The Minimum Significant Difference at the NOEC calculated with a non-parametric test

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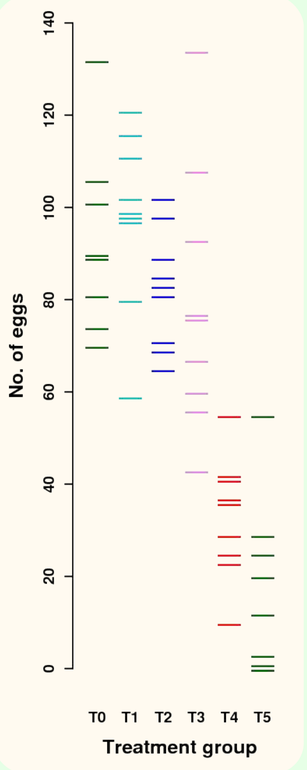
The MSD: What is it, why use it.

In ecotoxicology the result of a statistical significance test tells you whether or not a significant difference between the treatment and the control can be observed. The fact that you cannot see a difference, does not prove that there is no difference. It only indicates a lack of power to observe a difference. The Minimal Significant Difference (MSD) is the smallest difference between treatment and control that, if observed, would have been considered significant. If the MSD is small, the absence of a significant difference indicates that the treatment effect, if any, is small. If, however, the MSD is large and you are not able to see a difference, the treatment effect might still be quite large.
For statistical tests based on the normal distribution (Student-*t*, Dunnett's, ...) the MSD can be calculated routinely in many software packages. For non-parametric tests, a method is presented in van der Hoeven (2008). This method will be illustrated and explained with an example.

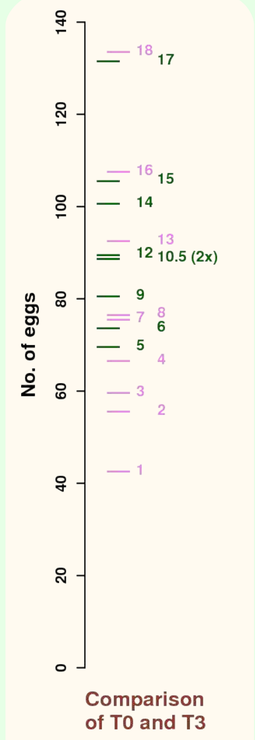
NOEC testing with pair-wise comparison



The number of eggs produced in a control (T0) and five increasing concentrations (T1, .. , T5) will be used as example. The No Observed Effect Concentration (NOEC) will be calculated using sequential pair-wise comparison between a concentration and the control using the non-parametric Wilcoxon's test. Sequential testing implies that the concentrations are tested starting with the highest concentration. The effect of a concentration is considered significant at level α if the *p*-value for the pair-wise comparison test at that concentration and at all higher concentrations is less than α.

The data used as example were simulated with expected number of eggs per treatment level: 100.0 (T0), 97.3 (T1), 90.0 (T2), 69.2 (T3), 36.0 (T4) and 12.3 (T5).

The Wilcoxon's test

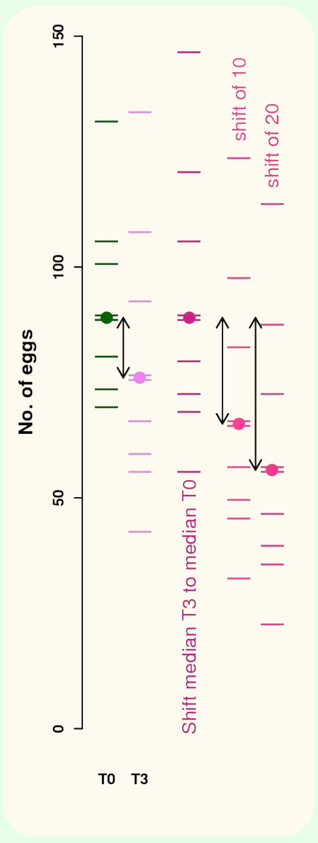


The Wilcoxon's test enables us to test whether the two samples might be drawings from the same distribution, the alternative hypothesis being that the distribution of the second sample is shifted compared to the first one. The test assumes that the observation in the two samples is the only thing known about that distribution. If both samples have the same distribution, the rank-order of the data would be completely at random.
If the distribution of the second sample is shifted compared to the first one, the median of the second sample is shifted. The difference between the medians can be used as an indication of the difference between the samples. In the data set, the median is 89, 99, 83, 76, 36 and 12 for T0, T1, T2, T3, T4 and T5, respectively.
The NOEC* for the data set is treatment T3 with a median of 13 eggs less than the median of the control (T0).

