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2021

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- Βιβλιογραφική έρευνα και “Ερευνητική Υπόθεση”.
- Συγγραφή δοκιμίου βιβλιογραφικής ανασκόπησης
- Η διεξαγωγή της έρευνας--Θεμελιώδεις αρχές.
- Εισαγωγή στη “Εναρκτήρια Έκθεση”--- Καθοδηγητικά βήματα για την παραγωγή της “Εναρκτήριας Έκθεσης”.
- Δειγματοληψία, Σχεδιασμός πειραμάτων, Συλλογή δεδομένων, Πειραματικές προσεγγίσεις.

Βιβλιογραφική έρευνα και “Ερευνητική Υπόθεση”

Literature research for proposals,
reviews and discussions

Scientific Hypothesis

- Observation / research
- Construct a hypothesis
- Experiment
- Conclusion

Researching the Hypothesis

- What is known about the subject?
- What is known about the specific question?
- Who are the experts in the field?
- What direction is the research being taken?
- Is your question still valid?
- How will the current understanding help you to answer your question?
- How will the answer to your question advance understanding in the field?
- What are the current debates in the field?

Sources of Data

- Library: text books and Journals
- Lecturers
- Peers
- Internet databases to find: Peer reviewed journals, curated data archives, organization webpages, online analysis tools
 - Millions of science articles published each year so computer aided research is critical

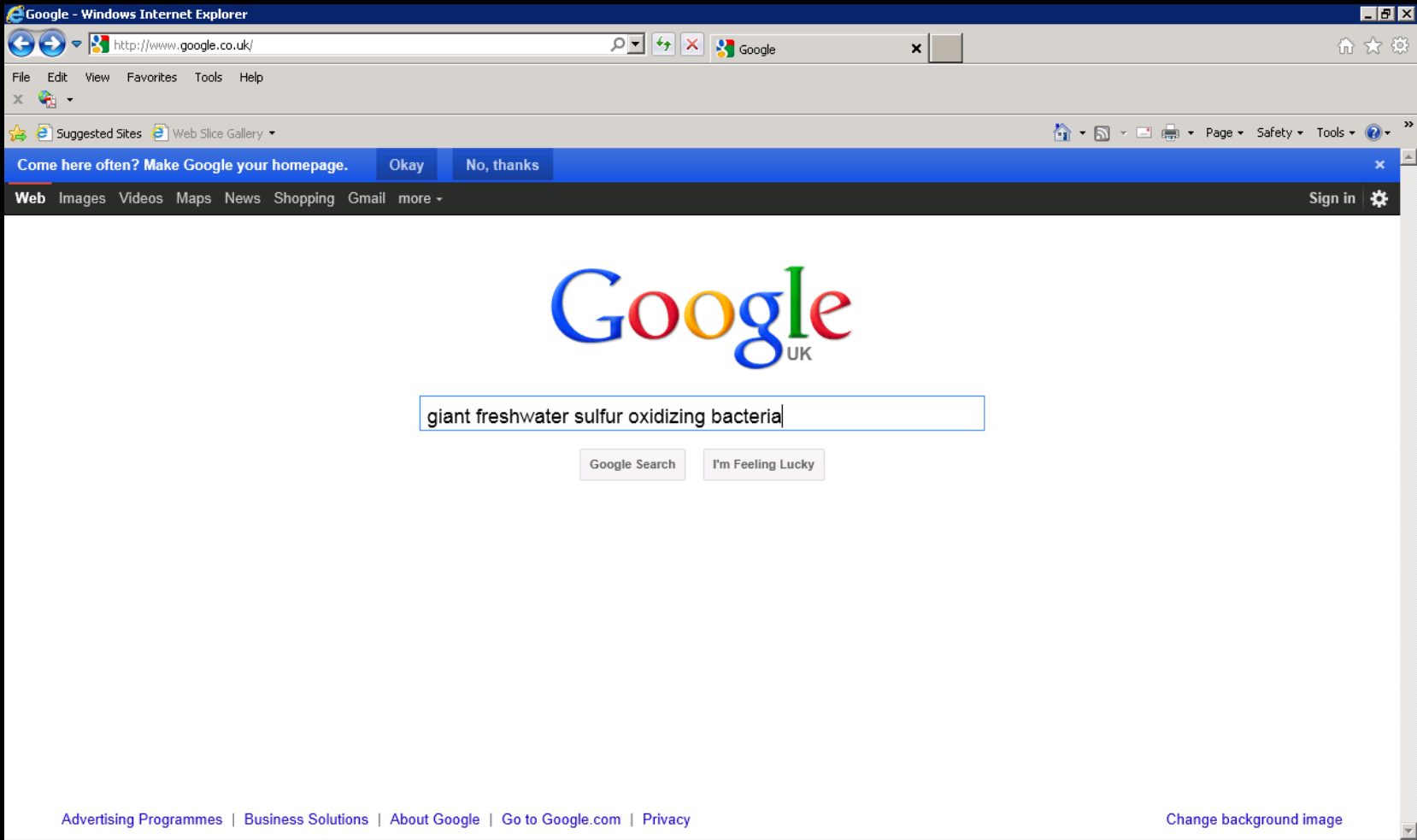
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N. D. Gray,^{1,2} R. Howarth,^{1,2} R. W. Pickup,³ J. Gwyn Jones,⁴ and I. M. Head^{1,2,*}

Fossil Fuels and Environmental Geochemistry Postgraduate Institute (NRG)¹ and Centre for Molecular Ecology,² University of Newcastle, Newcastle upon Tyne NE1 7RU, and Institute of Freshwater Ecology³ and Freshwater Biological Association,⁴ Windermere Laboratories, Far Sawrey, Ambleside, Cumbria LA22 0LP, United Kingdom

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Microbial energetics applied to waste repositories

K. W. Hanselmann

Institute of Plant Biology, Microbiology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich (Switzerland)

Summary. Through their catalytic abilities microbes can increase rates of chemical reactions which would take a very long time to reach equilibrium under abiotic conditions. Microbes also alter the concentration and composition of chemicals in the environment, thereby creating new conditions for further biological and chemical reactions. Rates of degradation and possible indirect consequences on leaching rates in waste repositories are a function of the presence or absence of microbes and of the conditions which allow them to become catalytically active.

Microbially mediated reactions are no exception to the rule that all chemical processes are basically governed by thermodynamic laws. Naturally occurring processes proceed in the direction that leads to the minimal potential energy level attained when equilibrium is reached. A continuous supply of energy to an ecosystem in the form of biochemically unstable compounds maintains non-equilibrium conditions, a prerequisite for all chemotrophic life. Energy is released as a chemical reaction progresses towards equilibrium. Microbes scavenge that portion of the free energy of reaction (ΔG) which can be converted into biochemically usable forms during the chemical oxidation processes. As 'electrontransfer catalysts', the microorganisms mediate reactions which are thermodynamically possible thereby stimulating reaction rates. Decomposition and mineralization in systems without a continuous supply of substrates and oxidants will lead to equilibria with minimal free energy levels at which point further microbial action would cease. The differences in the free energy levels of reactions (ΔG), represent the maximal energy which is available to microorganisms for maintenance and growth. How much of the released free energy will be conserved in energy-rich bonds, compounds (e.g. ATP), and chemical potentials (e.g. emf) useful for biosynthesis and biological work is characteristic for the microbes involved and the processes and metabolic routes employed.

