



EIS 1437

AA068049

A study of algal growth inhibition using artificial shading

NSW DEPT PRIMARY INDUSTRIES



AA068049



LAND & WATER
CONSERVATION



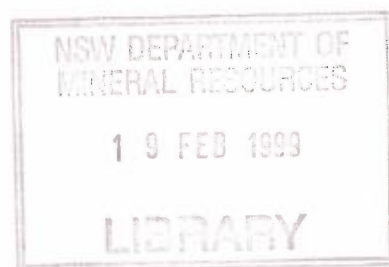
M10813

**DEPARTMENT OF LAND AND WATER CONSERVATION
URBAN WATER MANAGEMENT**

**A STUDY OF
ALGAL GROWTH
INHIBITION
USING
ARTIFICIAL SHADING**



**LAND & WATER
CONSERVATION**



Report No. SD RD 12

CNR97.107

WATER QUALITY SERVICES UNIT, CENTRE FOR NATURAL RESOURCES

NOVEMBER 1998

ISBN 0 7313 0342 3

This report forms part of a series which presents information resulting from research projects supported by the Department of Land and Water Conservation. It is published as a record of the work undertaken and as a means of disseminating the findings.

This work is copyright. The *Copyright Act* 1968 permits fair dealing for study, research, news reporting, criticism or review. Selected passages, tables or diagrams may be reproduced for such purposes provided acknowledgement of the source is included. The entire document or major extracts may not be reproduced without the written permission of the Director, Urban Water Management, Sustainable Water Management Division, Department of Land and Water Conservation.

SUMMARY

Urban Water Management (the former Sewerage and Drainage Section) engaged the Water Quality Services Unit (Environmental Services Branch) to undertake a trial at a sewage treatment plant (STP) examining algal control by artificial shading. Stroud STP was subsequently chosen as the most suitable site for this study. This report presents findings from this pilot study.

The pilot study was conducted between December 1996 and April 1997. Four degrees of shading were investigated: 0%, 50%, 80% and 100%.

The shade cloth was suspended above effluent maturation pond surfaces by support poles and high tension wire. Sampling occurred fortnightly at three locations within the maturation ponds: influent to, effluent from and midpoint within each of the treatment compartments. Water quality parameters measured were: Temperature, dissolved oxygen (DO), pH, suspended solids (SS), faecal coliforms (FC) and full algal count (FAC).

Results from the pilot study showed that:

- Shading had some effect on algal populations, but consistently significant decreases in algal numbers were not evident for any treatment. Some reduction in algal populations was achieved with the 100% shade cover. Algal concentrations decreased using this treatment and a distinct algal community developed but this did not prove to be statistically significant from the other treatments,
- The effluent retention time of approximately nine days for each treatment compartment may have been an insufficient period in which to reduce algal concentrations. Algal cell increases in the Pasveer channel and Pond 1, which were not shaded, may have contributed to increased algal concentrations in the 0%, 50%, 80% and 100% treatment compartments,
- The power of statistical analysis was limited because of insufficient data,
- Artificial shading was not found to adversely affect the ability of the maturation ponds to significantly reduce FC concentrations in the ponds. This implies that UV light is not the most important factor for microbial reduction in maturation ponds; there may be several factors involved,
- pH, DO, and SS trends through the treatment compartments were similar for all levels of shading, including the 0% shade treatment. DO concentrations showed a downward trend in the 0%, 50% and 100% treatments. Substantial duckweed growth on these treatment compartments may account for the decrease in DO levels. The 80% treatment compartment with limited duckweed cover did not exhibit similarly large decreases in DO levels,
- The mosquito population was observed to increase in the enclosed environment and this may be a potential management issue if artificial shading is conducted on a large scale.

If artificial shading is to be pursued as a potential algal reduction technique, further work will need to be conducted to more closely examine the issues raised in this study. Recommendations for future studies are to:

1. Investigate the effect of retention times required for significant reduction of algal populations at different degrees of shading;
2. Determine water quality and algal concentrations of the inflow and assess the use of shading at this point; and
3. Sample the full water column and/or replicate water quality variables to determine and eliminate the variability of individual grab sample results.

TABLE OF CONTENTS

1.0 INTRODUCTION	1
2.0 OBJECTIVES.....	1
3.0 METHODS	2
3.1 SAMPLING SITE	2
3.2 SAMPLING DESIGN	2
3.3 SAMPLING DATES	3
3.4 DATA ANALYSIS	5
4.0 RESULTS.....	7
4.1 FIELD OBSERVATIONS.....	7
4.2 ALGAL COMMUNITIES.....	10
4.3 PHYSICOCHEMICAL VARIABLES	16
4.3.1 <i>Light</i>	16
4.3.2 <i>Temperature</i>	16
4.3.3 <i>Dissolved Oxygen (DO)</i>	17
4.3.4 <i>pH</i>	18
4.3.5 <i>Suspended Solids (SS)</i>	18
4.3.6 <i>Faecal Coliforms (FC)</i>	19
4.3.7 <i>Full Algal Count (FAC)</i>	20
5.0 DISCUSSION	22
6.0 CONCLUSIONS & RECOMMENDATIONS.....	25
7.0 REFERENCES	26
APPENDIX A: TIME SERIES PLOTS.....	28
APPENDIX B: SUMMARY STATISTICS.....	34
APPENDIX C: PARAMETRIC STATISTICS/HYPOTHESIS TESTING.	35
APPENDIX D: NMDS PLOTS.	38
APPENDIX E: EXPLANATION OF STATISTICAL TECHNIQUES.....	42
BOX PLOTS (TUKEY, 1977)	42
HIERARCHICAL AGGLOMERATIVE CLUSTERING (CLARK AND WARWICK, 1994)	43
NON METRIC MULTI-DIMENSIONAL SCALING ORDINATIONS (NMDS)	43
SIMPER PROCEDURE	43
ANALYSIS OF SIMILARITIES (ANOSIM).....	43
BIOENV PROCEDURE.....	44
ANALYSIS OF VARIANCE AND NON PARAMETRIC ANALYSIS	44

LIST OF FIGURES

FIGURE 1: STROUD STP PLAN.....	4
FIGURE 2: SHADE CLOTH STRUCTURE OVER AN EFFLUENT MATURATION POND.....	5
FIGURE 3: CLUSTER DENDROGRAM - FAC IN STAGE 2.....	10
FIGURE 4: 3D NMDS PLOT - STAGE 2 (STRESS 0.13).....	11
FIGURE 5: CLUSTER DENDROGRAM - FAC IN STAGE 3.....	12
FIGURE 6: 3D NMDS PLOT - STAGE 2 (STRESS 0.18).....	13
FIGURE 7: CLUSTER DENDROGRAM - FAC IN STAGES 2 AND 3 AND ALL TREATMENTS (ALL SAMPLING OCCASIONS SUMMED).....	14
FIGURE 8: NMDS PLOT OF FAC MEDIANS FOR ALL TREATMENTS IN STAGE 2 AND 3 DATA (STRESS 0.01).....	15
FIGURE 9: BOXPLOT OF WATER TEMPERATURE.....	16
FIGURE 10: BOXPLOT OF DO.....	17
FIGURE 11: BOXPLOT OF PH.....	18
FIGURE 12: BOXPLOT OF SUSPENDED SOLIDS.....	19
FIGURE 13: BOXPLOT OF FAECAL COLIFORMS.....	20
FIGURE 14: BOXPLOT OF FULL ALGAL COUNTS.....	21
FIGURE 15: TIME SERIES PLOTS OF TEMPERATURE FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	28
FIGURE 16: TIME SERIES PLOTS OF DISSOLVED OXYGEN FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	29
FIGURE 17: TIME SERIES PLOTS OF PH FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	30
FIGURE 18: TIME SERIES PLOTS OF SUSPENDED SOLIDS FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	31
FIGURE 19: TIME SERIES PLOTS OF FAECAL COLIFORMS FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	32
FIGURE 20: TIME SERIES PLOTS OF FULL ALGAL COUNTS FOR 0%, 50%, 80% AND 100% SHADE TREATMENTS AND ALL STAGES.....	33
FIGURE 21: NMDS PLOT - STAGE 2 CONTROL (0% SHADE). STRESS 0.05.....	38
FIGURE 22: NMDS PLOT - STAGE 2, 50% SHADE TREATMENT. STRESS 0.11.....	38
FIGURE 23: NMDS PLOT - STAGE 2, 80% SHADE TREATMENT. STRESS 0.15.....	39
FIGURE 24: NMDS PLOT - STAGE 2, 100% SHADE TREATMENT. STRESS 0.07.....	39
FIGURE 25: NMDS PLOT - STAGE 3, CONTROL (0% SHADE). STRESS 0.05.....	40
FIGURE 26: NMDS PLOT - STAGE 3, 50% SHADE TREATMENT. STRESS 0.03.....	40
FIGURE 27: NMDS PLOT - STAGE 3 80% TREATMENT, STRESS 0.08.....	41
FIGURE 28: NMDS PLOT - STAGE 3 100% TREATMENT, STRESS 0.08.....	41

LIST OF TABLES

TABLE 1: WATER QUALITY VARIABLES MEASURED.	3
TABLE 2: SAMPLING DATES AT STROUD.	3
TABLE 3: FIELD OBSERVATIONS SUMMARY.	7
TABLE 4: SIMPER RESULTS FOR STAGE 2 FAC.	11
TABLE 5: SIMPER RESULTS FOR STAGE 3 FAC.	12
TABLE 6: LIGHT LEVELS (LUX) RECORDED FOR EACH TREATMENT.	16
TABLE 7: AIR TEMPERATURE DATA - 18/03/97.	17
TABLE 8: TEMPERATURE - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 9: DO - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 10: PH - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 11: SS - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 12: FC - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 13: FAC - MEAN AND STANDARD DEVIATION (SD).	34
TABLE 14A: ANOVA RESULTS FOR TEMPERATURE.	35
TABLE 14B: KRUSKAL-WALLIS RESULTS FOR TEMPERATURE.	35
TABLE 15A: ANOVA RESULTS FOR DISSOLVED OXYGEN.	35
TABLE 15B: KRUSKAL-WALLIS RESULTS FOR DISSOLVED OXYGEN.	35
TABLE 16A: ANOVA RESULTS FOR PH.	36
TABLE 16B: KRUSKAL-WALLIS RESULTS FOR PH.	36
TABLE 17A: ANOVA RESULTS FOR SUSPENDED SOLIDS.	36
TABLE 17B: KRUSKAL-WALLIS RESULTS FOR SUSPENDED SOLIDS.	36
TABLE 18A: ANOVA RESULTS FOR FAECAL COLIFORMS.	36
TABLE 18B: KRUSKAL-WALLIS RESULTS FOR FAECAL COLIFORMS.	37
TABLE 19A: ANOVA RESULTS FOR FULL ALGAE COUNT.	37
TABLE 19B: KRUSKAL-WALLIS RESULTS FOR FULL ALGAE COUNT.	37

LIST OF PHOTOGRAPHS

PHOTOGRAPH 1: POND CONFIGURATION AT STROUD BEFORE THE PILOT STUDY BEGAN (NOVEMBER 1996).	8
PHOTOGRAPH 2: 18/2/97 - DUCKWEED COVERING THE SURFACE OF THE PONDS.	8
PHOTOGRAPH 3: 18/2/97 - 100% AND 80% TREATMENTS - WATER POOLING ON THE SURFACE OF THE 100% COVER WHICH REQUIRED DRAINING.	9
PHOTOGRAPH 4: 18/2/97 - UNDERNEATH THE 80% SHADE CLOTH. NOTE ALGAL SCUMS ON THE SURFACE, BUT NO DUCKWEED.	9

GLOSSARY

algal community	A collection of algae, living and growing together, which possesses a certain unity and individuality. A community comprises a typical species composition that has resulted from the interaction of populations over time.
Chi square	Relates to the statistical distribution of values. The Chi square distribution is a probability density function whose values range from zero to infinity. Unlike the normal and t distributions, the function approaches the axis only at the right hand tail of the curve, and not both tails. A chi square value is found in non-parametric analyses and is used to calculate whether a pattern of values is significantly different from a random pattern.
degrees of freedom	Defined as $n-1$ where n is the number of samples (used in many formulae for statistical analysis).
eutrophic	Describing a body of water with an abundant supply of nutrients which often stimulates excessive growth of algae.
faecal coliforms	Includes all gram negative, non spore-forming, aerobic and facultative anaerobic rod-shaped bacteria that ferment lactose with gas and acid formation within 48 h at 44.5°C. <i>E.coli</i> and <i>Klebsiella</i> spp. predominate in this subset. Generally used in sewage treatment plant monitoring.
full algal count	A count of all algal species detected in a water sample, extrapolated to the number of algal cells occurring in 1 mL.
maturation ponds	Surface ponds or lagoons that collect treated sewage effluent for a number of days (retention period) before it is discharged into the environment. Maturation ponds (often less than 1.5 m deep) are designed primarily to disinfect secondary effluent.
mean	Arithmetic average.
mean square	Variance. Measure of the dispersion of a data set.
median	The value of the variable (in an ordered array) that has an equal number of items on either side of it. The median divides a frequency distribution into two halves.
Pasveer channel	An open canal for the periodic aeration of sewage (secondary treatment).
pH	A measure of the acidity or alkalinity of a solution.
primary treatment	Removal of coarse particulate matter in a sewage treatment plant.
secondary treatment	Involves the addition of a biological treatment phase following primary treatment. This treatment removes about 85% to 95% of the organic matter in waste water but has little effect on dissolved materials or on the nutrients that stimulate the growth of algae in receiving waters.
standard deviation	A statistical measure of the dispersion of data, weighting each datum by its deviation from the mean.
suspended solids	Particulate matter dispersed in a liquid medium (water body).
Note:	For all statistical analyses performed and graphs produced, See Appendix D for a detailed description of interpretation of results.

ABBREVIATIONS

Analysis of similarities.....	ANOSIM
Analysis of variance.....	ANOVA
Blue-green algae.....	BG
Chi square.....	CHISQU
Conductivity.....	Cond
Degrees of freedom.....	df
Department of Land and Water Conservation.....	DLWC
Dissolved oxygen.....	DO
Environment Protection Authority.....	EPA
Equivalent persons.....	EP
Faecal coliforms.....	FC
Full algal count.....	FAC
Mean sum.....	MS
Non blue-green algae.....	non BG
Non-metric multi dimensional scaling.....	NMDS
Probability.....	P
Standard deviation.....	SD
Suspended solids.....	SS
Sewage treatment plant.....	STP

Contributors

Members of Water Quality Services Unit contributing to this study were Michael Moroney, Adam Boey, Bruce Chessman, Douglas Westhorpe, John Graice, Monika Muschal, Simon Mitrovic and Meredith Royal.

Acknowledgments

This project was funded by Urban Water Management (Sewerage and Drainage Section), Department of Land and Water Conservation.

Thanks are extended to the Directorate's officers Bob Budden, Bruce Murray, Michael Poon and Lance Walker for their contributions to the project.

Special thanks to MidCoast Water (Great Lakes Shire Council) for permitting the experiment to be conducted at Stroud Sewage Treatment Plant, and to its staff, in particular Tom Baldwin, for assisting in the field work.

1.0 INTRODUCTION

Maturation ponds, used in sewage treatment, are an effective means to partially disinfect secondary treated effluent. Discharges from sewage maturation ponds sometimes do not meet effluent suspended solids requirements because of significant algal concentrations. Blooms of some algal species can cause odours or toxicity that detrimentally affects receiving waters. Algal growth in maturation ponds is generally limited by either light or carbon, as nutrients such as nitrogen and phosphorus are usually in plentiful supply. Artificial shading could therefore be one means to reduce algal concentrations in maturation ponds.

Bowling and Mitrovic (1996) reviewed the treatment processes available to operating authorities for reduction of algae in STP effluent. No information was found on algal reduction by artificial shading of maturation ponds. However, shading by a variety of means has been used successfully to reduce algal growth in drinking water reservoirs. Griffith (1988) reported satisfactory algal control with synthetic rubber covers. These spanned 11.75 ha over a large reservoir in California, USA, and were used to inhibit algal growth in conjunction with a change in chlorination techniques.

Shading techniques other than artificial covers have been used to reduce algal growth in maturation ponds. Floating water plants, such as duckweed or water hyacinth, have been used extensively for this purpose. Water plants are also valuable in the removal of nutrients from the water column as long as the plants are harvested from the system to prevent anoxic conditions developing below the root zone (Hillman and Cullry, 1978). Plants totally covering the surface of the water may produce odours as well as increasing the suspended solids in the water.

Urban Water Management engaged the Water Quality Services Unit (Environmental Services Branch) to undertake a pilot study of algal removal by artificial shading. The purpose of this study was to investigate biological interactions that occur when covers of different strength are used over maturation ponds. Results would add to the current limited knowledge concerning shading and STPs. From this study the cost effectiveness of artificial shading could be evaluated.

2.0 OBJECTIVES

The objectives of this project were to:

- Determine the effectiveness of various shading materials to control algae while maintaining adequate disinfection;
- Measure and examine the effect of the materials on all relevant effluent characteristics; and
- Consider the needs of NSW country sewerage authorities when delivering findings.

3.0 METHODS

3.1 Sampling Site

Five possible sites were investigated for this pilot study. These included: Frederickton, Delungra, Scone, Stroud and Singleton Sewage Treatment Plants (STPs). Stroud STP was chosen due to its manageable size and regular shaped maturation ponds and its geographical location on the north coast where many algal blooms in STPs have been reported.

Stroud STP is situated close to the centre of Stroud surrounded by grazing land and local dwellings. The existing plant comprises a Pasveer Channel, sludge lagoon and three effluent maturation ponds. The STPs design capacity is rated at 1000 EP and is based on primary and secondary treatment in a Pasveer Channel. Primary treatment comprises basic solids removal, secondary treatment consists of activated sludge treatment (intermittent aeration) and aeration in the Pasveer channel. Effluent then passes into the three maturation ponds in series and discharged into Lamans Creek. Stroud STP output was approximately 120 000 Litres/day.

3.2 Sampling Design

The maturation pond configuration at Stroud STP was three ponds in series. The STP plan detailing the configuration of the pilot study is shown in Figure 1. The first pond was used for settling out and removal of organic matter before flow into the second and third ponds. The first pond was unsuitable for the study due to its role as a settling pond and the presence of a sludge layer on the bottom which could affect comparisons between treatment types. The second and third ponds were thought to have significantly less organic matter, hence no sludge removal would be necessary for these ponds. This was confirmed by Great Lakes Shire Council staff when the ponds were drained to examine possible water plant contamination, sludge depth and to install the baffles and pipes.

As shown in Figure 1, the inflow (stage 1) was split four ways from the first pond to the four treatment compartments in ponds two and three (stage 2). Baffles were installed to divide the ponds in halves which created four separate treatment compartments over two ponds. The ponds were drained and the baffles and pipes installed between 12th and 16th November 1996. The baffles consisted of corrugated iron sheeting joined together by pot rivets and sealed with silicon. The four treatment types were:

<i>Compartment</i>	<i>Treatment</i>	<i>Pond</i>
1	0% shade (control)	2
2	50% shade	2
3	80% shade	3
4	100% shade	3

The ponds were 35.3m square and 2.1m deep. Area and volume were 1 246m² and 617m³ respectively. The total storage capacity of the three maturation ponds was 2.8 ML and the retention time for the three ponds was 14.2 days. Assuming there is complete mixing within each of the treatment compartments and the flow was split evenly from the flow divider (pond 1) into each of the treatments, the approximate retention time for each treatment compartment was 9.3 days.

