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STATISTICAL DESIGNING OF FISH NUTRITION EXPERIMENTS

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It is the pioneering work of two renowned statisticians
Prof. R.A. Fisher and Dr. F. Yates in the nineteen thirtees
which laid the foundation for the development of the subject
'design of experiments'. The subject had a phenomenal
growth to cope up with the fast increasing needs of experi-
mental sciences. Today it is a very well established branch
of applied statistics and there are several text books and a
large number of theoretical and applied papers in literature.
The present note discusses briefly the need for statistical
designing and presents some simple designs useful for fish
nutrition experiments.

1. Need for Statistical Designing

The validity of the findings of scientific experiments
depends on the type of data collected and their amenability
to statistical analysis. Considering the importance, stati-
stical methods are now taught as compulsory subjects in all
the professional courses. Even then, many a research worker
feels hesitant to avail of the appropriate statistical
back-up for his experimental programmes. No doubt a stati-
stical design has an underlying theoretical model and assu-
mptions. But the applicational part is relatively simple
and easy to implement. A little experience would convince

that designing ensures objectivity in the procedures and adds confidence to the ensuing results.

Statistical designing involves the formulation of a scheme or a lay-out plan where the placements of treatments in experimental units are specified keeping in view the objectives of the programme and the statistical requirements. Here the word 'treatments' is used in a general sense and may mean factors like levels of feeding, doses of stimulae and stocking densities.

Consider 4 feeds denoted by A, B, C and D in a fish culture experiment for comparison of growth rates. Let there be 20 ponds/sub-ponds which can be taken to be homogeneous. Under the simplest design, namely, 'Completely Randomised Design' the feeds are randomly allotted to the different ponds and a lay-out is as follows.

D	B	D	C	A
A	C	A	B	C
A	B	C	B	D
C	D	D	A	B

Each feed is replicated five times and they are randomly allotted. Do you need these replications and the randomisation process bringing in constraints and affecting the freedom of the experimenter? Here comes the basic question, namely, why statistical designing?

Variability in experimental material is an inevitable feature in any field of research. Consider for example two fish culture ponds kept under conditions as similar as possible with the same area, species, stocking density etc.

At the time of harvesting one would find that the yield of one pond is different from the other. This may be attributed to the uncontrolled variation inherent in the production process. Consider another two ponds kept under almost identical conditions except that in one pond supplementary feed is given. Here again, at the time of harvesting the yields would be found to be different. Can we attribute the difference to the effect of levels of feeding? We cannot. May be the supplementary feed did not contribute anything to the difference in yield and the difference could be purely due to the inherent uncontrolled variation. Differences are expected even when similarity is maintained in the two ponds.

Thus variation introduces a degree of uncertainty into the conclusions that are drawn from the results. The observed variation between treatments may be partly due to real treatment differences if there are any and partly to the uncontrolled factors (commonly called experimental error) which influence yield even in the absence of any real treatment differences. It is therefore necessary to evaluate the magnitude of variation due to experimental error and compare with it the observed variation between treatments through an appropriate test of significance to conclude whether the experiment indicates any real differences in the effects of treatments. Only a statistically designed experiment can result in the estimation of the different components of variation and permit valid test of significance involving probability statements whether a particular difference is due to chance causes or can be attributed to the real difference between treatments.

2. Principles of Designing

Two primary requisites in designing experiments are replication and randomisation. Replication or repetition of treatments provide stability to the mean but more than that makes it possible to estimate the experimental error by say, considering the differences between units under the same treatments in different replications as in a completely randomed design. It also increases the precision of the estimates of both the treatment mean and the experimental error.