Materials whose elements are not present in the most oxidized form attainable in the oxic environment of our planet are potentially reactive. Microbial activities are associated only with chemical reactions whose free energy changes are exergonic. This should be kept in mind for all investigations related to the role of microbes in repositories or in the layout of proper waste storage conditions. Rigorous application of thermodynamic concepts to environmental microbiology allows one to develop models and design experiments which are often difficult to conceive of in complex natural systems from physiological information alone. Thermodynamic considerations also aid in selecting proper deposition conditions and in carrying out thoughtful experiments in areas related to microbial ecology of waste repositories.

Key words. Microbial ecology; ecosystems; state parameters; microbial activity; thermochemical values; group increment method; Gibbs free energy of reaction; bitumen degradation; nuclear waste repositories.

Introduction

This presentation is an attempt to apply chemical thermodynamics to microbial ecology. I would like to illustrate, how simple concepts taken from the theory of chemical equilibrium thermodynamics might enable one to explain microbial action in natural and artificial ecosystems. Emphasis is placed on three aspects:

1) the microbial mediation and the consequences on habitat conditions of the degradation of organic substrates with the multitude of oxidants which are accessible to microbes; 2) the meaning of the basic laws of thermodynamics in ecophysiology and 3) evaluating and summarizing a consistent dataset of thermochemical values of inorganic and organic compounds involved in microbially mediated processes.

It seems appropriate to consider in this discussion the broad spectrum of enzymatic abilities present in the microbial communities of particular environments as a whole rather than looking at single physiological traits.

This geo-microbiological approach can be employed to understand the multitude of microbially mediated oxidation-reduction reactions, their sequence under environmental conditions, their synergistic coupling and the consequences for dissolution and precipitation processes. Thus, chemical thermodynamics becomes a tool to make predictions about the behavior of microbial communities involved in biogeochemical processes in nature. It can also help to develop a unifying view about the role in geobiochemical processes of the great metabolic diversity of microbes.

The basic conclusions drawn from the 'eco-thermodynamic' thoughts might be helpful in designing material repositories in such a way that chemical reactivity and microbial action on the components are minimal or maximal. These conclusions might also be useful in predicting the long-term behavior of potentially reactive wastes stored under particular conditions. It should become clear that by understanding the few basic concepts to be

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FIG. 2. Kinetics of toluene (A) and *o*-xylene (B) degradation. Experimental data were plotted with Monod kinetic model predictions. The biomass yield (Y) and the initial biomass concentration (X_0) were measured experimentally. The Monod parameters K_s (half-saturation constant) and μ_{max} (maximum specific growth rate) were approximated by nonlinear regression of the data from substrate depletion curves to the Monod model. The Monod model parameters for toluene were as follows: $Y = 17$ g of cells per mol of toluene, $X_0 = 6.5$ mg/liter, $K_s = 30$ μ M, and $\mu_{max} = 0.11$ day $^{-1}$. The Monod model parameters for *o*-xylene were as follows: $Y = 17$ g of cells per mol of *o*-xylene, $X_0 = 8.4$ mg/liter, $K_s = 20$ μ M, and $\mu_{max} = 0.07$ day $^{-1}$.

FIG. 3. Rate of degradation versus initial substrate concentration in mixed methanogenic cultures enriched with either toluene or *o*-xylene.

we found that near stoichiometric amounts of methane were produced in experiments with unlabeled substrates, we calculated the theoretical yield of radiolabeled methane from the measured amount of labeled toluene degraded at each time point and plotted these calculated [14 C]methane values in the same figure (Fig. 1). A near perfect mass balance was obtained upon summing up the radioactivity in the various fractions (measured and theoretical). Experiments with methyl-labeled toluene or methyl-labeled *o*-xylene also yielded near stoichiometric amounts of labeled CO $_2$.

Kinetics. Over a period of 2 years, the rate of degradation in enrichment cultures increased 10-fold predominantly as a result of the increased biomass concentration. From protein measurements, we estimated the cell yield (Y) to be about 17 g of cells per mol of toluene or *o*-xylene (standard deviation = 6.2, $n = 8$). The observed yield of 17 g/mol is marginally greater than the predicted theoretical yield of 11 to 13 g/mol and may indicate that the actual energy transfer efficiency is greater than 60% (assumed in theoretical calculations) and might be closer to 80%. Anaerobic systems have been shown to have higher energy transfer efficiencies (26). Substrate depletion curves for toluene and *o*-xylene were obtained for a range of initial substrate concentrations and for a given initial biomass concentration. The initial biomass concentration in these substrate depletion experiments was estimated from protein measurements and was assumed to be the active biomass concentration (X_0). We measured an initial biomass concentration of 6.5 mg/liter and 8.4 mg/liter for the toluene and *o*-xylene depletion experiments, respectively. The data from substrate depletion curves for toluene and *o*-xylene were fit to the Monod kinetic model (without a decay term) by nonlinear regression (Fig. 2) to derive values for the half-saturation constant (K_s) and the maximum specific growth rate (μ_{max}). This procedure yielded estimates for K_s of 30 and 20 μ M ($\pm 30\%$) and for μ_{max} of 0.11 day $^{-1}$ and 0.07 day $^{-1}$ ($\pm 20\%$) for toluene and *o*-xylene, respectively. The doubling times for the stable consortia growing on toluene or *o*-xylene were therefore about 6 and

concentration (based on equations in Table 1) for both toluene and *o*-xylene in 22 different incubations over the three years of this study. Radiolabeled toluene and *o*-xylene were used to confirm the formation of CO $_2$ (or HCO $_3^-$). The 14 C label distribution shown in Fig. 1 was obtained from duplicate cultures fed [ring- 14 C]toluene. The radioactivity in the volatile fraction (toluene), the nonvolatile fraction, and the CO $_2$ fraction was measured over time. For the volatile compounds CO $_2$ and toluene, the total mass in the bottle (aqueous phase and headspace) was the sum of the counts obtained in samples from the liquid phase and the corre-

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- **NARROW YOUR SEARCH:**
- **ANALYZE YOUR ARTICLES:**
 - Are the articles relevant, what are the key points, do the conclusions fit the data. Does it make sense to you
- **WRITE IT:**
 - Plan the review, don't simple list the papers.
 - Beginning, middle and end
 - Group articles together
 - Reference everything you have said.

Quote references Correctly

- Innes et al. (1997) investigated the hopanoid signatures of the sediment from a fresh water lake, Priest Pot.....
- The hopanoid signatures of the sediment from a fresh water lake, Priest Pot (Innes et al., 1997)....
- What is the preferred reference style
- Different journals – different styles, be consistent
- Organic Geochemistry style
- Innes, H.E., Bishop, A.N., Head, I.M. Farrimond, P. 1997 Preservation and diagenesis of hopanoids in recent lacustrine sediments of Priest Pot, England. *Organic Geochemistry* 26, 565-576.

Summary

- Ensure you understand your idea
- Find a good start point
- Use reputable sources of data
- Plan your literature review based on what you have found out
- Get the references right

Carrying out research

Η διεξαγωγή της έρευνας--
Θεμελιώδεις αρχές

Scientific experiments

- Observation / research
- Construct a hypothesis
- **Experiment or Laboratory Analysis**
- Presentation of results (dissertation, papers...)

Analytical approaches

- The experimental approach you take will be dictated by the research type
- **Exploratory research** will likely focus on representative sampling and measurement of system properties perhaps compared to a benchmark system. e.g. Contaminated land assessment
- **Hypothesis driven research:** Once hypothesis (predictions!) are made, they can be sought by analyses with state-of-the-art analytical techniques.