The shade cloths were rolled over the pond at bank height which left approximately 30 to 100cm of air space between the water surface and the material. The shade cloths did not reach the top of the bank of each of the ponds, however complete shading of the water surface was achieved for all treatment compartments. Tripods were inserted into the centre of the ponds and high tension wire, attached to star pickets, stretched underneath the cloths to support the material. The material was fastened to the banks of the maturation ponds by guy ropes and pegs. Figure 2 details the design of one of the ponds, the shade cloth on the other pond was erected similarly. Effluent from the four treatment compartments was brought to a single junction in the existing effluent pipe which runs into Lamans Creek. The junction was the final point (stage 3) at

which water quality was measured and samples were taken. The configuration allows the study to be conducted at one location, removing the problem of spatial variation in the study. There was no replication (other than time). Replication was prohibited by construction and analysis costs, which was deemed acceptable for a pilot study of this nature.

Water quality variables that were measured during the study are presented in Table 1.

Table 1: Water quality variables measured.

<i>Parameter</i>	<i>stage 1</i>	<i>stage 2</i>	<i>stage 3</i>
temperature	✓	✓	✓
dissolved oxygen (DO)	✓	✓	✓
pH	✓	✓	✓
suspended solids (SS)		✓	✓
faecal coliforms (FC)	✓	✓	✓
full algal count (FAC)		✓	✓

Definitions: stage 1 - the effluent before it reaches the different treatment compartments, stage 2 - the effluent within the treatment compartments and stage 3 - effluent when it has left the ponds. Light readings, for two occasions were also taken for each treatment. The study was conducted under normal STP operating conditions.

3.3 Sampling Dates

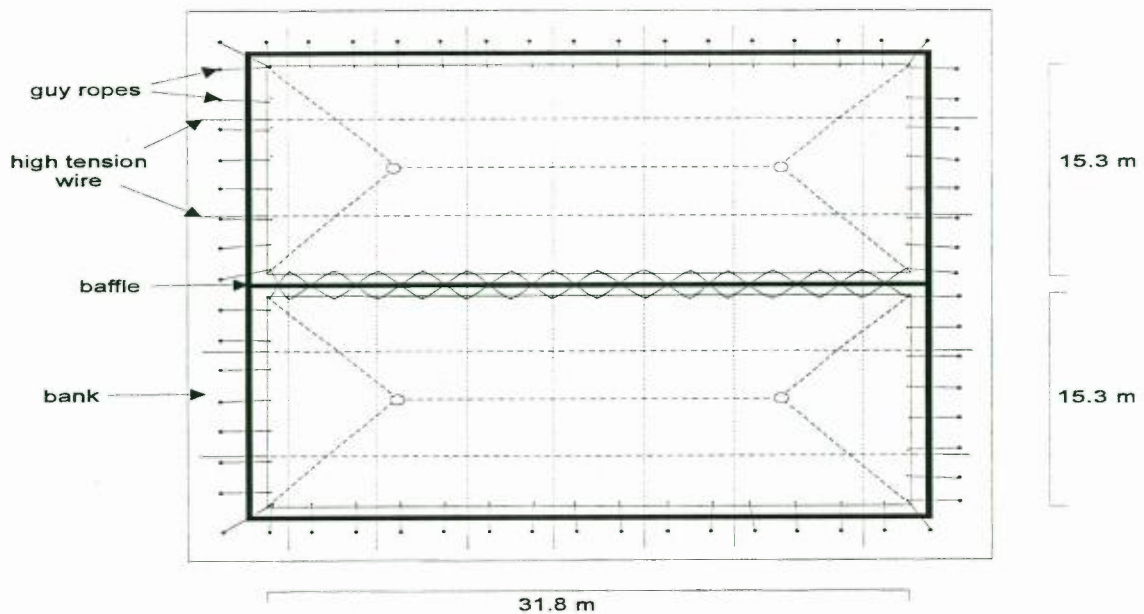
The field trial was programmed to allow sampling and observations to be made during summer when algal blooms were most likely to occur. The sampling dates are shown in Table 2.

Table 2: Sampling dates at Stroud.

<i>Sampling Occasion</i>	<i>Date</i>
1	12th December 1996
2	23rd December 1996
3	6th January 1997
4	21st January 1997
5	4th February 1997
6	18th February 1997
7	4th March 1997
8	18th March 1997
9	1st April 1997

The sampling dates corresponded to sampling every fortnight. Water quality analysis was performed at the DLWC Water Environment Laboratory (WEL) or at Australian Laboratory Services P/L and EML Consulting Services Pty Ltd.

TOP VIEW:



CROSS SECTION:



Figure 2: Shade cloth structure over an effluent maturation pond.

3.4 Data Analysis

Temperature, pH, DO, SS, FC and FAC data are summarised using boxplots and time series graphs. Analysis of variance (MANOVA) and non-parametric analysis (Kruskal-Wallis) was performed on all the above variables to determine if there was a significant difference between:

- treatments (0%, 50%, 80% and 100% shade),
- stages of water quality collection (stage 1, stage 2 and stage 3), and
- dates (sampling occasions).

All values were log transformed where appropriate. Statistical significance was assessed at a probability level of 5%.

Non-parametric multi-dimensional scaling (NMDS) was performed on the Full Algal Count data (stages 2 and 3) expressed as a rank similarity matrix using the Bray-Curtis similarity measure as outlined in Clarke (1993). The data was initially fourth root transformed. Two dimensional ordination plots were produced to display the community pattern. Hierarchical agglomerative clustering was used to define the different groups in the ordination diagrams. The clustering technique was used to enable a clear representation of the interactions between the various groups which would not be achieved using the NMDS procedure along. The NMDS ordination plots were therefore only used for individual treatments. When groups were found to be different, the taxa contributing most to the observed differences were determined using the similarity percentages (Simpser). Where a distinct grouping was found to occur in an ordination plot, a two-way nested analysis of similarities (ANOSIM) procedure was used.

Labelling for NMDS (ordinations) and cluster diagrams is as follows. For ordinations, the day and month is shown on the plot, the year has been left out to reduce the size of the unique identifier. If stage 2 and stage 3 results are on the same plot, 's2' denotes stage 2 results and 's3' denotes stage 3 results. For both cluster and ordination diagrams the labels are divided up as follows. The 't' or 'c' represents a treatment or the control, respectively. If a 't' is present, it is then followed by the appropriate treatment, i.e. 50%, 80% or 100% shade. For cluster plots only, the last digit signifies the sampling occasion, i.e. 1 to 9.

Linking of biotic community structure to environmental parameters was carried out using the BIOENV procedure. This technique defines an optimal subset of environmental variables which best explains the biotic structure using rank correlation (Clarke and Ainsworth 1993). pH was not analysed in the BIOENV procedure as there were incomplete data.

Univariate procedures (ANOVA) and summary statistics (boxplots) were carried out using the SAS statistical software package. All multivariate procedures (e.g. analysis of algal data) were carried out using the community analysis package PRIMER v.4 developed by the Plymouth Marine Laboratory, UK.

For detailed descriptions and interpretations of the above analyses see Appendix E.

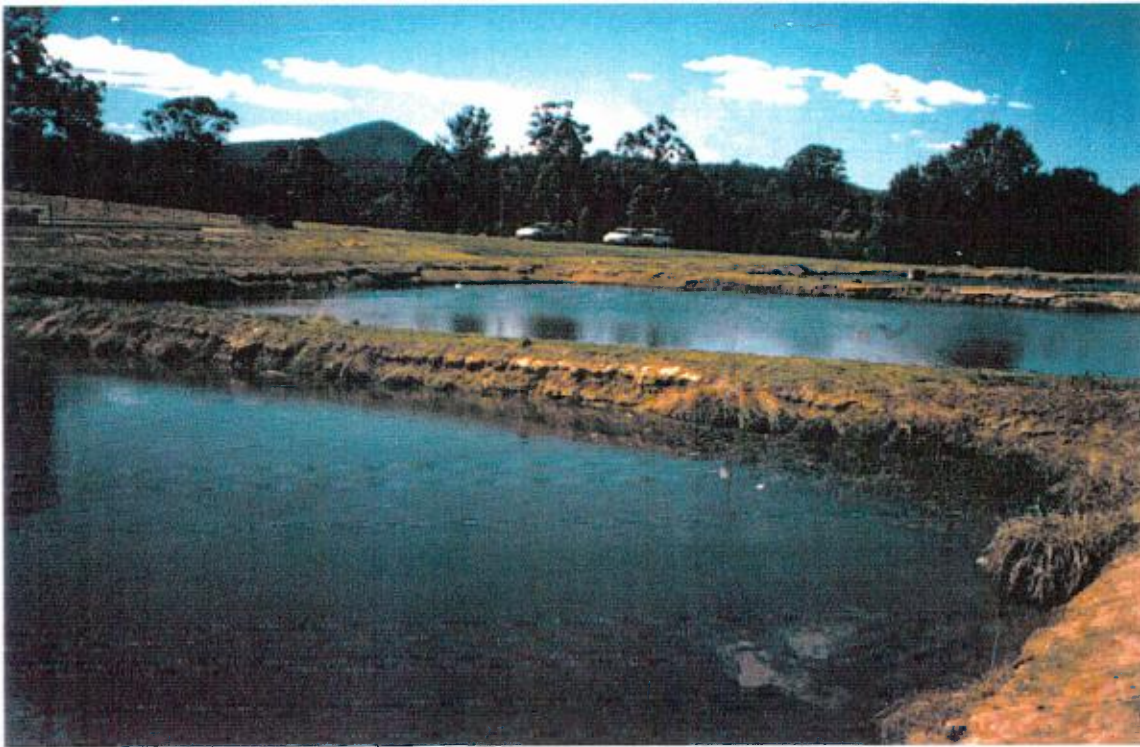
4.0 RESULTS

4.1 Field Observations

A summary of all field observations can be seen in Table 3. Photographs 1-4 show the effluent retention ponds and the erection of shade cloth over the ponds.

Table 3: Field observations summary.

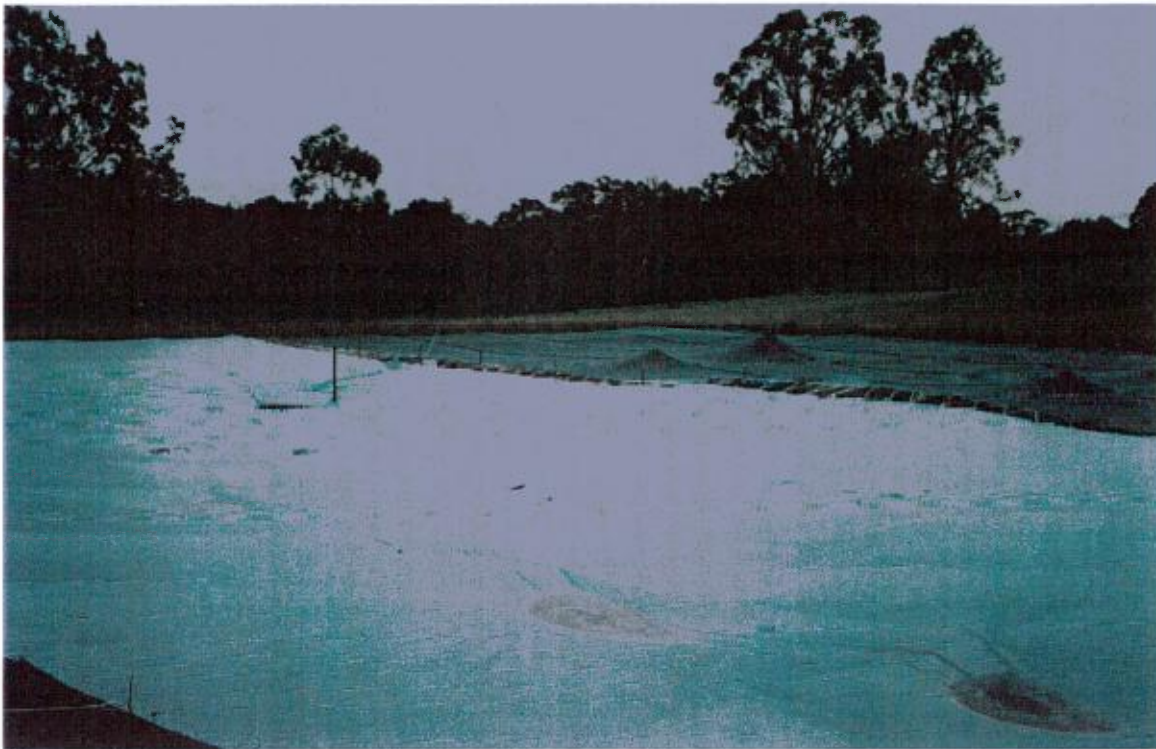
DATE	POND	OBSERVATION	RESULT/EXPLANATION
9/12/96	3	<ul style="list-style-type: none"> • Low water level 	<ul style="list-style-type: none"> • No water quality samples taken for stage 3 80% and 100% treatments. Effluent was not flowing through to final junction point.
23/12/96	3	<ul style="list-style-type: none"> • Water level at same height as Pond 2 	<ul style="list-style-type: none"> • Effluent flowing through to final junction point - stage 3 80% and 100% treatment samples taken.
6/1/97	1,2 1,2,3	<ul style="list-style-type: none"> • Water level very high compared to pond 3 • Duckweed growth 	50% shade cloth slightly underwater - heavy rain.
4/2/97	1,2 1,2,3	<ul style="list-style-type: none"> • Water level at bank height • Continued duckweed growth 	<ul style="list-style-type: none"> • 50% shade cloth mostly submerged.
18/2/97	3 1,2 1,2,3	<ul style="list-style-type: none"> • Mosquitoes underneath 100% shade cloth, cover punctured by tripod. • Very high water levels still but not up to bank height • Duckweed covering all ponds, except 80% treatment. 	<ul style="list-style-type: none"> • Perfect conditions for mosquito growth - humid, dark and no air movement. • Duckweed growth on top of submerged portion of 50% shade cloth - guy ropes were tightened and accessible areas cleaned of duckweed. -
4/3/97 to 4/4/97	1,2 2 1,2,3	<ul style="list-style-type: none"> • Water level dropped but still higher than Pond 3 • 80% shade cloth compartment with small algal scums floating on water surface. Duckweed covering 15-20% water surface. • Duckweed cover 1cm thick on 0%, 50% and 100% treatment compartments. 	<ul style="list-style-type: none"> • Possible seepage from Pond 1 into Pond 2 then Pond 3.



Photograph 1: Pond configuration at Stroud before the pilot study began (November 1996).



Photograph 2: 18/2/97 - Duckweed covering the surface of the ponds.



Photograph 3: 18/2/97 - 100% and 80% treatments - water pooling on the surface of the 100% cover which required draining.



Photograph 4: 18/2/97 - underneath the 80% shade cloth. Note algal scums on the surface, but no duckweed.

Results for the repeated measures analysis models are summarised below, then results for non-parametric algal community analysis (ANOSIM, SIMPER and BIOENV), followed by results for individual water quality variables. Appendix A contains time series plots (Figures 15-20), Appendix B contains summary statistics (Tables 8-13), Appendix C contains ANOVA results (Tables 14a-19a) and Kruskal-Wallis results (Tables 14b-19b).

Repeated measures analyses were performed; first using only algal data, then physicochemical data and finally, all data. The three data sets revealed significant time effects ($P < 0.06$, $P < 0.01$ and $P < 0.04$, respectively), significant time by stage effects ($P < 0.01$, $P < 0.01$ and $P < 0.01$, respectively) and significant time by treatment effects ($P < 0.07$, $P < 0.01$ and $P < 0.01$, respectively).

4.2 Algal Communities

The cluster dendrogram of stage 2 FAC results shows three distinct large groupings at the 37% similarity level (Figure 3):

1. Group 1 (sampling occasions from 4/2/97 onwards, low cell counts);
2. Group 2 (early sampling occasions 12/12/96 to 21/1/97, high cell counts); and
3. Group 3 (sampling occasions from 4/2/97 onwards, low cell counts).

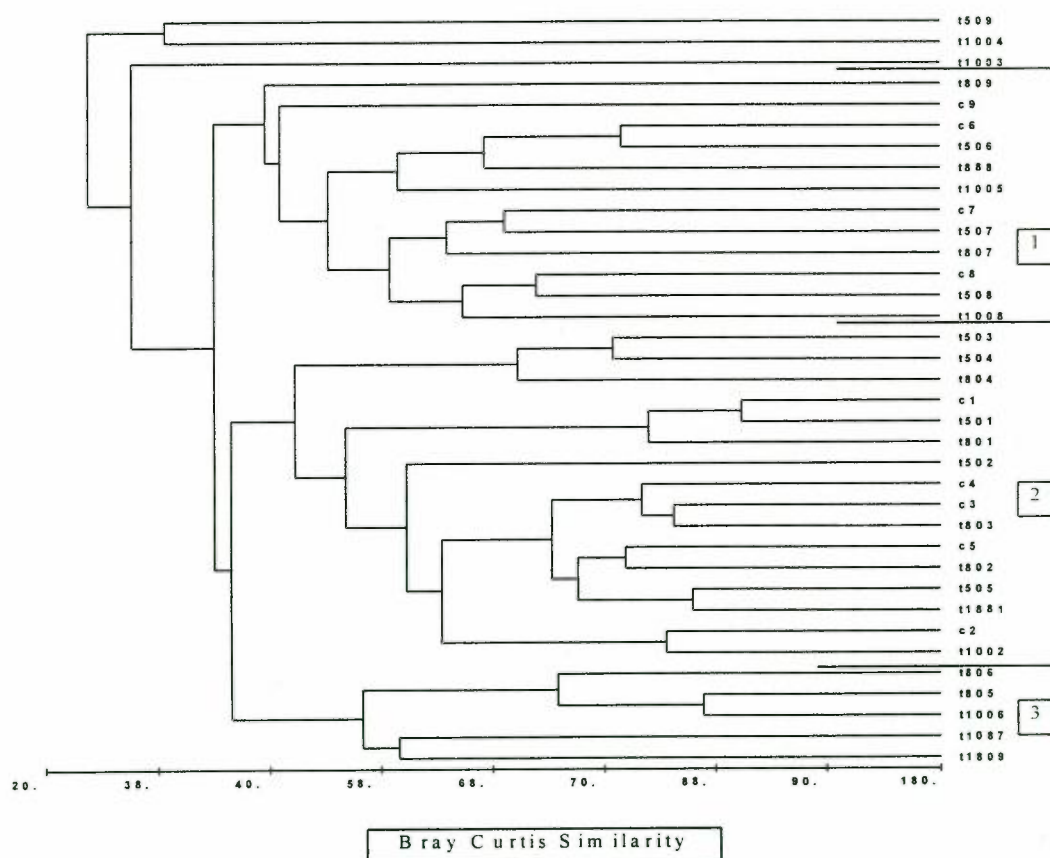


Figure 3: Cluster dendrogram - FAC in stage 2.

Note: The x-axis represents the similarity level and the vertical branches represent fusion of objects or subgroups (at a particular similarity level). The horizontal lines separate each major group. t = treatment (50%, 80% and 100% shade), c = control (0% shade), 1 to 9 = sampling occasion.

Samples from the 4/2/97 onwards were divided into two distinct groups. The SIMPER procedure (Table 4) showed that the absence of *Cryptomonas* sp. (a green, flagellated alga) characterised group 2, whereas the presence of *Ankistrodesmus* sp. (a green, unicellular alga) characterised groups 2 and 3. Ordination

separated the sampling times into two major groups: 12/12/96 to 21/1/97 and 4/2/97 to 1/4/97. Duckweed was visually most abundant from the 4/2/97 onwards. However, the 80% treatment, which was relatively duckweed free, did not have a distinct algal assemblage.

Table 4: Simper results for stage 2 FAC.

