LARVAL SURVIVAL: EVALUATION AND STATISTICAL ANALYSIS FOR ITS DETERMINATION IN FISH CULTURE.

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ABSTRACT.-

Different methods of sampling to estimate mortality in larval culture tanks are presented and evaluated. Different statistical treatments specially designed to analyse survival are also described; direct, Kaplan-Meier, and actuarial or Cutler-Ederer. Additionally, the most commonly used tests of survival comparison between different groups are included (Breslow and Mantel-Cox, Log-Rank, Lee & Desu). A final evaluation is made of the suitability of the application of these methods against other statistical calculations with the aim of a standardisation of criteria in the analysis of larval survival.

INTRODUCTION.-

There are numerous publications on fish culture which refer to survival, above all in those dealing with larval culture. The method of estimation varies from one author to another; most use methods of estimation by syphoning from the bottom and counting dead larvae, while the rest use methods involving a count of live larvae through sampling through the use of beakers, in which, clearly only in the case of all the larvae being transferred by beaker to another tank and being counted in turn does the most exact method result. The estimation is expressed as a number or a percentage and the statistical analysis is performed in different ways (ANOVA, Student t-test, Pearson's correlation, etc.).

The aim of this work is to propose a specific methodology for the determination of survival commonly applied in biomedical studies, but which is equally applicable in
survival studies of any species in experimentation; these are procedures which deal directly and specifically with survival analysis, in which we propose the creation of a standard for application as well as a method of sampling permitting the use of these analyses.

**METHODOLOGY.**

**Sampling methods.**

Knowing the exact initial number of larvae, different samplings were made in tanks with an average capacity of 1000 l, 1.20 m in diameter, and with a bottom slope of 3%, with the purpose of estimating the error committed and to find out the reliability of each. The first estimation consisted of sampling the number of live larvae through use of beakers. This was carried out with different concentrations of larvae and by different persons, at the same time procuring that larval distribution was as homogeneous as possible. The second type of sampling consisted of syphoning the bottom, also using different larval densities and different persons, and counting the total of dead larvae collected, or counting the aliquotes, according to the methods established for zooplankton samples (Frontier, S., 1969 and Frontier, S., 1972).

**Methods of statistical analysis.**

The statistical technique is called follow-up life table. This basically consists of establishing periods of time from the beginning of observation. Once the time intervals have been established, which must be considered as periods of one day in the case of larval experimentation, the probabilities of an individual surviving from the beginning of observation until the end of an experimental interval, and of surviving until the end of the experiment are established. According to Matthews D. & Farewell V., Carrasco J. and the manuals of statistical packages (specified later), the probability of surviving to the end of a time period \( k \) will be equal to the probability of surviving to the end of the period \( k-1 \) multiplied by the probability of surviving to the end of interval \( k \) after having survived to the end of the interval \( k-1 \):

\[
P(k) = P(k-1) \times P(k/k-1).
\]

There will be individuals which survive to the end of the study, and others which survive a determined period of time, a period which will be common to other individuals (Fig 1). Those individuals which have been observed for \( t \) units of time without having died are denominated as censored and are considered to have a censored time of survival. To analyse survival rates several methods are presented: direct, Kaplan-Meier, Actuarial. Each method is briefly described, in accordance with the bibliography cited above. The direct method is the least precise, despite being the simplest. The survival rate is calculated as
a probability by dividing the number of individuals which survive to the end of the experiment by the number at the beginning of the experiment, ignoring the missing cases. The method of Kaplan-Meier (1958) divides time into irregular intervals, defined by successive deaths. The method uses the concept of conditioned probabilities, and the product of these provides the cumulated survival rates calculating the standard error as a function of the cumulated rate, the number of cases exposed at the beginning and the number of deaths at the end of each interval, according to Greenwood's formula.

\[ EE = \sqrt{\frac{\sum \text{Death}}{\text{Exposed}(\text{Exposed} - \text{Death})}} \]

The Actuarial method (Cutler and Ederer, 1958) follows a statistical procedure like that described above, but permits the predetermination of the time intervals in such a way that the duration of each is constant. The probability of survival is calculated as cumulated probability, this being the product of successive conditioned probabilities.

\[ \text{COND. PROB.} = \frac{[\text{EXP. ST. INTV.}] - [\text{DEATH, INTV.}]}{[\text{EXP. ST. INTV.}]} \]

COND. PROB. = CONDITIONED PROBABILITY
EXP. ST. INTV. = EXPOSED TO RISK START INTERVAL

The graphical result of a survival curve will be similar to that shown in fig. 2. In general, although the sample may be small, the calculations become very complex and it is necessary to carry them out through the use of a computer. Among the statistical packages dealing with survival, the three most important stand out; SPSS, BMDP and SYSTAT.