State-of-the-art: the latest and most sophisticated or advanced stage of a technology, art, or science

- **If the test results contradict the predictions, the hypotheses which entailed them are called into question and become less tenable. Sometimes the experiments are conducted incorrectly or are not very well designed, when compared to a crucial experiment. If the experimental results confirm the predictions, then the hypotheses are considered more likely to be correct, but might still be wrong and continue to be subject to further testing.**
- **The experimental control is a technique for dealing with observational error. This technique uses the contrast between multiple samples (or observations) under differing conditions to see what varies or what remains the same. We vary the conditions for each measurement, to help isolate what has changed.** will focus on Document the micron-sized spheroid hematite occurrences
- Investigate for organic content with sensitive techniques (ToF-SIMS)
- Identify possible biomarkers:
- morphologic (i.e. fossil microbes, filaments, or biofilm)
- chemical (i.e. organic)
- Attempt to decipher the origin of hematite.
- experiments that make comparisons of replicated altered systems against unaltered

Analytical approaches

- The experimental approach you take will be dictated by the research type
- **Hypothesis driven research:** Once hypothesis (predictions!) are made, they can be sought by analyses with state-of-the-art analytical techniques.

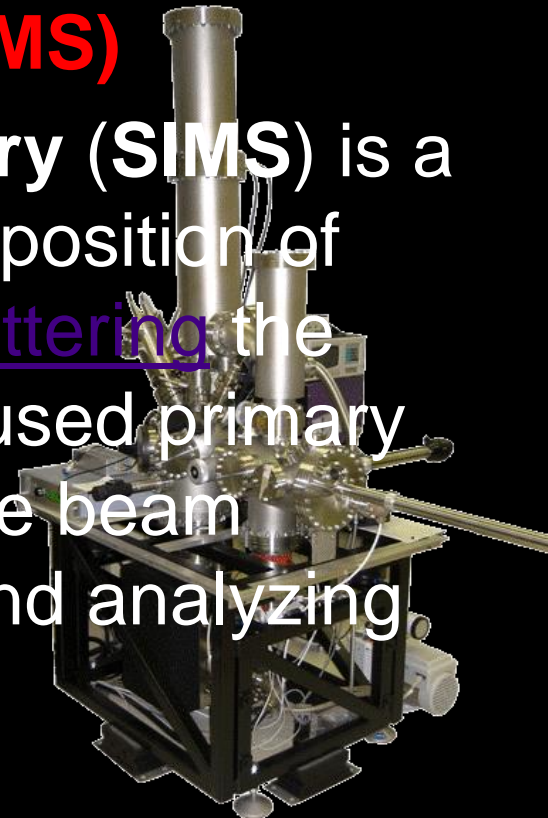
State-of-the-art: the latest and most sophisticated or advanced stage of a technology, art, or science

- If the test results contradict the predictions, the hypotheses which entailed them are called into question and become less tenable. Sometimes the experiments are conducted incorrectly or are not very well designed, when compared to a crucial experiment. If the experimental results confirm the predictions, then the hypotheses are considered more likely to be correct, but might still be wrong and continue to be subject to further testing.

Materials & Methods

Time of Flight –Secondary Ion Mass Spectrometry (ToF-SIMS)

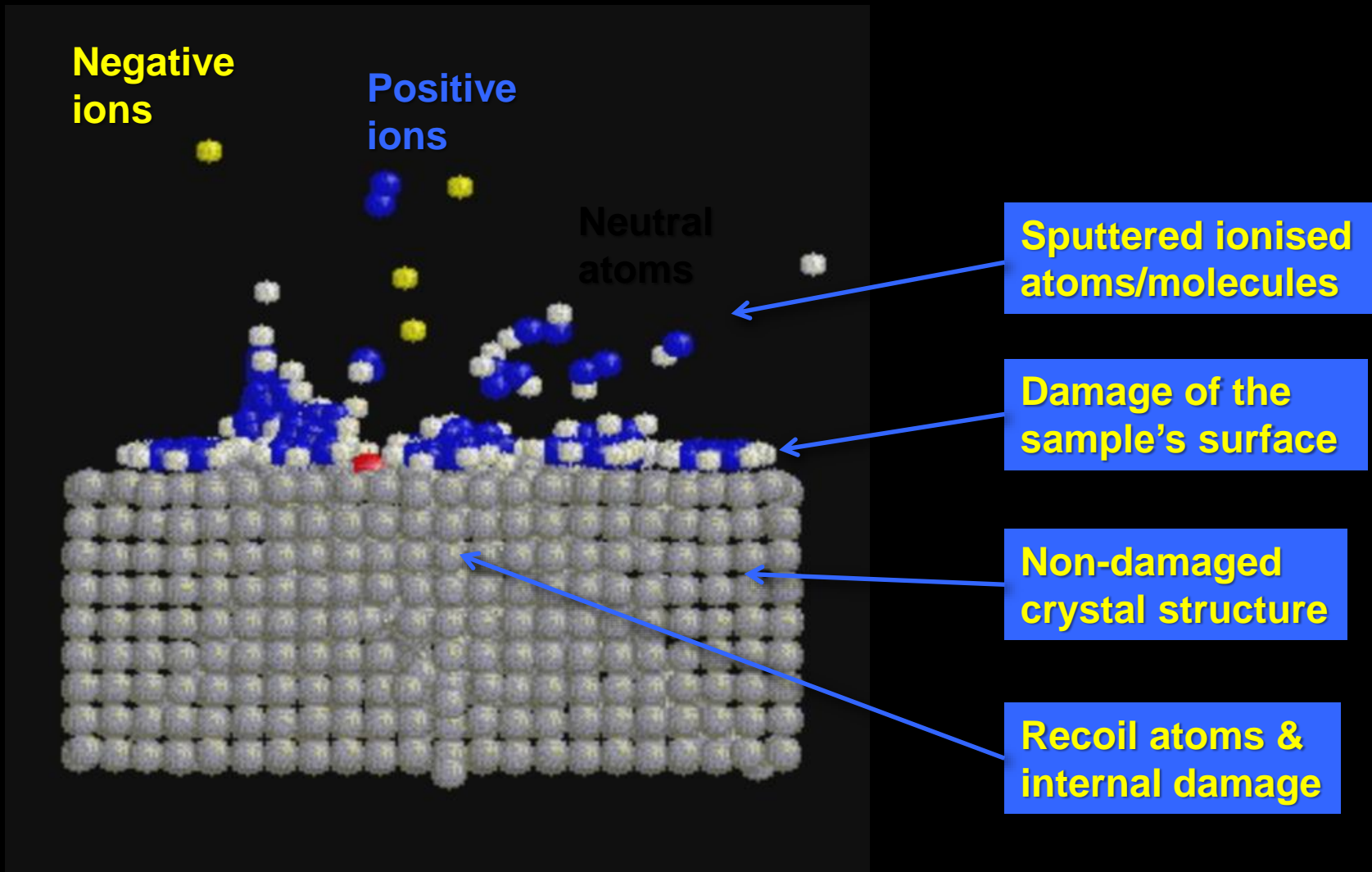
- **Secondary ion mass spectrometry (SIMS)** is a technique used to analyze the composition of solid surfaces and thin films by sputtering the surface of the specimen with a focused primary ion beam (a type of charged particle beam consisting of ions) and collecting and analyzing ejected secondary ions.