Group	Taxon (Genus)	Mean Abundance (cells/mL)	Ratio (mean dissimilarity/SD)	Contribution %	Cumulative %
1	<i>Cryptomonas</i>	71.6	4.7	33.4	33.4
	<i>Rhodomonas</i>	169.0	6.4	31.3	64.8
2	<i>Ankistrodesmus</i>	4787.4	3.4	30.3	30.3
	<i>Oocystis</i>	5043.6	3.0	25.1	55.5
	<i>Rhodomonas</i>	1418.6	4.0	21.1	76.6
3	<i>Ankistrodesmus</i>	248.3	5.3	24.7	24.7
	<i>Cryptomonas</i>	759.3	3.5	22.3	46.9
	<i>Chlamydomonas</i>	2426.3	1.3	17.1	64.1
	<i>Sphaerocystis</i>	1018.3	0.8	11.8	75.9

NMDS of the individual treatments for stage 2 showed a distinct difference between the 100% treatment and the 0%, 50% and 80% treatments (Figures 21-24, Appendix D). The 0%, 50% and 80% treatments generally formed two distinct groups based on sampling occasions: 12/12/96 to 21/1/97 and 4/2/97 to 1/4/97. The 100% treatment showed a different pattern. Four groups were formed where initial sampling occasions are divided into two and the latter sampling occasions predominantly form a single group. This indicated that algal populations under different shade cloths behaved differently. The algal community under 100% shade exhibited the largest departure from algae in the control.

NMDS results for FACs (stage 2 - all treatments and sampling occasions) were also plotted in 3 dimensions to improve interpretation (Figure 4). It showed that treatments and sampling occasions were not well separated; no definite groups were formed. There were instances, within the plot, where several samples of a particular treatment (e.g. a long 'crescent' band of samples in the 100% shade treatment on the left-hand side of the plot) were grouped together, and also where certain sampling times were grouped together (e.g. a group of samples taken on the last three sampling times, 7, 8 and 9 were situated on the right-hand side of the plot).

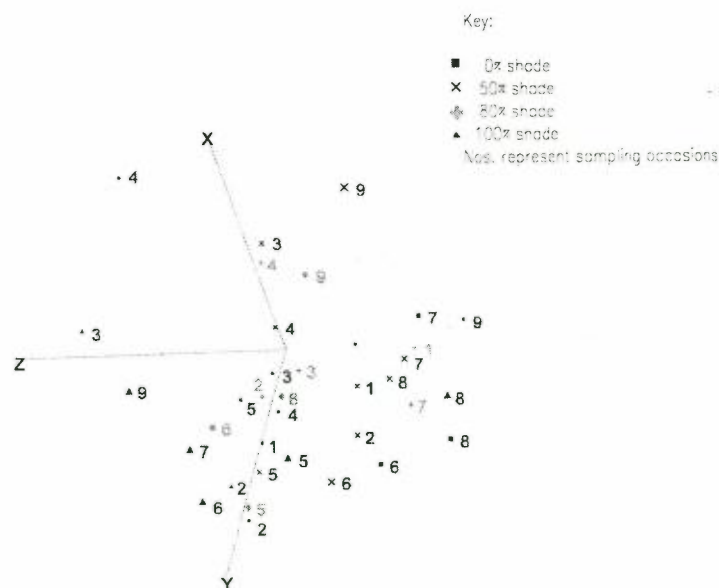


Figure 4: 3D NMDS plot - stage 2 (stress 0.13).

Note: Nos. represent sampling occasions.

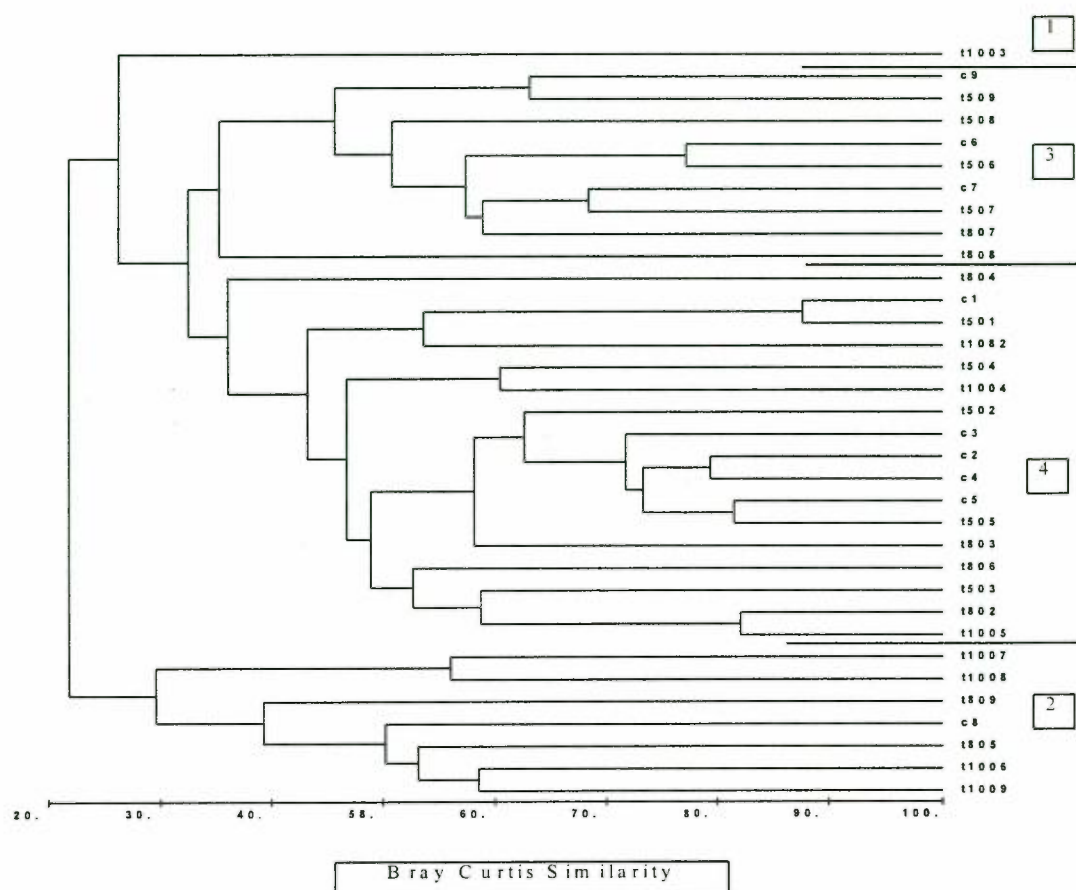


Figure 5: Cluster dendrogram - FAC in stage 3.

Note: The x-axis represents the similarity level and the vertical branches represent fusion of objects or subgroups. The horizontal lines separate each major group. t = treatment (50%, 80% and 100% shade), c = control (0% shade), 1 to 9 = sampling occasion.

Table 5: SIMPER results for stage 3 FAC.

Group	Taxon (Genus)	Mean Abundance (cells/mL)	Ratio (mean dissimilarity/SD)	Contribution %	Cumulative %
1	<i>Fragilaria</i>	7391.3	3.7	67.0	67.0
2	<i>Ankistrodesmus</i>	2557.0	1.9	23.0	23.1
	<i>Rhodomonas</i>	765.2	2.2	18.5	41.6
	<i>Oocystis</i>	3091.1	1.5	18.4	60.0
	<i>Chlamydomonas</i>	188.0	1.5	13.5	73.4
3	<i>Ankistrodesmus</i>	179.9	3.7	18.7	18.7
	<i>Cryptomonas</i>	468.9	4.7	18.3	37.0
	<i>Sphaerocystis</i>	929.4	0.7	13.9	50.9
	<i>Rhodomonas</i>	559.8	1.1	10.4	61.3
	<i>Chlamydomonas</i>	300.0	1.1	10.3	71.6

Three major groups and one small group were separated at the 30 and 33% similarity levels for stage 3 FAC (Figure 5). The groups are numbered according to increasing similarity. These groups were not separated according to sampling occasions as for stage 2 FAC results. The SIMPER procedure (Table 5) showed that *Fragilaria* sp. contributed a large percentage to the similarity in group 3, and groups 4 and 2 were defined by various percentages of genera such as *Ankistrodesmus* sp., *Rhodomonas* sp. and *Chlamydomonas* sp. Note that the Simper procedure cannot identify contributing algal taxa for a single observation, i.e.

characteristic taxa for group 1 (with only one 'observation') could not be determined. The dendrograms for both stage 2 and stage 3 showed no clear distinction on the basis of individual treatments. The groups in the dendrograms were defined predominantly on changes in the algal community over time.

NMDS analysis of stage 3 FAC results (Figures 25-28, Appendix D) showed that the 0%, 50% and 100% treatments were divided into two or three distinct groups based on sampling occasions (12/12/96 - 21/1/97 and 4/2/97 - 1/4/97). The 80% treatment showed no distinct division between early and latter sampling occasions. Note that for the 80% and 100% treatments no sample was taken on the first sampling occasion.

NMDS results for FACs (stage 3 - all treatments and sampling occasions) were also plotted in 3 dimensions, to improve interpretation (Figure 6). It showed that treatments groups were more separated; groups were apparent with samples taken from the 100% shade treatment situated on the right side of the plot. Moving right to left, most of the 80% shade treatment samples were grouped together, then the 50% shade treatment, then the control. This plot has shown that treatment groups were defined by algal communities.



Figure 6: 3D NMDS plot - stage 3 (stress 0.18).

Note: Nos. represent sampling occasions.

Considering the above dendrograms do not compare results for stages 2 and 3, FAC results for both stages were combined. Also, it was difficult to interpret these previous dendrograms (Figures 3 and 5) as each of the sampling occasions were not pooled, masking any obvious patterns that may have emerged. Therefore, the dendrogram was simplified by summing results for each sampling occasion (Figure 7).

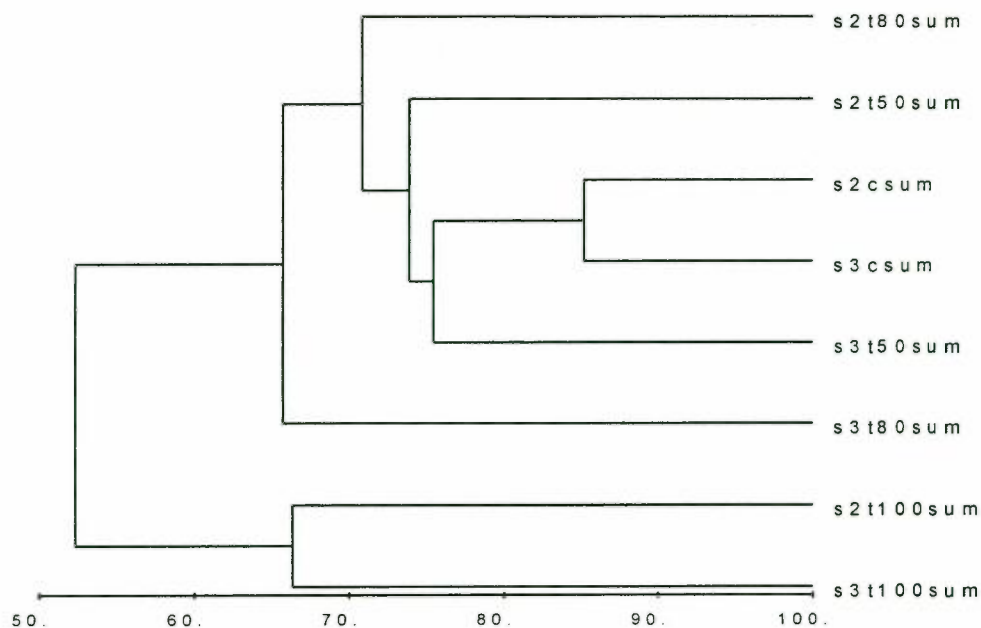


Figure 7: Cluster dendrogram - FAC in stages 2 and 3 and all treatments (all sampling occasions summed).

Note: The x-axis represents the similarity level and the vertical branches represent fusion of objects or subgroups. The horizontal lines separate each major group. s2 = stage 2; s3 = stage 3; t = treatment (50%, 80% and 100% shade); c = control (0% shade).

The cluster dendrogram of FACs in both stages and all treatments shows two groupings at the 65% similarity level (Figure 7):

1. Group 1 (100% shade in stages 2 and 3) and
2. Group 2 (all other treatments: 0%, 50% and 80% shade).

At the 70% level, Group 2 (from above) showed further separation into 3 groups:

1. Group 1 - Stage 2, 80% shade;
2. Group 2 - Stage 3, 80% shade; and
3. Group 3 - 0% and 50% shade (stages 2 and 3 for both treatments).

Finally, at the 80% level (from above) showed separation into 3 groups:

1. Group 1 - Stage 2, 50% shade;
2. Group 2 - Stage 3, 50% shade; and
3. Group 3 - 0% shade (stages 2 and 3).

These groupings show that according to algal counts, the 100% shade treatment was different to the other 3 treatments. Subsequently, each treatment separated at distinct similarity levels: the 80% shade treatment at 70% similarity, and the 0% and 50% shade treatments at 80% similarity. Treatments (i.e. 50% shade in stage 2 and in stage 3) appeared to be more similar to each other compared to stages.

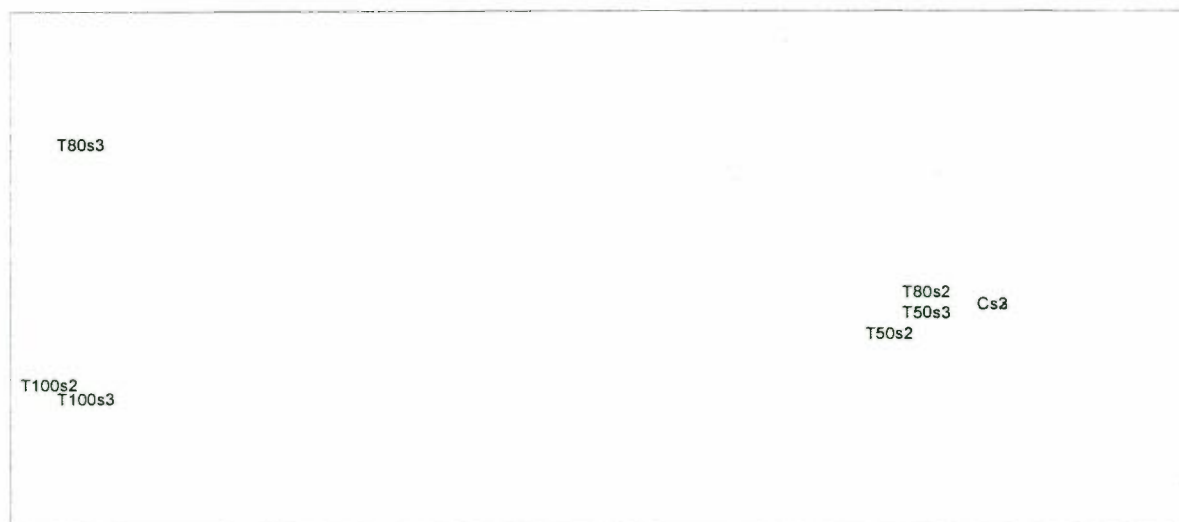


Figure 8: NMDS plot of FAC medians for all treatments in stage 2 and 3 data (stress 0.01).

Note: Distances between groups in the ordination diagram reflect the similarity of the algal communities comprising the group. T = treatment (50%, 80% and 100% shade), C = control (0% shade) and s2/s3 = stage 2/stage 3.

Ordination of median FAC data for stages 2 and 3 produced three distinct groups (Figure 8). Note that the 0% shade treatment in stage 2 (Cs2) was very closely associated with the 0% shade treatment in stage 3 (Cs3), such that one is overlayed on the other. Separation of the 100% treatment from all other groups was clearly evident. From this NMDS plot, each treatment was more closely related to the other for stages 2 and 3, i.e. algal communities in the 100% treatment at stage 2 were similar to those of stage 3. Groupings by stage were not apparent. This indicates that differences in algal communities lie in shade treatment conditions and not stage. Shade treatments may have some effect on algae.

In contradiction, the ANOSIM procedure using the same data (median FAC) found no significant differences between treatments. The power of the test may have been too low because of the small number of possible permutations. However, the high sample statistic ($R=1$) from this analysis indicated that the 100% treatment stage 2 and 3 algal community were very similar to each other. Likewise, the algal community for the 0%, 50% and 80% (stage 2 only) treatments were also similar to one another. In summary, the procedure has shown that the 100% shade cloth changed the algal species and algal numbers present in the pond but not to the extent where the two groups were statistically different from one another.

Similar distributions of sampling occasions were not evident in the NMDS plots for each treatment in stages 2 and 3. This could have been due to different sampling techniques; surface sampling for stage 2 water quality collection as opposed to sampling from conduits extracting effluent from the bottom of the treatment ponds for stage 3. Also, for most treatments, from the 4/2/97 (5th sampling occasion) a marked change in algal populations occurred with different algal species becoming dominant.

The BIOENV procedure, which links the biological community structure to environmental parameters, found the correlation between the algal genera found (FAC) and environmental variables tested to be very low (stage 2: $SS = -0.081$, stage 3: $SS, FC, \text{temperature and } DO = 0.015$). Therefore, no correlation could be drawn between the environmental variables tested and the algal community.

4.3 Physicochemical variables

4.3.1 Light

The light readings taken on two sampling occasions indicated the reduction in light reaching the pond surface according to shade strength (Table 6).

Table 6: Light levels (lux) recorded for each treatment.

Dates	0% shade	50% shade	80% shade	100% shade
4/3/97	2340	650	450	36
18/3/97	1830	550	470	64

4.3.2 Temperature

The 80% treatment had the lowest median water temperature of all treatments and stages. Median temperatures in stage 3 were higher than median stage 2 temperatures (Figure 9). As would be expected when autumn approached, temperature gradually decreased over time for stages 2 and 3 (Figure 15, Appendix A). Water temperature decreased from the 6th sampling occasion 18/2/97) in the 0% and 50% shade treatments, but not until the 7th occasion (4/3/97) in the 80% and 100% treatments, perhaps heat retention with these shade cloths was higher than with the other two cloths. There was a significant difference in water temperature over time indicating the influence of season (samples were taken in summer to the start of autumn) and perhaps the effects of continuous input from effluent processing (ANOVA, $P < 0.0001$). However, the differences of temperature between stages and treatments were not statistically significant (MANOVA, $P = 0.38$ and 0.36 , respectively, for stage and treatment). This suggests that the time effects were more influential. Temperature was also positively correlated with algae (FAC; $r^2 = 0.80$).

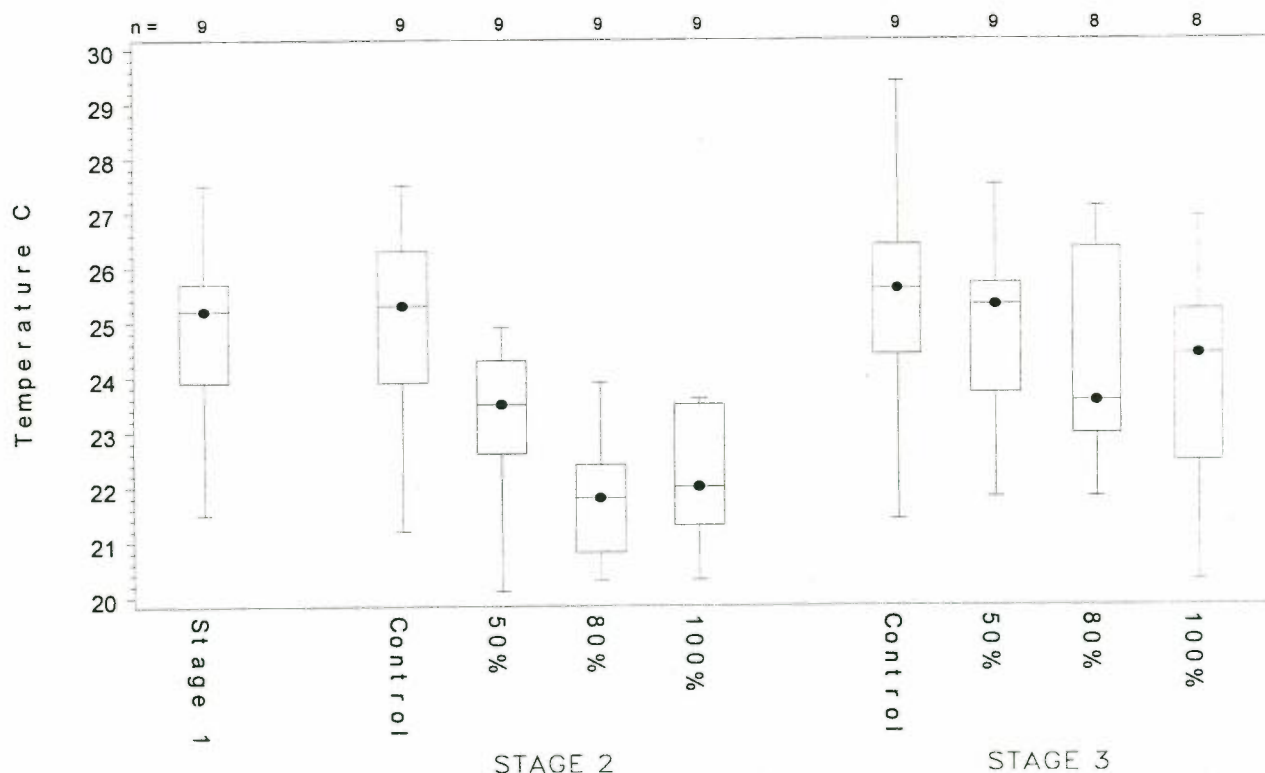


Figure 9: Boxplot of water temperature.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers. n represents the number of observations within a group.

