $P<0.05$). No significant r values are found in farms 3 and 4. The graphs of CIRCM against growth rate in farms 1 and 2 appeared linear (Fig.4.3a,b). A negative relationship was found in farm 4. Comparison of Tables 4.1 and 4.2 shows that CIRCM is positively correlated with growth rate only in the farm-strain combinations where growth was most rapid. All the significant correlations in Table 4.2a come from the three highest DL and G values in Table 4.1. This is consistent with earlier findings (Talbot and Doyle 1992) that the CIRC technique breaks down when there are large numbers of slow-growing or non-growing fish.

## ESTIMATES OF POWER IN RICE-PADDIES

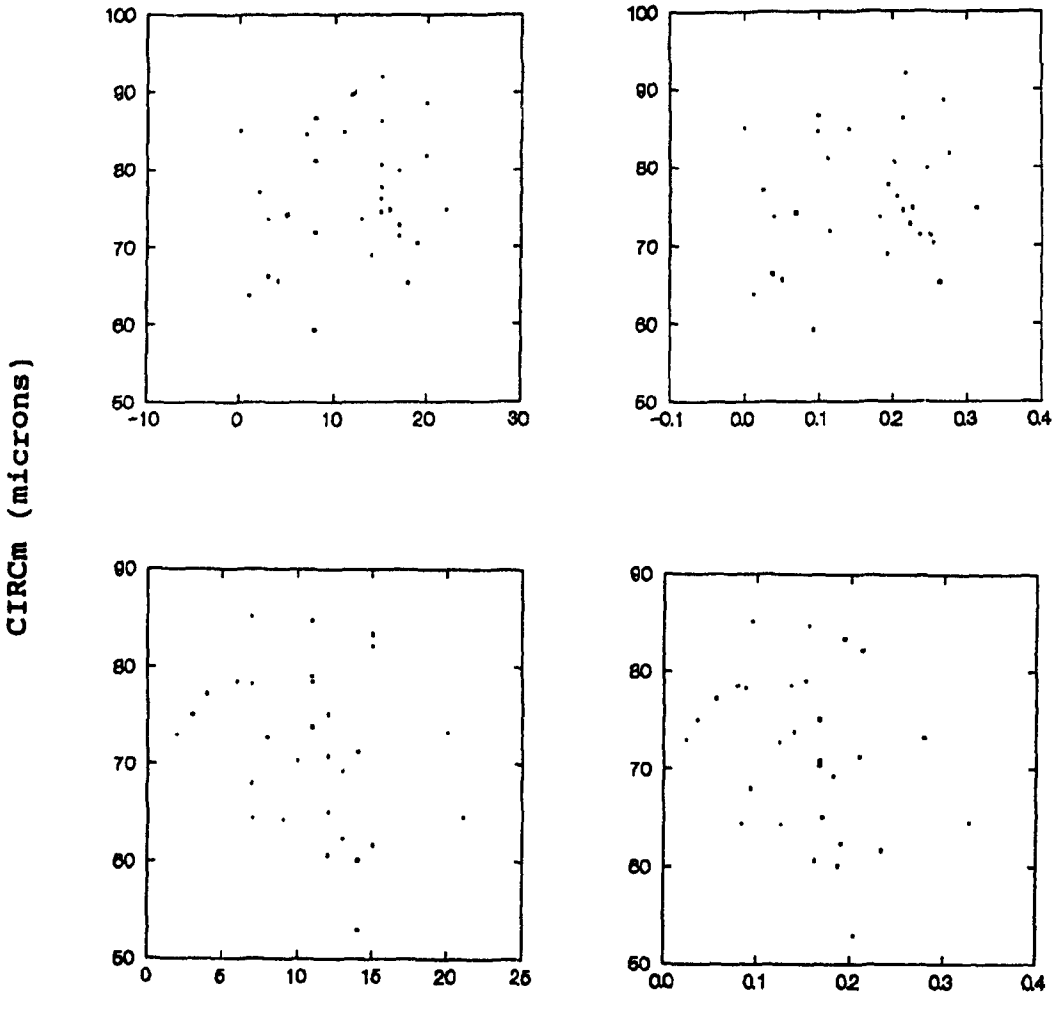
The major use of Power and Sample Tables is in planning experiments. When the significance criterion ($\alpha$), the effect size (ES) and the sample size are specified, these Tables are also used for post hoc analysis of power after the experiment is done. I have adopted the Power and Sample Tables of Cohen (1988) to illustrate the concept of power analysis when strains are compared in rice-farms.