• What is Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)

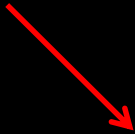
- Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is a surface-sensitive analytical method that uses a pulsed ion beam (Cs or microfocused Ga) to remove molecules from the very outermost surface of the sample. The particles are removed from atomic monolayers on the surface (secondary ions). These particles are then accelerated into a "flight tube" and their mass is determined by measuring the exact time at which they reach the detector (i.e. time-of-flight). Three operational modes are available using ToF-SIMS: surface spectroscopy, surface imaging and depth profiling.
- Analytical capabilities of ToF-SIMS include:
 - Mass resolution of 0.00x amu. Particles particles with the same nominal mass (e.g. Si and C₂H₄, both with amu = 28) are easily distinguished from one another because as Mr. Einstein predicted there is a slight mass shift as atoms enter a bound state.
 - Mass range of 0-10,000 amu; ions (positive or negative), isotopes, and molecular compounds (including polymers, organic compounds, and up to ~amino acids) can be detected.
 - Trace element detection limits in the ppm range.
 - Sub-micron imaging to map any mass number of interest.
 - Depth profiling capabilities; sequential sputtering of surfaces allow analysis of the chemical stratigraphy on material surfaces (typical sputtering rates are ~100 Å/minute).
 -

A few words on the ToF-SIMS analytical technique



ToF-SIMS instrument used in this work

IDLE 1



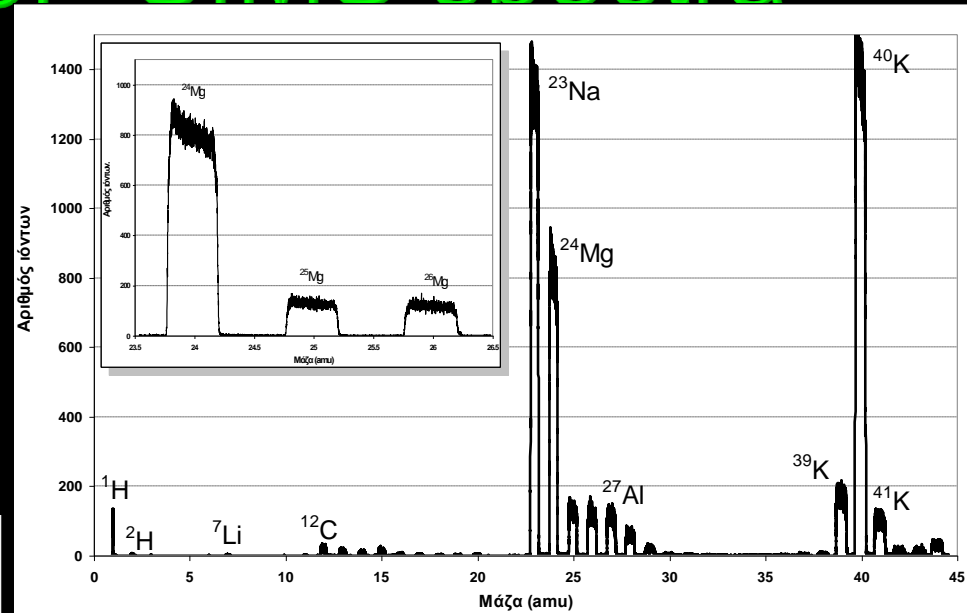
IDLE 2



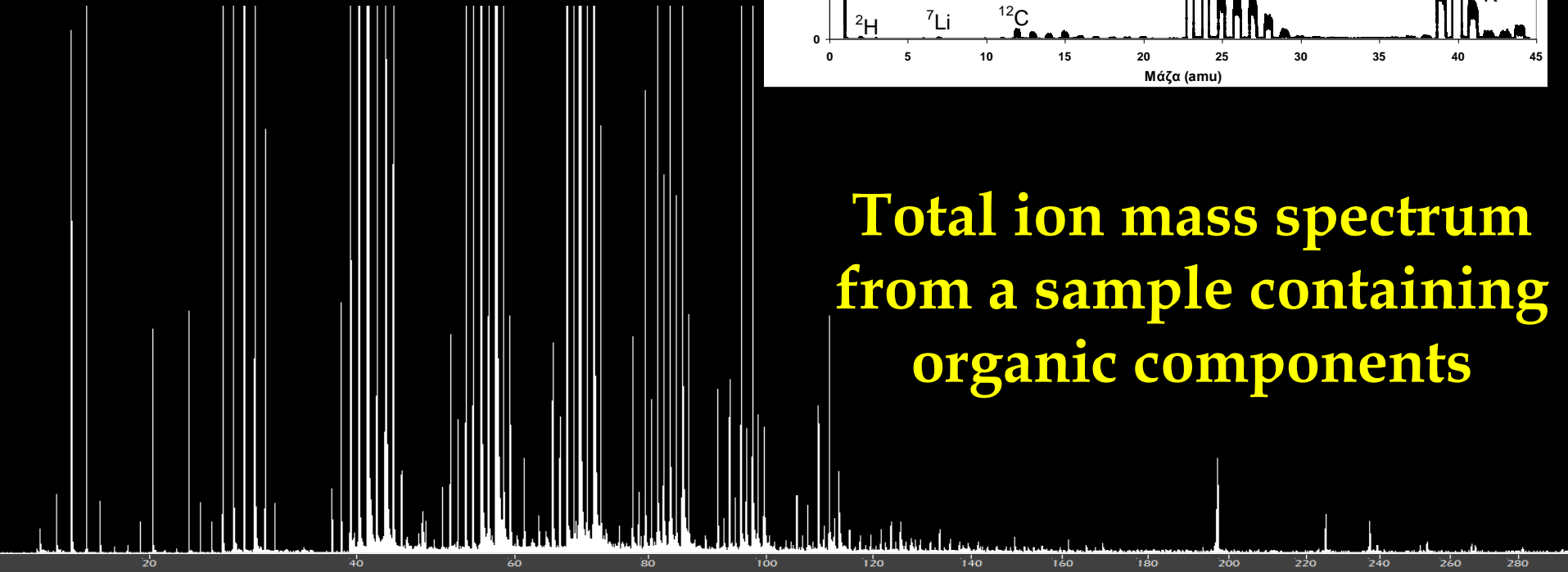
**University of Manchester – School of Earth, Atmospheric and Environmental Sciences
Group of Isotope Geology**

Examples of ToF-SIMS spectra

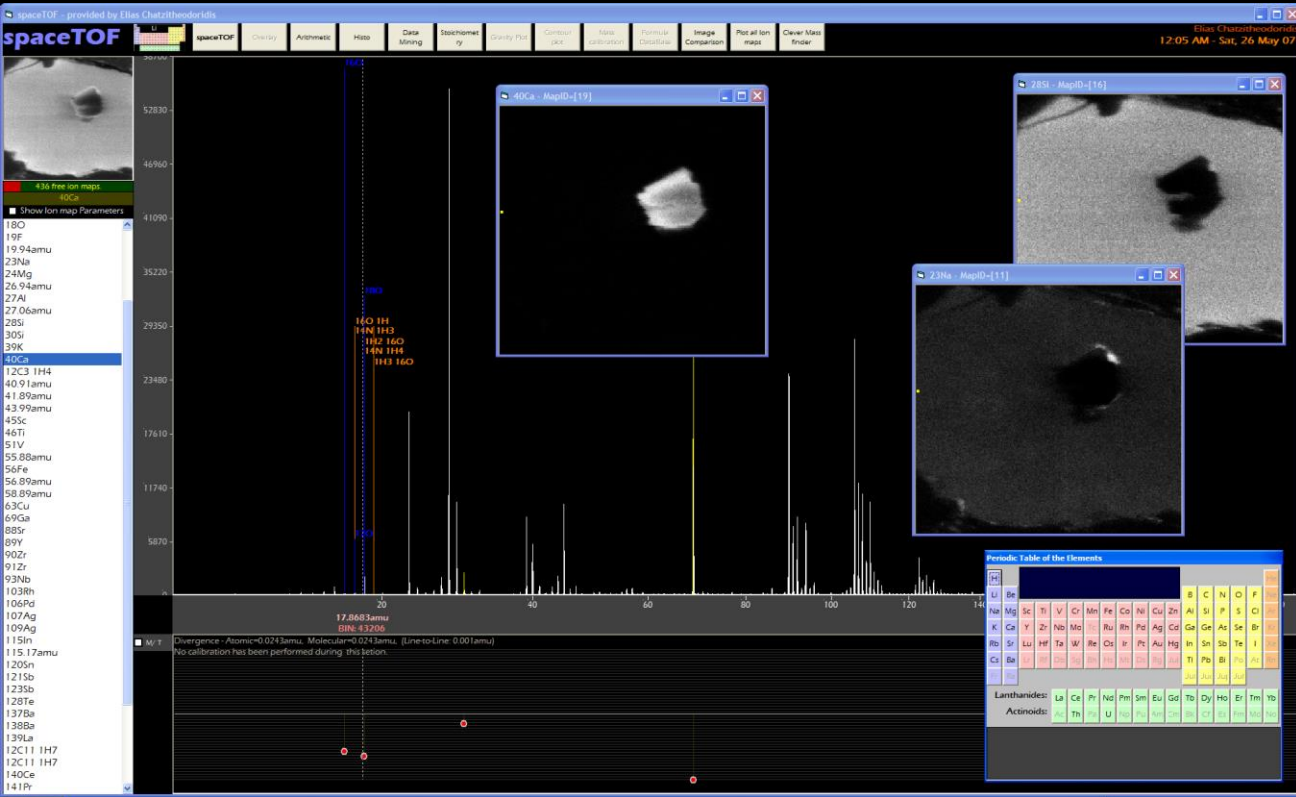
Magnification of a mass spectrum in the range of 1-45amu and on the Mg isotopes (inset)