The effect of shading was seen in the air temperatures measured on one sampling occasion underneath the shade cloth treatments (Table 7):

Table 7: Air temperature data - 18/03/97.

Treatment	Temperature (°C)
0%	24.3
50%	23.5
80%	21.5
100%	23.9

The lowest reading, from under the 80% shade cloth (Figure 7), was probably due to the combined effects of insulation and air flow.

4.3.3 Dissolved Oxygen (DO)

DO levels gradually decreased throughout the trial for stages 2 and 3, especially during January, when the amount of duckweed (*Lemna* spp.) rapidly increased on the ponds. Stage 3 DO readings were much higher than those of stage 2 readings because of oxygenation at stage 3 in the agricultural pipes. Stage 3 readings fluctuated more than stage 2 readings, also due to oxygenation in the pipes. DO in all treatments (all stages) tended to decrease over time (Figure 16, Appendix A). As with temperature, the differences of DO between stages and treatments were not statistically significant (MANOVA, $P=0.22$ and 0.49 , respectively). However, a significant difference was found between sampling occasions (ANOVA, $P<0.0001$).

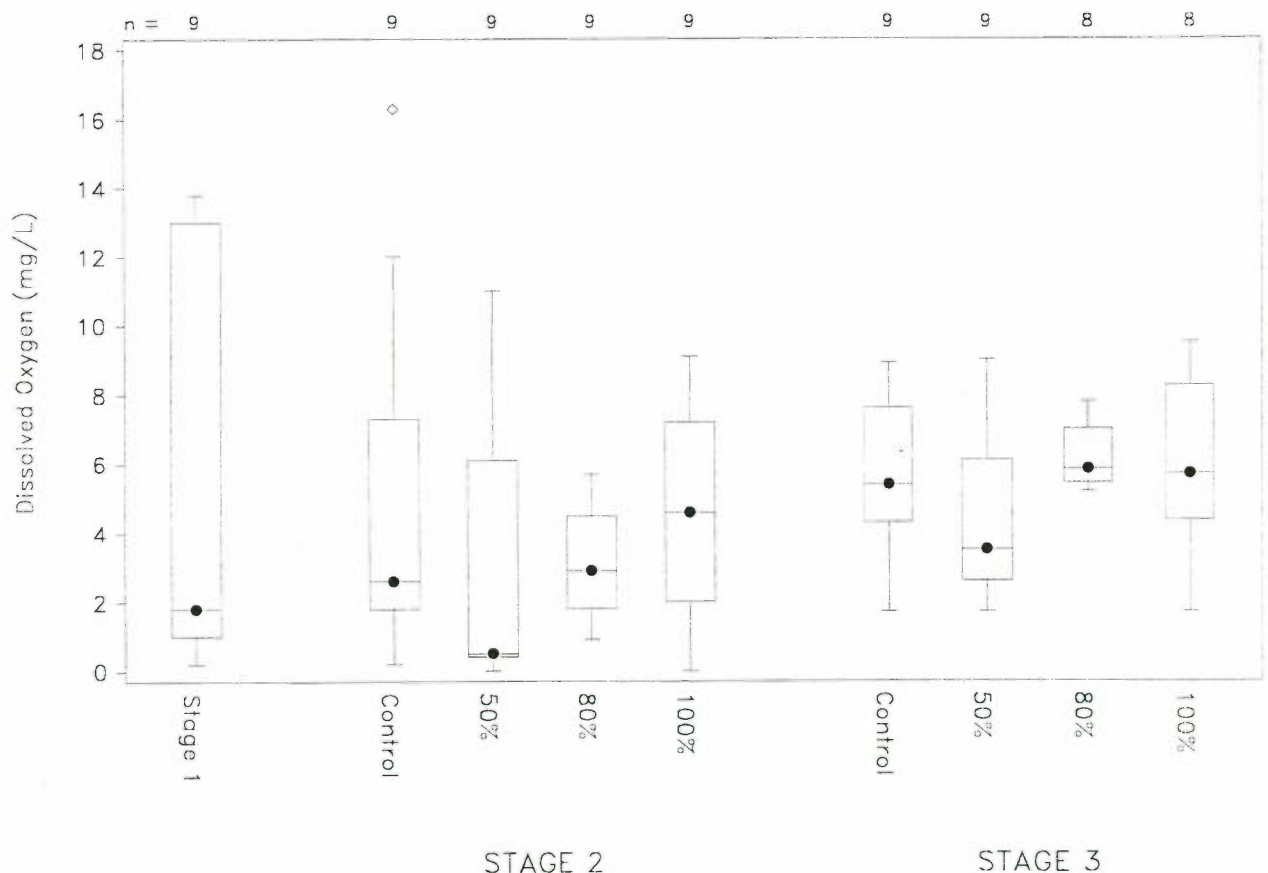


Figure 10: Boxplot of DO.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers. n represents the number of observations within a group.

4.3.4 pH

Stage 1 had the lowest median pH (6.6) and the 100% treatment at stage 3 had the highest pH (7.5). For both stage 2 and 3, there was a slight increase in pH from 0% shading to 100% shading. pH remained stable for stages 2 and 3 after the first sampling occasion (12/12/96) where pH was elevated (Figure 17, Appendix A). The 0% and 50% shade treatments gradually decreased through time for both stages 2 and 3; this was not evident for the 80% and 100% treatments. As with temperature and DO, the differences of pH between stages and treatments were not statistically significant (MANOVA, $P=0.18$ and 0.90 , respectively). However, a significant difference was found between sampling occasions (ANOVA, $P < 0.0001$), probably indicating the variable nature of effluent entering the pond from secondary treatment.

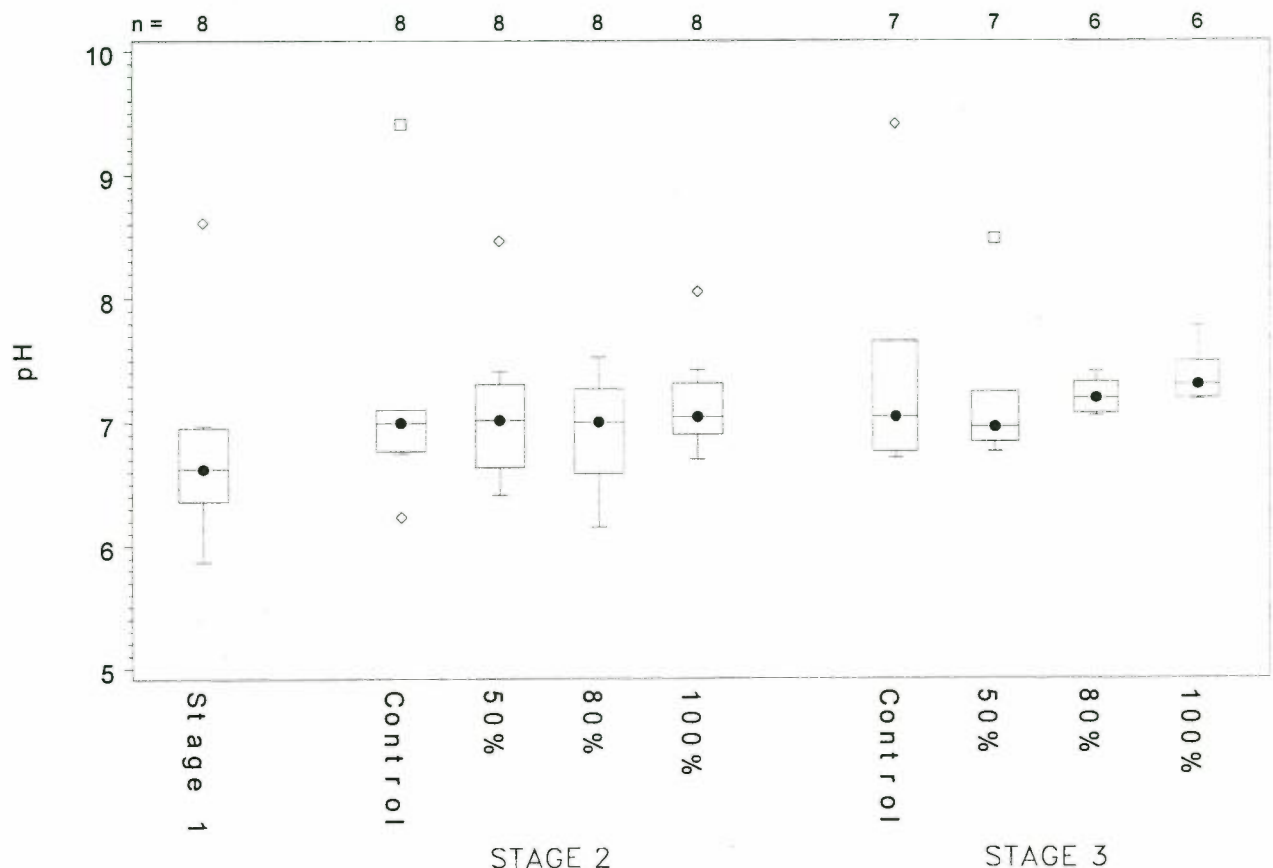


Figure 11: Boxplot of pH.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers. n represents the number of observations within a group.

4.3.5 Suspended Solids (SS)

SS collected in previous years (1990-1995 mean - 25 mg/L) were comparable to SS measured in stage 2 of this study (Table 11, Appendix B). The lowest median SS occurred in stage 2 in the 100% treatment, but the highest median also occurred in the 100% treatment (stage 3). The outlet pipes in stage 3 for both 80% and 100% shade treatments were close to the pond bottom. It was observed that sediment on the bottom of the pond was being drawn into these pipes when water was allowed to drain from the pond for sampling. Particulate matter accumulating on the sides of the agricultural pipes also may have been resuspended when effluent samples were taken for some of the treatments. Significant differences in the logarithmic values of SS were found for both stage and treatment (MANOVA $P < 0.01$). SS concentrations were also positively correlated with algae (FAC; $r^2=0.69$).

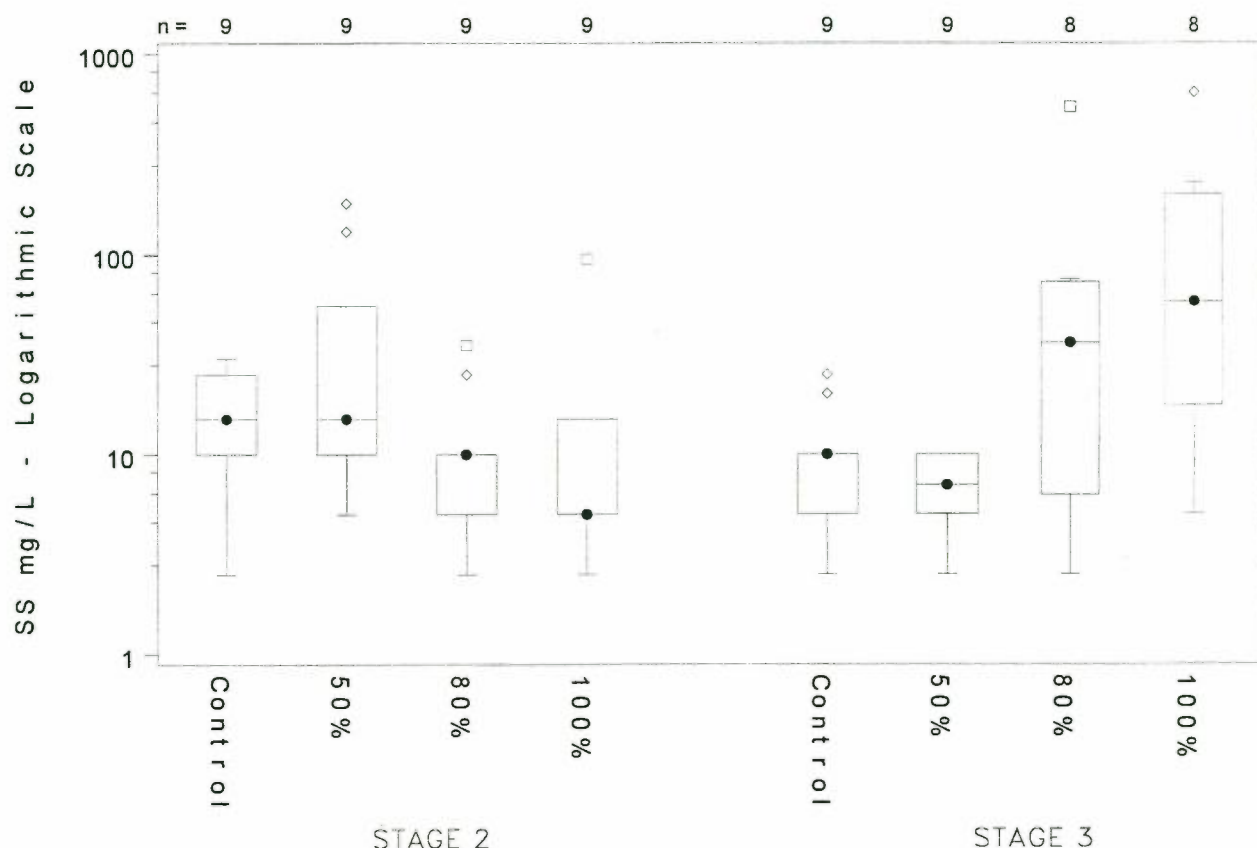


Figure 12: Boxplot of suspended solids.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers or diamonds (extreme outlier points). n represents the number of observations within a group.

4.3.6 Faecal Coliforms (FC)

The median value for stage 1 (8000 CFU/100mL) was much higher than stage 2 and 3 medians for all treatments (Figure 13). Perhaps retention of effluent and not shading treatments, effectively reduced FC concentrations (FC medians in the 0% shade treatment in stages 2 and 3 were lower than that of stage 1). FC medians for the 80% and 100% shade treatments in both stages were lower than those of the 0% and 50% shade treatments. As with temperature, DO, pH and SS, the differences of FC between stages and treatments were not statistically significant (MANOVA, $P=0.15$ and 0.33 , respectively). There was a significant difference through time (ANOVA, $P < 0.008$).

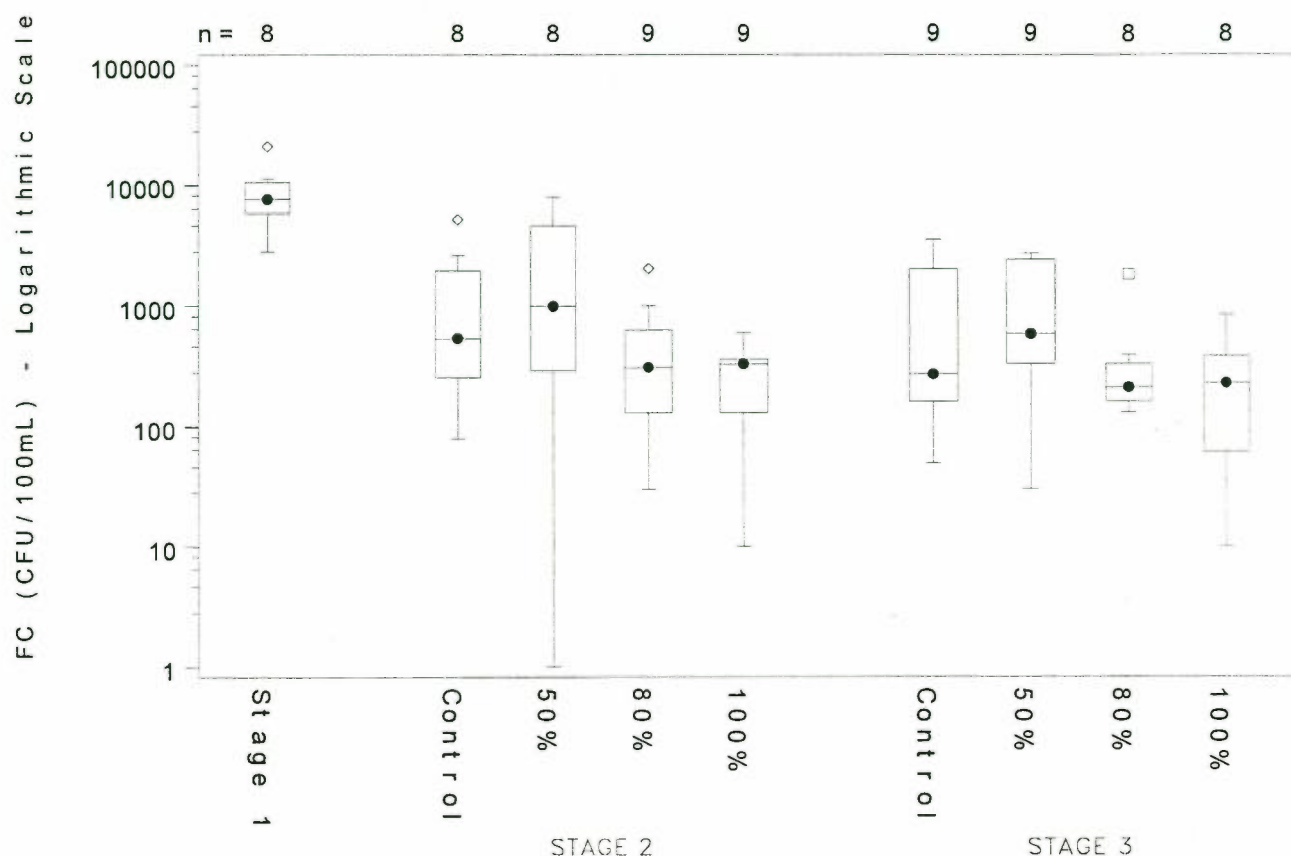


Figure 13: Boxplot of faecal coliforms.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers or diamonds (extreme outlier points). n represents the number of observations within a group.

4.3.7 Full Algal Count (FAC)

Median algal concentrations were lowest for the 100% treatment for stage 2 (Figure 14). Stage 3 results did not show the same pattern; the 100% treatment median being the highest. This could be because of the different sampling techniques recovering different algal concentrations and species. Stage 2 sampling was essentially at the surface whereas stage 3 sampling was of water that was drained from the bottom of the different treatment compartments. Significant differences in the logarithmic values for FAC were found for both stage and treatment (MANOVA $P < 0.01$). A significant difference in FAC was found to occur over time for stage 2 only ($P < 0.01$).