Fig. 4.3a. Validation of CIRCm as an estimate of absolute increase in length (left-hand graphs) and specific growth (right-hand graphs) in farm 1 (upper graphs) and farm 2 (lower graphs).




Growth Rate
Fig. 4.3a

Fig. 4.3b. Validation of CIRCm as an estimate of absolute increase in length (left-hand graphs) and specific growth (right-hand graphs) in farm 3 (upper graphs) and farm 4 (lower graphs).




Growth Rate

Fig. 4.3b

The estimates of the population means, common standard deviations, effect size (d), number of samples needed to derive power are given in Table 4.3.

Farm 1 (Post hoc power analysis)

Post hoc power analysis can be done for completed experiments. When the null hypothesis is not rejected the researcher might want to determine the power which a given statistical test had, before making sweeping conclusions that two strains of fish are not different. Post hoc power analysis will be illustrated with the preliminary data from one rice-fish farm. Power tables are used when the significance criterion ($\alpha$), the effect size (ES), and the sample size are known.

Step 1. Specify the level of $\alpha$

Step 2. Calculate the effect size (d) = $\frac{|\mu nifi - \mu com|}{\sigma}$

$\mu$nifi= 20.94 mm (estimte of change in length)

$\mu$com = 19.60 mm (estimate of change in length)

$\sigma$ = 6.63 mm (estimate common standard deviation)

d = 20.94 - 19.60/6.63 = .20 mm

Step 3. Since there are unequal number of samples in the two populations , the harmonic mean of the samples (n') is computed Cohen (1988)

n'= 2n1n2/(n1+n2)

Table 4.3. Estimates of population means of absolute change in length (in mm), common standard deviation ($\sigma$), effect size (d), number of fish for each strain in each farm (N)and harmonic mean of sample size (n') of NIFI and COM strain

| Farms | Strains | Means | $\sigma$ | d | N | n' |
|---|---|---|---|---|---|---|
| 1 | NIFI | 20.94 | 6.63 | .20 | 18 | 16 |
| | COM | 19.60 | | | 15 | |
| 2 | NIFI | 21.75 | 8.26 | .46 | 16 | 19 |
| | COM | 17.91 | | | 22 | |
| 3 | NIFI | 14.18 | 5.94 | .99 | 17 | 16 |
| | COM | 8.25 | | | 16 | |
| 4 | NIFI | 13.44 | 3.08 | 2.18 | 18 | 14 |
| | COM | 6.73 | | | 11 | |

Effect size = $d = \left|\frac{\mu nifi - \mu com}{\sigma}\right|$ where

$\mu_{nifi}$ = estimates of population mean of NIFI
$\mu_{com}$ = estimates of population mean of COM

$n' = \frac{2N_{nifi}N_{com}}{N_{nifi}+N_{com}}$ where

$N_{nifi}$ = number of NIFI in each farm
$N_{com}$ = number of COM in each farm

$n' = 2NnifiNcom/(Nnifi+Ncom)$

Nnifi = 18, Ncom = 15,

$n' = 2(18)(15)/(18+15) = 16$

Step 4. Use Table 4.4 (Cohen 1988)

For a two tailed t test and at an $\alpha = .05$, the statistical power of the test to detect an effect size of .20 was only about .08. The power to detect a difference of .20 was very low.

The fish were not size graded but the initial size difference of the two strains at the start of strain testing was almost the same (73.55 mm and 73.87 mm for the NIFI and COM strains, respectively).

Farm 1 ( A priori power analysis)

Power analysis is more useful in planning an experiment in an economic context. Once $\alpha$ and the magnitude of difference (ES) are set a priori, e.g. on economic grounds such as acceptable opportunity lost of Type II error, then the sample size that will generate a desired power to detect the effect size can be derived from Sample Tables.