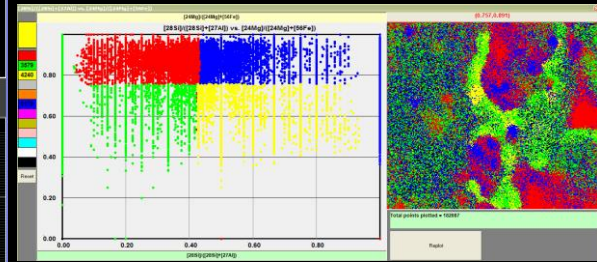
Total ion mass spectrum from a sample containing organic components



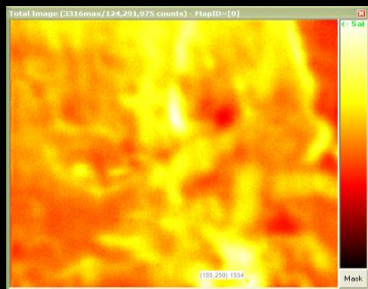
The "spaceTOF" software



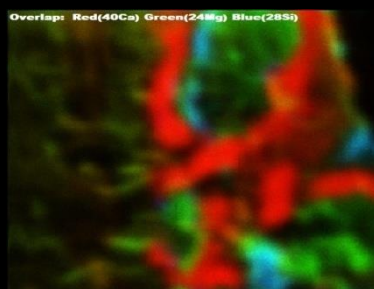
- Interactive environment
- Database assisted interpretation
- Ion signatures patterns
- Image calculations



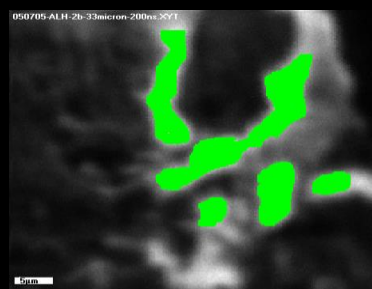
Data mining



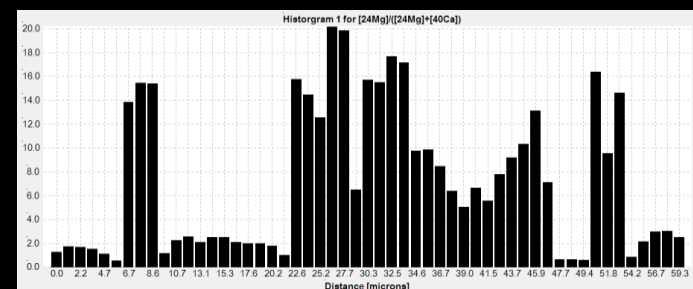
Ion mapping



Map overlaps



Masking



Profiling

What are you going to measure?

- Most important things that you need to do in planning an experiment are:
 - Find out which staff or technicians can help you
 - Work out how long it will take to complete an assay
 - Who else might want to use the machine
 - Therefore is the number of analyses planned realistic
 - Start in time to be able to carryout all work twice

Carrying out Research

- All research is time limited (i.e. 3 years or 3 months depending).
- Make sure that the work you intend to do can fit in to the available time
- Write a realistic plan with (if possible) key objectives along the way
- Make sure you know when your plan is going wrong and correct.
- BE IN CONTROL

Carrying out Research

- Research rarely proceeds smoothly
 - Machines break down, samples don't arrive.....
- The trick is to be organised so that
 - You have time to cope with the problems
 - You have fully assessed risks and have backup ideas and plans

‘They planned their campaigns just as you might make a splendid piece of harness. It looks very well; and answers very well; until it gets broken; and then you are done for. Now I made my campaigns of ropes. If anything went wrong, I tied a knot; and went on’.