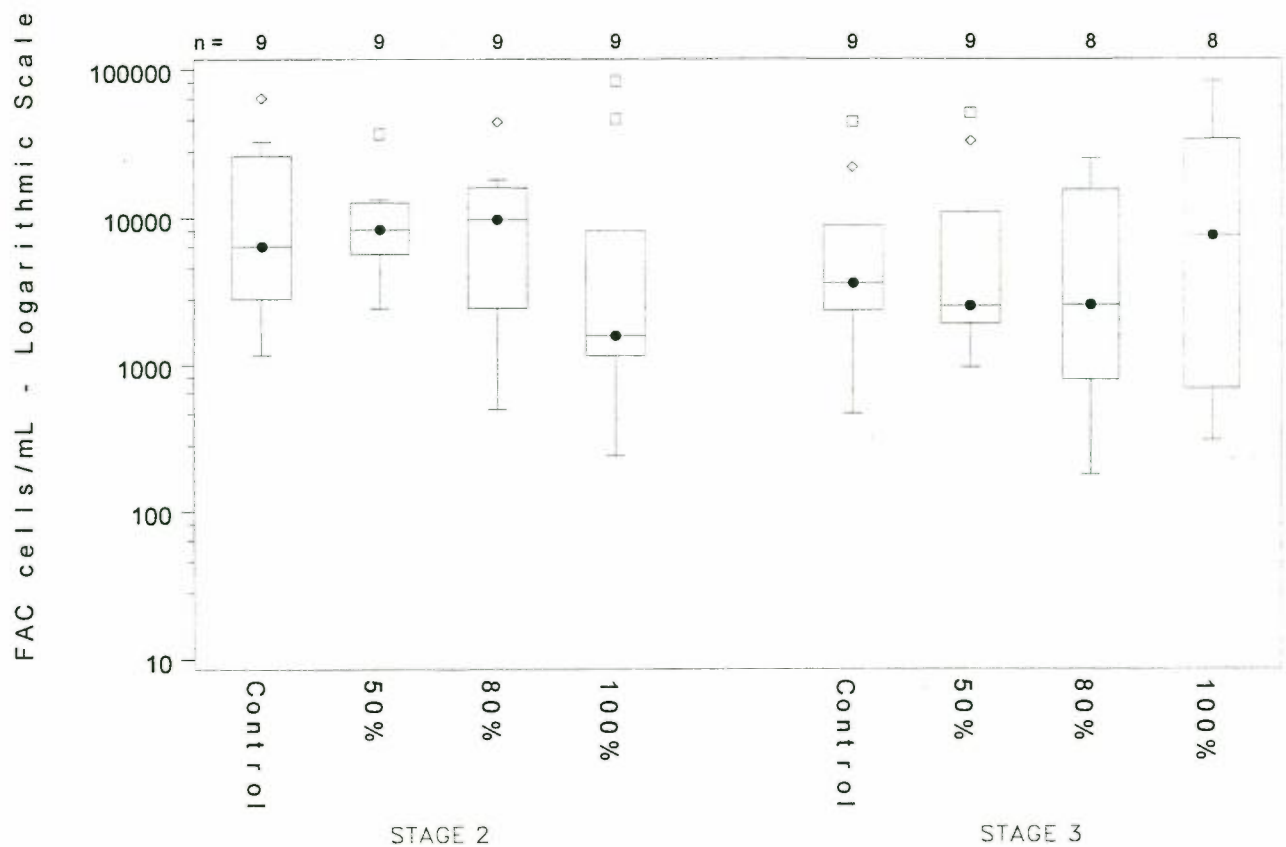


Figure 14: Boxplot of full algal counts.

Note: The median is represented by the central line and dot in each of the boxes. 50% of collected data are represented by the length of the box. The rest of the data are shown by the use of whiskers or diamonds (extreme outlier points). n represents the number of observations within a group.

5.0 DISCUSSION

'Repeated measures' analyses revealed that there were significant time effects on temperature, dissolved oxygen, pH, suspended solids, faecal coliforms, and algal counts. The effect of time on these variables was significantly different for stages 2 and 3. The effect of time on these variables was also significantly different for the 4 treatments (0%, 50, 80 and 100% shade). Water quality and algal communities changed over the study period. This was most likely a seasonal effect, with temperature decreasing over time for all stages and treatments. Temperature can often affect other water quality variables, as well as biological activity (ANZECC 1992; SEAC 1996; Walling and Webb 1992).

Multi-variate analysis of stages 2 and 3 data clearly showed a change in the algal communities in the ponds with varying algal dominance over time. It was not possible to determine if certain algal genera characterised different treatments or stages. This was due to the lack of replication and this lowered the power of statistical analysis and eliminated the possibility of performing certain multivariate tests.

The constant flow of effluent may be a crucial factor in determining whether shade treatments in maturation ponds are an effective method for algal removal. The constant influx of heterogeneous effluent indicates that more rapid control methods may need to be employed. Hansen *et al.* (1992) clearly stated the advantages of long retention times on algal growth if this is combined with well mixed deep ponds. The mixing results in the algae being transported to the darker regions of the pond, thus inhibiting growth. Mixing will also stop the formation of a warm surface layer which could increase the algal growth. Mixing can either be achieved by wind motion or by artificial means if the ponds are too small for wind action to be effective. Griffith (1988) reported the use of a synthetic rubber membrane in a storage reservoir in Orange County, US in a bid to reduce trihalomethane (THM) levels without increasing algal growth. The long storage time in the reservoir would lead to an appreciable decrease in algal concentrations. In comparison, maturation ponds are eutrophic and have significantly shorter retention times. Algal reduction might be achieved with large, deeper ponds and if the retention period was extended. The time for substantial algal death would need to be determined if artificial shading was to be qualified as a successful method of algal removal.

The highly fluctuating nature of algal populations during the pilot study was partially responsible for the difficulty in characterising treatments and stages with algal taxa. Shillinglaw and Pieterse (1977) reported similar problems with their observations of algal populations on experimental maturation ponds which exhibited marked fluctuations throughout their study. Large algal concentrations could only be maintained for brief occasions followed by drastic declines. This was due to sedimentation or by removal through outflow. The large standard deviations for FAC results showed the highly variable nature of algal populations. The use of median FAC results for NMDS and ANOSIM partially eliminated this problem. The constant inflow of heterogeneous effluent probably contributed to the high variation in algal numbers and taxa, though determining the mechanisms involved were well beyond the scope of this study.

The FAC box plot for stages 2 and 3 data showed a decrease in median algal levels for the 100% shade cover. The ordination of median FAC data also showed the separation of the 100% treatment from other treatments. However, this did not prove to be statistically significant using ANOSIM, i.e. based on algal community composition. The analysis did indicate however that algae occurring in the 100% treatment was unique (algal species composition and algal numbers) compared to the 0%, 50% and 80% treatments. ANOSIM showed that the number of permutations involved for calculating significance levels was very small (i.e. the power of the analysis was low). The power of the ANOSIM could be increased through replication of samples within treatment compartments; the resulting model would become more robust. The test may also have resulted in more conclusive explanations with an extended effluent retention time in the treatment compartments. The increased retention time may allow for a reduction in algal cells.

Data analysis results of water quality monitoring at Stroud STP may have been confounded by the extensive growth of duckweed occurring on three of the four treatments commencing from the third sampling occasion (6/1/97). Duckweed had not been present at Stroud STP for 5 years (pers. comm. Tom Baldwin) and it may have been introduced by ducks. Hillman and Culley (1978) suggested that duckweed grow at a rapid rate, confirmed by our field results where from the 6/1/97 duckweed rapidly covered the surface on the 0%, 50% and 100% treatments. Duckweed appeared to be at its highest levels on the 4/2/97 at which time algal counts were lowest.

Algae occurring in maturation ponds are those capable of adaptation to high nutrient conditions and a wide range of organic compounds. Species present are usually associated with polluted water bodies. The most common genera are *Anabaena*, *Anacystis*, *Ankistrodesmus*, *Chlamydomonas*, *Chlorella*, *Euglena* and *Scenedesmus* (Williams 1980). Six genera characterised algal populations in the effluent ponds: *Ankistrodesmus*, *Chlamydomonas*, *Cryptomonas*, *Oocystis*, *Rhodomonas* and *Sphaerocystis*. *Ankistrodesmus* sp. and *Chlamydomonas* sp. were found to dominate water quality samples taken in all the treatments. The change in the algal community (evident from the 5th sampling occasion) can be attributed to seasonal changes which influence all natural water bodies, together with inflow of heterogeneous effluent. The factors responsible for the change are light, temperature, grazing and organic pollution (White 1975; Rodgi and Patil 1971; DeNoyelles 1967).

Similar to fluctuating FAC levels, SS concentrations were also found to vary considerably. SS summary statistics showed that standard deviations were greater than means for many treatments. However, all SS concentrations for treatments were comparable to previous routine sampling results. Since SS was positively correlated with FAC, a decrease in algal cell numbers would result in a similar decrease in SS levels. The 100% treatment showed a decrease in median FAC and SS concentrations but both did not prove to be statistically significant. To increase the statistical power of analyses to account for the inherent variability of sampling maturation ponds, replication of all water quality variables would be needed to confirm any associations between those variables. Alternatively a profile sample representing the entire depth of the maturation pond could be taken. This option would be less expensive than sample replication. Person *et al.* (1987) found that full water column sampling for COD, SS, FC, chlorophyll and ammonia provided reasonably accurate mean daily effluent values compared to grab sampling. The vertical distribution of algae within the water column of a pond varies with organic loading, time of day and algal species. It can range between homogenous distribution of algae with respect to depth, to algae concentrated in a narrow mobile layer which moves through the water column in response to certain environmental factors such as light. This uneven distribution was observed in the 80% shade cloth where small algal scums were found to occur on the water surface. This was not obvious for the other treatments due to the duckweed covering. Alternate to profile sampling may be to simply sample more intensively at stage 1. If continued variability was observed at this point of the treatment phase, then sampling of stages 2 and 3 would serve no purpose; variability would exist at these two stages as well.

The large decrease in DO levels for stage 2 sampling, with the exception of the 80% treatment compartment, up until the fifth sampling occasion (4/2/97) may reflect the impact of duckweed, combined with high algal numbers, on the ponds. Duckweed, if not continually removed, may form anoxic conditions in the root zone (Lewis and Bender 1961) and algae can also deplete oxygen levels in their diurnal cycle of respiration.

FC concentrations were not found to be significantly different between stages 1, 2 and 3 and treatments, even though medians for the 80% and 100% shade treatments were lower than those of the 0% and 50% treatments. Stage 1 FC concentrations were much higher than any of the treatments. From this, it would appear that variations in FC concentrations within treatments were more influential than those between treatments and reasons for this significant variation probably lie in the heterogeneous nature of the incoming effluent. Mean FC concentrations had an approximate log reduction from stage 1 to stage 2 treatment levels. The absence of sunlight in this current pilot study was not detrimental to FC removal. This implies that UV light is not the major agent for microbial reduction in maturation ponds in this study. Maturation ponds are designed to be relatively shallow (1.0-1.5m) allowing light to penetrate deep into the water column (i.e. a large photic zone) so that bacteria can be degraded. This design is ideal for algal growth.

Curtis *et al.* (1992) states that visible light is more important than U.V. light and that light alone is not responsible for FC removal. A combination of light, high pH and high oxygen is required for light to have any effect on FC concentrations. The retention times for effluent in this study were probably insufficient to allow significant decreases in microbial populations. Ellis and Rodrigues (1985) reported that FC levels in maturation ponds were principally affected by loading, retention time, pond depth and effluent electrical conductivity. Higher loadings and increased retention periods were found to improve FC removal. Studies by both Ellis and Rodrigues (1985) and Curtis *et al.* (1992) indicate that light is not the dominant factor affecting FC removal.

The guidelines for FC discharge into Class C receiving waters for recreational use are 200 CFU/100mL (EPA 1980). This level (set for bathing purposes only) was exceeded 65% of the time. Effluent from the treatments did not undergo the full retention period of 15 days, therefore FC levels were higher than under normal operating conditions. pH levels were also found to exceed levels for Class C waters. At stage 3, the discharge water would have failed to meet EPA guidelines 23% of the time. Wastes are not to be discharged into Class C waters if the pH value of the waste is less than 6.5 or more than 8.5 (EPA 1980).

The occurrence of mosquitoes predominantly under the 100% treatment is worth comment. The lack of air movement caused by the tarpaulin over the pond creates the perfect environment for mosquito populations. Mosquitoes do not normally occur in areas where there is water movement, wave action or wind. Mosquitoes also prefer humid warm conditions in their adult phase. Warm temperatures in ponds can also promote the development of larvae (Merritt and Cummins 1996). They feed on microorganisms, detritus, zooplankton and inert particles from the water column by filtering, gathering and collecting (Merritt and Cummins 1996). STP ponds would provide an abundant source of food for mosquito larvae. If 100% shading was to be considered as a viable option in the future, the presence of mosquitoes in such a man-made environment would need to be considered as a management problem.

6.0 CONCLUSIONS & RECOMMENDATIONS

Results from the pilot study have shown that, generally, shade treatments had some effect on algal communities in the ponds. However, the power of statistical analyses was limited because of the lack of replication and statistical models tested may not truly reflect the biological activity in the effluent ponds. Some reduction in algal populations was achieved with the use of the 100% shade cloth. Algal concentrations decreased and a distinct algal community developed but this did not prove to be statistically significant from the others. The effluent retention time of approximately nine days for each treatment compartment was also too short to reduce algal concentrations. Growth of algae in areas which were not shaded (Pasveer channel and pond 1) may have contributed to increased algal concentrations in the 0%, 50%, 80% and 100% shade treatment compartments. If all open areas were shaded, the 15 day retention period may be sufficient to substantially reduce algal numbers. Investigation of optimal retention times for considerable algal reductions would answer this question. This would determine if the expense of constructing large covers would be offset by substantial reductions in algal concentrations. The increase in mosquito populations in an enclosed environment, such as under 100% shading, also warrants further investigation and could be a potential management problem if artificial shading is pursued.

The presence of duckweed, a floating macrophyte, on three of the four treatment ponds may have confounded some of the outcomes of this study. Duckweed may have been responsible for the large decline in DO levels in the treatments.

Artificial shading, even with 100 % shading, was not found to detrimentally affect the ability of the maturation ponds to significantly reduce FC concentrations in the ponds. This implies that UV light is not necessarily the major agent for microbial reduction in maturation ponds which may enable their design to be re-examined. Temperature was different for each of the treatments, the difference was most likely a result of the shade provided and restriction of air movement particularly for the 100% treatment. pH, DO and SS concentrations were not affected by different levels of shading.

If artificial shading is to be pursued further as a potential algal reduction technique, further work will need to be conducted to more closely examine the issues raised in this study. Recommendations for future studies are to:

1. Determine effective retention times required for significant decreases in algal and microbial populations subjected to various degrees of shading;
2. Assess water quality at the inflow point and assess the use of shading at this stage; and
3. Sample the full water column and/or replicate water quality variables to reduce the variability of individual grab sample results.

7.0 REFERENCES

- ANZECC. (1992). Australian Water Quality Guidelines for Marine and Freshwaters. National Water Quality Management Strategy.
- Bowling, L. and Mitrovic, S. (1996) **Literature review: algae control and removal from tertiary sewerage treatment ponds. Management options for algal control and removal.** *Department of Land and Water Conservation.*
- Clarke, K.R. (1993) **Non-parametric multivariate analyses of changes in community structure.** *Aust J. Ecol.* **18**, 117-143.
- Clarke, K.R. and Ainsworth, M. (1993) **A method of linking multivariate community structure to environmental variables.** *Mar. Ecol. Prog. Ser.* **92**, 205-219.
- Clark, K.R. and Warwick, R.M. (1994) **Change in marine communities: an approach to statistical analysis and interpretation.** *Plymouth Marine Laboratory.*
- Curtis, T.P. (1992) **The effect of sunlight on faecal coliforms in ponds: implications for research and design.** *Wat. Sci. Tech.* **26**:7-8, 1729-1738.
- Curtis, T.P., Mara, D.D. and Silva, S.A. (1992) **Influence of pH, oxygen and humic substances on the ability of sunlight to damage faecal coliforms in water stabilisation pond water.** *Appl. Envir. Microbiol.* **58**, 1335-1343.
- DeNoyelles, F. (1967) **Factors affecting phytoplankton distribution in double-cell sewage lagoon.** *J. Phycol.* **3**, 174-181.
- Ellis, K.V. and Rodrigues, P.C.C. (1985) **Multiple regression design equations for stabilization ponds.** *Wat. Res.* **29**:11, 2509-2519.
- EPA (1980) **An atlas of classified waters in NSW.** Environmental Protection Authority.
- Grabhi, A., Ferchichi, M. and Drakides, C. (1994) **Treatment of wastewater by stabilisation ponds - applications to Tunisian conditions.** *Wat. Sci. Tech.* **28**:10, 193-199.
- Griffith, C. (1988) **Floating reservoir cover controls algae growth, allows lowered THMs.** *Jour. Amer. Water Works Assn.* **Oct**, 66-69.
- Hansen, P.J., Crockett, J.A. and Hartley, K.J. (1992) **Algae in lagoon effluent.** *ANRC and Melbourne Water, Lagoon Technology Seminar, Melbourne.*
- Hillman, W.S. and Culley, D.D. (1978) **The uses of duckweed.** *American Scientist* **66**, 442-451.
- Larson, T.E. (1939) **Bacteria, corrosion and red water.** *Jour AWWA.* **31**:1, 1186.
- Lewis, W.M. and Bender, M. (1961) **Effect of a cover of duckweeds and the alga *Pithophora* upon the DO and free CO₂ of small ponds.** *Ecology.* **42**, 602-03.
- Mandi, L, Ouazzani, N., Bouhoum, K. and Boussaid, A. (1995) **Wastewater treatment. Stabilisation ponds with and without macrophytes under arid climate.** *Wat. Sci. Tech.* **28**:10, 177-181.

-
- Merritt, R.W. and Cummins, K.W. (1996) **An introduction to the aquatic insects of North America, third edition.** *Kendal and Hunt Publishing Company.*
- Mitchel, W.D. ed. (1980) **An ecological basis for water resource management.** *ANU Press.*
- Person, H.W., Mara, D.D., Konig, A., de Oliveira R., Mills, S.W., Smallman, D.J. and Silva, S.A. (1987) **Water column sampling as a rapid and efficient method of determining effluent quality and the performance of waste stabilisation ponds.** *Wat. Sci. Tech.* **19**:12, 109-113.
- Rodgi, S.S. and Patil, M.S. (1971) **Ecological adaptations of algae in an oxidation pond at Dharwar.** *Ind. Zool.* **2**, 105-12.
- SEAC (1996) **Australia: State of the Environment 1996.** State of the Environment Advisory Council. CSIRO Publishing, Collingwood.
- Shillinglaw, S.N. and Pieterse, A.J.H. (1977) **Observations on algal populations and experimental maturation pond system.** *Wat. Sth. Africa* **3**:4, 183-192.
- Sokal, R.R. and Rohlf, F.J. (1995) **Biometry, third edition.** *W.H. Freeman and Company.*
- Tukey, J.W. (1977) **Exploratory data analysis.** *Addison-Wesley, Reading, Massachusetts* (Chapters 2,3,4 & 7).
- Walling, D.E. and Webb, B.W. (1992) **Water Quality I. Physical Characteristics.** In: *The Rivers Handbook.* Vol. 1. (eds. P. Clow and G. Petts). Blackwell Science, Oxford.
- Williams, W.D. ed. (1980) **An ecological basis for water resource management.** *ANU Press.*
- White, R.W. G. (1975) **An ecological study of fish and phytoplankton populations in sewage effluent lagoons.** Ph.D thesis. *University of London.*
- Wolfe, R.L., Means, E.G., Davis, M.K. and Barrett, S.E. (1988) **Biological nitrification in covered reservoirs containing chlorinated water.** *Jour AWWA* Sept, 109-114.