I will use the estimates I obtained from farm 1 to illustrate a priori power calculation. In our example, suppose that a geneticist wants to stock another strain of tilapia in farm 1 and he wants to detect a 10% difference between the new strain and the NIFI strain because he

## Table 4.4. Power table

Power of t test of $m_1 = m_2$ at $a_2 = 05$

| | | d | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n | $d_c$ | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| 8 | 1.07 | 05 | 07 | 09 | 11 | 15 | 20 | 25 | 31 | 46 | 60 | 73 |
| 9 | 1.00 | 05 | 07 | 09 | 12 | 16 | 22 | 28 | 35 | 51 | 65 | 79 |
| 10 | .94 | 06 | 07 | 10 | 13 | 18 | 24 | 31 | 39 | 55 | 71 | 84 |
| 11 | .89 | 06 | 07 | 10 | 14 | 20 | 26 | 34 | 43 | 61 | 76 | 87 |
| 12 | .85 | 06 | 08 | 11 | 15 | 21 | 28 | 37 | 46 | 65 | 80 | 90 |
| 13 | .81 | 06 | 08 | 11 | 16 | 23 | 31 | 40 | 50 | 69 | 83 | 93 |
| 14 | .78 | 06 | 08 | 12 | 17 | 25 | 33 | 43 | 53 | 72 | 86 | 94 |
| 15 | .75 | 06 | 08 | 12 | 18 | 26 | 35 | 45 | 56 | 75 | 88 | 96 |
| 16 | .72 | 06 | 08 | 13 | 19 | 28 | 37 | 48 | 59 | 78 | 90 | 97 |
| 17 | .70 | 06 | 09 | 13 | 20 | 29 | 39 | 51 | 62 | 80 | 92 | 98 |
| 18 | .68 | 06 | 09 | 14 | 21 | 31 | 41 | 53 | 64 | 83 | 94 | 98 |
| 19 | .66 | 06 | 09 | 15 | 22 | 32 | 43 | 55 | 67 | 85 | 95 | 99 |
| 20 | .64 | 06 | 09 | 15 | 23 | 33 | 45 | 58 | 69 | 87 | 96 | 99 |
| 21 | .62 | 06 | 10 | 16 | 24 | 35 | 47 | 60 | 71 | 88 | 97 | 99 |
| 22 | .61 | 06 | 10 | 16 | 25 | 36 | 49 | 62 | 73 | 90 | 97 | 99 |
| 23 | .59 | 06 | 10 | 17 | 26 | 38 | 51 | 64 | 75 | 91 | 98 | * |
| 24 | .58 | 06 | 10 | 17 | 27 | 39 | 53 | 66 | 77 | 92 | 98 | |
| 25 | .57 | 06 | 11 | 18 | 28 | 41 | 55 | 68 | 79 | 93 | 99 | |
| 26 | .56 | 06 | 11 | 19 | 29 | 42 | 56 | 69 | 80 | 94 | 99 | |
| 27 | .55 | 06 | 11 | 19 | 30 | 43 | 58 | 71 | 82 | 95 | 99 | |
| 28 | .54 | 07 | 11 | 20 | 31 | 45 | 59 | 73 | 83 | 96 | 99 | |
| 29 | .53 | 07 | 12 | 20 | 32 | 46 | 61 | 74 | 85 | 96 | 99 | |
| 30 | .52 | 07 | 12 | 21 | 33 | 47 | 63 | 76 | 86 | 97 | * | |
| 31 | .51 | 07 | 12 | 21 | 34 | 49 | 64 | 77 | 87 | 97 | | |
| 32 | .50 | 07 | 12 | 22 | 35 | 50 | 65 | 78 | 88 | 98 | | |
| 33 | .49 | 07 | 13 | 22 | 36 | 51 | 67 | 80 | 89 | 98 | | |
| 34 | .48 | 07 | 13 | 23 | 37 | 53 | 68 | 81 | 90 | 98 | | |
| 35 | .48 | 07 | 13 | 23 | 38 | 54 | 70 | 82 | 91 | 98 | | |
| 36 | .47 | 07 | 13 | 24 | 39 | 55 | 71 | 83 | 92 | 99 | | |
| 37 | .46 | 07 | 14 | 25 | 39 | 56 | 72 | 84 | 92 | 99 | | |
| 38 | .46 | 07 | 14 | 25 | 40 | 57 | 73 | 85 | 93 | 99 | | |
| 39 | .45 | 07 | 14 | 26 | 41 | 58 | 74 | 86 | 94 | 99 | | |
| 40 | .45 | 07 | 14 | 26 | 42 | 60 | 75 | 87 | 94 | 99 | | |
| 42 | .43 | 07 | 15 | 27 | 44 | 62 | 77 | 89 | 95 | 99 | | |
| 44 | .42 | 07 | 15 | 28 | 46 | 64 | 79 | 90 | 96 | * | | |
| 46 | .41 | 08 | 16 | 30 | 48 | 66 | 81 | 91 | 97 | | | |
| 48 | .41 | 08 | 16 | 31 | 49 | 68 | 83 | 92 | 97 | | | |

considers a 10% difference economically beneficially to the farmer. How many individual fish would he require to generate a power of 0.80?