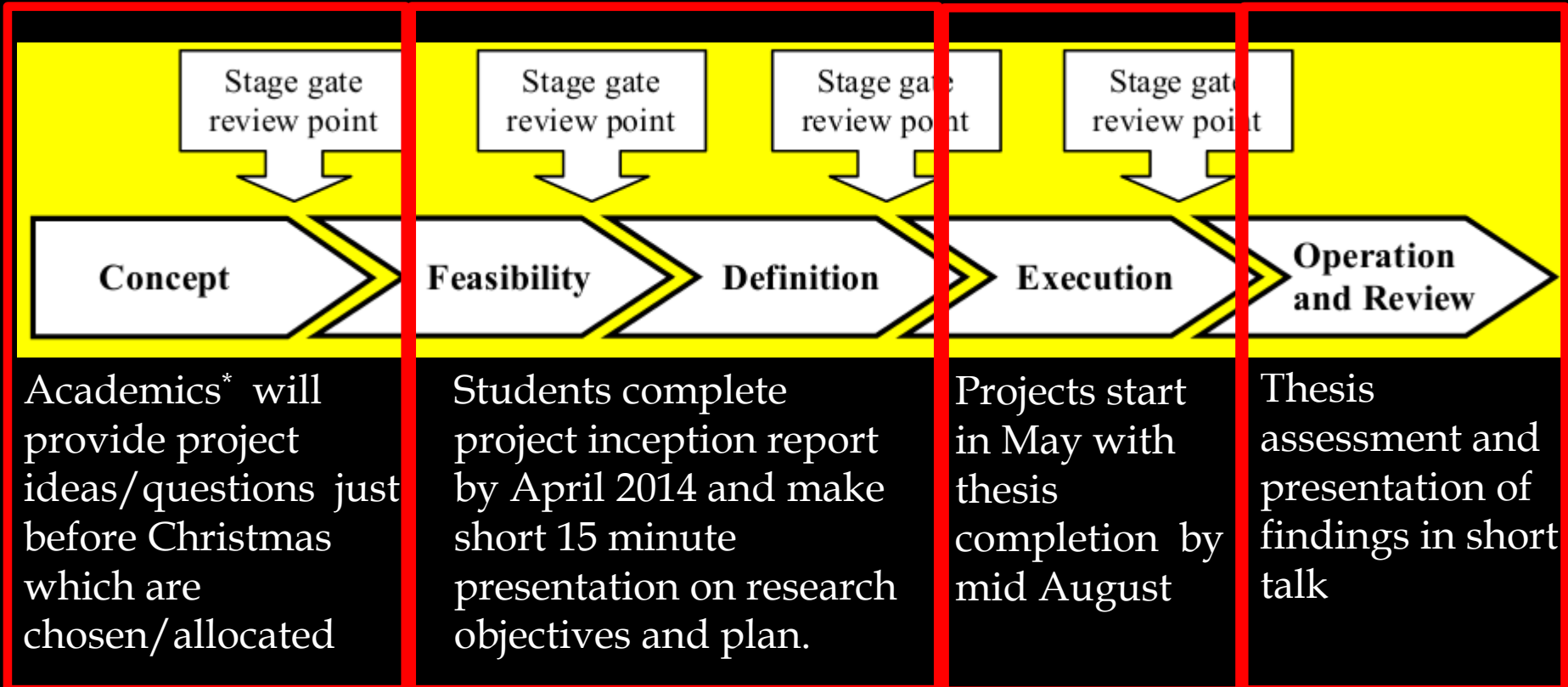
Sir Arthur Wellesley



Project planning

‘All projects have a project life cycle’ WG Morris “Science, objective knowledge, and the theory of project management”

The masters projects are just the same with stage gates throughout



*ideas for projects are welcome from students

Project inception report

- The PIR is essentially a document that should facilitate the MSc/Diploma dissertation and is developed in consultation with the project supervisor. The primary purpose of the PIR is to:
 - Prevent delays in thinking about the specification, development, planning, and undertaking of the research involved in the project, and the writing-up of the project into a dissertation of an acceptable standard;
 - Allow time to develop the skills to undertake the project and to ensure that necessary resources (e.g. computational and/or experimental, consumables, etc.) are in place.
 - Allow any required equipment to be manufactured in time for the start of the project.
 - Allows laboratory space and technician support to be allocated and planned (e.g. a laboratory schedule) efficiently.

Εισαγωγή στη
“Εναρκτήρια Έκθεση”
(Project Inception Report)

Καθοδηγητικά βήματα για την
παραγωγή της
“Εναρκτήριας Έκθεσης”

Project inception report structure

- Supervisor:
- Student:
- Project title:
- Introduction:
- Aim:
- Objectives and hypotheses:
- Scope:
- Initial Literature Review
- Proposed Methods
- Skills requirements
- Resource requirements
- Programme of work
- Anticipated outcomes
- Risk Assessment
- Project risk assessment

Scope

- what will and will not be investigated.
- The project must be manageable, not too ambitious nor trivial.
- Since the aims and objectives indicate largely what will be covered, the importance of scope is to give bounds to the project and why certain aspects have **not** been investigated.
- Restrictions might be based on time for one sample set, access to samples, experimental design focus on a specific question

Methods -samples?

- Where from? Field location, archive.....
- What type? e.g. cores, water samples....
- When taken
- How many samples and their relationship?
 - Sample replication/spatial distribution (**based on objectives and strategy**)
- How samples will be or, were, removed
 - Sample handling (**contamination**)
 - Sample storage (**cold room for live experiments, freezer for physico- chemical analysis**)

Methods –experimental setup

- What will be the structure of your experiment or, field survey be
 - Different Treatments, locations and sampling times
 - Replication
 - Controls
- or, what is the structure of previously obtained data sets
- what will or was the ‘conditions’ and type and frequency of sub-sampling

Methods- measurements ?

- What and how are you going to measure
- e.g. Fe speciation, bulk chemistry of major, minor, trace and RE elements, stable isotopes $\delta^{13}\text{C}$, δD , $\delta^{18}\text{O}$, $\delta^{34}\text{S}$, $\delta^{30}\text{Si}$..
- What method are you going to use LA-ICP-MS, EMPA, Synchrotron, ToF-SIMS
- What equipment do you need?
 - Do you need to build or buy anything?

Methods- data analysis ?

- Raw data will need some form of processing
 - How will you present and assess the accuracy and significance of your findings
 - Need to think about this before not after project e.g. data manipulation software, statistical tests and likely graph types
 - Follow lectures on data analysis (statistics) and presentation

Skills requirements?

- Hopefully many of the techniques and approaches required will be acquired during the course
 - What sampling and experimental skills do you need to learn to sample? (trench/channel sampling, chip sampling,)
 - What analytical skills do you need to learn?
 - What computational and data analysis skills do you need to learn

Resource requirements?

- Staff support –who are key people
 - Academics, researchers, technicians, industry contacts

Analytically equipment and its availability

GC
GCMS
HPLC-MS
ICP
IC
Microbiological
Titration techniques
AA
Porosimetry
software

Other support equipment

Sampling equipment
Storage facilities (if unusual)
Glassware
Ovens
Transport
Reagents
standards

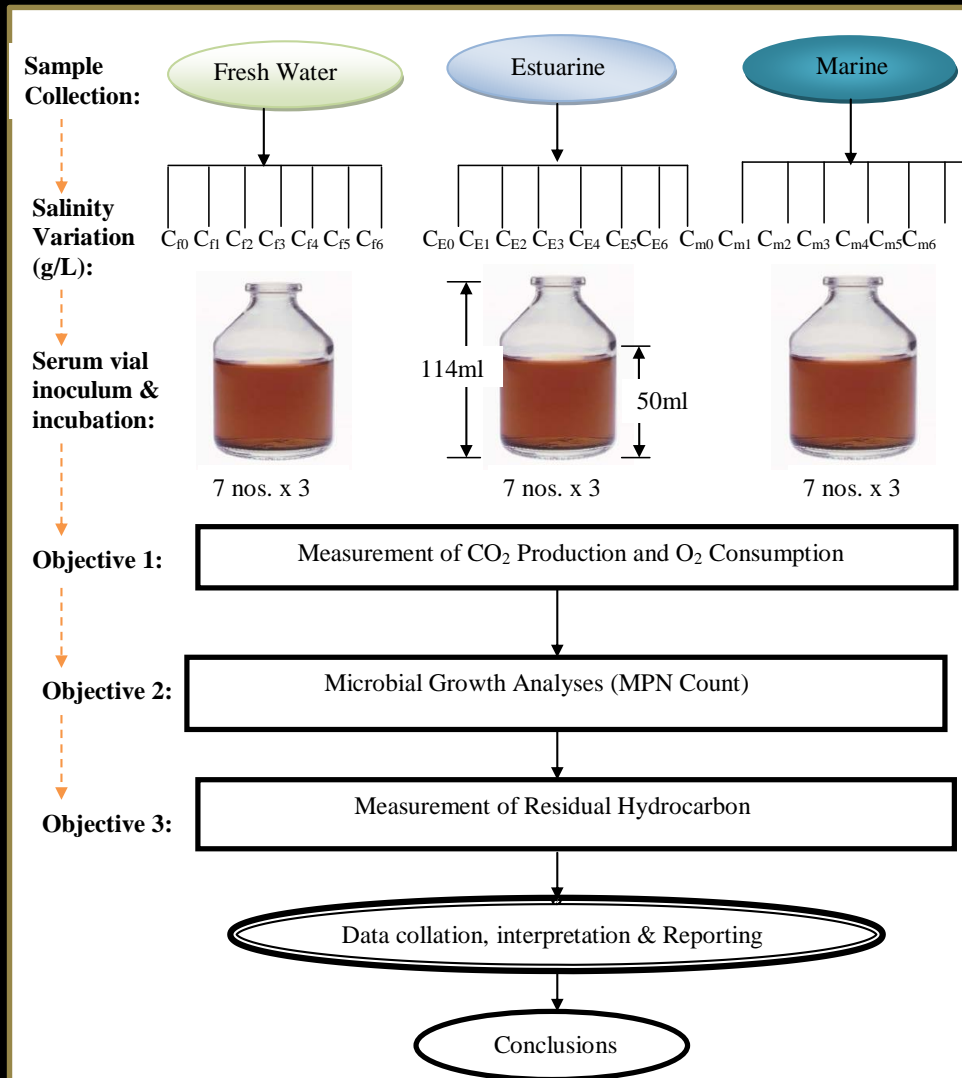
Work programme

- In what order should things happen in the project- Usually a logical flow to a research programme
 - Sampling (if necessary) or obtaining data
 - Analytical Method development (if necessary)
 - Experimental setup (if appropriate for project)
 - Analytical work (if appropriate for project)
 - Data analysis, representation, interpretation
 - Thesis writing
 - **New research questions for next year from results**