To compare possible differences between survival in different groups different statistical tests are used. The first test was proposed by Mantel, (1966). More recently and increasingly used are the Breslow test (1970), Mantel-Cox (1972), Log-Rank and that of Lee and Desu (1972). In the Mantel-Cox test, it is established that this follows a normal typified distribution through obtaining a statistical value C, under the null hypothesis. Once obtained, if this figure is greater than 1.96 this hypothesis is rejected and the significant differences in survival rates are confirmed (p<0.05). Breslow (1970) uses a similar statistical method to the Kruskal-Wallis test. The tests differ in the way of evaluating the observations, both being valid in large samples whether the censoring models are the same or not. The Log-Rank test is based on obtaining points depending on the logarithm of the survival function. In the same way, in the Mantel-Cox method a statistic, L, is calculated, which, under the null hypothesis, is shown to be distributed according to a
normal typified rule and survival rates can be checked to see if they behave in a significantly different way. The Lee-Desu test (1972), especially useful with small samples, establishes average points calculating them by comparing each case with the rest and increasing the points figure by 1 if the case has a longer survival time than another, and reducing by 1 if it has a shorter survival time, resulting in a non-parametric test to check whether k samples come from the same distribution. It is an extension of the Kruskal-Wallis test, and serves to compare the effect that k treatments may have on survival. The test can be used in cases in which censoring has been applied in the same way for all samples (Lee and Desu, 1972).

**DISCUSSION.**

From the sampling methods tried, the method of counting through the use of beakers has the highest error of estimation due, among other factors, to the lack of homogeneity in the distribution of larvae in the tank and the difficulty of achieving this given the particular shape of the tank; the fact that the number of larvae is normally small in relation to the volume makes the distribution curve similar to that of Poisson. The errors of estimation found in our experiment varied from ±3 to 20 larvae per litre depending, in addition to the reasons described above, on the number of larvae, their condition and specific behaviour, and so the method can be considered neither precise nor reliable. The syphoning method, used by most authors allows the collection of 93 to 97% of the dead larvae, and in addition to causing less "stress" in the live larvae, the number of samples does not need to be increased to reduce the standard error; furthermore it is enough to perform the syphoning over an adequate period of time and to cover the bottom of the tank collecting the samples. The incidence, in the case of being used only in censored cases, on the final percentage of survival is minimal if the syphoning is carried out well; nevertheless, to estimate real survival it will be necessary to apply a correction factor according to the error committed. Syphoning gives better results if it is done at least twice daily with the aim of avoiding the onset of decomposition in some larvae. References using the method of transferring and counting all the larvae are very scarce, and this method would produce better results, but the difficulty of using it with large samples is evident.

When referring to statistical treatment, the use of the methods proposed seems more satisfactory given that they compare survival behaviour in determined time periods of the duration of the treatment; as non-parametric methods they are also more satisfactory for the treatment of number of cases than those which are designed to analyse measurements (T-TEST, ANOVA; etc.). On the other hand the use of methods like regression, etc. to compare frequencies, we consider less satisfactory than the use of the Kruskal-Wallis test or Chi-squared for example, reaffirming the above, since it deals with the comparison of
attributes. Finally, the use of statistical and specific treatments for the analysis of survival, extracted from the scientific literature and briefly described, permit not only the comparison of frequencies but also the survival behaviour in determined time periods during the treatment, as already stated.

REFERENCES.-


FIG.- 1

PERCENT. OF SURVIVAL

FIG.- 2

(Taken from Fernández-Pato and Martínez Tapia 1991)