Step 1. Set $\alpha = .10$

Step 2. Compute a 10% difference using the mean estimate of the NIFI strain and compute effect size using the formula

$$(d) = \left|\frac{\mu new - \mu nifi}{\sigma}\right|$$

$$d = \left|\frac{23.03 - 20.94}{6.63}\right| = .32$$

Step 3. Use Table 4.5

Since the geneticist wants to know whether the new strain is better growing than NIFI, then his statistical test is a one-tailed test. At $\alpha = .10$, the sample size required of each strain is 100 individual fish to generate a power of .80. A power of .80 means that there is an 80% chance (probability) that an effect size of .32 will be detected. Conversely, the probability of a Type II error is .20. There is only a 20% chance of not detecting a true difference. At a lower power of .70, the sample size required is only 73 individuals of each strain. At a very high power of .99, the sample size required is 290 individual fish. A power of .99 means

## Table 4.5. Sample size table

| | $a_1 = .01$ ($a_2 = .02$) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 547 | 138 | 62 | 36 | 24 | 17 | 13 | 10 | 7 | 5 | 4 |
| .50 | 1083 | 272 | 122 | 69 | 45 | 31 | 24 | 18 | 12 | 9 | 7 |
| .60 | 1332 | 334 | 149 | 85 | 55 | 38 | 29 | 22 | 15 | 11 | 8 |
| 2/3 | 1552 | 382 | 170 | 97 | 62 | 44 | 33 | 25 | 17 | 12 | 9 |
| .70 | 1627 | 408 | 182 | 103 | 66 | 47 | 35 | 27 | 18 | 13 | 10 |
| .75 | 1803 | 452 | 202 | 114 | 74 | 52 | 38 | 30 | 20 | 14 | 11 |
| .80 | 2009 | 503 | 224 | 127 | 82 | 57 | 42 | 33 | 22 | 15 | 12 |
| .85 | 2263 | 567 | 253 | 143 | 92 | 64 | 48 | 37 | 24 | 17 | 13 |
| .90 | 2605 | 652 | 290 | 164 | 105 | 74 | 55 | 42 | 27 | 20 | 15 |
| .95 | 3155 | 790 | 352 | 198 | 128 | 89 | 66 | 51 | 33 | 23 | 18 |
| .99 | 4330 | 1084 | 482 | 272 | 175 | 122 | 90 | 69 | 45 | 31 | 23 |
| | $a_1 = .05$ ($a_2 = .10$) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 189 | 48 | 21 | 12 | 8 | 6 | 5 | 4 | 3 | 2 | 2 |
| .50 | 542 | 136 | 61 | 35 | 22 | 16 | 12 | 9 | 6 | 5 | 4 |
| .60 | 721 | 181 | 81 | 46 | 30 | 21 | 15 | 12 | 8 | 6 | 5 |
| 2/3 | 862 | 216 | 96 | 55 | 35 | 25 | 18 | 14 | 9 | 7 | 5 |
| .70 | 942 | 236 | 105 | 60 | 38 | 27 | 20 | 15 | 10 | 7 | 6 |
| .75 | 1076 | 270 | 120 | 68 | 44 | 31 | 23 | 18 | 11 | 8 | 6 |
| .80 | 1237 | 310 | 138 | 78 | 50 | 35 | 26 | 20 | 13 | 9 | 7 |
| .85 | 1438 | 360 | 160 | 91 | 58 | 41 | 30 | 23 | 15 | 11 | 8 |
| .90 | 1713 | 429 | 191 | 108 | 69 | 48 | 36 | 27 | 18 | 13 | 10 |
| .95 | 2165 | 542 | 241 | 136 | 87 | 61 | 45 | 35 | 22 | 16 | 12 |
| .99 | 3155 | 789 | 351 | 198 | 127 | 88 | 65 | 50 | 32 | 23 | 17 |
| | $a_1 = .10$ ($a_2 = .20$) | | | | | | | | | | |
| | d | | | | | | | | | | |
| Power | .10 | .20 | .30 | .40 | .50 | .60 | .70 | .80 | 1.00 | 1.20 | 1.40 |
| .25 | 74 | 19 | 9 | 5 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |
| .50 | 329 | 82 | 37 | 21 | 14 | 10 | 7 | 5 | 4 | 3 | 2 |
| .60 | 471 | 118 | 53 | 30 | 19 | 14 | 10 | 8 | 5 | 4 | 3 |
| 2/3 | 586 | 147 | 65 | 37 | 24 | 17 | 12 | 10 | 6 | 4 | 3 |
| .70 | 653 | 163 | 73 | 41 | 27 | 19 | 14 | 11 | 7 | 5 | 4 |
| .75 | 766 | 192 | 85 | 48 | 31 | 22 | 16 | 13 | 8 | 6 | 4 |
| .80 | 902 | 226 | 100 | 57 | 36 | 26 | 19 | 14 | 10 | 7 | 5 |
| .85 | 1075 | 269 | 120 | 67 | 43 | 30 | 22 | 17 | 11 | 8 | 6 |
| .90 | 1314 | 329 | 146 | 82 | 53 | 37 | 27 | 21 | 14 | 10 | 7 |
| .95 | 1713 | 428 | 191 | 107 | 69 | 48 | 35 | 27 | 18 | 12 | 9 |
| .99 | 2604 | 651 | 290 | 163 | 104 | 73 | 53 | 41 | 26 | 18 | 14 |