Randomisation which means random allocation of treatments to various experimental units, insures that a treatment will not be unduly favoured or handicapped in successive replications. It ensures unbissedness of the estimates of experimental error and provide for valid treatment comparisons against the experimental error (Fisher, 1954). When treatments are replicated and allocated randomly to the various units we are in a position to test the significance of observed treatment differences by the use of test of significance procedures. Thus it is essential to provide for adequate number of replications and ensure proper randomisation at the planning stage (Panse, et al., 1964).
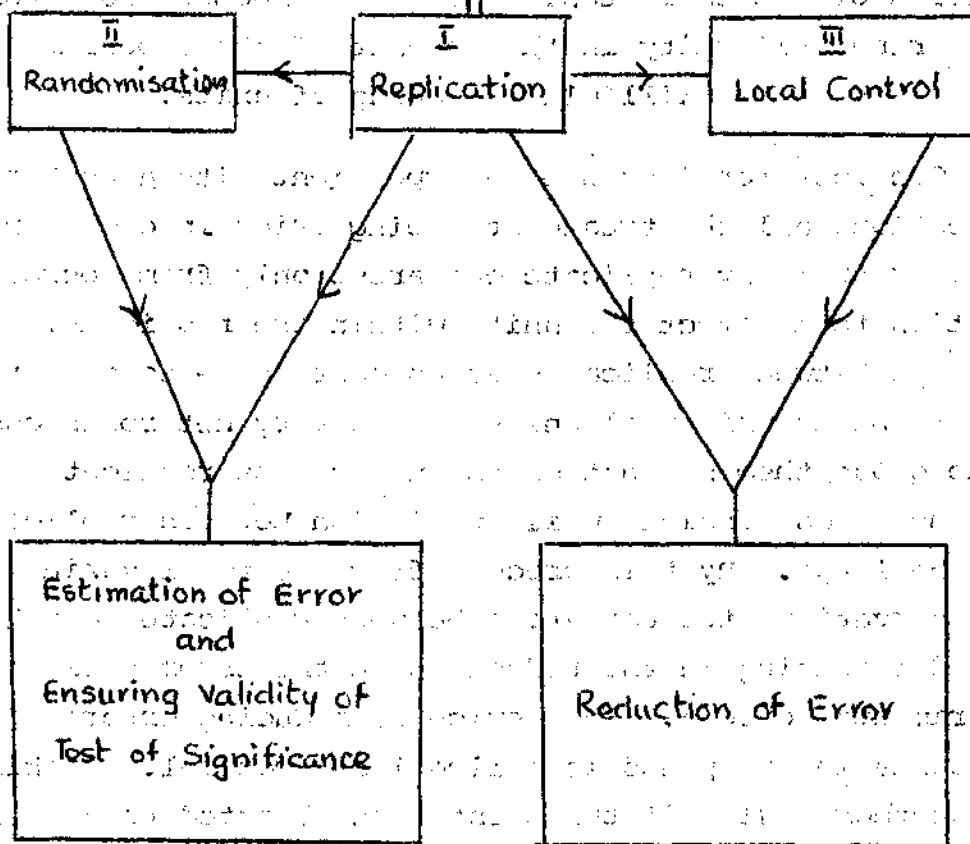
As indicated earlier the results of an experiment are affected not only by the action of treatments but also extraneous variation which tend to mask the effects of treatments. This extraneous variation is conventionally termed as experimental error (or sometimes called 'error'), where the word 'error' is not synonimous with mistakes but indicates all types of extraneous variation (Cochran, et al., 1973). There are two types of experimental errors, one refers to the inherent variability in the experimental material or units to which the treatments are applied and the other type

refers to the failure to standardise the experimental technique. It is desirable that the experimental error is kept as minimum as possible as otherwise a large difference in the treatment means will only be detected as significant. Reduction of experimental error automatically increases the precision of the estimates. One way to reduce the error is by ensuring uniformity in the conduct of the experiment. Another way is by skillfully grouping of units.