Work programme

- It is fundamental to good research that you have a plan.
- The plan links all the steps together.
- In some cases your plan will be wrong. The trick is to know when your plan goes wrong and compensate
- It is a bad plan that can not be changed
 - Publilius Syrus (1st century AD)

Work programme diagram



Timetable

For an MSc project there is a deadline – end of year 2.

- This is fixed, although in certain circumstances it can be moved
 - Illness
 - Problems that are out of your control
- The more in control you are the sooner you will know if you can hit your deadline.
- The earlier you tell us the more we can help you to make the deadline or get the extension.
- Extensions are bad as they are expensive and delay your career

Timetable

- producing a timetable ?
- Step 1 – how long will the different work programme components take to do
- Step 2 – cumulatively do these fit in with the allowed time
- Step 3 – if yes construct final timetable
- If no revise work programme and go back to step 1

Timetable

- Time table milestones - a **milestone** is the end of a stage that marks the completion of a work package or phase – a time for review and reflection
- Examples
 - Agreeing plan with supervisor
 - Obtaining samples
 - Completing experiments on samples
 - Submitting a chapter for review by supervisor
 - The timetable should include milestones and highlight likely bottlenecks.

Risk to human health

- Outline the key risks associated with conducting the work and the necessary precautions.
- Produce risk assessments for laboratory and field/site work. Produce COSHH forms for any hazardous substance to be used.
 - site specific sampling dangers (i.e. rivers in Africa have dangers not typical of the Tyne e.g. Crocodiles, hippos, buffalo....)
 - sample dangers
 - Inoculation to prevent disease
 - Reagents
 - Explosive, corrosive, toxic risks

Project risk

- Identify the risks that affect the management and completion of the project, giving possible solutions to avoid such risks e.g.
 - Difficulty getting samples (alternative sources)
 - Difficulty with access to equipment (prompt start to project, use of booking forms, cooperation)

Efficient execution of project plan

- Do pre-project equipment and consumables inventories
- Have an awareness (and use) of equipment booking sheets (talk to technicians)
- Get specialist software training early
- Where appropriate for experimental studies carry out data monitoring and rapid analysis to assess experimental progression
- Link project milestones\decision gates to supervisor meetings

Effective management of experiment and analyses

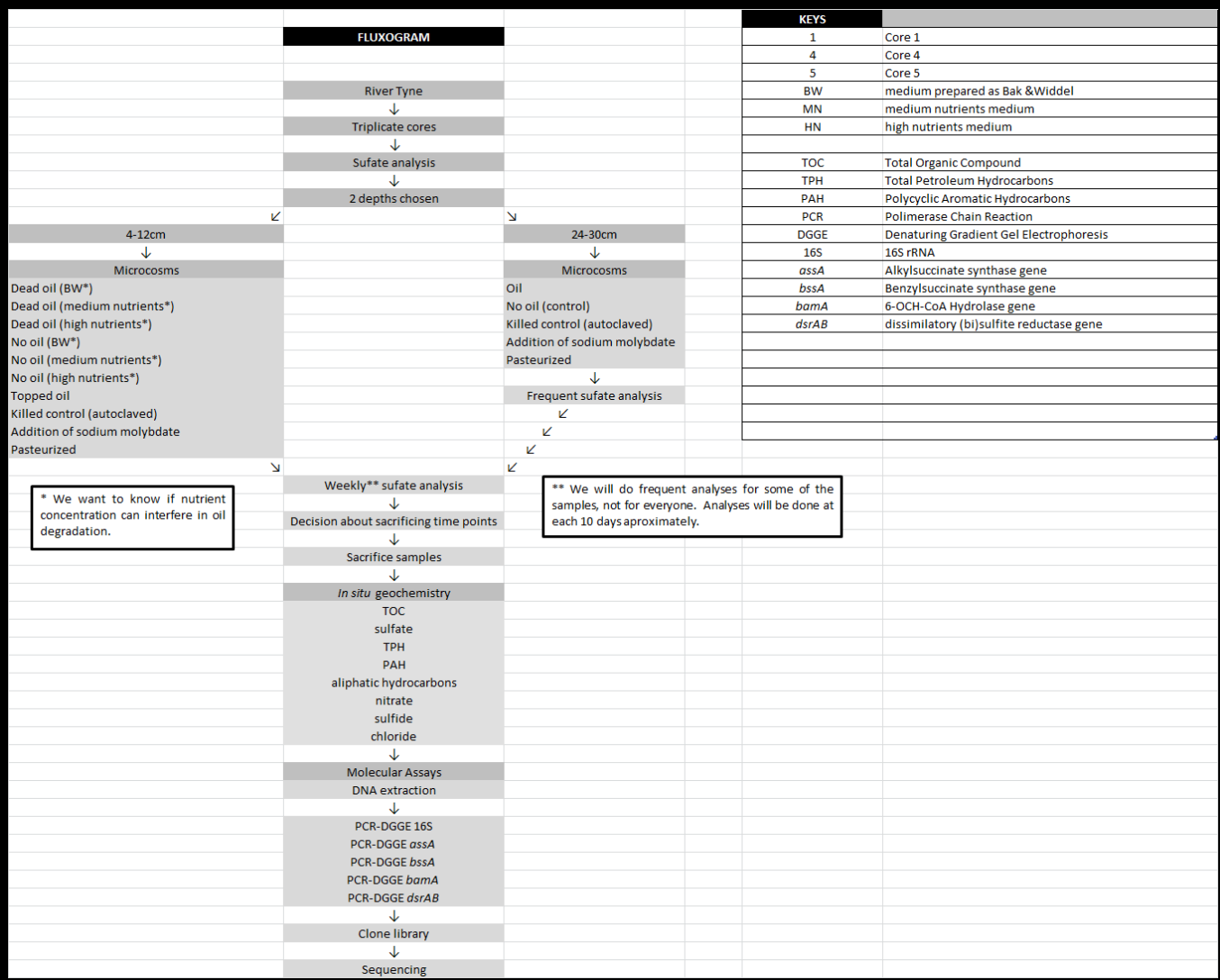
- Use of work programme developed in PIR to inform project progression
- Use of linked excel spread sheets to document and track systematically labelled samples, experiments, datasets, analyses
- Use of excel to carryout basic data summarization i.e. averages, errors calculation
- Use excel to provide basic graphical representations of the data and the data error
- Use excel to manipulate data for export to other data analyses packages