that there is a 99% chance of detecting an effect size of 0.32 if it exists.

Farm 3 (Post hoc analysis)

Suppose that the difference obtained between the NIFI and COM strains was a true difference, the power of the test to have detected the difference can also be derived from Power Tables.

Step 1. Specify the level of $\alpha$

Step 2. Calculate the effect size (d) = $\left|\frac{\mu nifi - \mu com}{\sigma}\right|$

$\mu$nifi= 11.18 = population mean of NIFI

$\mu$com = 8.25 = population mean of COM

$\sigma$ = 5.94 = common standard deviation

d = 14.18 - 8.25/5.94 = .49

Step 3. For unequal number of samples in the two populations , the harmonic mean of the samples (N') will be computed according to the formula

n`= 2NnifiNcom/(Nnifi+Ncom)

Nnifi = 17, Ncom = 16, substitute in the formula

n'= 2(16)(22)/(16+22)= 16

Step 4. Use Table 4.4

The test was a non-directional test since it was not set a priori which strain will grow better. So, at a two tailed t test and at an $\alpha$ = .05, the statistical power of the test to detect an effect size of .49 was a power of .29. There was only 29% chance to detect an effect size of 0.49 mm.

I have shown with the examples above that power analysis can be computed after the experiment is done (post hoc analysis) However, the more important aplication of power analysis is in designing experiments. The smaller the effect size, the larger the necessary samples and large power requires a large number of observations.

Farm/strain comparison

Significant F-ratios were obtained in the two-way ANOVA with absolute growth (DL) and specific growth (G) as dependent variable and farm and strain as independent variables (Table 4.6). CIRCM did not show any significant F-ratios. There was no significant farm and strain interaction but the hypothesis command in the SYSTAT module showed that both strains were faster in farms 1 and 2 than in farms 3 and 4.

In farms 3 and 4 the NIFI strain were initially smaller than the COM strain (Table 4.1) After 96 days of culture in these farms, the NIFI strain grew faster than the COM strain. This growth difference was reflected in

Table 4.6. Two-way factorial ANOVA of change in length (DL), specific growth (G), and CIRCM by farm and strain and the interaction between farm and strain

$DLijk = \mu + Fi + Sj + (FS)ij + Eijk$

| Source | df | MS | F | P |
|---|---|---|---|---|
| Farm | 3 | 946.839 | 22.895 | 0.000 |
| Strain | 1 | 638.344 | 15.435 | 0.000 |
| Farm/strain | 3 | 45.017 | 1.089 | 0.357 |
| Error | 125 | 41.356 | | |

$R^2 = .391$

$Gijk = \mu + Fi + Sj + (FS)ij + Eijk$

| | | | | |
|---|---|---|---|---|
| Farm | 3 | 0.111 | 15.631 | 0.000 |
| Strain | 1 | 0.139 | 19.654 | 0.000 |
| Farm/strain | 3 | 0.012 | 1.680 | 0.175 |
| Error | 125 | 0.007 | | |