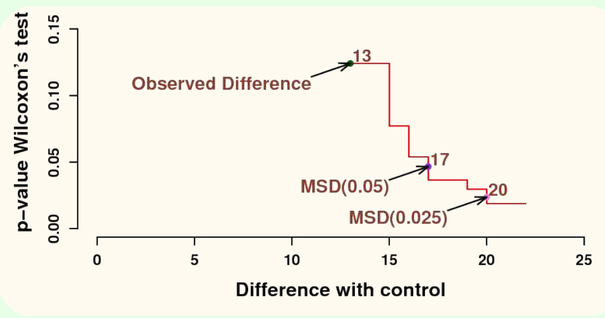
* at a = 0.05, one-sided test

Comparison of T0 with	p-value Wilcoxon test
T5	0.000
T4	0.000
T3	0.124
T2	0.115
T1	0.187

Shifting the median



The complete observed data set in the treatment can be shifted. By testing the significance of the difference between the shifted treatment data and the control data, the significance can be tested of a difference smaller or larger than the observed one. The measure for the difference between the observed control data and the (shifted) treatment data is the difference between the medians of these data sets. The MSD is the smallest difference leading to *p*-value smaller than the significance level (commonly set at 0.05).



Calculating the MSD

To calculate the MSD at the NOEC, determine the median of the control observations, X_1, ..., X_n, and of the observations at the NOEC, Y_1, ..., Y_m. Let the control median be $\boldsymbol{X}$ and the median at the NOEC be $\boldsymbol{Y}$. For the calculation, it is assumed that the effect of the chemical, if any, is a linear downward shift in the distribution of the observed variable (e.g. egg production). In van der Hoeven (2008) the method for an upward shift and for a proportional shift in the observed variable is also given.
Calculate for each combination of X_i and Y_j

$$Z_{ij} = (X_i - \boldsymbol{X}) - (Y_j - \boldsymbol{Y})$$

Order all observations Z_{ij} from smallest to largest and determine the rank number of Z_{ij}, R(Z_{ij}). The ordered Z-values are indicated as $Z[0] \le Z[1] \le \ldots. \le Z[n \times m\text{-}1]$.

For small samples sizes:

Look in a table for the α critical values of the one-sided Wilcoxon test at m and n observations. The largest tabulate rank sum with a *p*-value less than α is the critical value $W_{m,n}$.
Calculate $Q = W_{m,n} - m(m+1)/2$
Note 1: The usual Wilcoxon tables assume that $n \ge m$. If $n<m$, look up the critical value $W_{n,m}$ and calculate $Q = W_{n,m} - n(n+1)/2$.
Note 2: If the Mann-Whitney-U statistic U is tabulated instead of the Wilcoxon's statistic W, $Q=U$.

The MSD is now slightly less than $Z[Q]$, that is between $Z[Q\text{-}1]$ and $Z[Q]$.

For large sample sizes:

Use the normal approximation. Determine the α critical value for the standard normal distribution, $z=z_\alpha$.

Calculate $$Q^* = \frac{mn}{2} - z\sqrt{\frac{mn(m+n+1)}{12}}$$

and take Q as the largest integer below Q^*.
The MSD is now $Z[Q]$.

Some general remarks

The MSD is a measure for the ability to distinguish a treatment group from the control group. As such, it is required in many ecotoxicological test guidelines. For normal-distribution based tests, standard methods are available to calculate the MSD. For non-parametric tests, such a method was until recently missing. In the above, it is illustrated how to calculate the MSD for the non-parametric Wilcoxon's test (for a more detailed version, see van der Hoeven, 2008).
This method could be incorporated in standard ecotoxicological software packages. On the ecostat website, a simple R-program is made available to calculate the MSD for the one-sided Wilcoxon rank sum test (www.ecostat.nl).

References

van der Hoeven, N., 2008. Calculation of the minimum significant difference at the NOEC using a non-parametric test. *Ecotox Environm. Safety* **70**: 61-66