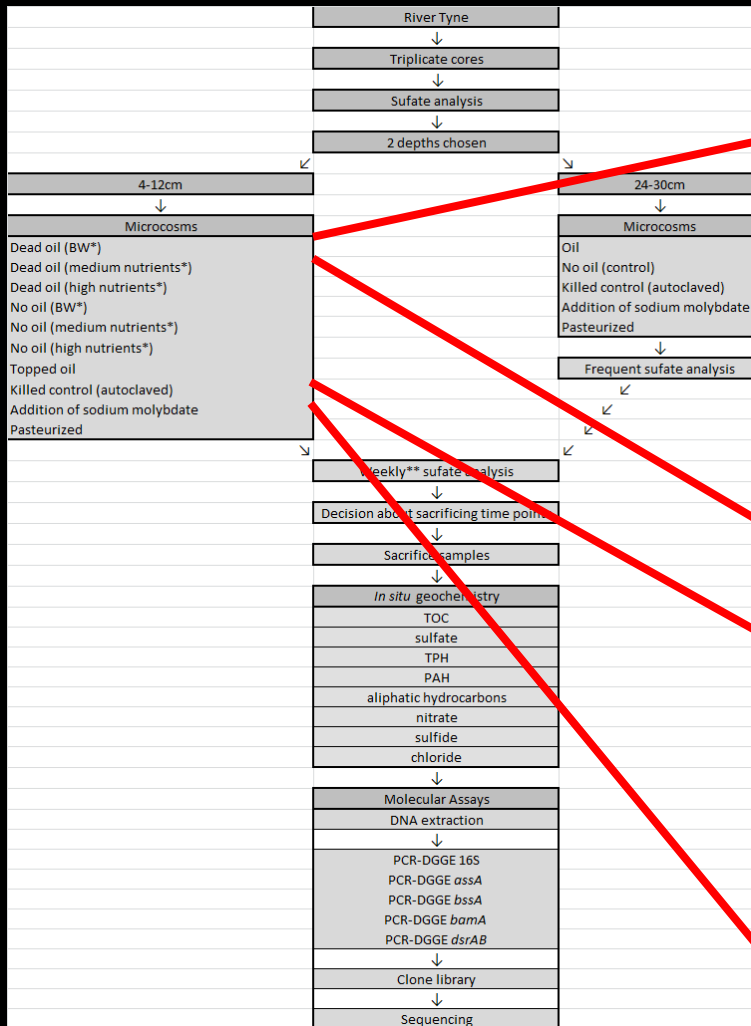
Effective management of experiments and analyses

Details of samples taken

Details of experimental microcosms to be setup

Details of analysis to be carried out on some microcosm samples



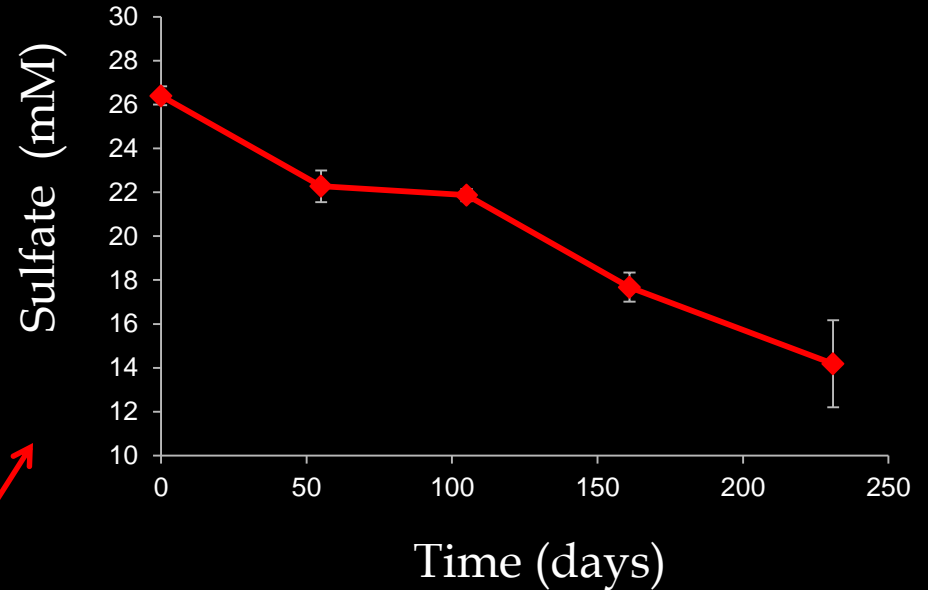


4-12cm/dead oil/ BW/RT						
Bottle	Depth	Oil condition	Nutrients	Temperature	Time zero	Sacrificed
1	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011
2	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011
3	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011
46	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011
47	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011
48	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011
91	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011
92	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011
93	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011
136	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010
137	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010
138	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010
181	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010
182	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010
183	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010
226	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010
227	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010
228	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010

4-12cm/dead oil/ BW/killed						
Bottle	Depth	Oil condition	Nutrients	Temperature	Time zero	Sacrificed
23	4-12cm	dead oil	BW	Killed	06/09/2010	17/08/2011
24	4-12cm	dead oil	BW	Killed	06/09/2010	17/08/2011
67	4-12cm	dead oil	BW	Killed	07/09/2010	17/08/2011
68	4-12cm	dead oil	BW	Killed	07/09/2010	20/04/2011
69	4-12cm	dead oil	BW	Killed	07/09/2010	20/04/2011
22	4-12cm	dead oil	BW	Killed	09/09/2010	20/04/2011
113	4-12cm	dead oil	BW	Killed	09/09/2010	11/02/2011
114	4-12cm	dead oil	BW	Killed	09/09/2010	11/02/2011
112	4-12cm	dead oil	BW	Killed	14/09/2010	11/02/2011
157	4-12cm	dead oil	BW	Killed	14/09/2010	21/12/2010
158	4-12cm	dead oil	BW	Killed	14/09/2010	21/12/2010
159	4-12cm	dead oil	BW	Killed	14/09/2010	21/12/2010
202	4-12cm	dead oil	BW	Killed	17/09/2010	08/11/2010
203	4-12cm	dead oil	BW	Killed	17/09/2010	08/11/2010
204	4-12cm	dead oil	BW	Killed	17/09/2010	08/11/2010

4-12cm/dead oil/ BW/RT								
Bottle	Depth	Oil condition	Nutrients	Temperature	Time zero	Sacrificed	days	sulfate mM
1	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011	351	
2	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011	351	
3	4-12cm	dead oil	BW	RT	31/08/2010	17/08/2011	351	
46	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011	231	17.91
47	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011	231	13.52
48	4-12cm	dead oil	BW	RT	01/09/2010	20/04/2011	231	11.12
91	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011	161	17.45
92	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011	161	16.65
93	4-12cm	dead oil	BW	RT	03/09/2010	11/02/2011	161	18.94
136	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010	105	21.91
137	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010	105	22.34
138	4-12cm	dead oil	BW	RT	07/09/2010	21/12/2010	105	21.37
181	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010	55	20.84
182	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010	55	22.92
183	4-12cm	dead oil	BW	RT	14/09/2010	08/11/2010	55	23.07
226	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010	0	25.81
227	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010	0	27.24
228	4-12cm	dead oil	BW	RT	16/09/2010	17/09/2010	0	26.15

4-12cm dead oil BW RT



SACRIFICED SAMPLES	SACRIFICING TIME POINT				
	0	55	105	161	231
4-12cm dead oil BW RT	25.81	20.84	21.91	17.45	17.91
4-12cm dead oil BW RT	27.24	22.92	22.34	16.65	13.52
4-12cm dead oil BW RT	26.15	23.07	21.37	18.94	11.12
average	26.40	22.28	21.87	17.68	14.18
std error	0.43	0.72	0.28	0.67	1.99

Leave time to write up

- Finish the data acquisition/analysis stage early enough to allow ample time to write your thesis- it takes time to do your findings justice
- Negotiate with supervisors about their input i.e. chapter read through.
- Start writing some chapters e.g. methods and literature review before end of laboratory work