$R^2 = .338$

$CIRCMijk = \mu + Fi + Sj + (FS)ij + Eijk$

| | | | | |
|---|---|---|---|---|
| Farm | 3 | 456.923 | 7.127 | 0.000 |
| Strain | 1 | 23.479 | 0.366 | 0.546 |
| Farm/strain | 3 | 60.749 | 0.948 | 0.420 |
| Error | 125 | 64.110 | | |

$R^2 = .160$

the analysis of variance (Table 4.7a,b,c). However, analysis of covariance showed a highly significant initial size effect. The variance due to initial length was very high especially in farm 3 ($P<.0001$). Use of initial length as a covariate did not remove the significant strain effect in farm 4, however. The negative Pearson correlation coefficient between growth and initial length in farms 3 and 4 is shown in Table 4.2b. The r values ($r= -.844$ and $-.718$, in farms 3 and 4) are highly significant ($P<.001$). It is apparent from the results that under rice-fish condition, smaller fish grow faster than bigger fish. It can be seen in Table 4.1 that in farms 3 and 4, the difference in size between strains was much less at the end of the grow-out period than at the beginning (compensatory growth). This was not true in farms 1 and 2.

## DISCUSSION

### Validation of the CIRC technique

Circulus spacing at the growing margin of the scales (CIRCM) of the NIFI and COM strains was significantly correlated with growth rates in rice-fish farms 1 and 2 but not as high as the 0.7 to 0.8 range reported in previous studies of tilapia (Matricia et al., 1989; Sriputinibondh 1988; Doyle et al., 1987). A low but positive correlation was also shown in farm 3. When all

Table 4.7a. ANOVA and ANCOVA of DL by farm. Len0 was used as covariate in the analysis

| | ANOVA | | | ANCOVA | | | |
|---|---|---|---|---|---|---|---|
| Farm 1 | | | | | | | |
| Source | DF | MS | F | Source | DF | MS | F |
| Strain | 1 | 14.789 | 0.336 | Strain | 1 | 11.937 | 0.333 |
| | | | | Len0 | 1 | 286.469 | 7.896* |
| Error | 31 | 43.953 | | Error | 30 | 35.969 | |
| $R^2$ = .011 | | | | $R^2$ = .219 | | | |
| Farm 2 | | | | | | | |
| Strain | 1 | 136.656 | 2.002 | Strain | 1 | 89.944 | 1.440 |
| | | | | Len0 | 1 | 271.038 | 4.340* |
| Error | 36 | 68.245 | | Error | 35 | 62.451 | |
| $R^2$ = .053 | | | | $R^2$ = .157 | | | |
| Farm 3 | | | | | | | |
| Strain | 1 | 289.499 | 8.207* | Strain | 1 | 8.151 | 0.629 |
| | | | | Len0 | 1 | 704.545 | 54.345* |
| Error | 31 | 35.273 | | Error | 30 | 12.964 | |
| $R^2$ = .209 | | | | $R^2$ = .719 | | | |
| Farm 4 | | | | | | | |
| Strain | 1 | 308.063 | 32.412* | Strain | 1 | 59.220 | 7.178* |
| | | | | Len0 | 1 | 42.128 | 5.106* |
| Error | 27 | 9.505 | | Error | 26 | 8.250 | |
| $R^2$ = .546 | | | | $R^2$ = .620 | | | |

*Significant at P<.0001

Table 4.7b. ANOVA and ANCOVA of G by farm. Len0 was used as covariate in the analysis