APPENDIX A: Time series plots.

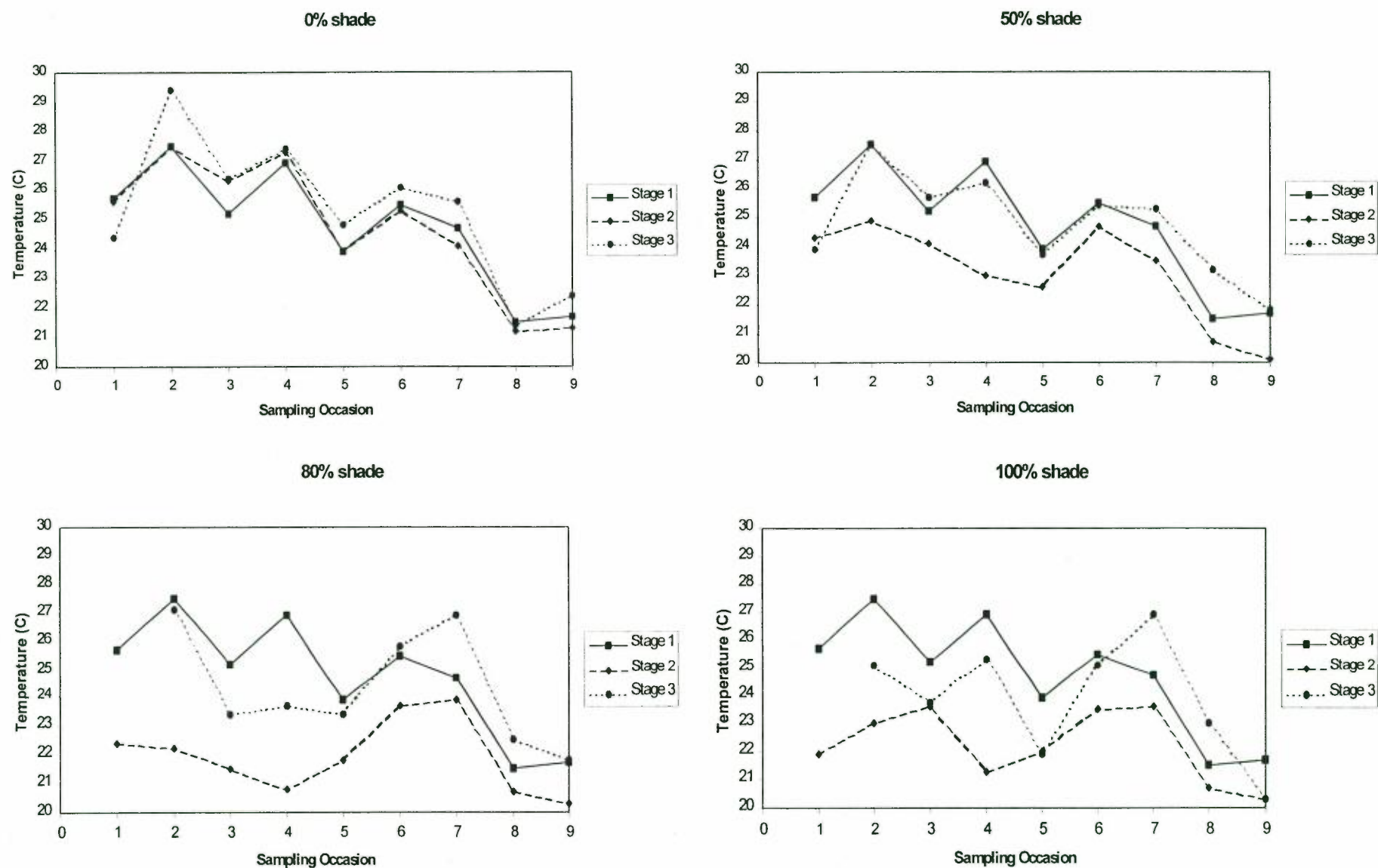


Figure 15: Time series plots of temperature for 0%, 50%, 80% and 100% shade treatments and all stages.

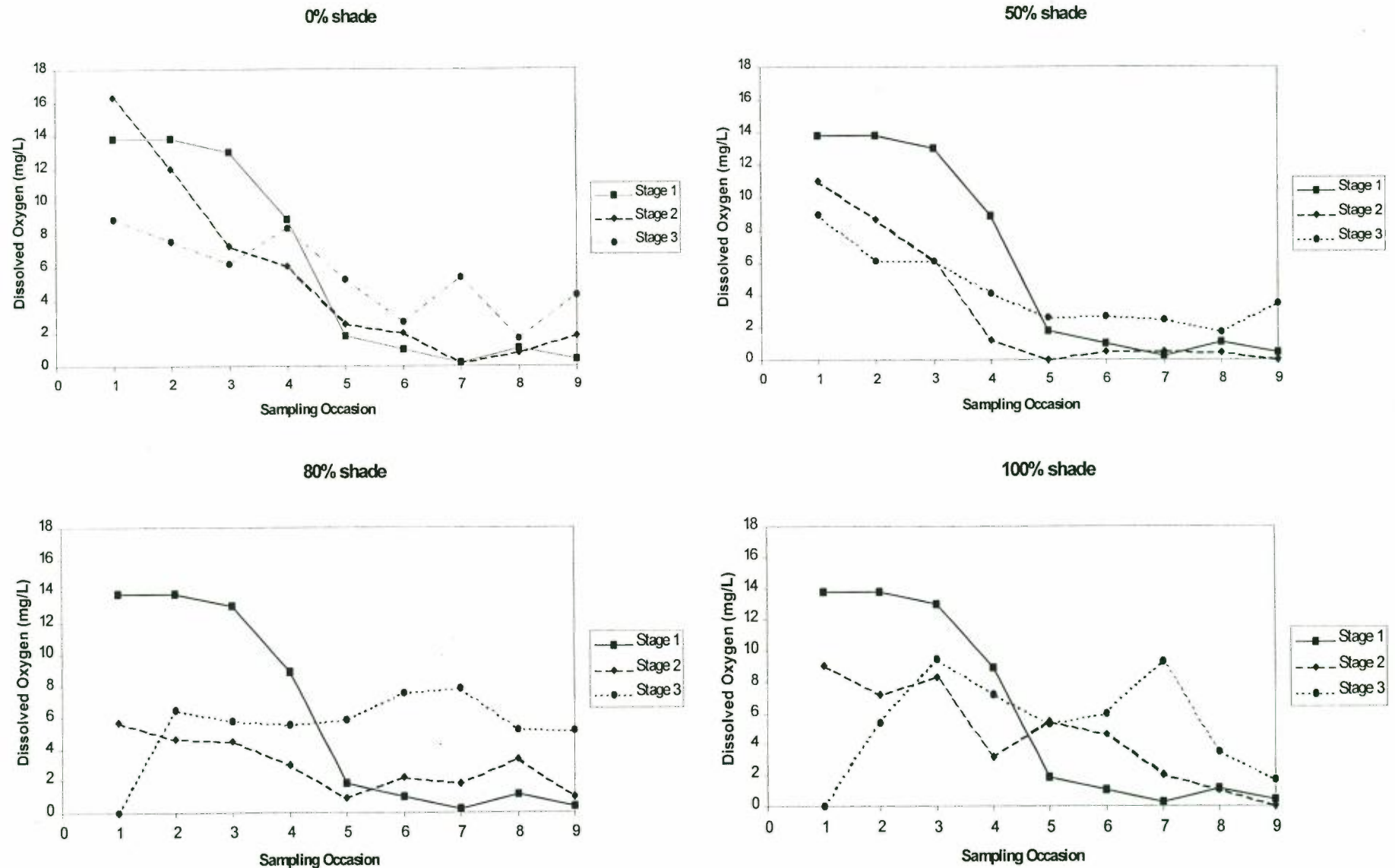


Figure 16: Time series plots of dissolved oxygen for 0%, 50%, 80% and 100% shade treatments and all stages.

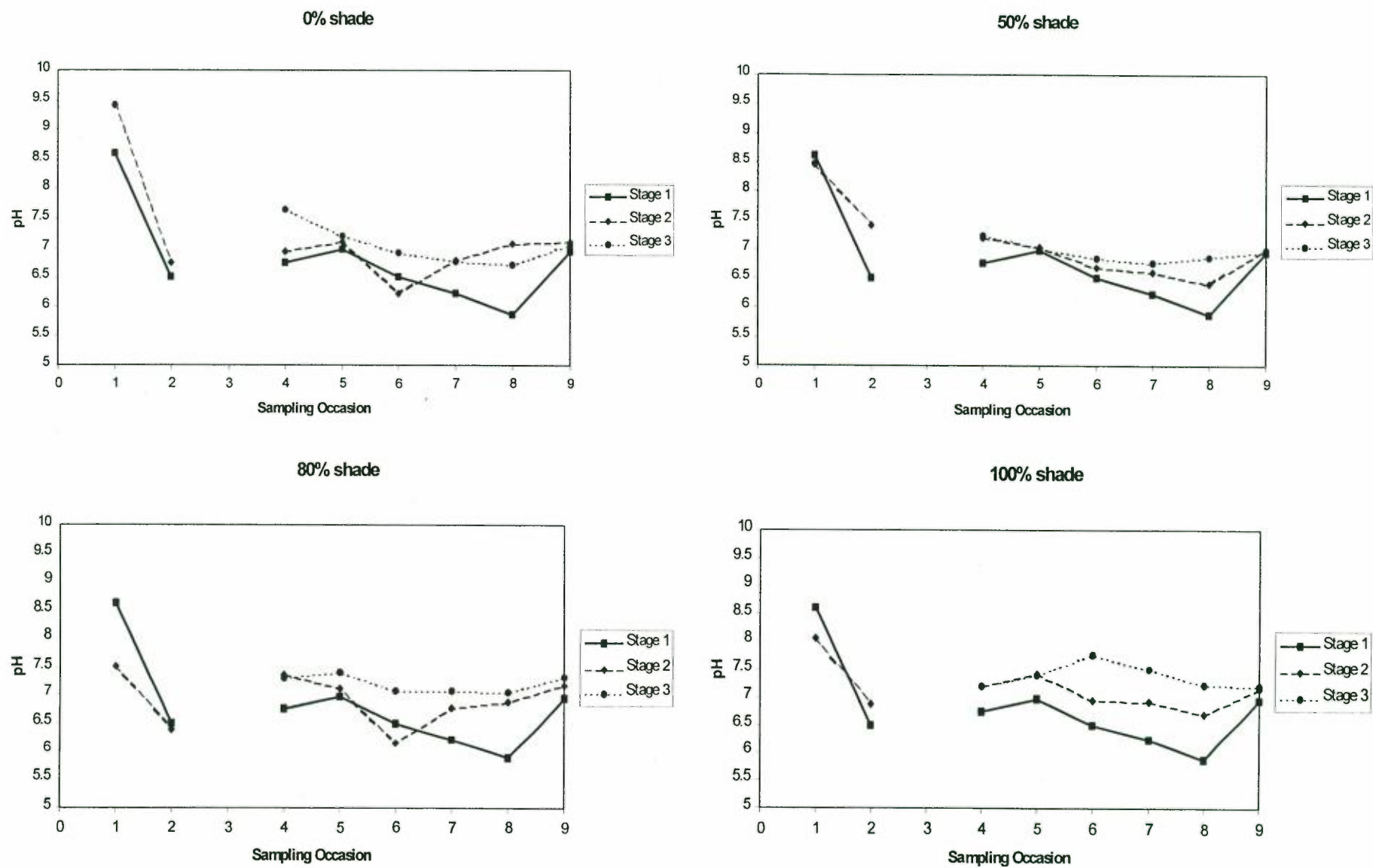


Figure 17: Time series plots of pH for 0%, 50%, 80% and 100% shade treatments and all stages.

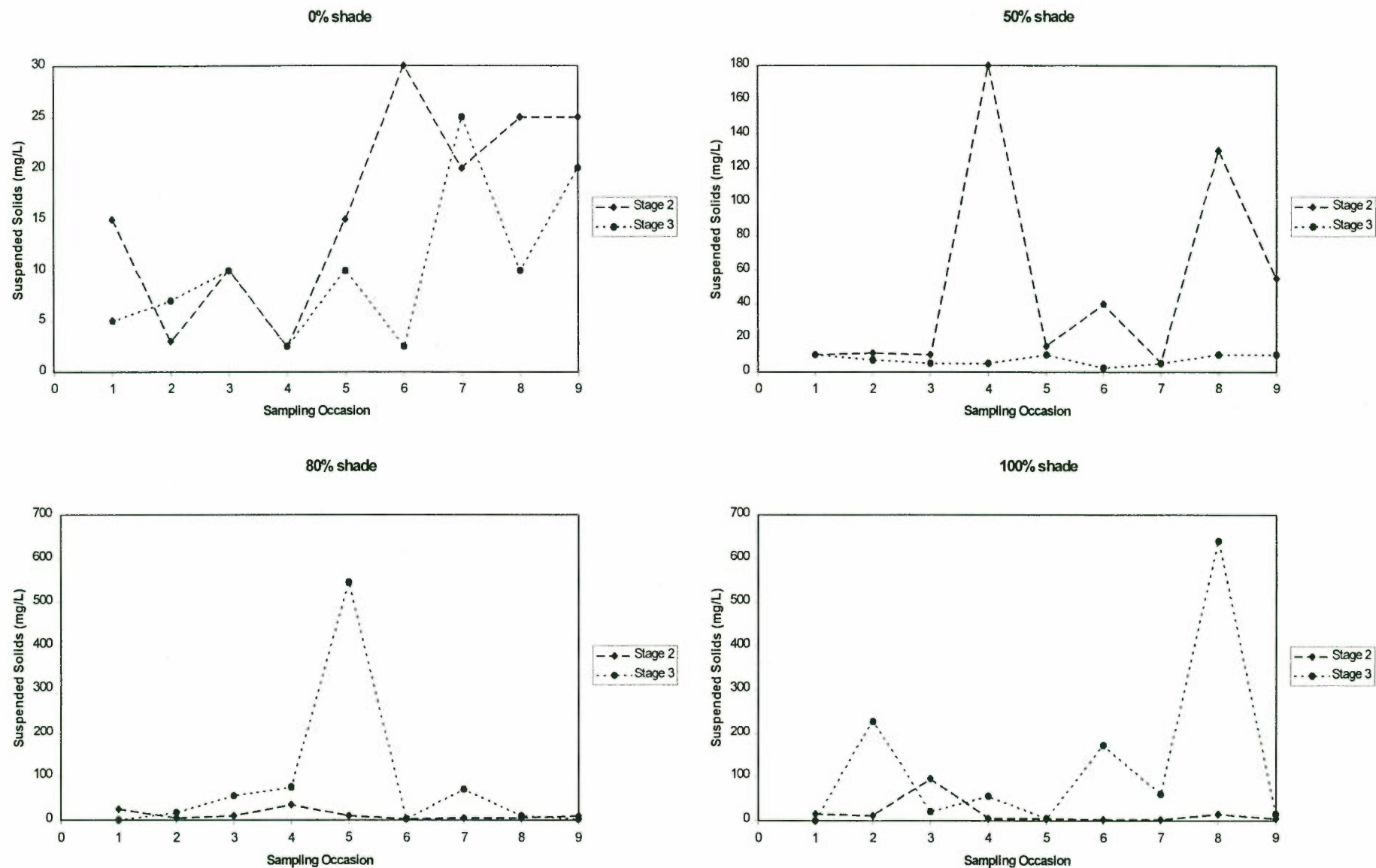


Figure 18: Time series plots of suspended solids for 0%, 50%, 80% and 100% shade treatments and all stages.

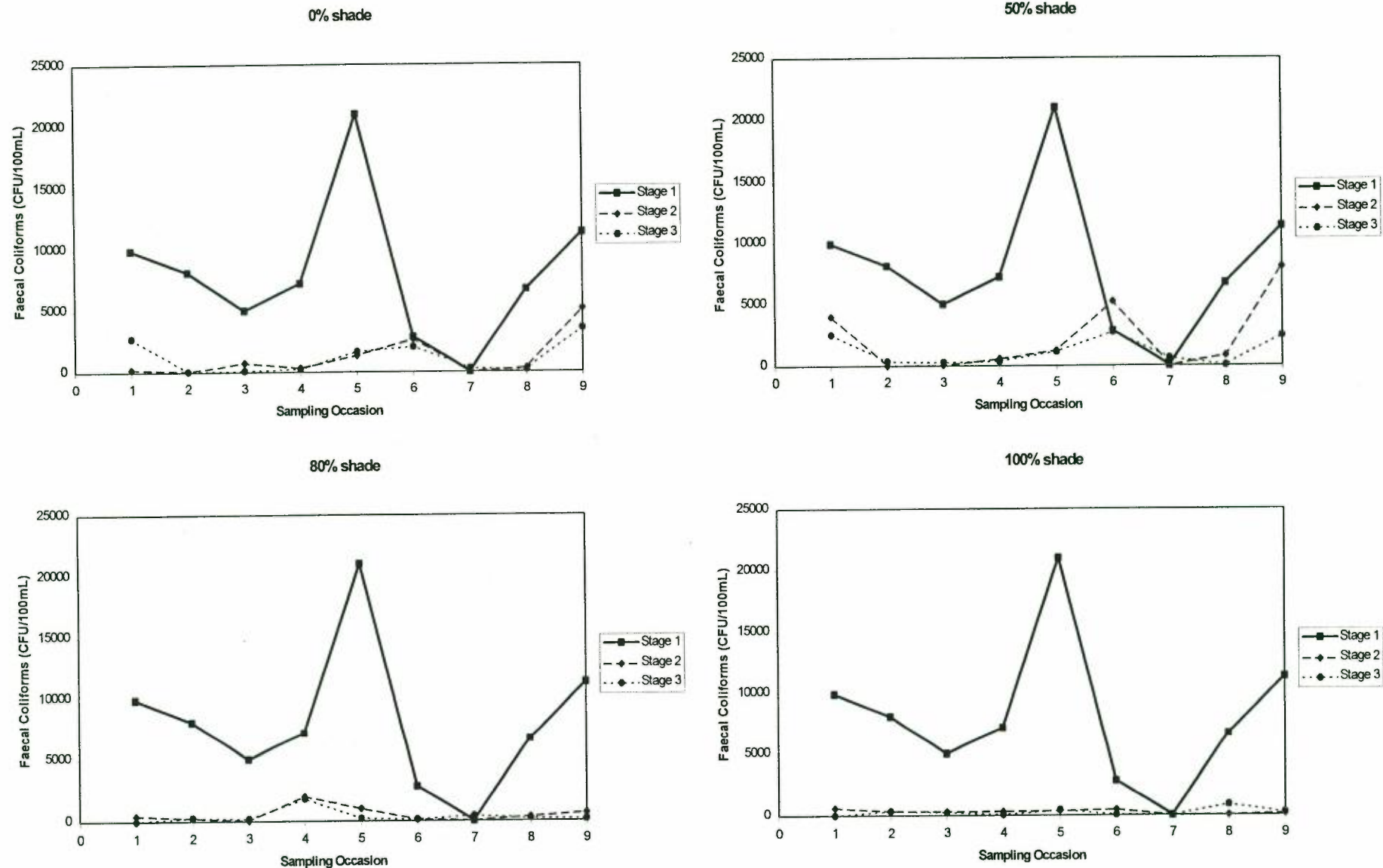


Figure 19: Time series plots of faecal coliforms for 0%, 50%, 80% and 100% shade treatments and all stages.

