

## Introduction & Aims

As part of an Environment Risk Assessment, short-term fish pair breeding assays, e.g. Ref 1, may be used to examine the effects of chemicals exerting adverse impacts on sexual development and reproduction. Incorporation of pre-exposure data from such fish breeding assays into the analysis may increase statistical power and hence reduce the number of organisms required to be tested. The work has reviewed established texts and relevant current literature, obtained and analysed a number of datasets and subsequently assessed the most appropriate statistical methods and endpoints.

## Test Design

A typical fish pair breeding assay incorporates the following;

- Flow-through dosing system
- Control + 1 or more exposure treatments
- 1 tank per breeding pair with spawning tile (Fig 1)
- 6 to 8 breeding pairs per treatment
- Pre-exposure phase (e.g. 21 day for Fathead Minnow)
- Exposure phase (e.g. 21 day for Fathead Minnow)



Figure 1 – tanks containing breeding pairs

## Test Endpoints

The following observed or calculated endpoints were obtained for both the pre-exposure and exposure phase of the study;

- Total egg production
- Number of spawnings (egg batches)
- Average egg number per spawn
- Area Under Curve (AUC) of cumulative egg production

Reproductive endpoints exhibit high variability between organisms, resulting in less powerful statistical tests. Reducing, or accounting for the variability in some way can result in more powerful statistical tests.

## Pre-exposure data

Although primarily used to establish the satisfactory performance of the organisms prior to dosing and also confirm equivalence between treatment groups, analysis of pre-exposure and exposure data from control group and exposure groups shows that, for some endpoints, a relationship exists. This relationship may be utilised to account for the variability between organisms. Pre-exposure data may be incorporated in a number of ways, e.g.;

- **Difference scores** [ $X_{exp} - X_{pre}$ ]: An absolute measure of the effect of the exposure treatments.
- **Relative change scores** [ $(X_{exp} - X_{pre})/X_{pre}$ ]: Accounts for variability by describing treatment effects relative to the pre-exposure value.
- **Blocking**: Subgroup data will be less variable, therefore analysis of the subgroups should provide more powerful statistical tests.
- **Covariates**: Utilises the pre-exposure and exposure values to account for variability.

## Statistical Analysis

Data from three paired fish reproduction assays have been analysed, taking account of the pre-exposure data;

- The relationship between the the pre-exposure and exposure data was assessed.
- Exposure data only, difference scores and relative change scores for each endpoint were analysed according to the approach outlined in Figure 2 (based on Ref 2).
- Analysis of Covariance (ANCOVA) was undertaken to incorporate the pre-exposure data as a covariate. Where parametric analysis was not appropriate ANCOVA was undertaken on the rank transformed data.
- Blocking and subsequent analysis, e.g. by 2-Way ANOVA, was not undertaken since there were insufficient replicates in the studies for the data to be satisfactorily split into subgroups.

For each endpoint and statistical approach, the Minimum Detectable Difference (MDD) from the control has been determined for a significance level of  $p=0.05$  and a statistical power of 0.8.

The average MDD for each statistical approach was then calculated and compared using 1-Way ANOVA.

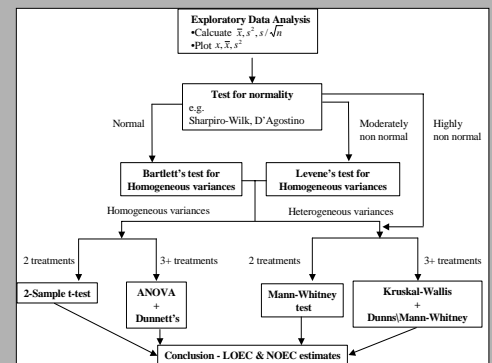
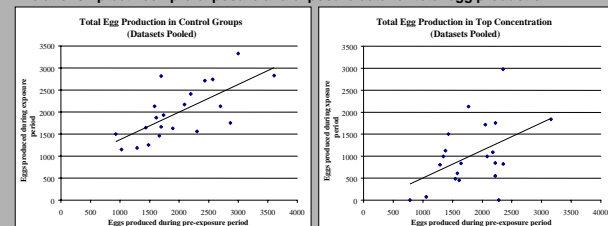


Figure 2 – Summary of statistical approach

## Results

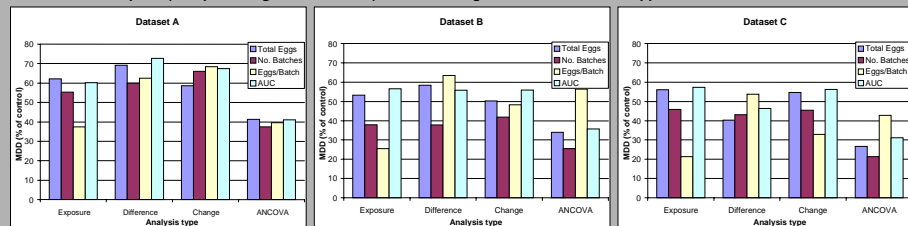
### Relationship between pre-exposure and exposure data for total egg production:



### Regression analysis of exposure data against pre-exposure data:

Endpoint	Treatment	All Datasets Pooled	
		Regression coefficient	Significance (p-value)
Total Eggs	Control	0.62	<0.01
	Top Conc.	0.63	<0.05
Number of Batches	Control	0.95	<0.01
	Top Conc.	0.89	<0.01
Eggs per Batch	Control	1	<0.01
	Top Conc.	0.61	0.06
AUC	Control	Not calculated	-
	Top Conc.	Not calculated	-

### MDD for each endpoint (as a percentage of the control) obtained using each of the statistical approaches:



### Comparison of the average MDD obtained using each of the statistical approaches:

Comparison	Dataset A		Dataset B		Dataset C	
	Difference between means	p-value	Difference between means	p-value	Difference between means	p-value
ANCOVA vs Diff Scores	17.18	0.0441	23.72	0.0009	21.74	0.0005
ANCOVA vs Rel Change	26.81	0.0034	17.64	0.0014	12.63	0.019
ANCOVA vs Exposure only	19.24	0.0248	20.40	0.0005	25.24	0.0002
Rel Change vs Diff Scores	9.628	0.3349	-1.081	0.9845	-9.107	0.0916
Rel Change vs Exposure only	2.061	0.9795	1.679	0.9466	3.505	0.7283
Diff scores vs Exposure only	-7.567	0.5231	2.760	0.8103	12.61	0.0192

## Discussion

Results suggest that for the endpoints considered, a relationship exists between pre-exposure and exposure data.

For all endpoints, ANCOVA produced an MDD, as a percentage of the control, between 20 - 30% smaller than other statistical approaches, whilst change and difference scores did not result in an MDD that was significantly smaller than analysis of exposure data alone.

None of the endpoints considered resulted in a consistently smaller MDD, although a significant difference in MDD between endpoints was found for Dataset B.

## Conclusions

Pre-exposure data may be usefully employed in increasing the statistical power of tests used to derive NOEC and LOEC values from paired fish reproduction assays.

Gains in statistical power may be utilised to either detect smaller but biologically relevant treatment effects or to reduce the number of organisms employed within the study.

No single endpoint produces a consistently more powerful statistical test

A larger number of datasets needs to be analysed to further confirm these findings.