| | ANOVA | | | ANCOVA | | |
|---|---|---|---|---|---|---|
| Farm 1 | | | | | | |
| Source | DF | MS | F | Source | DF | MS | F |
| Strain | 1 | 0.002 | 0.360 | Strain | 1 | 0.002 | 0.450 |
| | | | | Len0 | 1 | 0.090 | 22.538* |
| Error | 31 | 0.007 | | Error | 30 | 0.004 | |
| $R^2$ = .011 | | | | $R^2$ = .436 | | | |
| Farm 2 | | | | | | | |
| Strain | 1 | 0.025 | 2.231 | Strain | 1 | 0.014 | 1.563 |
| | | | | Len0 | 1 | 0.093 | 10.377* |
| Error | 36 | 0.011 | | Error | 35 | 0.009 | |
| $R^2$ = .058 | | | | $R^2$ = .274 | | | |
| Farm 3 | | | | | | | |
| Strain | 1 | 0.065 | 9.559* | Strain | 1 | 0.002 | 1.124 |
| | | | | Len0 | 1 | 0.150 | 72.952* |
| Error | 31 | 0.007 | | Error | 30 | 0.002 | |
| $R^2$ = .236 | | | | $R^2$ = .719 | | | |
| Farm 4 | | | | | | | |
| Strain | 1 | 0.075 | 35.889* | Strain | 1 | 0.009 | 6.169* |
| | | | | Len0 | 1 | 0.019 | 13.037* |
| Error | 27 | 0.002 | | Error | 26 | 0.001 | |
| $R^2$ = .546 | | | | $R^2$ = .620 | | | |

*Significant at P<.001

Table 4.7c. ANOVA and ANCOVA of CIRCM by farm. Len0 was used as a covariate in the analysis

| ANOVA | | | | ANCOVA | | | |
|---|---|---|---|---|---|---|---|
| **Farm 1** | | | | | | | |
| Source | DF | MS | F | Source | DF | MS | F |
| Strain | 1 | 25.180 | 0.531 | Strain | 1 | 21.538 | 0.537 |
| | | | | Len0 | 1 | 266.501 | 6.644* |
| Error | 31 | 47.411 | | Error | 30 | 40.108 | |
| $R^2$ = .017 | | | | $R^2$ = .195 | | | |
| **Farm 2** | | | | | | | |
| Strain | 1 | 0.365 | 0.005 | Strain | 1 | 13.774 | 0.211 |
| | | | | Len0 | 1 | 589.718 | 9.017** |
| Error | 36 | 79.965 | | Error | 35 | 65.401 | |
| $R^2$ =.000 | | | | $R^2$= .205 | | | |
| **Farm 3** | | | | | | | |
| Strain | 1 | 0.006 | 0.0001 | Strain | 1 | 28.502 | 0.468 |
| | | | | Len0 | 1 | 137.422 | 2.255 |
| Error | 31 | 63.409 | | Error | 29 | 60.942 | |
| $R^2$ = .000 | | | | $R^2$ = .070 | | | |
| **Farm 4** | | | | | | | |
| Strain | 1 | 170.803 | 2.714 | Strain | 1 | 34.469 | 0.534 |
| | | | | Len0 | 1 | 21.483 | 0.333 |
| Error | 27 | 62.945 | | Error | 25 | 64.540 | |
| $R^2$ = .091 | | | | $R^2$ = .103 | | | |

*Significant at 0.015 P level
**Significant at 0.005 P level

fish were pooled in farm 4 (Table 4.2b), there were low negative correlations between growth rates and CIRCM. These low negative correlations (-0.114 and -0.180 for absolute and and specific growth rates, respectively) were due to the NIFI strain in farm 4. In this farm, there was also a low and non-significant positive correlation between CIRCM and initial size within the NIFI strain. In contrast the negative correlations between growth rates and initial size in farm 4 were highly significant. This negative correlation is expected of fish growing at maximum rates (Brett 1979). In farm 4, fish from the NIFI strain may have been growing faster than the COM strain.

When growth is reasonably good, it is correlated with CIRC (farms 1 and 2). When growth is poor (farms 3 and 4) the correlation breaks down either because, when smaller fish are growing faster they may be forming circuli more frequently than larger fish growing at the same rate, or that the correlation breaks down when there are large numbers of slow-growing or non-growing fish (Talbot and Doyle 1992). In a previous study on tilapia, Sinclair (1989) reported a positive correlation between CIRCM and size but the relationship was attributed to the growth advantage gained by larger fish over smaller fish within a mixed-size group of tilapia.

Size-dependence of growth

In all four farms, there was a negative correlation between growth rates and initial size. This negative correlation was, however, much higher in farms 3 and 4 (Table 4.2b). Negative correlation is an indication of growth compensation when small fish are growing close to their maximum rates (Brett and Shelbourn 1975; Elliot 1975; Jobling 1983). The observed differences in the growth rates of the NIFI and COM strain in farms 3 and 4 were just environmental artifacts and not true genetic differences. The NIFI strain was also smaller than the COM strain in farms 1 and 2 but the mean size difference was negligible and the size range of individuals of the two strains in both farms were the same. The growth of fish in farms 1 and 2 was dominated by the effect of the final size, whereas in farms 3 and 4, growth was dominated by the strong initial size difference.