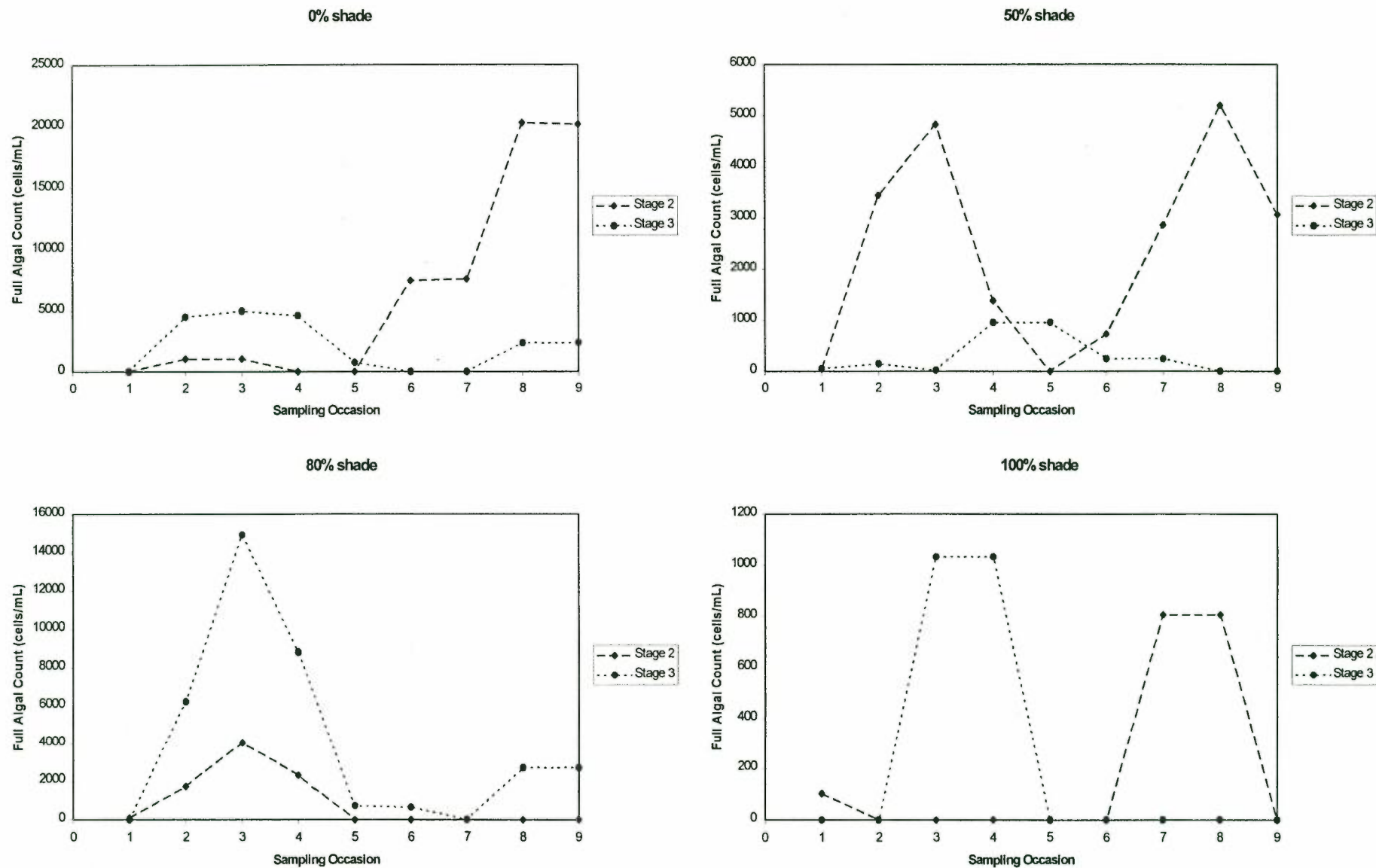


Figure 20: Time series plots of full algal counts for 0%, 50%, 80% and 100% shade treatments and all stages.

APPENDIX B: Summary statistics.

Table 8: Temperature - mean and standard deviation (sd).

Treatments	Stage 1 temperature (°C)		Stage 2 temperature (°C)		Stage 3 temperature(°C)	
	mean	sd	mean	sd	mean	sd
0% shade	24.7	2.0	24.7	2.3	25.3	2.5
50% shade	n/a	n/a	23.1	1.7	24.7	1.7
80% shade	n/a	n/a	21.9	1.2	24.3	2.0
100% shade	n/a	n/a	22.2	1.2	23.9	2.1

Table 9: DO - mean and standard deviation (sd).

Treatments	Stage 1 DO (mg/L)		stage 2 DO (mg/L)		stage 3 DO (mg/L)	
	mean	sd	mean	sd	mean	sd
0% shade	6.0	6.2	5.5	5.6	5.6	2.5
50% shade	n/a	n/a	3.2	4.3	4.3	2.4
80% shade	n/a	n/a	3.0	1.7	6.2	0.9
100% shade	n/a	n/a	4.5	3.3	5.9	2.7

Table 10: pH - mean and standard deviation (sd).

Treatments	Stage 1 pH		Stage 2 pH		Stage 3 pH	
	mean	sd	mean	sd	mean	sd
0% shade	6.8	0.8	7.2	0.9	7.4	0.9
50% shade	n/a	n/a	7.1	0.6	7.2	0.6
80% shade	n/a	n/a	6.9	0.5	7.2	0.2
100% shade	n/a	n/a	7.2	0.4	7.4	0.2

Table 11: SS - mean and standard deviation (sd).

Treatments	Stage 2 SS (mg/L)		Stage 3 SS (mg/L)	
	mean	sd	mean	sd
0% shade	16.2	9.8	10.2	7.7
50% shade	50.7	62.6	7.2	2.9
80% shade	11.9	10.9	97.1	183.4
100% shade	17.3	29.5	149.1	213.7

Table 12: FC - mean and standard deviation (sd).

Treatments	Stage 1 FC (CFU/100mL)		Stage 2 FC (CFU/100mL)		Stage 3 FC (CFU/100mL)	
	mean	sd	mean	sd	mean	sd
0% shade	9000.0	5527.6	1332.4	1734.3	1182.1	1308.6
50% shade	n/a	n/a	2470.3	2941.2	1142.2	1085.3
80% shade	n/a	n/a	535.3	630.1	416.3	565.3
100% shade	n/a	n/a	283.3	196.9	273.4	271.2

Table 13: FAC - mean and standard deviation (sd).

Treatment	Stage 2 FAC (cells/mL)		Stage 3 FAC (cells/mL)	
	mean	sd	mean	sd
0% shade	17015.0	20815.9	10182.4	14266.6
50% shade	10939.6	10333.5	11973.0	17674.6
80% shade	11777.8	13749.9	19514.8	40834.8
100% shade	16457.9	28983.7	20902.3	29322.8

APPENDIX C: Parametric statistics/hypothesis testing.

Table 14a: ANOVA results for temperature.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stages 1 & 2	4	0.03	<0.01*
treatments	stage 2	3	0.03	<0.01*
treatments	stages 1 & 3	4	0.003	0.7
treatments	stage 3	3	0.005	0.6
treatments	stages 2 & 3	3	0.03	0.02*
treatments	stages 1, 2 & 3	4	0.02	0.02*
stages	stages 1, 2 & 3	2	0.05	<0.01*
dates	stages 1, 2 & 3	8	0.04	<0.01*

N.B. * = significant difference.

Table 14b: Kruskal-Wallis results for temperature.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stages 1 & 2	4	14.4	<0.01*
treatments	stage 2	3	9.5	0.02*
treatments	stages 1 & 3	4	2.0	0.7
treatments	stage 3	3	1.9	0.6
treatments	stages 2 & 3	3	10.2	0.02*
treatments	stages 1, 2 & 3	4	11.9	0.02*
stages	stages 1, 2 & 3	2	11.3	0.04*
dates	stages 1, 2 & 3	2	42.1	<0.01*

N.B. * = significant difference.

Table 15a: ANOVA results for dissolved oxygen.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stages 1 & 2	4	0.7	0.8
treatments	stage 2	3	0.9	0.6
treatments	stages 1 & 3	3	1.1	0.2
treatments	stage 3	4	1.1	0.2
treatments	stages 2 & 3	3	0.8	0.4
treatments	stages 1, 2 & 3	4	0.9	0.5
stages	stages 1, 2 & 3	2	3.5	0.03*
dates	stages 1, 2 & 3	4	0.9	0.5

N.B. * = significant difference.

Table 15b: Kruskal-Wallis results for dissolved oxygen.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stage 1/ stage 2	4	1.6	0.8
treatments	stage 2	3	1.8	0.6
treatments	stage 1/ stage 3	4	2.3	0.7
treatments	stage 3	3	3.2	0.4
treatments	stage 2/ stage 3	3	2.2	0.5
treatments	stage 1/ stage 2/ stage 3	4	7.9	0.1
stages	stage 1/ stage 2/ stage 3	2	3.8	0.2
dates	stage 1/ stage 2/ stage 3	9	42.0	<0.01*

N.B. * = significant difference.

Table 16a: ANOVA results for pH.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stages 1 & 2	4	0.005	0.7
treatments	stage 2	3	0.002	0.9
treatments	stages 1 & 3	3	0.001	0.9
treatments	stage 3	4	0.009	0.3
treatments	stages 2 & 3	3	0.003	0.8
treatments	stages 1, 2 & 3	4	0.008	0.4
stages	stages 1, 2 & 3	2	0.02	0.1
dates	stages 1, 2 & 3	8	0.04	<0.01*

N.B. * = significant difference.

Table 16b: Kruskal-Wallis results for pH.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stages 1 & 2	4	3.6	0.5
treatments	stage 2	3	0.7	0.9
treatments	stages 1 & 3	4	10.5	0.03*
treatments	stage 3	3	4.6	0.2
treatments	stages 2 & 3	3	3.1	0.4
treatments	stages 1, 2 & 3	4	7.9	0.1
stages	stages 1, 2 & 3	2	0.02	0.1
dates	stages 1, 2 & 3	8	36.5	<0.01*

N.B. * = significant difference.

Table 17a: ANOVA results for suspended solids.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stage 2	3	2.4	0.1
treatments	stage 3	3	8.9	<0.01*
treatments	stage 2/ stage 3	3	1.7	0.4
stages	stage 2/ stage 3	8	0.8	0.9
dates	stage 2/ stage 3	1	1.1	0.4

N.B. * = significant difference.

Table 17b: Kruskal-Wallis results for suspended solids.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stage 2	3	5.2	0.2
treatments	stage 3	3	10.5	0.02*
treatments	stage 2/ stage 3	3	1.3	0.7
stages	stage 2/ stage 3	1	0.04	0.8
dates	stage 2/ stage 3	8	3.5	0.9

N.B. * = significant difference.

Table 18a: ANOVA results for faecal coliforms.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stage 1/ stage 2	4	18.9	<0.01*
treatments	stage 2	3	6.3	0.05
treatments	stage 1/ stage 3	4	18.4	<0.01*
treatments	stage 3	3	3.4	0.2
treatments	stage 2/ stage 3	3	9.1	<0.01*
treatments	stage 1/ stage 2/ stage 3	4	23.4	<0.01*
stages	stage 1/ stage 2/ stage 3	2	33.4	<0.01*
dates	stage 1/ stage 2/ stage 3	8	6.8	<0.01*

N.B. * = significant difference.

Table 18b: Kruskal-Wallis results for faecal coliforms.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stage 1/ stage 2	4	21.8	<0.01*
treatments	stage 2	3	7.1	0.07#
treatments	stage 1/ stage 3	4	22.0	<0.01*
treatments	stage 3	3	5.01	0.2
treatments	stage 2/ stage 3	3	10.9	0.012*
treatments	stage 1/ stage 2/ stage 3	4	28.7	<0.01*
stages	stage 1/ stage 2/ stage 3	2	20.1	<0.01*
dates	stage 1/ stage 2/ stage 3	2	18.4	0.03*

N.B. * = significant difference (0.05) and # = approaching significance.

Table 19a: ANOVA results for full algae count.

Data Analysis	Data Source	df	MS	F (P<0.05)
treatments	stage 2	3	1.9	0.5
treatments	stage 3	3	0.5	0.9
treatments	stage 2/ stage 3	3	1.1	0.7
stages	stage 2/ stage 3	1	1.5	0.5
dates	stage 2/ stage 3	8	9.3	<0.01*

N.B. * = significant difference

Table 19b: Kruskal-Wallis results for full algae count.

Data Analysis	Data Source	df	CHISQ	P>CHISQ
treatments	stage 2	3	2.0	0.6
treatments	stage 3	3	0.5	0.9
treatments	stage 2/ stage 3	3	1.1	0.8
stages	stage 2/ stage 3	1	0.8	0.4
dates	stage 2/ stage 3	8	31.0	<0.01*

N.B. * = significant difference.

Appendix D: NMDS plots.

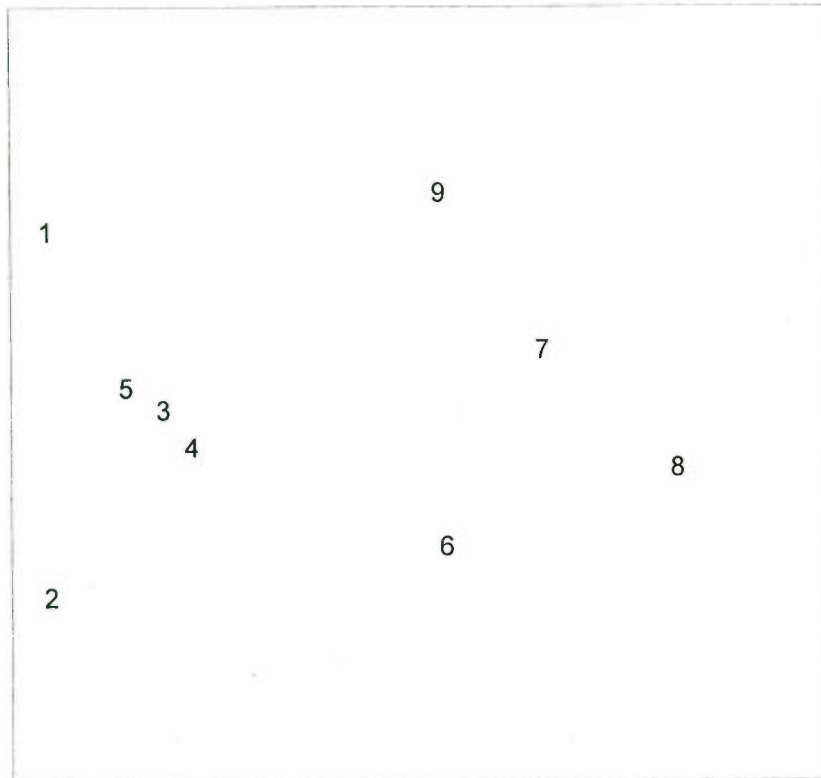


Figure 21: NMDS plot - stage 2 control (0% shade). Stress 0.05.
N.B. Nos. represent sampling occasions.

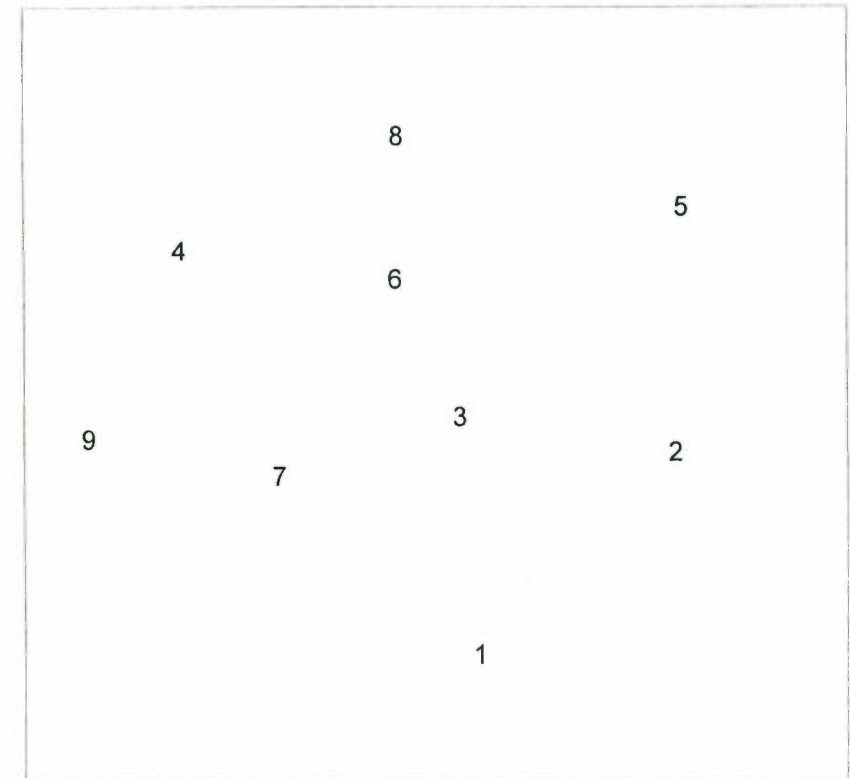


Figure 22: NMDS plot - stage 2, 50% shade treatment. Stress 0.11.
N.B. Nos. represent sampling occasions.

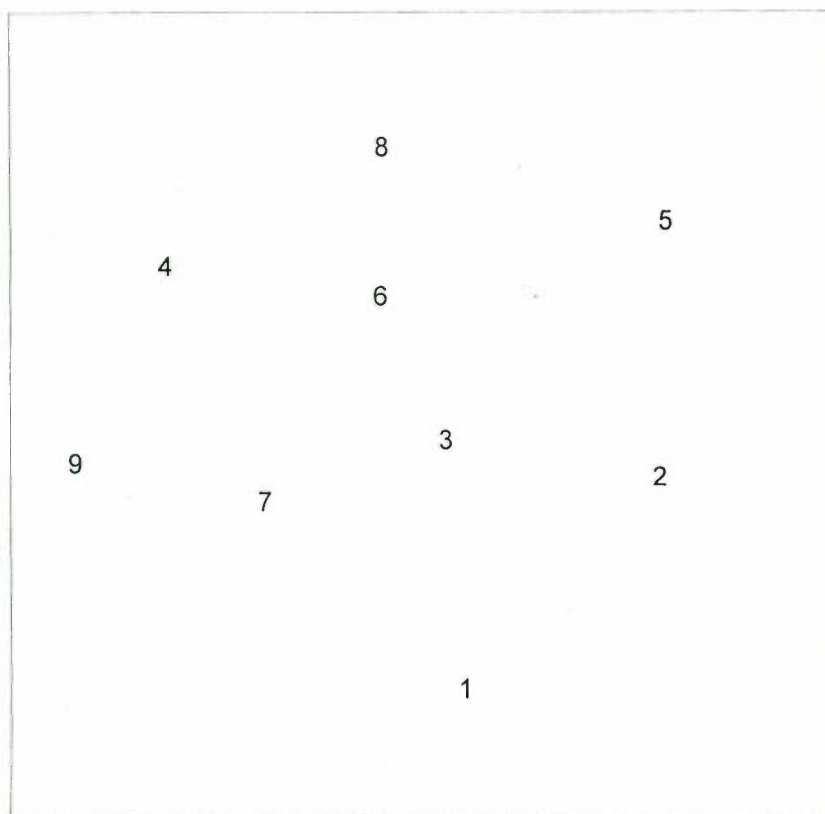


Figure 23: NMDS plot - stage 2, 80% shade treatment. Stress 0.15.
 N.B. Nos. represent sampling occasions.