Consider for instance an experiment with a number of replications all the treatments being tried in each replicate. The error from any replicate can arise only from sources of variation that affect the units within the replicate. Variation between replicates do no contribute to the error. Thus if the experimental units form a very heterogeneous set, try to group them so that units in the same replicate is as homogeneous as possible while variation between replicates could be large. By this process from the total variation in the observations the variation between replicates can be removed resulting in the reduction in the error variance (experimental error). The device of reducing errors through such suitable groupings is called local control. Looking from another angle, if treatments are allotted to a replication with homogeneous units their differences indicate the real variations between the treatments. The principle of local control is the basis for experimental designs such as 'randomised blocks' and 'latin squares'. Where the number of treatments to be accommodated in a replicate becomes large, the homogeneity within a replicate tends to be lost and can be restored by dividing the replication into smaller blocks which is the basis of confounding in factorial experiments and also various incomplete block designs.

A flow - chart indicating the three principles of designing and their functions is shown below:

Three Principles of Designing and their Functions

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┌──────────────────┐      ┌──────────────────┐      ┌──────────────────┐
│       II         │ ←─── │        I         │ ───→ │       III        │
│  Randomisation   │      │   Replication    │      │  Local Control   │
└──────────────────┘      └──────────────────┘      └──────────────────┘
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┌─────────────────────────┐        ┌─────────────────────────┐
│  Estimation of Error     │        │                         │
│         and              │        │                         │
│  Ensuring Validity of    │        │   Reduction of Error    │
│  Test of Significance    │        │                         │
└─────────────────────────┘        └─────────────────────────┘
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3. Some Designs Useful For Nutrition Experiments

(i) Randomised block

One of the most commonly used plans is the randomised block design where experimental material is divided into block each of which constitute a single replicate in such a way that the units within a block is as homogeneous as possible. The treatments are now randomly allotted to the experimental units within a block. This increases the comparability of treatment effects

as they act under conditions which are similar except for the treatments. For instance in an experiment to select an economic supplementary feed mixture from among 4 prepared mixtures for prawn culture, 4 ponds all located by the side of the main water body like the backwater or estuary could be grouped as one block or replication and allot treatments at random. The next 4 could be ponds running paralled to the first set but more inside the land so that within a block salinity and associated features are likely to be similar. This arrangement takes care to a good extent salinity gradient likely to be reducing when moved away from the main water body. In the experiments if there are 5 replications there will be total 20 ponds. If all the 20 ponds are more or less similar no blocking or stratification is required and the treatments could be randomly allotted over the entire range of the 20 ponds. Such a design is called completely randomised design. However if heterogeneity in the features in the ponds is suspected it is desirable to provide blocks which may help in reducing the experimental error. A lay-out plan for randomised block design is given below

Block

I	II	III	IV	V
B	A	B	C	D
D	C	C	D	A
C	B	D	A	B
A	D	A	B	C

(ii) Latin square

In randomised blocks one-way restriction is imposed. If heterogeneity is suspected in two directions the experimental area can be divided into rows and columns and treatments are applied in such a way that a treatment appears only once in a row and once in a column. Such an arrangement is called a latin square design. Through elimination of row and column effects the residual error variance may be very much reduced. With two-way stratifications the latin square controls more variation than randomised block design resulting often in smaller error mean square. However the number of treatments is limited to the number of rows or columns and for large number of treatments it is no preferred.

(iii) Factorial experiments in complete and incomplete blocks

Consider an experiment to study the effect of different levels of protein and energy on weight of fish in culture ponds. If there are say 2 levels for each factor there will be in all 4 (2^2) treatment combinations. A group of treatments which contains two or more levels of two or more factors in all combinations is known as the factoral arrangement. The different combinations could be allotted as in a randomised block design. The experimenter could try a one-factor-at-a-time approach. The advantage in factorial experiments is that not only the main effects but also the interactions between factors can be studied and tested for statistical significance.

If the number of factors and levels are large, say, 3 factors salinity, temperature and oxygen content at 3 levels each, the number of treatment combinations will be 27 (3^3). It may be difficult to get 27 experi-

mental ponds, which are more or less homogeneous with regard
to factors other than being tested so that the principle of
stratification to reduce experimental error cannot be
implemented. An ingenious device to overcome this situation
is called confounding where a homogeneous block will not
accommodate the full replication. One replication is
divided into say, 3 compact blocks such that the units in
the smaller blocks are homogeneous. The 27 treatment
combinations can be divided into 3 groups of 9 each and
allotted to the 3 compact blocks (Cochran and Cox, 1973).