Results from farms 1 and 2 (same size range) were analogous to the results obtained when fish were size-graded and compared under experimental conditions (Chapter 3). In both situations, no significant differences were found between the two strains. Results from farms 3 and 4 were likewise analogous to results of mixed-size groups under experimental conditions. Signficant differences were observed whenever there was a big initial size difference between the two strains. Under experimental conditions, the strain that had an initial

size advantage grew fastest. Growth depensation was indicated when the smaller strain remained smaller. In rice-paddies, on the other hand, smaller fish grew fastest (growth compensation). In both situations, the observed differences between the two strains was an environmental artifact induced by initial size difference. In fish, size is generally considered more important than age as a determinant of growth (Larkin et al., 1956; Parker and Larkin 1959; Doyle et al., 1987).

Between-farm variation

Farming practice played an important role in the oberved between-farm variation in growth rates of fish. Both NIFI and COM strains were growing faster in farms 1 and 2 than in farms 3 and 4. These four farms are located in four different localities in the same geographical area in Central Luzon, Philippines. Farms 1 (850 sq. m.) and 2 (600 sq. m.) are smaller in size than farms 3 (1,200 sq. m.) and 4 (1,250 sq. m.). In all four rice farms only 10% of the total area is used as pond refuge for fish. Water management differed in these four farms. Farms 1 and 2 had more access to irrigation water and the depth of water at the pond refuge was maintained at about 1 meter. Water source was a problem with the other two farms, especially farm 3. Water was maintained at less than a meter deep in farm 3 and about half-meter deep in farm 4. Farm 3, which was managed by a senior high school student,

seemed to be the most artisanal in terms of logistic support like water quality maintenance, fertilizers etc. Farms 1 and 2 are both owned by farmers who are economically well off relative to farmers 3 and 4. Except for farm 2, all the farms are practically backyard farms where each farm is located beside the farmer's house.

Rice-fish culture had a long tradition in Asia before the Green Revolution but is a relatively new farming system introduced in the Philippines only about 15 years ago (Bimbao et al 1990). It is a sustainable aquaculture that offers a viable option for rural inhabitants to increase protein production and income. About 80% of the fish produced in Philippine rice farms are consumed by the farmer's household (Tagarino 1985). A tilapia selected for fast growth in rice farms will definitely improve the small farmer's protein requirement. Excess fish not consumed by the family can be marketed and become an extra source of income.

## Implications of the size-dependency of growth in aquaculture

Initial size differences which can lead to either growth depensation which seemed to occur under experimental conditions (Chapter 3 of this thesis) and growth compensation which was shown in the rice-paddy data may have important bearing on genetic improvememt

programmes in aquaculture. In growth depensation, growth differences will have strong non-genetic components because fish that are relatively small for environmental reasons (asynchronous spawing, maternal effects) will remain small and will not reflect a true genetic difference ( Wohlfarth and Moav 1972). Likewise, if growth compensation is occuring as shown in the current data, smaller fish will be growing the fastest which is also an environmental artifact. In either case, an initial size difference between genotypes will have detrimental effects on the success of artificial selection because it will affect the results of genetic experiments. This exaggeration or compensation of initial size differences could make strain evaluation difficult if the initial size differences are not genetically based.

Size-grading or having almost the same size range among genotypes may help minimize environmentally induced variation like initial size differences.

# Chapter 5

## Conclusions

1. Initial size differences can induce non-genetic significant differences in growth rates.
2. Growth depensation was apparent under experimental cage and tank environments.
3. Growth compensation seemed to occur under rice-fish farm conditions.
4. Communal rearing of mixed-size strains can magnify initial size differences between strains and lead to environmentally induced variation in growth rates.
5. Use of an internal control fish as a covariate to reduce environmental error is inefficient when fish are of mixed-sizes and ages.
6. Use of the absolute increase in growth as a measure of growth can give misleading results because of initial size differences.
7. Mixed-size rearing of fish can lead to Type I error (strains are taken to be different when they are not) and Type II error (do not detect a true difference).

8. The problem of initial size variation can be overcome by size-grading or size-matching. Size-grading appears to be the most efficient method of minimizing environmental sources of variation in strain testing. Size-grading is more powerful than mixed size rearing at detecting true differences.

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