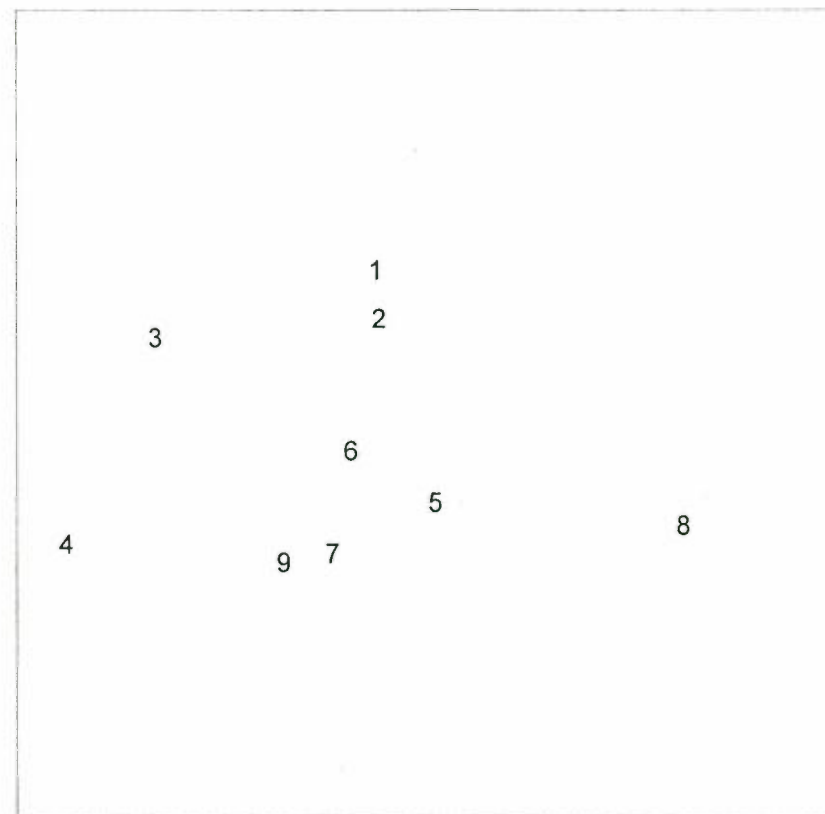


Figure 24: NMDS plot - stage 2, 100% shade treatment. Stress 0.07.
 N.B. Nos. represent sampling occasions.

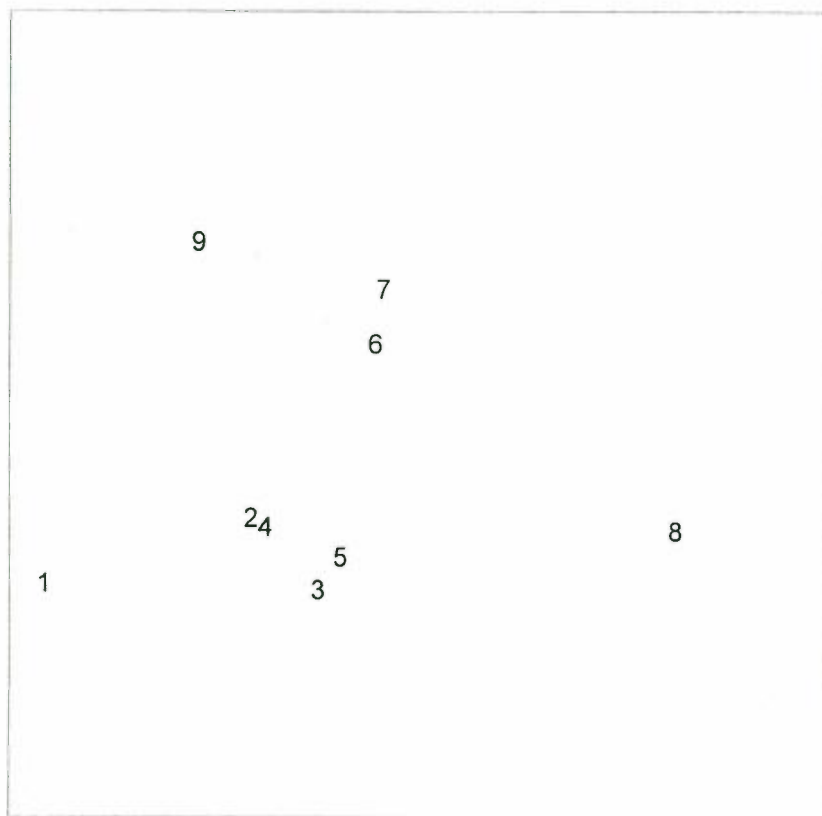


Figure 25: NMDS plot - stage 3, control (0% shade). Stress 0.05.
 N.B. Nos. represent sampling occasions.

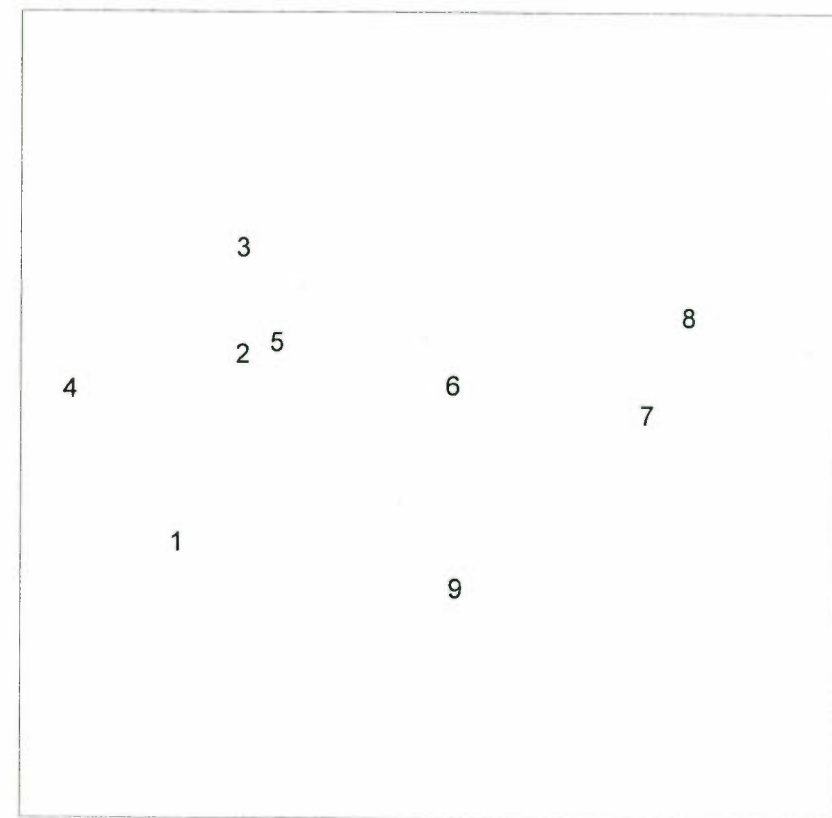


Figure 26: NMDS plot - stage 3, 50% shade treatment. Stress 0.03.
 N.B. Nos. represent sampling occasions.

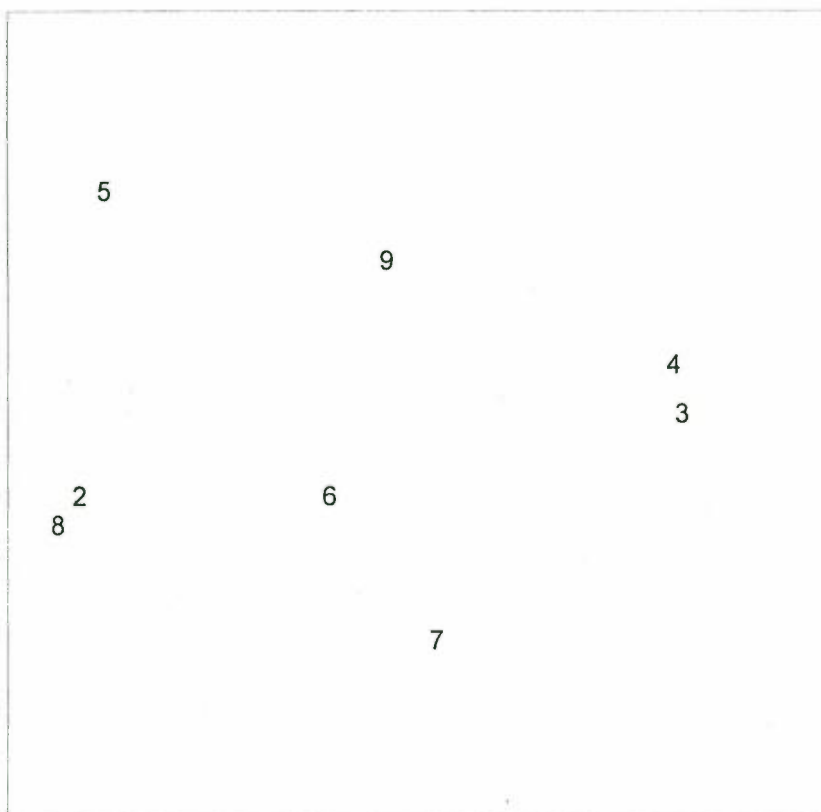


Figure 27: NMDS Plot - stage 3 80% Treatment, Stress 0.08.
 N.B. Nos. represent sampling occasions.

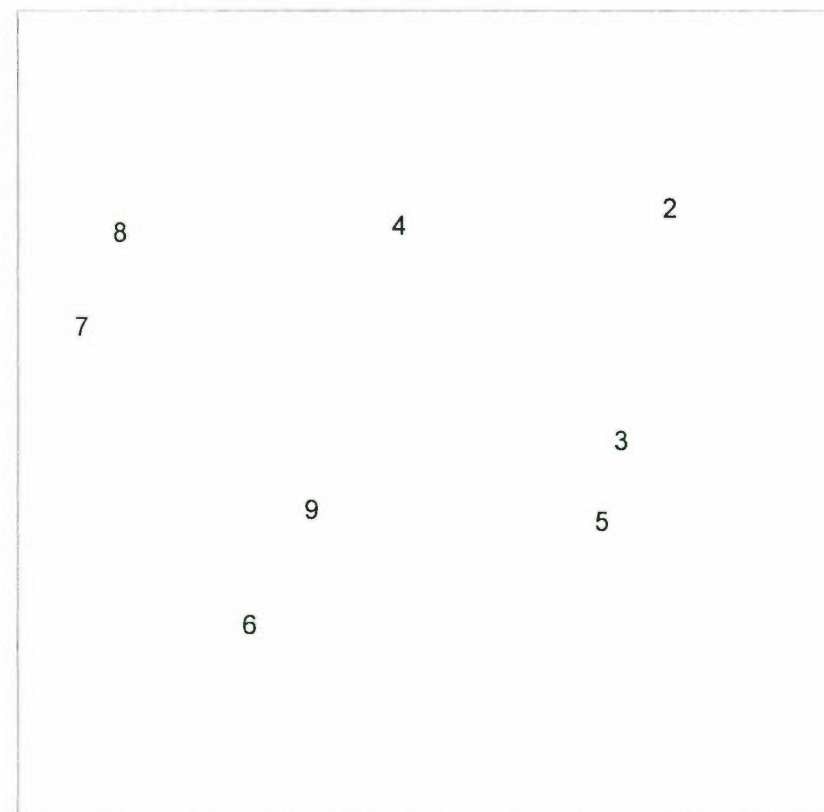


Figure 28: NMDS Plot - stage 3 100% Treatment, Stress 0.08.
 N.B. Nos. represent sampling occasions.

APPENDIX E: Explanation of statistical techniques.

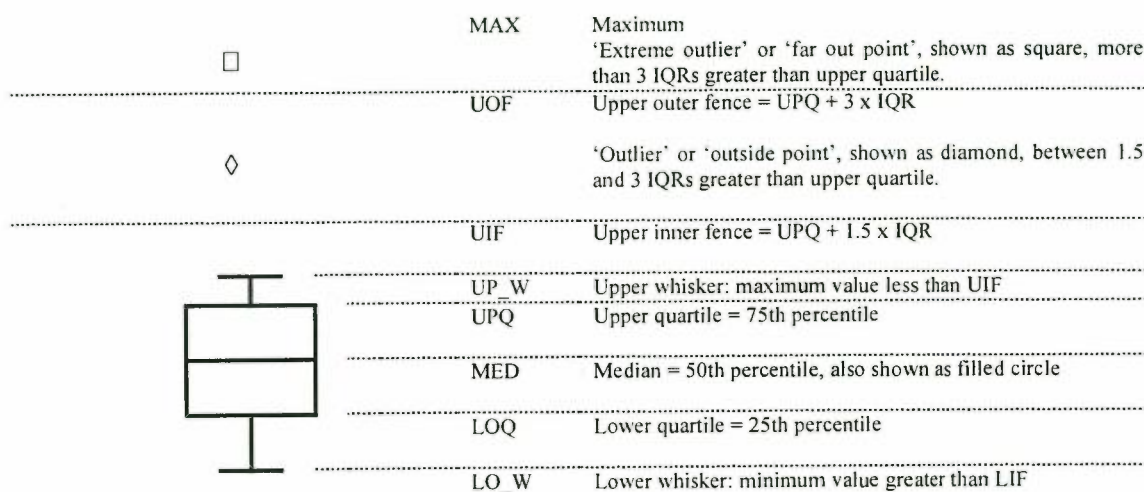
Box plots (Tukey, 1977)

Box plots provide a useful means of displaying a summary of a group of data, allowing meaningful comparisons to be made between groups. Box plots possess a high degree of resistance to outlying points and focus attention on five important properties of a group of data:

- typical or central value
- spread or variability
- shape - symmetry or skewness
- outlying data points
- behaviour of the tails.

The central box of the box plot delineates the 25th percentile (lower quartile), the 50th percentile (median) and the 75th percentile (upper quartile). Inner fences are then defined, 1.5 times the inter-quartile range (IQR) above and below the box. Whiskers are added to the box, drawn from the top and bottom to the most extreme value inside the fence. All data points outside the inner fence are individually identified, either as 'outliers' or 'extreme outliers'.

The following diagram outlines the principal components and underlying statistics of a box plot, for a moderately symmetric distribution.



While boxplots are useful for looking at a single data set, they are a powerful tool for comparing groups of data. Boxplots are conceptually simple to use and provide a non-parametric and graphical alternative and/or adjunct to classical techniques such as analysis of variance (ANOVA). Even when the data comply with none of the basic ANOVA assumptions, rendering that technique essentially useless, boxplots will at least indicate basic differences and similarities.

At the simplest level, one can simply compare the central value of data from several groups, using a robust measure such as the median (cf. a mean which is not robust, but is sensitive to skewed distributions, and particularly to extreme outlier points). The median is the central line of the box. Here, a dot has been used to reinforce its location.

Extreme and outlying points are clearly indicated. The extreme points (i.e. maxima and minima) indicate the range of data. Outliers are defined as being more than 1.5 IQR above or below the upper and lower quartiles respectively. Points more than 3 IQR outside the quartiles are classified as extreme outliers. Where a data distribution is highly skewed, sometimes it can show features such as a maximum, upper quartile and median all having the one data value.

References

- Chambers, John M, Cleveland, William S, Kleiner, Beat, and Tukey, Paul A. (1983). **Graphical methods for data analysis**. Wadsworth and Brooks/Cole Publishing Co. Pacific Grove, California. 395 pp.
- McGill, R., Tukey, J.W. and Larsen, W. (1978). **Variations of box plots**. *The American statistician*, 32:12-16
- SAS Institute Inc. (1985). **SAS[®] procedures guide for personal computers, version 6 Edition**. Cary NC: SAS Institute Inc. 373 pp.
- Tukey, J.W. (1977). **Exploratory data analysis**. Addison-Wesley, Reading, Massachusetts.

Hierarchical agglomerative clustering (Clark and Warwick, 1994)

The most commonly used clustering techniques are the *hierarchical agglomerative* methods. These usually take a similarity matrix as their starting point and successively fuse the samples into groups and the groups into larger clusters, starting with the highest mutual similarities then gradually lowering the similarity level at which groups are formed. The process ends with a single cluster containing all samples.

The result of hierarchical clustering is represented by a tree diagram or *dendrogram*, with the y axis representing the full set of samples and the x axis defining a similarity level at which two samples or groups are considered to have fused. There is no firm convention for which way the dendrogram should be portrayed (increasing or decreasing y/x axis values) or even whether the tree can be placed on its side. The branches of the dendrogram represent the level of similarity for particular groups. The lower the similarity percentage for a particular branch, the lower the similarity between species of Group A to Group B.

Non metric multi-dimensional scaling ordinations (NMDS)

Non-metric multi-dimensional scaling ordinations (Clark and Warwick, 1994) are a technique to summarise and graphically represent (usually in two dimensions) multivariate data. In this type of ordination the distances between samples in the ordination reflect the similarity of the species composition of samples. Stress is the measure of how accurately the underlying (or raw) data is presented in the ordination. In general:

Stress<0.05 gives excellent representation

Stress<0.1 corresponds to a good ordination

Stress<0.2 still gives potentially useful 2-dimensional plots, but not too much reliance should be placed on the detail of the ordination

Stress>0.3 indicates points are close to being arbitrarily placed in the ordination and not used for interpretation.

In general, samples are not as distinct in ordinations, but still provide information on a gradient of change in community composition.

SIMPER procedure

Programs such as NMDS can be used to analyse for differences between sites, however these analyses do not tell us which species are responsible for any differences that occur. The procedure SIMPER ('Similarity percentages') (Clark and Warwick, 1994) does this by examining the contribution of each species to the average Bray-Curtis dissimilarity between two groups of samples and to the average similarity within a group (Clark, 1993). The procedure lists the species which most contribute to the uniqueness of that particular group with their relative percentage and cumulative percentages.

Analysis of similarities (ANOSIM)

The One-way ANOSIM Procedure (Clark and Warwick, 1994) tests for differences between groups of community samples using a randomisation method on similarity matrix produced by the CLUSTER procedure. The acronym ANOSIM is very similar to ANOVA, the differences between the two methods is that ANOSIM is a multivariate technique where ANOVA is univariate. Univariate techniques analyse data which is a single reading (where replicates can be involved). Multivariate techniques analyse data which involve more than a single value which represents one sample i.e. FAC data where many species names comprise the one sample.

The ANOSIM procedure computes a test statistic which reflects the differences between and within sites. This statistic can be used to determine if a significant difference occurs between the specified groups when pairwise tests (ie. Between groups 1 and 2) are performed. If the test statistic is equally to or less than the given pairwise test, then this result is significant.. The procedure also calculates a sample statistic R which is a measure of the similarity between samples in the specified groups. R lies between the range of -1 and 1. If R=1 then replicates within groups are more similar to each other than replicates from different groups. If R=0 then all samples from all groups are approximately the same. The ANOSIM procedure is most powerful when there are a large number of permutations within each of the pairwise comparisons.

BIOENV procedure

The BIOENV Procedure (Clark and Warwick, 1994) analyses environmental data such as FC and DO and links it with the species community data. The premise for this procedure is that if a suite of environmental variables responsible for structuring the community were known, then samples having similar values for those variables would be expected to have a similar species composition. The procedure gives the environmental variables (this is usually more than one) which best correlate with the species community data.

References

Clark, K.R. and Warwick, R.M. (1994) Change in marine communities: an approach to statistical analysis and interpretation. Plymouth Marine Laboratory.

Analysis of variance and non parametric analysis

Analysis of variance is used to test for the differences among sample means and differences among linear combinations of means. Its name is derived from the fact that variances are used to measure differences among means. All ANOVAs require that sampling of individuals be random and thus normally distributed (ie. when all points are plotted out for a variable it should approximate a bell shaped curve). If the evidence indicates that the assumptions for an analysis of variance can not be maintained, we can either carry out a different test not requiring the assumptions such as a distribution free test (non-parametric test) or we can transform the variable to be analysed in such a manner that the resulting transformed variates meet the assumption of the analysis (Sokal and Rohlf, 1995). Common transformations are to either log or double square root the variables. Non-parametric analyses such as Kruskal-Wallis are not concerned with mean values but only with the distribution of the variates. The analyses are based on the rank of the variates in a data set.

References

Sokal, R.R. and Rohlf, F.J. (1995) Biometry, Third Edition. W.H. Freeman and Company.