(iv) Switch-over

There are occasions in which treatments are applied in
sequence over several periods on a group of individuals.
Consider an experiment to study the effect of mineral
supplementation of two types on lobsters kept in artificial
tanks. If there are say, 12 groups of lobsters separated
and kept in tanks with sub-partitioning, then the two types
of supplementations are given such that half the groups
received say, type A and the other half type B in period 1.
The lobsters receiving type A in period 1 will get type B
in period 2 and vice versa. Such a design is called switch-
over or change-over design (Federer, 1973). On the other
hand if a time trend is expected in the character under
study a switch-back or a double reversal design will have to
be used. In these procedures a rest period is to be provided
between two treatment periods so that there is no carry-over
effect or residual effect influencing the treatment
during the second period. However if a reasonably long rest
period is not feasible or the residual-effect is itself a
topic of interest the procedure is to be modified so that
direct and residual effects of treatments can also be
measured.

4. Statistical Analysis

Once the data become available it is essential to follow appropriate statistical procedures for analysis. The type of analysis basically depends on the design used. Computational details for forming the analysis of variance table and for performing test of significance are available in several publications (Cochran and Cox, 1973).

Some experimenters do not bother to follow a design but try to analysis the data statistically. Some others follow a design but do not care to follow the appropriate procedure of analysis. It is essential in scientific experimentation to follow a suitable design and analyse the data through appropriate procedures.

5. Number of Replications

One aspect need to be stressed here, namely, the provision of enough number of replications in an experiment. Consider the earlier example of 4 feed mixtures which are tried for economic evaluation in prawn culture. If the mixtures are allotted only one each in four ponds without replication we will get only a single figure on, say, cost of production of a unit weight, for one mixture. Thus with four treatments the character under study will have only four values, a single value for each, and no statistical analysis is possible (Jacob et al., 1978). One way is to partition the ponds into 4 sub-ponds which may provide 20 values, 5 each for one treatment for analysis. It may be stressed that apart from reducing experimental error replication of treatments alone can provide an estimate of the experimental error essentially needed for treatment comparisons.

The question of minimum number of replications required is of great importance in aquaculture experiments because of the cost involved and the inherent special problems compared to experiments on land. An important consideration in determining the minimum number of replications is that the test of significance should be sufficiently sensitive to detect real difference between treatments as distinguished from variation due to chance causes. The sensitiveness of the test will depend primarily on the magnitude of variations in the experimental units with regard to the character under study. If the magnitude is known the number of replications required for detecting a particular difference with certain confidence can be worked out (Fedever, 1967, Panse et al., 1964). In the absence of any knowledge regarding the magnitude of variability the number of replications could be decided in such a way that at least 12 degrees of freedom are ensured for error. This is inferred from the fact that the tabulated value of 'F' at the conventional level of significance of 5 per cent ceases to fall off rapidly for degrees of freedom beyond 12. On this basis the minimum number of replications can be worked out for a particular design.

6. Concluding Remarks

The need for statistical designing and some guidelines for planning experiments have been dealt with in the preceeding pages. Some of the simple designs which can be used in fish nutrition experiments have been presented. The references given at the end would provide a wide range of useful designs. However, it may be observed that considering the resources available and the special nature of certain problems some amount of tailoring may have to be resorted to, to suit particular situations. Once the data are acquired, statistical analysis appropriate to the design employed has to be carried out so as to arrive at conclusions relating to the hypothesis under test.

To sum up, statistical designing of experiments attempts to minimise the effects of heterogenity in experimental units from treatment comparisons, reduce experimental error, provide unbiassed estimates and ensure validity in test procedures. The test of significance emanating from the design exerts a sobering influence on the type of experimenter who jumps to exciting conclusions that can as well be ascribed to the natural variation inherent in the experiment (Cochran, et al., 1973).

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